U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOMASS PROGRAM

2009 Biochemical Conversion Platform Review Report:

An Independent Evaluation of Platform Activities for FY2008 and FY2009

December 2009

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Dear Colleague:

This document summarizes the recommendations and evaluations provided by an independent external panel of experts at the U.S. Department of Energy Biomass Program's Biochemical Conversion platform review meeting, held on April 14-16, 2009, at the Sheraton Denver Downtown, Denver, Colorado.

All programs in the Department of Energy's Office of Energy Efficiency and Renewable Energy are required to conduct a biennial peer review of their project portfolios, and this report is intended to officially document the process utilized by the Biomass Program, the results of the review, the program's response to the results and recommendations, and a full compilation of information generated during the review of the Biochemical Conversion platform. Additional information on the 2009 platform and program review meetings—including presentations for all of the individual platforms and the program review—is available on the program review Web site at www.obpreview2009.govtools.us.

The Biomass Program peer review process included a systematic review of the project portfolios in the six separate technology platforms managed by the program and a separate meeting where the program is comprehensively reviewed. The Biomass platform reviews were conducted between March and April 2009 in the Washington, D.C., and Denver, Colorado, areas. The platform reviews resulted in the peer review of the program's projects in applied research, development, and demonstration, as well as analysis and deployment activities. The program peer review held in July 2009 was conducted to evaluate the program's overall strategic planning, management approach, priorities across research areas, and resource allocation.

The recommendations of these expert reviewers are routinely used by the Biomass Program staff to conduct and update out-year planning for the program and technology platforms. The review results are reviewed in combination with other critical project information to result in a complete systematic evaluation of the accomplishment of programmatic milestones, project goals, and objectives.

I would like to express my sincere appreciation to the reviewers. It is they who make this report possible, and upon whose comments we rely to help make project and programmatic decisions for the new fiscal year. Thank you for participating in the 2009 Biochemical Conversion platform peer review meeting.

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U.S. Department of Energy

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Executive Summary

2009 Biochemical Conversion Platform Peer Review
U.S. Department of Energy
Biomass Program

On April 14–16, 2009, the U.S. Department of Energy (DOE), Office of Energy Efficiency and Renewable Energy (EERE), Biomass Program held a peer review of its biochemical and thermochemical conversion platforms. These peer review meetings were collocated, but held in separate, adjoining rooms. Both meetings featured introductory presentations by program staff to provide information on the platform and presentations by the principal investigators of the federally funded projects that make up the conversion platforms project portfolio. Approximately 200 people attended the conversion platform review meetings and learned about the state-of-the-art research, development, and deployment activities being performed by the programs Biochemical and Thermochemical conversion platforms to address both strategic OBP decision-making and to support biomass industry developments Among the attendees were two separate and individual panels of independent experts from outside the program who were tasked with reviewing the research, development, and demonstration (RD&D) activities managed by the Conversion platforms. This report is specific to the review of the Biochemical platform.

Presentations given during each of the platform review meetings, as well as other background information, have been posted on the registration Web site: www.obpreview2009.govtools.us. Additional information—such as the reviewer comments, recommendations, meeting agendas, and a list of attendees—can be found in the individual platform reports.

Biochemical Conversion Platform Peer Review Process

The Biochemical Conversion platform review was one of six platform reviews and one program review held as part of the 2009 Biomass Program peer review. The peer review is a biennial requirement for all EERE programs. The results of the peer review are used by Biomass Program technology managers in the generation of future work plans and in the development of Annual Operating Plans, Multiyear Program Plans, and potentially in the redirection of individual projects.

The goals of the independent review panel were to provide an objective and unbiased review of the individual projects in the platform portfolio as well as the overall structure and direction of the biochemical conversion platform. In forming its review panel, the biochemical conversion platform evaluated a total of 18candidates from industry, academia, and government, with a range of experiences in the technical areas related to the biochemical conversion. An outside, objective steering committee established to help ensure the independence and transparency of the overall peer review process reviewed available biographies for review panel candidates during the planning process and provided feedback and recommendations to the platform teams. Seven reviewers were selected to ensure a breadth of experience and expertise relevant to the platform portfolio. A list of review panel members for the biochemical conversion Platform can be found on page 4 of this report.

At the platform review meeting, project principal investigators (PIs) presented their project budgets, goals, accomplishments, challenges, and relevance to the biochemical conversion platform and answered questions from the review panels and general audience. Projects were evaluated by the review panel solely on the basis of information that was either presented by the PI or contained in a standard program management plan. Reviewers used a software tool developed to facilitate both scoring and constructive comments on a range of evaluation criteria. The results of these evaluations (along with those of the other five platforms) formed the basis for the overall Biomass Program review meeting, which was held on July 14–15, 2009.

Biochemical Conversion Platform Information

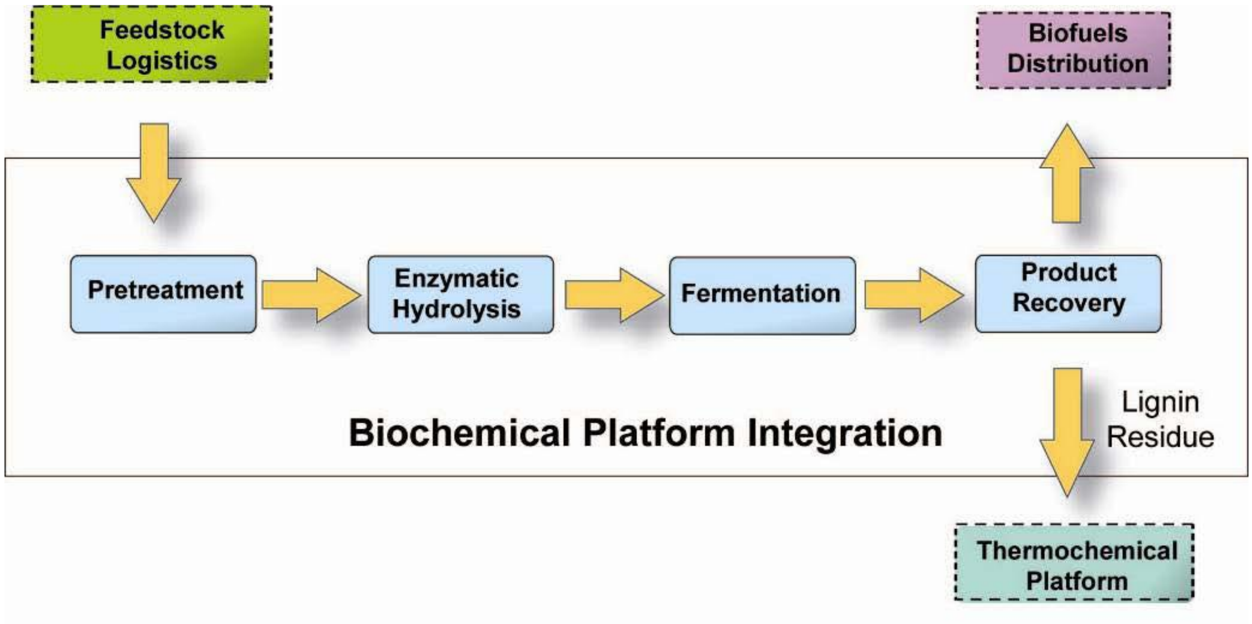
The Biochemical platform is focused on reducing the cost of converting lignocellulosic biomass to mixed, dilute sugars and their further conversion to liquid transportation fuels, such as ethanol, to enable successful integrated biorefineries. Biochemical conversion uses biocatalysts, such as enzymes and microorganisms, in addition to heat and chemical catalysts, to convert the carbohydrate portion of the biomass (hemicellulose and cellulose) into an intermediate sugar stream. The biomass sugars act as intermediate building blocks which are then fermented to ethanol and other products. The remaining lignin portion of the biomass can be used for heat and power, or alternatively used to produce additional fuels and chemicals via thermochemical processing.

Biochemical platform R&D will make further improvements to feedstock interface, pretreatment and conditioning, enzymes and fermentation processes, in addition to process integration in order to reduce sugar costs; these economically viable technologies will act as the springboard to launching the next generation technology to produce ethanol and other products from a wide range of cellulosic feedstocks.

Biochemical Platform Unit Operations

Exhibit 1 outlines the main technologies/unit operations of the baseline biochemical biomass-to-ethanol process. Process details are available in the most recent design report.

Exhibit 1 – Biochemical Platform Integration



Pretreatment (Prehydrolysis): In this step, biomass feedstock undergoes a thermochemical process to break down the hemicellulose fraction of the feedstock into a mixture of soluble five-carbon sugars—xylose and arabinose, and soluble six-carbon sugars—mannose, galactose, and glucose. This partial solubilization makes the remaining solid cellulose fraction more accessible for enzyme saccharification later in the process. A small portion of the cellulose is often converted to additional glucose in this step, and a portion of the lignin fraction may also be solubilized. The specific mix of sugars released depends on the feedstock used and pretreatment.

Conditioning *(Optional)***:** In some process configurations, the pretreated material goes through a hydrolyzate conditioning process which removes undesirable byproducts from the pretreatment process that are toxic to the fermenting organism.

Enzymatic Hydrolysis: In the enzymatic hydrolysis step, the pretreated material, with the remaining solid carbohydrate fraction being primarily cellulose, is saccharified with cellulase enzymes, releasing glucose. Addition of other enzymes, such as xylanases, in this step may allow for less severe pretreatment, resulting in a reduced overall pretreatment and hydrolysis cost. Enzymatic hydrolysis requires several days, after which the mixture of sugars and any unreacted cellulose is transferred to the fermenter. The process concept under development assumes that the cellulase enzymes are purchased from enzyme companies, like other consumable catalysts and chemicals. The current concept may also combine the enzymatic hydrolysis and fermentation steps.

Fermentation: In the fermentation step, an inoculum of a fermenting organism is added and fermentation of all sugars to ethanol is carried out while continuing to utilize the enzymes for further glucose production from any remaining solid cellulose. After a few days of fermentation and continued saccharification, nearly all of the sugars are converted to ethanol. The resulting beer (low-concentration ethanol) is sent to product recovery.

Product Recovery: Product recovery involves distilling the beer to separate the ethanol from the water and residual solids. A final dehydration step removes any remaining water from the ethanol. Residual solids are composed primarily of lignin which can be burned for combined heat and power generation or thermochemically converted to synthesis gas or pyrolysis oil intermediates for other uses. This process is part of the Thermochemical platform focus.

Biochemical Platform Interfaces

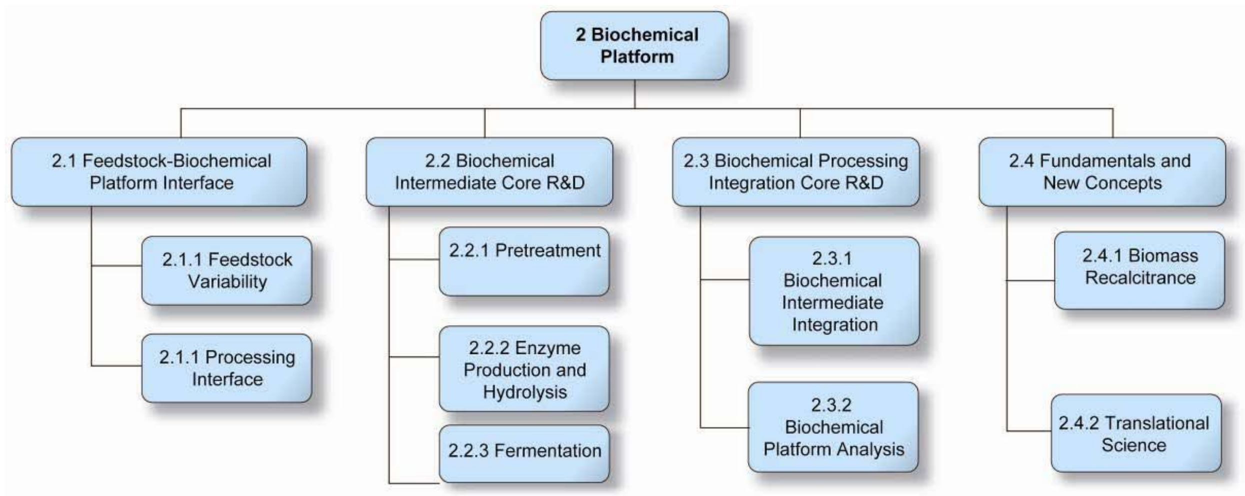
Feedstock Logistics Interface: The Feedstock platform provides preprocessed feedstock that meets the requirements (composition, quality, size, etc.) as defined by the specific biochemical conversion process configuration. Close coordination between the Feedstock and Biochemical Conversion platforms is required to ensure that the feedstock and the process are optimized together for the lowest overall cost and highest conversion efficiency of the biomass.

Thermochemical Platform Interface: Lignin and other byproducts/residues of the biochemical conversion process can be used to produce the electricity required for the production process. Lignin can also be thermochemically converted to fuels and chemicals.

Biofuels Distribution Interface: The next step in the biomass-to-biofuels supply chain is the biofuels distribution step. Biofuels leaving a biorefinery must meet all applicable federal, state, and local codes and standards.

Exhibit 2 summarizes each task element's work as it relates to specific platform barriers and biorefinery pathways. At the peer review meeting, the biochemical conversion platform R&D portfolio was presented in seven technologies area groupings.

Exhibit 2 – Work Breakdown Structure for Biochemical Platform Core R&D



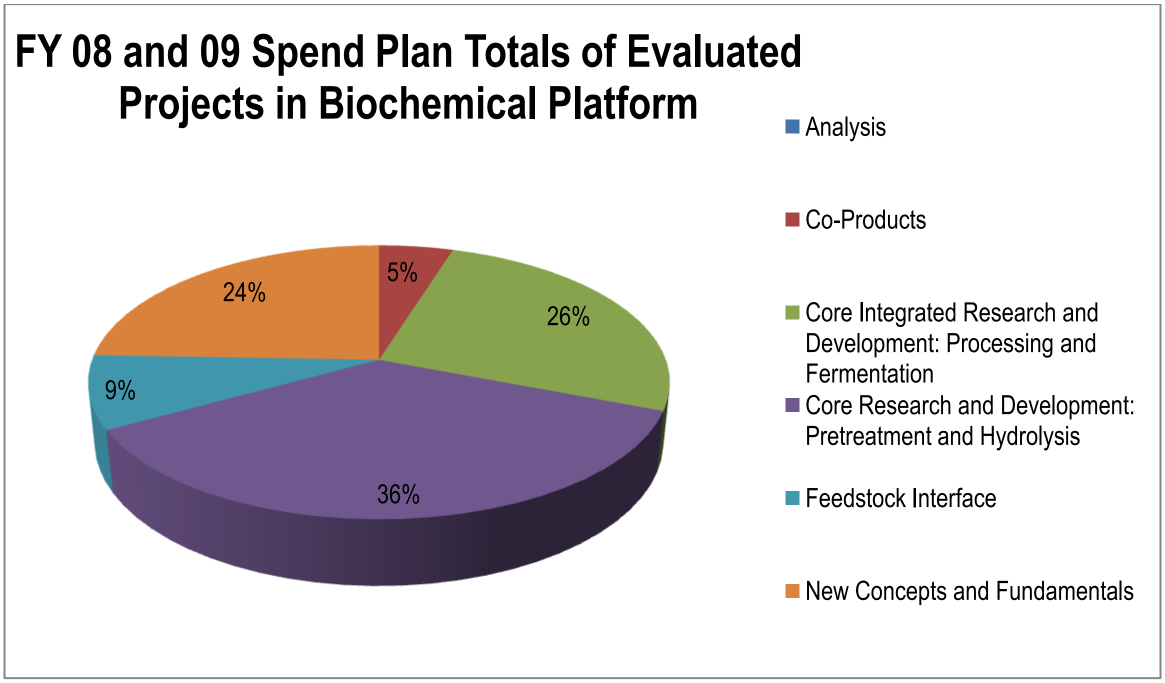
FY 2008 and FY2009 Budgets

The Biochemical platform R&D portfolio was presented in six Technology Area groupings. The total spend plan allocation of projects in each Area is given in Exhibits 3 and 4.

Exhibit 3: Biochemical Platform FY2008 and 2009 funding breakdown for evaluated projects by focus area.

FY 2008 and 2009 Spend Plan Totals of Evaluated Projects in Biochemical Platform	
Technology	**Allocation**
Analysis	$1,600,000
Co-Products	$4,264,150
Core Integrated Research and Development: Processing and Fermentation	$23,032,711
Core Research and Development: Pretreatment and Hydrolysis	$31,865,719
Feedstock Interface	$7,635,205
New Concepts and Fundamentals	$21,605,868

Exhibit 4 – Graphical Representation of Total Spend Plan Allocations; Peer Reviewed Thermochemical Conversion Project Portfolio, FY2008 & FY 2009



Platform Direction for FY 2010

In FY 2010, the Biochemical Conversion Platform will continue its research and development activities with heavy focus on meeting the 2012 cost targets. This will include activities that pursue new biomass pretreatment reactors designs, and improved process integration steps (i.e., pretreatment and downstream processing). In FY2010, the biochemical conversion pathway will also begin to transition its activities toward the pursuit of infrastructure compatible biofuels using alternative biochemical pathways.

Summary from the Review Panel

Integration and Synergies among Projects

The review panel concluded that the projects are effectively buying down the risk for the establishment of a biochemically based biofuels industry. There are several approaches to the various goals and a balance needs to be struck between realizing the benefits of revolutionary technologies and the needs to get a new industrial sector established in a timely way. Choosing winners too early in the cycle can potentially compromise later options but more conservative choices are needed for early systems. An improved set of standards and analytical criteria need to be established to better gauge progress against overall OBP goals. With time the research projects should embrace a wider range of feedstocks and end products, applying the learning that has derived from the current focus on ethanol from corn stover and switchgrass. Analysis of the issues of sustainability, productivity, cost, and scaling potential of feedstock and end product choices should guide system choices. In general there needs to be more outreach and technology transfer to allow industry to benefit from modeling and analytical capabilities and new process IP.

Algal based fuels research

The Algae projects do not seem to be a good fit for the platform until there is a better understanding of the research and development pathways required to make algae competitive. Specifically the algae program should be subject to the same requirements and metrics as the ethanol or other fuels projects. There must be a more robust understanding of the role of DOE funding in advancing the algae industry and of the strategic criteria for DOE long-term investments.

Pretreatment and Enzymatic Hydrolysis

There is a robust variety of approaches to solving the enzyme problem which makes a nice portfolio. The skill set in metabolic engineering and enzyme optimization is impressive. Much of the work is dependent on Mother Nature however, utilizing screens of natural systems, and manipulation to achieve the needed results. This needs to evolve to a more rational approach, more effort in understanding enzyme mechanistics, and more high risk research in design and engineering of enzymes. Such work will of necessity be multidisciplinary in nature and require substantial teams. While the validation techniques now in use were impressive there needs to be a new generation of diagnostic capabilities, especially to support process integration efforts going forward.

Process Integration and Fermentation

Process integration research is critical as new technologies are incorporated into process streams. There is an impressive set of efforts that are well on target to bring the needed elements together and to develop the modeling and diagnostics to support this essential step. These efforts need to be expanded to new feedstocks, processing technologies, and end products and the program expands as discussed above.

Fermentation (Ethanologens)

The projects in this area are well chosen. With time there should be more analysis of mixed cultures and the focus on C5 and C6 sugars can be expanded to perhaps C1. Metabolic engineering capability development will be crucial for success, and consideration should be given to both split or combined streams. It is also important to continue work on inhibitors. Commercially driven projects are important since they are closer to endpoints that will reduce the risk for meeting near and mid-term OBP goals. Key to a process environment is better diagnostics (throughout the process chain) –development of analytical tools that can help support more real-time diagnostics should be encouraged.

Platform Discussion

This is an impressive overall portfolio that was well chosen and has been productive. Interactions across private and public entities have provided energy and have kept all parties advancing. With the enormity of the challenge current funding is not adequate to fully meet the congressionally mandated technical goals. Under current funding efforts must be focused to better guarantee success and to take advantage of progress. New resources must be used to expand the range of targets and encourage further collaboration. Continuity and expansion of current successful efforts is critical for success. To fill gaps in the portfolio investments should be made in alternative outputs, new tool sets, broadly available systems biology databases, and an expanded set of analyzed organisms and plants supported by a curation effort to warehouse ranges of standardized materials and analyzed samples. This can include expanded outreach in many forms, examination of the next generation of problems, and better integration with for example the Office of Science Bioenergy Research Centers.

Platform and Project Evaluation Results

The Biochemical Conversion platform management actively uses the qualitative and quantitative information resulting from the review process to consider the future direction of the platform RDD&D activities, and project and platform goals, approach, and targets and milestones. The numerical rating scale used for this review was a whole number scale, where 5=Excellent, 4=Good, 3=Satisfactory, 2=Fair, and 1=Poor.

Overall, the platform activities were evaluated positively. The overall average score given to the platform was a 4.07. The average of the 39 project score was 3.81. Copies of the platform and project evaluation forms can be found in Attachments 1 and 2 at the end of this report.

Platform Evaluation

At the conclusion of the project review, the review panel evaluated the overall platform management on the basis of the five evaluation criteria, listed below.

Platform Evaluation Criteria and Rating System

Goals *– Are platform goals, technical targets and barriers clearly articulated? Are platform goals realistic and logical? Do the platform goals and planned activities support the goals and objectives of the Biomass Program as outlined in the MYPP? How could the platform change to better support the Biomass Program's goals?*

Approach *– How well does the platform approach (platform milestones and organization, RD&D portfolio, strategic direction) facilitate reaching the Program Performance Goals for each platform as outlined in the MYPP? What changes would increase the effectiveness of the Platform?*

RD&D Portfolio *– The degree to which the Platform RD&D is focused and balanced to achieve Biomass Program and Platform goals? (WBS, unit operations, pathway prioritization)*

Progress *– Based on the presentations given, how well is the platform progressing towards achieving Biomass Program and Platform goals? Are we meeting our performance targets? Is it on track to meet the goals presented? Please provide recommendations on improvements for tracking progress in the future.*

A summary of the reviewer evaluation scores of the Biochemical conversion platform is presented in Exhibit 5. The average score represents an equally weighted average of the four scored platform evaluation criteria. In addition to the platform evaluation scores, an evaluation of the subplatform areas was performed by aggregating individual project scores.

Exhibit 5 – Average Evaluation Scores of the Biomass Program Biochemical Conversion Platform for Each of the Four Scored Criteria

Evaluation Criteria	Average Score*	StdDev
Platform Goals	4.14	0.69
Platform Approach	4.14	0.69
Platform RD&D Portfolio	4.14	0.69
Platform Progress	3.86	1.07

** Average represents mean of individual reviewer scores. Review panels did not develop consensus scores.*

Please see Section IIB for detailed explanations of the criteria. Please see the detailed responses to each evaluation criteria throughout Section IIB as well as Section IIC for the full summary response.

Project Evaluations

The review panel evaluated individual RD&D projects in the six subplatform technology focus area (Analysis, Co-Products, Core Integrated Research and Development: Processing and Fermentation, Core Research and Development: Pretreatment and Hydrolysis, Feedstock Interface, New Concepts and Fundamentals). This breakdown of work mirrors the platform management for the current review period. Each project was evaluated on both the strength of the work and the relevance of the work to the platform objectives. Five scored evaluation criteria were used, applying the same 1–5 whole-number rating system used for the platform evaluations.

Project Evaluation Criteria

Relevance *– The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program MYPP. Market application of the expected project outputs has been considered.*

Approach *– The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.*

Technical Progress *– The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the Biomass Program MYPP and overcoming technical barriers outlined in the MYPP.*

Success Factors *– The degree to which the project has identified critical success factors (technical, business, and market factors) that will impact technical and commercial viability of the project and the degree to which the project has identified potential show-stoppers (technical, market, regulatory, legal) that will impact technical and commercial viability.*

Future Research *– The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off-ramps, or identified other opportunities to build upon current research to further meet Biomass Program goals and objectives.*

The evaluation scores were aggregated at the technology focus area level. Overall, the strength of work of the individual projects was clear—as, on average, the RD&D work in the four focus areas was evaluated as highly relevant to platform objectives, of sound technical approach, making good technical progress, aware of challenges and success factors, and generally on track for the future. The project presentations are available in PDF format at http://www.obpreview2009.govtools.us/biochem/. Each project was reviewed by 3–7 reviewers in five scored review criteria. The overall average scores of projects in each technology focus area are given in Exhibit 6.

Exhibit 6 – Review Panel Average Scores* for Biochemical Conversion SubPlatform Areas for Each Project Evaluation Criteria

Technology Area	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Analysis	4.2	4.15	3.95	3.8	3.7	4
Co-Products	2.94	3.21	3.06	2.94	2.94	3.01
Core Integrated Research and Development: Processing and Fermentation	4.25	4.13	3.97	3.69	3.79	3.97
Core Research and Development: Pretreatment and Hydrolysis	4.39	4.16	3.85	3.71	3.96	4.01
Feedstock Interface	3.88	3.65	3.80	3.50	3.63	3.70
New Concepts and Fundamentals	4.35	4.35	4.25	3.98	4.025	4.20
	4.00	3.94	3.81	3.60	3.67	3.81

** Average scores represent the mean of individual reviewer scores. Review panels did not develop consensus scores.*

Detailed explanations of the project evaluation criteria are can be found in Section IIIA with the individual project evaluations. The scores presented below are the mean scores of the all the projects evaluated in the IBR platform.

Summary Platform Management Response

The platform Management Team appreciated the comments and recommendations provided by the reviewers through this review process and will consider and utilize this information to shape platform activities in the future.

Platform goals will continue to be evaluated regularly to ensure that the thermochemical platform responds appropriately to changing feedstock types and availability.

Exhibit 7 lists each project that presented at the review and a summary of next steps determined by the platform management.

Exhibit 7 – Summary of Evaluation Scores of Projects in the Thermochemical Platform Portfolio

WBS Number	Project Title; Presenting Organization; PI Name	Final Average Score	Next Steps: Continue Project	Next Steps: Continue w/ possible adjustments to Scope	Next Steps: Other	Technology Manager Summary Comment
2.6.1.1	Biochemical Platform Analysis, NREL, David Hsu, Ph.D.	4.26	X			This project quantifies the platforms technical targets and progress towards achieving that goal. In 2009 this task with support from others will develop an updated biochemical conversion design report.
2.6.1.2	Analysis for Production—Technical and Market Analysis, PNNL, Sue Jones	3.66		X		This project provides analytical guidance on the potential of future research and development pathways. The subtasks of this task are agreed upon yearly between PNNL and headquarters. Reviewer comments will be taken into consideration while choosing FY10 scope.
2.4.1.2	Fungal Genomics, PNNL, Scott Baker	4.26	X			The fungal biotechnology project provides knowledge and technology for filamentous fungal systems to provide industry with the enabling tools to rapidly and effectively develop many new processes.
2.1.1.1	Storage Systems, Feedstock Supply,	4.30	X			The project is to optimize the

and 2.1.1.3	Etc., Nick Nagle, NREL						characteristics of the feedstock for the process and vice versa.
2.1.1.6	Extremophilic Microalgae: Advanced Lipid and Biomass Production for Biofuels and Bioproducts, Montana State University, Brent M. Peyton, Ph.D.	3.49	X				This project is to focus on determining growth and lipid production of existing alkaliphilic populations with intent to utilize selected alkaliphilic algae for lipid production in open test ponds.
2.1.1.7	Improving Cost Effectiveness of Algae-Lipid and Biomass Production for Biofules and Bioproducts, University of Georgia Research Foundation, KC Das, Ph.D., P.E.	3.20	x				This project is attempting to reduce costs associated with algae production and establish the viability of carbon capture technologies for providing CO2 at high-rate to algae ponds.
7.2.1.1	Bioenergy Demonstration Project: Value-Added Products from Renewable Fuels, University of Nebraska-Lincoln, Paul Blum, Ph.D.	4.0				X	This project is investigating thermoacidophilic microbes for establishment of the deconvolution and saccharification of lignocellulose to maximize biofuel yields.
2.2.1.1	Pretreatment and Enzymatic Hydrolysis, NREL, Rick Elander	4.37	X				This task investigates and evaluates pretreatment approaches that are aimed at increasing the digestibility of residual cellulose.
2.2.1.2	Value Prior to Pulping, CleanTech Partners, Carl Miller, Ph.D.	3.54				X	This project is fully funded and will be closing out in fiscal year 2010.
2.2.2.2	Energy Corn Consortium, Edenspace Systems Corporation, Michael J. Blaylock, Ph.D.	3.37				X	This project is fully funded and will be closing out in fiscal year 2010.

2.3.1.4	Integration of Leading Biomass Pretreatment Technologies with Enzymatic Digestion and Hydrolyzate Fermentation, CAFI, Charles E. Wyman, Ph.D.	4.66		X	The CAFE3 project examined the effectiveness of multiple pretreatments on several different batches of switchgrass feedstock. This project is coming to its natural end in FY10.
2.2.2.3	Enzyme Solicitation Support and Validation, NREL, James D. McMillan, Ph.D.	4.49	X		This project continues to monitor and evaluate the developments within the enzyme projects.
2.2.2.5	Enhancing Cellulase Commercial Performance for the Lignocellulosic Biomass Industry, Danisco USA, Mike Arbige, Ph.D.	3.83	X		This is one of the four projects selected from the Enzyme Solicitation. This project is ongoing and supporting the programmatic cost targets.
2.2.2.6	Development of a Commercial Enzyme System for Lignocellulosic Biomass Saccharification, DSM Innovation, Manoj Kumar	3.89	X		This is one of the four projects selected from the Enzyme Solicitation. This project is ongoing and supporting the programmatic cost targets.
2.2.2.7	Project Decrease: Development of a Commercial-Ready Enzyme Application System for Ethanol, Novozymes, Paul Harris, Ph. D.	4.37	X		This is one of the four projects selected from the Enzyme Solicitation. This project is ongoing and supporting the programmatic cost targets.
2.2.2.8	Commercialization of Customized Cellulase Solutions for Biomass Saccharification, Verenium Corporation, Justin Stege, Ph.D.	4.03	X		This is one of the four projects selected from the Enzyme Solicitation. This project is ongoing and supporting the programmatic cost targets.

2.2.2.9	Addressing the Recalcitrance of Cellulose Degradation through Cellulase Discovery, Nano-scale Elucidation of Molecular Mechanisms, and Kinetic Modeling, Cornell University, Larry Walker, Ph.D.	3.71	X			The purpose of this task is to identify other potential available cellulases found in the community of highly virulent plant pathogenic fungi and bacteria
7.2.2.2	Advancing Texas Biofuel Production, Baylor University, Kevin Chambliss, Ph.D.	3.80			X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. The project is focused on fundamental information on plant variety and relative amounts of degradation products
2.3.1.1	Biochemical Processing Integration Task, NREL, Dan Schell	4.60	X			The overall objective of this project is to investigate enzymatic cellulose hydrolysis-based biomass-to-ethanol conversion process technology based on a large-scale domestic feedstock (corn stover is the model feedstock)..
2.3.1.5	Integrated Biorefinery—Separations/Separative Bioreactor—Continuous Bioconversion and Separations in a Single Step, ANL, Seth Snyder	4.0	X			The project objective is to address the cost of production of organic acids separation of organic acids and amino acids.
2.3.2.7	Lab Validation for Organism Development Solicitation Recipients, NREL, Nancy Dowe Farmer	4.31	X			This project continues to monitor and evaluate the developments within the ethanologen projects.

2.3.2.1	Biocatalyst for Fermenting Hydrolyzate at Low pH and High Temperature, Cargill, Gary Folkert	4.26	X		This is one of the five projects selected from the Ethanologen Solicitation. This project is ongoing and supporting the programmatic cost targets. This project will receive additional review at the 18 month point of the project.
2.3.2.2	Improvement of Zymomonas Mobilis for Commercial Use in Corn-based Biorefineries, DuPont, Bill Hitz, Ph.D.	4.43	X		This is one of the five projects selected from the Ethanologen Solicitation. This project is ongoing and supporting the programmatic cost targets. This project will receive additional review at the 18 month point of the project.
2.3.2.3	Development of Thermoanaerobacterium Saccharolyticum for the Conversion of Lignocellulose to Ethanol, Mascoma, David Hogsett, Ph.D.	4.17	X		This is one of the five projects selected from the Ethanologen Solicitation. This project is ongoing and supporting the programmatic cost targets. This project will receive additional review at the 18 month point of the project.
2.3.2.4	Improvements in Ethanologenic Escherichia Coli and Klebsiella Oxytoca, Verenium Corporation, David Nunn, Ph.D.	3.91	X		This is one of the five projects selected from the Ethanologen Solicitation. This project is ongoing and supporting the programmatic cost targets. This project will receive additional review at the 18 month point of the project.

2.3.2.5	Further Improvement of the Robust Recombinant Saccharomyces Yeast for the Conversion of Lignocellulosic Biomass to Ethanol, Purdue University, Nancy Ho, Ph.D.	3.91	X	This is one of the five projects selected from the Ethanologen Solicitation. This project is ongoing and supporting the programmatic cost targets. This project will receive additional review at the 18 month point of the project.
2.3.2.8	A Novel Simultaneous-Saccharification-Fermentation Strategy for Efficient Cofermentation of C5 and C6 Sugars Using Native, non-GMO Yeasts, University of Toledo, Patricia Relue	3.89	X	The objective of this project is to develop cost-effective biocatalysts capable of increasing utilization of C5 and C6 sugars by native yeast in the conversion of lignocellulosic biomass to ethanol.
2.3.3.1	Production of Higher Alcohol Liquid Biofuels via Acidogenic Digestion and Chemical Upgrading of Organic Industrial Wastes, University of Maine, Peter van Walsum, Ph.D., P.E.	3.6	X	This project is trying to determine the optimal yield and productivity of high potential bacteria at moderate to high temperatures, on regionally available feedstock.
7.2.3.1	BioEthanol Collaborative, Clemson University, Mike Henson, Ph.D.	2.57	X	This project assesses the use of regional feedstocks, switchgrass and sorghum varieties in South Carolina and the Southeast for production of cellulosic-based ethanol.
2.4.1.1	Targeted Conversion Research, NREL, Mike Himmel, Ph.D.	4.77	X	This project focuses on developing higher efficiency technologies for sugar generation from lignocellulose, with focus on reduced costs of feedstock, pretreatment (prehydrolysis), and enzymes.

2.4.1.3	Lignin as a Facilitator, not a Barrier, during Saccharification by Brown Rot Fungi, University of Minnesota, Jonathan Schilling	4.03	X			This project characterizes the approach taken by brown rot fungi to enhance C5 and C6 sugar release from biomass
7.2.4.1	Ethanol Fuel Development, Arkansas State University, Elizabeth Hood, Ph.D.	3.66			X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. The project is focused on improving recovery of cellulase enzymes from transgenic corn seed, lowering the cost of production by increasing the amount of enzyme per dry weight of production material and/or enhancing activity
7.4.1.2	Biofuel Production Initiative, Claflin University, Dan Page	2.09			X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. This project is to develop cellulosic processes for utilizing sugarcane grown in the state to produce biobutanol as an alternative fuel.
7.4.1.4	Sustainable Energy Center Biodiesel from Algae, Western Michigan University, John B. Miller, Ph.D.	2.83			X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. The project explores the technical and economic feasibility of converting two waste streams into fuels that can be used with existing transportation infrastructure and vehicles

7.4.2.4	Bioeconomy Initiative, MBI International, Susanne Kleff, Ph.D.	3.69		X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. This project is investigating the feasibility of producing and recovering organic acids through fermentations using an industrially stable strain for the production of organic acids
7.4.2.6	Intermediary Biochemicals, Doug Burdette, Ph.D.	3.06		X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. The project is developing platform systems to cost effectively produce intermediate chemicals from renewable feedstocks using sustainable processes.
7.4.5.2	Development of Applied Membrane Technology for Processing Ethanol from Biomass, Compact Membrane Systems, Sudip Majumdar, Ph.D.	3.23		X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. The project focuses on developing separations technologies for separation of ethanol and water
7.4.1.6	Snohomish County Biodiesel Project, Snohomish County, Deanna Carveth	2.94		X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. This project focuses on development of catalyst for biodiesel production.
7.4.3.7	Connecticut Biodiesel Power Generator, Greater New Haven Clean Cities Coalition, Carla York and Robert Schmitz	3.31		X	THIS IS A CONGRESSIONALLY DIRECTED PROJECT. The project focuses on working with local, state and regional officials to identify and streamline regulations for biodiesel power facilities.

** Average represents mean of individual reviewer scores. Review panels did not develop consensus scores.*
Each project is identified by a unique code (WBS Number), as well as the project title, presenting organization, and PI name. Projects are listed in the chronological order by which they presented at the review meeting. The average overall score is the mean of the five evaluation criteria scores. The Next Steps column is a summary of the management response to the evaluation.

I. Introduction

On April 14–16, 2009, the U.S. Department of Energy (DOE), Office of Energy Efficiency and Renewable Energy (EERE), Biomass Program held a peer review of its Biochemical Conversion platform. The platform review was part of the overall 2009 program peer review implemented by the Biomass Program. The peer review is a biennial requirement for all EERE programs to ensure:

"A rigorous, formal, and documented evaluation process using objective criteria and qualified and independent reviewers to make a judgment of the technical/scientific/business merit, the actual or anticipated results, and the productivity and management effectiveness of programs and/or projects."

The results of the peer review are used by Biomass Program Technology Managers in the generation of future work plans and in the development of Annual Operating Plans, Multiyear Program Plans (MYPPs), and potentially in the redirection of individual projects.

Leslie Pezzullo was designated by the Biomass Program as the lead for the Biochemical Platform. Ms. Pezzullo was responsible for all aspects of planning and implementation including coordinating the review panel, coordinating with principal investigators, and overall planning for the Platform Review.

Approximately 200 people attended the Conversion Platform Review meeting, as the Biochemical and Thermochemical Platform reviews were held concurrently. The project and platform review forms that were used to collect information from the reviewers are presented in Attachments 1 and 2 of this report. An agenda for the meeting is provided in Attachment 3. A list of attendees is provided in Attachment 4. Presentations given during each of the Platform Review meetings as well as other background information are posted on the registration website: www.obpreview2009.govtools.us.

The remainder of this section provides a brief description of the implementation process for the Platform Review meetings, identifies the Biochemical Conversion Platform Review Panel, and describes the role of the Steering Committee.

A. Biomass Program Peer Review Process

The 2009 Biomass Program peer review process consisted first of a series of six platform peer review meetings followed by the overall program review meeting. The six platforms that were peer reviewed matched the manner in which the Biomass Program organizes its research and analysis activities. The platforms are Integrated Biorefinery, Infrastructure, Analysis, Feedstocks and Sustainability, Biochemical Conversion, and Thermochemical Conversion. The platform review meetings were held during the February–April timeframe.

The six platform review meetings consisted of technical project-level reviews of the research projects funded in each of the six Biomass technology platform areas. The overall structure and direction of the platform was also reviewed. A separate review panel and chair were formed for each platform review. Review panels were comprised of independent, external technical reviewers with subject matter expertise related to the platform being reviewed.

The program review was held in July 2009 following each of the six platform reviews. During the program peer review, an independent external panel evaluated the strategic organization and direction of the Biomass Program, using the results of the platform reviews and presentations from each of the platform review chairs as input. The panel for the Biomass Program review consisted of a steering committee formed to provide overall oversight of the program peer review process. The program review panel also will include the chair from each platform review panel.

This report represents the results of the Biochemical Conversion platform review and evaluation of the platform and the individual projects in its research portfolio. A separate program review report has been prepared for each platform review and the program review meeting. The program review report may also include additional comments related to the Biochemical Conversion platform.

The Biomass Program followed guidelines provided in the EERE *Peer Review Guide* in the design and implementation of the platform reviews and program peer review. An outside steering committee was established to provide recommendations and help ensure an independent and transparent review process. A description of the general steps implemented in each of the program peer review process is provided in Exhibit 4.

Neil Rossmeissl of the Biomass Program was assigned by the Biomass Program Manager as the peer review leader. Mr. Rossmeissl managed all aspects of planning and implementation. He was supported by a planning team comprised of staff from the Biomass Program, DOE Golden Office, National Renewable Energy Laboratory (NREL) Systems Integrator and contractor support. BCS, Incorporated was the lead contractor responsible for organizing each of the peer review. The team held weekly planning meetings beginning September 2008 to outline the review procedures and processes, to plan each of the individual platform reviews and subsequent program review and to ensure that the process followed EERE peer review guidance.

B. Biochemical Conversion Platform Review Panel

Each platform portfolio was reviewed by a review panel of experts from outside the program. The purpose of the review panel is to provide an objective, unbiased and independent review of the individual research, development, and deployment (RD&D) or analysis projects as well as the overall structure and direction of the platform. One member from each review panel also served as the panel chairperson and was responsible for coordinating review panel activities—ensuring independence of the panel, overseeing the production of the platform review report, and representing the panel at the program peer review in July.

In forming its review panel, the Biochemical Conversion platform evaluated 18 candidates for its review panel. Candidates were evaluated based on their subject matter knowledge in the technology platform area, willingness to commit the time and energy needed to serve on the panel, and lack of a conflict of interest (COI), as represented by receipt of their COI form. An outside, objective steering committee—established to help ensure the independence and transparency of the overall peer review process—reviewed available biographies for review panel candidates during the planning process and provided feedback. Platform review planning teams considered the steering committee feedback in making final decisions on its review panel. Exhibit 8 lists review panel members for the Biochemical Conversion platform. Per steering committee guidance, at least three of the Biochemical Conversion platform reviewers were assigned to review each project. Reviewer assignments were based on reviewer expertise and to avoid conflict of interest.

Exhibit 8 – Basic Steps in Implementing the Biomass Program Peer Review

1. The program's RD&D and Analysis project portfolio was organized by the six platform areas.

2. A lead was designated for each platform review. The platform review lead was responsible for all aspects of planning and implementation including coordinating the review panel, coordinating with principal investigators, and overall planning for the platform review.

3. Each platform identified projects for review. Target: review at least 80% of program budget.

4. A steering committee of external, independent experts was formed to provide recommendations for designing and implementing the review and the scope, criteria and content of the evaluation.

5. Draft project-level, platform-level and program-level evaluation forms were developed for the 2009 platform review meetings. Similarly, a draft presentation template and instructions were developed. EERE *Peer Review Guidelines* and previous forms were evaluated in developing the drafts. Separate forms were used for RD&D and Analysis projects. The forms were reviewed and modified by the steering committee before being finalized.

6. Each platform lead identified candidate members for the platform review panel. The peer review lead requested steering committee feedback of candidate reviewers. Biographies that were available were provided to the steering committee for review. Committee provided *Yes/No* recommendations on candidates and recommended other candidates for the platforms to consider. Results were provided to platform leads for consideration in final selection of review panels.

7. Upon confirmation, each review panel member was provided background information on the review, instructions, evaluation forms, presentation templates and other information needed to perform his or her duties. Project lists and COI forms were provided to each reviewer in advance of the review meeting and COI forms were collected. At least one conference call was held for each review panel to provide instructions, discuss panel member responsibilities and to address any questions. To the extent possible, steering committee members participated in those calls.

8. The Biomass Program performed outreach to encourage participation in each of its platform review meetings by sending announcements to over 3,000 program stakeholders, principal investigators, and attendees at previous program events. The program reviews were also announced on the Biomass Program Web site.

9. Platforms invited PIs to present their projects at the platform review. PIs were provided with presentation templates and instructions, reviewer evaluation forms, and background information on the review process. Follow-up calls were held with PIs to address questions. If PIs chose not to present they were requested to submit a form stating such.

10. Platform review meetings were held according to guidelines developed by the peer review lead and planning team, platform lead, and steering committee. Members of the steering committee participated in each review to ensure consistency and adherence to guidelines.

11. Review panel evaluations were collected during each platform review meeting using an automated tool. These evaluations were posted to a password-protected Web site following each review and review panelists were provided approximately 10 working days to update and edit their comments. PIs were then provided approximately 10 working days to go to the same password-protected Web site and see comments on their projects. PIs were given the opportunity to respond to review panel evaluations.

12. Results of review panel evaluations and PI responses were provided to each platform review lead for overall evaluation and response. The compilation of these inputs was then used to develop this report.

Exhibit 9 – Biochemical Conversion Review Panel

Name	Affiliation/ Title	Expertise
Carl Anderson	Senior Geneticist and Biology Chairman, Brookhaven National Laboratory	Genetics
David Berry	Partner, Flagship Ventures	Cell Biology
Mike Cotta	Supervisory Microbiologist , U.S. Department of Agriculture	Fermentation
Mike Knotek	Consultant, Knotek Scientific Consulting	Transitional science
Mike Penner	Associate Professor, Oregon State University	Enzymes
Jan Pero	Director of Specialty Chemicals, BioEnergy International, LLC	Metabolic engineering of microorganisms
Lise Raleigh	Chief Technology Development Officer, New England Biolabs	Enzymes and strains

**Review Chair*

C. Organization of This Report

The remainder of this document provides the results of the Feedstock platform review meeting, including the following:

- Results of review panel comments on the overall Biochemical Conversion
- Results of review panel comments on projects evaluated during the platform review and PI responses to reviewer evaluations for their projects
- The Biomass Program Biochemical Conversion platform Technology Manager response to review panel comments and discussion of next steps for each project.

II. Platform Overview and Evaluation Results

A. Platform Overview

A. Platform Goals and Objectives

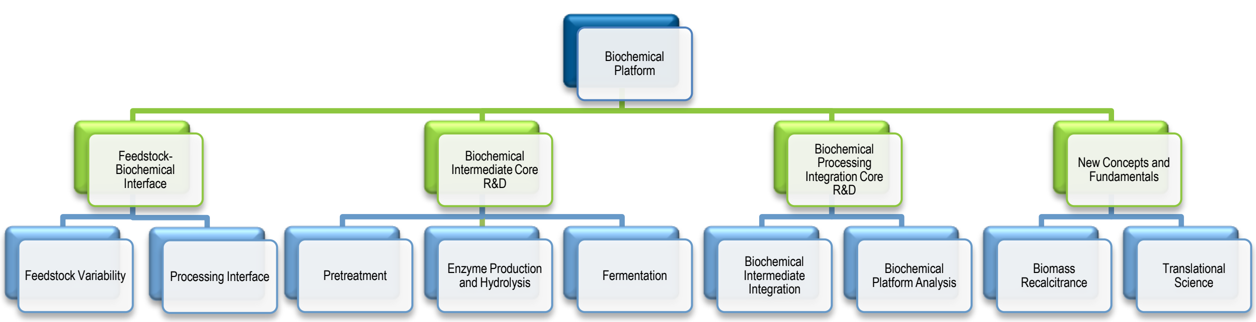
The fundamental goal of the Conversion platform (biochemical and thermochemical) is to cost-effectively convert feedstocks into transportation fuel, bioproducts, and biopower. In particular, the Biochemical platform aims to reduce the modeled processing cost of converting feedstocks to ethanol to $0.92 per gallon by 2012 and $0.60 per gallon by 2017 (2007 dollars). Other measures of performance for the platform include:

- Validate integrated pretreatment and enzymatic hydrolysis of corn stover (dry and wet) at pilot scale by 2012. In addition, validate or optimize integrated production of ethanol from corn stover derived sugars at pilot scale by 2012.
- Validate integrated pretreatment and enzymatic hydrolysis of switchgrass and integrated production of ethanol from switchgrass derived sugars at pilot scale by 2017.

B. Platform Work Breakdown and Major Milestones:

There are four sub-platforms under the Biochemical platform: Feedstock-Biochemical Interface; Biochemical Intermediate Core Research and Development; Biochemical Processing Integration Core Research and Development; and New Concepts and Fundamentals. The following table, Exhibit 10, breaks down the Biochemical R&D portfolio by sub-platform.

Exhibit 10: Biochemical Platform Core R&D



Feedstock-Biochemical Interface

Activities under the first sub-platform, Feedstock-Biochemical Interface, are designed to keep or improve feedstock yield potential through the biochemical conversion process. Major milestones outlined in the 2008 Multiyear Program Plan (MYPP) include:

2007-2012

- Characterize or optimize lignocellulosic biomass feedstocks
- Assess or mitigate impacts of biomass characteristics on downstream unit operations
- Determine process sensitivity to differences in feedstock type and quality
- Identify required process modifications to accommodate feedstock differences
- Evaluate technology options and trade-offs with respect to feedstock assembly and preprocessing with biochemical conversion processes
- Validate feedstocks as received from feedstock logistics systems at pilot scale

2013-2017

- Design and manipulate plant cell wall composition and structure to maximize yield of fermentable sugars
- Continue efforts with new or emerging feedstocks

Biochemical Intermediate Core Research and Development

There are three main components to this platform: processing, enzyme production and hydrolysis, and fermentation. Projects are centered on lowering the operating costs, particularly pretreatment. They also have the objective of increasing the enzymatic digestibility of residual cellulose and hemicellulose in pretreated biomass material. The following milestones were listed in the 2008 MYPP:

2007-2012

Pretreatment:

- Evaluate and compare lignocellulosic biomass pretreatment options with respect to hemicellulose conversion, cellulose digestibility, and ethanol production
- Further develop pretreatment options with the highest likelihood of success
- Validate targeted performance in pilot- scale pretreatment reactor systems

Enzyme Production and Hydrolysis:

- Lower enzyme cost through development of high-activity enzyme mixtures and low-cost production processes
- Define optimum enzymatic hydrolysis conditions or reactor design to reduce enzyme utilization requirements
- Enumerate effects of enzyme loading, strain inoculation time, and inoculum charge on integrated hydrolysis or fermentation process performance
- Validate targeted enzymatic hydrolysis performance of pretreated biomass in scalable system configuration

Fermentation:

- Develop multi-sugar fermenting organisms that can tolerate impurities in biomass hydrolysate
- Validate targeted organism performance on pretreated hydrolysate in scalable system configuration

2013-2017

Pretreatment:

- Map structures and chemistries of native and prehydrolyzed plant cell walls to better understand cell wall deconstruction

Enzyme Production and Hydrolysis:

- Develop improved enzymes for advanced biochemical conversion technologies

Fermentation:

- At lab scale, develop organism for single-step processing that compares to commercial fermentative organisms and enzymes.

Biochemical Processing Integration Core Research and Development

The Biochemical Processing Integration Core Research and Development sub-platform has two components: Biochemical Intermediate Integration and Analysis. The first component, Biochemical Intermediate Integration, funds projects that help to ―define, coordinate, or consolidate” the interfaces within the Biochemical platform. The other component of the sub-platform, Analysis is designed is designed to support ongoing research of the Biochemical platform. As it does for the Thermochemical platform, the objective of Analysis is to monitor research improvements in the conversion of lignocellulosic biomass for a Biorefinery, locate opportunities for significant cost reduction, and provide analytical support for deployment and transition. Milestones for the Biochemical Processing Integration Core Research and Development sub-platform include:

2007-2012

Biochemical Intermediate Integration:

- Integrate pretreatment and enzymatic hydrolysis with biomass sugar fermentation to maximize cellulose hydrolysis and sugar fermentation cost, rates, and yields
- Validate targeted integrated process performance in pilot-plant-scale system

Analysis:

- Prepare annual *State of Technology* estimates to show progress to the 2012 performance targets
- Develop conceptual process design and mature technology cost estimates for other feedstocks, including wet corn stover and switchgrass, based on the dry corn stover baseline model

- Validate 2012 performance target using pilot plant data and baseline process design and mature technology cost estimate

2013-2017

Biochemical Intermediate Integration:

- Identify optimized pretreatment technology for use with single-step biological processing

Analysis:

- Complete conceptual design reports on advanced conversion technology configurations including significant process consolidation

New Concepts and Fundamentals

In general, projects funded under this sub-platform assist in developing and communicating information on biomass or biological systems to develop technologies that will increase conversion efficiency while decreasing conversion costs. Projects that fall under the Biomass Recalcitrance component of this sub-platform help to identify and determine which causes contribute to biomass recalcitrance in addition to figuring out how best to deconstruct plant cell walls. The other component of the sub-platform, Translational Science, develops and employs systems biology methods for improved understanding of basic conversion mechanics. New Concepts and Fundamentals milestones include:

2007-2012

Biomass Recalcitrance:

- Define the relationships between pretreatment conditions and biomass structural changes to selectively remove sugars
- Determine how cellulase enzymes move along cellulose chains
- Define how cellulases and other enzymes interact with plant structure
- Examine the basic mechanisms that will provide the framework for future deconstruction technologies

Translational Science:

- Develop systems biology methods for strain improvement of enzyme producing and fermentative microorganisms

2013-2017

Biomass Recalcitrance:

- Investigate the basic mechanisms of deconstructing plant cell walls in bioenergy feedstocks

Translation Science:

- Apply systems biology methods to identify and improve enzyme producing and fermentative microorganisms for use with a wide range of feedstocks.

Co-products

An unofficial sub-platform, Co-products covers byproducts from the creation of a Biofuel that have additional use and economic value such as grains, animal feeds, oils, and glutens. Projects funded under this category are designed to find additional uses for co-products. There are no established milestones for Co-products.

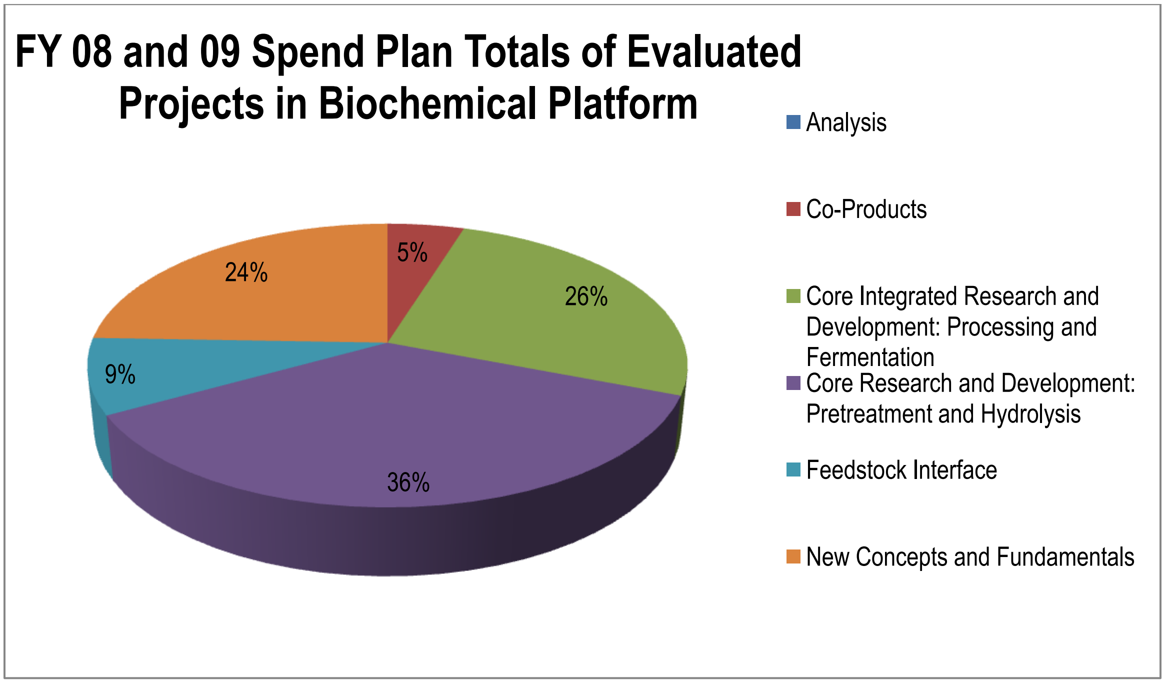
C. FY 2008 and FY 2009 Budget by Technology Area

The following table and chart provide FY 2008 and FY 2009 data on evaluated projects as listed in the 12-31-08 Spend Plan.

Exhibit 11: Biochemical Platform FY2008 and 2009 Total Funding for Evaluated Projects

FY 2008 and 2009 Spend Plan Totals of Evaluated Projects in Biochemical Platform	
Technology	**Allocation**
Analysis	$1,600,000
Co-Products	$4,264,150
Core Integrated Research and Development: Processing and Fermentation	$23,032,711
Core Research and Development: Pretreatment and Hydrolysis	$31,865,719
Feedstock Interface	$7,635,205
New Concepts and Fundamentals	$21,605,868

Exhibit 12: Biochemical Platform FY2008 and 2009 funding breakdown for evaluated projects by focus area.



D. Platform Direction for FY 2010

In FY 2010, the Biochemical Conversion Platform will continue its research and development activities with heavy focus on meeting the 2012 cost targets. This will include activities that pursue new biomass pretreatment reactors designs, and improved process integration steps (i.e., pretreatment and downstream processing). In FY2010, the biochemical conversion pathway will also begin to transition its activities toward the pursuit of infrastructure compatible biofuels using alternative biochemical pathways.

B. Results of 2009 Biochemical Conversion Platform Evaluation

The review panel evaluated the platform on criteria such as goals, approach, RD&D portfolio, and progress, and also provided comments on the strengths and weaknesses of each. The following are questions posed to each of the reviewers followed by average scores, reviewer comments, and the Biochemical Conversion Platform Technology Manager responses to those comments. The 7 independent evaluations of the Biochemical Conversion platform as a whole are summarized numerically in Exhibit 7. In addition to the numerical scores, each reviewer provided written comments, which have been reproduced below. Additionally, the section provides verbatim results of the review panel evaluation of the Biochemical Conversion platform.

Exhibit 13 – Average of Reviewer Platform Evaluation Scores

Evaluation Criteria	Average Score	Standard Deviation
Goals - Are platform goals, technical targets and barriers clearly articulated? Are platform goals realistic and logical? Do the platform goals and planned activities support the goals and objectives of the Biomass Program as outlined in the MYPP? How could the platform change to better support the Biomass Program's goals?	4.14	0.69
Approach - How well does the platform approach (platform milestones and organization, RD&D portfolio, strategic direction) facilitate reaching the Program Performance Goals for each platform as outlined in the MYPP? What changes would increase the effectiveness of the Platform?	4.14	0.69
RD&D Portfolio - The degree to which the Platform RD&D is focused and balanced to achieve Biomass Program and Platform goals? (WBS, unit operations, pathway prioritization)	4.14	0.69
Progress - Based on the presentations given, how well is the platform progressing towards achieving Biomass Program and Platform goals? Are we meeting our performance targets? Is it on track to meet the goals presented? Please provide recommendations on improvements for tracking progress in the future.	3.86	1.07

Rating System: 5=Excellent; 4=Good; 3=Satisfactory; 2=Fair; 1=Poor

The following sections provide the full written comments of the review panelists for each of the five evaluation criteria.

i. Platform Goals

Are platform goals, technical targets and barriers clearly articulated? Are platform goals realistic and logical? Do the platform goals and planned activities support the goals and objectives of the Biomass Program as outlined in the MYPP? How could the platform change to better support the Biomass Program's goals?

Exhibit 14 – Platform Goals: Strengths and Weaknesses

Strengths	Weaknesses
Well-focused platform given available resources.	More resources would allow a wider focus that could reduce risk of not meeting goals within give timeframe.
The goals and plans are very clearly defined and articulated.	The plans cover a set of relevant platform goals. There are no innovation focused goals. There are also a number of approaches that are being pursued commercially with significant distinctions from what the OBP has considered. Broadening the approach would be quite useful for the OBP to help facilitate the development of the best technologies to carry forward.
The Biochemical Platform goals are clearly delineated and focused OBP goals. The program correctly identifies barriers requiring R&D effort to overcome these and is supporting these research and commercialization efforts.	Quantitative targets may be somewhat optimistic. While the individual R&D activities may be largely successful, the overall platform may collectively find these difficult to achieve. The time frame that some of these are scheduled to achieved within (e.g. 2012 goals) may be extremely challenging in part due to delayed starts on some of the projects. Some of the targets could well be outside of the program's control (e.g. feedstock costs).
This is a very well thought out portfolio that takes a broad approach which attempts to explore an optimum set of options.	With existing resources it will be tough to meet all of the goals that congress has set.
well articulated, logical	---
Goals are well articulated.	Technical targets are overly ambitious
Well focused work plan on the	Too narrow to give high probability of meeting targeted renewable

pathway most likely to succeed with available resources in the mandated timeframe	fuel standard. A wider search for optimal input and output materials would increase the probability

Technology Manager Response

The Biochemical Conversion Platform Technology Manager (TM) appreciates the reviewer's comments about the Platform having a clear, well thought planning effort, diverse portfolio and delineated goals. Efforts continue within the Program to ensure that the platform goals are succinct and transparent in how they contribute to the overall Program goal. The Platform team has worked with Program Management and industry and academic stakeholders to assemble a robust portfolio of R&D activities that is responsive to the development of cost-effective means to convert biomass into biofuels via biochemical methods. As noted in our Platform Overview presentation, our Platform activities are focused on achieving the 2012 targets and is managed and organized to address and overcome the related R&D challenges. The activities funded by the Platform represent projects that are selected based on a multitude of parameters, including innovation, technical merit and technology diversity with an end game goal of commercialization.

Although, the TM welcomes the suggestions that a broader focus and approach would be useful in mitigating the risk associated with the stated ―optimistic goals", the platform is restricted by available funding. The Program continues to explore ways to maintain a diverse, but goal oriented, portfolio that will in the near-term meet the technical milestones and targets associated with the 2012 goals. This holds true for the development of goals based on innovative technologies or technical approaches. While the Platform portfolio is focused on the achievement of 2012 technical goals due to the high priority placed on cost-competitive biofuels by this administration, the Platform continues to seek innovative projects to better balance the portfolio. As 2012 approaches, there will be a renewed focus on long-term, high-risk, high-reward activities, for which new goals will be developed.

ii. Platform Approach

How well does the platform approach (platform milestones and organization, RD&D portfolio, strategic direction) facilitate reaching the Program Performance Goals for each platform as outlined in the MYPP? What changes would increase the effectiveness of the Platform?

Exhibit 15 – Platform Approach: Strengths and Weaknesses

Strengths	Weaknesses
Most projects are well-focused and showed excellent progress.	---
There are clear milestones and a clear plan. Several participants have been engaged in the development	Several of the milestones are inconsistent with the aims of the participants (i.e., the enzyme

of the milestones.	companies). It is not clear that the timeline is grounded with the rate of accomplishment in academia and the industry. It would be useful to have more contribution from industry in helping to create the goals. There needs to be a well defined set of standards for various parties to engage in the definition of the performance goals; this seems to be missing.
Program goals (and I suspect the RFPs that come from these) clearly focus the research efforts on OBP and platform objectives. Validation programs for the enzyme and biocatalyst development efforts will hopefully provide unbiased, commercially relevant assessments of the progress of these R&D efforts.	There may be a challenge in trying to integrate the divergent research activities into an integrated "whole" process. There is strong support for integration R&D, so hopefully this will overcome this.
Making a valiant attempt given the resources and the state of the science.	---
the approach is appropriate, the addition of the auditing activities is a big plus	---
Platform approach is excellent and well focused	---
Very targeted to applied goals, broken down into comprehensible units with an overall order that seems well conceived.	Since the targets are narrow, there is less likelihood of finding novel long-term.

Technology Manager Response

The TM welcomes the reviewers' positive comments on the effectiveness of the approach, management of the Platform, and composition of the portfolio. The Platform team has worked with Program Management and industry and academic stakeholders to develop a comprehensive and inclusive approach to portfolio management, utilizing suggestions from the 2007 Platform Peer Review. We routinely review our timelines and milestones and will consider the reviewer comments as part of our 2010 platform planning cycle.

The timeline and the approach followed by the solicited projects are based on the targets associated with meeting the 2012 Program cost goal for the modeled minimum ethanol selling price of $1.76 per gallon and subsequent commercial success. Development of these first-of-a-kind products will spur the transformation of the marketplace and enable commercialization. The TM recognizes these are aggressive targets and that they push the awardees to achieve technical targets that support the successful development of the second generation biofuel industry.

The TM thanks the reviewers for their recognition of the enzyme and ethanologen validation efforts. These activities have been developed in response to recommendations provided by Program stakeholders during workshops and at previous reviews. These efforts serve to validate the current state of technology, and the advancements achieved by the portfolio of work. We will explore ways to continue to improve our methods of tracking the accomplishments of the Program's Biochemical project portfolio, and those achieved from all disciplines in industry and academia. In this manner, we will be better able to constantly gauge our relevance to wider biochemical conversion community.

iii. Platform RD&D Portfolio

The degree to which the Platform RD&D is focused and balanced to achieve Biomass Program and Platform goals? (WBS, unit operations, pathway prioritization)

Exhibit 16 – Platform R&D Portfolio: Strengths and Weaknesses

Strengths	Weaknesses
The program is well balanced and systemically covers the goals and targets identified. The distribution has been covered reasonably well.	Certain aspects of the program should probably have more support to have a greater likelihood of achieving the goals. The enzymatic support does not feel as balanced, especially as all the technologies that are supported are dated, with no support of new technologies that can overcome the barriers these approaches have often and repeatedly encountered.
The platform R&D is made up of a good balance of activities that in addition to the major thematic activities also incorporates a number of alternative lines of research. This expands the diversity and scope of approaches to overcome the technical barriers. The program is also strengthened by excellent fundamental scientific elements that provide understanding and new avenues for future R&D efforts.	---
As things progress refinements and portfolio adjustments will suggest themselves.	---
Good focus with an appropriate amount of diversity	---
Good balance and focus to RD platform	---
Very high quality projects, especially the benchmarking and analysis projects at NREL. Outstanding industrial partners for the major competitive proposals.	Inadequate consideration of non-ethanol output stream

Technology Manager Response

The reviewers' comments on the well-balanced nature of the platform validate the Platform's approach to engaging its stakeholders, a diverse group of partners with varied expertise and disciplines. The reviewer comment praising the strength of our partners is a testament to the growing strength of the biofuels community. The DOE is fortunate to have stakeholders and partners' with the willingness and enthusiasm to partner with us in these endeavors. Additionally, we appreciate the reviewers' acknowledgment of the biochemical conversion basic research efforts, knowing that they serve to guide future innovative conversion research paths.

Although the Platform agrees with the recommendation that the focus should be expanded beyond cellulosic ethanol, at this point the Program is tied to 2012 goal of cost-competitive ethanol. As 2012 approaches, there will be a renewed focus on long-term, high-risk, high-reward activities, in which a broader suite of biofuels will be targeted. Currently, the Recovery Act funds managed by the Program include several efforts focused on non-ethanol biofuels. The Platform fully expects that biochemical processes that produce infrastructure-compatible biofuels will be included in those efforts.

The TM appreciates the reviewer's concern that there is an in-balance in the enzyme activities, and acknowledges their point of view. The program sought to include some diversity in the selection of these projects, (i.e., DSM), to address this exact concern, but the intent of the solicitation was to focus on the development of mature enzymes systems that would enable the budding cellulase market in the near-term, in support of our 2012 goal. The platform intends to continue our support of alternative enzyme systems as funding allows.

iv. Platform Progress

Based on the presentations given, how well is the platform progressing towards achieving Biomass Program and Platform goals? Are we meeting our performance targets? Is it on track to meet the goals presented? Please provide recommendations on improvements for tracking progress in the future.

Exhibit 17 – Platform Progress: Strengths and Weaknesses

Strengths	**Weaknesses**
Most presentations were very good to excellent and demonstrated excellent progress.	A few week sisters and/or presentations did not adequately show the progress that had been made to an outsider.
It is clear that most of the projects are progressing within their plan. Some are ahead of schedule and some have had some surprisingly promising results. Some very promising technologies have been supported that could be one-offs that single handedly reach the goals.	There is no punitive mechanism to discontinue failing or ineffective projects to increase the likelihood of success. Most of the participants appear to reflect the goals set by OBP for the sake of getting funding, but the plans of the grantees, for the most part, do not have a clear and practical plan of meeting these milestones.
The platform R&D efforts are making good progress toward achieving the technical goals delineated. There is still much technical advancement needed to accomplish the ultimate goals.	Accomplishing the technical goals may not assure commercial success which is the ultimate goal of this program.

Very strong and productive portfolio, great people and institutions	May need to focus more as time toward goal dates diminishes
Making good progress	---
Auditing excellent addition to meet and standardize targets and achievements	Goals ambitious, going to be difficult to achieve by 2012.
The progress being made is very impressive given the resources.	---

Technology Manager Response

As the reviewers identified, ―There is still much technical advancement needed to accomplish the ultimate goals.” With that said, the TM appreciates the comments regarding the progress that the Platform is making and their recognition of the promise that some projects hold in reaching Platform goals.

The Platform and Program review period offers the OBP Team the opportunity to conduct an independent and rigorous review of project, platform, and program level activities. It also affords us the opportunity to consider adjustments to the competitively awarded projects. The executive summary of the final review report contains a table with our next-step recommendations for each project. The reviewers project level comments resulting from this process are considered and recommendations to project scope, funding, and targets can be made if deemed necessary to insure that projects achieve successful outcomes. Some projects will reach a natural conclusion and will be scheduled for close-out, independent of whether or not they achieve successful outcomes.

As stated in earlier responses, as we approach the 2012 target dates, there will be a renewed focus on long-term, high-risk, high-reward activities, and new goals will be developed. The TM acknowledges that the accomplishment of technical goals alone will not assure commercial success of the cellulosic biofuel industry, though we are hopeful that we can make an impact and assist the industry in transforming the marketplace.

v. Portfolio Gaps

Are there any gaps in the Platform RD&D Portfolio? Do you agree with the RD&D gaps presented by the Platform Manager?

Exhibit 18 – Platform Gaps: Reviewer Comments

Reviewer Comment
Additional resources would allow development of more tools and to included work on additional feedstocks.
The lack of a plan on algae is notable. While this reviewer does not believe algae should be supported but OBP, a more explicit plan needs to be defined. There are a number of approaches looking at other fuels that are widely accepted fuel molecules that are not being included. Also, the program is focused on a set of biofuel based standards that have been viewed as "the way to do things" for some time. There is no avenue for

innovative projects that may be able to disrupt the standard way biofuels have been thought about.
The program focuses heavily of corn stover and to a lesser degree switchgrass as feedstocks, it seems likely that if the industry is successful many other biomass materials will be used. Hopefully the technologies being developed here translate well to these other materials.
As this field progresses there will be a growing need for capabilities such as developing more complete models and modeling environments, and developing and providing access to databases and tools.
make comments regarding the use of standardized models, methods, and materials
If there were enough money, investigating additional feedstocks and output streams would increase the likelihood of meeting targets. Additional research in the external community could be leveraged by founding a collection of standardized stocks of treated feedstocks available for purchase, to increase the role of organism and process discovery compatible with favored biomass resources.

Technology Manager Response

The TM appreciates all of the suggestions from the reviewers on the future direction and gaps of the Platform.

The TM recognizes the importance of investigating additional feedstocks, and would like to acknowledge the activities of the CAFI pretreatment project on switchgrass as an example of our dedication to this future endeavor. It is the intent of the Platform to utilize the knowledge gained in our current activities on the model feedstock to inform future work on other agricultural residues and energy crops.

The Program has recognized the growing need for additional models available to the community will be necessary as the industry develops. We are working with our Analysis lead to ensure that advanced biochemical technologies will be included in those modeling efforts.

The Program is currently developing a management plan for our algae effort. There is a draft roadmap available that was developed in response to a barriers workshop that was held last December by DOE, that is currently being reviewed. A significant amount of funds ($50 million) are expected to be spent on algae over the next several years as a result American Recovery and Reinvestment Act.

vi. Additional Recommendations, Comments, and Observations

Exhibit 19 – Other Reviewer Comments

Reviewer Comment
The review was a very valuable experience.
It would be useful if the OBP could engage with programs with some punitive capabilities for those who are not meeting milestones. Keeping the projects competitive even after granting will help to ensure success of the various projects.

The OBP appears to be adding algae as a biomass feedstock? Although the prospects of using algae as a biodiesel feedstock seem overly optimistic, there is significant commercial activity in this arena. Given this and the fairly mature technology of converting algal lipids to biodiesel, what should be the role of the biochemical platform in advancing this technology? Much of the research conducted under this platform will not be published. This unfortunate, but understandable because of the commercial interest in the technologies under development.
Find a way to more closely link to the Bioenergy Research Centers and have a greater impact on their portfolios and priorities
Audit programs particularly strong addition.
Money. Staff of the program are doing a truly excellent job with limited resources.

Technology Manager Response

The TM appreciates the additional comments provided by the reviewers and acknowledges the opinions submitted as part of this evaluation. The EERE, has supported the use of Peer Reviews to gather independent stakeholder opinions on its Program activities for many years. We agree that Peer Review process is an important part of the Program and Platform management, and the results are routinely used to modify our Program efforts and direction. We also consider other factors in making Program decisions such as Stage-gate reviews, technology audits, third party engineering assessments, and other types of analyses and assessments.

The OBP is charged with the responsibility to investigate all biofuel commodities. The recent interest in the potential of Algal conversion processes for the production of biofuels is now included among the alternative biomass-based fuels being considered. In that regard, the Algal biofuel conversion activities are looking at commodities such as renewable gasoline and renewable diesel which are considered to be ―infrastructure-compatible" or ―drop-in" fuels. These fuels could be shipped in the existing petroleum products pipeline and other infrastructure assets without any modifications. The conversion technology associated with the production of the renewable gasoline and diesel are not yet commercially mature and certainly not as mature as the commercial scale, base-catalyzed transesterification process that is used to produce almost all of today's biodiesel available in the marketplace. As such, there is a Federal government role in considering their economic and societal potential.

There was not much information provided on the interagency level coordination between the OBP and Office of Science, Bioenergy Research Centers (BRC) at the Platform Review. Since the collaboration with the Office of Science occurs at the interagency level, information on these activities will be presented at the Program review meeting. The BRCs were awarded through a competitive awarded solicitation sponsored through the Office of Science, and OBP must work through the appropriate channels to access information, talent, and results that could impact the efforts of the conversion platforms. OBP has made a significant effort to stay informed of the progress being made at the BRCs. It should be noted that, a significant amount of American Recovery and Reinvestment Act funds ($20 million) will be directed from OBP to Lawrence

Berkeley National Laboratory to be spent on a biomass process development unit (PDU) to be managed by Lawrence Berkeley National laboratory. This award is something that has occurred since the Platform Review meeting in March. The PDU will be available for use to the public and the other BRCs through an application process.

C. Overall Technology Manager Response

The TM appreciates the participation, hard work, and excellent review comments and recommendations from this year's esteemed review panel. The Platform is indebted to the review panel members for their inputs and will use the information and comments to the extent possible in the future management of the Platform. The Biochemical Conversion Platform is managed as an integrated component of the Biomass Program and its activities are aligned with the MYPP with set targets and milestones for management of the research activities. Since the management of any R&D program is an iterative process, the overall positive feedback from the review panel this year is rooted in the quality of our past review efforts and adjusts that have been made. These adjustments will be incorporated into future versions of the MYPP that will be produced by the Program. The detailed comments that have been provided and responded to in this report will be useful in improving the overall Platform and its future direction. The Platform will continue to pursue its goals and objectives by adjusting the Platform activities in a logical and consistent manner and incorporating the comments and recommendations into the projects and the platform.

In response to the review, the Platform will:

- Continue to assess the balance and focus of countless potential conversion technologies and foster technology and technology integration improvements in the conversion of biomass into biofuels.

- Continue to have a ―balanced" portfolio of data collection and analyses, technology development, testing, and demonstration, and model development. Efforts will be made to have the portfolio flexible enough to adapt to changes and sufficiently staunch enough for Program planning and management. When there are data voids, efforts will be made to obtain the needed data. Models will be developed as appropriate to provide input for policy development or as tools for decision-making. Attempts will be made to ground truth and validate models through field tests whenever resources are provided.

- Conduct additional efforts to better select and manage the overall portfolio of projects. This will be accomplished by more comprehensive planning, better tracking of milestones and monitoring of progress, and stronger collaborative efforts with DOE's Office of Science, USDA and other federal agencies, and involvement in the Interagency working groups help identify both research needs and collaborators. More efforts will be made to strengthen collaborations with industry and academia.

- Integrate the Congressionally Directed Programs into the overall Platform as feasible.

Infrastructure Platform thanks the Peer Reviewers for their valuable comments, time, and expertise. The concerns and opinions express to us throughout the platform and program review proceedings will be considered as the program reviews its strategic plan and planning activities, and assist in guiding the program and program accomplishments. Since each successive review looks at previous peer review platform and project results for improvements and adjusts, the platform manager hopes that the PI's take the Peer Reviewers comments seriously and work to incorporate this information to improve project performance and results.

III. Project Review

The Analysis Platform supports research and development projects with the National Labs, University and Industry partners, non-governmental organizations, and other entities. Projects funded through the Analysis Platform align their activities with the Biomass Program Multi-Year Program Plan (MYPP) goals. At the February 19, 2009 Review, 9 projects gave 20-30 minute presentations that focused presenting how project results would help achieve the Biomass Program objectives. Projects were evaluated by a subset of the Feedstock Platform Review Panel, in accordance with the reviewers' areas of expertise.

A. Evaluation Criteria

Each project was evaluated systematically by set of criteria developed in conjunction with the Biomass Program peer review steering committee. The evaluation criteria were provided to the project PIs ahead of time. The five criteria are provided below:

Relevance - *The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs has been considered.*

Approach - *The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.*

Technical Progress - *The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.*

Success Factors - *The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.*

Future Research - *The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.*

Rating System – 5=Excellent; 4=Good; 3=Satisfactory; 2=Fair; 1=Poor

B. Project Scoring

Exhibit 20 – Project Scoring Summary Table

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Analysis	2.6.1.1	Biochemical Platform Analysis	4.4	4.3	4.3	4.3	4.0	4.3
Analysis	2.6.1.2	Analysis for Production-Technical and Market Analysis (PNL)	4.0	4.0	3.6	3.3	3.4	3.7
Co-Products	7.4.1.2	Biofuel Production Initiative Claflin (SC)	3.0	2.3	1.9	1.3	2.0	2.1
Co-Products	7.4.1.4	Sustainable Energy Center Biodiesel from Algae (MI)	3.3	3.1	2.3	2.7	2.7	2.8
Co-Products	7.4.1.6	Snohomish County, Biodiesel Project (WA)	2.6	3.0	3.3	3.1	2.7	2.9
Co-Products	7.4.2.4	Bioeconomy Initiative at MBI International	3.3	4.0	3.7	3.6	3.9	3.7
Co-Products	7.4.2.6	Intermediary Biochemical's (MI)	2.7	3.1	3.3	3.1	3.0	3.1
Co-Products	7.4.3.7	Connecticut Biodiesel Power Generator	3.0	3.6	3.6	3.4	3.0	3.3
Co-Products	7.4.5.2	Development of Applied Membrane Technology for Processing Ethanol from Biomass	2.7	3.4	3.3	3.4	3.3	3.2
Core Integrated Research and	2.3.1.1	Biochemical Processing Integration Task	4.9	4.6	4.7	4.1	4.7	4.6

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Development: Processing and Fermentation								
Core Integrated Research and Development: Processing and Fermentation	2.3.1.5	Integrated Biorefinery-Separations/Separative Bioreactor-Continuous bioconversion & separations in single step	4.0	4.0	4.3	3.7	4.0	4.0
Core Integrated Research and Development: Processing and Fermentation	2.3.2.1	Biocatalyst for Fermenting Hydrolyzate at Low pH and High Temperature	4.4	4.7	4.4	4.0	3.7	4.3
Core Integrated Research and Development: Processing and	2.3.2.2	Improvement of Zymomonas Mobilis for Commercial Use in Corn-Based Biorefineries	4.7	4.4	4.7	4.0	4.3	4.4

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Fermentation								
Core Integrated Research and Development: Processing and Fermentation	2.3.2.3	Development of Thermoanaerobacterium saccharolyticum for the conversion of lignocellulose to ethanol	4.5	4.2	4.2	4.0	4.0	4.2
Core Integrated Research and Development: Processing and Fermentation	2.3.2.4	Improvements in Ethanologenic Escherichia coli and Klebsiella oxytoca	4.3	4.1	3.7	3.9	3.6	3.9
Core Integrated Research and Development: Processing and Fermentation	2.3.2.5	Further Improvement of the Robust Recombinant Saccharomyces	4.3	4.1	4.0	3.7	3.4	3.9
Core	2.3.2.7	Lab Validation for Organism Development	4.6	4.6	4.1	4.1	4.1	4.3

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Integrated Research and Development: Processing and Fermentation		Solicitation Recipients						
Core Integrated Research and Development: Processing and Fermentation	2.3.2.8	A novel simultaneous-saccharification-fermentation strategy for efficient co-fermentation of C5 and C6 sugars using native, non-GMO yeasts	4.3	4.0	3.6	3.7	3.9	3.9
Core Integrated Research and Development: Processing and Fermentation	2.3.3.1	Production of higher alcohol liquid biofuels via acidogenic digestion and chemical upgrading of organic industrial wastes.	3.6	4.0	3.6	3.3	3.6	3.6
Core Integrated Research and Development:	7.2.3.1	BioEthanol Collaborative (SC)	3.1	2.7	2.4	2.1	2.4	2.6

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Processing and Fermentation								
Core Research and Development: Pretreatment and Hydrolysis	2.2.1.1	Pretreatment and Enzymatic Hydrolysis	4.7	4.6	4.4	3.9	4.3	4.4
Core Research and Development: Pretreatment and Hydrolysis	2.2.1.2	Value Prior to Pulping	3.7	4.1	3.0	3.6	3.3	3.5
Core Research and Development: Pretreatment and Hydrolysis	2.2.2.2	Energy Corn Consortium	3.6	3.9	3.1	3.0	3.3	3.4
Core Research and	2.2.2.3	Enzyme Solicitation Support and Validation	4.7	4.4	4.0	4.6	4.7	4.5

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Development: Pretreatment and Hydrolysis								
Core Research and Development: Pretreatment and Hydrolysis	2.2.2.5	Enhancing Cellulase Commercial Performance for the Lignocellulosic Biomass Industry	4.6	3.7	3.3	3.4	4.1	3.8
Core Research and Development: Pretreatment and Hydrolysis	2.2.2.6	Development of a Commercial Enzyme System for Lignocellulosic Biomass Saccarification	4.6	3.9	3.6	3.7	3.7	3.9
Core Research and Development: Pretreatment and Hydrolysis	2.2.2.7	Project Decrease: Development of a Commercial-Ready Enzyme Application System for Ethanol	4.9	4.4	4.3	4.0	4.3	4.4
Core	2.2.2.8	Commercialization of Customized Cellulase	4.6	4.3	3.9	3.9	3.6	4.0

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Research and Development: Pretreatment and Hydrolysis		Solutions for Biomass Saccharification						
Core Research and Development: Pretreatment and Hydrolysis	2.2.2.9	Addressing the Recalcitrance of Cellulose Degradation through Cellulase Discovery, Nano-scale Elucidation of Molecular Mechanisms, and Kinetic Modeling	3.9	3.9	3.9	3.0	4.0	3.7
Core Research and Development: Pretreatment and Hydrolysis	2.3.1.4	Integration of Leading Biomass Pretreatment Technologies with Enzymatic Digestion and Hydrolyzate Fermentation	4.9	4.6	4.9	4.3	4.7	4.7
Core Research and Development: Pretreatment and Hydrolysis	7.2.2.2	Advancing Texas Biofuel Production	4.1	4.0	3.9	3.4	3.6	3.8

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
Feedstock Interface	2.1.1.1 and 2.1.1.3	Storage Systems, Feedstock Supply, Etc.	4.6	4.0	4.0	3.9	4.1	4.1
Feedstock Interface	2.1.1.6	Extremophilic Microalgae: Advanced Lipid and Biomass Production for Biofuels and Bioproducts	3.6	3.4	3.7	3.4	3.3	3.5
Feedstock Interface	2.1.1.7	Improving cost effectiveness of algae-lipid production through advances in nutrient delivery and processing systems	3.3	3.1	3.4	3.0	3.1	3.2
Feedstock Interface	7.2.1.1	University of Nebraska, Lincoln, Bioenergy Demonstration Project: Value-Added Products from Renewable Fuels (NE)	4.0	4.1	4.1	3.7	4.0	4.0
New Concepts and Fundamentals	2.4.1.1	Targeted Conversion Research	4.7	4.7	4.9	4.7	4.9	4.8
New Concepts and Fundamentals	2.4.1.2	Fungal Genomics	4.6	4.4	4.6	3.9	3.9	4.3
New Concepts and Fundamentals	2.4.1.3	Lignin as a Facilitator, not a Barrier, during Saccharification by Brown Rot Fungi	4.4	4.4	3.6	4.0	3.7	4.0

Technology Area	WBS	Title and Project Information	Relevance	Approach	Technical Progress	Success Factors	Future Research	Overall
New Concepts and Fundamentals	7.2.4.1	Arkansas State University Ethanol Fuel Development	3.7	3.9	3.9	3.3	3.6	3.7

C. Biochemical Conversion Platform Individual Project Reviews

The following 39 projects were evaluated by three to seven reviewers. The number of reviewers for each project is listed for each project. Each evaluation provides a summary table of the evaluation scores provided by the review panel followed by a verbatim reproduction of the full written comments provided by the review panel. The written comments do not in any way reflect an official opinion of the U.S. Department of Energy. Following the review, each project Principal Investigator was given an opportunity to review and respond to the written evaluation provided by the review panel. These responses are provided in full below. The Principal Investigator responses do not reflect an official opinion of the U.S. Department of Energy.

This section will provide review results for each project in the sub-platform and PI response.

Biochemical Platform Analysis

Technology Area: Analysis
Project Number: 2.6.1.1
Performing Organization: National Renewable Energy Laboratory
Number of Reviewers: 7

1. Summary of Project Scores

Evaluation Criteria	Average Score*	Standard Deviation
Relevance	4.43	0.79
Approach	4.29	0.49
Technical Progress	4.29	0.76
Success Factors	4.29	0.76
Future Research	4.00	0.58

* Average represents mean of individual reviewer scores. Review panels did not develop consensus scores.

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs has been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Strong commercial company	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are consistent with the offices program.	Goals represent a singular approach without an opportunity to assess alternative approaches Fundamental goals are limited and not necessarily consistent with the end-points of the program
PI Response: No response to this comment has been provided by the Principal Investigator.	
Targets developing a robust ethanologen that can ferment c5 and c6 biomass sugars, at high temperatures, low pH and in presence of	---

acetate.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
an important pathway to develop	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal is to develop robust ethanol producing organisms capable of converting C5 and C6 biomass sugars.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Uses proprietary yeast that ferments at low pH and high temperature and is resistant acetic acid.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Called in by FOA and subject to validation	---
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development, and Demonstration (RDD&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant

contributions progress.

Strengths	Weaknesses
Using and developing a proprietary, non-conventional yeast for converting biomass C5 and C6 sugars to ethanol at elevated temperature (~40C) and low pH in the presence of inhibitors (e.g. acetate). Strain engineering will be used to add an ability to ferment arabinose. The approach is based on the use of genomic and good molecular biology tools for pathway integration and development.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Company is using a proprietary organism and working with it to improve net performance. Plans reveal a high level plan to get to ethanol. Approach is well controlled	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Uses Cargill's yeast strain that has tolerance to variety of adverse conditions . Plan to engineer the yeast for xylose fermentation by incorporating xylose isomerase followed by introduction of arabinose fermentation genes.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
straightforward methods; tested Cargill advanced strain; utilized modern tools and genomic information	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
- use of Cargill's proprietary non-conventional yeast - use genomics and genome wide tools - introduce xylose isomerase path	---
PI Response: No response to this comment has been provided by the Principal Investigator.	

---	No work with hydrolysates. Needs more economic analysis with potential ethanol concentrations
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well-defined objectives Proprietary yeast acid tolerant and impurity tolerant, EtOH tolerant. Genomics and genome-wide tools to look at metabolic flux and carry out metabolic engineering.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
The project began in July 2007 and will end in February, 2010. It has achieved two initial significant milestones which are to be validated soon; e.g. excellent conversion of sugar; showed that the introduction of XI	Path to achieve inhibitor resistance not well described. Scale and feed stock not clearly described.

expression does not degrade performance, and have shown that the process works at a higher temperature. They have Integrated an arabanos conversion pathway and showed it can make ethanol. The yield exceeded target and is 96% of theoretical.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have met internal milestones. Appear to be on timeline Temperature adjustment is valuable Validation of c5/c6 approach in hand Data is quite promising that project will be on target.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Yeast produces ethanol from hexoses and xylose in good yield in the presence of acetate at low pH and elevated temperature.	Investigators claim co-utilization, but this is hard to see from data presented. How will the strain perform at high glucose and xylose concentrations. This is frequently a problem and incomplete xylose use often results. Were all experiments performed in complex synthetic media? This can have a signification impact on tolerance to adverse conditions. What is the level of inoculum used for these experiments and how was it prepared? Were the organisms grown up under the same conditions and how much did they grow over the time course of these studies?
PI Response: No response to this comment has been provided by the Principal Investigator.	
have shown good progress in yield, rate, titer, and robustness	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
achieved xylose isomerase incorporation, achieved incorporation of arabinose utilizing genes, - demonstrated ability of yeast to perform at pH 4.5 - demonstrated that incorporation of xylose	---

isomerase did not change hexose fermentation parameters - demonstrated co-utilization of glucose and xylose - engineered bacterial arabiniose pathway and showed constructed strain could ferment arabinose to ethanol' - tested acetate resistance, found yeast could do well in presence of 10g/L acetate -	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Runs at 40C and does metabolize xylose. Succeeded in genetic engineering of strain.	Higher tolerance to inhibitors discussed but no examples presented other than acetic acid.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Bring in xylose isomerase, Bring in arabinose pathway show EtoH could be made Ara to EtoH could be made Achieved these targets remainder with by combining xylose and ara in one strain. Audit results shown. 70 g/L EtoH. (v high tolerance) Show result is robust in XI host. Also show glucose/xylose mix is giving equiv results. Arabinose from bacteria to fungal. Also showed working on arabinose. EtoH titer better than targets x 6 reps rate per h similar of 2X EtoH % theoretical beat targets in both cases improved conversion and presence of acetate.	All in pure mixed C-source, not hydrolysate.
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and

strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Key critical success factors identified including robustness in commercial performance.	Challenges in combining arabinose and xylose utilization and alternative approaches not well described.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Recognition of what is needed for success and challenges that go with it. Historical performance is consistent with ability to likely deal with challenges.	No clear demonstration of plan to deal with risks.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Recognition of the need to demonstrate robustness, achieve yield, rate, and titer targets and whether this can be translated from the lab to the plant	How will this strain perform in real biomass hydrolysates containing multiple inhibitors?
PI Response: No response to this comment has been provided by the Principal Investigator.	
reasonable lists, few details	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
- shooting for balance between rate, yield, and titer along with robustness of organism	scale-up to commercial operations can be difficult—industrial applications can require even more robustness
PI Response: No response to this comment has been provided by the Principal Investigator.	

	Showstoppers not adequately discussed.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Done well on rate yield and titer. Robustness tbd commercial operations tbd	---
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Will combine arabinose and xylose utilization. They state that they have methods but that achieving success isn't trivial.	No details provided on methods for achieving success and alternatives if plans fail.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear future goals with a development timeline and milestones.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Researchers plan further strain development;	Is additional assessment of inhibitor tolerance

combining xylose and arabinose fermentation capability into single strain.	planned? So far the focus has been largely on acetate, but how about other inhibitors in hydrolysates. How do these strains produce in "real" biomass hydrolysates where there are multiple inhibitory compounds present which can act in concert to accentuate toxic effects?
PI Response: No response to this comment has been provided by the Principal Investigator.	
	A set of difficult next steps, but few details as to what will actually be done.
PI Response: No response to this comment has been provided by the Principal Investigator.	
- enhance robustness of strains - combine xylose and arabinose utilization in one strain - continue strain and fermentation development to achieve proposed targets	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Validation, gate reviews, get to the next step	---
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
There is no basis for tech transfer in this plan.	
How will they transfer this strain to others??? First idea was apparently to outsource this organism and license it to others. Is this the current plan?	
plan is to out-license this technology	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Well thought out plan and project	

Analysis for Production-Technical and Market Analysis (PNL)

Technology Area: Analysis
Project Number: 2.6.1.2
Performing Organization: Pacific Northwest National Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.00	0.58
Approach	4.00	0.58
Technical Progress	3.57	0.53
Success Factors	3.29	0.49
Future Research	3.43	0.79

Overall Principal Investigator Response(s)
We appreciate the reviewers' comments. Several reviewers had similar observations, to which we have a few clarifications: 1) <u>Focus needs to be broader, particularly for hydrocarbon compatible fuels</u>: we agree, and were already planning to expand the work on hydrocarbon fuels by directly interacting with additional players in the hydrocarbon compatible fuels field. Our general focus in the bioconversion task is to assess alternative processing options and longer-term processing routes that provide OBP with information to look beyond a single process. 2) <u>Diverse Subtasks</u>: The current project, as noted by the reviewers, contains two technoeconomic tasks that were described at this review, plus a third task of GIS mapping to assist in determining opportunities for algal biofuels. Only the first two of these tasks were reviewed at this time. The GIS work was reviewed approximately two weeks earlier and received a score of 4.04 on the same 5-point scale. One of the objectives of the PNNL analysis is to assess alternative processing and engineering options available to DOE-OBP, and that translates into a wider range of analytical activities.

3) <u>Dissemination of results</u>: we agree that public dissemination is important and the detailed results of all the work will be made publically available.

1. Relevance to overall Program objectives and market need

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs has been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Critical for assessing research directions and assessing future progress.	Project recently refocused—too early to judge how well it will be implemented.
PI Response: No response to this comment has been provided by the Principal Investigator.	
This is a very valuable piece of research that both academics and industry can use.	A plan to engage with industry can help achieve the goals of this project and disseminate the results beyond straightforward publishing (and more limited on the website).
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project plans to provide needed analysis tools to help PNNL projects evaluate alternative	Lots of speculation on which direction this might go in leaving all doors open. Not completely obvious why these processes were

bioconversion routes to fuels.	selected. Assume these fit with ongoing research at PNNL and Washington State University
PI Response: No response to this comment has been provided by the Principal Investigator.	
has the scope that could be useful for the analysis of alternative pathways and technology choices	Not being developed as a community useful model, too focused on local work and goals. One would assume that if an institution is doing research on a feedstock, that the value of that research has been predetermined. The balance between analyses of scenarios and development of the modeling capability was unclear.
PI Response: No response to this comment has been provided by the Principal Investigator.	
necessary to have such techno-economic analyses if successful, will help decision making	limited number of systems can be evaluated—that is the nature of the work, but difficult to know which systems are worth this analysis
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Focus for analysis directed primarily for PNNL use
PI Response: No response to this comment has been provided by the Principal Investigator.	
I understand that this model supports a recently revived project	Unfamiliarity with the original work made it very hard to follow. Showing an example of a particular salient variable, how it's determined and what difference it makes, would make the presentation more educational. However, that's not the point of the exercise.
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and *implemented effective project management practices. Difficult for the approach to be improved significantly.*

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Good to identify unprofitable pathways but need to be sure of assumptions in case these need to be revisited.	Limited data from fungal systems but analysis needed to guide research. May be too PNNL centric.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Broad based approach. Fungal work is an important contribution as an angle that has not been explored by many. Lignin contribution is essential for understanding corn and cellulosic feedstock net potential.	Hydrocarbon milestones are thin and unclear how the project will progress.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The approach used considers alternative conversion strategies [e.g. lignin combustion v. pyrolysis oil conversion from corn stover]. This is a nice integration	Three distinctly different tasks considered. It is somewhat unclear which direction is a priority
PI Response: No response to this comment has been provided by the Principal Investigator.	
good scope, good interactions, good potential impacts	not taking a broad enough view of problems or of use of analyses by community
PI Response: No response to this comment has been provided by the Principal Investigator.	
seem to be working with researchers to get	difficult to know if what applies to one system

appropriate data models appear appropriate	will apply to others (e.g. using NREL 2002 Model)
PI Response: No response to this comment has been provided by the Principal Investigator.	
Task design may be well designed. Steps to be combined by fungi consolidate the economics. Doing limit examination. Lab validation feedback	---
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Program just started but seems to have a good handle on what is needed.	Algae GIS subject of recent effort but data not presented here so can't evaluate.
PI Response: No response to this comment has been provided by the Principal Investigator.	
First order analysis on pyrolysis provides good initial conclusions. Butanol and lipid models are good starts, and connect well to the other aims	Understanding other approaches on lignin under a similar approach would be useful in supporting the conclusion. The amount of

of the study.	experimental/empirical evidence in fast pyrolyssis is seemingly limited for the conclusions made. Limited view on hydrocarbon fuels—multiple paths are being pursued with only a limited view being provided here—an unbiased comparison of these approaches (esp. for hydrocarbons) would be valued by the industry.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project is still in planning stages.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Capable looking system wide and providing valuation of pathways.	Needs to look more broadly.
PI Response: No response to this comment has been provided by the Principal Investigator.	
progress is made in specific areas (fungal work) and the development of the model	model is applied to specific system, so critical to choose appropriate system
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Most progress is on-going
PI Response: No response to this comment has been provided by the Principal Investigator.	
Serving to provide a connection to real world economics for variety of projects	Disparate tasks make overall rating hard I don't see how the topics hang together
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear

strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Good knowledge of success factors and challenges.	Need to develop a more proactive communication plan to inform researchers in the field. May need to be more proactive to keep up with rapidly changing data; literature review only a start and always a year or two behind current status.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals well defined. Fungal work is clearly important, though early with interesting results like to come.	Would be beneficial to define how to engage with industry to get better access to organisms, data, progress, process information, etc. Butanol and hydrocarbon projects are early with little data to date and an unclear process of how to mitigate the risks
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clearly identifies the lack of needed data to support analysis effort as a challenge toward moving forward.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
The focus on fungal fermentation and integrated bioprocessing should be in the context of a broad and comprehensive set of analyses.	There needs to be a more coherent long term vision for what this research is producing—is it a set of analyses or a capability for such analyses, or some combination. Models are needed by the industry.
PI Response: No response to this comment has been provided by the Principal Investigator.	

are aware of limitations and make attempts to account for uncertainties	ability to get appropriate data for the modeling work must keep working to keep model relevant
PI Response: No response to this comment has been provided by the Principal Investigator.	
---	Vague and wordy
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project)

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Working on process modeling and additional fungal scenarios, and co-products. Co-products may be critical for commercial successes.	Few specifics regarding plans. Not clear how customer feedback will be incorporated or how models will be compared to real world scenarios.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals of the future work are important in defining the economic potential of various areas.	Future scope seems less defined than ideal. While there is recognition of the methodology, the ways of getting data, the partnerships to provide data, and the details of the analyses to

	do (i.e. different ways of producing hydrocarbons) are lacking.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project plan has identified a path constructing models to provide researchers with cost evaluations to help direct research priorities.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Useful products from the reviews and the report.	Products will be of fleeting value in a rapidly progressing field. The capability for further analyses should be the prime deliverable.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Many areas could benefit from this work, those proposed seem appropriate	difficult to know which projects have highest priority
PI Response: No response to this comment has been provided by the Principal Investigator.	
---	Should try interact more with industry which has probably done extensive work on biobutanol models
PI Response: No response to this comment has been provided by the Principal Investigator.	
This seems straightforward listing of projects due.	Not enough concrete information on what the criteria are for ending projects
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Interactions with others are seemingly limited outside of the fungal approaches	

Tools are intended to assist researchers in planning program: not currently intended to be used commercially. I would be nice if the project moved this direction in the future. Works closely with PNNL and wash state; however, doesn't appear to interact with NREL folks working on modeling cost analysis efforts	
Too much focused on the local research efforts.	
work with WSU but have not made information available to public at this point	
Recommend wider-based collaborations	
Is there a research literature on "analysis" methods? I'm not clear on what appropriate modes of publication or sharing are.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
The scope of the project is broad and important. A more focused methodology to engage broadly in collaborations would help the project.	
More focus on broader OBP goals, less on the local context.	
this type of work would greatly benefit from outside input, with respect to models and data used in model development	
I'm not sure the "task" structure gives enough long-term focus to the lab. Is this a tool-generating shop?	

Biofuel Production Initiative Claflin (SC)

Technology Area: Co-products
Project Number: 7.4.1.2
Performing Organization: Claflin University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.00	0.82
Approach	2.29	1.11
Technical Progress	1.86	0.90
Success Factors	1.29	0.76
Future Research	2.00	0.82

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs has been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Private university in collaboration with bioenergy consultant. Emphasis on sugarcane to produce biobutanol.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
investigation of butanol from cellulose is consistent with OBP goal	No clear innovative angle to producing biobutanol relevance of project beyond SC is not clear
PI Response: No response to this comment has been provided by the Principal Investigator.	
Production of butanol from cellulose in a cost competitive process. Project just started and still in the early planning phase	no actual experimental plan proposed
PI Response: No response to this comment has been provided by the Principal Investigator.	
looking at butanol	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- biobutanol may be an important co-fuel with ethanol - planning to establish a pilot plant for commercial evaluation - demonstrate butanol benefits relative to ethanol	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Sugar cane and bagasse to biobutanol Economic focus	
PI Response: No response to this comment has been provided by the Principal Investigator.	

Co products are not in the plan. Should they be given a separate set of specific goals	
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Combining academic and professional engineers to derive economic process for butanol—second generation biofuel. Developing a two-step process.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
---	Approach does not leverage innovations in cellulose capture or butanol synthesis. Process design is highly inefficient. Absence of an economic model is evident by choices in approach.

PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan to produce butanol from sugar cane and bagasse start with conventional ABE fermentation then look to use extreme thermophiles as cellulose conversion organism.	Very vague how actually plan to execute project.
PI Response: No response to this comment has been provided by the Principal Investigator.	
---	poorly defined
PI Response: No response to this comment has been provided by the Principal Investigator.	
- two step process where butanol produced	- approaches to be employed were not effectively communicated
PI Response: No response to this comment has been provided by the Principal Investigator.	
---	Approach was not detailed; what organisms are they using? How do they plan to produce biobutanol? What is the IP?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Many goals are education and local business development-related. Different industrial structure model	Small-scale distributed energy generation is one approach but not clear how it articulates with DOE plan.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP

goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Project start October 1 for 1 year.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
No data provided—progress could not be assessed	
PI Response: No response to this comment has been provided by the Principal Investigator.	
none to date	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	no DOE funding in place yet
PI Response: No response to this comment has been provided by the Principal Investigator.	
- have not received funding from DOE	does not have
PI Response: No response to this comment has been provided by the Principal Investigator.	
	No data presented on progress to date.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Haven't started yet	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Not discussed	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
not identified	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
looking at cellulose to biobutanol -- many plentiful local feedstocks	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
cost effective butanol	success factors and showstoppers were not effectively presented
PI Response: No response to this comment has been provided by the Principal Investigator.	
not addressed	Fungible fuel status of butanol not addressed
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project)

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
The entire project is future work as it appears	No discussion on milestones, goals, or timing. No discussion on key barriers outside of recapitulating work that has been done by others.
PI Response: No response to this comment has been provided by the Principal Investigator.	
an outline of planned research but not much detail provided	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	still defining program
PI Response: No response to this comment has been provided by the Principal Investigator.	
complete project as proposed	
PI Response: No response to this comment has been provided by the Principal Investigator.	

	Did not present details of plans; appears to plan to use old ABE technology
PI Response: No response to this comment has been provided by the Principal Investigator.	
rather undefined	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) <u>Technology Transfer/Collaborations</u>

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Well positioned for tech transfer given collaborations. Interactions, however, are narrowly focused related to execution.	
Organic BioEnergy	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Use modern butanol approaches. Need some inclusion of separations if dealing with butanol	
Really cannot grade the project at this early stage of development. Although just a one year project	
Needs to present much more specific information on the approach to be taken and results to date.	

Sustainable Energy Center Biodiesel from Algae (MI)

Technology Area: Biomass Program
Project Number: 7.4.1.4
Performing Organization: Western Michigan University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.29	0.95
Approach	3.14	1.07
Technical Progress	2.29	0.76
Success Factors	2.71	0.76
Future Research	2.71	0.76

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs has been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

<table>
<tr><th>Strengths</th><th>Weaknesses</th></tr>
<tr><td>Western Michigan in cooperation with county and environmental company.</td><td>---</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>Well defined goals. Goals are consistent with OBP Approach is unique</td><td>---</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>The project strives to capture value from municipal wastes in the form of useful transpiration fuel. The plan is to produce biodiesel from waste grease [trap grease] and microalgae that can be harvested from runoff and municipal waste systems.</td><td>---</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>a niche approach applicable to many locales</td><td>---</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>production of biofuels from waste streams
- biodiesel production systems
- algae biofuels using open wild ponds or pond clean-up</td><td>---</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>Environmentally conscious</td><td>Regulatory issues not precleared.</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
</table>

2. Approach to performing the Research, Development and Demonstration (RDD&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Fuels from wastes: Make biodiesel from trap grease; Make biofuels for algae in open systems.	Highly variable feedstock.
PI Response: No response to this comment has been provided by the Principal Investigator.	
High level approach recognizes the practicalities of the feedstock and attempts to deal with rationally. Plan is geared towards commercial applications Inclusion of an economic analysis is essential Benthic culture approach is very worth exploring	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
The immediate research plan calls for collection of raw feedstock, conduct conversion	Grease is high in free fatty acid and water content and is challenging to convert. "Wild" algae are generally low in lipids and what to do

chemistry, scale this up to 50L.	with residual cellular biomass; Ferment it to biofuels. How much can really be produced form the residue?
PI Response: No response to this comment has been provided by the Principal Investigator.	
haven't started	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Production of diesel from food oil (trap grease). Production of biofuel with algae on municipal wastewater—outreach component regarding production and use of biofuels—will try to apply methods that can be used in both systems. Plan to do economic and energy analyses for production of both fuels. Plan to use benthic algal communities for ease of harvesting.	very few details, so difficult to ascertain limitations
PI Response: No response to this comment has been provided by the Principal Investigator.	
Working to adopt the research plan model. This is an approach to smaller-scale distributed production of fuels rather than centralized large-scale production. Educational component of mission is a piece of the effort. Using attached algae community to improve recovery.	Most of the critical issues to be dealt with are regulatory and logistical rather than scientific. No clear plan for collection and transport--clarifying the issues. Issues related to waste disposal another barrier not wholly integrated into the process.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Contract still being finalized.	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
DOE project has not yet started Other work has provided a significant baseline to grow upon and justifies approach.	Would like to see an early process flow diagram with economics against which progress can be explored.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have gathered information on the composition of raw materials and developed strategies for working with materials.	Have yet to receive DOE funding so just planning.
PI Response: No response to this comment has been provided by the Principal Investigator.	
0 % progress and funding	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
project is just starting—no work on project to date	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
No funding received yet	---
PI Response: No response to this comment has been provided by the Principal Investigator.	
Not started yet	---
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Algal turf easier to harvest.	Trap grease handled differently in different places. Difficult to control algal metabolism in open system. Primarily using biomass not oil.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well described and understood success factors and risks Good understanding of commercial implications of project	Key risks are inherent that are fundamental to this project and represent significant risks without a well defined plan to address
PI Response: No response to this comment has been provided by the Principal Investigator.	
Ultimate goal is to scaling up to industrial scale. Achieving a favorable energy balance is necessary.	Not really specific, identifiable items.
PI Response: No response to this comment has been provided by the Principal Investigator.	
favorable energy balances economic viability	- may not have considered major limitations,

water content limitations markets for byproducts	such as breaking out overall economics of system
PI Response: No response to this comment has been provided by the Principal Investigator.	
To address collection and transport issues and regulatory issues will be a major goal.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research Approach and Relevance (as defined in the project)

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Understanding logistics of grease collection. Build pilot plant. Initial fermentation to ethanol and eventually butanol.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined plan for future work.	Would like to see timelines and expected milestones given scope of work remaining
PI Response: No response to this comment has been provided by the Principal Investigator.	

Implement research plans and processes outlined. Investigate conversion of residual algal biomass to fuels [esp. butanol]	Conversion of the residue is not trivial. Certainly more complex than producing biodiesel. How much is likely to be generated?
PI Response: No response to this comment has been provided by the Principal Investigator.	
compete project looking at feasibility of trap grease and wastewater/algae biofuel processing	
PI Response: No response to this comment has been provided by the Principal Investigator.	
More collaborators	Dewatering is a topic to address.
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Well thought through collaborations are being well used to ensure commercial relevance of study.	
I believe there is a company in Philadelphia that does the waste grease conversion (Black Gold Biofuels, formerly Fry-O- Diesel). Investigators may want to investigate this.	
project has private industry and municipality collaborators	
Need a business school partner	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Not sufficient common science to support waste	

stream utilization link—break into two and associate with trap grease projects in thermal conversion platform.	
Not clear how the two projects build off each other. Ensuring that there is internal leverage would be valuable.	
Conduct some simple process and economic modeling/ estimation. What is really the potential for fuel production from these resources? How much?	

Snohomish County, Biodiesel Project (WA)

Technology Area: Biomass Program
Project Number: 7.4.1.6
Performing Organization: Snohomish County
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	2.57	0.98
Approach	3.00	0.58
Technical Progress	3.29	0.76
Success Factors	3.14	0.69
Future Research	2.71	0.95

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs has been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
County effort to minimize costs and create/preserve jobs.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined project to assess fuel development in a local economy. Important case study consistent with OBP goals	Without an understanding of region characteristics, the data may not be extendable to other regions.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Develop a local, renewable fuel capacity to operate municipal vehicles.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	dealing with a local set of goals and contingencies
PI Response: No response to this comment has been provided by the Principal Investigator.	
- production of biodiesel for municipal use—possible worthwhile test of looking at biofuel implementation at a local scale, or biofuel at the local level -	not particularly relevant to commercialization of fuel ethanol
PI Response: No response to this comment has been provided by the Principal Investigator.	
Community project	Not new technology for commercialization Not a match with MYPP objectives
PI Response: No response to this comment has been provided by the Principal Investigator.	
Local control of fuel, business development	Market analysis locally driven by municipal government. Not very clear how this relates to

motivation	DOE platform goals
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Produce from canola biodiesel for local consumption.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Execution focused approach provides good empirical data Recognition of pros and cons on biodiesel.	Slow iteration time implicit in design. Several factors (i.e. market dynamics), which can greatly impact the project are not able to be influenced by the project.
PI Response: No response to this comment has been provided by the Principal Investigator.	

Construction of a plant to produce biodiesel for municipal vehicle fleet using locally grown canola as a feedstock. Canola is a good rotational crop in Washington State. Can pay growers $0.25 per pound which it a profitable option.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
solving a practical problem	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- tested different oil seed crops in various plot sizes - worked with processing of canola (chosen crop) - worked with community on logistics	- seems could have brought in more outside expertise and, if possible, obtained sufficient funds to purchase all necessary equipment (rather than splicing older pieces together)
PI Response: No response to this comment has been provided by the Principal Investigator.	
logistics issues dried seed with biogas from dump	Hard to displace working economic activity
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

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and may contribute to overcoming technical barriers.

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Strengths	Weaknesses
Built plant	GMO barriers—acceptance by locals.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Data to date has identified several key challenges. Key challenges have been systematically addressed. Data has led to efficient movement towards a successful local operation. Rapidly learning key lessons about use of biodiesel in a community. Proven capability to install necessary pieces as well as to interconnect them, which of course is important on an execution project. Data is trending to support local use of biodiesel, and environmental data will be generally valuable Project has been de facto successful	Certain data and processes are raising local and potentially global concerns. Several technical challenges, but were typically overcome.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have setup infrastructure to do this; growers, harvesters, etc. Facility is also using landfill gas to run driers. They also conducted a required air quality and emission assessment.	What are the anticipated costs and economics of this program? Is this something that could be transferred to other localities?
PI Response: No response to this comment has been provided by the Principal Investigator.	
scrounged together the needed equipment and materials	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- have decided upon a crop - have obtained land for production and related equipment - have obtained some processing equipment,	

including dryers powered by landfill biogas - have obtained storage equipment - trying to get things all integrated - have began monitoring biodiesel performance of vehicles - past year harvested over 300 acres of canola for project - have done air permit calculations	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Is achieving demonstration of locally providing fuel. Have put "steel in the ground".	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Working if subsidized. Potential to work as a local integrated close-the-loop process.	Not very committed to economic analysis for long run success
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

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2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Acceptance by locals; economics.	Small scale that works locally—may be different in other places.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Recognition of certain risks Have been able to systematically deal with challenges as they present	Certain key risks (i.e. volcanoes, earthquakes) are not addressable Have not predicted several challenges
PI Response: No response to this comment has been provided by the Principal Investigator.	
Local farmer acceptance seems to be the biggest challenge to success. It appears that these concerns have been resolved.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
the processing facility must be up and running and viable	
PI Response: No response to this comment has been provided by the Principal Investigator.	
$160/acre for growers	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

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2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Add crusher and combustion engine to make electricity.	Low relevance to MYPP
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focused future work plan Clear time lines and milestones	Significant variability is intrinsic in plan.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The oilseed crusher has delivered and will get operational soon and combustion engines for power generation from landfill gas.	Not really relevant to OBP and Biochemical Platform milestones but certainly important to this project
PI Response: No response to this comment has been provided by the Principal Investigator.	
an interesting local project with a positive impact	
PI Response: No response to this comment has been provided by the Principal Investigator.	
get crusher functional, produce biodiesel, and maintain system	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Built the refinery. First crush May 15. Working with biogas from landfill to finish drying and cogeneration.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Project is well connected between key players, and designed to enable local commercial success. Tech/knowledge transfer is a clear goal.	
built in	
this is a validation project, so technology transfer is really not an issue	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Characterization of general localized parameters would allow for a potential generalization to other locales.	
Good model for other communities.	

Bioeconomy Initiative at MBI International

Technology Area: Biomass Program
Project Number: 7.4.2.4
Performing Organization: MBI International
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.29	0.95
Approach	4.00	0.00
Technical Progress	3.71	0.49
Success Factors	3.57	0.98
Future Research	3.86	0.90

Overall Principal Investigator Response(s)
We fully understand the difficulty of evaluating a project, the progress, the approaches when the products cannot be disclosed. We had offered before the review in Denver to disclose the products and the organism to the review panel, but not to the public. We would be happy to provide the information to the reviewers, please let us know.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
High value co-products may be important to commercial success.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Program is relevant to the goals of OBP Organic acids represent an important avenue of exploration, especially given previous publications from OBP Broad opportunities that can result from this	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Research plans to use Actinobacillus succinogenes for production of other organic acids other than succinate. Make value added products in corn based biorefinery/mill	Not directly focused on Biochemical Platform goals, but could be a source of bioproducts as replacements for petroleum based chemicals.
PI Response: Organic acid production is very suitable for the integration into ethanol biorefineries creating value-added by-products. Our approach to develop a fermentation process for A. succinogenes that utilizes biorefinery by-products as the major medium component (Thin Stillage), and some of CO_2 produced in ethanol fermentations, we are producing a bio-product that is part of the corn wet mill and corn dry mill improvement pathway, stated in the MYPP. The change in organism used in the fermentation has eliminated the use of Thin stillage, but the other benefits still apply.	
test feasibility of producing organic acids with A. succinogenes—develop fermentation process and assess performance	diversion of readily fermentable sugars to organic acids may not promote biofuels production, but good for biochemicals

	production
PI Response: The production of organic acids as a new bio-product in ethanol producing bio-refineries could be a valuable, profitable by-product . It would increase the diversity to the bio-refinery products and has the potential to improve the profitability of the corn mill operations. Furthermore, the initial focus on modifying A. succinogenes was based on the organism's propensity to utilize multiple carbon sources simultaneously. This would allow the use of C5-sugars that are not consumed by the current ethanol -producing yeast strains, when non-food, biomass-derived sugar streams become the feedstock for ethanol fermentations.	
Switched to more effective microorganism for organic acid A	Why the focus on A. succinogenes? What are the advantages over other possible bacteria?
PI Response: The focus on A. succinogenes was based on the organism's desirable trait that it is omnivorous, i.e. it consumes multiple 5-carbon sugars, 6-carbon sugars, and glycerol simultaneously. This feature would greatly facilitate utilization of non-food, biomass-derived sugars streams.	
Alternate products proposed; Includes cost analysis. Organism has been known before.	Principal value is to be derived from co-products, not energy fuels.
PI Response: True, organic acids would be a co-product of bio-refinery operations. However, the utilization of bio-refinery by-products may enhance their profitability, and reduce their carbon footprint through CO_2 incorporation into the product.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Make organic acids co-products of value using herterologus expression of pathway genes.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Broad approach covers several organic acids Leveraging previous knowledge to move up the value chain in an efficient way.	The market interest of the various organic acids is recognized as mixed.
PI Response: We used the market interest for the various acids to guide our research efforts and utilize our resources most efficiently.	
Capitalizes on MBI experience with this organism to produce succinic acid. Builds up this as a starting point for production of other products. Will metabolically engineer A. succinogenes to produce different organic acids.	Products of interest not disclosed, just ranked in relative value and market so it is difficult to judge the details of the approach
PI Response: That is a very valid comment, and could be considered for future review processes. I had offered and supplied a PMP to the DOE office that specified the products to facilitate the reviewer's understanding. The secrecy was necessary for the public presentation.	
Developing low cost paths to co-products would enhance the product stream of ethanol biorefineries.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
-chose organic acids based on market interest organism - A succinogenes, expression of heterologous pathways and genes - looked at different organisms for production of different acids	
PI Response: No response to this comment has been provided by the Principal Investigator.	

Metabolic modification by genetic engineering. Established QC targets with industrial partners
PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

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2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Developed clean (not sterile) fermentation process for acid production. Failed on acid A&B. Did make acid C but market interest is low. Then identified suitable organism that uses C5 and C6 sugars for acid A, which yielded 77g/l. Recovery excellent (96%) at lower cost. .	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Validation of fermentations is an important milestone Titers and productivities are consistent with a project that has moved directionally well. Follow on high value project	Targets are appropriate for exploratory work, but not clear if enough head room for an industrialized commercial process. Failed metabolic engineering project is of note. Would

with very significant data.	have liked to understand why this didn't work. Probably a lot to be learned there. Good that the project was discontinued, however with failure.

PI Response: The economic targets for organic acid A are clearly defined. It is currently a product in a niche market with a known price. MBI has an economic model for the fermentative production and recovery of organic acid A that projects the manufacturing cost. The model identified the sugar as the largest cost contributor to the manufacturing cost, which led to the conclusion that yield improvements should be the target for future improvements. Reducing the amount of sugar needed, while maintaining other performance factors of the current process, would reduce the manufacturing cost by 27 cents/lbs. This would bring us a big step closer to entering high volume markets, although additional smaller changes may be necessary.

The production of other organic acids in A. succinogenes was a high-risk project. A.succinogenes is geared towards succinic acid production and the anaerobic process derives a significant portion of its metabolic energy from fumarate respiration. It is reasonable that any alteration affecting the energetics may disable the organism limination. A. succinogenes is not a widely used organism, and our knowledge of molecular tools, tuning of expression levels for multiple proteins in the organism are limited. It seemed beyond the scope of the program to elucidate in further detail.

Most importantly, we learned only during the last quarter that MBI may have restricted IP freedom to use A. succinogenes for the production of organic acids A and B.

It looks like the project has made good progress toward determining that A. succinogenes is not a viable candidate for production of higher value organic acids. A decision was made to switch to production of product A with another organism which makes this in high yields from c5 and c6 sugars in simple medium	Use of a secret bug to product a secret product makes it difficult to more fully evaluate.

PI Response: I had offered to identify the products and organism for the reviewers, see response in section 2.

This project had a very organized and fruitful approach, in both successful and unsuccessful trials.	

PI Response: No response to this comment has been provided by the Principal Investigator.

80% complete - developed fermentation process using	

available carbon Iraw starch from dry milling) sources and modified strains to produce other acids - worked out test process, including liquefaction, saccharification and pasteurization components -attempted introduction of novel pathways for acids A & B but organism continued to produce succinic acid, so chose to not continue working with A. succinogenes - found new organism that could be developed to produce organic acid A -demonstrated recovery improvements to obtain 96% recovery with 95% purity -	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Lack of knowledge of product make difficult to evaluate results.
PI Response: I had offered to identify the products and organism for the reviewers but not the public, see response in section 2, reviewer 17902.	
Parallel development of existing organism, fermentation and metabolic manipulation. Need pasteurized sugar stream; thin stillage gave inhibitory components upon sterilization; used pasteurization instead. Expect same fermentation in this. Antisense RNA used for manipulation of genetic system. Heterologous expression. These did not work. Milestone block. Can make better succinate-->acid C stream with heterologous additions Chose a different organism. Need IP assessment Economic target also available. Examined fermentation conditions, medium; got 77 g/L; fed batch got 1.6 g/L-h Recovery process demonstration. Purity increased from 85% to 95%, 95% recovery; capital cost 7.3/MM	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
	Chemical companies usually don't have fermentation facilities or experience.
PI Response: We are aware of this. Our marketing strategy involves contacts with producers and end-users.	
Commercial focused success is thought through Challenges focus on industrialization. Clear plan to address scaling issues, and are well suited to do so.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Logical list of challenges and barriers to further development and commercialization.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
good grasp of what it will take to get this to market and the market segments that are	

potential	
PI Response: No response to this comment has been provided by the Principal Investigator.	
working out potential pitfalls	providing system that chemical companies accept (finding adopters)
PI Response: We have contacts with producers and chemical companies. Samples to assess product quality and purity, produced in our own pilot facility, have been provided to chemical companies. Finding the adopters, identifying their concerns and system requirements is ongoing but we have had some positive feed-back.	
De risking and scaleup. Price points accessible to high-end/low volume markets. Many chemical companies tend not to have fermentation; so producer and end-user integration Are ready to go to pilot scale.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Plan scale up and metabolic pathway development.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan to scale is important for industrialization Appropriate goals and timeline set for scaling and potential commercialization plans.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Scaling up of product A and exploration for new organisms for product B appear to be productive lines of research.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
strongly focused on bringing this concept to the commercial stage	
PI Response: No response to this comment has been provided by the Principal Investigator.	
scale-up for organic acid A continue to improve production/recovery acid B establish metabolic models	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Scale up, establish metabolic model and reduce by-products attack acid B.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response

Clear plans for commercialization, though partners remain to be determined.	MBI has developed a succinic acid production process using A. succinogenes. We are actively marketing this product and identified producers as well as end-users. We know that other organic acids, especially organic acid A, may involve the same industrial partners.
Clearly trying to look to chemical companies for	Chemical companies have been identified as the end-users, and we are supplying some of them with product samples to assess product quality and purity. We are also in contact with potential producers, i.e. companies that have large fermentation facilities.
Assess IP situation. Looking for partner; patent application in progress	Development of our IP position on fumaric acid is a high priority currently.

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Would be good to understand how process improvements impact the economic model	A moderate increase in yield to 0.95 g product / g glucose consumed, which is below the theoretical yield for organic acid A, would reduce our manufacturing costs by 27 cents/lbs, leaving other parameters at the current level. In itself, this reduction would not be sufficient to enter the commodity market, but it is the biggest leap that can be achieved altering one aspect. The next step would involve increase of productivity, which should bring us close to the envisioned target.
A logical execution of research plan but difficult to ascertain with so many secrets	We certainly are aware of the situation and I had made the suggestion to provide the information on the specific organic acids and the organism to the reviewers.

Intermediary Biochemical's (MI)

Technology Area: Biomass Program
Project Number: 7.4.2.6
Performing Organization: Intermediary BioChemicals (MI)
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	2.71	1.25
Approach	3.14	0.90
Technical Progress	3.29	0.76
Success Factors	3.14	0.69
Future Research	3.00	0.82

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Working with MSU	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focused on OBP relevant goals. CO2 fixation has not been listed as a defined goal of OBP, but is a valuable add. Goals focus on a commercial operation consistent with OBP focus	
PI Response: No response to this comment has been provided by the Principal Investigator.	
An ambitious program of research to produce cost competitive bioproducts.	While worthwhile, not much focus on overcoming Biochemical Platform barriers to production fuels. Could potentially serve as bioprocesses as alternatives to petrochemical processes.
PI Response: No response to this comment has been provided by the Principal Investigator.	
A potentially useful sidestream -- aspartate use is cost limited	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Adapt A. succinogenese to produce aspartate and fix CO2 develop electrochemical bioreactor - prepare stable alkaline phosphatase for analytical use - develop electrochemical reactor for manitol production	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Limited market for aspartate Alkaline

	phosphatase unrelated niche market? Relationship to DOE? Not good match to MYPP objectives
PI Response: No response to this comment has been provided by the Principal Investigator.	
Coherent goals	Not aimed at fuels
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Adapt A. succinogenes using molecular genetics to fix CO2 and reduce cost of aspartae production by replacing fumerate with bioreactor produced fumerate; develop bioreactor for manitol from glucose; improving alkaline phosphatase. Aspartate identified as	

building block but too expensive.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focus on aspartate is a useful program. Using existing strains.	Requires development of tools, which is an unpredictable process. Bioreactor approach represents a clear uphill battle
PI Response: No response to this comment has been provided by the Principal Investigator.	
Really three independent projects: 1) Redirection of Actinobacillus succinogenes metabolism to produce fumarate instead on succinate, 2) Over expression alkaline phosphatase with more desirable temperature activity and stability properties, and 3) development of an electrochemical bioreactor (here used for production of mannitol). Approaches described to advance development of these are logical	Development of the electrochemical reactor appears too much further along than the other projects which are in much earlier stages of development.
PI Response: No response to this comment has been provided by the Principal Investigator.	
	A small project with some difficult goals
PI Response: No response to this comment has been provided by the Principal Investigator.	
Use bacterial strain already adapted for fumerate production, introduce appropriate genes for aspartate production (aspartase) and use renewable surface technology with electrode for bioreactor	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Too many activities to achieve real results with any of three different projects. Project needs focus on one activity.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Fix Co2 to aspartate; replace existing fumarate-->aspartate with biological process from A. succinogenes. Need to develop molecular	Somewhat disparate goals

genetic tools.
PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Recent start. Current bench scale fermentations yields are cost effective.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have clear gene identified. Organism choice has clear advantages. Validated assays Early safety related data for bioreactor is promising. Have identified model and limiting factors which will allow for a go-no go decision	No successful gene transformation into host organism as of yet. No clear integrative data. No clear interconnect between projects AlkPhos data has not validated success of project.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Research on the aspartate and alkaline	Further progress will hinge on whether genetic

phosphatase projects are progressing with the development of the molecular approaches needed to advance these projects. Investigators were able to demonstrate operation of the electrochemical reactor.	tools being developed perform as needed. Difficult to predict success at this stage of the research. Yields for mannitol [example used here] will need to be improved to make this process competitive.
PI Response: No response to this comment has been provided by the Principal Investigator.	
	a one year project with few goals met and one month to go
PI Response: No response to this comment has been provided by the Principal Investigator.	
- screened different expression systems for alkaline phosphatase - validated assay used to develop system - achieved glucose to manitol yields of 50%, have developed a bench-scale bioreactor, surveyed different electrode surface interfaces	
PI Response: No response to this comment has been provided by the Principal Investigator.	
--Bench-scale fumarate fermentation Genetic manipulation has been demonstrated. Low efficiency; need selectable markers; shuttle vectors in testing. Improved growth on C5 --Alk Phos. More stable reagent than CIP wanted. Do modification of gene form thermophile to make expressible. Gene synthesis. Constructs in testing PNPP activity assay validated. Automated mutagenesis system has been developed and in testing. --Electrochemical bioreactor; need renewable carbon electrode surface chemistry for enzyme immobilization and electron transfer; a bench-scale bioreactor to demonstrate feasibility of conversion of fructose to mannitol; glucose to mannitol Immobilized enzyme system driven by electrons. Series of electron donor/acceptors. Maximum performance determined by number of layers. Have a balance equation.	Too many projects

PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Clear description of critical success factors and potential challenges.	Limited market for AP.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear understanding of needs to be success. Clear understanding of challenges and are well aligned with success factors. Market opportunity has been reasonably characterized Clear market risks	Plans to leverage a developed organism are not discussed. Means of solving real showstopper problems are not evident. Working on an old project that has been unsolved—no clear plans on how to overcome well established problems.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Identification of cost competitiveness and customer acceptance are logical success factors.	What are the anticipated lifetime of electrodes and enzymes?

PI Response: No response to this comment has been provided by the Principal Investigator.	
- must get genetic tools to work on succinogenese - generate purified aspartate - must prepare alkaline phosphatase system of considerable benefit at lower cost - must produce manitol at lower costs than presently available	- get new alkaline phosphatase accepted by industry currently using existing system
PI Response: No response to this comment has been provided by the Principal Investigator.	
--Identified technical barriers for A. succinogenes --Alk Phos needs to fit established conditions. Neogen is partner --carbon electrode performance needs demo; cost of fabrication; confidence in scale-up	--Little demonstrated expertise in metabolic engineering. --Niche market Alk Phos.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Clear plan for molecular approach to increasing yields.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals and milestones well defined and consistent with making this a successful project.	No data to suggest that key milestones will be achieved. The project may be limited in even the earliest milestones.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Continued development of genetic tools for the organism and enzymes work and scaling of the electrochemical reactor are logical lines of investigation	
PI Response: No response to this comment has been provided by the Principal Investigator.	
reasonable list	this project is effectively finished (June 2009)
PI Response: No response to this comment has been provided by the Principal Investigator.	
continue application of genetic tools to improve aspartate production - continue work to develop stable AP for commercial use electrochemical bioreactor - improve electrode performance for lower costs of bioelectrode processing	
PI Response: No response to this comment has been provided by the Principal Investigator.	
--Focus on genetic tools for A. succinogenes. Developing auxotrophic markers. --screening want 20% CIP sp act at 25%C with room temp stabil advantage.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations

Does the project adequately interact, interface, or coordinate with other institutions and *projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?*

Reviewer Comment	PI Response
Neogen identified as commercial partner.	
Clearly have plans for collaborations and technology transfer, but not well defined	
There is a commercial partner for AP work	
currently looking at partners for application of these technology	
This is basically a technology development shop associated with university. MSU. Knows what he's talking about scientifically	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Projects are not clearly related—no necessary synergism. Given challenges associated with each, would be more productive to focus on one or two rather than the breadth of them.	
Electrochemical Bioreactor and Aspartate Production do not belong in the same project, especially one with limited funding.	

Connecticut Biodiesel Power Generator (CT)

Technology Area: Biomass Program
Project Number: 7.4.3.7
Performing Organization: The Greater New Haven Clean Cities Coalition, Inc.
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.00	1.00
Approach	3.57	0.79
Technical Progress	3.57	0.98
Success Factors	3.43	0.53
Future Research	3.00	1.29

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
City coalition. May contribute to streamlining regulatory requirements.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Commercial scale demonstration project Clearly aligned with DOE goals. Addressing sensors and controls issues directly as a valuable cross-cutting technologies	Not clear that the program is geared to address standards outside of establishing parameters for local execution.
PI Response: No response to this comment has been provided by the Principal Investigator.	
BioWatz project seeks to overcome regulatory and commercialization/demonstration issues. Producing power from biodiesel. Investigate economic benefits from carbon offset.	Project doesn't target many Biochemical Platform objectives but could serve as technology demonstration and implementation template.
PI Response: No response to this comment has been provided by the Principal Investigator.	
	a useful project at some level, but not for this program
PI Response: No response to this comment has been provided by the Principal Investigator.	
- Evaluate industry standards and regulations - commercial-scale demonstration facility for electricity from biodiesel - sensors etc. for biofuel processing	topics are of general interest to the commercialization of biofuels but do not specifically address biofuel topics
PI Response: No response to this comment has been provided by the Principal Investigator.	
Full information disclosure. Model for others.	Not good match to MYPP
PI Response: No response to this comment has been provided by the Principal Investigator.	

Not clear relevance to DOE program Clear attempt to identify where it fits
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
6 tasks. Build power generator using local biodiesel that has novel control system that is scalable and commercial. Using and integrating mostly off-the-shelf components. Collecting lots of data.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focus on scalability and commercial capabilities Using off the shelf components for repeatable design as well as risk mitigation. Employing utility grade instruments	Broad partnerships and dependencies pose execution risks.

Transparency and open architecture is useful as a development and demonstration project. Approach has proven successful	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Integration of bioenergy based system into power generation system using biodiesel as the feed material. Lots of controls and monitoring. Participation of many stakeholders.	What are the economics associated with using biodiesel for electricity generation?
PI Response: No response to this comment has been provided by the Principal Investigator.	
- integrate best-in-class technology for electricity generation - considerable data collection for performance evaluation - system is open to public via the web using fully integrated approach to allow very diverse members of the team to communicate	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Addressing the non-scientific barriers to local distributed fuel/power generation Working to make a replicable result. Utility grade materials. Data collection posted on internet	
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and

objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
2 year project; about half cost from DOE; 40% complete. State of the art systems control.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Successful procurement Safety a clear priority Demonstrated operations Successful build	Unclear implications of dependency on CPL for successful operations via permitting
PI Response: No response to this comment has been provided by the Principal Investigator.	
All equipment has been procured, site improvements performed, and now commissioning is underway.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
procurement on path, some testing finished	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- 40% of way through project timeline - obtained all necessary equipment, integrated equipment, tested performance, - have prepared considerable documentation for commercialization by others, as well as technical documentation - now waiting to test it with the grid	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Developing standards for local integrated systems. Utility is an effort Equipment installed.	

Utility upgrade to 3 phase power; need final grid interconnect. In progress of commissioning the system. Educational and outreach materials in production for education.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Experience potentially valuable to other cities.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Recognition of needs and process for success. Good recognition of regulatory issues as well as technical issues	Challenges are hard to mitigate and can prove unpredictable Assessment of market opportunity is limited in scope.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Technical and commercial viability of system	

overcoming regulatory issues would certainly constitute success. Short of that, this could serve as a model for others and may streamline this process for others wishing to do something like this.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
practical to get systems up, detail to support marketing concept	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- need streamlined regulatory process requirements - need to achieve desired results (power outputs) - need to be sustainable, viable, technology	possible regulatory issues
PI Response: No response to this comment has been provided by the Principal Investigator.	
Identified regulatory process requirements, integrated system, approachable for the utility comfort. Lots of moving parts	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Commissioning, developing contracts for power and validating technical and economic costs. Complete reports. Baslinine with #2 diesel then working to biodiesel for various sources.	Future work not related to MYPP goals.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear plan for project competition Process is well underway	Unclear timeline.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Demonstration phase to determine system's technical and economical performance. Testing on other oil based feedstocks which may prove to be more economical and increase flexibility is a good idea.	Assess engine durability and function with variable feed materials. They can monitor a lot of engine performance parameters.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The effort to document this process and the results will be valuable for others who can take advantage of biodiesel opportunities and applications.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- just bringing the system on-line, need to demonstrate/validate performance - complete commercialization issues - currently use #2 diesel, will begin testing other feedstocks	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Will compare #2 diesel with all varieties of	

biodiesel. Principal output is to be electricity?
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Project is an execution project with no clear plans for information dissemination of tech transfer beyond the participants Documentation could provide a basis for this, but the plans for the documentation is not well defined	
The goal is to commercialize this approach. They are working as team with all partners and will provide data to all who wish to receive this.	
excellent plan to promulgate this model	
systems are installed and open for inspection by others	
All information will be available of web. Considerable effort on education/explanation to assist others.	
Publication and web-enabled.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Would be useful to understand the implications of a successful demonstration-i.e. are there plans to replicate, scale, etc, or is this a one-off.	

Plans as to how to generalize learnings would be very beneficial	
Seems important to consider economics beyond this specific setting, such that others may use this model for assessing feasibility of such power-generating systems for other situations.	

Development of Applied Membrane Technology for Processing Ethanol from Biomass

Technology Area: Biomass Program
Project Number: 7.4.5.2
Performing Organization: Compact Membrane Systems
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	2.71	0.49
Approach	3.43	0.79
Technical Progress	3.29	0.49
Success Factors	3.43	0.53
Future Research	3.29	0.49

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Dupont spin-off company.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are consistent with OBP program focus Can facilitate ethanol isolation which has potential cost benefits	No understanding as to the potential impact of this avenue of research relative to total cost was offered
PI Response: In our economic evaluation studies we have shown that for a typical 70 MM gallon/year ethanol plant when comparing 1-stage CMS membranes to pressure swing absorption (PSA) molecular sieves there is both a capital and energy cost savings. This has been presented in Slide 19. The CMS membrane system is estimated at less than $2.5MM for capital costs for the final drying of fuel grade ethanol (90% to 99.5% ethanol) while PSA molecular sieve systems are estimated at $4.4MM of capital costs for the same 70 MM gallon/year plant. Energy change in this 1-stage membrane system is 7 billion BTU/yr. A second case evaluated a 2-stage membrane process and compared against PSA system and poly vinyl alcohol (PVA) membrane process. While this 2-stage process added some capital costs it significantly reduced the percentage of water (permeate containing ethanol) being returned to the rectifier column. Therefore our 2-stage membrane system added some capital costs while further reducing the energy consumption. While the 2-stage CMS membrane process provides high energy savings, these savings are not quite as high as those of PVA membranes but the PVA membranes are simply not attractive compared to PSA since capital costs are too high for PVA.	
The goal is to apply a low cost, high flux membrane system for recovery of ethanol from azeotrophic ethanol water mixtures. Successful development may lead to reduction in the cost of product recovery.	This technology is commercially available
PI Response: The technology is commercially available in the form of PVA membranes, which are hydrophilic. This membrane becomes very inefficient at low water concentration (e.g., >90% ethanol), which is the focus of membrane technology use. CMS membranes, which are	

hydrophobic/organophobic, perform particularly well in the range of 90 to 100% ethanol, namely, the range of interest for making FGE.	
membranes could be part of economic refineries	
PI Response: No response to this comment has been provided by the Principal Investigator.	
membrane systems for post-distillation purification of distillate for separation of fuel-grade ethanol (e.g. 95 to 99% ethanol)—membrane systems have applications to many related areas	
PI Response: No response to this comment has been provided by the Principal Investigator.	
No cost extension. Material separations target of the work	No stage gate identified. No customers identified
PI Response: We have partners in various upstream material suppliers (potting, membrane support, coatings) as well as downstream membrane systems users and engineering design firms.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant

contributions progress.

Strengths	Weaknesses
Developing separation systems for removing water using fluorinated polymer membrane technology. Membrane modeling.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Evaluating potentially best in class membranes, which can define viability of the proposed approach. Background data provides significant basis for water-ethanol separations May have applications to other fuels. Rational approach for the task at hand using harnessing previously developed technologies for relevant applications	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Use fluorinated polymers to construct chemical and thermal resistant membrane system that support high fluxes	
PI Response: No response to this comment has been provided by the Principal Investigator.	
very straightforward assembly and testing	not much innovation
PI Response: Up to 2009 the only commercially available membranes for Ethanol drying are hydrophilic, which have performance problems at low water concentration. We are introducing novel hydrophobic/organophobic membranes, which are chemically and thermally resistant. These membranes have extremely high fluxes and operate quite well in the full ethanol concentration range.	
- use fluorinated polymers for membrane development, - have access to membranes for gas separations, degassing liquids and dehydration assisted chemical reactions - gases to be separated by molecular size - will attempt to selectively permeate ethanol from water	

- find appropriate support materials for membranes - demonstrate separation process and do modeling	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Looking for industrial application for existing membrane platform. A number of commercial application for the existing membrane are identified Gas-phase separation--using for dewatering from fuel grade ethanol, biodesel, lubricating oil for wind turbines. Build suitable membrane	Competition not identified.
PI Response: The conventional technology in use is PSA. Our technology is more energy efficient and more cost effective than PSA. CMS membrane performance is superior to the commercially available PVA membrane. There are other membrane companies at various stages of development, but not commercial yet.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP *objectives and technical barriers.*

Strengths	Weaknesses
2 year project now finished. Made prototype membrane for ethanol-water separation and operated system. Demonstrated drying from 75% to 95.5%. Preliminary cost analysis indicate potential saving.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Data suggestive of significant forward progress System well set up for analysis association with commercial potential is potentially valuable Tests in relevant conditions provide reasonable basis for project to continue Key early data validates approach Good baseline comparison between this and other approaches.	Economic analysis is preliminary. Unclear justification of assumptions in economic model. No access for large scale testing—not clear how reflective recirculation is of scaling.
PI Response: The PSA cost data came from NREL Report # NREL/TP-510-32438. We used consistent assumptions with NREL report for the membrane process, e.g., materials of construction, labor, installation cost factors, etc. We are talking to downstream companies about testing on a real ethanol-water stream produced from biomass for a pilot scale demonstration. Membrane processes are modular. The results obtained from a single module in recirculation mode or on a single-pass mode can be easily used for scaling up to a larger system.	
Investigators constructed hollow fiber membrane cartridge system and tested this over a wide range of ethanol and water concentrations: achieving dry ethanol (to 99+%) from 74% solutions. Economic analysis suggests these have the potential for capital cost and energy savings over conventional molecular sieve based systems.	What is the potential lifespan of these membrane systems?
PI Response: It is projected to be at least 5 years. Follow-up test work includes a several-month exposure of the membrane module to 100% ethanol at 120C and 65 psi and verification that the	

it retains its performance.
assembled and tested modules and demonstrated performance
PI Response: No response to this comment has been provided by the Principal Investigator.
project from 2006-2008 - built and operated ethanol-water lab separation system - tested wide range of conditions using test membrane modules - have done economic analyses, comparing their membrane systems with alternatives
PI Response: No response to this comment has been provided by the Principal Investigator.
created hollow fiber porous support, build a lab separation system for etoh-wter sep. Started and operated, collected data Dried from 75 to 99.5% etoh; prelim economic anal. Fuel-grade ethanol in recirculation mode.
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome *showstoppers are not well developed.*

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Potential need—Biodiesel can contain significant water (1500 ppm); US regulation limits to 400 ppm.	Membrane lifetime not evaluated.
PI Response: This will be addressed in follow-up test work: module will be exposed for several months to 100% ethanol at 120C and 65 psi. We will verify that performance is retained.	
Identification of basic success factors Challenges well understood. Experienced team that can likely solve problems as they develop. Well understood market opportunity and plan to get to market.	Did not address scale-up
PI Response: Membrane systems are modular. Scale-up is straightforward. We are talking with engineering design companies about partnering to run a pilot scale demonstration unit to produce FGE.	
Investigators indicated that cost and reliability in real commercial application need to substantially reduce cost to gain acceptance.	Although potentially cheaper, is this great enough to get industry to change at this point?
PI Response: Short term strategy is to incrementally increase existing plant capacity production with membrane systems retrofit at no risk to customer. Long term, new plant construction will occur with the introduction of cellulose ethanol. By then, we will have demonstrated the value of CMS membranes.	
Adequate identification of factors that must be dealt with to demonstrate market viability.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
lifetime of operating modules production costs of modules	potential problems in use with "real" distillates, currently done with only water/ethanol mixtures
PI Response: As part of the follow-up work, a test will be run with an ethanol-water mixture coming from the distillate of a fermenter. We will verify that the performance is not affected by the actual bio-ethanol feed.	

Lifetime of membrane resources for validation at biorefinery manufacturing cost. Reliability of operation with other gas mixtures. May use to reduce Nox from tailpipe emissions.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Expect future funding. Apply to other separations. Test new (improved)P membrane. Test in commercial setting.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well described future goals	Timeline would help to assess milestones.
PI Response: A general timeline was presented in the conference (Chart 27). A more detailed timeline will be presented in the follow-up work proposal.	

Long term testing and scaled up test models at commercial plants are the next phases for evaluation of this technology.
PI Response: No response to this comment has been provided by the Principal Investigator.
Product development through manufacturing.
PI Response: No response to this comment has been provided by the Principal Investigator.
testing with industrially relevant samples long term testing of modules scale-up work test new membranes continue optimizing models for performance and economics
PI Response: No response to this comment has been provided by the Principal Investigator.
To update against economics optimize performance, find partner to test real stream.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Project is focused towards commercialization of these membranes. Company is planning for commercialization. Publication is not expected.	S. Majumdar, D. Stookey and S. Nemser, ―Dewatering Ethanol with Chemically and Thermally Resistant Perfluoropolymer Membranes", Presented at the 2008 International Congress on Membranes and Membrane Processes (ICOM 2008), Honolulu, Hawaii, July 12-18, 2008. S. Majumdar, D. Stookey, S. Nemser, D. Campos and K. Pennisi, ―Perfluoropolymer Membranes for Dehydration of Ethanol", Presented at the AIChE Centennial Annual

	Meeting, Philadelphia, PA, November 16-21, 2008.
Commercial entity with experience in membrane separations, especially dehydration.	
no partners	We have partners in various upstream material suppliers (potting, membrane support, coatings) as well as downstream membrane systems users and engineering design firms.
currently working with industry to find applications	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Development of scale-up plan would be beneficial.	This will be addressed in follow up work.

Biochemical Processing Integration Task

Technology Area: Biomass Program
Project Number: 2.3.1.1
Performing Organization: National Renewable Energy Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.86	0.38
Approach	4.57	0.53
Technical Progress	4.71	0.49
Success Factors	4.14	0.38
Future Research	4.71	0.49

Overall Principal Investigator Response(s)
We appreciate the reviewer's candid remarks and we have tried to address their concerns with our individual comments below. The near-term focus of this project is to demonstrate OBP's 2012 conversion target goals. This approach is being done in parallel to commercial development projects in an effort to mitigate risk to the Program. This task provides valuable supporting integrated performance data for the major unit operations, but also generates information on important but less exciting unit operations such as recycle water, emissions and waste treatment. In addition, we develop new analytical methods directly applicable to commercialization efforts of the biomass industry.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Strong NREL group with subcontract to Colorado State for evaluating membrane for removing acetic acid. Also working with Membrane Applied Science and Technology (MAST) center.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are central to the goals of the biomass program. Provides a pathway to think about execution towards discrete goals of ethanol production by 2012. Goals are quantitative and consistent with programmatic goals. Focus on on-line control and analytical is critical to provide. Clear recognition of challenges to be addressed.	No independent analysis of where goals should be focused on otherwise standardized goals.
PI Response: No response to this comment has been provided by the Principal Investigator.	
This research provides for important integration and demonstration of biochemical platform technologies to meet targeted technical goals.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Needed integrated test toward the 2012 cost	the integration aspect was not that well

goal.	presented
PI Response: No response to this comment has been provided by the Principal Investigator.	
- address barriers facing 2012 biofuel production goals in integrated way - demonstrate 2012 near-term biochemical targets - develop analytical tools for performance evaluation	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Process Integration critical	
PI Response: No response to this comment has been provided by the Principal Investigator.	
General utility critical information development and custody	
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
The team is conducting bench- and pilot-scale research to understand the impact of integrated operations on process performance. They also are developing analytical methods for wet chemical and rapid analysis of biomass feed stocks with emphasis on accuracy and automation. The current emphasis is on corn stover; they then plan to transition to other feed stocks.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focus on process integration is important and unique relative to other projects. Waste stream and emission focus will be important given policy trends. Pilot plant inclusion important to industrial relevance. Development of analytical techniques is of great importance to the industry. Plan to improve throughput of analytical techniques and interface with process control is a need of industry	Limitation to bench scale and pilot scale renders this project behind industrial and commercial development, and will limit the applicability of data. No plans to create standards, though the project is well positioned to do so.
PI Response: We are exploring issues, such as, the impact of waste streams on process economics, process emissions and power requirements that commercial projects have yet to rigorously explore. By doing this work, we will be positioned to offer solutions and independent data to commercial developers on a time line that fits their commercialization plans and achieves OBP's 2012 goals.	
Research is planned to evaluate new technologies at the bench scale on corn stover as they are developed [e.g. enzymes and new microbes]. The eventual goal is to Integrate processes and scale up to pilot scale in NREL pilot plant. The project also provides large amounts of materials to other researchers [pretreated feedstock] and develops and automates analytical tools to support platform	

research

PI Response: No response to this comment has been provided by the Principal Investigator.

Integrated testing of multiple options from bench to pilot. Developing supporting compositional analytical techniques.

PI Response: No response to this comment has been provided by the Principal Investigator.

- start with bench scale systems
- move to pilot-scale systems
- develop analytical tools in parallel with testing integrated processing
- prehydrolysate conditioning with ammonium hydroxide has been looked at to try to lower costs
- via subcontract, working with membrane systems for removal of inhibitors (primarily acetic acid, which also increases pH)
- currently evaluating commercial enzyme performance

seems essential to work with best unit operations for each step in the integrated process—requires constant updates using date generated within NREL as well as outside

PI Response: We agree that it is essential to work with the best technology option for each unit operation. Information from internal NREL work in the Pretreatment and Enzymatic Hydrolysis Task and work in the CAFI group are being continually monitored to determine the best pretreatment technology. In addition, we are monitoring new advances in both cellulytic enzymes and fermentative microorganisms and we will use the best available biocatalysts that we are allowed access to for future work. We have incorporated key dates into our research plan to select the best available technology options to demonstrate OBP's 2012 goals.

Well described multiple projects

PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
The project began in FY 2001 and is scheduled to end in FY 2012. They have made strong progress toward several goals with good data. They are developing an automated workstation for high throughput substrate characterization. They have the instrumentation and are now working out methods. It is expected to be operational in the fall of 2009. This should dramatically reduce sample costs. They looked at pre-extraction which gave some improvement but it is not quite there yet. They are developing NIR compositional methods and have made progress in relating feedstock composition to conversion efficiency. They have demonstrated about a 10% increase in yields over past 4 years. They have a good publication record.	There is difficulty in closing lignin mass balance.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Mass balance work is important Continued analytical development may be posed to achieve key goals including closing mass balance. NIR compositional analysis has shown exciting data validating approach and suggesting the potential that this could become a more pervasive	Inability to close mass balance raises questions about the potential for the analytical methods being employed Unclear why this project also includes enzyme evaluation given other NREL projects on the same. Goals are highly

approach. Understanding of variability of material provides an important baseline for understanding feedstock and pretreatment implications Studies providing insights into effective feedstock utilization in a process setting Early data consistent with process improvements Membrane studies potentially promising avenue to provide an alternative conditioning technology and provides a basis for further evaluation. Process performance studies showing consistently improving yields Reaction time decreases are promising.	overlapping with that project.
PI Response: Regarding the mass balance closure issue, we were the first research group to quantitate the inaccurate lignin mass balances closures around the pretreatment process when using the standard Klason method for measuring lignin. Since that time, we have been working on new analytical methods to resolve this problem. Nevertheless, the standard Klason method is still the only method being routinely used by all research groups. We hope to be able to disseminate a new and more accurate method in the near future. Regarding the enzyme evaluation work, efforts in the Targeted Conversion Research Task are assessing fundamental mechanisms controlling enzymatic hydrolysis, while work in the Pretreatment and Enzymatic Hydrolysis (P&EH) Task studies the rheology of enzymatically hydrolyzed slurries and uses enzymatic hydrolysis assays to understand the effectiveness of pretreatment processes. P&EH is not concerned with the performance of various enzyme preparations during integrated processing. Our work evaluates the available enzyme preparations that achieve high cellulose conversion yields during integrated processing. While the work on commercial enzymes ended about a year ago, we anticipate continuing work to evaluate and then select the best advanced enzymes that achieve the 2012 cellulose conversion target and then use this enzyme during pilot-scale demonstration runs.	
An automated analytical workstation developed that expedites analysis of the large number of samples that can be generated in this research. The development of NIR tools for compositional analysis will facilitate rapid analysis. Testing of process steps and options has identified sources of variability and optimization of these processes.	How does analysis of lignin compare to Klason ADL?? Klason lignin content has been found to have some value for feedstock quality and conversion efficiency. Can't close mass balance on lignin yet.
PI Response: No response to this comment has been provided by the Principal Investigator.	

closing mass balances, using NIR for component analysis, analyzing effect of feedstock variability on results; identifying factors determining performance; membranes; enzyme performance; improved overall performance
PI Response: No response to this comment has been provided by the Principal Investigator.
2001-2012 - customized automated system for biomass analysis, currently applying to macrocomponent analyses - working to improve lignin mass balance values for dilute acid pretreatments - rapid analysis of pretreated slurry, liquid extraction by transmission NIR and solids extraction by reflectance NIR - evaluating impact of stover variability on pretreatment performance - currently testing performance parameters of different process schemes
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

<table>
<tr><th>Strengths</th><th>Weaknesses</th></tr>
<tr><td>Comprehensive approach to success factors. Critical success factors aligned with technical barriers. Include environmental impacts.</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>Key success factors identified and are well aligned with obp goals with a reasonable time scale built in. Recognition of the importance of environmental issues is an important additional success factor. Key challenges have been identified with short term plans to approach.</td><td>Longer term potential challenges are lacking, as are plans to reduce.</td></tr>
<tr><td colspan="2">PI Response: We agree that longer term challenges are missing, but they are perhaps not within the scope of this project, which is focused on meeting the 2012 goals. However, we will begin efforts to address the longer term challenges for the technology.</td></tr>
<tr><td>Good alignment with OBP performance goals. Targets for success.</td><td>Approach has focused on examination of individual process steps. How do these perform when physically combined in an integrated process?</td></tr>
<tr><td colspan="2">PI Response: Our approach is focused on evaluating and improving integrated process performance, but perhaps this was not clearly conveyed. The integrated data we presented at the meeting was for a process configuration in which each major unit operation (i.e., pretreatment, liquor conditioning, enzymatic hydrolysis and fermentation) was performed individually because this process configuration optimized overall economic performance, but was still integrated because material from each unit operation was fed directly to the next sequential operation. We have evaluated other process options (e.g., whole slurry conditioning and variations of separate enzymatic hydrolysis and fermentation or simultaneous saccharification and fermentation), but did not have time to present all of our data and results.</td></tr>
<tr><td>success factors align with biochemical platform goals since this project attempts to tie it all together -must demonstrate integrated</td><td>unit operations chosen for this approach may be out-dated by the time results are obtained</td></tr>
</table>

performance hitting targeted cost parameters— development of rapid reliable methods
PI Response: We remain open to various technology options and have built into our research plans key decisions dates to select technology options that will be used to demonstrate the 2012 goals. Although our selections may not be the same as eventually selected by commercial developers, we believe the other information (e.g., waste stream composition and emission information, power requirements, etc.) generated by this project beyond demonstrating achievement of conversion targets will be valuable to commercial developers.

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Robust plans for future improvements in analytical techniques, process integration and pilot studies. Includes looking a power requirements and low cost treatment of waste water.	It was unclear from the presentation how well and quickly information becomes available to potential commercial companies.
PI Response: We try to quickly distribute and publish our results at conferences, in journal	

publications and at these review meetings so that the biomass industry is aware of our work. Per input from a previous review meeting, we also distribute a quarterly newsletter to academic and industrial stakeholders that highlights recent task work and major findings. In addition, much of the knowledge we gain is also transferred to industrial partners during execution of cooperative research and development agreements. Many times, information developed during execution of these agreements, to the extent possible, is used to keep our work relevant to the needs of the biomass industry.	
Clear plan to engage plan against economic factors. Solid understanding of primary contributing factors Well designed future sets of plans focused towards key goals. Plans to increase the breadth of the project such that it can continue to maintain or increase the industrial relevance of the project.	Would like to see connections being made between this pilot plant work and that being done by other DOE supported programs to ensure that generalizations can be drawn and relevance to industrial processes can be maintained.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Future research plan is flexible allowing it to be guided by improvements and new developments. Continued improvement of analytical methods (including inhibitors) will provide more details on compositional changes and improve ability to close mass balances. Evaluating new ethanologens with regards to inhibitor response in commercially relevant media and growth conditions will be valuable.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Very extensive scope.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
evaluate process configurations evaluate new thanologens - understand factors limiting overall performance - evaluate low cost media for fermentations - evaluate power requirements and ways to reduce energy use - new pilot plant is under construction which will allow expanded testing of process	

performance - improved analytical methods for raw feedstocks and processing intermediates
PI Response: No response to this comment has been provided by the Principal Investigator.
Integrate new ethanologens Work on assumptions--e.g. power requirement which depends on residence time. To include in the model? Thinking ahead to waste treatment and media aspects. Membrane development Pilot critical additions and upgrade. Assay sample methods to be developed for automation.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Good academic collaboration base with a solid publication record. The project would benefit from collaborations with industry.	
results to be made available through interaction with collaborators, and public dissemination via meetings and publications	
Good publication record.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Scope is quite broad, though apparently well	

covered.	

Integrated Biorefinery- Separations/Separative Bioreactor- Continuous bioconversion & separations in single step

Technology Area: Biomass Program
Project Number: 2.3.1.5
Performing Organization: Argonne National Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.00	0.58
Approach	4.00	0.00
Technical Progress	4.29	0.76
Success Factors	3.71	0.49
Future Research	4.00	0.82

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project

align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
The project is aimed at economical co-product (organic acids) production to provide higher economic yields, which may be crucial for commercial success, it least in the near term and involves a CRADA with Archer Daniels Midland, Co., a strong experienced commercial partner.	Does not directly address to goal of reducing the cost of producing ethanol from cellulosic feedstocks.
PI Response: The technology increases the economic viability of co-products in an integrated biorefinery and would therefore reduce the MESP	
Focus on organic acids is an important component of the OBP Represents a unique set of goals within the set of projects funded. Focus on integrated production ensures relevance of project as well as its industrial applicability.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This is an interesting technology that could be used to clean up/ condition biomass hydrolysates prior to fermentation and recover an additional organic acid product from the process. Use of this and other similar approaches would move the overall process toward an integrated biorefinery that would produce multiple bioproducts from a biomass feedstock.	Adds another operation to an already costly process. Would need to reduce costs and/or produce additional income to offset this.
PI Response: There will be an economic balance between an additional upstream unit operation to concentrate and clean-up the sugar streams and reduced capital in the downstream	

fermentation and product recovery. As we evaluate performance of the technology we will develop a process economics model. We expect to be able to make a go/no go decision before the next review cycle.	
Developing the concept of a separative bioreactor which could be part of a low cost processing strategy. Separating organic acids electrochemically	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- integrated processing for biobased products is likely to be a key component of economically feasible biomass processing schemes—Separative Bioreactor—coproduct production to go along with fuels; seems coproducts are going to be essential for cost-effective biofuel processing	feasibility of byproducts may be largely dependent on the processing approach for fuel ethanol (pretreatments, etc)—so may not be highest priority until fuel ethanol processing parameters are decided on—although significant byproducts could drive the ethanol processing scheme
PI Response: If the value stream from co-products can reduce MESP, then this well help guidance process approach decisions.	
Developing interesting new technology	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This project is aimed at recovering other products as well as biofuel. This is to help hit cost targets.	The bioreactor seems to have been repurposed from another application. I was left without a clear picture of where this technology fits with other pieces of the platform.
PI Response: The reviewer is correct that the project was originally conceived for another process (sugar desalination). With technology development, the team identified more valuable applications (production of organic acids) and have focused there.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Working at pilot scale. Separation base on resin wafor technology for electrodeionization to remove organic acid from pretreatment. Improved power consumption. Potential applications to other areas such as cleaning wastes.	Issues with conversion efficiency.
PI Response: The performance is within the range we targeted for economic viability. We continue to work on improving conversion efficiency.	
Highly collaborative approach leveraging skill sets of various participants. Distributed study of various process components allows for a broad project to be studied systematically. Well described experimental plan with significant novelty.	Scalability risks only being addressed empirically without a clear model to define risks as well as test hypotheses.
PI Response: From the bench to the pilot was a ~twenty fold scale-up. Except for the lower performance of the two cell pairs at each end of the stack, scale-up is linear. One risk that we identified is contamination. When we have had contamination problems they occurred rapidly. We address them and were able to run campaigns well in excess of our one month target for the pilot campaign.	
The process uses Argonne developed wafer membrane system/ electro dialysis to recover	Has been used on relatively clean feedstocks to date. Will need to be evaluated using more

lactic and gluconic acids.	complex hydrolysates in biomass to ethanol process.
PI Response: We agree that more complex hydrolysates are important. They are planned for the next year.	
All is based on the development of the membranes for the separations.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- their intellectual property relates to use resin wafer technology to recover acids from pretreatment so get neutralization with addition of lime - have considerable interaction with partners for testing their system, so collaborative effort -	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Started by desalting corn syrup, moved over to work on immobilized enzymes. Not so clear to me what the process is as opposed to who is going to do it. Side products recovery to reduce environmental impact and cost structure is in principle an attractive idea but complicated to manage.	How scalable would the immobilized enzyme be.
PI Response: We will have the answer to the scalability of the enzyme system by the end of FY2009.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and

objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Bench scale system installed at ANL and run 3000 hours. Scaled resin wafer to pilot scale. Run at pilot scale by ADM with improve performance in power used, yields compared to bench scale. Good IP position.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Completion of bench scale bioreactor and experience in operations provides a basis for validation and scale-up Successful scaling to pilot. Validation at ADM confirms usability in an industrial setting Significant improvement demonstrated in process economics. Validated scaling without clear limits.	Would like to see more involvement from Argonne on pilot. Would be useful to understand potential concerns relating to scaling and plan to address. Surprising that aspen model was not built until after pilot was built
PI Response: Argonne is operating the pilot at our facility right now. Simplified Aspen models we built early in the project to guide understandings. There was not enough experience with linking biochemical and electrochemical systems to make more robust Aspen models. They will be completed in early FY2010	
A bench scale enzymatic separative bioreactor has been constructed and operated successfully for long periods of time. Demonstrated at ADM for organic acid production in real commercial setting . ADM staff performed operations demonstrating the robustness of technology. 100's gallons of organic acids per day. Demonstrated scalability of process.	

PI Response: No response to this comment has been provided by the Principal Investigator.	
Good progress on all goals. Titer, yield, power, continuous operations. Pilot scale test at Adm under ANL guidance and participation. Great performance at pilot relative to goals.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have demonstrated their acid recovery system at bench scale and pilot scale. - are evaluating the entire process of sugar processing - have exceed proposed goals in several areas	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Separations met challenges so far. 1200 h of continuous operation, 3000 h without process upsets. Using design model (Aspen) to get economics on the process. So far membrane separation at lab scale, with IP on fabrication of the membranes. The goal is to do a continuous process to reduce loss of sugars, reduction of toxin generation. . Long-term operation (>600h) Run by technicians at ADM with telephone advice. In progress of development Enable recycling acid for pretreatment or conditioning steps anticipated.	Not clear that membrane will scale up. Membrane needs to be manned continuously--have done with partner to monitor for long times.
PI Response: The scale-up from the bench to the pilot is ~twentyfold. Argonne's pilot system (other processes) have been operated on a 24 hour day using only one shift to monitor.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Good plans and understanding of challenges. Could form a company for marketing reactor.	Need to demonstrate process and economics with more complex feedstocks. Depends on success of commercial biorefinery efforts.
PI Response: We agree that more complex feedstocks should be tried and will work on that in FY2010	
Recognition of key factors required for success	Challenges are described in only limited terms with a limited plan to reduce the risks.
PI Response: We have additional risk reduction plans in terms of feedstock choices, products, configurations, and membrane vendors. The CRADA partner considers the details proprietary.	
Practical requirements are identified. Long term performance of the bioreactor needs to be determined how long they can perform: So far months, how about years?	Performance needs to be evaluated using biomass hydrolysates: I believe this is planned for
PI Response: No response to this comment has been provided by the Principal Investigator.	
Getting this technology into biorefinery designs. IP development	
PI Response: No response to this comment has been provided by the Principal Investigator.	
they control the IP and background for this technology	- application dependent on finding an appropriate product

PI Response: We agree selection of the target is important for success. We have used the OBP's Top Chemicals for Biomass as a public resource for this decision process.	
	Life of cells and/or enzymes with biocapture wafers
PI Response: We have significantly exceeded our one month targets for the pilot scale. Biocatalyst activity is an important part of the continued technology development	
Finding a builder of the membrane processor	ADM has right of first refusal for their purpose. Unclear how this will affect generalization
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
They are building an Aspen model for the process and have a plan for moving to commercial validation.	

PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined plans for continuing scale up and supporting integrated system. Well defined timeline and milestones. Clear plans to mature technology for applications	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Scale up and completion of process and economic modeling should be completed as planned	
PI Response: No response to this comment has been provided by the Principal Investigator.	
insertion, commercialization, manufacturing technologies	need to examine more complex streams; other processes for application
PI Response: We agree that and plan to evaluate more complex feedstocks in FY2010	
- complete pilot scale enzymatic runs, are currently completing set-up of Enzyme recovery system at Argonne - complete economics models will be developed for these systems	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Process economics to be done, and automation of fabrication. Project has a good eye for other applications to promote investor interest	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response

Industry partner with significant project experience to increase likelihood of technology transfer. Project is lending itself to IP that is being pursued. Technology transfer is occurring directly within this project Questions about broad access to technology given ADM involvement	
Lots of IP on the technology. Working to identify commercialization opportunities for technology.	
partnership with ADM	
closely work with collaborators for tech transfer	
Works in CRADA with ADM	
IP issued and in progress, which will enable public disclosure of the process.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Very well designed project with demonstrated success at development and scale-up. Would be interested in seeing how well this deploys at the appropriate time.	
cleaning up glycerin streams project sound like a good idea	This work was done with separate funding. We did not believe that it addressed the OBP mission

Biocatalyst for Fermenting Hydrolyzate at Low pH and High Temperature

Technology Area: Biomass Program
Project Number: 2.3.2.1
Performing Organization: Cargill
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.43	0.79
Approach	4.71	0.49
Technical Progress	4.43	0.53
Success Factors	4.00	0.58
Future Research	3.71	0.76

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Strong commercial company	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are consistent with the offices program.	Goals represent a singular approach without an opportunity to assess alternative approaches Fundamental goals are limited and not necessarily consistent with the end-points of the program
PI Response: No response to this comment has been provided by the Principal Investigator.	
Targets developing a robust ethanologen that can ferment c5 and c6 biomass sugars, at high temperatures, low pH and in presence of acetate.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
an important pathway to develop	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal is to develop robust ethanol producing organisms capable of converting C5 and C6 biomass sugars.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Uses proprietary yeast that ferments at low pH and high temperature and is resistant acetic acid.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Called in by FOA and subject to validation	

PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Using and developing a proprietary, non-conventional yeast for converting biomass C5 and C6 sugars to ethanol at elevated temperature (~40C) and low pH in the presence of inhibitors (e.g. acetate). Strain engineering will be used to add an ability to ferment arabinose. The approach is based on the use of genomic and good molecular biology tools for pathway integration and development.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Company is using a proprietary organism and working with it to improve net performance.	

Plans reveal a high level plan to get to ethanol.
Approach is well controlled

PI Response: No response to this comment has been provided by the Principal Investigator.

Uses Cargill's yeast strain that has tolerance to variety of adverse conditions . Plan to engineer the yeast for xylose fermentation by incorporating xylose isomerase followed by introduction of arabinose fermentation genes.

PI Response: No response to this comment has been provided by the Principal Investigator.

straightforward methods; tested Cargill advanced strain; utilized modern tools and genomic information

PI Response: No response to this comment has been provided by the Principal Investigator.

use of Cargill's proprietary non-conventional yeast
- use genomics and genome wide tools
- introduce xylose isomerase path

PI Response: No response to this comment has been provided by the Principal Investigator.

No work with hydrolysates. Needs more economic analysis with potential ethanol concentrations

PI Response: No response to this comment has been provided by the Principal Investigator.

Well-defined objectives Proprietary yeast acid tolerant and impurity tolerant, EtOH tolerant. Genomics and genome-wide tools to look at metabolic flux and carry out metabolic engineering.

PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
The project began in July 2007 and will end in February, 2010. It has achieved two initial significant milestones which are to be validated soon; e.g. excellent conversion of sugar; showed that the introduction of XI expression does not degrade performance., and have shown that the process works at a higher temperature. They have Integrated an arabanos conversion pathway and showed it can make ethanol. The yield exceeded target and is 96% of theoretical.	Path to achieve inhibitor resistance not well described. Scale and feed stock not clearly described.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have met internal milestones. Appear to be on timeline Temperature adjustment is valuable Validation of c5/c6 approach in hand Data is quite promising that project will be on target.	

PI Response: No response to this comment has been provided by the Principal Investigator.	
Yeast produces ethanol from hexoses and xylose in good yield in the presence of acetate at low pH and elevated temperature.	Investigators claim co-utilization, but this is hard to see from data presented. How will the strain perform at high glucose and xylose concentrations. This is frequently a problem and incomplete xylose use often results. Were all experiments performed in complex synthetic media? This can have a signification impact on tolerance to adverse conditions. What is the level of inoculum used for these experiments and how was it prepared? Were the organisms grown up under the same conditions and how much did they grow over the time course of these studies?
PI Response: No response to this comment has been provided by the Principal Investigator.	
have shown good progress in yield, rate, titer, and robustness	
PI Response: No response to this comment has been provided by the Principal Investigator.	
achieved xylose isomerase incorporation, achieved incorporation of arabinose utilizing genes, - demonstrated ability of yeast to perform at pH 4.5 - demonstrated that incorporation of xylose isomerase did not change hexose fermentation parameters - demonstrated co-utilization of glucose and xylose - engineered bacterial arabiniose pathway and showed constructed strain could ferment arabinose to ethanol' - tested acetate resistance, found yeast could do well in presence of 10g/L acetate -	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Runs at 40C and does metabolize xylose.	Higher tolerance to inhibitors discussed but no

Succeeded in genetic engineering of strain.	examples presented other than acetic acid.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Bring in xylose isomerase, Bring in arabinose pathway show EtoH could be made Ara to EtoH could be made Achieved these targets remainder with by combining xylose and ara in one strain. Audit results shown. 70 g/L EtoH. (v high tolerance) Show result is robust in XI host. Also show glucose/xylose mix is giving equiv results. Arabinose from bacteria to fungal. Also showed working on arabinose. EtoH titer better than targets x 6 reps rate per h similar of 2X EtoH % theoretical beat target in both cases improved conversion and presence of acetate.	All in pure mixed C-source, not hydrolysate.
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Key critical success factors identified including robustness in commercial performance.	Challenges in combining arabinose and xylose utilization and alternative approaches not well described.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Recognition of what is needed for success and challenges that go with it. Historical performance is consistent with an ability to likely deal with challenges.	No clear demonstration of plan to deal with risks.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Recognition of the need to demonstrate robustness, achieve yield, rate, and titer targets and whether this can be translated from the lab to the plant	How will this strain perform in real biomass hydrolysates containing multiple inhibitors?
PI Response: No response to this comment has been provided by the Principal Investigator.	
reasonable lists, few details	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- shooting for balance between rate, yield, and titer along with robustness of organism	scale-up to commercial operations can be difficult—industrial applications can require even more robustness
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Showstoppers not adequately discussed.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Done well on rate yield and titer. Robustness tbd commercial operations tbd	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Will combine arabinose and xylose utilization. They state that they have methods but that achieving success isn't trivial.	No details provided on methods for achieving success and alternatives is plans fail.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear future goals with a development timeline and milestones.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Researchers plan further strain development; combining xylose and arabinose fermentation capability into single strain.	Is additional assessment of inhibitor tolerance planned? So far the focus has been largely on acetate, but how about other inhibitors in hydrolysates. How do these strains produce in "real" biomass hydrolysates where there are multiple inhibitory compounds present which can act in concert to accentuate toxic effects?
PI Response: No response to this comment has been provided by the Principal Investigator.	

	A set of difficult next steps, but few details as to what will actually be done.
PI Response: No response to this comment has been provided by the Principal Investigator.	
- enhance robustness of strains - combine xylose and arabinose utilization in one strain - continue strain and fermentation development to achieve proposed targets	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Validation, gate reviews, get to the next step	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
There is no basis for tech transfer in this plan.	
How will they transfer this strain to others??? First idea was apparently to outsource this organism and license it to others. Is this the current plan?	
plan is to out-license this technology	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Well thought out plan and project	

Improvement of Zymomonas Mobilis for Commercial Use in Corn-Based Biorefineries

Technology Area: Biomass Program
Project Number: 2.3.2.2
Performing Organization: DuPont
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.71	0.49
Approach	4.43	0.53
Technical Progress	4.71	0.49
Success Factors	4.00	0.82
Future Research	4.29	0.76

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Strong company with excellent track record.	Is the commitment to produce biofuels or other chemical feedstocks?
PI Response: The main commitment is to produce biofuels. Ethanol as the main target and the one that is the focus of this project. Other fuel alcohols are a possibility but to move to that state the first goals are still effective use of sugars from biomass hydrolysate at commercially relevant solids and degree of processing.	
Well defined goals and objectives consistent of OBP goals Project directly address key goals of the program Project focused towards commercial success.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project focuses in improving Zymomonas to meet targets of Biochemical Platform goals: High productivity; complete xylose, glucose, and arabinose fermentation to10% ethanol under commercially relevant [media] conditions.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
exercise in strain development and refinement	
PI Response: No response to this comment has been provided by the Principal Investigator.	
clearly there is importance in improving the performance of ethanologens, to develop a "fuels organism" that fits within commercially realistic biomass-to-ethanol processing - goal to get strain to use both arabinose and xylose in prehydrolysate	

PI Response: No response to this comment has been provided by the Principal Investigator.
Starting strain works in hydrolysate to produce 80 g/l ethanol Strain suitable for genetic engineering
PI Response: No response to this comment has been provided by the Principal Investigator.
A critically important aspect of the platform is to have fermentation strains that use resources to the maximum with low-cost inputs.
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
The approach uses iterative metabolic engineering followed by selective strain adaption under specific growth conditions to	Zymomonas is not an accepted feed supplement. Not clear how the biomass will be

achieve complete utilization of glucose and xylose by Zymomonas mobilis.	used.

PI Response: In the integrated biomass to fuels process the only co-products are other energy forms. The incoming cellulosic biomass is of relatively low feed value other than energy to ruminants. Once the process has fermented those sugars the remaining matter is mostly lignin plus non-fermentable sugars along with the added biomass from the fermenting organism. That combined stream is sufficiently de-watered to allow it to burn with net energy gain and the co-products are process steam and steam of sufficient energy to be used for electricity generation. There is no animal feed stream.

Approach is driven to carry a specific organism to key milestones. High level plan is consistent with traditional integrated design planning.

PI Response: No response to this comment has been provided by the Principal Investigator.

Metabolic engineering of Zymomonas for improvement of characteristics. Use iterative approach to identify bottle necks, sequence analysis of adapted strains to gain information to direct desirable changes.

PI Response: No response to this comment has been provided by the Principal Investigator.

iterative method application in directed changes and adaptation

PI Response: No response to this comment has been provided by the Principal Investigator.

-use a range of genetic engineering approaches
- target directed changes based on key performance analyses
- whole organism sequencing where feasible helpful for informed directed changes

PI Response: No response to this comment has been provided by the Principal Investigator.

Using several techniques to improve Clear pathway to commercialization

PI Response: No response to this comment has been provided by the Principal Investigator.

Metabolic engineering approach using gene addition, deletion and re-regulation, together

with classical strain improvement (random mutagenesis and selection) and sequencing. Metabolite flux explicitly addressed.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Project started September 2007 and ends September 2010. Good progress toward state goals. Integrated and improved strain with arabinose pathway. Improved sugar transport to get co-fermentation. Improved robustness; sequenced strain and identified rate limiting step in xylose utilization. Achieved 100g/liter ethanol. Accomplished 200 L fermentation.	Not clear how well strain will work with different and/or more complex feedstocks.
PI Response: We have worked mostly with corn cob hydrolysate and that is a fairly mild feedstock as it comes from the ammonia pretreatment and enzyme hydrolysis. We have	

fermented hydrolysates from switch grass and from sorghum, both pretreated and hydrolyzed by the same process as cob. They fermented well. We have done no work looking at fermentation of hydrolysates coming from acid pretreatments.	
Promising arabinose data has led to early accomplishment of a goal. Co-fermentation data is quite promising in ability to use sugar High sugar loading has been employed Clear progress against inhibitor removal Data consistent across scaled reaction conditions	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project is making good progress toward developing strains that meet the fermentation and substrates utilization goals in commercially relevant growth media using reasonable inocula. Investigators successfully demonstrated that the process can be scales from 150ml to 200L easily with equivalent performance.	Improvements in sugar transport improved co-utilization of glucose and xylose but at the cost of extending the fermentation time. Likewise for adaptation for improved arabinose use: sacrificing rate of xylose use
PI Response: In the case of glucose and xylose co-utilization the overall fermentation time did not increase but the time to full glucose use did. We did not speed up xylose use rate, we slowed glucose use rate. That is a limitation of doing this only by changing transporter affinity and we hope to be able to combing the equal affinity transporter with faster xylose metabolism to get an overall faster rate. At the present co-utilization seems to give us about a 5% improvement in the extent of xylose use in cob hydrolysate but no improvement in rate. Strategies that give very complete use of arabinose are currently slowing xylose and glucose use. We can get about 50% use of arabinose without impacting the use rate of other sugars but not arabinose use in the range of 90%. Getting high use o arabinose without impacting the other sugars is a goal for the second half of the project.	
Have met most of their task goals and established methodology for further such transformations.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- integrate arabinose utilization into xylose fermentor, showed arabinose utilization but some slowing of xylose utilization - improved sugar transport for co-fermentation of glucose and xylose	

- improve robustness in hydrolysate, attempting this by trying to improve rates of xylose fermentation - analysis of metabolic by-products to get understanding of limiting factors - sequence changes Z. mobilis strains adapted to growth in high concentrations of acetate -	

PI Response: No response to this comment has been provided by the Principal Investigator.

Excellent, multiple real results

PI Response: No response to this comment has been provided by the Principal Investigator.

The project has made good progress toward intermediate goals. Arabinose pathway addition has been done, genetic manipulation methods developed. However, a new problem is that this affects rate of xylose utilization. Separately, cofermentaion of glucose and xylose was achieved--getting over 95% use of both. To improve robustness--increase rate of xylose metabolism. Sequence showed what the slow step was, got faster enzymes for it. Analyzed byproducts to identify metabolic interactions--imbalance in pentose-P. Goal is to prevent dephosphorylation and excretion. Acetate resistance now under study. The cost of sequence is not a problem but understanding the result is. 9 changes now. Achieved 100 g/L EtOH in defined medium with 10 g/L acetate. Doing continuous culture to adapt to hydrolysate. Scale-up to 200L is now continuously integrated into the project. Can get superimposed 1L and 200L graphs.	Cagey about what enzymes were identified as limiting xylose utilization.

PI Response: We did not identify limiting steps. We are still in the process for finding an effective way of removing the first limiting step and that solution may give an intellectual property possibility. This review format required that all information be available for public release and for that reason we could not give details that might be patentable in the future. Sorry

for not fully explaining the reason for withholding information.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Good understanding of factors and challenges. Metabolic yield increase may be a challenge.	Need to improve water-use footprint to control costs.
PI Response: Growth at high solids content and to high ethanol titer are specific targets and they are one of the ways that the organism can contribute to low water use. We will test for effective fermentation in re-cycled process water as the process becomes sufficiently integrated to provide representative process water. This may require further adaptation of production strains but it could also be that extensive adaptation to first pass hydrolysate will provide most of the resistance to inhibitors that will be required.	
Clear identification of factors to support project success. Recognition of where project has been lagging.	No discussion of potential limits that the organism may suffer from
PI Response: Z. mobils is inherently faster at anaerobic metabolism than yeast since it produces 1/2 the energy per mole of sugar consumed and that is positive. That may make it slightly more	

susceptible to growth in high metabolic stress environments but that does not appear to be a current limitation vs. C5 using yeast today. Yeast has more room for improvement and could become preferred it that improvement occurs. Since Z. mobilis is a bacteria ,the prophylactic antibiotics that are available for use in yeast systems to counteract infection are much more restricted. Again because it is a bacteria Z. mobils is likely to be more susceptible to phage infection that is yeast. Process design has to be in place to counter the limitations as much as possible.	
Quantifiable and practical goals and challenges are identified that serve as useful benchmarks.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
well defined	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- success in areas of glucose and xylose fermentations - improved success in hydrolysate performance (robustness)	utilization rates are below targeted rates at this point (glucose and xylose)
PI Response: Rates of use on a fermenter basis are slightly below target and well below the end of project target. There are two means of achieving the target: increase the specific use rate and increase the ending cell mass in an economical manner. We think that is important to improve specific use rate as part of the research path to get good growth in biomass hydrolysate to achieve the second. Both the metabolic engineering work and the adaptation work is focused on faster rates as the project goal.	
The presentation did a very good job of identifying where each aspect of the project fits with platform goals.	Did not discuss in much detail how the pentose pathway integrates with glycolysis. Is it possible to use all sugars for conversion without poisoning the anabolic pathways needed to grow the cells?
PI Response: The organism will grow in clean media on either xylose or arabinose as the only carbon source. Getting catabolism to occur does not seem to be a problem. Growth on either of those is much slower than growth on glucose and it is possible that the rate of production of some intermediate required for growth is slow when 5 carbon sugars are the main carbon source. At present we feel that just slow metabolism of 5 carbon sugars is more of a problem in media that presents a high metabolic stress is of more importance than catabolic bottle necks so	

that is our main thrust.

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Combine arabinose and glucose/xylose co-metabolism—essentially completed. Need Increased tolerance to hydrolysate. Minimize nutrient input.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear plans and milestones built into program. Understanding of key focal areas for future work Exploration is clearly focused towards achieving a commercial organism.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
A clear path is outlined for future strain development: combine arabinose pathway into	

xylose using strain, increase hydrolysate tolerance, faster fermentation rates minimize nutrient input, and reduce seed cost regulatory challenges summary is good
PI Response: No response to this comment has been provided by the Principal Investigator.
well thought out to meet all task goals
PI Response: No response to this comment has been provided by the Principal Investigator.
- improve rates of fermentation in co-fermentors - characterize co-fermenting strains with arabiose utilization strains incorporated - continue to try to increase tolerance of hydrolysates - characterize nutrient needs
PI Response: No response to this comment has been provided by the Principal Investigator.
Identify losses; redox imbalance plays a role. Combine the two aspects (co-fermentation of glucose and xylose, utilization of arabinose). Will move to low complex-nutrient condition. Improve rate need to do things a bit at a time--too much overproduction of enzymes is a problem. Want more cell mass during the production fermentation to decrease seed cost more; need information on specific nutrient needs.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
No partners. No clear plans for technology	While we have no partners in doing the research

transfer or collaboration	or the strain development, we do have a first commercialization partner. We will begin use of one a Z. mobilis strain in a pilot plant that we are jointly developing with Danisco and that use will start late in 2009. Strains coming from this program will be tested at that facility and assuming success will be used in commercial plants to follow.
Path to commercialization outlined.	
commercialization is planned through DuPont/Danisco Cellulosic Ethanol	
Will be used in JV with Danisco. Pilot plant ready 12/09.	
Danisco collaboration for piloting; to choose the first strain this year. Model is internal commercialization of the processing plant	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Well designed project focused on central project aimed at core OBP goals.	

Development of Thermoanaerobacterium saccharolyticum for the conversion of lignocellulose to ethanol

Technology Area: Biomass Program
Project Number: 2.3.2.3
Performing Organization: Mascoma
Number of Reviewers: 6

Evaluation Criteria	**Average Score**	**Standard Deviation**
Relevance	4.50	0.55
Approach	4.17	0.41
Technical Progress	4.17	0.41
Success Factors	4.00	0.63
Future Research	4.00	0.00

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Experienced company partnered with ORNL for omics work.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
PROJECT NOT REVIEWED DUE TO COI	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Proposes the use of a novel thermophilic ethanologen to convert biomass sugars to ethanol (grows at 55 deg). The ability to grow at elevated temperatures and reduced pH complements the activity of commercial cellulase preparations in a way that should allow for reduced enzyme loading and biological integration. The organism also grows on xylan and other plant carbohydrates already (but not cellulose).	In its current form, the ethanol yields are probably too low for practical application
PI Response: No response to this comment has been provided by the Principal Investigator.	
development of commercial strain utilizing genomics and modern techniques - metabolic engineering; at temp and pH optimized for cellulases	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Are focused on fuels organism development, with some emphasis on cellulase loading and biological process integration. All stated objectives are relevant to the biochemical platform.	

PI Response: No response to this comment has been provided by the Principal Investigator.
Strain grows low pH, high temperature, on xylose, etc. Have developed genetic engineering
PI Response: No response to this comment has been provided by the Principal Investigator.
This project approaches the ethanologen problem using a novel organism, and a distinct feedstock (wood chips). Metabolic engineering and classical strain improvement methods, as with other projects in this group, but starting from a very different place.
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Approach involves development of proprietary	

strain using genetic engineering of T. Thermoanaerobacterium saccharolyticum for conversion of lignocellulose from hardwoods. Organism is a strict, thermophilic anaerobe that is compatible with thermotolerant deconstruction enzymes.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Using their metabolic engineered strain that has competing product pathways knocked out, assess capability for growth on biomass hydrolysates and continue strain development and improvements. The goal is to improve shortcomings of the organism while capitalizing on above characteristics. Technical approaches described are appropriate.	What is the upper limit for ethanol production by this organism?
PI Response: No response to this comment has been provided by the Principal Investigator.	
genomic and metabolic engineering approach	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- use Thermoanaerobacterium saccharolyticum, has range of lignocelluose components it can utilize (not cellulose) - employ metabolic engineering, using basic tools - looking at lowering cellulase loadings -employ proprietary bacterium - using resequencing techniques to look at changes to tolerant/robust strains	
PI Response: No response to this comment has been provided by the Principal Investigator.	
55-60C organism that carries out mixed acid fermentation and with pH and temperature optimum is like that of commercial cellulases. Publication in PNAS rational and classical improvement integrated with bioprocess development	Tool and literature background small. Organism is strict anaerobe.

PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Project began in December 2007 and will end in November, 2010. Markerless strains that have mutations to block lactic and acetic acid production, which compete with ethanol production were developed as were new plasmids and selection tools. The genome sequence and annotation was completed. They state they are on schedule for understanding performance barriers. Several overexpression and knockout strains were developed. Metabolite and transcriptome analysis in response to furfural was accomplished. A three-fold increased ethanol tolerance in defined media was achieved but the	Performance barriers have yet to be defined so that an approach to overcoming them can be developed. Strain development used defined media which does not have components that contribute to performance in the real world. Can omics be used to identify complex real-world barriers?

increased tolerance in complex media was much less. The goal is for a five-fold increase in ethanol tolerance.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project has made good progress toward identifying performance barriers and developed approaches to attack these. Use of targeted molecular approaches as well as more classical mutagenesis and selection have generated improvements in inhibitor tolerance. Good simultaneous use of biomass sugars simultaneously, now 4% ethanol conduct SSF, look at performance w added cellulases.. Not dynamic completed genome sequence, map pathways look at expression/ transcriptional responses to inhibitor tolerance¦ targets for gene improvements Panlabs classical strain improvements.. Improvements in inhibitor tolerance analyze mutations, id candidates for exploitation developed genetic system/tools to work with this organism performs well in marker less strains adaptation to HMF and furfural some success?	Need to improve ethanol yield.
PI Response: No response to this comment has been provided by the Principal Investigator.	
have improved inhibitor tolerance and performance; resequencing to determine mechanisms	
PI Response: No response to this comment has been provided by the Principal Investigator.	
2008-2011 -demonstrated simultaneously uses all sugars (glucose, xylose, arabinose) - expanded genetic tool set to allow genetic development - identified some inhibitors and tested performance against these inhibitors - developed informative knock-out and over-expression libraries	

- developed genome sequence and designed microarrays - established a strain improvement program which is being employed in studies on inhibitor resistance - have evaluated organisms in pretreated hardwood prehydrolysates in SSCF type experiments	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Developed genomic tools successfully through collaborations. Worked with inhibitors.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Engineering tools were developed and used to remove lactate, acetate production.	
Shows audit with 5 sugars. Sugars coutilized. Needs knowledge base, tool set, no genome, increase inhibitor tolerance. Markerless strains, develop new plasmids and tools 5 single sugar strains grows well with different lags. Focusing on hardwood feedstocks. Found conditions for metabolic stasis to allow looking at other blocks. Kos and overexpression libraries to inform understanding of metabolism. ID a KO to figure out what would prevent lag to growth in furfual. Completed genome sequence and annotation, created a metabolic model, designed and produced microarrays, did gene expression and metabolomic analysis. Classical improvement also done. Met intermediate goals. Rich media protects against inhibitors. Resequencing to identify the relative important things. 6 isolates, looking for shared mutations. Fewer than thought 25 total shared between more than one strain. Most have quite a few but less than 100. Some optimization of the cellulase digestion conditions. No scaleup yet.	Having to invent the wheel. Resequencing can be less informative than might be hoped.

PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
	Obtaining robust performance under real-world process conditions will be a challenge.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Investigators understand the critical factors that will be barriers to the commercial development of this organism.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
overall ethanol production performance robustness performance under processing conditions scale-up	- obtaining robust strains may be difficult - integrating new enzyme systems in this organism may be difficult
PI Response: No response to this comment has been provided by the Principal Investigator.	

Not strict anaerobe	Ethanol titers need to be improved. Strain does not like high sugars.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Thinks will need less cellulase (a critical cost factor) but this was not shown for improved strains.	much less detailed evaluation of process factors than Dupont
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Future plans involved continued mutagenesis and screening; continued adaptation; evaluate targets identified through omics.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Proposal to continue development of strains	This project is moving towards the development

using the tools developed is logical and should produce much valuable data for improvement of this strain and/or other thermoanerobic biocatalysts.	of CBP strategy and could be of great benefit in the next generation of improvements in biochemical conversions. The project has been making excellent progress in developing approaches toward improving this interesting organism. Considerable gap still exists between the current stage of development and commercialization.
PI Response: No response to this comment has been provided by the Principal Investigator.	
finishing it up for final validation	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- continue mutagenesis and screening evaluations -continue screening libraries - integration of pieces	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Strain development will continue; process development plans to be finalized.	no process economics discussed
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Yes—Mascoma, but publications, if any, not given.	
Oak Ridge NL Panlabs	
not discussed	
Benefits from interactions with Oak Ridge and	

Pan Labs. Has published some work.	
Publication of basic information is occurring; endogenous commercialization anticipated	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Project should include real goals that have commercial relevance.	

Improvements in Ethanologenic Escherichia coli and Klebsiella oxytoca

Technology Area: Biomass Program
Project Number: 2.3.2.4
Performing Organization: Verenium Corporation
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.29	1.11
Approach	4.14	0.69
Technical Progress	3.71	1.25
Success Factors	3.86	1.07
Future Research	3.57	0.98

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Strong company partnered with excellent academic labs at University of Florida and MIT.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Important overarching goal defined for ethanologen development Good justification of organism being employed	No granularity in goals. No clarity as to how goals relate to the OBP goals. No defined success metrics.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project capitalizes on pioneering work at the University of Florida to produce a recombinant ethanologen capable of fermenting mixed biomass sugars.	Although considerable prior art exists for improvement of these strains their performance with regard to ethanol yield and inhibitor resistance will still need to be improved.
PI Response: No response to this comment has been provided by the Principal Investigator.	
utilizing advanced techniques to develop strains and insert into process environment	
PI Response: No response to this comment has been provided by the Principal Investigator.	
To generate improved E. coli and K. oxytoca strains able to ferment sugars in a relevant process—this is germane to obtaining an economically relevant biomass-to-ethanol process -	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Working to improve growth on hydrolysates. Strain already well-developed for growth on C-	Work focused on just bagasse.

6, C-5 sugars.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Another arrow in the quiver for a critical piece of the puzzle, able to leverage a huge amount of background knowledge on the starting organism.	E. coli might not have been the immediate choice for ethanologenesis.
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
The approach is to generate improved E. coli and K. oxytoca strains able to ferment pentose and hexose biomass sugars in a simultaneous saccharificationcofermentation (SScF) in an economically viable and commercially relevant process by identifying genes and metabolic	

pathways that have increase tolerance to hydrolysate. Company has fully integrated R&D platform. Using energy cane and bagass hydrolysate from demo plant rather than artificial feedstocks. Strains have been approved.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear basis for organism selection Approach includes several collaborators with various levels of expertise Verenium well positioned for information capture.	Program seems quite decentralized with much of the work being done separately by various contractors without a method for groups to mutually learn from one another. No clear techniques described to be used.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Initially proposed as separate hexose and pentose fermentations; the plan is to ultimately combine the desired characters into a single strain to ferment all the sugars provided. Multiple approaches are being employed to identify genetic characters for inhibitor and ethanol tolerance and incorporate these into production strains. Collaborators for strain improvement are experts and the prospects for some level of success are high.	Focus is on improving tolerance. Are there plans to improve ethanol titer produced by these strains?
PI Response: No response to this comment has been provided by the Principal Investigator.	
broad approach to achieve multiple capabilities	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- using organism capable of using all sugars - use steam/dilute acid process, prehydrolysate fermented by E. coli, cellulose used in SSF with K. oxytoca - have complete biomass-to-ethanol process under company umbrella which will foster very practical approach - organisms generate ethanol in near theoretical yields	

- produce hydrolysates from energy cane, analyze and send to sub-contractors, including MIT (global regulators), Univ. Florida (serial enrichment), etc.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good collaborations with U. of Florida and Genomatica.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
An important goal of this project not attempted by others is to incorporate fermentation with the saccharification process. The use of two different organisms for C5 and C6 metabolism minimizes the complexity of metabolic jiu-jitsu needed to address both sources in one metabolic implementation, although there are plans to combine the results in one strain. Identify genes, pathways, and strain variants that confer tolerance to hydrolysate inhibitors and ethanol. Incorporate into a production strain, then validate at stagegate and validate with final process of simultaneous saccharification and fermentation. Vertical integration of work with enzymes and strains, strains adapted to separate solid/pretreated stuff so there are two fractions to digest. Normally mixed acid fermenters, now produce Ethanol. Near theoretical yields in the hydrolysate, seed grown on hydrolysate. Groundwork by Lonnie Ingraham. reduce inputs	This process is focused on energy cane, rather than more widely applicable feedstocks. Not clear to me why Klebsiella was included rather than carrying out parallel C5 and C6 investigations in E. coli.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Recently completed metabolic pathway map and have RNA array data from University of Florida. Identified 15 genes that confirm increased growth under stree under hydrolysate or ethanol challenge conditions.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Work has been done on hydrolysates Ingram has clearly done work relating to resistance development, that appears to be focused to understanding resistance. Data has produced three targets, suggesting potential relevance of this approach. Verenium work has identified mutants with the potential to improve various genes leading to improvements in tolerance	Progress seems limited on hydrolysates limited to compositional analysis. Data disclosure is significantly limited compared to peer organizations raising questions about progress and level of accomplishment Stephanopolous shows approximately no gain at ends of run—differences only at time=0. Raises questions about relevance of this work. Ingram/genomatica data has identified three targets of unknown significance. No clear benefit from these three changes despite claims of incorporation. No clear impact as to genes

	identified for tolerance. No understanding of synergy or impact on productivity. Data appears cursory at best with only early targets identified.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Research has successfully identified multiple targets for improvement and these are now being introduced into ethanologenic strains.	Organisms' optimal growth conditions don't coincide with optimal enzyme saccharification conditions for typical fungal enzyme products. This will require some sacrifice in process performance when integrating this process. How stable are adapted strains??
PI Response: No response to this comment has been provided by the Principal Investigator.	
progress using a wide array of empirical and modeling techniques—very informative	
PI Response: No response to this comment has been provided by the Principal Investigator.	
2007–2010: hydrolysates produced at different severities, then analyzed and have been sent to collaborators - MIT has been working on ethanol tolerance and production by looking at global regulators - U. Florida looking at tolerance to inhibitors using serial dilutions and genomic sequencing - Genomatica is looking at sequence data to develop metabolic models to target inhibitor resistance - Verenium using metagenomic libraries to test ehtanologen performance of the organism	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Analysis of hydrolysate in progress as well as . MIT is looking global hydrolysis. Genomatica is doing metabolic modeling. Hydrolysate with different severity treatments analyzed by subcontractors. MIT working to increase tolerance to EtOH. 8% tolerance is what is needed. 15% increase in EtOH production was achieved. U. Fla is doing classical strain	As with others, not much detail was provided on assays or identified genes.

improvement approach for different inhibitors. Process involves carrying out transcriptome, sequence, analysis, Send to Genomatica for metabolic modeling, then look for meaning of the modifications. Three possible targets identified, then validated the changes with directed modification. All changes were made in genome not plasmids, so markerless and stable. Libraries Bioscreen is used (which is what?). 15 genes confer improved growth in challenge, classed as stress proteins, DNA repair, efflux, dehydrogenases. Simply making more of a particular gene product is not necessarily the requisite, need to tune the expression.
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses

Minimizing inhibitors without increase nutrient requirements.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Describes standard factors that all organisms need for success in this field. Focus on scale-up for mitigation	No identification of product specific success factors or showstoppers. No clear plan to mitigate risks especially given stated timeline of "now" Clear internal factors (organizational) that are limiting development.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan identifies specific challenges such as achieving inhibitor tolerance in minimal medium and moving strains to demonstration in a pilot plant ASAP. These should be readily addressed given Verenium facilities and capabilities.	The project has focused inhibitor tolerance work on well know, high concentration compounds [e.g. furfural], how about other compounds present in hydrolysates? It does appear that some of this work was done with "hydrolysates."
PI Response: No response to this comment has been provided by the Principal Investigator.	
major factor --> improve tolerance to inhibitors while keeping nutrient levels low - reiterative approach to continued improvement	incorporation of input knowledge/technology into ehtanologen strains already on commercialization path consistency of feedstock
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well-considered list of barriers and approaches to them.	
Robustness to accident. Challenge is to coordinate all the different inputs. Lockdowns limit ability to transfer new things to the process. Commercialization license.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Focused on successful completion of intermediate stage gate targets. Select single ethanologen. Continue to identify beneficial targets. Increase robustness.	Few details as to how decisions will be made or what specifics will be done. Bacteria are less ethanol tolerant than yeasts; ethanol tolerance is determined by many genes. How many and what genes affect tolerance to other inhibitors? What are the likely reasonable limits.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Future work plays out conceptually ideas as to how to move the project forward.	No clear milestones or timelines. No clear role for various players going forward outside of continued work. No identification of clearly removing barriers.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal is to get characters into a single, ethanologenic organism [not two stage as initially described], single pot system. Improve robustness of strains.	

PI Response: No response to this comment has been provided by the Principal Investigator.	
working toward introduction into a commercial environment	the industry has been resistant to these concepts—i.e., replacing yeast
PI Response: No response to this comment has been provided by the Principal Investigator.	
- Continued focus on obtaining single SSCF process, would like to use a single organism. - continue to incorporate new findings into existing strains	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focusing on high-solid fermentation single ethanologen. Combined process is giving more activity to the cellulase because draws off product	Didn't discuss economic model very specifically
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Verenium is major biofuel relevant company. Two publications apparently being submitted for publication.	
Involvement of academic collaborators helps to facilitate publication and information distribution. Technology transfer is aimed at in to Verenium	
Excellent collaborative effort. Availability of Verenium commercial facility offers unique opportunity to integrate biocatalyst	

development with process development.	
these organisms are already on the path to commercialization	
Uses extensive collaborators well.	
Internal development anticipated; Stephanopolous, Genomatica are partners.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Project should include real goals that have commercial relevance.	

Further Improvement of the Robust Recombinant Saccharomyces Yeast for the Conversion of Lignocellulosic Biomass to Ethanol

Technology Area: Biomass Program
Project Number: 2.3.2.5
Performing Organization: Purdue University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.29	0.49
Approach	4.14	0.69
Technical Progress	4.00	0.58
Success Factors	3.71	0.49
Future Research	3.43	0.79

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project

align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Ho's lab has extensive experience in engineering yeast strains for fermentation and the utilization of different sugars. Ho-Purdue yeast strains have been used by a number of companies for commercial operations and one Canadian company for cellulosic ethanol fermentation from wheat straw.	
PI Response:	
Clear focus on using well established yeast to produce ethanol. Project is directly in line with goals of OBP. Well defined plan with clear tasks that are relevant to industrialized goals. Building off strong technology platform.	Strain is proprietary, raising questions about how broadly this academic work can be leveraged.
PI Response: The original parent strain is not proprietary. The engineered yeast is patented by Purdue University. Purdue has the full rights to license the yeast to any company able to demonstrate the capability of industrial scale production cellulosic ethanol. The improved strain developed in this project will follow the same mechanism of licensing. It will license to any company that will produce industrial scale of cellulosic ethanol.	
Goal of developing a robust ethanologen based on improvement of a commercial yeast strain. Builds on pioneering work of this group in the development of xylose fermenting yeast which is now identified as "Purdue Yeast"	
PI Response: No response to this comment has been provided by the Principal Investigator.	

a multifunctional robust yeast strain	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Aims to improve a commercially acceptable yeast strain for lignocellulose ethanol production. - goal to make industry-ready Purdue Yeast - must incorporate adaptation to inhibitors	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Aligns with DOE objectives	Purdue xylose degrading yeast been around long time with no commercial use?
PI Response: This reviewer seems to have missed some of the PI's presentation including those presented in additional slides provided. Please see reviewer # 17900's comments on this matter above.	
Responds to the need for improved utilization of sugars in the fermentation process, making use of a workhorse of fermentation that has a large catalog of manipulation tools already available.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are *developed. Improvements in approach would improve project quality.*

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
The approach is to modify Purdue yeast strain by adaptation, random mutagenesis and testing, for improved inhibitor tolerance (e.g. acetic acid, furfural, ethanol), and to provide pathways for e.g. arabinose utilization.	Limited knowledge-based improvements based on presentation.
PI Response: Our approach includes both “irrational” (adaptation, mutagenesis, and selection) as well as rational (directed molecular biology) to inhibitor improvements. Our presentation focused on current successes which are primarily in the “irrational” improvement tasks. One of the goals of the microarray and metabolomics efforts is to expand the knowledgebase of yeast for future directed improvements.	
Approach is broad-based and covers a path to improving organism both rationally and non-rationally. Program incorporates several techniques to improve organism. Approach recognizes fundamental elements of a successful industrial organism.	Approach is highly conserved with industry. Would like to see more innovation in the process of improvement from an academic based group. Plan is lacking discrete goals in the process of improvement, which is again striking given that the group is academic, especially when industrial groups are providing this information.
PI Response: Please see our response above.	
A number of approaches are designed to overcome current limitations of the xylose fermenting yeast strain. These include adaptation to inhibitors, mutant screening, and targeted genetic modifications of the "Purdue yeast." The research group as well as other Purdue collaborators are experts in biomass pretreatment and fermentation.	

PI Response: No response to this comment has been provided by the Principal Investigator.
adaptations, directions, mutagenesis screening, and systems biology
PI Response: No response to this comment has been provided by the Principal Investigator.
use yeast strain that is already well characterized - evaluate adaptation to inhibitors - use direct modifications - employ random mutagenesis - using system biology analysis of robust strains will provide information for further direct modification, an iterative process - do collaborative studies with ADM
PI Response: No response to this comment has been provided by the Principal Investigator.
This approach is to separately address each of 15 goals and then hope to reintegrate them into one strain.
PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP

goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Obtained significant increased tolerance to inhibitors (acetic acid, furfural, ethanol) and Improved arabinose utilization. One publication in current period with several more to be submitted.	Strain not sequenced?
PI Response: The genome of Saccharomyces cerevisiae has been sequenced. However, we expect that many of the changes in the adapted strains are likely in gene and metabolic regulation and not due to mutation in specific genes. We plan to determine this through expression profiling and metabolomics.	
Data is showing solid improvement of organisms by well accepted measures. Sugar utilization with significant improvement Good adaptation to ethanol and other inhibitors is emerging. Good success in broad-based sugar utilization Significant metabolic yields that are approaching best-in-class Transport project showing interesting data suggesting significant value in experimental pathway	PPP pathway improvements are not as strong as other portions of the program
PI Response: We agree. While we have improved the expression levels in the PPP pathway, this did not significantly improve our yeast performance in fermenting pentose sugars such as xylose. This confirms that our yeast strain is effective for PPP metabolism and rate limitations for xylose fermentation lie elsewhere. We intend to refocus our efforts for the remainder of this project on the tasks showing greater promise in improving performance.	
Adaptation produced a moderate improvement in inhibitor tolerance and good improvement in acetate tolerance. Improved ethanol tolerance [issue with xylose fermentation] also achieved. A number of metabolic improvements were also achieved using targeted engineering approaches	Growth is somewhat slow in some of the experiments requiring nearly 100h to complete the mixed sugar fermentations and in the presence of inhibitors. In other experiments the initial cell concentrations were very high. Are these practical or is further development needed

(e.g. GDP1 as a redox balancer, galactose transporters). Some of this was accomplished in other strains and will need to be transferred to Purdue yeast. ADM mutations, screening?? Improvement in inhibitor tolerance?	to improve inoculum development?
PI Response: We have done some studies on inoculum and it can be substantially lowered without affecting the overall fermentation. We used high inoculum for these studies, mainly because initial and validation experiments were run under these condition and our milestones and benchmark are related to these conditions. Overall, we believe high inoculum might not be too costly for the production of cellulosic ethanol and may provide substantial advantages for the overall production. One advantage for using yeast as the microorganism to produce ethanol is that ethanol production by the sacchromyces yeast is not dependent on the growth of the cell, unlike other microorganisms. Thus, we can take advantage of using higher inoculum for fermentation.	
achieved improved tolerance to inhibitors and arabinose fermentation; reduced xylitol formation	
PI Response: No response to this comment has been provided by the Principal Investigator.	
10/07–8/10: have improved xylose utilization kinetics - have increased resistance to furfural - have significant improvement in ethanol tolerance - yeast was constructed to ferment arabinose - have obtained information, from metabolic analysis, on genes likely modified for enhanced performance - considerable improvements in xylitol utilization	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Demonstrated many improvements	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Individual goals are being addressed.	Metabolite analysis has not been carried out, nor is it clear how the separate changes that have been obtained will interact when

combined.
PI Response: The metabolite analysis has been carried out on schedule but they have not been totally completed. When all the improvements to be combined into a single yeast strain, we believe the resulting yeast should perform much more effectively than any strain with single improvements. The metabolic analysis will be an important tool to understanding those interactions.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Seems on track to meet project goals.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Success factors geared towards milestones Clear understanding of relevance to commercial paths and goals for industrial development	Challenges are generic comments about organism improvements. Important to understand benefits of this organism, and also intrinsic challenges to it.

PI Response: No response to this comment has been provided by the Principal Investigator.	
Combine improvements into and individual strain of the industrial yeast in a genetically stable form.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
good list but few details	
PI Response: We proposed eleven different tasks to improve the yeast. We have limited time and space to present and it was not possible to provide more detail.	
fulfill other milestones - incorporate improvements into a single commercially reasonable strain	must combine all improvements achieved into a single yeast strain and make these improvements genetically stable
PI Response: With our patented technology for integrating genes into the yeast chromosome, we do not expect significant problems in incorporating all the improvements into a single, improved strain. However, this remains a major challenge. An alternative solution is to incorporate the major improvements into one yeast strain first. The improvements to acetic acid tolerance and the increased tolerance to ethanol together will have significant impact on the economics of using this strain without being combined with the other improvements. We can continue to further improve the yeast for even greater performance and use other existing techniques to incorporate the desired new traits into the already improved yeast.	
	Showstoppers not well defined. Route to commercialization not clear.
PI Response: Our parent yeast strain is already in industrial use. The results we obtained so far from this study showed that our yeast can be further improved for the production of cellulosic ethanol, and the uncertainty only lies in if all of these improvements can be incorporated into a single strain. Nevertheless, we do not envision it will be a serious problem. Purdue already has an established mechanism for marketing the yeast. For example, Purdue has already licensed the yeast to Iogen and other companies for the production of cellulosic ethanol. There are no legal barriers preventing licensing of the original as well as the improved Purdue yeast strains to various companies.	
	Combining the various changes while maintaining strain stability is indeed a major challenge. The analysis focused on bureaucratic targets,

	not scientific or economic issues
PI Response: Some of the reply to the comments to reviewer # 17905 can also be used for responding to reviewer # 17907. We are confident we can incorporate all the individual important improvements into a final yeast. Please see our response to reviewer #17905 above.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Increased ethanol tolerance and improved arabinose utilization using same approaches..	A number of yeast mutations have been identified that increase ethanol tolerance—no obvious plan to utilize this information.
PI Response: We will try to incorporate most important improvements into one yeast before the end of this project. The improved strain will be available for licensing on a non-exclusive basis as soon as possible. The yeast might be able to be further improved for even better performance and we will seek funding for such a purpose. Also in our rational design we are incorporating targeted improvements (by cloning specific genes such those known to improve ethanol tolerance) to our strain(s) however at the time of the platform review we did not have results	

from those studies to present.	
Future work is set out by goals. Final goal lends itself towards a commercial organism Plans are clearly industrial in nature.	No detail provided about timeline, milestones, and plans for adaption with unforeseen challenges.
PI Response: We have provided clear timelines and milestones for each tasks in our original proposal. The time line and milestones were provided to DOE in the form of PMP that was supposed to be available to reviewers. Since we have the results from 11 different tasks to report, we could not provide details on all of them during our presentation. There is no unforeseen challenges could make the entire effort fail as progress has already been demonstrated. However, some proposed task may create better improvements than others, thus we are using go/no go decision points to determine when to abandon ineffective strategies and redirect resources to the most promising ones. For an example see our comment above regarding PPP improvement.	
Plan to complete tasks outlined, combine improvements, and make these genetically stable [permanent] is sound.	Are there any plans to test newly developed strains at the pilot scale? Perhaps with ADM collaborator?
PI Response: Purdue will provide the improved yeast to ADM as well as other companies that want to test the yeast at pilot scale. There is a company in Indiana that will also carry out such a test.	
appropriate but little detail	
PI Response: No response to this comment has been provided by the Principal Investigator.	
complete tasks as proposed - integrate changes into starting strain (single strain) - work to make constructs genetically stable	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	No economic analysis was proposed.
PI Response: Economic analyses are included in the project as required by the contract. Our economic analysis method has been independently validated by DOE. Improvement in economic performance due to improved yeast performance will be evaluated in our upcoming stage gate review. The results of this stage gate analysis of economic performance will be independently validated by DOE.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Prior strains have been used for commercial fermentations. Good publication record.	
Partnership with ADM provides direct avenue for technology transfer	
ADM, Purdue collaborators very strong in this area. Investigators plan to make strains available to anyone who wants them.	
plan is to make improved strain immediately available for industrial cellulosic ethanol production	
Working with ADM; nature of relationship not clear.	We have made it clear during our presentation, ADM, like many other companies, appreciated our work and wanted to help us to further improve the yeast. ADM agreed that it would not receive any special advantages in using the Purdue yeast than other companies. ADM cannot dictate which company can or cannot use the Purdue yeast. This is the major reason we chose to collaborate with ADM. However, ADM will be able to use our patented technologies to develop its own cellulosic ethanol producing yeast.
licensing model; publication of results	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Project is comprehensive in its view of improving an organism for industrial use.	
Yeast are the commercially preferred organisms. This industrial strain is already very robust and has been used in pilot scale production of ethanol from wheat straw (Iogen).	

Lab Validation for Organism Development Solicitation Recipients

Technology Area: Biomass Program
Project Number: 2.3.2.7
Performing Organization: National Renewable Energy Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.57	0.79
Approach	4.57	0.79
Technical Progress	4.14	0.69
Success Factors	4.14	0.69
Future Research	4.14	0.69

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Provides critical support to validate results from ethanologen providers. Strong, experienced NREL team. Working with major ethanol producers.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plays an important role by validating ethanologen award winners. Helps to support goals, but does not directly achieve goals in its own right.	Unclear end deliverables outside of merely visiting labs.
PI Response: The project's deliverables consist of conducting initial, 18-month and 36-month audits and generating reports summarizing those audits. The initial audits verified awardees' organism performance claims in the original proposals and set the criteria by which organism improvements will be measured. The prestage gate audits will verify organism improvements which will aid the stage gate review panel in their decision to recommend further project funding. Final audits will verify whether the awardees' project goals were completed. This is a new model for the DOE's Biochemical Platform in assessing success of funded projects.	
Supports the ethanologen solicitation to ensure R&D targets/ benchmarks are met. This will provide an unbiased validation and assessment of fermentation organisms performance with regard to yield, c5 and c6 fermentation, and inhibitor tolerance.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Crucial for meaningfully determining status and progress, and to measure the results of research.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

This type of validation work is essential for monitoring the progress of funded projects and merits of funding. - important for fuels organism development
PI Response: No response to this comment has been provided by the Principal Investigator.
Essential Auditing; important to evaluate organisms.
PI Response: No response to this comment has been provided by the Principal Investigator.
Important part of providing competition and alternatives that have credibility.
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Onsite visits to validate results from	

ethanologen solicitation awardees.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear focus on playing soup-to-nuts role throughout process of companies developing ethanologens. Clear endpoints and timelines in project planning.	Standards against which validations will be made appear to be lacking.
PI Response: A validation protocol describing the audit procedures was provided in the original FOA. Each proposal had to include data from three fermentation tests: 1) Fermentation with the five most common monomeric sugars found in biomass at specified concentrations to show substrate use. 2) Fermentation with the five sugars and an inhibitor (acetic acid) commonly found in biomass to show sugar utilization in presence of to an inhibitor. 3) Fermentation with high sugar concentrations (sugar composition of the awardees' choice) with acetic acid to demonstrate ethanol production and ethanol tolerance in the presence of an inhibitor. In addition, the topic 2 proposals were required to include fermentation data from real hydrolyzates. Results were to be summarized in specific tables included in the FOA. All awardees were required to repeat these fermentations during the initial audit within a 5% variance. Awardees were given two chances to meet the audit criteria or risk losing funding. The awardees will repeat these tests comparing new and old strains for the prestage gate and final audits. It is up to the merit review committee to decide if the projects should continue to be funded after the 18-month audit.	
NREL and GO staff will conduct on-site validations to confirm baseline performance, facilities, progress toward final achievement of project goals	It appears that each project has different performance targets and reviewed using separate criteria. Will there still be an "apples to apples" comparison with regard to meeting Biochemical Platform benchmarks? Will NREL provided hydrolysate be used in evaluation so that performance will conform with this process? Is any scale up planned. Performance of biocatalyst in commercially relevant pilot plant using practical growth media and controls?
PI Response: Some ~~-a~~pples to apples" comparisons can be made between the different projects using the results generated from the common tests required for the audits. We recognize the need to demonstrate the Biochemical Platform goals using these improved strains. DOE GO will try to negotiate at the stage gate for access to those strains for testing on NREL's hydrolyzate at the pilot scale to demonstrate DOE's 2012 ethanol cost target.	
Development of techniques and having a set	These on-site analyses need to be efficiently

schedule for these analyses is critical.	performed to avoid interference with research to any extensive degree.
PI Response: The on-site audits take a week to perform with two weeks of preparation and analysis. We try to keep the individual tests at a manageable number. There are three audits; the initial, prestage gate at 18 months and a final audit at 36 months.	
-do on-site validation visits - do in-house testing at NREL - must evaluate each project due to the uniqueness of each project - looking for confirmation of improvements in overall fermentation performance - develop performance criteria - on-site all groups must do pure sugar demonstrations to verify what was in original application, then work with prehydrolysates	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Different criteria for different organisms and different processes; not all comparisons of apples to apples
PI Response: No response to this comment has been provided by the Principal Investigator.	
Criteria and auditing strategy onsite visit are very important to verify intermediate targets	How is the process observed?
PI Response: We travel to the laboratories and watch the inoculation of the seed and fermentation vessels, all sampling relevant to calculating sugar utilization, process yields and productivity and any sample analysis. We record data that is generated during sampling and from the analytical equipment. We also verify calibrations by checking verification samples. Final performance tables are generated the last day of the audit. The audit usually takes a week to complete.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Initial validation of all awardees completed in FY2008 for baseline performance. Current work aimed a pre-stage-gate validation (18-22 months later). All groups did pure sugar fermentation as well as process fermentations. All awardees moved to stage 2.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Work has apparently established a baseline for production and process economics.	Not clear how consistent standards are.
PI Response: No response to this comment has been provided by the Principal Investigator.	
all passed initial assessment	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Benchmarking initiated; stage 1 reviews complete, all passed on to next stage.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- have completed first round of on-site validations - now in process of doing validations for	

intermediate performance targets -
PI Response: No response to this comment has been provided by the Principal Investigator.
Call for applications required pure sugar fermentations, process fermentations to work on hydrolysate, and process details to validate economics
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Clear need for unbiased validation and confirmation of commercial readiness.	Different requirements for different organisms have required flexibility in developing validations.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focus is on an unbiased approach. Recognition	Consistent metrics are not clearly present in this

of challenges.	analysis. No clear project goals. Challenges are merely doing the work.
PI Response: This project's goals are to audit the performance of improved ethanologens based on a set of criteria for the DOE and delivering audit reports at the beginning of the projects, at 18 months and after 36 months. After the 36 months, the project ends.	
To provide unbiased validation of strain performance and confirm commercial readiness. It will be challenging to meet doe goals within the time constraints of fy11 so they are ready for 2012 target of OBP goal.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good lists.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
obtain unbiased validation of strain improvements—confirm commercial readiness	- understand the results from validations, applicability to DOE-relevant questions - keeping validation visits on schedule
PI Response: No response to this comment has been provided by the Principal Investigator.	
Validation is critical. Are the improvements important for DOE (meaning of general utility) or only the proprietary process?	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or *diminishing OBP MYPP barriers in a reasonable period.*

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Continue validations.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Role is understood going forward.	Project is lacking detailed future goals and milestones. Timelines have been slipping against monitoring status.
PI Response: The project's future goals and milestones are to complete the 18 and 36 month audits and submit an audit report to DOE GO. After the 36 months, the project ends. NREL's validation schedule is dependent on the schedule of each of the individual ethanologen projects. The ethanologen projects are to be completed by the end of 2010 in order to meet DOE's 2012 ethanol cost target goals. Slippage in the schedule may affect the DOE's cost target goal.	
Perform validations at critical points as planned.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
advancing the procedures	May need to develop methods to validate organisms identity and genetic makeup.
PI Response: This is a valid concern and NREL should look into this further. Currently, we are using fermentation performance and repeatability of results to verify the strain, with the assumption that repeatable results equal the same strain. We recognize the awardees could substitute a different strain demonstrating the same fermentation performance without our knowledge.	
- complete validations as proposed to verify strain improvements	

PI Response: No response to this comment has been provided by the Principal Investigator.	
Straightforward task to repeat what was done before, and is critically needed, if can keep on schedule.	What is the contractors incentive to fulfill the gate?
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Program apparently eschews technology transfer	
Are partners required to commercialize strains?? Will the results be published like the enzyme validation work?	Commercialization is the goal of the FOA. The ethanologen development project predates the enzyme development project, so testing of the strains on a common feedstock was not written into the FOA. DOE GO will try to negotiate access to the strains for testing on NREL's pretreated corn stover at the stage gate review with the intent of publishing results in a nonattributed manner.
By the nature of the project this is highly collaborative (validator and validatee). The indication is that success/failure results of validation tests are public information	
Commercialization is a required part of the FOA	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Developing standards would be a valuable addition.	
Need a method to assure that organism being tested are indeed those asserted by researchers.	

A novel simultaneous-saccharification-fermentation strategy for efficient co-fermentation of C5 and C6 sugars using native, non-GMO yeasts

Technology Area: Biomass Program
Project Number: 2.3.2.8
Performing Organization: The University of Toledo
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.29	0.49
Approach	4.00	0.00
Technical Progress	3.57	0.53
Success Factors	3.71	0.49
Future Research	3.86	0.69

Overall Principal Investigator Response(s)
We would like to thank the reviewers for their insightful comments and suggestions. Since the project is in the beginning stage, we will incorporate these suggestions into individual tasks within the PMP as appropriate.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project

align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Goals are consistent with the OBP. Focus on improving catalysis and fermentation of hydrolysis is of clear relevance to the OBP goals. Economic component is a differentiating factor of this program.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project employs a novel approach to accomplish c5 and c6 ethanol fermentation using non-GM commercial yeast by conversion non-fermentable xylose to fermentable xylulose in the fermentation medium thereby bypassing the xylose fermentation bottle neck. While conversion of xylose to xylulose for fermentation has been previously demonstrated there is need to overcome equilibrium issues and pH optimum issues. The researchers developed a plan for this using urease containing enzyme pellets to generate a more ideal localized pH environment and a borate shuttle that helps to overcome equilibrium problem.	While a clever idea, I am not sure that it accomplishes all the Biochemical Platform envisions for a robust fermentation process [all sugars, tolerance to inhibitors, etc.].
PI Response: The proposed technology has two components: (1) using non-GMO, commercial yeast strains and (2) the immobilized enzyme pellets. With respect to the yeast strains, we believe that non-GMO commercial yeast strains are more likely to adapt and thrive under the harsh conditions encountered in biomass hydrolysates with minimal added nutrients. These yeasts are capable of fermenting all C6 sugars as well as xylulose. Arabinose is the only sugar that is not utilized by native yeasts, but represents a minor component in most feedstocks. The	

pretreatment method that we are using for our studies does not generate fermentation inhibitors. However, we have tested our immobilized enzyme pellets in the presence of acetic acid and do not see any adverse effects. We have not tested our pellets with other fermentation inhibitors such as furfural and HMF, but they are unlikely to have an adverse effect on the enzymes. We fully expect that our immobilized enzyme technology is compatible with hydrolysates generated with other leading pretreatment methods.
This is a novel approach to optimizing a multistep process
PI Response: No response to this comment has been provided by the Principal Investigator.
develop cost-effective biocatalysts capable of increasing utilization of C5 and C6 sugars by native yeasts in the conversion of lignocellulosics to ethanol - this presents an alternative approach to xylose utilization that is worth consideration
PI Response: No response to this comment has been provided by the Principal Investigator.
Novel approach merits further work high risk
PI Response: We have demonstrated this technology at a laboratory scale. We acknowledge that there can be issues to be resolved during scale-up.
A novel approach, to manipulate externally
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified *but management approaches may not be fully developed.*

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Aimed at allowing xyluose utilization by natural yeast by development of reusable biocatalyst. Insert Xylose isomerase to convert xylulose and then on to ethanol. Immobilization of XI on solid support (Sweetzyme) because XI has pH optimum of 7-8 where as next step is pH 4-5. Cleaver approach using borate to enhance reaction.	Reusability and cost not yet clear.
PI Response: Reusability of the pellets forms two of the principle tasks of the proposed work and will be addressed during the course of the project. While preliminary cost justification for the technology was not presented at the review, cost estimates were part of the written proposal and indicate that the technology should be cost-competitive with other approaches.	
Approach is differentiated relative to its fermentation pathway (i.e. metabolism approach) pH approach represents in intriguing approach. Solving this problem would have broad applicability well beyond this problem. Clear use of go-no go decisions.	Solving a problem with very challenging approaches. Immobilization approach may solve multi-zone problem, but raises questions as to scalability.
PI Response: Once we have finalized the methods for robust co-immobilization of the enzymes, we do not anticipate issues with scalability; immobilized XI pellets are already used at commercial scale for production of high fructose corn syrup.	
Using exogenous xylose isomerase to convert xylose to xylulose, demonstrate that a pelletized , urease containing form can be used to efficiently convert biomass sugars to ethanol using conventional fermentation yeasts. Use	

ionic liquids as a pretreatment agent to generate biomass hydrolysates.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The development of an immobilized catalyst and controlled pH as a method to accommodate native yeast is useful as an alternative to modifying the yeast.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
use supplemental xylose isomerase to convert xylose to xylulose and then have native yeast ferment the xylose - pH disparity between xylose isomerase (pH 7-8) and that of yeast fermentation (pH 4.5) - use pellet approach to create optimum environment for XI while in fermentation broth at pH 4.5 - using borate to shift equilibrium of reaction toward xylulose - hope to develop SIF (simultaneous isomerization and fermentation) systems - using ionic liquid pretreatment -	
PI Response: No response to this comment has been provided by the Principal Investigator.	
GMO organisms objected to. Extending an existing high value technology (used industrially for corn syrup production) to another use. Use external enzyme (xylose isomerase) to allow crossfeeding Equilibrium issues; pH optimum drastically different than fermentation optimum Uses catalyst pellet to shield pH distribution Borate shift the enzyme properties toward desired product. "Transport" in and out of pellet biases toward the right product. Trying to work with both unfiltered hydrolysate and filtered hydrolysate (getting rid of lignin)	Reuse of the enzyme pellets? Big addition to enzyme need.
PI Response: Unlike cellulases, which are not presently reused, the co-immobilized enzyme	

pellets will be used for isomerization of large volumes of hydrolysate for extended time periods. The high fructose corn syrup industry reuses XI pellets for 6-9 months.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Relatively "new" project but obviously have been working on project with other support. Proof of concept demonstrated.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Proof of concept experiments validate experimental approach Diversity of feedstocks employed will help to increase general relevance of program. Internally generated data has yield much higher than other approaches in certain cases, showing very significant promise.	Data is done at much lower sugar concentrations than other approaches
PI Response: We are currently generating data on hydrolysate with higher sugar concentrations.	

Investigators successfully demonstrated the concept: immobilized enzyme pellets and borate shuttle. Process demonstrated at laboratory scale with conventional yeast and glucose and xylose mixtures and biomass hydrolysate could be converted to ethanol in high yield.	The use of ionic liquids as pretreatment agent is questionable. Looks like they work well when enzymatically convert of some material to sugars however this was true in all cases. What are the costs of these materials and can they be practically applied at the commercial scale.
PI Response: We are using hydrolysate generated from ionic liquid pretreatment because this pretreatment method was developed in our laboratories and is readily available to us. However, the proposed isomerization/fermentation technology is general and is applicable to hydrolysates from other pretreatment methods as well. We will do such testing in the future.	
just started, Feb. 2009 - completed proof of concept work with pellets, urea and borate - demonstrated ionic liquid pretreatment feasibility	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Beginning results very positive	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Two-pH validation--shows the urea (pH modification) and borate tricks work. Test with poplar and corn stover (spezyme to solubilize) Ionic liquid pretreatment (what is IL?) Enzyme pellets are compatible with poplar hydrolysate. Hollow fiber membrane fermenter in use.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and

strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Good understanding of barriers.	Challenges include scaling to industrial process and pellet lifetimes. GMO yeast acceptance would obviate need for biocatalysts.
PI Response: Immobilized XI pellets are already used at commercial scale with an average life time of 6-9 months. Even if GMOs do become accepted, their licensing and propagation costs versus the cost of our technology will decide which approach will be industrially adopted.	
Good identification of key success factors. Challenges have been reasonably explored Rapid plan for commercialization	Certain challenges, such as scalability, may have been overlooked. Would be good to figure out how to mitigate this and similar challenges. Extensive stress placed on GMO component. Commercial implementation plan is unrealistic, merely given time to build a plant, much less the absence of a pilot or demo scale of the process.
PI Response: The scope of this project is at the research and development level, not at a commercial scale. However, since the fermentation is based on native yeast, the technologies developed in the corn-to-ethanol industry are readily scalable to this technology. In addition, the use of immobilized XI pellets at commercial scale (in high fructose corn syrup production) also provides much insight needed for scale-up of the pellet technology. Moreover, the approaches that we are exploring in developing the robust, co-immobilized enzyme pellets are those that scale easily to industrial quantity production.	
The ability to develop robust co-immobilized pellets will certainly be critical to further	Pellet lifetime could be an issue. What are the potential costs associated with this approach?

development of the proposed process.	
PI Response: Reusability of the pellets forms two of the principle tasks of the proposed work and will be addressed during the course of the project. While preliminary cost justification for the technology was not presented at the review, cost estimates were part of the written proposal and indicate that the technology should be cost-competitive with other approaches.	
The major need is to determine the relative productivity of this method to accommodate native yeast to that for GMO yeast.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
robust immobilization technologies applied to current system are critical	- viability of containment design in prehydrolysate liquors - using yeast strains not adapted for biomass-to-ethanol systems
PI Response: One of our proposed tasks is to develop a containment design for the pretreated biomass that is compatible with simultaneous isomerization and saccharification. This task represents a Go/No Go for SSF in our project. In SSF, cellulase enzymes are typically added prior to microorganisms to allow partial liquification and improve mixing prior to fermentation. We expect that with our technology, cellulases would be added to liquify, our pellets would be added to isomerize, and the yeast would be added to ferment, all in the same vessel, but likely in a fed-batch operation. We fully anticipate that the native yeast strains used in our approach will be adapted to the pretreatment method used to generate the hydrolysate.	
Well documented the many risks	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Has identified what the issues are	No cost analysis yet.
PI Response: While preliminary cost justification for the technology was not presented at the review, cost estimates were part of the written proposal and indicate that the technology should be cost-competitive with other approaches.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Biocatalyst production and testing with filtered and unfiltered hydrsylate.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well thought through work plan with clear accomplishment based stage-gates	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Continued biocatalysts development and fermentation work is planned. Biocatalyst development should focus on economical products.	It would useful to evaluate how the pellets perform with more conventionally produced biomass hydrolysates [e.g. dilute acid pretreated corn stover]
PI Response: We have proposed using hydrolysate generated from ionic liquid pretreatment as this pretreatment method was developed in our laboratories and is readily available to us. However, the proposed isomerization/fermentation technology is general and is applicable to hydrolysates from other pretreatment methods as well. We will test our technologies on more conventionally produced hydrolysates as we progress.	
This is a good set of experiments to determine the viability of this method. There are significant opportunities to enlarge this concept to other systems.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

biocatalyst production SIF design and implementation scale-up for commercialization	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plans to address many issues	More early on economic analysis critical
PI Response: While preliminary cost justification for the technology was not presented at the review, cost estimates were part of the written proposal and indicate that the technology should be cost-competitive with other approaches. We will continue to refine our cost estimates as we develop our pellet production methods.	
to compare cost structure with GMO+licensing and propagation	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Commercial partner identified.	
Work has been licensed to SuGanit, demonstrating a direct path for commercialization and technology transfer.	
Project has an industrial collaborator to do pilot scale up.	
not discussed	
Limited, one commercial partner	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Plan is well thought through with a number of project directions included.	
This is a very clever idea based on sound biochemical principals. Excellent progress for this early in the project.	Thank you.

Production of higher alcohol liquid biofuels via acidogenic digestion and chemical upgrading of organic industrial wastes.

Technology Area: Biomass Program
Project Number: 2.3.3.1
Performing Organization: University of Maine
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.57	0.53
Approach	4.00	0.58
Technical Progress	3.57	0.53
Success Factors	3.29	0.49
Future Research	3.57	0.53

Overall Principal Investigator Response(s)
I am very pleased with the care reviewers have taken to read this proposal and make their suggestions. I recognize that this project is somewhat outside the usual technology box and so appreciate the efforts of the review panel to evaluate the project with their fresh impressions.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project

align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Focus of the program is consistent with OBP goals. Product focus is differentiated relative to several other approaches.	Exploring multistep process that has intrinsic inefficiencies compared to other approaches that are being developed commercially.
PI Response: Multi step processing is certainly an issue, and evaluating the trade-offs is an important issue in this work. In support of this approach is the familiarity and relative ease of design for several of the processing steps, which should give more confidence for the performance of a commercial scale design.	
This project uses a somewhat different approach to overcome technical barriers to Biochemical Platform goals such as reducing costs associated with enzymes and complete conversion of all biomass carbohydrates. Basically a mixed culture anaerobic digestion where conversion to methane is inhibited. The mixed organic acids products are converted catalytically to alcohols. This type of system is flexible in feedstocks that can be used and most likely resistant to the presence of fermentation inhibitors.	The mixed products generated in this process may present challenges for ultimate use.
PI Response: Currently, mixed higher alcohols have been approved as a gasoline additive. I expect that as butanol and F-T routes to mixed alcohols approach commercial reality that there will be more interest and support for approving mixed alcohols for larger scale fuel use. There may also be lower marketing thresholds to displacing heating oil.	
a niche application, but an interesting trial and utilizes sound technique	
PI Response: Yes, this is a niche application, and we hope a good opportunity to demonstrate	

the complete process from raw material to final product.
attempting to demonstrate conversion of chosen industrial waste streams to cartboxylate salts (organic acids) using mixed culture acidogenic fermentation (value-added processing of waste streams at existing industries)
PI Response: No response to this comment has been provided by the Principal Investigator.
Dealing with real existing industry waste
PI Response: No response to this comment has been provided by the Principal Investigator.
Feedstocks contemplated are waste from carrageenan production and paper pulp. Alternate end product exploration 50 tons/day available. A primary focus on co-products rather than energy fuels.
PI Response: For the applications targeted in this work, production of chemicals may well be a preferred route to commercial application. But the technology is applicable at larger scale, as well--what we are seeking to do in this project is to demonstrate a complete conversion from raw material to final product, which has not yet been reported. As an example of scalability, there is currently a 400 ton/batch pilot digester operating at Texas A&M that is loaded with sorghum (as representative energy crop) that they plan to run in the carboxylates-ketones-alcohols (CKA) mode to produce mixed alcohols.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Designed to make higher alcohols from xylan in hardwood pulp using acidogenic consortium for digestion of pulp. Also from seaweed sludge. Adds value to Kraft pulp and seaweed waste. Evolving culture system; not sterile.	Complex system.
PI Response: Indeed, it is a more complex system than the more conventional sterile fermentation. I believe however, that the benefits of non-sterile operation are worth the extra downstream complexity. We avoid expensive stainless steel construction, seed fermentor trains and sterile handling of solids--which is very tricky. When completed this project should give some valuable data toward assessing this trade-off.	
Clear goals and objectives to explore the breadth of the approach of producing the chemicals of interest. Clear understanding of what the program can leverage from other pre-existing studies, and what is new. Focused on early decisions to rule in or rule out various paths of work. Inclusion of economic analysis at various stages and a focus on revenue adds to the industrial credibility of the project.	Would be good to explore how well ethanol and other existing facilities can be used as an alternative avenue to reduce cost requirements.
PI Response: Currently, there is a funded DOE project, nearly underway, to convert the pulp mill extract stream to ethanol. Results from this study will be compared to this other work to make a direct "apples to apples" comparison on this one feedstock application.	
Digest feedstocks to mixed acids using anaerobic digester system. Upgrade these to alcohols thermochemically. The plan will focus on hemicellulose rich pulp and carrageenan wastes. These have been already subjected to alkali pretreatment. The anaerobic	Reproducibility and stability can be potential issues. What sorts of controls can you envision to monitor performance and make adjustments as needed?

digestion/fermentation approach is auto hydrolytic [no additional enzymes needed], Thermochemical conversion processes have apparently been well worked out; just need to decide on which to use and optimize this.	
PI Response: Reproducibility could well be an issue. In the past I have conducted fermentation experiments on dairy manure and did find relatively good reproducible results from one batch to the next. I believe the greatest challenge in this project will be consistency of the feedstock qualities, particularly with the seaweed sludges. A benefit of a non-aseptic fermentation is that the culture can adapt to variations in feed quality. An important insight we hope to gain in this project is how dependent the downstream processes will be on variations initiating from the feedstock, and how well the front end adaptable digestion can dampen these variations.	
A good set of methods to solve a local problem and create an opportunity for some new product streams.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- working with hardwood pulp mill pre-extractant and Algal carrageenan waste streams - using acidogenic digestion - separation and conversion of resulting chemicals to value-added chemicals - composition of pulp pre-extract is primarily xylan, acetic acid, and uronic acid - composition of carrgeenan processing waste contains galactose - will make use of natural flora - will use three different chemical processing approaches - many go/no-go decision points, to be resolved as project progresses - attempting to minimize risks and investments	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Evolved ecosystem needs more documentation of past results by others Needs more information on how purify pure carboxylates from fermentation waste and cost of process

PI Response: Numerous references are available documenting the performance of the mixed culture digestions. I can supply additional sources. The purity needs for the carboxylate salts are an open question at this time, and one of our main interests in demonstrating the complete process from start to finish. I expect that the robustness of the process to impurities will be a key issue in the viability of the technology. Studies have been conducted on the cost of this process, and all show positive economics. The lack of a complete raw material to final product demonstration throws some doubt on these predictions, however—a situation that should be improved with the results of this project.	
Would give second income stream to pulp mill. Extraction composition has been analyzed. Second income stream to algae harvest. High galactose. Waste disposal problem for carrageenan production. Goals well articulated. Process models included early. Not a sterile digestion; evolving autohydrolytic, buffered pH, repeated inoculation. Low CH4 production. Sour digester, get organic acids. Recover carboxylate sals, convert to ketones and esters, convert to alcohols: ethanol, propanol butanol. Chemical market rather than energy might be the final destiny.	Potential variability of the input composition, output composition and consortium composition
PI Response: Please see 17902 (above) for some thoughts on the variability issue.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Project just starting. Characterization of hydrosylate. Extraction of acedic acid.	Many options to try; both strength and weakness.
PI Response: We plan to reduce the downstream processing options relatively quickly. For example, the algae sludge comes fully treated with lime, hence it is very likely that a calcium rather than ammonia buffered fermentation will be best for this feedstock. The use of calcium buffer will in turn eliminate the CHA processing option on this feedstock.	
Work is progressing along a number of avenues simultaneously to address several key barriers. Tasks appear to be mostly on schedule.	Most work is seemingly preliminary, though this is not surprising given the start time of the project. Manpower may be ultimately limiting, given breadth of the project.
PI Response: Manpower may indeed become limiting if we fail to select between our processing options on time. As mentioned above (17900), some simplification of options is already becoming apparent.	
It is very early in the project. The bench scale fermentation of pulp extract have been initiated and thermal conversion equipment is being set up.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
very early but have scoped the ensuing activities well	
PI Response: No response to this comment has been provided by the Principal Investigator.	
project has just started - started bench-scale fermentations of pulp hemicellulose streams - have ordered relevant equipment	

- have previous experience with acid extraction and now applied that experience to this system	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Just started not possible to evaluate	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Higher value products expected.	Not really started yet
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Task is to achieve integration. Working with Old Town Fuel.	Many potential challenges
PI Response: No response to this comment has been provided by the Principal Investigator.	
Solid recognition of key success factor. Recognition of value of opportunity within	Limited plans to overcome challenges Limited assessment of the market opportunity for this

industry. Identification of key issues within the industrial applications Well thought through challenges. Have identified ways to harness previous developments efficiently Recognize strengths and weaknesses of the program as well as their implications.	space. For example, the major players in higher alcohols (Shell, P+G, etc have been excluded).
PI Response: As the project progresses it will be important to investigate markets. As a chemical producer, FMC may have an interest in possible chemical products.	
Challenge will be to integrate process. The ultimate process needs to be attractive economically.	Product recovery may be complex. Evaporation of water may be costly. What are the markets for the mixed products that will be the product of this process?
PI Response: Evaporation could be an important issue. This may be less of a problem at a pulp mill, where low grade heat is likely to be available. One task of the modeling work is to look at integrating the final technology with the existing processes.	
well thought out set of go or no-go decision points and tests	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- must develop integrated system from proven unit operations, requires parallel development and dovetailing of several diverse operations	system is dependent on widely different operations, so problems in one area may greatly slow progress of overall system
PI Response: Indeed, this is one of the major motivators for conducting the process from start to finish.	
Investigating many steps	Many new processes to investigate
PI Response: No response to this comment has been provided by the Principal Investigator.	
Many options for economic pursuit; a sensible approach to the basic problem of waste stream treatment and adding value.	Many options, choices. Very early in the process.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Early days. Good list of tasks.	Not clear how future work will be prioritized.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined plans and goals for future work. Clear discrete milestones over the next six months	Milestones beyond the next six months do not have a crisp timeline associated.
PI Response: The options for completion of the project are still numerous, and so concise milestones are difficult to map out very far into the future. I am expecting that by the end of the first year we will have narrowed processing options and will be in a better position to clearly identify future milestones remaining.	
Execute the project plan. Tasks are delineated.	Establishing stable fermentations may prove challenging. Can these be reproduced? What will be the definition of a consistent final product. For complete anaerobic digestion it is maximum conversion to methane. Is there a desired end-product mix composition? What is the most desirable output?

PI Response: Anaerobic digestion does normally give an end result of methane and CO_2. In this case we inhibit the methanogenesis and accumulate acids. Reproducibility will be an important performance metric to monitor. I expect that the pulp mill extract will be more reproducible than the seaweed sludge, due to its more consistent composition. As mentioned above, an important determination will be the extent to which the non-asceptic mixed culture can dampen variations in the feedstock quality prior to the downstream processing.
seems to be well in hand
PI Response: No response to this comment has been provided by the Principal Investigator.
to do the project as proposed
PI Response: No response to this comment has been provided by the Principal Investigator.
Essentially an integration proposal. Looked at waste water stream from pulp mill for mixed inoculum; also to other environmental sources. Manure, sewage sludge possible nutrient feeds. Not aseptic system.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Connections with several potential local commercial partners.	
No plans yet in place for technology transfer, though apparently ready to engage on this axis.	
Project has industrial partners on the feedstock side.	
working with pulp mill to incorporate this stream on site	

project includes industrial partners	
Working with Texas A&M who has experience in some of these areas and collaborating with local pulp industry and alga harvesting industry	
Publication expected	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
This is a well designed project. It will be interesting to see how it progresses.	Thank you, and we agree--we are very interested to see how the project progresses.

Technology Area: Biomass Program
Project Number: 7.2.3.1
Performing Organization: Clemson University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.14	0.69
Approach	2.71	0.49
Technical Progress	2.43	0.79
Success Factors	2.14	1.07
Future Research	2.43	0.79

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
University research groups working with SRNL and Dyadic International.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal is focused on the local relevance of various feedstocks. The goals are consistent with aims of the OBP	While the aims are consistent, the generalizabilty of this study remains unclear, given that it is not clear how representative SC is of other states that produce the same feedstocks.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Research will focus on the biochemical conversion of switchgrass and sorghum.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
A niche application -- utilizing switchgrass locally grown to develop a process stream.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- pretreatment chemistry - enzyme development - fuels organism development - use of switchgrass and sorghum for fuel ethanol production based on ammonia-based pretreatments using various biocatalysts and organisms - ultimately want to get to pilot scale	- seems could be considerable overlap with other more directed biochemical platform projects
PI Response: No response to this comment has been provided by the Principal Investigator.	

Focused on switchgrass from South Carolina	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Process development to be examined Pretreatment Enzyme biochemistry Fuel organisms (ethanologens)	It might be simpler to cast this as a training project employing an available pilot plant and existing methods rather than trying to address too many topics with an unfocused project.
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
One year of funding. Developing a process and cost model of pretreatment of switchgrass and possibly sorghum. Evaluate pretreatment, hydrolysis and fermentation potential. Optimize fermentation; evaluate and select pretreatment technologies.	Many tasks for a relatively small, short project.

PI Response: No response to this comment has been provided by the Principal Investigator.	
Approach represents an avenue to explore for the ultimate production of ethanol, with thoughts about the industry preferred methods. Goals are in line with industrial standards.	Approach is limited to one specific switchgrass source. Given how other projects have demonstrated the variability between other feedstock sources, it is not clear the relevance of this approach. Scale-up is limited in intent. It is hard to say a bioprocess is optimal based on the results from a 5L scale.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Researchers plan to examine pretreatment , saccharification and fermentation of switchgrass to produce ethanol using non-conventional microbes including thermophiles. Also planning to examine sorghum as a biomass feedstock	Somewhat vague on actual experimental strategies. The plan seems very ambitious list of activities for the timeframe of project. There appear to be lots of independent research activities planned. Is there any coordination of these activities?
PI Response: No response to this comment has been provided by the Principal Investigator.	
	it would be difficult for this group to meaningful solve the fundamental problems in the processing of switchgrass in a commercially viable way
PI Response: No response to this comment has been provided by the Principal Investigator.	
- use switchgrass from existing university farms milled with various methods in pretreatments - will use lab-scale pretreatment systems - using Trichoderma and Thermotoga neapolitana biocatalysts - also studies with a Clostridium for direct conversion of switchgrass to ethanol -	- not clear how results will be evaluated for decisions regarding pilot plant implementation of optimum processes
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Research activities need more focus.
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Effort diffused over the entire production process

PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Started October 2008. Evaluating pretreatment of switchgrass using ammonia and low temperature - promising but preliminary; deconstruction using fungal cellulases in 5 L bioreactor; evaluating ethanol production using yeast.	Still early days but only one year project.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have laid out clear milestones for project that is just beginning. Milestones are consistent with programmatic goals. Initial work provides a baseline against which the future work can be measured.	No progress is evident based on the data presented.
PI Response: No response to this comment has been provided by the Principal Investigator.	

Switchgrass grass was grown and collected and some bioreactor studies on Thermotoga as enzyme producers when screened on switchgrass.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
high throughput analyses have some promise for screening for e.g., ethanol tolerance of biosystems	Fairly superficial analyses—not going to contribute to the frontiers of this science
PI Response: No response to this comment has been provided by the Principal Investigator.	
in start of one year project - have looked at cellulase enzyme production on pretreated switchgrass - have evaluated Thermatoga performance under proposed conditions - have started looking at cellulase activities on different substrates - have started preliminary studies with Clostridium in present of switchgrass preparations - looking at ethanol production by Saccharomyces under different conditions - have applied microtiter plate assays for fermentation performance	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Sampling many organisms; needs to define focus and goals
PI Response: No response to this comment has been provided by the Principal Investigator.	
Cellulases--look at in-house cellulases from known sources. Evaluated production from Trichoderma; looking at commercial cellulase as well. Thermotoga neapolitana as novel source. Visible growth of T. neapolitana; 10% dw consumed. Searching for more organisms using microplates	in part duplicative; local implementation of a standard process Examination of the entire production train dissipates effort/

PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
	Not clear that they will be ready to enter pilot phase in 2 years.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are well described. Focus towards pilot as success provides industrial relevance	Challenges have been only minimally identified. No technical goals are described. No recognition of potential showstoppers.
PI Response: No response to this comment has been provided by the Principal Investigator.	
High kinetic conversion rates are needed to optimize hydrolysis and fermentation.	These are too general. Performance metrics used to make decisions unclear. These need to be developed to assist in focusing future research efforts.
PI Response: No response to this comment has been provided by the Principal Investigator.	

Reasonable set of problems, but will be very difficult with these resources to reach pilot scale.	This effort is far too small in scale and capability to solve the field to biorefinery problems for switchgrass.
PI Response: No response to this comment has been provided by the Principal Investigator.	
seem to have multiple investigators working in their areas of expertise	not clear how different segments of project will dovetail
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Challenges general and not directed toward this project.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Demonstrates diffusibility of existing models	Too many parts of the process addressed in one project Target aspects not described yet
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
	Priorities, values and metrics for go/no go not clear. Alternatives if efficiencies not sufficient?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined plan with several discrete milestones and points for evaluation of progress.	Would be useful to have a timeline of expected completion of the various milestones, and a plan describing the interdependencies of the various aspects of the program given the mention of how one aspect can hold the whole plan up.
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Again. Very general. Hard to know exactly what is planned.
PI Response: No response to this comment has been provided by the Principal Investigator.	
	unrealistic
PI Response: No response to this comment has been provided by the Principal Investigator.	
- continue aspects for which they have preliminary data - develop conceptual pilot-scale process	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Future work needs more focus. What are the primary, specific goals of this work?
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Well established academic and industry connections. Strong potential for publication.	

Dyadic represents the potential for a direct path to market with appropriate development	
Dyadic assists in enzyme discovery and application work.	
not discussed	
Collaboration between Clemson University, Savannah River National, and Dyadic International	
Should replicate some of the processes already demonstrated, show diffusibility of knowledge accumulated. Pilot plant should provide opportunity to focus on one aspect once it's running	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Explicit requirements for a pilot operation would be valuable to this plan. In the absence, it is not clear whether or ton this project will have been successful.	
Project a bit unfocussed. Individuals working on lots of little bits that are interesting, but not too well coordinated.	
Choose one area to focus on	

Technology Area: Biomass Program
Project Number: 2.2.1.1
Performing Organization: National Renewable Energy Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.71	0.49
Approach	4.57	0.53
Technical Progress	4.43	0.79
Success Factors	3.86	0.90
Future Research	4.29	0.76

Overall Principal Investigator Response(s)
We thank the reviewers for their specific and thought-provoking comments. There appear to be some common themes identified by the reviewers, especially in regard to ways to improve this project. These include: a wide breadth of activities, with some uncertainty as to how these activities tie together; the impact of other factors, such as variability of feedstock, that may overwhelm process improvements that are achieved; the applicability of the process development strategies and evaluation tools being developed to pretreatments other than dilute acid; and the continued need to publish findings and methodologies to enable comparisons to other process approaches and configurations. We will address these comments, as well as other key comment from the reviewers on an individual basis in the following sections.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

<table>
<tr><th>Strengths</th><th>Weaknesses</th></tr>
<tr><td>Progress is critical for overall goals of OBP program. Supported by strong CAFI teams.</td><td>May be nearing point of diminishing returns (effort required to get last 5%) in some areas.</td></tr>
<tr><td colspan="2">PI Response: Conversion targets have been developed in conjunction with overall programmatic conversion and cost targets for 2012 demonstration. While the final 5% may seem relatively trivial and does perhaps represent a point of diminishing returns as a stand-alone target, there are several other similar conversion targets throughout the Biochemical Platform that, if not met collectively, would represent a significant shortfall in achieving the overall programmatic objective.</td></tr>
<tr><td>Project focuses on a clear need of the biochemical platform. Understanding economic targets is a clear need. It is of critical importance to tie feedstock breakdown to a economic analysis that can demonstrate a path to commercial success.</td><td>Goals are limited to techniques and technologies known in the art. Exploration is not designed to elucidate innovative and potentially better approaches to process feedstock</td></tr>
<tr><td colspan="2">PI Response: This task works in coordination with more fundamental pretreatment kinetics studies and evaluations of structural effects of selected pretreatment chemistries and conditions on biomass that are being conducted within the Targeted Conversion Research Task at NREL. We anticipate that knowledge gained through these activities will provide a rational basis for exploring changes in pretreatment conditions that are perhaps substantial. The Pretreatment and Enzymatic Hydrolysis Task then conducts process development activities on potentially improved pretreatment approaches in a process-relevant manner and identifies potential impacts on subsequent biochemical conversion steps.</td></tr>
</table>

The testing of pretreatment and enzyme hydrolysis concepts in process relevant context is a central component of the biochemical conversion platform. Highly focused on meeting OBP targets.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
focuses on all the key issues in making cellulosic ethanol viable	
PI Response: No response to this comment has been provided by the Principal Investigator.	
pretreatment is fundamental to bioconversion systems, this project is mainline to their objectives - looking at how pretreatment impacts feedstock properties - trying to make results process relevant	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Little discussion of customers and markets; perhaps obvious
PI Response: No response to this comment has been provided by the Principal Investigator.	
Results of this project, which addresses properties of enzymes and their operation on a variety of substrates and under various operating conditions, will be critical both to near term goals and longer term biomass energy production even if feedstock changes from the acid-treated corn stover.	There are a lot of separate projects in this presentation. I did not fully grasp how they are related
PI Response: We feel that all activities are directed toward selection of pretreatment conditions to achieve 2012 programmatic targets in a manner to allow us to better understand the impacts of the pretreatment conditions on subsequent enzymatic hydrolysis and hydrolysate fermentation. Understanding of these impacts will also be helpful in future efforts on other feedstock and for other potential sugar-derived advanced biofuels.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Moving from tool development to pilot scale analysis. Have identified windows where improvements can be made. Open to exploring additional processes to solve problems.	Will real world variation in feedstocks undo "tweaking" of hydrolysis conditions.
PI Response: Feedstock variability and its impact on specific conversion targets is important, as shown in NREL's Biochemical Processing Integration Task presentation that revealed the influence of various corn stover sources on xylose yield in pretreatment. We feel that it is important to be able to maximize yields for a particular, well-controlled feedstock batch while also understanding the effect of and reasons for performance differences as a function of feedstock variability.	
Described tasks broadly cover the breadth of many of the approaches being currently commercially considered. Approach can define baseline comparators for feedstock breakdown. Key intent to identify requirements for success. Operations in a pilot scale reactor apply more relevance to resultant data. Targets are	Plan does not incorporate potential new innovative techniques that may emerge over the timeline. Unclear of relevance of data to non-ethanol fuels given problem set stated.

consistent with NREL and other industry goals. Demonstration plan ensures focused timeline and execution	
PI Response: First part of comment has been addressed in the Section 1 response above. With regard to relevance to non-ethanol fuels, we believe that the activities in this task (to achieve high yields of sugars from pretreatment and enzymatic hydrolysis processes) will be highly relevant for process development of other sugar-derived biofuels, although there will likely be some different process considerations for other products.	
Comprehensive evaluation of parameters to optimize pretreatment and saccharification of biomass. Coordinated development of fundamental and applied knowledge to meet targets.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
comprehensive, high quality science and technology	
PI Response: No response to this comment has been provided by the Principal Investigator.	
pretreatment systems take into account broad picture (such as impact on ethanologens) trying to move into more process relevant areas	emphasis stays on limited number of pretreatments (dilute acid related)
PI Response: Comparative data for a range of other pretreatment process is being developed and provided by the CAFI project. NREL's efforts in CAFI are funded through the Pretreatment and Enzymatic Hydrolysis Task. We are developing knowledge and capabilities for enzymatic conversion of hemicellulose-derived oligomers (and, in the Targeted Conversion Research Task, for enzymatic conversion of unhydrolyzed xylan). This is an essential process capability that must be understood and established to enable less severe and possibly less costly pretreatment processes (including less severe dilute acid pretreatment conditions and/or alkaline or non-catalyzed pretreatments) that can achieve the high overall monomer xylose yield targets.	
This is a planning and support project not a performance project--performance is in the next two talks	Changing targets (sources and final product) make it hard to keep the picture in focus
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Real data shown. Efforts appear to be well integrated. Identified short window for high solids conditions and potential solution by lowering temperature. Good development of alternate strategies.	Unclear whether benchmarks will hold for real world feedstocks. Toxic effects were not distinguished from inhibitory effects. Not clear if there is a well designed program to understand and overcome toxic and inhibitory effects.
PI Response: Our efforts to understand toxicity effects of hydrolysates on ethanologen growth and fermentation are focused first on identifying compounds in hydrolysates (either those liberated from the feedstock during hydrolysis or those generated via carbohydrate degradation reactions) and then determining which compounds are toxic and at what concentrations they are toxic. This is a new area for the task within the past 2 years and it has made much progress in developing tools and assays (much of which was not able to be presented at the review meeting due to time limitations). These tools are now just starting to be applied to process-relevant hydrolysates (first from high solids dilute acid pretreatment of corn stover). A variety of hydrolysates from different pretreatment conditions are planned to be evaluated in this manner over the next year.	
Early data has validated each of the areas of exploration. Early data is promising that goals	Data is based on lab scale analysis. Plan to develop standards is not clearly articulated, and

will be met. Use of commercial tools is invaluable.	would be a valuable component of this project. Hydrolysate date has not clearly progressed the state of the art given conclusions that are being drawn.
PI Response: Pretreatment performance targets from 2008 forward are based upon operation at high solids loadings in pilot-scale continuous pretreatment reactor systems. We are attempting to set an example (and perhaps a future standard) that the ultimate performance measure for all major conversion steps (pretreatment, enzymatic hydrolysis, and fermentation) must be determined at process relevant solids loadings and associated liquid-phase concentrations. Hydrolysate toxicity characterization work is evolving from a tools development stage to a process-relevant hydrolysate characterization stage, as described in the previous response.	
Research has produced a long list of technical findings. Key areas; solids loading and hydrolysate toxicity. Xylose conversion and recovery. Determination of xylan hydrolysis products may provide interesting insights into improving the bioconversion of these polysaccharides.	Findings seem largely empirical. Is it possible to develop some predictive measures for conversion (e.g. based on feedstock composition).
PI Response: An ultimate goal would be to predict conversion (i.e. overall reactivity of feedstock in pretreatment and enzymatic hydrolysis) based upon feedstock composition or other measureable properties, such as pretreated solids composition (an initial approach based on Principal Component Analysis is presented in supplemental slide #33, although preliminary results have not been conclusive). Tools and approaches being developed in the Targeted Conversion Research Task on structural effects and component rearrangement impacts of pretreatment (especially on lignin) will contribute additional needed knowledge and understanding. We are attempting to utilize fundamental data (from this task and other projects) to develop or improve kinetic and mechanistic models for both pretreatment and enzymatic hydrolysis, especially at higher solids loadings and concentration conditions than have typically been developed in the past.	
significant capability developed and wide range of systems and phenomena examined	
PI Response: No response to this comment has been provided by the Principal Investigator.	
nice work on lignin chemistry during pretreatments kinetics of pretreatment reactions with respect to xylose yields is important -acid	only limitation is if dilute acid pretreatments are not what is eventually used by industry

impregnation etc. all good—impressive analytical data on the components of prehydrolysates	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Many dimensions of concrete data were presented. At least some results seem to be of general utility. Others are critical to getting data on a pilot plant, which is critical to the overall platform and will have general utility in that context.	Hard to evaluate overall because so many different projects were presented at once.
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Good understanding of challenges. Strong team with good partners.	Will changes in upstream (feedstocks) or downstream (saccharification) affect benchmarked processes.

PI Response: No response to this comment has been provided by the Principal Investigator.	
Understanding of key goals to achieve success of the defined project. Recognition of key risks and mitigation approaches is included.	Plan to overcome lacks granulity. Flexibility of approach cannot be assessed.
PI Response: Much of the work to date in this task has been developing tools based upon a model pretreatment system (dilute acid pretreatment) on a particular feedstock (single batch of corn stover). We are developing performance data for the individual conversion steps to characterize current performance levels and to serve as a baseline for targeting process improvements. This data is also helping to identify implications on subsequent conversion steps, such as pretreatment severity impacts on enzymatic hydrolysis, types and amounts of enzymes needed to achieve high monomeric glucose and xylose yields, and hydrolysate inhibition and toxicity effects of ethanologen fermentation. These tools and approaches will enable consideration of other process alternatives in a more modular and flexible manner. Future pilot-scale equipment capabilities at NREL will also permit greater process development flexibility.	
Translation of fundamental findings to practice is a laudable goal. Timely dissemination of information will benefit many researchers in the field. High xylose yields in high solids pretreatment remains a challenge.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
systems approach, fundamental plus applied methods, publish results for community, tied to OBP and DOE goals	
PI Response: No response to this comment has been provided by the Principal Investigator.	
good collaborative efforts, particularly with imaging capabilities, also demonstrated good collaborative work with prehydrolysate inhibitors	Very few, if an, "showstoppers" for this type of optimization work—problems may be more in coordinating all collaborators. Specific challenges include xylose yield, etc., but that is the point of the research
PI Response: No response to this comment has been provided by the Principal Investigator.	
Providing public parameters for evaluation and design.	Some of the knowledge is specific to a particular incarnation of the pipeline (acid treated corn stover). How to focus on generalizable problems without getting too far

	from implementation?
PI Response: Our approach to being able to focus on generalizable problems is to utilize the current process configuration that NREL knows best (based upon a dilute acid pretreatment of corn stover) to develop tools for characterizing process-relevant performance data for the key conversion steps and understand the implication of the properties of process streams on subsequent conversion steps. We can then use these tools to help guide improvements in that process configuration and also apply those tools to other potentially attractive configurations. Plans to implement greater flexibility in pilot-scale pretreatment and high solids enzymatic hydrolysis capabilities will also allow us to evaluate other selected configurations in a process-relevant manner (including other feedstock types).	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Clear plan focused on near-term targets.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Future work is clearly defined at a high level and consistent with the project goals. Timeline	Milestones are vague and not well defined. Key decision points are not defined. Project would benefit from a better understanding of how well

is reasonable given the scope of the work.	the work is tracking against quantitative goals.
PI Response: Key milestones are outlines in chart on Slide 4 of presentation. These serve as the basis for several (generally 6-12) annual milestones to gauge progress toward the key milestones.	
Decision point coming up. Identify the pretreatment process to go forward with research that will identify this [85% xylose yield]. Build on existing research program to incorporate these into a conversion SYSTEM that will maximize sugar yields and minimize costs.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
going after the key issues at both a fundamental and practical level	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Must make major decision on pretreatment process for use in 2012 pilot operation, using existing data, as well as collect further data to support decision.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good progress.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Pilot scale reactor to is be done by 2012, and a main focus will be what configuration and process to implement. Now have output of the pretreatment vessel work to disseminate to others.	A large number of goals--presenting too many lines at once.
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and

projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
15% of budget goes to ~10 academic and commercial partners.	
Well defined collaboration basis. Strong publication record.	
Cooperates extensively with subcontractors and external collaborators (i.e. CAFI). Recognition of the need to communicate results in the form of scientific publications and presentations.	
Tech transfer appears good as they seem to work with many groups outside NREL and have good track record of publishing their work.	
Many partners, good publication record	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
While integration is important for results, should consider separately review engineering and biochemical efforts.	
A plan to include emergent technologies would be greatly beneficial.	Many of the potential emergent technologies lack publicly-available process-relevant data. As stated in earlier responses, we feel that the tools and approaches for evaluating process performance and unit operations interactions are being developed in this task and can be applied to other process configurations. We will strive to publish our findings and methodologies to help enable those evaluations.
Program has understandably focused on	Evaluation of improved cellulose enzyme

conversion of corn stover; I will be interesting to see how this translates to other feedstocks. The cooperation with CAFI will undoubtedly prove valuable. Are there plans to incorporate new enzymes being developed into processes?	products is being conducted within NREL’s Biochemical Processing Integration Task. We are working to identify hemicellulase and other –accessory” enzyme requirements and effectiveness on milder pretreatment approaches that may generate a significant amount of hemicellulose-derived oligomers and/or insoluble xylan that survives pretreatment under these conditions. These are now beginning to be evaluated on process-relevant hydrolysate slurries.
Hard to grasp the whole set--there could be something to remove or downplay.	

Value Prior to Pulping

Technology Area: Biomass Program
Project Number: 2.2.1.2
Performing Organization: CleanTech Partners, Incorporated
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.71	0.76
Approach	4.14	0.69
Technical Progress	3.00	1.00
Success Factors	3.57	1.13
Future Research	3.29	1.25

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Synergistic with pulp and paper industry. Could provide ethanol to areas that do not produce grains. Understands challenges.	No sure all challenges were described.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Industrially relevant goals Can expand feedstock opportunities	Goals are focused towards the pulp and paper industry, which represents an important feedstock source and an industry in need of clean-up, but limited in focus relative to the biomass program goals. The paper industry and its profitability appears to be the primary focus of this project.
PI Response: Although the primary intent of VPP is to improve the profitability of US pulp mills (which have been under considerable pressure from foreign competitors), the technology could have utility outside the P&P industry. The VPP program will demonstrate the potential and value of producing ethanol from hemicellulosic feedstocks.	
The project builds on an existing industrial platform and could add income to this process and biomass to ethanol helping to achieve early OBP production goals.	The ultimate contribution to ethanol production is Will contribute a fairly minor amount of fuel ethanol if taken collectively which may not be in line with OBP goals.
PI Response: True, the quantity of ethanol produced from a relatively small hemicellulosic stream at a pulp mill is minor relative to the biofuel needs of the country. However, the technology could be used to produce biofuel from any hemicellulosic feedstock AND if cellulose conversion is delayed and/or proven not to be economical, the conversion of hemicellulose to ethanol could represent a substantial contribution to biofuels,	
improves pulp mill efficiency, profitability, and viability by producing biofuels in addition to fiber -- could produce up to 1.5BGal per year	

PI Response: True—1.5BGY if only used for conversion of hemis from pulp mills.	
remove hemicellulolse from wood for ethanol production, process remaining wood as usual for pulp -- obviously has merit for the pulping industry, stated that major objective is to improve profitability of pulp mills can produce significant amount of ethanol and acetic acid from process	may not have that big of impact on overall US ethanol production per se
PI Response: Again, the demonstration of the profitability of converting hemicellulose to ethanol could create a new industry and have great utility outside of the P&P industry.	
Uses by-product of pulp & paper industry to produce ethanol. Feedstock available and on site.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This project would divert what is now potentially waste to exportable fuel. Hemicellulose removed from wood first, leaving cellulose for papermaking. It requires using hemicellulose as a feedstock in the fuel process. This would add value to the pulp industry and convert solid fuel to liquid fuel. 1500 ton per day kraft mill could produce 15E6 gal ethanol per year. Combined capacity 1.5 E9 in US.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved *significantly.*

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Separation of hemicellulose may solve some downstream processing issues for lignocellulose. Excellent set of partners. Aimed at wood feedstocks, which is relevant to much of the US east.	Aware of issues such as water use but do not appear to have thoroughly evaluated issues.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Broad-based approach that represents all key process elements with a variety of technical solutions. Correlation with modeling and business case is central to the relevance of the other components and their perceived value to the whole of the project. Detailed planning and organization evident.	Focus on biomass program related goals are limited.
PI Response: Conversion of hemicellulose from any feedstock to biofuels could have a considerable impact on the US transportation fuel needs. This technology represents a fallback position if cellulosic conversion is delayed or not possible.	
Takes advantage pretreatment like extraction of hemicellulose from wood chips that exits in plants and ferments this. This leaves behind a cellulose rich fraction which would require milder alkaline pulping process. Roles of collaborators clearly identified.	Method calls for membrane clean up of hydrolysate to produce fermentable HC hydrolysate. This may prove to be a challenge.

<table>
<tr><td colspan="2">PI Response: The VPP team recognizes the potential obstacles with membrane processing and is evaluating alternatives. However the hydrolyzate from any biomass hydrolysis will need to be concentrated and inhibitors removed. This is a large cost in any biomass to biofuels process. The use of membranes will accomplish this and IF fractionation and recovery of acetic acid enabled by membranes is proven to be technically and economically possible, the value in the fractionation will "pay" for the concentration and purification process.</td></tr>
<tr><td>Fractionation and processing of xylose from hemicel. Several feedstocks and associated research groups. Highly coordinated. 4 product streams without effecting paper quality -</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>project has considerable collaboration, with different teams using their expertise—appears the pre-extractions, details not presented, appear to be mile pretreatments</td><td>not clear that all the parts of the projects can be dovetailed, maybe this will be clear as more results are obtained</td></tr>
<tr><td colspan="2">PI Response: The presenter apologizes that this was not more clear. The project is very well coordinated and the output of the separate all workgroups have been well integrated.</td></tr>
<tr><td>Well-coordinated team work</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>Well-considered approach to evaluating a new feedstock for the industry, with recovery of acetic acid as a second product from conditioning step. Working on four forest tree types. Extraction, fractionation/conditioning; fermentation; process design, modeling and business case.</td><td>Water use per gallon of ethanol.</td></tr>
<tr><td colspan="2">PI Response: Water usage per gallon of ethanol is an issue in any biomass to biofuel process. The VPP technology may use less water per gallon than a cellulosic process and since VPP is to be implemented in an existing facility, there are considerable opportunities to recycle the water plus low pressure steam available to is evaporate the water.</td></tr>
</table>

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Showed that 40% hemicellulose can be extracted from hardwood pulp without effect on paper quality.	Little (no) data shown to validate fraction and removal of inhibitory compounds with membranes. Fermentation was only with simulated extracts. No discussion of barriers and potential solutions for softwoods.
PI Response: The presenter apologizes for not making it more clear that the fermentation trials were actually done with authentic feedstock that was processed by membranes. The simulated feedstock results discussed were only to demonstrate the ability to ferment higher concentration of sugars.	
Fundamental tenants of program with cursory results across the board. Validation of approach in hand	Would expect more data on front end hydrolysis and separations given time underway in project Fermentation data has limited novelty relative to traditional ethanol production studies.
PI Response: The guidelines given to the presenter suggested detailed technical results were not to be presented. The project is on schedule and has made considerable progress. The presenter believes the fermentation results are indeed novel and will have considerable value to the goals	

of the biomass program.	
Research was able to demonstrate high ethanol yields on simulated extracts.	The membrane system remains unproven.
PI Response: No response to this comment has been provided by the Principal Investigator.	
use membrane conditioning; high conversion of concentrated extract; integrated team;	
PI Response: No response to this comment has been provided by the Principal Investigator.	
started in 2007 showed potential for use of membranes to remove inhibitors have done some model fermentations with pentoses with good yields	do not appear to have made a lot of progress in many areas
PI Response: The presenter apologizes for giving this impression. The project is on schedule and has generated a tremendous quantity of data. The sequential staging of tasks may have given the impression that little work has been done in some areas.	
Good results with hardwoods	Requires recovery and sale acetic acid. Needs additional research to work with softwoods
PI Response: Solving the softwood issue is the current focus of the project and results look very promising.	
Have identified promising (hardwood) and unpromising (softwood) sources, useful ethanologens, and parameters of a successful economic process. For example.40% removal of hemicellulose successfully extracted. Economic and benefit analysis is in progress. Three different ethanologens tested--Z mobilis S.ce, pichia stipitis. Fermentation trials with simulated hemicell substr--10-12% ethanol good. Base case model in Aspen analysis system validated.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Serious partners who understand the economics.	Need to show it can work in continuous systems. How much water is needed?
PI Response: The project team recognizes the need for continuous processing and will be demonstrated during scaleup. The project first needs to prove the feasibility of VPP in batch operations. The engineering and process models will assist in addressing the most economical means to recycle water.	
Recognition of broad factors for commercial success. Recognition of where challenges have been,	Clear quantitative factors are absent, and given the broad nature of the collaboration, would be expected. Unclear plan for progress against soft wood goal. Similarly unclear plan for reduction in water use. No plan in place to deal with the recognized volatility of the industry.
PI Response: The presenter apologizes for not making it clear that well defined targets for all elements of the project are indeed in place. The softwood study was not completed at the time of presentation. The study is now complete and a road forward is in place. The project team, having strong industrial representation is very focused on "real world" commercial and technical issues. Again, the presenter apologizes to the project team if he inadequately represented the	

industrial relevance of the structure and output of the project.	
Research clearly under the need to balance between pulp loss and ethanol value. The potential for co-products to pay for the process [e.g. acetic acid] could improve the attraction of this technology.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
economics, yield, coproducts such as acetic acid, minimize capital by working at highest possible concentration; worry about softwood extension	highly dependent on the pulp market variations
PI Response: No response to this comment has been provided by the Principal Investigator.	
idea seems reasonable, but difficult to see the success factors at this point	do not appear to have demonstrated feasibility with real samples for several phases of the study
PI Response: The presenter apologizes for not making it more clear that the ONLY simulated study done in the entire project was with one case of fermentation at high sugar concentrations.	
Well-defined strengths and weaknesses. Includes adequate modeling and economic analysis.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear parameters have been identified to identify whether an acceptable economic balance can be obtained. Water is a killer.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to *address one or more key technical barriers in the OBP MYPP in a timely manner.*

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Understands the importance of softwoods as feedstock. Open to additional processes.	Still need to produce a compelling business case. Economics depends on both the value of ethanol and the value of pulp, both of which can be volatile.
PI Response: The strong industrial representation on the project team does indeed understand the importance of rigorous process and economic models. These tools will be generated during the course of the project.	
Well focused to meet stated milestones	Scope of modeling is limited in its ability to achieve a comprehensive model. Unclear plans on how to move to pilot plant Would benefit from timeline on goals set out as milestones.
PI Response: The presenter apologizes for not making the structure and control of this very complex project more clear. The project plan has 45 milestones, 37 deliverables and 32 go-no go decision points.	
Complete work on softwood, other bits. Compile info into a business case, if negative recommend alternatives	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Very practical, extend to softwood? Modeling of process scenarios—compelling business case or alternatives	

PI Response: No response to this comment has been provided by the Principal Investigator.
if project ends in 2009, then appears they will not be able to complete project as presented—based on progress to date
PI Response: The project as outlined in the PMP is on schedule and will end in 2010.
Working on business design in a targeted manner.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Good connections with industry but not sure how they view the project.	The industrial members devote considerable time to this effort and are intimately involved in all aspects of the project.
Eight industrial partners providing technical oversight. Valuable collaboration is helping to keep directionality focused and valuable with relevance to challenges the pulp and paper industry is facing. Strong academic collaborations help to ensure dissemination of information.	
The projects combines the expertise of an impressive list of collaborators. Lots of technical oversight	
strong ties to pulping industry	
does not appear to have had much result dissemination at this point	Due to the potential of generating intellectual property, there have been no plans to publish

	results at this stage of the project.
Good collaborations	
The project involves a mixed group. Publications not part of the picture.	There are plans in place for the academic side of the project to publish their results.

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
The process design work should be built out more comprehensively to understand what the business case looks like. The project, given its lack of focus on innovating new organisms, could also include incorporating established organisms that produce other fuels to see if ancillary benefits may be achievable based on process commonalities.	There is a plan in place to produce rigorous process and economic modeling tools to support a solid compelling business case if the technical results warrant. It is not in the scope of the project (and lack of adequate funding would not allow it) to evaluate other microorganisms for non-ethanol biofuels. The project team would welcome this opportunity if funds were made available.
Interesting project with real opportunity for success.	

Energy Corn Consortium

Technology Area: Biomass Program
Project Number: 2.2.2.2
Performing Organization: Edenspace Systems Corporation
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.57	0.79
Approach	3.86	0.38
Technical Progress	3.14	0.90
Success Factors	3.00	0.58
Future Research	3.29	0.76

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Potential to solve downstream processing issues in advance.	Early stage. Probably needs more fundamental research to avoid trial and error approach. Interesting approach that has potential to add value but may not be critical for overall OPB program success unless downstream processes are unable to meet goals.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear, well defined goals. Highly relevant to biomass program goals with the potential to simplify the total process.	Would be useful to understand the potential benefits that may be achievable.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The development of custom crop lines for targeted use may have value if other agronomic properties not sacrificed. In this case the addition of lignocellulose degrading enzymes could reduce the cost of conversion of corn stover to ethanol.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
extension of corn is an interim strategy toward goals	Corn has fundamental sustainability limitations, and when/if a value is put on the carbon that goes into e.g., fertilization, tillage, and irrigation it will be less attractive.
PI Response: No response to this comment has been provided by the Principal Investigator.	
improved corn plants for ethanol production are important	

PI Response: No response to this comment has been provided by the Principal Investigator.	
	Multiple questions still need to be addressed
PI Response: No response to this comment has been provided by the Principal Investigator.	
This project might ease processibility of feedstock by including cellulases in plant tissue already when harvested.	Manipulation methods tied up in patents, meaning high IP costs out of the gate. There are Intrinsic limits on plant choice due to technical barriers even without IP barriers
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Good set of partners. Systems approach. Working with corn which has a near-term payoff.	Lacks sufficient fundamental science underpinning to insure success. There are many potential technical barriers.
PI Response: No response to this comment has been provided by the Principal Investigator.	

Clear, directed approach focused on fundamental capabilities of the collaborators and program participants.	Approach is limited in scope with single enzyme goals. Given successes of Mendel and Monsanto, targets for more optimized systems would be expected.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Use of transgenic corn as a means to introduce biomass converting enzymes (cellulases) that will facilitate the subsequent conversion of stover to fermentable sugars.	The enzymes will need to survive subsequent processing and pretreatment of biomass. May also require conditional expression of genes so that they are produced at time needed for conversion.
PI Response: No response to this comment has been provided by the Principal Investigator.	
incorporate enzyme genes in corn to self process -- create energy corn; utilizing systems design perspective	Need to analyze the effects on soil ecosystems of such strategies.
PI Response: No response to this comment has been provided by the Principal Investigator.	
systems engineering approach --> identify enzymes, consider potential, introduce promising ones in model systems, evaluate impact on growth etc, consider impact on processing	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Has an industrial partner	Not easy to determine what the actual work plan is. Talk sounded more like a pitch to VC community
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the *barrier(s) will be overcome.*

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Impressive set of glycomes and enzymes tested.	Apparent significant value at low enzyme loading but data still preliminary. No data given on effects on plants. Would same effort to increase starch or pectin in stalks/leaves give similar or better cost reductions?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good progress on screening sequences and gene functions. Fundamental work accomplished for expression in plants Good data on glucan conversion suggestive of baseline project success. Data validates basis for considering this approach.	A broader screen would have been expected at this stage of the project. Plant transformation system would have been expected prior to work starting.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The researchers have achieved transgenic expression of endoglucanase genes in corn. The transgenic plants required lower exogenous enzyme loadings and/or reduced pretreatment severity.	What are the agronomic characteristics of these varieties? Is the effect observed due to increased enzyme activity or other [non-target] changes in the transgenic plants. CMC is a relatively easy substrate to hydrolyze and its hydrolysis often does not correlate with natural cellulose conversion.
PI Response: No response to this comment has been provided by the Principal Investigator.	
bringing to bear technologies of the industry to	Improving corn could be a rapid source of gain,

transform one of the most highly utilized crops; isolated multiple enzymes and genes for analysis; test implant with success	but is not part of the long term solution.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Identified gene sequences of interest (1400 sequences), characterized selected ones (46), promising ones cloned in plants. Have now progressed to testing some plants in the field showed some promising studies looking at gucan conversion data for wildtype and transgenic strains	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Effect on conversion in corn stover--20% increase in sugar utilization, or lower SpeZyme use, or temperature for preprocessing.	Not impressive result for the technical challenges of getting there
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no *recognition of relative importance or prioritization of activities.*

Strengths	Weaknesses
Good knowledge of issues.	Need knowledge of effect of remaining stover on field parameters. Demonstrated effect is incremental. Will strains be accepted by farmers. GMO concerns could present issues, especially in other countries.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Recognition of important factors	Focus appears on commercialization rather than success of the project at hand. Technical risk factors not addressed. No plan for overcoming risks presented.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The investigators point to regulatory challenges as potential impediment to implementation. Their proactive stance towards this issue appears to be a good idea.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The GMO regulatory hurdles need to be understood and dealt with.	There needs to be an analysis of the ultimate potential of this approach.
PI Response: No response to this comment has been provided by the Principal Investigator.	
have obtained considerable outside funds to expand project	regulatory challenges may be an issue
PI Response: No response to this comment has been provided by the Principal Investigator.	
Regulatory challenges are acknowledged	True technical issues were not described.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or

identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Many parameters that could be evaluated.	Priorities not clear.
PI Response: No response to this comment has been provided by the Principal Investigator.	
High level goals presented	Milestones (i.e. pilot) do not seem achievable in timeline remaining Little to no granularity on timing of milestones.
PI Response: No response to this comment has been provided by the Principal Investigator.	
A more detail examination of enzyme activities in plants appear to be planned. This may will help elucidate what is actually occurring in this plants and help identify future targets for improvement.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This program has substantially come to an end. Further work needs to accommodate progress in many aspects of feedstocks and processing.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
project was from 2006-2009 propose to test	

more transformed plants
PI Response: No response to this comment has been provided by the Principal Investigator.
More enzymes with different specificities to be tested.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Apparent strong interactions with industrial partners and Oklahoma State University; not clear if there is strong communication with end users.	
Clear focus with Edenspace as source for technology commercialization	
Good collaboration with partners who can test this from bench to commercialization.	
Ties into the large energy corn community.	
was not discussed	
ICN is a partner for early reality check. This may limit further dissemination of lessons learned from the project.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Given the amount remaining, it does not appear that the company will be able to meet the goals of the project in the stated timeline.	
Would be attractive to have these enzymes expressed at plant death or harvest—is that part of the plan?	

Enzyme Solicitation Support and Validation

Technology Area: Biomass Program
Project Number: 2.2.2.3
Performing Organization: National Renewable Energy Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.71	0.49
Approach	4.43	0.79
Technical Progress	4.00	0.58
Success Factors	4.57	0.53
Future Research	4.71	0.49

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Critical support for enzyme solicitation that is aimed at reducing cost of enzymes for deconstruction to validate manufacturer's accomplishments.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plays a critical role in serving as a central supporter and evaluator of enzyme solicitation	Not clear what end-points are: i.e. how NREL can work with projects not achieving goals
PI Response: No response to this comment has been provided by the Principal Investigator.	
Enhanced enzyme performance is central to biochemical process improvement.. Enzymes are still among the most expensive costs of conversions and impacts overall performance of the process (e.g. rates, fermentation, etc.)	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This is a critical function in establishing the true state-of-the-art to gauge progress against.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Auditing function for DOE to monitor improvements in enzyme attributes; including cellulase enzyme costs, required loading, potential for integration. Topic is critical to biochemical platform, provided we do not go to consolidated saccharification and fermentation	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Benchmark auditing excellent method to	

monitor progress.
PI Response: No response to this comment has been provided by the Principal Investigator.
Validation role is critical for integrity of the program, DSM, Genecor, Novozyme, Verenium contractors. Very clear presentation
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Assembled an experienced, multi-talented support team. NDAs in place with enzyme manufacturers.	Should stored standards be coordinated with feedstock storage at NREL?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Important enzymatic focused goals Clear plan	Corporate focus makes cost numbers hard to

in place to get from present to cost goals financially with centrally understood targets	define especially given oversight role
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project will validate enzyme improvement achievements of contractors [four companies selected] using a standard NREL produced, pretreated corn stover. Are they meeting the benchmarks? Send personnel to monitor progress and performance. This is critical.	Assessment concentrates on corn stover [consistent with Biochem. Platform objective]: is there any consideration for evaluation on other feedstocks?
PI Response: No response to this comment has been provided by the Principal Investigator.	
This process and resource is critical for progress. The industry must have a vetted analysis of the state of the art in the area of enzymes. Important curation of substrate standards.	Does this overlap with the storage curation effort for materials?
PI Response: No response to this comment has been provided by the Principal Investigator.	
validate enzyme improvements including on-site testing/inspections target to have by 2011 enzyme costs of $0.12/gallon based on 90% enzymatic hydrolysis of sugar yield	Seems appropriate confidentiality arrangements must be In place, such that private industry is not able to use the "Proprietary" claim to avoid an actual audit.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good approach. Comparisons excellent information.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Option to use non-NREL feedstock leads to additional paperwork for a producer. This seems inevitable, however.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Validation plan authored . They have produced standard materials for companies and standards for analyses. Have completed visits to 3 of 4 companies.	Project started in October, 2009. Early days for determining how well teams will carry out tasks.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Having validation plan in place in key step. Detailed focus is very relevant to stated goals. Connection to net total cost is maintained. Development of standard feedstocks and enzymatic standards is an essential development.	Dependency on corporate matters for success can make progress unpredictable
PI Response: No response to this comment has been provided by the Principal Investigator.	
An enzyme validation plan developed and corn stover hydrolysate as a standard feedstock lot produced that will allow for and "apples to apples" comparison and how contractors achieve program goals even though they may	It is not clear by what metrics will be used to measure "performance." It appears they may differ with contractor?

seek to optimize for other biomass feedstocks.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Data and materials and infrastructure for access is a good start.	Contracts took a little long to get in place.
PI Response: No response to this comment has been provided by the Principal Investigator.	
10/09 --> 9/12 have enzyme validation plan in place, includes documentation about pretreatments, enzyme loading, costs, etc. - have prepared pretreated substrates - have performed benchmarking audits on 3 of 4 companies	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good progress; three of four companies audited	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Benchmarking audits on the standardized substrate to published for four competitors. Publication of results will be anonymous as to enzyme source.	Genecor not audited yet.
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to *overcome showstoppers have been proposed.*

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Independent, experienced National Lab team should be capable of validating results to provide believable assurance for commercialization companies. Requires good interactions with companies.	No consensus on how to make some measurements. Genencor contract still needs to be completed.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well characterized success factors. Good recognition of challenges	Unclear plan to tackle challenges. Certain corporate challenges, if manifest, may be hard to mitigate NREL is in a valued position to set standards, and should view its role here as an opportunity rather than a challenge.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan recognizes the technical challenges of performing the audits in time to meet program requirements and considers alternatives.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Rightly aimed at rigorous analytical capabilities and auditing of progress.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
NREL should have ability to monitor these companies based on independent testing	can they actually audit these companies specific methods need to be developed for comparative testing
PI Response: No response to this comment has been provided by the Principal Investigator.	
Understands difficulty of determining protein levels, enzyme loading. Focused on important	

issues.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well identified--as simple as how to determine protein concentration.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Will review companies quarterly. Expect to publish comparison of benchmarks.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well designed future work plan.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan outlined.	

PI Response: No response to this comment has been provided by the Principal Investigator.
NREL will organize a collaborative study looking at the different enzyme preparations NREL will publish findings of final comparisons of enzyme preparations
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Will validate data from the four major commercial enzyme companies.	
Publication is important. Lack of attribution raises questions about importance of information actually disseminated—the greater industry cannot benefit from the information gained.	
A plan to publish comparative data on enzyme performance even without identifying the source is laudable; however this may have limited scientific value without knowing the technologies used to achieve the improvements.	
linked to the community and developing a resource for the community	
the plan is to publish comparative studies looking enzyme performance	
Auditing four companies with contracts, and making non-confidential information public.	
Reports will be released presumably on web.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
An ability to have some punitive element in conjunction with the audits would give this process more teeth Part of the mandate as a universal player between industry leaders should be to develop the standards by which things are measured.	
This is an important aspect of DOE mission—it seems essential to keep this type of auditing in place to assume DOE funds are going toward directed activities	
Keep the auditing function!	

Enhancing Cellulase Commercial Performance for the Lignocellulosic Biomass Industry

Technology Area: Biomass Program
Project Number: 2.2.2.5
Performing Organization: Danisco USA, Incorporated
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.57	0.53
Approach	3.71	1.25
Technical Progress	3.29	1.25
Success Factors	3.43	1.27
Future Research	4.14	0.38

Overall Principal Investigator Response(s)
We appreciate the comments from the reviewers, and have endeavored to address the points that were not clear in the project presentation

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
This effort is critical to meet US and DOE cellulosic ethanol price targets.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal of developing enzymes for enhanced degradation is consistent with program goals. Protein engineering capability is consistent with company's capabilities Diversity of approaches is important in expanding success potential	Unclear plan to advance goals of the program
PI Response: Project target objective is to reduce the cost of enzymes for biomass conversion by enhancing the efficiency of the cellulase enzyme complex, to enable feasible manufacturing economics for large scale production of ethanol.	
Enzyme improvement critical to reducing costs of conversion. Perform in commercial relevant setting. Project is well aligned with program objectives.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
this capability must be developed if progress is to be had in the improvement of enzymes	
PI Response: No response to this comment has been provided by the Principal Investigator.	
improving cellulase performance for commercial applications is important for economic performance—looking for more efficient enzymes	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Genencor has a excellent track record in	

enzyme production.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This project is an in-house pilot plant in joint venture. The company plans to implement the process internally, and possibly also supply enzyme to other processors.	Data sharing plans are not clear
PI Response: Although not specifically addressed in our review, presentation at conferences and publications in scientific journals are part of our plan.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Multinational, experienced company with excellent track record in developing commercial enzymes. Partnered for this project with NREL for enzyme characterization. Strong plan focused on changing the rate limiting step in each enzyme set in a set of cellulose	Using "old" protein engineering technology and methods which work, but may not be as efficient as newer methods. Concentrating on improved enzymes rather than improved enzyme production.

<table>
<tr><td colspan="2">hydrolysases.</td></tr>
<tr><td colspan="2">PI Response: Genencor is one of the world’s leading companies in creating and commercializing engineered enzymes and enzyme systems. We have continued to develop state of the art methods of protein engineering, realizing that the best approach is through small scale perturbations of protein structure, combined with massively parallel high throughput screens to improve multiple properties of proteins simultaneously. In our proprietary approach (PCT WO2008002472, WO2008153925), we assess the effect of changing every amino acid in a target molecule individually to each of its 19 variants, measuring the consequences in performance and stability, and accumulating the data in fungal systems within weeks. We then combine the positive effects through combinatorial, intelligent design, reapplying the massively parallel screens and delivering the improved products through the correct selection of screening conditions, many of which include real world substrates at realistic concentrations. Our technology is more than an order of magnitude faster than the best competing technologies. Today we are one of only two companies in the world that consistently deliver 8-10 protein engineered enzyme commercial products per year. Based on our strategy we deliver generations of products to our commercial customers.</td></tr>
<tr><td>Protein engineering is well established technique</td><td>No plan for what enzymes are set. Protein engineering techniques described are dated with no cutting edge engineering techniques included. Very generic plan that may not even be being applied to relevant enzymes</td></tr>
<tr><td colspan="2">PI Response: We have defined our specific target enzymes of the Trichoderma enzyme mix, as well as identified the critical activities to improve in those target enzymes for this project, even though we did not specify them in this presentation, due to the early status of the project. We refer you to the response above regarding cutting edge protein engineering technology.</td></tr>
<tr><td>Investigator plans to employ an approach that has been successfully applied by them toward the improvement of other enzymes [e.g. proteases]. The approach identifies key factors limiting enzyme performance and will apply protein engineering techniques to overcome these. Namely: produce enzyme variants, evaluate the impact every amino acid position, change virtually all of these. Assay all of these individually and identify best. Evaluate combinatorial library for all the characteristics of interest.. Stability, activity, etc. Their experience has been that this approach works</td><td>The approach seems to focus on enzymes working singly. How about enzymes working in concert with each other? I believe they plan to monitor this by evaluating improved candidates in context of overall mix performance. This could result in a complex, iterative process (produce new limitations and targets). How many proteins do they plan to "improve?" Which enzymes will be improved? While not "random" it may begin to approach this in permutations.</td></tr>
</table>

<table>
<tr><td>better than random combinations.</td><td></td></tr>
<tr><td colspan="2">PI Response: We agree that the biomass conversion performance of a cellulase enzyme mix is a result of the multiple enzyme activities acting in concert on a complex substrate, and our experienced practice is to generate improvements measuring performance on real world substrates, in real world conditions, in the context of the whole cellulase mix. It is our business model to deliver enzyme system products whose components have been co-optimized for performance in processes which convert complex, insoluble substrates to products.</td></tr>
<tr><td>A fairly sophisticated screening and evolution method.</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>- identify key enzymes
- protein engineering to improve activity
- evaluate cocktails using modified enzymes
- consider each amino acid in protein with substitutions
- will try to evaluate several parameters per enzyme, using several different assays
- will eventually have a combinatorial library</td><td>considerable funding has already been spent on this type of work and the improvements have been relatively small with respect to cellulase performance in biofuel applications</td></tr>
<tr><td colspan="2">PI Response: Previous DOE projects delivered over a 30 fold reduction in the cost of enzymes in biomass conversion. A large part of that reduction was due to the modification of the enzymes’ characteristics, temperature profile, enzyme kinetics, etc. Those results laid a strong foundation, upon which this presented project is based..</td></tr>
<tr><td>Plans to use its established protein engineering on large scale to screen for improved enzymes.</td><td>Appear to be using only one approach.</td></tr>
<tr><td colspan="2">PI Response: The approach we tried to outline is a systems approach, examining multiple catalysts with multiple activities, in a complicated milieu.</td></tr>
<tr><td>Very little specificity was provided in part due to incomplete negotiations on the audit process.

Nevertheless, the company is experienced in working to improve specific activity, and will implement a HTP approach, with combinatorial libraries after initial evaluation. An example was given for a soluble enzyme for which the procedure had worked to improve detergent</td><td>No discussion of actual activities proposed to be measured. All data presented relates to an unrelated protein. Implementation of relevant activity assays will be critical to utility of the project.

The goal will be to ameliorate the limiting activity, but there was no description of how the limitation determined (i.e. what are the</td></tr>
</table>

stability. Relative free energy is reported, a useful approach. Target was an unidentified protease (subtilisin was extensively investigated by Genecor, with published results).	surrogate assays?).
PI Response: We wholeheartedly agree the right activity assays will be critical, and that is why we have developed the methods to screen performance on complex, real substrates, not simple models, in high throughput examining the impact on multiple properties. We presented some graphs with results for some of our screens specifically from this project.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Will evaluate the impact of nearly every amino acid in enzyme with about 15 aa substitutions, analyses of which are used to generate a combinatorial library for further evaluations. Developed and validated good high throughput micotiter plate assay with dilute acid-treated corn stover as the relevant substrate.	Contract not yet awarded; thus too early in process for progress evaluation.

PI Response: Response to all: Reviewers are correct in that we are in the early stage of the project, have established our targets and performance screens, and have a well defined and established methodology for delivering improved enzymes.	
The grantee has clearly done screening work The company has validated that site directed mutagenesis works and is better than random libraries Company has assembled core enzymatic assays	It is unclear how the data relates to goals given the lack of information provided on goals or relation of experiments to goals.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have started working on Trichoderma enzymes, developed and validated their assays; micro titer assays work well. It appears they are now ready to process site directed variants.	Too early to judge if approach will be successful.
PI Response: No response to this comment has been provided by the Principal Investigator.	
An important high throughput set of methods that can be further applied.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
project has not yet started - using Trichoderma reesei enzymes, noting two exo, three endo, and one betat-glucosidase - developed and validated assays in microtiter plates - ready to process/assay combinatorial libraries	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project has not yet started, so progress cannot be evaluated	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project hasn't actually started. List of enzymes to be worked on were given with Trichoderma preparation. Using the NREL standard feedstock, enzyme vs. growth phase was shown. Variability well-to-well done.	

PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Looking at enzyme set as a system. Oriented toward near-term market of corn stover. Significant added value from company experience and resources.	Not directly looking at effect of changes on possible effects on enzyme production. Change in feedstock or pre-treatment could change rate limiting step in hydrolysis.
PI Response: The objective of maintaining reasonable protein production is embedded in all of our protein engineering projects, including this project, due to the direct impact on the cost of the enzymes. As part of the engineering strategy we screen for protein expression, and we get very reliable data in fungal expression. The evaluation of the impact of different feedstocks or pretreatment changes is not a part of the FOA or project scope, but as a company we actively evaluate multiple substrates, outside of this project.	
It is identified that there are project risks.	The risks read like an S1 with no clear project related risks No clear strategies are identified

<table>
<tr><td colspan="2">PI Response: We understand that there are a number of technical risks, including those mentioned by other reviewers. The risks include potential effects on protein production, pretreatment effects on the enzymes in saccharification, effect of new enzyme activities on the ethanologen or downstream processes, alterations to the synergies of the complex enzyme mix, etc. As with every enzyme engineering program, there are assumptions that must be experimentally checked and monitored throughout the project, and we do this through the use of performance tests, with correlations to applications performance, as well as through our task called Systems characterization. As we enter the project we will be regularly updating the DOE team on these assumptions.</td></tr>
<tr><td>Success will be defined by ability to deliver enzymes that overcome barriers, lower enzyme dose while working on commercially relevant substrates. Plan identifies a few technical (and non-technical) challenges to overcome.</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td></td><td>not enough detail to make a judgment</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>"You get what you screen for"—quote used in presentation—suggests that they are well aware of the need for multiple assays looking at many parameters.</td><td>shot-gun approach may not be most cost effective way to improve enzyme activity
- although this reviewer does not pretend to know the details enzyme improvement better than the enzyme companies
- how specific will improvements be with respect to different substrates</td></tr>
<tr><td colspan="2">PI Response: We agree that a shot gun approach would not be effective, and refer you to the response about our systematic approach under Section 1.

In this project, improvements will be measured on the NREL standard substrate, as per the funding announcement, but we will monitor for performance on other substrates outside of this project.</td></tr>
<tr><td></td><td>Not fully discussed</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>Acknowledges the difficulties of immobilized enzyme</td><td></td></tr>
</table>

PI Response: No response to this comment has been provided by the Principal Investigator.

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Strong experience at task.	
PI Response: We agree with these comments. Reflection of the early status of the project.	
The project is early, so the plan of developing enzymes has been set out using a generalized genencor approach.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Overall work plan is outlined and includes an economic evaluation.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
all work discussed above is "future" in that project has not yet started	

PI Response: No response to this comment has been provided by the Principal Investigator.
Essentially the same as presented at the beginning.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Danisco/Genencor is one of the big successful enzyme producing companies.	Thank you.
Given the complete absence of disclosure, it is hard to imagine how anyone can benefit from this project. With no clarity on the goals, the project appears to be funding internal R+D in the way that traditional private investment has been used in the past.	In support of the developing Biomass market, our ultimate goal is to drive this project to completion and commercialize the output of this R&D through enzyme products for the Biomass market. Part of our commercialization drive is to seed the industry with the newest enzyme materials for use in optimization of the complete ethanol production process. This is underlined by our recent launch of 4 new products in the Accellerase® line of products for the Biomass conversion market.
Working with NREL and DuPont which will allow them test enzymes in commercial demonstrations. Company has history of cooperation with many academic, government, and industrial researchers working in this field.	
Work done in private industry will, by the nature of the situation, not be as forthcoming as work done in government labs. This is a limitation of funding industrial projects. However, private enterprise appears to be in the	In addition to our commercial endeavors, our scientific staff strives to help drive the industry forward through support and active participation in scientific conferences (e.g. >10 presentations at SIM's 31st Symposium on Biotechnology).

best position to make timely significant improvements toward the production of lower priced ethanol.	Furthermore we collaborate with and support numerous academic labs through funding and provision of enzyme materials.
Provision of enzyme to the community may be limited by the competitive interest of the industrial partner. However, chances are very good that the technology will be used if development is productive.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Without even classes of enzymes described, it is hard to say how relevant any scope of this project is.	As we enter the award, the updates to DOE will make this clear.
Since this project has not yet started, evaluation is difficult, but Genencor has an excellent record for developing and producing improved enzymes and is likely to be a key player in lowering enzyme costs for cellulose hydrolysis.	Thank you for the vote of confidence.

Development of a Commercial Enzyme System for Lignocellulosic Biomass Saccarification

Technology Area: Biomass Program
Project Number: 2.2.2.6
Performing Organization: DSM Innovation, Incorporated
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.57	0.53
Approach	3.86	0.69
Technical Progress	3.57	0.98
Success Factors	3.71	0.76
Future Research	3.71	0.76

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Global enzyme company with strong toolbox and experience. Partnered for this project with Sandia National Laboratory and Los Almos National Laboratory.	Relatively late entry into cellulose deconstruction area.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined goals. Goals are consistent with cellulosic goals of the biomass program. Well defined programmatic targets that have industrial relevance Plan to scale is clear.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Enzymes are a central component of the biochemical conversions process and their improvement critical to the success of the biochemical platform. Need to reduce enzyme loading, production costs, improve enzymatic performance [e.g. double specific activity].	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	we were not told what the team actually did to meet their goals
PI Response: No response to this comment has been provided by the Principal Investigator.	
Cellulase enzyme development is critical for biomass-to-biofuel improvements. - cost reduction for enzymes - developing higher activity enzymes	
PI Response: No response to this comment has been provided by the Principal Investigator.	

This company has strength in fermentation and strain development, more than in enzyme development specifically. With extensive experience in screening for and purifying enzymes at large scale for sale in food and industrial processing, it is well-positioned to contribute to implementation of the goals of the platform. Its strain development process is genomics-enabled.	This is another example of package competition, where the enzyme and process will be internally optimized rather than made aimed at external use by the larger community.
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Company has large collection of enzymes for cellulose processing and developed fermentation processes for different business lines. Strong partnerships with DOE National Labs for molecular characterization and with	

Abengoa, an established biofuels company for pilot facilities testing.. Have developed in-house codon and gene optimization algorithms. Use proprietary fungal expression systems—looking for improvements in host system as well as improvement to enzyme. Plan to harness genome information for finding and developing more efficient enzymes.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
High level approach captures protein engineering capabilities Plan is defined at a high level to achieve focused goals Logical design to ensure project remains on track Leverages core strengths of various participants Clear plans to utilize core technologies in a systematic way.	Little to no focus on standards or on analytical
PI Response: No response to this comment has been provided by the Principal Investigator.	
Investigators plan to uses a relatively straight-forward, classical approach to enzyme improvement. This will capitalize on their experience with Penicillium and Aspergillus as an enzyme producers and provides an alternative to Trichoderma based systems currently en vogue. The plan is heavy dependent on partnering for component tasks leveraging their considerable expertise in these areas E.g. using Abengoa pretreated wheat straw, another for protein engineering, directed evolution. Benefits from in- house expertise on fungal physiology, genetics, and molecular biology.	Seems to be relatively uninspired approach to enzyme improvement. The project seems to focus solely on cellulase and is not very interested in hemicellulase which can improve bioconversion efficiency.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Powerful team of performers to cover the scientific breadth of this very aggressive program. Very powerful and modern approach.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- DSM is working in several areas related to this	Since project is done in different sites, must

project, so could be some synergism - project is being done in several sites, so may get considerable viewpoints for improvements use several metabolic engineering approaches - initially feasibility studies, plan to move to pilot scale. - looking at expression, host improvements, enhanced specific activity	have good management to insure projects dovetail—goals are impressive, seems a likelihood that all will not fall into place as presented since tasks appear to be dependent on one-another to a certain extent
PI Response: No response to this comment has been provided by the Principal Investigator.	
Working with thermally stable cellulases.	Still developing host fungal production systems. General technology for all protein production, not focused on cellulose-degrading enzymes.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The major focus is on host and expression improvement, with a smaller effort at enzyme specific activity. Four projects are aimed at general and specific host expression, one to specific activity improvement. A fairly complete list of aspects of making the enzymes was presented. Sandia is partner for the protein structure determination, which will inform directed-mutagenesis approaches. Los Alamos is expected to do protein engineering for thermal stability.	As above, this is aimed at internal use, not provision as external supplier.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and

objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Have partnerships in place for molecular characterization of enzymes. A two-fold improvement in enzyme expression has been achieved. New proprietary enzymes with improved performance. Have achieved simultaneous saccharification and fermentation with DSM enzymes (at what scale?).	No discussion of assays, which are critical for real-world performance.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Given time of project, clear progress has been made validating basis of approach Early data showing significant positive movement of project Enzyme cocktail data is promising	Standards for comparison do not appear to be the industrial standards. An incorporation of other commercial enzymes would provide further validation of progress.
PI Response: No response to this comment has been provided by the Principal Investigator.	
It is early in research. The researchers have tools available from previous work PluGBug as a protein expression host and will use other as well. To date they have been able to gain cellulase production improvements of 2x level via in-house comparison.	It is not clear how the results of the enzyme improvement compare to others already available. Their comparisons are all with in-house variants.
PI Response: No response to this comment has been provided by the Principal Investigator.	
substantially improved enzymes; and production rate	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- 10/08- 9/12; are approximately 10% into	Not clear if DSM enzymes are significantly

program - DSM has considerable expertise in relevant areas that will be applied to this project - currently trying to develop host strains for enzyme production - are working with A. niger for enzyme production - DSM has enzymes that perform well in SSF	better than those from other suppliers
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project started 6-months ago.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Metabolic engineering is integrated with previous experience at very large scale. The marker-free gene system in Aspergillus niger allows multiple modifications, although I believe marker replacement is not efficient in this system, so that most manipulation is additive. Expression cassettes add heterologous enzymes by insertion to preserve fermentation characteristics. Interesting work on secretion and the stress induced by hyper secretion was presented, with approaches to relieve that problem.	Feedstock to be used is different than the NREL standard. I'm not sure this is a problem, since it's likely that multiple feedstocks will be needed in the long run.
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear

strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Experienced company with good understanding of challenges and potential routes to success.	High-throughput assay not yet developed.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined path to success. Key factors identified technologically and related to internal standards for decision making. Well defined risks with a plan to overcome	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan identifies some technical challenges that need to be overcome and have a conceptual framework that they envision the technology fitting into (Vertical integration of process).	Many of the challenges identified are business related and probably cannot be resolved via technical research.
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Not at all clear exactly what is being proposed as the program proceeds. Successful, but why?
PI Response: No response to this comment has been provided by the Principal Investigator.	
must have good interaction between different components within DSM working toward the goals of this project	- still trying to develop assays, this is essential - also still working on hosts for enzyme production, this could be a limiting factor in doing the rest of the proposal
PI Response: No response to this comment has been provided by the Principal Investigator.	
A basic claim is that the same company has to	Much of discussion is related to the entire

produce the enzymes and implement the production strategy, that vertical integration of the process including enzyme production and ethanolic fermentation in one stream is the way forward. DSM is not convinced that there are general solutions. This remains to be seen.	project rather than the task they signed up to.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Propose harnessing genome information to find and develop new enzymes.	Presentation gave few approach specifics to technical challenges.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Much of the work is ahead, though plans reveal a detailed systematic plan.	More details on the timeline and expected milestones would be useful.

PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan to continue improvement of enzyme cocktails toward commercialization using "proprietary dilute acid pretreated" wheat straw.	Will have to validate with use on NREL corn stover please publish results, make publically available
PI Response: No response to this comment has been provided by the Principal Investigator.	
	not many details were provided
PI Response: No response to this comment has been provided by the Principal Investigator.	
essentially all of the project needs to be completed, so future work is as outlined in tasks above	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Fairly non-specific, probably due to commercial considerations.
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Yes. A global company with strong experience in the field.	
Broad-based collaborations leverages a series of expertises to ensure that developments are shared.	
Lots of expert partners in project: Abengoa, Sandia, los Alamos. Abengoa fermentation, pilot plant, biomass experience will give the opportunity to test enzymes in commercially	

relevant setting.	
not clear how tech transfer will occur other than, if successful, will provide improved enzyme preparations	
Good and necessary collaborations with Abengoa, Sandia National Labs, and Los Alamos National labs	
By design the process is very self-contained, not for dissemination. Actual results are to be protected by IP and thus made public. A comment on the competition: there is a necessary tension between DOE desire for a general solution and the specific choices made by real-life competitors that are optimizing for a specific commercial implementation.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Well defined project both in scope and execution.	
Investigators would like to capitalize on their fungal genomics and fermentation experience, but will have to validate with use on NREL corn stover.	
As usual, it is imperative for DSM to make as much information public as possible.	
Project in early stages, difficult to evaluate, considerable general enzyme production technology being developed in addition to some work on cellulases.	

Project Decrease: Development of a Commercial-Ready Enzyme Application System for Ethanol

Technology Area: Biomass Program
Project Number: 2.2.2.7
Performing Organization: Novozymes, Incorporated
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.86	0.38
Approach	4.43	0.53
Technical Progress	4.29	0.49
Success Factors	4.00	0.58
Future Research	4.29	0.49

Overall Principal Investigator Response(s)
I thank the reviewers for their time and effort and appreciate the generally positive comments about the direction of our research. Hopefully I have responded adequately to the perceived weaknesses in our project. It is important to emphasize that DECREASE is only a part of our overall research effort in this area. There are many areas outside the scope of the project such as strain development, low-cost fermentation research and process integration research that synergize with and support DECREASE but are not directly supported. With well over 100 researchers working in this area, Novozymes is absolutely committed to enabling the cellulosic biofuels industry.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Highly experienced enzyme company with strong, experienced academic individuals as partners as well as PNNL.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are consistent with biomass program goals. Intent to commercialize with a market appropriate timeline is included.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Enzyme improvement and cost reduction is critical to success of biochemical conversion of lignocellulosic biomass to ethanol. This project targets reduction of enzyme loading and improvement of enzyme biochemistry to achieve this.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Methods must be developed (like these) to screen for better enzymes.	What if mother nature hasn't solved the problem?
PI Response: Another question might be, what if mother nature has solved the problem and we can't find the answer. In either event, we are fully prepared to take what mother nature has provided (and we can find) and improve on it further through protein engineering. We believe	

that our extensive diversity screening will provide us not only with the best possible backbones for protein engineering but also allow us to utilize techniques such as family shuffling to recombine the best features from multiple backbones. Our capabilities in this area were not emphasized during my talk, but Novozymes has vast experience in engineering proteins for improved characteristics. We are currently using those capabilities in this project and will ramp up those activities if and when diversity screening reaches the point of diminishing returns.	
enzyme-related barriers are critical to biofuel production are important—goal to improve biomass enzyme components for production of commercial enzyme cocktails	seems to be considerable overlap between DOE-funded enzyme projects at major industrial enzyme players
PI Response: This is more a question for DOE to answer, but in general I would respond that the approaches of the different enzyme developers differ enough that they are all likely to come up with different solutions to the problem, and it is prudent for the DOE to "spread the risk" and also the potential rewards.	
Proven track record, closely aligned with DOE goals.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This project addresses a showstopper problem with a well-considered approach to analyzing activities of the component enzymes and improving their performance. Unlike the other projects in this area, the resulting enzymes are explicitly aimed at external markets rather than internal use.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Several parallel approaches. Exploiting a new activity (GH61) that has been found to dramatically enhance cocktail efficiency. The focus on replacing the "weak link" in an established enzyme cocktail for lignocellulose deconstruction. Using a large scale screen to identify new enzyme components that are synergistic.	Somewhat based on luck in finding better enzymes. Additional activities may have tradeoff between increased activity and additional cost for new component.
PI Response: Luck is undeniably a component of this research, but the same can be said for most scientific endeavors, including protein engineering. However, our search is attempting to stack the odds in our favor by sampling natural diversity in places where new and improved enzymes are most likely to be found, e.g. rotting corn stover, compost piles of mixed agricultural waste, etc. Cost is an issue if we have difficulty expressing the new activities in our high-level expression hosts such as Trichoderma reesei. We can often increase expression levels by manipulating the gene (e.g. changing codons), swapping signal peptides or making gene fusions. Also, often when we have difficulty expressing a particular gene, we can find a close homologue that expresses well.	
Clear focus on optimizing components of the enzyme system in a directed manner. Exploring both new approaches and optimization approach gives the project a better chance to success.	No innovation in protein engineering evident. Techniques being employed are not including recent technological improvements in protein engineering.
PI Response: I did not emphasize our protein engineering capabilities since it is not our primary focus during the first year of work. Novozymes has an unparalleled success rate in optimizing enzymes for industrial conditions using a variety of technologies including DNA shuffling and "sloning" coupled with high-throughput assay capability. We are currently performing protein	

engineering on one of our "weak links" and intend to employ these capabilities whenever suitable targets present themselves as weak links in our current system.	
Research plans to expand on earlier development program by exploiting GH61 improvement of cellulase function, screen for more synergistic complimentary proteins and replacement of weak/missing link in enzyme components. Although the details of these are vaguely described this appears to be a productive avenue of exploration.	How will results translate from screening conditions to more commercial high substrate loads?
PI Response: A very good question and one that we have considered in some detail. Novozymes has multiple projects running in support of developing a commercial enzyme mixture for cellulosic biomass hydrolysis. One of those projects, not supported by DOE funding, is to develop an efficient process that mimics what we believe will be relevant industrial conditions. On a regular basis we submit our experimental enzyme mixtures to this project for validation of performance. The process conditions employed include up to 30% total solids. We also work with our industrial partners to validate enzyme improvements under pilot plant conditions. Even though not funded under this project, we will likely present these data during our Stage Gate Review. In general we have found that enzymes that work well under our screening conditions also work well at high solids, although the optimum ratio of activities is sometimes altered.	
Diversity screening—when that reaches diminishing returns they will switch to protein engineering	What comes next?
PI Response: We view diversity screening and protein engineering as long-term activities that will incrementally improve our enzyme systems and further reduce cost to the point that their cost is not an issue with respect to commercialization for at least some cellulosic substrates. However, other substrates (such as softwoods) may remain less tractable and considerable effort will be needed to create new enzyme mixtures and probably new pretreatments for these substrates.	
- exploit famil of glycosyl hydrolases (GH61) - replace weak links in enzyme system - doing diversity screening and genetic engineering - working toward identifying synergistic systems	

PI Response: No response to this comment has been provided by the Principal Investigator.	
Excellent approach; concentrating on weak link and missing link enzyme activities. Already has excellent expression/production enzyme systems.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This is primarily a screening project, looking for additional enzymes to improve complete utilization of the substrate available. This is an important addition to the quiver, since enzyme-optimization (Genecor, Verenium) and production-optimization (DSM) may increase activity and availability at some steps but not address the whole cellulose-degradation problem. An additional focus is to improve on GH61 family of enzymes, to understand what this mysterious enzyme family does. Markers for abandonment if no progress is made were articulated.	
PI Response: I would add that production optimization is an ongoing project at Novozymes, but is not supported by the DOE.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP

goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
They have Identified a better GH61 enzyme and have screened several hundred enzymes for sygeneric effect on activity with good thermal stability on acid treated corn stover. Similar screen of new fungal systems for activity and synegerism and "missing links", Have also looked at several other substrates (no details)	
PI Response: I did mention in response to a question that we have a large array of substrates on hand including wheat straw, rice straw, lodgepole pine, poplar, sugarcane bagasse, switchgrass and others, pretreated under various conditions. We do not routinely screen with most of these substrates unless we have a commercial partner who is interested in them, but we do use them for follow up and validation of progress.	
Early data on GH61 is promising and validates the basis for spending time and money on this project. May offer an unique way to get towards NREL defined project goals. Protein engineering approach and screening is showing continued progress and continued head-room for further R&D. Early data has shown some potential for weak link replacement. Data on missing links is showing substantial synergy. Analysis has been systematic with valuable data and potential for IP emerging.	Unclear how the various enzymes compare to other standards. The experimental design would benefit from inclusion of generalized standards. Important to have a mathematic definition of synergy, as this is often as misused term. Weak link replacement data shows only minimal synergy thus far, which is hard to assess in the absence of error, which is essential in synergy analyses. Screening of cellulytic systems has not shown significant progress at present, though more work clearly to come.
PI Response: The standard which we always include is our own commercial cellulase preparation, Cellic CTec. The synergy assay is based on adding monocomponents (or whole fermentation broths of wild-type organisms) to this standard enzyme preparation. We make two synergy ratio calculations: The first is the cellulose conversion observed with a fixed amount of Cellic CTec plus a fixed amount of the monocomponent under test divided by the conversion observed for the same amount of each alone. The second has the same numerator but the	

denominator is the conversion observed with an amount of Cellic CTec that is equivalent to the sum of both. The second calculation is a more stringent indicator of the benefit we would expect to see by including this component in our cellulase mix. As for assay error, we typically run the assays in triplicate with a coefficient of variation averaging approximately 1-2%. While the synergy effects may seem small, a small change in percent conversion can translate to a significant effect on the total dose of protein required to reach a specific level of conversion. This is because the conversion versus protein loading plot has an increasingly small slope as conversion levels reach our target of 80-90%. We always follow up our synergy assay results with complete protein loading profiles so that we can accurately gauge the fold-reduction in total protein loading required to achieve a given level of cellulose hydrolysis.	
Results to date are predominantly from earlier work but show promise toward improving current Trichoderma enzyme products,	
PI Response: The results with GH61 are largely from previous work, but much of the monocomponent synergy work is quite current.	
quite successful in isolating improved enzymes and in improving cocktails -	Not looking at metagenomic libraries because they can't be expressed.
PI Response: Actually we do look at metagenomic libraries and have attempted to express some genes from those libraries. In general we have found this a less productive approach than targeted screening.	
- GH61 dramatically improves cellulse performance (GH61 is expressed by Trichoderma along with the cellulases) - currently screening other GH61 proteins and studying their expression - weak links have been identified in cellulase mixtures and are being tested using monocomponent enzyme supplements to the cellulase mix (considering activity & temperature stability) - missing link activities are also considered and have been demonstrated - are doing screening of natural cellulolytic systems and using biochemical fractionation to determine identity of interesting enzymes	not clear that mechanistic information is being gathered and shared with public (to the extent possible for private industry)
PI Response: A manuscript describing much of our GH61 work is currently in preparation. We	

are obviously restrained by IP considerations from publishing some information, but in many cases that information is available to the public in our patent applications even if no publication eventually ensues. Very little of the information we generate is treated as a trade secret. All work performed with DOE funding will be made public within a maximum of 5 years.	
Showed examples of real progress. Tested multiple feedstocks.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Three subprojects were described, one based on looking for improvement in the specific component GH61 screened for improved activity mutants or other genomic sources; a second examining specific component additions to the starting enzyme cocktail; the last using spent growth media from fungal organisms as additives, which would then allow fractionation and identification of the active component to allows cloning and expression (avoiding production issues with wild organisms).	As with the other presentations, little was disclosed about what the assays were in detail. For example, the "monocomponents" added in the second project were not identified, nor were the "weak or missing" links in the enzymatic activity profile described. Time was short, but everyone is working in a haze not really knowing what the substrates and products are.
PI Response: As mentioned above, much of the relevant information is eventually made public in patent applications. I believe that Novozymes was much more forthcoming with real data than the other enzyme companies. It is policy at Novozymes and almost certainly at other companies that proprietary information is not made public until, at the very earliest, patent applications have been filed.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Strong, experienced enzyme company with good understanding of issues and challenges. Have surveyed a number of substrates some of which may better represent real-world substrates than NREL standard. Have introduced enzyme set with current highest activity on market.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Identification of key needs consistent with NREL goals and recognition of implications for the industry Clear recognition of challenges likely to face in the path forward and a recognition of the concerns related to the NREL feedstock standard as potentially not industrially relevant. Clear recognition of a need to more commercially and industrially relevant standards. Well defined timeline to achieve goals	Strategies to overcome some inherent problems could be better defined—in particular, the relevance of samples being used to evaluate and normalize progress.
PI Response: I agree. In many cases, agreements with our commercial partners prohibit us from disclosing anything about the substrates being evaluated. The fact that we are working with companies such as POET and other potential second-generation ethanol producers should speak to the fact that some of the substrates we are evaluating are of commercial interest. We are contractually obligated to use NREL dilute acid-pretreated corn stover as our benchmark substrate, but our enzyme developments are validated on additional substrates. We have additional collaborations with pre-treatment experts such as Jack Saddler at the University of British Columbia. His laboratory uses our enzymes to assess various pretreatment methods on several biomass substrates. Some of this material is made available to us for additional testing.	
Goal is to achieve reductions is enzyme costs to	Program could be developing optimized

<table>
<tr><td>allow meeting goals of OBP and allows progression of biofuels industry.</td><td>enzymes for single feedstock. May not be the ultimate winner. Don't have a commercial process out there yet to allow for iterative approach to application improvement.</td></tr>
<tr><td colspan="2">PI Response: The lack of a true commercial process is a limitation for all enzyme developers. However, as indicated in my response to reviewer 17901 above, we have commercial partners producing alternative substrates and these are used for benchmarking and in some cases also for screening. Since that activity is largely outside the scope of the DOE funding, we typically do not report it. To date, the majority of enzyme improvements appear to be robust across substrates and pretreatments. Certainly there are some substrates, for example those with high hemicellulose content that require a different mix of enzymes than acid-pretreated corn stover, and we are fully aware of this and have other projects directed toward those substrates.</td></tr>
<tr><td></td><td>Not much technical content to the success factors and challenges.</td></tr>
<tr><td colspan="2">PI Response: We believe that the primary technical challenges at this point are as much substrate and pretreatment-related as they are enzyme-related, and therefore we emphasized the wide range in observed hydrolyzability of substrates as a significant technical challenge for enabling the industry. We obviously have technical challenges with respect to lowering enzyme dosage and cost, but the progress that has already been made in enzyme development and the progress that we foresee in the near future will, we believe, shift the commercialization barrier more towards the substrate and pretreatment.</td></tr>
<tr><td>- success factor is to achieve reduced costs
- Novozymes has extensive background in this area</td><td>ability to translate information obtained with model feedstock (e.g. dilute acid treated corn stover) to other feedstocks/pretreatments</td></tr>
<tr><td colspan="2">PI Response: This comment was addressed in previous responses, and we agree that it is an issue for the entire cellulosic biofuels community.</td></tr>
<tr><td>This project presented results with NREL model to determine enzyme cost though with high error bars. Pretreatment was cause of big error bars, indicating that developing enzymes in a vacuum is difficult; shows 20X variation over a set of about 15 stocks. Feedstock-robust enzymes are needed, or the process needs to be customized to each particular chosen feedstock. The latter is the choice of the other three participants in this area.</td><td>Asserted that enzyme availability will not be a showstopper by 2012, but it's not clear why the window for enzyme improvement closes in 2012. That may be true for this MYPP to achieve its goals, but investment in understanding the enzymatic process is likely to be a productive endeavor for much longer than that.</td></tr>
</table>

PI Response: I did not mean to imply that enzyme development will halt in 2012. We expect that the enzymes for cellulosic biomass hydrolysis will evolve continuously as they have for the starch ethanol industry and as enzyme systems have in other industries such as detergent proteases. The industrial enzyme market is highly competitive and very much cost-driven and we work continuously to improve the performance and lower the cost of our enzyme products. The 2012 date is perhaps somewhat arbitrary, but we believe that cost-competitive enzymes (and pretreatments) must be available by then if this nascent industry is to have a good chance of getting off the ground and competing with petroleum-derived transportable fuels in the near future. I would also add that some of the uncertainty in our cost estimates derives from the uncertainty surrounding development of good C5-fermenting organisms.

As mentioned in another response, most of our enzyme developments appear to be feedstock-robust, but it is also true that certain specialized enzymes may be required for some substrates and feedstocks. For example, we have introduced Cellic HTec along with our Cellic Ctec in order to improve the hydrolyzability of hemicellulose-rich substrates.

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**

Will continue to screen for new enzymes and will integrate PNNL into workflow.	No details given on number and sources of enzymes being screened. Difficult to judge probability of success.
PI Response: To date several hundred individual enzymes have been screened and several thousand wild-type organisms have been surveyed for cellulolytic activity.	
Well defined goals consistent with grantee and program goals. Leveraging past successes Incorporating economic models with progress. Clear quantitative goals have been set.	Given timeline of industrial need, correlation of milestones to that would be beneficial.
PI Response: Based on both internally funded and DOE-funded work we are committed to introducing an improved enzyme mix into the marketplace in 2010 and have formally announced this intention publicly. Assuming that our DOE-funded work is successful, we anticipate that additional improvements will be incorporated into a commercial product no later than 2012.	
Decisions on future direction of research are clearly outlined and will help focus efforts toward the most productive activities in the time frame required. Decision points for additional research on GH61 proteins and synergistic proteins from natural hosts will help focus this. Alternative strategies consider for improvement of missing/weak links.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
GH61 protein work continue screening work continue synergism studies	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response

"Novozyme is an experienced global company.	
Well defined partner/collaborator groups focused on development and investigative targets.	
Cellic-tm enzyme product is out may be close to targeted cost/performance goals already. Investigators don't appear to have a commercial fermentation partner to work with (although I am sure they must be working with a number of industrial partners) but plan to partner with PNNL on fermentation/enzyme production piece. Plan to team up with PNNL for fermentation work	Novozymes does its own fermentation and is the largest producer of industrial enzymes in the world. We are currently constructing a production plant in Blair, Nebraska to serve the enzyme needs of both first- and second-generation ethanol. Our collaboration with PNNL is for fermentation of wild-type fungi for our biochemical fractionation work. We have limited capacity for this type of fermentation at our facility in California, hence the outsourcing.
provide low cost enzyme to industry	
Good collaborations; already selling commercial cellulase.	
Good collaboration with other actors. Model is provide enzymes, not internal use.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Comprehensive program covers several angles for potential success. Contingency plans for discontinued programs would be useful to understand, else, it is unclear why program support should continue for such subprojects in the absence of continued company work.	We expect that some of our approaches such as GH61 diversity screening will reach a point of diminishing returns. If that occurs, resources would be diverted to efforts such as determining the GH61 mechanism of action and engineering our existing GH61 proteins for enhanced thermostability and functionality. Similarly, if our wild-type organism screening reaches a point of diminishing returns,

	additional effort would be focused on areas such as a detailed proteomic, genomic and biochemical analysis of the cellulolytic systems of those cellulase producers that surpass the performance of our commercial Trichoderma reesei system. Currently that effort is limited by the need for resources to screen large numbers of organisms rather than drilling down more intensively on a few species. This focus can readily be shifted if and when diversity screening is reduced. The same is true for monocomponent screening wherein detailed biochemical characterization and protein engineering will gradually supplant diversity screening.
please publish results, make publically available	We will do so as allowed by our management and IP considerations. As indicated in a previous response, there is often more information available in patent applications than in journals, and in this rapidly developing area that may be the best source for cutting edge information (assuming that you can wade through the legalese).
Novozymes, like other companies working in this area, are encouraged to make as much information public as possible	
Strongest presentation by companies funded by Enzyme Solicitation	
A limitation of screens is that the substrate load is low for a commercial process. Have implemented a second step to look at commercially relevant situation.	As indicated, we have implemented additional screening and validation steps at commercially relevant substrate loadings, and we regularly adjust our lower-loading screening assays to better reflect the results from those validation steps. In general we find a good relative correlation between the different assays.

Commercialization of Customized Cellulase Solutions for Biomass Saccharification

Technology Area: Biomass Program
Project Number: 2.2.2.8
Performing Organization: Verenium Corporation
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.57	0.53
Approach	4.29	0.49
Technical Progress	3.86	0.90
Success Factors	3.86	1.07
Future Research	3.57	0.79

Overall Principal Investigator Response(s)

We would like to point out that this grant for enzyme evolution is just one component of Verenium's R&D pipeline that is staged to deliver continuous improvements to our process technology. New and improved technologies like these advanced enzymes will be scaled up through our existing pilot plant and demonstration plant facilities. Successfully demonstrated technologies will be implemented in existing and new commercial facilities in partnership with BP. The Verenium-BP partnership has already initiated the process of constructing our first commercial plant in Florida. Cost-reduction activities like this program are essential for reducing the ethanol production cost to accelerate adoption and commercialization to meet the muti.

The FOA and subsequent award negotiations required this enzyme development be integrated in a technically and economically viable biomass conversion process. The agreed upon scope of this grant award is focused on evolving enzymes to reduce their dose as part of a defined process and economic model based on dilute acid-pretreated bagasse. Multiple comments were directed at the relevance and utility of this program towards other types of processes or feedstocks. Where possible we have tried to anticipate and incorporate the requirements of other processes and we believe most of our improvements will have generally applicability. These and other reviewer comments are important and insightful, but being addressed by Verenium activities outside of the scope of this grant program.

We would also like to point out that as part of a corporate research program, we are unable to disclose specific details on methodologies and results. To do so would compromise our competitive position and our ability to file patents. We acknowledge that the lack of details makes a comprehensive evaluation challenging, but believe these concerns are addressed by the audits and oversight provided by NREL and DOE under confidentiality agreements.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
The focus is on the major cost factor for lignocellulose conversion to fuel. Major enzyme company with over 10 years experience with cellulases, strong analytical capabilities and ongoing cellulosic ethanol commercialization effort.	No partners.
PI Response: Verenium has end-to-end capabilities for research and development for biomass	

processing and enzyme evolution in particular. We are fortunate to have a defined and established cellulosic ethanol process in which to develop improved cellulase enzymes.	
Program goals to introduce enzymes with less net requirement is consistent with goals. Project is well defined with clear goals.	The proposed advances may have little impact on how to reduce cost of producing fuels.
PI Response: Enzyme cost is widely acknowledged as one of the most expensive components of biomass conversion processes. Within the guidelines of this grant, we are projecting that our target of reducing the cellulase dose by 4-fold will result in similar reduction in enzyme cost. Even without knowing our current enzyme costs, a 4-fold cost reduction will undoubtedly be hugely beneficial.	
Project plans to reduce enzyme loading and cost. Alignment with other parts of the company will promote coordination of activities of enzyme business unit with the biofuels business unit.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
screening and molecular evolution techniques -- extensive results from 10 years of discovery	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- decreasing enzyme costs - some focus on pretreatment chemistry & process integration - trying to make 2nd generation enzyme preparation - trying to make minimal cocktail of enzymes (4-enzyme cocktail) to get job done - lower enzyme costs = low doses, high rates, low lignin binding - maintain/improve process compatibility	considerable overlap with work done by other enzyme producers that are also receiving DOE funding
PI Response: No response to this comment has been provided by the Principal Investigator.	
Using Diversa's extensive collections and molecular evolution experience to improve cellulase enzymes.	Initially enzymes will be used only by Verenium and only later be available to other companies.

PI Response: No response to this comment has been provided by the Principal Investigator.	
This project complements the three others in this group, adding a directed evolution approach to those aimed as designed mutation (Genecor), expression enhancement via metabolic engineering (DSM) and screening de novo for additional enzyme components that increase overall effectiveness (Novozyme). This project focuses on improvements in four defined cocktail components, aiming to increase specific activity and other properties.	By choosing only four activities, the opportunity to recover a larger fraction of the total sugar content may be foregone. However, the Novozyme project has the opportunity to provide those.
PI Response: We have found that only 4 enzyme activities are required to effectively digest the pretreated bagasse that is the target feedstock for this application. These results are consistent with published studies by other groups. We acknowledge that other pretreatments may produce a substrate requiring more enzyme activities. Inclusion of additional enzymes to address alternate processes or feedstocks will be staged according to our commercial priorities, but is outside of the scope of this grant program.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant

contributions progress.

Strengths	Weaknesses
Developing a discrete cocktail with a minimal 4 enzyme set. Their approach is based on gene discovery, combinatorial gene reassembly, and iterative evolution for enhanced performance and the effort is guided by rational approach. Verenium pioneered bioprospecting. They have a large catalogue of enzymes.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Approach leverages the technology base defined by the Diversa heritage. Approach leverages well defined technology cores that have been proven with several other enzymes, increasing the likelihood of success. Potential to scale can ensure industrial relevance of data (though not clear that this is part of the plan).	Path and process has no granularity. Analytical approaches are not described, but are essential for demonstrating progress.
PI Response: Methods and analytical approaches are proprietary to Verenium, but have been audited by NREL.	
Research builds on gene discovery and enzyme evolution experience of Verenium [Diversa] and plans to apply these approaches to cellulase improvement in the current context. Plans to apply 2 rounds of evolution on each of the 4 cocktail enzymes. The company is highly skilled in molecular evolution and high throughput screening.	Bagasse is used as the biomass feedstock. How enzyme mixtures optimized for this substrate will perform with others [e.g. corn stover] is unknown.
PI Response: This grant program and associated metrics for enzyme evaluation are specific to bagasse pretreated under defined conditions. Performance on a prioritized list of feedstocks is part of Verenium's overall R&D program, but is outside the scope of this grant. Generally, we have found that, with the appropriate pretreatment conditions, different feedstocks do not require specific enzyme solutions.	
great application of modern techniques for evolution and screening	

PI Response: No response to this comment has been provided by the Principal Investigator.	
have enzyme unit and biofuel production unit that work together - considerable enzyme discovery and enzyme improvement - different evolution techniques - strong analytical capabilities - dovetail with ongoing cellulosic ethanol commercialization program (Jennings, LA) -	seems to be some overlap with competing enzyme producers
PI Response: No response to this comment has been provided by the Principal Investigator.	
The main focus appeared to be mutagenesis and iterative directed evolution aimed at particular properties thought to be relevant to the commercial process for the four chosen components. The company has a strong background in enzyme design and evolution as well as assay design.	As with the other projects in this group, the enzyme assays were not described in detail, so it's hard to make a judgment on their relevance to the commercial process.
PI Response: Assays for evaluation of enzymes are proprietary to Verenium, but have been audited by NREL. We feel that the process-relevance of our assays is a point of differentiation for Verenium due to our in-house process development programs and R&D activities at bench, pilot plant and demonstration plant scale.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP

goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
The project began in October, 2008. They have developed a high throughput screening assay and have discovered >50 beneficial variants for each component of their cocktail. The best variants reduced the enzyme load 2-fold. They are strongly leveraging of previous experience.	Assay based on bagass and not on NREL standard.
PI Response: The grant is seeking to develop enzymes as part of an integrated process using bagasse. As described by Jim McMillan at the start of the session, our enzyme will be benchmarked at NREL using their standard corn stover and assay conditions. We have every confidence that the improvements to our enzymes will translate to NREL corn stover and other dilute-acid pretreated feedstocks.	
Heritage data provides a good basis for this project. Recognition of discovery limits emphasizes programmatic approach. DirectEvolution technology is well described. Analytical approaches are included, and co-analyzed. Approach has clearly identified mutants	Data has shown only minimal progress. No composite data is evident. Much of the data appears to come from old experiments. Technical barriers have not necessarily been defined, and a plan to optimize is not evident based on the progress to date.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The first round of evolution is complete and apparently identified 50 beneficial mutations for each of the 4 enzyme activities. These will serve as the basis for next round of evolution. The finding that the location of mutations is not very rational illustrates the need these more random approaches.	Results presented are only vaguely quantitative and expressed relative to current in-house products.
PI Response: We have developed proprietary procedures to precisely define the amount of enzyme required to meet our performance targets that are based on an integrated process and	

economic model. These methods have been audited by NREL. Using these methods, we have shown our first enzyme mutants reduced the required enzyme dose by 2-fold and expect even more improvements from our ongoing efforts.	
probably discussing work that was extant when program was awarded	
PI Response: No response to this comment has been provided by the Principal Investigator.	
9/08–6/10: completed first round of evolution with several key enzymes - have ongoing enzyme biochemistry studies - previously done bio-prospecting have resulted in large number of biomass degrading enzymes - done considerable evolution studies, techniques well worked out (GSSM) - continue developing 96-well plate assays relevant to larger scale systems - demonstrated improved dose response - demonstrated improved temperature stability - have found >50 beneficial mutations per enzyme evaluated (evaluating 4-enzyme cocktail) -	difficult to determine what work has been done under the present DOE award,
PI Response: No response to this comment has been provided by the Principal Investigator.	
The starting materials have been audited, a very good step. The genomic library approach has reached diminishing novel enzyme discovery returns (apparently before the start of the funding period), so directed evolution technology is the main focus, aiming to relieve feedback inhibition, improve substrate binding or improve process compatibility. Good results with thermotolerance, finding 50 beneficial mutations for each of the 4 enzymes with up to 2X improvement; next to do combinations and repeat.	In order to optimize, inevitably the researchers have to choose a defined process condition. Whether this will be of general utility is not clear at this point.
PI Response: The feedstock and process conditions have been defined for this grant program. Outside of the scope of this grant, Verenium will validate performance of the resulting enzyme cocktails under alternative conditions or feedstocks. We expect the improvements to be general.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Good knowledge of challenges.	It is not clear what may limit enzyme performance. Performance may be different on different substrates or with different pretreatments.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Success factors are consistent with goals. Challenges and potential limits are recognized as challenges.	No plan to overcome or mitigate showstoppers. No plan for early identification of emergent showstoppers.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The overriding goal is to reduce enzyme loading and thus reduce cost. Investigators recognize the need to demonstrate performance in commercially relevant setting.	Company is finding that there is not much diversity left to look at for sources of new genes. Are the prospects for molecular evolution better?

PI Response: No response to this comment has been provided by the Principal Investigator.	
Looking for the limits of natural evolution. Then use lab evolution techniques	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- Major success factor is reducing enzyme costs - important that they have commercial ethanol production facility to test enzyme preparations.	may be limits to cellulase evolution—not clear how improvements for enzymes with one substrate will translate to new substrate
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Unspecified substrate properties (feedstock) make life difficult for everyone for the general case.
Described the landscape well (for all competitors)	Like two of the three projects in this group, the enzyme work may be tailored to a particular implementation of the feedstock/fermentation pipeline. The presentation does imply that the enzymes might be provided for general use at the end.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on *removing/diminishing key OBP MYPP barriers in a reasonable timeframe.*

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Verenium is undertaking a second round of evolution they the hope will give a 4x reduction in enzyme loading. They are leveraging several in-house capabilities and expect to continue beyond DOE funding.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Future work is defined as meeting goals. Achievement of goals will ensure success in the project	No clear decision points or milestones are defined. No clear oversight of progress against plan and plan to adapt methodology
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plan to build on current plan and complete second round of evolution. Place enzymes into commercial production strains. Adapt enzymes to fit new processes and incorporate additional enzymes as needed.	What is the expression/production host? Lots of options to consider.
PI Response: Expression and manufacturing of these enzymes is outside of the scope of this program. Outside of this award, Verenium is pursuing a number of options to produce these enzymes in large volumes and at low cost.	
	Path forward is a little less clear. Not clear what the DOE money is paying for.
PI Response: The DOE award is funding the evolution of the 4 enzyme cocktail to meet our biochemical targets. The award is NOT funding the efforts required to express and manufacture these enzymes and to validate their performance at large scale in Verenium's pilot and demonstration plants. These are critical activities that will be initiated by Verenium when the biochemical targets have been demonstrated.	
- do second round of evolution - demonstrate 4-fold reduction in required enzyme dosage	

- move enzymes into commercial production strains	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project has a well defined strategy with a good prospect of successfully obtaining improved enzymes for a particular implementation. Whether those will be sufficient is hard to evaluate. Phase II will be internally funded.	Cost structure analysis was not an explicit part of the project unlike Novozyme, and not using the NREL substrate for this.
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Verinium is a major enzyme company with an excellent track record.	
Lack of partners raises questions of likelihood for dissemination of information and developments. Clear focus on commercialization.	
Internal cooperation between business units allows application in commercial setting	
Technology transfer will likely occur as lower cost enzyme preparations come available.	
The availability of enzymes for external use not assured. In-house project gave head start from internal funding.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
It's not clear that the funding for this project has fostered direct development of anything. The project, given the amount of funding directed to this company should be broad in scope and deep in expectation, which it is not. A plan for commercial demonstration should be included to ensure that there is some ultimate relevance here.	Verenium will demonstrate and commercialize the product of this project, but these activities are outside the scope of this grant. Verenium has a well defined R&D and commercialization pipeline as technologies scale up from the laboratory into our pilot plant and demonstration plants. Once successfully demonstrated, new technologies (like these enzymes) will be implemented in new or existing commercial facilities.
As much as possible, information garnered from this award should be made available to the public.	
Cost structure analysis to be added	

Addressing the Recalcitrance of Cellulose Degradation through Cellulase Discovery, Nano-scale Elucidation of Molecular Mechanisms, and Kinetic Modeling

Technology Area: Biomass Program
Project Number: 2.2.2.9
Performing Organization: Cornell University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.86	0.90
Approach	3.86	0.90
Technical Progress	3.86	0.69
Success Factors	3.00	0.58
Future Research	4.00	0.82

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project

align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Experienced academic team in both enzymology and nanoscience.	While natural diversity is large, there is strong competition from commercial companies for discovery of new enzymes. There is little precedence for rationale design based on molecular understanding of enzyme-solid substrate interactions in the development of better cellulases.

PI Response:

Although there has been extensive research on cellulases since the end of World War II, there are still some major gaps in our understanding of the mechanism by which they catalyze the hydrolysis of crystalline cellulose [1]. One gap is information on the mechanism by which a cellulase binds a segment of a cellulose chain from a microfibril into its active site. This is probably the rate limiting step for crystalline cellulose degradation, so that understanding the mechanism of this step is very important for trying to engineer cellulases with higher activity on real cellulose substrates. Another gap is understanding the way that certain free cellulose binding modules (CBM) stimulate cellulase hydrolysis [2,3]. It is possible that these domains modify the cellulose but exactly how is not known. Finally, while there are some plausible mechanisms for cellulase synergism, there is still much more to be learned about this important process , particularly how mixtures of cellulases hydrolyze both crystalline and amorphous regions in bacterial cellulose while most individual enzymes only seem to degrade amorphous regions [4]. In several cases mutant enzymes with higher activity on crystalline cellulose do not increase the activity of synergistic mixtures (5). At this time, it is not clear why this is happening but it has been shown for several exocellulases. Another surprising result is that an improved processive endocellulase catalytic domain, produced by combining two site directed mutations, that showed higher activity in synergistic mixtures then the wild type catalytic domain, did not show higher activity on crystalline cellulose then the wild type intact enzyme when the missing domains were added back to form the intact mutant enzyme. This result seems surprising but it shows that activity on crystalline cellulose may involve interactions between the catalytic domain and the carbohydrate binding module (CBM) that go beyond the CBM simply anchoring the catalytic domain to the cellulose [6]. The work in our project is designed to try to fill in in these gaps and then use the information to rationally design cellulases that will give mixtures with higher activity on specific pretreated biomass substrates.

Directed evolution of cellulases with improved activity on crystalline cellulose requires that the mutant cellulases be screened on a crystalline substrate not on CMC, as most mutations that increase CMCase activity decrease activity on crystalline cellulose. Furthermore the native enzyme should be utilized not the catalytic domain given the above result. Finally improved enzymes need to tested in the appropriate synergistic mixture on the actual substrate for the final process in order to be certain that they will be useful. A problem with directed evolution is that it can only be used to screen potential single, or with a massive screen, potential double mutations, since the mutant library size required to include most possible larger multiple mutations is too large. It is probable that more than two mutations will be required to significantly increase cellulase activity on crystalline substrates, Rational design does not have this limitation but it does require a detail understanding of structure-functional relationships for cellulase crystalline cellulose activity that is still lacking. If we can gain a clear understanding of exactly how cellulases mixtures hydrolyze crystalline cellulose, it should be possible to design enzymes with multiple changes that have higher activity on specific biomass substrates.

In this project, we are using T. fusca cellulases as a good model of the classical free cellulase system used by most cellulolytic fungi, as it only produces six cellulases, all of which are well characterized and can be expressed readily in E. coli. In addition 3-dimensional structures are available for four of the catalytic domains and extensive site directed mutagenesis has been carried out on three of the cellulases. Since two of the structures were funded by Genecor (now Denisco), the usefulness of information from this system for industrial cellulases is recognized by others.

1. Wilson DB. Cellulases. Chapter in Encyclopedia of Microbiology 3d Edition. M. Schaechter Ed. Elsever Inc, San Diego 2009

2. Wang L, Zhang Y, Gao P. A novel function for the cellulose binding module of cellobiohydrolase I. Sci China C Life Sci. 2008;51:620-9.

3. Moser F, Irwin D, Chen S, Wilson DB. Regulation and characterization of Thermobifida fusca carbohydrate-binding module proteins E7 and E8. Biotechnol Bioeng. 2008;100:1066-77.

4. Yao Chen, Arthur J. Stipanovic, William T. Winter, David B. Wilson and Young-Jun Kim. Effect of digestion by pure cellulases on crystallinity and average chain length for bacterial and microcrystalline celluloses. Cellulose 2007: 14: 283-293.

5. Zhang, S., Irwin, D.C. and Wilson, D.B. Site-directed mutation of non-catalytic residues of Thermobifida fusca exocellulase Cel6B. Eur. J. Biochem. 2000; 267, 3101-3115.

6. Esteghlalian AR, Srivastava V, Gilkes NR, Kilburn DG, Warren RA, Saddle JN. Do cellulose binding domains increase substrate accessibility? Appl Biochem Biotechnol. 2001;91-93:575-92.

This is core discovery work that is focused directly on the biomass program goals. The basic research described provides a strong basis for the potential of future development work. The work is well focused to key limiting factors in the industry. Focus of the work is not being done by many others rendering this an essential project.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This is a fundamental examination of enzyme function with the goal of elucidating enzyme mechanisms at the nano scale . The project also strives to discovery of new cellulolytic enzymes from plant pathogenic fungi: a potentially rich source of these activities.	While this research ultimately will provide some valuable fundamental insights into the action of cellulases; how this will lead to improved enzyme function is largely speculative at this point.

PI Response:

Although there has been extensive research on cellulases since the end of World War II, there are still some major gaps in our understanding of the mechanism by which they catalyze the hydrolysis of crystalline cellulose [1]. One gap is information on the mechanism by which a cellulase binds a segment of a cellulose chain from a microfibril into its active site. This is probably the rate limiting step for crystalline cellulose degradation, so that understanding the mechanism of this step is very important for trying to engineer cellulases with higher activity on real cellulose substrates. Another gap is understanding the way that certain free cellulose binding modules (CBM) stimulate cellulase hydrolysis [2,3]. It is possible that these domains modify the cellulose but exactly how is not known. Finally, while there are some plausible mechanisms for cellulase synergism, there is still much more to be learned about this important process , particularly how mixtures of cellulases hydrolyze both crystalline and amorphous regions in bacterial cellulose while most individual enzymes only seem to degrade amorphous regions [4]. In several cases mutant enzymes with higher activity on crystalline cellulose do not increase the activity of synergistic mixtures (5). At this time, it is not clear why this is happening but it has been shown for several exocellulases. Another surprising result is that an improved processive endocellulase catalytic domain, produced by combining two site directed mutations, that showed higher activity in synergistic mixtures then the wild type catalytic domain, did not show higher activity on crystalline cellulose then the wild type intact enzyme when the missing domains were added back to form the intact mutant enzyme. This result seems surprising but it shows that activity on crystalline cellulose may involve interactions between the catalytic domain and the carbohydrate binding module (CBM) that go beyond the CBM simply anchoring the catalytic domain to the cellulose [6]. The work in our project is designed to try to fill in these gaps and then use the information to rationally design cellulases that will give mixtures with higher activity on specific pretreated biomass substrates.

Directed evolution of cellulases with improved activity on crystalline cellulose requires that the mutant cellulases be screened on a crystalline substrate not on CMC, as most mutations that increase CMCase activity decrease activity on crystalline cellulose. Furthermore the native enzyme should be utilized not the catalytic domain given the above result. Finally improved enzymes need to tested in the appropriate synergistic mixture on the actual substrate for the final process in order to be certain that they will be useful. A problem with directed evolution is that it can only be used to screen potential single, or with a massive screen, potential double mutations, since the mutant library size required to include most possible larger multiple mutations is too large. It is probable that more than two mutations will be required to significantly increase cellulase activity on crystalline substrates, Rational design does not have this limitation but it does require a detail understanding of structure-functional relationships for cellulase crystalline cellulose activity that is still lacking. If we can gain a clear understanding of exactly how cellulases mixtures hydrolyze crystalline cellulose, it should be possible to design enzymes with multiple changes that have higher activity on specific biomass substrates.

In this project, we are using T. fusca cellulases as a good model of the classical free cellulase system used by most cellulolytic fungi, as it only produces six cellulases, all of which are well characterized and can be expressed readily in E. coli. In addition 3-dimensional structures are available for four of the catalytic domains and extensive site directed mutagenesis has been carried out on three of the cellulases. Since two of the structures were funded by Genecor (now Denisco), the usefulness of information from this system for industrial cellulases is recognized by others.

1. Wilson DB. Cellulases. Chapter in Encyclopedia of Microbiology 3d Edition. M. Schaechter Ed. Elsever Inc, San Diego 2009

2. Wang L, Zhang Y, Gao P. A novel function for the cellulose binding module of cellobiohydrolase I. Sci China C Life Sci. 2008;51:620-9.

3. Moser F, Irwin D, Chen S, Wilson DB. Regulation and characterization of Thermobifida fusca carbohydrate-binding module proteins E7 and E8. Biotechnol Bioeng. 2008;100:1066-77.

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6. Esteghlalian AR, Srivastava V, Gilkes NR, Kilburn DG, Warren RA, Saddle JN. Do cellulose binding domains increase substrate accessibility? Appl Biochem Biotechnol. 2001;91-93:575-92.

looking at plant pathogenic fungi and bacteria—
bringing to bear state-of-the art capabilities

PI Response: No response to this comment has been provided by the Principal Investigator.

important to understand the mechanisms of cellulase action - pore structure - mechanisms of processivity - mechanisms of synergy -	not sure about the priority of this aspect of mechanism, although clearly important
PI Response: We believe that much of the on-going cellulase/cellulose research is focused on assessing the products of hydrolysis. However, cellulose hydrolysis by cellulases is a heterogeneous catalysis problem where the physical structure of the cellulose defines the space where catalysis and synergism occurs. We believe that the imaging methods understand how cellulases access the physical structure of cellulose and move with-in the structure.	
Focused on understanding basic aspects of cellulase behavior	
PI Response: In particular, we need to have a better understanding of the molecular basis of synergisms to design more effective cocktails.	
Real science for understanding cellulases. Groundwork for others to make improvements via rational design	Not as close to market as the rating implies. Belongs in research space, not development space
PI Response: We routinely have conversations with scientists from Danisco and Novozyme about our work, and we are having collaboration discussions with one of these companies. We believe that this dialogue and collaboration are important for us to understand the challenges associated with the development of novel enzyme cocktails.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
The project has three components: discovery of new cellulases from e.g. plant pathogens; elucidating enzyme mechanisms using advanced tools and cutting-edge technology; integrating molecular mechanisms with mesoscale characteristics of feedstock.	Heavy competition in discovery and pathogens may not have evolved most robust enzymes. Feedstock is very heterogeneous so molecular understanding, which scientifically is interesting, may not always lead to understanding that can be readily translated into improved performance. It was not clear if the advanced fluorescence-based imaging system can give the resolution required to follow individual enzymes on the substrate. The cellulose substrate being used may quite differently than real-world substrates under real processing conditions.

PI Response: We are using "real" substrate, i.e., pretreated switchgrass, as the primary screening substrate, to be followed in our research plan focuses on analysis of the top candidates on pure substrates to further define the enzymatic components in the mixture. Our current data suggests that plant pathogens are copious producers of cellulytic enzymes; since they require plant materials for survival in the environment, it is logical that they would contain relevant enzymes for lignocellulose digestion.

The heterogeneity in biomass substrates will translate into heterogeneity in enzymatic action. While much can be assessed of enzymatic efficiency through ensemble measurements, the heterogeneity in the action of enzymes can only be measured through methods that address individuals, such as single molecule techniques. Precisely, the understanding of basic molecular interactions between cell-wall degrading enzymes and cellulose in its many isomeric forms is necessary to tackle the heterogeneity presented by lignocellulosic materials. Single molecule fluorescence spectroscopy in its many embodiments can span many length and time scales. In particular single molecule tracking can have accuracy better than one nanometer in localization,

<table>
<tr><td colspan="2">with millisecond accuracy. In addition, superresolution techniques can detail structural features with better than 10 nm resolution and with second accuracy. Thus, we believe the toolkit provided by high resolution fluorescence spectroscopy can yield a wealth of information not accessible with high temporal resolution by other methods such as X-ray diffraction. Finally, while the substrate used in the studies is not as complex as biomass, it is a good starting point for the development of the tools needed for such studies, with the crystalline structure that is most recalcitrant in lignocellulosic materials.</td></tr>
<tr><td>Fluorescent work has been well developed. Screening work is well defined. Molecular mechanism work is well designed and thoughtful with clear goals based on a strong core of research approaches.</td><td>Limitations in the data that can be obtained are recognized with no plan to expand.</td></tr>
<tr><td colspan="2">PI Response:

The heterogeneity in biomass substrates will translate into heterogeneity in enzymatic action. While much can be assessed of enzymatic efficiency through ensemble measurements, the heterogeneity in the action of enzymes can only be measured through methods that address individuals, such as single molecule techniques. Precisely, the understanding of basic molecular interactions between cell-wall degrading enzymes and cellulose in its many isomeric forms is necessary to tackle the heterogeneity presented by lignocellulosic materials. Single molecule fluorescence spectroscopy in its many embodiments can span many length and time scales. In particular single molecule tracking can have accuracy better than one nanometer in localization, with millisecond accuracy. In addition, superresolution techniques can detail structural features with better than 10 nm resolution and with second accuracy. Thus, we believe the toolkit provided by high resolution fluorescence spectroscopy can yield a wealth of information not accessible with high temporal resolution by other methods such as X-ray diffraction. Finally, while the substrate used in the studies is not as complex as biomass, it is a good starting point for the development of the tools needed for such studies, with the crystalline structure that is most recalcitrant in lignocellulosic materials.</td></tr>
<tr><td>Fungal plant pathogens are a potential new source of cellulose conversion enzymes. The research applies sophisticated [novel] labeling and microscopy techniques to visualize and elucidate mechanisms of cellulase action at the nanoscale.</td><td>Although labeling apparently does not influence the activity of the enzymes it may influence other characteristics [e.g. binding]. An unknown variable.</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>Using nanoscale characterization—this is a</td><td></td></tr>
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<table>
<tr><td>needed capability for progress across this field. Screening a unique set of organisms. Examining mechanistics at the right scale. Getting to dynamic representations.</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>nanoscale characterization is a valuable topic</td><td>Methodology may not allow type of information sought, but it will surely give indication of what is happening at the molecular level.</td></tr>
<tr><td colspan="2">PI Response: Binding is a key element that needs to be assessed in the labeling of enzymes. While activity measurements suggest that binding is not hindered (otherwise lower production of sugars would be observed), to have direct confirmation it is necessary to perform binding assays. These have been performed in our lab previously in a high throughput format.</td></tr>
<tr><td></td><td>No information on how basic discoveries will be used commercially.</td></tr>
<tr><td colspan="2">PI Response: The heterogeneity in biomass substrates will translate into heterogeneity in enzymatic action. While much can be assessed of enzymatic efficiency through ensemble measurements, the heterogeneity in the action of enzymes can only be measured through methods that address individuals, such as single molecule techniques. Precisely, the understanding of basic molecular interactions between cell-wall degrading enzymes and cellulose in its many isomeric forms is necessary to tackle the heterogeneity presented by lignocellulosic materials. Single molecule fluorescence spectroscopy in its many embodiments can span many length and time scales. In particular single molecule tracking can have accuracy better than one nanometer in localization, with millisecond accuracy. In addition, superresolution techniques can detail structural features with better than 10 nm resolution and with second accuracy. Thus, we believe the toolkit provided by high resolution fluorescence spectroscopy can yield a wealth of information not accessible with high temporal resolution by other methods such as X-ray diffraction. Finally, while the substrate used in the studies is not as complex as biomass, it is a good starting point for the development of the tools needed for such studies, with the crystalline structure that is most recalcitrant in lignocellulosic materials.</td></tr>
<tr><td>New enzymes from pathogens Mechanistic study via imaging analysis total internal reflection fluorescence microscopy kinetics with more imaging (confocal) controlling cellulose conformation for imaging study. Have screened 40 isolates with high sp. Act (defined how?) set up the microscopy system, controlled temp,</td><td>Lack of industry, national lab partners.</td></tr>
</table>

tracking software developed to be able to assess processivity (localization anyway). Fibil immobilization has been done with polymer lift-off technique Labeling the enzymes complete preliminary kinetics--images of indiv mol. Looking to do pretreatment. Label while bound to cellulose. Mixed pop of labels; can separate by degree of label. Need one fluorophore. How to tell when enzyme is coming off vs. moving along. 23-23 nm localiz	
PI Response: We are currently engaged in discussion with one enzyme company regarding the imaging work that is currently underway. This company is particularly interested in how we evaluate mutants in the imaging system.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**

They have developed and published a high throughput enzyme assays and have identify forty isolates with high specific activity. They are developing efficient labeling and purification techniques for fluorescent assays to identify single cellulase molecules. Current emphasize seems to be on nano-characterization of enzyme function; they have developed an immobilization method for cellulose.	No information on the value of enzymes high specific activity enzymes that were identified or how these will be tested and developed. Localization at 20-30 nm of enzyme on cellulose substrate but there may be significant signal to noise and photobleaching issues. How long/far can you track individual enzymes? Labeling may alter enzyme performance. They need to know high homogeneous the labeled enzymes are.
PI Response: Signal to noise ratio for highly labeled enzymes does not present an issue under the current experimental conditions, with each image taken for an experiment containing more than 10,000 photons. This number is considered a rule of thumb for the accurate localization of molecules with high spatial resolution. On bleaching or photodestruction of the fluorophores, many additives have been demonstrated to reduce photobleaching and extend the fluorophore's lifetimes 5-10 fold. We have used minimal buffer additives, restricting them to oxygen scavengers (see Moran-Mirabal et al. 2008 Biotechnology and Bioengineering). With such a minimal system we have been able to track enzymes for periods of time spanning up to 5 minutes with constant illumination or up to one hour with intermittent illumination. Labeling of enzymes is highly homogeneous, as shown by the data presented for the separation of labeled mixtures into highly homogeneous populations, and their characterization of the average photon output.	
Early progress demonstrates the potential of the approach. Techniques have been validated. Mutation development has demonstrated certain results providing a basis for the future work of the project. Much of the work of the project has been completed rapidly and at low cost.	Incorporation of commercial enzymes would provide a basis for direct industrial relevance. The implications of understanding cellulase movement remain to be seen
PI Response: Screening assay are using the industrial strain, T. reesei RUT-C30 as the benchmark for comparison to other fungi. Once the select enzyme candidates are established, our research plan will incorporate commercial enzymes as benchmarks. Incorporation of individual commercial enzymes into our high resolution imaging work is a possibility and one that is being explored.	
Research was successful in identifying cellulase producing candidate organisms. Successfully setup imaging system, tracking software, etc. to	This is impressive stuff, but I'm not sure what this is telling me. What have the researchers discovered using these approaches? How do the

study cellulase binding, movement, etc. Can now track movement of cellulases on cellulose.	enzymes from these new sources "measure up" with those currently available? How does one propose to use this information?
PI Response: No response to this comment has been provided by the Principal Investigator.	
screening and technology development has progressed rapidly	
PI Response: No response to this comment has been provided by the Principal Investigator.	
7/08–6/10: m stated 30% complete - got cellulase isolates from fungal pathogens - have set up TIRFM (TIRF microscopy) setup and refinement - completed cellulose fibril immobilization - have fluorescent tagged molecules (allows several types of mechanistic studies) - cellulose particle immobilization has been completed	not clear how all controls have been done to assure results are relevant to natural and/or industrial systems
PI Response: Screening assay are using the industrial strain, T. reesei RUT-C30 as the benchmark for comparison to other fungi. Once the select enzyme candidates are established, our research plan will incorporate commercial enzymes as benchmarks. Incorporation of individual commercial enzymes into our high resolution imaging work is a possibility and one that is being explored.	
Good progress in setting up experimental system imaging demonstrated.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and

strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Highly experienced group with excellent understanding of technical parameters.	While the team is developing good fundamental science that is pushing the physical limits, it is not yet clear that they can achieve sufficient resolution to contribute to practical successes or that the work will be relevant to real-world situations.
PI Response: Success factors for the imaging work are related to the resolution, temperature range that can be achieved and the information that can be extracted from high resolution measurements. These are all related to the robustness of the methods developed. The performance of control experiments, and comparison and validation of results with more conventional methods is necessary to avoid artifacts from measurements. Showstoppers to the progress of this program are intimately tied to the accessible information from the methods. Lack of sufficient temporal or spatial resolution can be showstoppers. From data reported in the literature, these experimental observables fall within the range accessible to optical fluorescence microscopy. A technical barrier and a limitation to optical systems is the range of temperature that can be achieved for the experiments, due to the high sensitivity of optical components. However, the enzymes used in industry tend to work at temperatures amenable to most optical systems, and the thermophilic enzymes we have chosen to work with can work efficiently at the upper range or the temperatures that we can achieve in our system.	
Clear understanding of requirements for success with a path to get there. The PI is clearly aware	No explicit definition of concerns, showstoppers, or risks is provided. No plan to

<table>
<tr><td>of the challenges and is working diligently against them, though these are only evident with direct inquiry to a specific set of risks offered by the reviewers.</td><td>overcome risks is presented.</td></tr>
<tr><td colspan="2">PI Response: Success factors for the imaging work are related to the resolution, temperature range that can be achieved and the information that can be extracted from high resolution measurements. These are all related to the robustness of the methods developed. The performance of control experiments, and comparison and validation of results with more conventional methods is necessary to avoid artifacts from measurements. Showstoppers to the progress of this program are intimately tied to the accessible information from the methods. Lack of sufficient temporal or spatial resolution can be showstoppers. From data reported in the literature, these experimental observables fall within the range accessible to optical fluorescence microscopy. A technical barrier and a limitation to optical systems is the range of temperature that can be achieved for the experiments, due to the high sensitivity of optical components. However, the enzymes used in industry tend to work at temperatures amenable to most optical systems, and the thermophilic enzymes we have chosen to work with can work efficiently at the upper range or the temperatures that we can achieve in our system.</td></tr>
<tr><td>Successful research accomplishments are identified.</td><td>Not really a list of critical factors to determine how this technology will be applied and barriers to success.</td></tr>
<tr><td colspan="2">PI Response: Success factors for the imaging work are related to the resolution, temperature range that can be achieved and the information that can be extracted from high resolution measurements. These are all related to the robustness of the methods developed. The performance of control experiments, and comparison and validation of results with more conventional methods is necessary to avoid artifacts from measurements. Showstoppers to the progress of this program are intimately tied to the accessible information from the methods. Lack of sufficient temporal or spatial resolution can be showstoppers. From data reported in the literature, these experimental observables fall within the range accessible to optical fluorescence microscopy. A technical barrier and a limitation to optical systems is the range of temperature that can be achieved for the experiments, due to the high sensitivity of optical components. However, the enzymes used in industry tend to work at temperatures amenable to most optical systems, and the thermophilic enzymes we have chosen to work with can work efficiently at the upper range or the temperatures that we can achieve in our system.</td></tr>
<tr><td colspan="2">They understand what has to be done.</td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>have made good progress in developing tagging</td><td>large potential for artifacts in such studies,</td></tr>
</table>

methods which seem to form the basis of these studies   artifacts could relate to binding, activity, etc.

PI Response: No response to this comment has been provided by the Principal Investigator.

Not really addressed--this was another accomplishment slide

PI Response: Success factors for the imaging work are related to the resolution, temperature range that can be achieved and the information that can be extracted from high resolution measurements. These are all related to the robustness of the methods developed. The performance of control experiments, and comparison and validation of results with more conventional methods is necessary to avoid artifacts from measurements. Showstoppers to the progress of this program are intimately tied to the accessible information from the methods. Lack of sufficient temporal or spatial resolution can be showstoppers. From data reported in the literature, these experimental observables fall within the range accessible to optical fluorescence microscopy. A technical barrier and a limitation to optical systems is the range of temperature that can be achieved for the experiments, due to the high sensitivity of optical components. However, the enzymes used in industry tend to work at temperatures amenable to most optical systems, and the thermophilic enzymes we have chosen to work with can work efficiently at the upper range or the temperatures that we can achieve in our system.

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP *barriers or advancing the program.*

Strengths	Weaknesses
They are pushing the frontier of enzyme-substrate characterization.	Success depends on usefulness of yet to be commissioned fluorescence-based characterization of enzyme-substrate interactions to guide mutation-based approach for development of better enzymes.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Future work is consistent with stated goals. Clear goals are stated and relevant. Progress to date is suggestive of a likelihood of future success.	Project would benefit from a timeline with better defined milestones. Risks of accomplishment are hard to assess.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Kinetic studies with labeled enzymes using the techniques developed could allow one to conduct experiments examining synergisms with different enzymes. It would be very interesting to see what sorts of results these types of experiments would produce. Experiments examining the effects of site directed mutagenesis on enzyme interaction with cellulose, examination of cellulase interactions with pretreated materials will be interesting.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This work will begin to provide a mechanistic basis for differentiating enzymes and feedstock materials.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- kinetic studies using the tools developed	

- characterization of cellulase interactions with cellulose particles
PI Response: We assumed that the reviewers has access to our quarterly reports where the milestones and goals are clearly stated and are commensurate with the proposed future research.
Good science inform the energy work at the level of rational design
PI Response: We are having fun!

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Route to demonstration of commercial value is not clear. No commercial partners listed.	
Strong group of academic collaborators with proven records and access to both traditional and non-traditional outreach. Proven publication record.	
Partnerships are primarily within Cornell collaborations	We are eager to work with enzyme companies. Also, we are beginning to engage other cellulase researchers in a dialogue. We are particularly interested in working with Ed Bayer of cellulosomes.
There is a good record of tech transfer at Cornell	Yes. However some companies find our intellectual property office difficult to work with!
not mentioned	

Project contained within Cornell University. No outside collaborations.	
Expect publication for dissemination of results	We are submitting papers to journal. One challenge is to balance the story between an molecular imaging or cellulase/cellulose story. We sometime get hit with the reaction that we are too focus on cellulases.

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
An exploration of a broader set of enzyme variants including industrial enzymes would be beneficial.	We agree! Our goal is to establish how far we can push are methods with the current set of T. fusca cellulases and mutants. Then we would like to work with industrial partners on exploring some of the industrial enzymes. We are very interested in observing the behavior of GH61 from Novozyme.
This is basic research that could or could not provide important information for the future of improving cellulases.	We agree! Our goal is to establish how far we can push are methods with the current set of T. fusca cellulases and mutants. Then we would like to work with industrial partners on exploring some of the industrial enzymes. We are very interested in observing the behavior of GH61 from Novozyme.

Integration of Leading Biomass Pretreatment Technologies with Enzymatic Digestion and Hydrolyzate Fermentation

Technology Area: Biomass Program
Project Number: 2.3.1.4
Performing Organization: CAFI
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.86	0.38
Approach	4.57	0.53
Technical Progress	4.86	0.38
Success Factors	4.29	0.49
Future Research	4.71	0.49

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Critical effort for the commercial utilization of switchgrass as an energy crop. The group has an outstanding track record and delivers quality data.	None obvious
PI Response: Thank you—we hope to provide valuable information.	
Clear, concise goals. Focus on education is of critical importance. Deep focus on a specific feedstock leverages previous studies for deep understanding. Comprehensive approach allows for the potential of clear conclusions to be drawn.	
PI Response: Thank you—we hope to provide valuable data and understanding.	
This large collaborative project provides an apples to apples comparison of the leading pretreatment technologies for pretreatment of Switchgrass. The data will be extremely valuable to researchers and commercial R&D efforts seeking to identify available pretreatment technologies for their particular application.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
science to application of a key set of issues; great team approach	
PI Response: No response to this comment has been provided by the Principal Investigator.	

This is important work, the comparison of pretreatment methods for conversion of Switchgrass in coupled pretreatment/enzyme saccharification systems (CAFI 3)	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Provides valuable information on switchgrass pre-treatment process for industry and commercialization	
PI Response: No response to this comment has been provided by the Principal Investigator.	
This project focuses on characterizing the substrates and products resulting from different feedstock preparations, pretreatments and enzymatic processing. This is critical information needed for all downstream uses.	Management of so many partners has to be a challenge.
PI Response: The CAFI Team works very well together in a truly cooperative approach that makes it quite manageable. Primary challenges are with respect to assuring flow of funds.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant

contributions progress.

Strengths	Weaknesses
Global integrated approach with experienced teams. Emphasis is on understanding mechanisms. Excellent partners. Strong advisory team. Very solid.	Projected started in September 2007 with first funds in July 2008; projects is expected to end in March, 2010. Thus, time short to accomplish goals.
PI Response: The time is short and the funds are very limited. However, the deep experience of the team with the technologies and with working together makes this manageable. Primary concern is that the limited time prevents us from attacking a wider scope or digging deeply into understanding the technologies.	
Strong basis of analysis to ensure a meaningful result. Recognized goal of providing information. Advisory group allows for industrial data to be incorporated. Broad and deep approach is well thought out. Best in class partners and advisors. Use of commercial "ingredients" enhances value of results.	Dependent on others for commercial scale validation.
PI Response: The resources for the project and the affiliation of the team limit commercial applications. However, we can provide strong data that allows commercial entities to understand what the reaction kinetics should be, helping them understand whether scale up issues in heat, mass, and/or momentum transfer are causing problems.	
Approach and research activities are well coordinated and conducted by the scientific leaders in this field. Material balances across the various methods is not a trivial accomplishment and the investigators are to be commended for this	
PI Response: No response to this comment has been provided by the Principal Investigator.	
highly integrated and practical; making available to industry to make key choices	Focuses on a small set of examples, which may have limited impact in an expanded field as research progresses.
PI Response: Not sure I understand this concern.	

Several labs collaborating with expertise in different pretreatment technologies using common substrates, analytical methods, etc. They have good support from advisory committees, NREL, and companies (e.g. Genencor provides the enzymes and Ceres provides the Switchgrass).	This appears to be the third iteration of this project, indicating specific labs are applying same techniques to different substrates. This approach leads to maximum expertise in applying specific pretreatments to different feedstocks, but it may limit the recognition of novel improvements that come from those new to the field.
PI Response: The CAFI approach takes advantage of the deep expertise of the team to avoid reinventing the wheel, thereby taking the technologies to the next level. In addition, CAFI 3 has been organized differently than the previous two to assure each team member looks across the technologies with respect to different aspects to understand their differences and gain new perspectives. Invention often comes from knowledge and insight more than from inexperience.	
Standardization of diverse materials and compositional analysis to provide basic understanding for others to use.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP *objectives and technical barriers.*

Strengths	Weaknesses
Working with 3 enzymes; all members use same enzymes and feedstocks. Three switchgrass species, 2 lowland and one upland. Strong analysis, believable, relevant data. Very strong record of publications.	Limited set of samples with which to disentangle factors such as harvest time and location.
PI Response: We would like to look at more samples but the time and funds allowed are insufficient to do this. However, we will gain important new insight because of the team's experience and coordination.	
Feedstocks well characterized. Well controlled data sets providing a good basis of analysis. Study design and results showing comprehensive basis of data generation. Recognition of the generation of measurements that are generalizable. Clear accomplishment towards central goals of biomass program	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Research plan appears to be executed as planned	
PI Response: No response to this comment has been provided by the Principal Investigator.	
bringing together all the pieces for switchgrass experiment; most useful to industry	
PI Response: No response to this comment has been provided by the Principal Investigator.	
2007-2009 (started late) extensive amount of quality data obtained/presented on several of the pretreatment strategies	The project is accomplishing its goal—that is to compare several methods
PI Response: No response to this comment has been provided by the Principal Investigator.	
Excellent progress; real data produced and	

available.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Many protocols evaluated, using materials from relevant participants (everyone is on the board). Genecor provided same lot to everyone. Ceres provided three strains of switchgrass. The result highlighted the significance of understanding harvesting and storage effects (which is in effect a "pretreatment"): summer harvested samples had a lot of free sugar, which may be more relevant than the point that a different strain of grass was used.	Speaker mentions that the process variation probably depends on time of harvest but keeps presenting as variation in cultivar. The problem is that too many parameters are varying at the same time.
PI Response: It would help to have more controlled samples, and we will try to get some that will better unravel the cause of differences. Free sugars are removed prior to pretreatment to avoid having their fate confuse the results.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Excellent understanding of success factors. Strong relevance to commercial decisions. Outstanding advisory board.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Success factors well understood and indentified. Recognition of key challenge and good data to support success here.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal is to reduce enzyme loading as much a possible while maintaining maximum sugar yields.	How will increasing the solids loading over those employed here influence the results obtained?
PI Response: Solids loading will affect yields due to end product inhibition. However, low solids loadings are used to assure that we see differences in pretreatments and not differences in enzyme inhibition. Ideally more resources could help understand better how loadings affect enzymes and yields and reasons for any differences among pretreatments.	
on track to provide an industry useful set of results	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Collaborative studies of this nature are extremely helpful provided all members play by the rules.	This project is so well worked out that it is unlikely to have major weaknesses
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Differences with different switchgrasses or time of harvest, more work to be done here.
PI Response: We would love to do more to understand reasons for differences if there were more time and money. However, we should gain very important new insight nonetheless.	
It's not clear that there are "showstoppers" for this project, since its goal is to provide information. The quality of analysis sets the	

standard for experimental design.
PI Response: No response to this comment has been provided by the Principal Investigator.

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Important to separate growth, place, and harvesting differences. They know what is important to accomplish with resources and timesets available. available.	Dependent on partners for feedstocks—limited
PI Response: Getting feedstock from well controlled circumstances in sufficient quantity does present challenges, but the team is gaining new insight with what is available.	
Well defined milestones and goals laid out. Clear timelines and deliverables Responsibilities well established. Clear relevance of pending	

work.
PI Response: No response to this comment has been provided by the Principal Investigator.
Completion of the economic modeling for these studies will of great value. Exploration of hemicellulase effects as more targeted enzymes become available will be very interesting.
PI Response: No response to this comment has been provided by the Principal Investigator.
Important set of goals to provide a model for analysis for industry.
PI Response: No response to this comment has been provided by the Principal Investigator.
complete proposed comparative studies
PI Response: No response to this comment has been provided by the Principal Investigator.
Importantly, this project includes plans to compare enzyme performance on standardized substrates from all participants. I'm unclear on whether all the developed enzymes will be commercially available or used as a proprietary reagent.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Good tech transfer and publication records.	
Broad consortium and advisory working efficiently to ensure industrial and commercial relevance, opportunities for technology transfer. Significant publication record.	

An impressive list of collaborators. Plan to publish the results and distribute these as widely as possible.	
great ties within a strong consortium, and providing end result and tools to industry	
They work with many companies through advisory boards, they also present annual symposiums, and they publish the majority of their work.	
Excellent collaboration of multiple partners	
A Herculean effort apparently is being made to keep everyone informed, which is reflected in the other talks.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
This program has been notable efficient in their use of funds and the quality/amount of data generated.	
Although outside the scope of these studies an examination of fermentation "inhibitors" formed during these pretreatments would be interesting. Have the investigators considered adding a source of ferulic acid esterase [e.g. pectinase in some preparations] to see this enhances saccharification?	We would like to incorporate more studies of interactions with other steps but resources are not sufficient. We have pectinases that will be applied in the near future.
Excellent project that fits OBP goals and produced real results.	

Advancing Texas Biofuel Production

Technology Area: Biomass Program
Project Number: 7.2.2.2
Performing Organization: Baylor University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.14	0.69
Approach	4.00	0.58
Technical Progress	3.86	0.38
Success Factors	3.43	1.13
Future Research	3.57	0.53

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the

project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Aimed at providing fundamental information at degradation products. Strong previous experience with biomass pretreatment. Good modern cutting-edge analytical methods for separation and detection.	Is sorghum a critical cellulosic feedstock?
PI Response: No response to this comment has been provided by the Principal Investigator.	
The goals of this project are well within the scope of the biomass program. This work is unique in looking at the non-fuel products, which is essential to the net value in biofuel production. The project is focused to provide a capability of achieving significant results over its duration.	A more direct connection with the CAFI projects could provide for a more comprehensive model of the results of various feedstocks—sorghum in this case.
PI Response: No response to this comment has been provided by the Principal Investigator.	
This research strives to improve process efficiencies by identifying and removing inhibitory materials that will negatively impact downstream process efficiency including enzymatic and fermentation processes. Although there is a reasonable understanding of the identity and processes that give rise to the predominant inhibitory compounds many other potential inhibitors are likely to exist and it may become more critical to identify these with increased water recycling in plants	

PI Response: No response to this comment has been provided by the Principal Investigator.	
developing a new generation of analytical technique which could be critical for understanding reaction intermediates from pretreatment and other processing; looking at a potential major feedstock for a agroeconomic region of the country	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- relevant analytical methods development is key to making advances in this field - focus is on degradation products occurring as a result of pretreatments - want to develop predictive understanding of the pretreatment chemistry that leads to degradation products	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Strong analytical analysis of inhibitors in treated biomass.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Pretreatment of sorghum to be investigated Interested in alternative degradation products not fermentable sugars for value added, and removing inhibitors.	Not one of the major feedstocks described by others
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

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3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Emphasis on degradation and focused on molecular mechanisms.	Identification of compounds needs to be correlated with effects on enzymes or microbes.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The approach of an additional high resolution component is of great value and leverages a previously defined infrastructure. Clear basis for the selection of the chosen analytical approach. The early data validates that the approach can work.	The scope of the approach is limited in its inclusions of novel analytical techniques. As the PI recognizes that analytical techniques only show you what you are looking for, including additional techniques with distinct capabilities would provide better insight into the unknowns. The amount of information that can be elucidated from this technique remains an unknown.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Development of analytic approach to understanding the identity and production of organic, potentially inhibitory, molecules in hydrolysates and process streams. Apply sophisticated MS techniques to perform this analysis. PI has high degree of technical expertise in the application of these techniques	
PI Response: No response to this comment has been provided by the Principal Investigator.	

Allaying a specific technique to this problem as a step toward understanding process fundamentals. Looking at degradation products. This is analytical chemistry.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- develop appropriate sample preparation protocols - uses several analytical approaches to enhance information gathering	although approaches are improvements to existing methods, still probably does not completely characterize system
PI Response: No response to this comment has been provided by the Principal Investigator.	
Identifying products of treatment--chemistry side; need this info for enzyme design. ESI MS method. With tandem MS for resolution. Limitation is finding what you're looking for. Have already methods in place to do this. Discovery set is new instrumentation. Sorghum is a new model system	Quantization challenge.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

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2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Relatively new project. Have set up instrumentation.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The work in this lab has provided a strong baseline for the proposed work that is now underway. The standards developed are critical for the advancement of analytical techniques, which are essential in the industry. Validation of the analytical techniques proposed has been provided.	It would be useful to understand the fundamental limitations of these analytical techniques. There are clearly difficulties that may be designed around, but the intrinsic physiochemical limitations would provide clear valuable insights.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Has established a methodological approach to identifying and quantitating these materials in sorghum hydrolysates. Can resolve and identify species of similar elution properties and masses	How do you evaluate the potential "inhibitory" nature of the compounds detected? Some of these may be neutral in effect and some may even be stimulatory.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Facing tremendous sample and spectrum complexity—ESI-MS; can now monitor 40 compounds; combines hplc, im, and ms; this provides a molecular signature of treatments.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
developed UPLC/MS system for analyses and trained individuals for use of analytical equipment	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project just started; acquired equipment	

PI Response: No response to this comment has been provided by the Principal Investigator.
Showing implementation of the instrument for resolving M/z 157 three compounds all present with this ratio; can resolve one of the three from the other two. Ion mobility to be tested to resolve the other two.
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

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4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Main success factor is sample complexity. Object is to characterize feedstock complexity.	Mass spec is not quantitative.
PI Response: No response to this comment has been provided by the Principal Investigator.	

Clear recognition of the challenges that must be overcome.	The apparent challenges are well understood and represent key challenges to be overcome for analytic development for the industry in general. Showstopper potential is recognized, though the expectations of them is limited, without a plan to overcome as they may hit; i.e. a plan to diversify analytical approaches etc.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Success is defined by the researchers as identification of optimum sorghum-variety and pretreatment chemistry combinations that minimize inhibitory degradation products and maximize value-added degradation products. The generation of data sets for predictive correlations between pretreatment and downstream inhibition will be extremely valuable.	Focus is on [forage] sorghum while OBP objectives calls for corn stover and switchgrass [?]. How well will this information translate to these feedstocks?
PI Response: No response to this comment has been provided by the Principal Investigator.	
need to link analytical results to processing effects—inhibitory and otherwise	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Have acquired equipment that looks appropriate for the proposed task	sample complexity is a difficulty
PI Response: No response to this comment has been provided by the Principal Investigator.	
Complexity is the biggest problem, hoping to overcome with this instrumentation	not presented before question
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or

identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Project work is defined consistent with the goals of the project. The project is clearly a discovery project to help define the directionality of future, and is well designed as such.	Clear milestones and deliverables would be valuable. Approaches to overcome technical barriers are not well defined.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The generation of data sets for predictive correlations between pretreatment and downstream inhibition will be extremely valuable. Identify potentially valuable co-products that may be generated in these hydrolysates could also be useful.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
The capability to predict the downstream effects of feedstock makeup from up front analysis would be a critically important technology for research and for refinery applications.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

evaluate sorghum hydrolysates using methods developed in initial phase of this study
PI Response: No response to this comment has been provided by the Principal Investigator.
Make a db of the products. Hope for ID inhibitory products and/or new value-added products
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
The implications for the industry are clear. The project would benefit from direct collaborators involved in other projects, though the collaboration with Bruce Dale may help to provide this bridge. The work is likely to be published. Development of these analytical tools is essential to the industry, with a logical, and likely directed path for dissemination via publications.	
PI is a recognized expert in this area and is frequently sought out to collaborate on studies of this nature.	
works with groups outside his group, participates in relevant symposia, and publishes his results	
Does interact with others.	
Long-term significance depends on being able to relieve inhibition efforts. Collaborations with many academics.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
A means to leverage this to other feedstocks would be valuable, and could serve as the basis for future projects.	
Too early in project to fairly evaluate.	

Technology Area: Biomass Program
Project Number: 2.1.1.1
Performing Organization: INL & NREL
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.57	0.53
Approach	4.14	0.69
Technical Progress	4.00	0.58
Success Factors	3.86	0.90
Future Research	4.14	0.69

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Important information for commercialization and for developing appropriate next generation feedstocks.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Clear, well articulated goals essential to tying the IBR to the feedstock component. Addresses important aspects of feedstock management and the limitations where work is needed to enable broader application.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Information gathered is important to the bioconversion process and provides a linkage to the feedstock platform. There is a need to know the feedstock characteristics for planning of subsequent pretreatment and saccharification strategies. Understanding feedstock variability with regard to composition and effect of storage important will be important determinants of subsequent processing and conversion technologies. Development of storage and preservation methods and their effect on composition/ quality noteworthy.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

Strongly linked to other parts of program. Library of data and materials are extensive. This is a critical link for success, and speaks to one of the major problems that biorefinery pilots are facing.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
important topic, biomass characteristics impact conversion systems	as expected there are infinite number of permutations to be tested --> so choosing the representative systems will be important
PI Response: No response to this comment has been provided by the Principal Investigator.	
This critical project addresses properties and parameters of materials from different feedstocks as they arrive for processing at the reactor door. No planning can be done without the information developed.	Presentation of 2.1.1.1 and 2.1.1.3 was combined, making it difficult to follow. The verbal presentation did not do justice to the topic, mostly because of insufficient delineation of different sections.
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant

contributions progress.

Strengths	Weaknesses
Good identification of critical parameters. Not flashy but solid. Strong partners.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Strong focus on key physiochemical on properties and their implications. Good breadth of properties being explored. Practical elements i.e. handling being addressed.	The details of what constitutes feedstock (per the analysis) are lacking. It is important to understand what the applicability of this is. Would be useful to explore how various views of the IBR and the implications on the feedstocks used (type and manner) impact these properties (and potentially require the introduction of other properties).
PI Response: No response to this comment has been provided by the Principal Investigator.	
The approach used is good and uses relevant analytical tools. There is a need to know physical and chemical properties of feed materials and their impact on conversion.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Establishing database and models, curation of samples—an important resource for the near and midterm. Efforts must be made to sustain this effort as feedstock and processing research progress.	The application of advanced analytical techniques would increase the value of curated materials.
PI Response: No response to this comment has been provided by the Principal Investigator.	
are clearly aware of the many factors that can impact conversion focus on coen stover supports other major efforts seem to have good collaborative system	not clear how they are choosing combinations of parameters to give maximum information per experiment

PI Response: No response to this comment has been provided by the Principal Investigator.	
Importantly, there is a source of standardized analyzed feedstock samples, which should enable planning for downstream uses and analytical activities. For example, it would be very important to understand that stratification of the materials by size changes glucan:zylan: galactan: arabinan and total sugars and that "fluid" flow will be impeded by too high a fraction of fines. The database implemented is a crucial asset, potentially including uses far in the future in addition to being essential to the present project.	Approach addresses too many issues for one presentation, without an opportunity to review ahead of time.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Good development of database with knowledge of importance for making information available. Dabase well populated for corn stover. Impressive progress in extending to other fee stocks.	Will need better measures of diversity for different suppliers.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Interesting early data on composition suggestive of the importance of this work and the need to continue. Good plan for how to utilize data and integrate. Producing an important database as a centralized reference to allow for broad understanding of feedstock attribute implications.	Little progress on engaging with industry members to ensure applicability. Data based been focused on a limited number of sources, and is incongruous with emerging/emergent industrial interests. Would be useful to incorporate new handling techniques into the analysis.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Data collection and dissemination look good. Ensiling work is important as a potential storage technology.	The results appear to be somewhat incomplete at this relatively early stage. One assumes that the gaps will be filled in with time.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Progress on ensiling process, databases, and curation.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
have characterized thousands of samples have completed ensiling study	do not appear to have looked a big picture of any one feedstock with respect to many parameters—that includes parameter properties and behavior in conversion systems
PI Response: No response to this comment has been provided by the Principal Investigator.	
The database was populated with corn stover, miscanthus, wheat straw for a total 10 sample sources. Ensiling is being tested now. Methods used with corn stover were successfully adapted	

to switchgrass. The ensilage storage method that reduces risk of combustion, gives a bigger harvest window, is familiar to the production community, lowers pH, and carries out predigestion with loss of 5-13% of var sugars. No increase in available sugar but importantly easier to handle
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Solid understanding of factors important to interface between feedstocks and bioprocessing.	Value may be decreased if feedstocks change significantly. Dependent on strains.
PI Response: No response to this comment has been provided by the Principal Investigator.	

Good identification of key factors and challenges. Well described risks.	Certain risks appear difficult if not impossible to mitigate. Will likely slow down the timeline. Can alternative approaches be developed?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Identification of key processing parameters influencing bioconversion quality will be of great value. These can also be included as a selection criterion for plant breeding. Feedstock variability is certainly a challenge to this.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
ensiling conditions seem to have been looked at with respect to optimum conditions, not clear how all other parameters related to ethanol production	thousands of parameters to evaluate, need to pick one system a limitation may be the apparent lack an effective method to correlate data on physical properties relates to actual performance in conversion systems
PI Response: No response to this comment has been provided by the Principal Investigator.	
Database in development seems to be a really good idea. The scale of the challenges identified is tractable to additional resources.	Issues related to making the database accessible to a potentially wide variety of researchers and users were not addressed.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Have identified critical issues and working to develop appropriate analytical tools.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well described and reasonable plan	More defined timeline would be useful as no milestones between now and 2012 described. Little to no plan to engage with commercial feedstock handlers/users to ensure relevance to the industrial goals.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Continuing work to identify key processes and components as determinants of feedstock quality is warranted. Developing high throughput NIR methods based on wet chemical analysis will expedite screening and assessment. Expanding the feedstocks to be evaluated will be of value to those interested in these other potential biomass resources.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
good system integration and partnerships	
PI Response: No response to this comment has been provided by the Principal Investigator.	
plan to continue the type of work they are doing	

PI Response: No response to this comment has been provided by the Principal Investigator.	
A sustainability focus is excellent for long-term utility of the results	Does this (or any project) include environmental issues of volume reduction?
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Good interactions across academia. No evident collaboration with industry.	
Excellent cooperation of DOE laboratories. Publishing databases online will provide a valuable tool for biomass conversion scientists and industry.	
heavily interacting across institutions and tasks	
plan to publish and make information available	
Excellent collaborations	
Good job so far. The model for the work entails good publication and interaction.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
Plans to use industrial samples or include them into the library would enhance the relevance of the project. An understanding of the distribution (geographically and farming method) would be beneficial to understand intrinsic properties.	

In addition to ensiling, has the application other "pretreatments" during storage been considered? Alkali treatments (e.g. ammonia, sodium hydroxide) have been used experimentally as a ways to improve forage feeding quality for livestock and may have value in this application if cost effective.	
Narrow scope some to make more progress in critical areas.	
Space dimension for storage to be included in tables. Address the possibility of a making the DB a publicly available resource.	

Extremophilic Microalgae: Advanced Lipid and Biomass Production for Biofuels and Bioproducts

Technology Area: Biomass Program
Project Number: 2.1.1.6
Performing Organization: Montana State University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.57	0.53
Approach	3.43	0.53
Technical Progress	3.71	0.49
Success Factors	3.43	0.98
Future Research	3.29	0.49

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Although at an early stage, algal production ultimately has a greater potential for solving liquid transportation fuel problem than cellulosic ethanol if costs can be reduced.	Need Identification of barriers to cost reduction.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are commercially oriented and aimed at driving towards a larger scale application.	Unclear how algae plays into the biomass program goals. A proposed viewpoint would be valuable. Goals are limited to screening and process definition in a limited scope of what is believed possible for algae. Justification for these approaches would be useful, especially given the similarity to previous studies that have been done. Assumptions of advantages to this type of system (carbon neutral etc) are not supported.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Alignment of this project with the overall program is unknown since this a new area of research for the Biochemical Platform. Never the less, the potential exists for algae to provide biomass for conversion to fuels [biodiesel]. There is significant industrial interest in developing algae as a biodiesel feedstock.	Despite the promise of a low input and high yielding product, algae don't seem to be able to deliver on this. I would think by now that we would be seeing more demonstrations of this technology.

PI Response: No response to this comment has been provided by the Principal Investigator.	
Introducing extremophile capabilities to this community is important.	needs to utilize more modern techniques
PI Response: No response to this comment has been provided by the Principal Investigator.	
Produce high lipid producing alkaliphilic algae, a worthwhile topic to investigate	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Novel approach	Open pond, high pH should help greatly
PI Response: No response to this comment has been provided by the Principal Investigator.	
Early stage evaluation of a new potential feedstock. High pH was a targeted growth parameter. Testing in field. (5000 gal)	Can the scale ever be enough?
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant

contributions progress.

Strengths	Weaknesses
Good understanding of the critical barriers to the economical productions of oils and biomass from algae.	It was not clear how extensive and representative the existing culture collections that will be tested are. Are culture properties stable after isolation and propagation in the laboratory? Need genomics to potentially identify differences between strains and to correlated with production. Pathway analysis will be key to identifying bottlenecks. Needs analysis of pros and cons of alkaliphilic strain approach vs. other methods to control contamination. Outdoor pond experiments may be pre-mature except to identify general barriers and problems. Ambitious project for a two year time frame.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Approach is aimed at getting a process to scale. Addresses key issues including water quality and system optimization. Extremophile focus is of value.	Issues address do not include several key factors such as total water use, validation of basic algae assumptions. Its well understood that secreted products are better than intracellular—a plan to explore that with significantly enhance the commercial implications of this work. Data would benefit from correlation to standard metrics like g/m2/day
PI Response: No response to this comment has been provided by the Principal Investigator.	
The plan to use alkaliphilic algae as a means of avoiding contamination and water quality issues seems a valid approach. The use of an alkaline growth "medium" should also improve availability of carbon dioxide to the organism.	

PI Response: No response to this comment has been provided by the Principal Investigator.	
Screening known systems will be useful	Need to drive the acquisition of a genomic and systems biology set of tools and data. This is pretty much a phenomenological approach -- needs more mechanistics.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Using real world systems	not clear if growth/lipid production parameters have been established in the laboratory
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined targets	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Percent of cropping area means what? Biological competition for accessibility to air will be critical. Water availability is also a limitation, though minimized by the high pH tolerated. Target is 15% total biomass as lipid.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives

and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
New project with reasonable plans. Screened several algal strains. Have method for physically selecting strains with increased growth/lipid production but not clear yet if trait is stable.	Preliminary data suggest that under alkaline growth conditions CO2 availability may still be a rate limiting factor. Long way to go to get lipid levels that are commercially viable (>40%).
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good baseline data, especially given length of project to date. Interesting early process data.	Would be useful to have a more comprehensive selection of algae, especially with team members that have experience in this area. Will be useful to understand how the early data correlates between species.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The investigators have worked out many of the analytic methods and screening tools that should assist them in their evaluation of candidate algal cultures	Although very early in the research; based on the current results, what is the potential productivity of these strains?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Some results for a new project.	not utilizing the latest systems biotech methods
PI Response: No response to this comment has been provided by the Principal Investigator.	
just started project, but still had reasonable amount of preliminary data -- have started screening tests	presented preliminary data are a long way from testing algae in open ponds, hopefully this transition can occur
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project just started, but off to strong start	
PI Response: No response to this comment has been provided by the Principal Investigator.	

The program has essentially just started. The assay for organisms with high lipid is easy, but not high throughput. Validated assay method--showed correlation of lipid (nile red) with time--growth limitation stimulates lipid production. Can stimulate production with bicarbonate. Found a good buffer (CHES) GC anal of fats--FA not increased, triacylglycerol -more with wt than CHES. Selecting cultures with more lipid by FACS. This is how it can work. Starting to screen, but very early.	Assay method limits the number of organisms screened.
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Good understanding of physical factors required for success.	Not clear the effort is large enough to sample biological diversity or to effectively identify biochemical bottlenecks. Development of commercially viable system will almost certainly required deep understanding of metabolism and its regulation in addition to growth/strain parameters.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focus on pH is a strong benefit.	Factors are limited to current work and are not geared towards long term success. How this work can be extended to larger scales would be an important thing to include. Means to mitigate risks not well elaborated—i.e. ways to screen etc. Commercial development should be an opportunity for collaboration rather than a risk factor.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Scale up certainly poses a challenge to translation of laboratory strain performance to pond scale	Provision and timing of nutrients to algae in a cost effective manner on a practical scale would seem to be critical to the success of this project. How do the investigators plan to assess this?

PI Response: No response to this comment has been provided by the Principal Investigator.	
Engineering approach will determine global parameters.	Don't see use of genomics and other modern tools but the option seems to be there. Algal analyses lag microbe equivalents.
PI Response: No response to this comment has been provided by the Principal Investigator.	
are making good use of high pH conditions for optimizing lipid production and increasing available ponds for lipid production	scale-up work may not be necessary if cannot find algae that produce enough lipid at higher pHs
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well documented	
PI Response: No response to this comment has been provided by the Principal Investigator.	
High pH gives a growth advantage, minimizes instability of the system to contamination. The process uses low value water, a big sustainability advantage.	Scale up; get faster growth rates.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on *removing/diminishing key OBP MYPP barriers in a reasonable timeframe.*

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	Weaknesses
Good plan to explore growth parameters.	Lacks pathway analysis.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Access to new strains and continued plans in that vein will be invaluable. Process work is a key component of the planned studies. Ability to scale will be crucial	Milestones appear in limited ambition given current state of the art. Plan to get to scale despite facilities being in place is striking—key areas for development will emerge from early runs even if not fully optimized.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Research will focus on kinetic analysis/ strain performance work.	A screen of 15 strains may not be adequate to obtain a hyper producing strain. May need to expand the scope and perhaps screen environmental samples for fresh isolates?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Looking at extreme environments will help exclude predator invasions. In general extremophile capabilities could transform this field.	need to integrate with systems biology approaches
PI Response: No response to this comment has been provided by the Principal Investigator.	
further work on screening/identification of good candidate algae demonstrating systems in raceway ponds	relevance of scale-up work may be dependent on finding appropriate algae
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Nothing addresses the scale issues for credibility.

PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Partnered with Greenfire Energy in Utah.	
While one industry collaboration is mentioned, the group is seemingly eschewing the industry. Given the amount of activity, more coordinated activity with various industrial partners would benefit this program.	
A partnership with Greenfire Energy has been developed to assist in transfer of technologies developed as part of this research.	
too early to tell	
early in project, so dissemination of information not issue yet	
Analysis of lipids is in progress. This will probably be the publication venue.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
More defined plans for organism discovery and screening would be invaluable. Exploring what extremophile traits are useful would be beneficial—seems limited to pH at this point. Economic analyses are essential to support the viability of algae in general. This should be included in a robust way much like it has been done with ethanol.	
Use the most modern tools—this field will grow exponentially and old techniques will not be capable of solving problems.	
Add economic analyses	
Sequence a few isolates.	

Improving cost effectiveness of algae-lipid production through advances in nutrient delivery and processing systems

Technology Area: Biomass Program
Project Number: 2.1.1.7
Performing Organization: University of Georgia Research Foundation, Incorporated
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.29	0.49
Approach	3.14	0.90
Technical Progress	3.43	0.53
Success Factors	3.00	0.58
Future Research	3.14	0.90

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project

align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Opportunity to integrate biofuels with existing poultry industry to provide added value. Potential to supply 1.5 B gal/yr ethanol.	Needs economic scale analysis.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal of nutrient cost reduction is of great importance. Approach is broad based and relevant to other environmental and industrial needs. Focus on carbon delivery is essential to the commercialization of algae. Focus on novel extraction techniques and related is valuable and should form the foundation for a number of potential avenues of deeper research for this group or others.	Would be useful to see how these factors are the key cost contributors based on an economic model.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Algae could serve as a biomass feedstock for production of biodiesel. If barriers to practical production can be overcome, economical methods for collection and processing this material will be needed.	Relevance somewhat unknown since no goals currently identified in the biochemical conversion platform.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Attacking current known limiters. Looking at specific nutrient sources. Poultry litter and nitrogen fixers	not using modern tools; limited strategic impact potential, but useful as a niche application

PI Response: No response to this comment has been provided by the Principal Investigator.	
all of the following have potential for use/improvement of algaculture: using poultry litter as nutrient for algaculture N-fixers in algaculture harvest systems improved lipid extraction systems	many research topics are proposed, but not real clear how all topics are related—are they dependent on each another
PI Response: No response to this comment has been provided by the Principal Investigator.	
Poultry waste potential good source of nutrients	More market/economic analysis would strengthen project
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focusing on cost, harvest, with local partners, which are other university departments.	Scale-up does not seem to be in the picture. The highly integrated process may be useful for distributed solutions in agricultural locations. Researchers have not clearly identified an "export" product--most of the energy produced is anticipated to be used on site.
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant *weaknesses.*

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Integrated plan.	Appears overly ambitious for size of effort. Needs to focus on critical process steps and to identify the critical barriers to make integration with poultry farm viable.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Approach is broadly applicable, but focused enough on specific projects to give results in a reasonable amount of time to inform directionality of these studies as well as relevance of future studies. Scope of work covers many of the key focal areas of algae research, with a recognition of what is not being covered.	Implications of a commercial process (i.e. flue gas and other CO2 sources) have not been included. Validation of the proposed work in the context of input CO2 would ultimately validate the commercial potential of this work.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Identifies potential low value nutrient supplement for growing algae and a process for harvesting biomass. Uses effluent from anaerobic digestion of poultry litter as a nitrogen and phosphorus supplement for cultivation of lipid accumulating algae.	The yield of algae uncertain. 8g/ meter**2 per day. Is this a good yield. 20 is their target. What does this translate to in terms of potential production of lipids/ biodiesel?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Very much a functional approach. No mechanistics	
PI Response: No response to this comment has been provided by the Principal Investigator.	
How concept of how finished system will work	difficult to see approach due to way presented, was essentially a large flow diagram, but major details were not presented

PI Response: No response to this comment has been provided by the Principal Investigator.	
Project started recently	Disconnected results
PI Response: No response to this comment has been provided by the Principal Investigator.	
Nutrient delivery $150/ton for existing system. Poultry litter and nitrogen fixers are sources CO2 capture to give higher yields. Process improvement--harvesting 1g/L at present. A problem is lack of good ways to harvest small algae; investigation is focusing on coagulation and fiber flocculation; lipid extraction methods.	8 g/m2/day lipid production; aiming for 20g/m2/day. So far the main roadblock is finding stable cultures not subject to contamination.
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
New project at an early stage. Explored	Algae strains not optimized. Not clear how

extraction process for litter and loadings for growth and are developing a flocculation technique using cellulose fibers and FeCO3 for harvesting.	contamination will be handled in open ponds. Not clear if balance between growth and oil production can be achieved. Only looking at one recovery method fiber flocculation, which has not been perfected and operates in a batch mode.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Data to date is notable given funding time. Results are suggestive of key avenues of research for the work to progress into.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Demonstrated that poultry litter extracts could serve as nutrient supplement for a variety of algal species. Prototype of harvesting and collection technique was developed.	Are these relevant species of algae? Did they produce lipids? The high turbidity of the medium may have a negative impact on light penetration in ponds.
PI Response: No response to this comment has been provided by the Principal Investigator.	
A useful analysis, but very phenomenological.	Hard to determine ultimate potential of this approach.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project just started recently worked with poultry litter as nutrient harvesting systems are being investigated	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project recently started	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Many uses are envisioned for the project--to create a closed loop system for production of other items (such as poultry). Existing technology is being hooked together in interesting ways, e.g. using anaerobic digestor with CO2 recaptured and injected into ponds--feedstock could be biogas or any other CO2 source; and lipids can be use directly onsite for	The process as envisioned is complicated. How much trained labor would be needed to keep the wheel turning and all input/output streams balanced?

power generation or used to make ethanol.
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Reasonable understanding of issues and factors.	Probably needs to be implemented locally and will need buy-in from poultry producers. Process potentially susceptible to disruption by poultry issues—e.g. disease. Needs cost analysis for individual steps so that opportunities for cost reductions can be identified and prioritized.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good understanding of key factors of success	Focus on poultry may bias results that prove most interesting. The study would benefit from

and risks.	other potential feeds. An association of factors with the economic analysis would be beneficial as it would provide a weighting to these factors. Key assumptions on opportunities have not been validated and the validation thereof does not appear to be part of the plan.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The plan identifies important barriers including whether can this information be applied broadly across variety of algal species and can the harvesting/ flocculation medium be recycled.	This process looks like it could work as an integrated "on-farm" system, but will this produce significant amounts of potential fuel. How does this scale up beyond this application?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Very practical and near term approach	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Shows strengths with respect to environmental issues and may lead to reduce costs for some processes (such as harvesting)	- economics of system could be a major limitation—system is very complex, so many parameters have yet to be worked out—not clear how readily this could be incorporated in on-site systems
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Show stoppers not well defined
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project is thinking on all levels, working on closing the loop. Success in at least one effort seems likely.	Consider disassembling the parts to make input/output industries? Might be better oriented to the waste management application.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or

identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
	May have cart before the chicken. Scaleup studies seem premature until appropriate strains are developed. Approaches to scaleup not well described.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined timeline	Numerical goals against the timeline would be valuable.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Researchers plan for scale up and integration of processes with the nutrient delivery technology the immediate focus.	The plan for use of nitrogen fixing organisms and other activities are not well described.
PI Response: No response to this comment has been provided by the Principal Investigator.	
completing the tests of chicken litter and nitrogen fixers, flocculation and other harvesting ideas	
PI Response: No response to this comment has been provided by the Principal Investigator.	

early in project so will continue with aspects outlined in original proposal	
PI Response: No response to this comment has been provided by the Principal Investigator.	
OK	Turnkey operation would be difficult. As stated, different goals are not independent of each other, reducing the value of multiple target choice.
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Have multiple relationships with the University of Georgia.	
Interactions are limited, and should be pursued.	
Partnerships are primarily with University of Georgia departments.	
A niche approach that will benefit a set of producers if algal techniques prove viable.	
Still early in project so dissemination of results not issue yet	
More collaboration would strengthen project.	
Not much so far, but expect publishable data	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response

Needs more focused approach.	
None	
Presentation could be improved.	
Reformulate goals so that each can contribute to external utility even if other parts of the project are not successful.	

University of Nebraska, Lincoln, Bioenergy Demonstration Project: Value-Added Products from Renewable Fuels (NE)

Technology Area: Biomass Program
Project Number: 7.2.1.1
Performing Organization: University of Nebraska-Lincoln
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.00	0.58
Approach	4.14	0.69
Technical Progress	4.14	0.69
Success Factors	3.71	0.76
Future Research	4.00	0.58

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project

align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Project is well focused on deconstruction of switchgrass with dilute acid only.	Is depended on dilute acid pretreatment.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are quite consistent with program goals and represent an important confluence of areas. Represents one of several paths to achieve fundamental goals. Unbiased approach is important in finding optimal ways of accomplishing these goals.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Application of thermoacidophilic archaea is a novel idea and opens up potentially new strategies for enhances conversion of acid pretreated biomass to fermentable sugars. The project capitalizes on these organisms natural ability to grow under these extreme conditions and produce lignocellulolytic enzymes thereby reducing the cost of biomass conversion.	Although novel and technically feasible, this may not offer any advantages over currently employed technologies
PI Response: No response to this comment has been provided by the Principal Investigator.	
Content doesn't match the name of the presentation. This is about extremophiles, T pH; archaea	

PI Response: No response to this comment has been provided by the Principal Investigator.	
worthy project looking at thermoacidophiles to be used following dilute acid pretreatments service component to establish a bioenergy faculty at U. Nebraska	may be duplicating industrial enzyme labs is such are funded—although funding academic lab has merit in that the findings will be made public
PI Response: No response to this comment has been provided by the Principal Investigator.	
Possibly cheaper source of in situ enzymes for degrading lignocellulose	No evidence presented for increased resistance to inhibitors
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**

Good understanding of the needs. Strong molecular genetic approach to development of improvements.	Organisms can be hard to grow. Too early to tell how approach will compare to traditional methods.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Logical and comprehensive approach to develop a universal solution.	Front end research to improve culturability would benefit screening, though this work clearly benefits from the PMs experience with these organisms.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Growth of these organisms is compatible with a leading biomass pretreatment. Identification of target organisms and strategies of manipulating these are well thought out. The principal investigator is expert in the molecular biology of this taxonomic group.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
approaching extremophiles as platforms; modern techniques	
PI Response: No response to this comment has been provided by the Principal Investigator.	
screening for cellulolytic thermoacidophilic organisms etc is appropriate and have experience in working with these organisms' genetic systems	Can this academic lab contribute beyond what the major commercial enzyme hunters are doing?
PI Response: No response to this comment has been provided by the Principal Investigator.	
Screening for platform for genetic engineering. Many technical strengths for the Sulfolobus organism, and compatibility with processing environment. Focus on inhibitors metabolically engineer to channel sugar flux and add other capacities.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Relatively new project. Have identified one organism with relatively good conversion of switchgrass feedstock. Have developed good analytical tools for analysis. Identified mutants that allow utilization of C5 or C6 sugars.	Methods for overcoming lignan toxicity not clear. Small genome organism may have less endogenous flexibility.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Power of genetic system is a critical accomplishment in focus organism. Understanding of metabolism of focal organism and early mutants validate promise of the research. Methyl depletion data validates ways of exploring limiting factors of organisms Analytical approach is essential to bring to this field and is showing good results already	Screening approach seems to be of limited focus of early work. Would be valuable to ensure that this stays as part of the research plan. Would be good to see follow up from small molecule work to genetic changes to eliminate limitations
PI Response: No response to this comment has been provided by the Principal Investigator.	
Research to date has made good initial progress	

toward the identification of biomass converting strains: those that produced lignocellulolytic enzymes and could grow on switchgrass and corn stover. The projects has also constructed strains blocked in ability to metabolize hexose sugars which will be critical to the use of these organisms as saccharification agents in acid pretreated biomass.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
amazing progress for such a new project; genomic methods and rational engineering approach	
PI Response: No response to this comment has been provided by the Principal Investigator.	
nine months into project (2 year project) have done some screening and molecular biology developing analytical methods for different metabolites	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Tree of thermoaciophilic archaea; hard to grow; culture collections. Based on field work. Screened around 25 orgs on consumption of lignocellulose of switchgrass; limiting diversity 7% rDNA divergence. Most not very good, a few do 25% consumption. Sulfolobus platform is OK genetically. Functional genomics via plasmid complementation. Can do transgenics. Metabolic engineering start. Already had catabolite repression mut. No hexose growth; hexose metab illust. Reduced expre glucose dH, kdg kinase--still grows on pentoses. Can inactivate pentose pathway. Analytical facil small molecules; Sam deficiency. MetK depleted. 2/3 of pool is gone where is the rest coming from? Agilent triple quadrupole MS. Looking at many of the sugar species--MS spectrum. Validated enzyme targets xylanase,	Early accomplishments may have got a jump start from earlier research related to other interests

xylosidase, xylanase, CelS, endo glucanase, var names and strains. No soluble protein in heterologous hosts. Not trying sol protein here, engineering the organism. Endoglucanases are focus, three in the chosen org. looking to up expr. (not yet done.)	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

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2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Model organism has small genome with genetic systems. Excellent progress in a relatively short time.	Reengineering microbe to commercial product is ambitious within 2 year timescale.
PI Response: No response to this comment has been provided by the Principal Investigator.	

Have recognized key challenges. Have proven successful at working quickly in a results-based manner	Several of the challenges are unknowns and thus uncharacterized
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goal for ideal strain is outlined: increased conversion to fermentable sugars.	Needs to have potential for lower cost than current methods. Neutralization and enzyme hydrolysis. It is unknown what the potential for this might be.
PI Response: No response to this comment has been provided by the Principal Investigator.	
tactical	
PI Response: No response to this comment has been provided by the Principal Investigator.	
high temperature systems are likely to be most successful in depolymerization systems, so this is on track with that objective	not clear how much sugar will be consumed during the saccharification of the lignocellulose
PI Response: No response to this comment has been provided by the Principal Investigator.	
Shown abil to work on genome design and small-molecule assay, Going after lignin (2/3 of lignocellulose), furfual is toxic; resistant mutants exist.	If block to all sugar use as planned, the organism might not be viable. PI says time is the challenge.
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or *diminishing OBP MYPP barriers in a reasonable period.*

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Good understanding of barriers.	Endogenous overexpression, as for heterologous expression, may not be without potential problems. May require a systems approach to identify unexpected consequences.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined goals with a track record of accomplishment.	Certain goals (i.e. lignin) are lofty, unprecedented, and without a plan.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Plans to extend metabolic engineering work to include blocking the use of pentoses good Evaluation of lignin toxicity and remediation are needed as outlined.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Pragmatic approach—has to work and at scale	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Optimize the system for maximum sugar yields as proposed	
PI Response: No response to this comment has been provided by the Principal Investigator.	

Opportunities for patents
PI Response: No response to this comment has been provided by the Principal Investigator.
Partners are in prospect. Implement engineered thermoacidophic strains with blocks in catabolism, Add genes (deconvolution) for lignin-related traits.
PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Passively seeking industrial partners. Given relevant activity by companies such as Mascoma, more commercial interactions would be beneficial to this group. Focus is clearly with industrial relevance.	
This project leverages U. Nebraska switchgrass research capacity. PI expert in biology of these thermoacidophiles. The project also establishing a bioenergy facility on campus to support this and similar research [e.g. algae] at U. Nebraska.	
strongly coupled to pragmatic perspective of Nebraska farmers and biotech; is being developed	
Are in the process of developing a bioenergy service center—seems it will be relatively small	
Reference to collaboration with local ethanol producers.	

Working on patents; Bioenergy facility is a service to univ community. Main focus of the facility is algal lipids support.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
The scope as proposed is reasonable with strong progress to date. The project will benefit from continued focus rather than increasing into a broad range of new projects.	
Add integration to pilot plant preparation,	

Targeted Conversion Research

Technology Area: Biomass Program
Project Number: 2.4.1.1
Performing Organization: National Renewable Energy Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.71	0.49
Approach	4.71	0.49
Technical Progress	4.86	0.38
Success Factors	4.71	0.49
Future Research	4.86	0.38

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Strong NREL research team with excellent NL, academic and industrial partners.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are aligned with OBP goals Understanding core mechanisms is essential for rational improvement of the system and represents a valuable approach	Separation of this from non-rational approaches (i.e. engineering, evolution etc), likely complicates the learnings to be gained. The goals can be better reached with more coordinated activities.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Targets major barriers of the platform. Provides fundament science underlying engineering to support and promote OBP goals. Publish the results to advance the science involved in these processes. Provide scientific understanding of the events being worked on by others	
PI Response: No response to this comment has been provided by the Principal Investigator.	
A comprehensive effort to utilize sophisticated analytical and theoretical methods to identify the essential features of cellulosic processing. Highly interactive with very capable groups around the country. Great mix of applied and fundamental	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- it is critical to get a better understanding of the	

chemistry of pretreatments - biomass recalcitrance is likely the most important barrier to biofuel production - provide basic/applied science knowledge for choosing optimum processing schemes - specifically looking at lignin deposition and redeposition impacts on saccharification
PI Response: No response to this comment has been provided by the Principal Investigator.
Solid, important basic research
PI Response: No response to this comment has been provided by the Principal Investigator.
Provides leadership and communication to develop information on the substrates and products for the bioreactors. This knowledge is essential and not provided by other projects.
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant

contributions progress.

Strengths	Weaknesses
New concepts. Important cutting edge research to move processing beyond the current, obvious approaches. Primarily working on 3 steps: xylose yields, glucose yields and enzyme costs using e.g. Advanced cell wall imaging, 2D tracking of CBM,	Hard to find any.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Broad-based approach allows for access and simultaneous exploration of several relevant aspects of the project. Clear path forward with focus on applying information to commercial benchmarks Approach is flexible in nature allowing for a number of unforeseen areas to be explored	Pathway to achieve economic goals is not clear. Economic goals also appear to be inconsistent with the motivations of the corporate participants.
PI Response: No response to this comment has been provided by the Principal Investigator.	
I don't think I can add anything to this. Excellent work that focuses on understanding the plant cell wall and its component chemistries. How enzymes actually interact with cell walls and cellulose and how understanding this can lead to strategies to improve catalysis.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Across the board approach—fundamental and applied, classic and modern methods, all relevant spatial and temporal scales	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- works with many collaborators - look at fundamental features of substrates and catalysts - advance cell wall characterization using	

impressive visualization tools - applying existing methods and developing new methods - all state-of-the-art methods appear to be applied to these studies
PI Response: No response to this comment has been provided by the Principal Investigator.
Uses latest technology and instrumentation to address issues
PI Response: No response to this comment has been provided by the Principal Investigator.
Providing fundamental understanding of relevant science and standardized samples. Understand structure of the substrate. Understand biological responses to the substrate (genomics and enzymology)
PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Comprehensive, fundamental analytical work using cutting-edge tools at the molecular/near-molecular level to make strong contributions to understanding of cell wall structure and substructures that is revealing mechanisms which contribute to recalcitrance and deconstruction concepts and increase understanding in a quantifiable way. Very strong publication record.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Cutting edge data is providing tremendous fundamental insight into the biology of cellulases and fiber degradation. This is clearly important work with a strong team executing well against its goals. All projects appear to be progressing well.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Coordinate practical work with scientific underpinnings and helps to focus future engineering research efforts. Provides new tools for examining plant cell polymers, their recalcitrance, and conversion.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
great progress on all fronts, reconciling scales, processes, and phenomena	
PI Response: No response to this comment has been provided by the Principal Investigator.	
project runs 2001-2015 - particle size studies showing relationship between particle size and saccharificaiton performance - recent studies with cellulose have shown that crystal structure is not all worked out	

- recent studies have developed methods for measuring dp of cellulose - have determined amorphous/crystalline content of man model celluloses - have done telling work on porosity of substrates and how to think about it - relating porosity to enzyme saccharification - have done enlightening work on lignin behavior during pretreatments - furthered current understanding of biphasic nature of xylose hydrolysis during pretreatments (not simply presence of lignin) - developed CARS microscopy probe for studying PCW behavior in processing - work with xylanases to demonstrate accessory enzyme behavior - looking a chemistry that could be relevant to high solids-loading pretreatment systems (reversion & degradation) - generated important fundamental knowledge on cellobiohydrolase I, what is thought to be the major cellulolytic enzyme	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Numerous accomplishments	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Providing imaging tools for cellulose characterization. Picture of how the reactions work is rudimentary. Still in phenomenology phase--correlating particle size with conversion. Only starting to make testable hypotheses (decouple size and xylan effects) Structural examination of the cellulases. Mine the databases. Community composition determination on rotting piles. Correlate structure function with standardized testing of different cellulases. Test enzymatic model. Twisting of fibrils inferred from microscopy causes question of interpretation of unit cell in	Not enough really basic research until recently? Presentation would benefit from focusing on a few key insights supported by the work that prepare the way for the next step.

x-ray structure of fibril Look at products of reaction Degree of crystallinity is critical in model studies--plants mostly crystalline, lab substrates mostly Effect on enzyme access of pore/spaces to allow enzyme in Lignin. Can come out of the wood at high temp; make droplets; some stuck to surface. MW of lignin only recently determined--droplets have smaller stuff than stuff stuck inside. Xylan can be inside the lignin droplets. Biphasic kinetics of xylan release with different treatments--goes away at very small particle size. Looking at various enzymes ability to release xylan; chemically formed disaccharides without biological linkages. High solids ratio decreases water activity may increase production of furfural etc.
PI Response: No response to this comment has been provided by the Principal Investigator.

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Strong understanding of fundamental tools and techniques, including computation, and how to apply them to give relevant information on mechanisms.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good understanding of success factors and risks. Analysis achieved both commercially and academically. The team has a track record supportive of being able to overcome challenges.	Not sufficient clarity on overcoming unforeseen challenges, which given the nature of the work, are sure to present.
PI Response: No response to this comment has been provided by the Principal Investigator.	
maintain fundamental effort to support process targets	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Right on!	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- generate fundamental knowledge to support biomass conversion systems	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Clear list of key goals and priorities.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well defined process for moving things forward and clear milestones and goals. Future work is very relevant to goals.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
continue doing good stuff	
PI Response: No response to this comment has been provided by the Principal Investigator.	
excellent suggestions on both local and national programs	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- develop "improved" CBH1 through rational understanding of how enzyme works, providing fundamental knowledge along the way - improve understanding of biphasic nature of xylose hydrolysis	

PI Response: No response to this comment has been provided by the Principal Investigator.

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Strong collaborative base built in. Work stems from academic core with interested industry partners clearly involved, and offering a direct avenue for tech transfer. PI etc are focused on publication record, and have a strong record there.	
Prominent scientific partnerships.	
a model of interaction at all levels—they are trying to move from science to use	
very active in dissemination of results—through peer-reviewed publications, participation in symposia/meetings, and working with industry/academic/government partners/collaborators	
Extensive collaboration with all leaders in field and work is published	
Publication record excellent, numerous collaborators.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
This is important, well designed work that is appropriately broad in scope. Connecting this to	

other similar projects would be invaluable.	
This is excellent research. I can add little to what they are accomplishing!! Program provides scientific understanding of biomass conversion technologies. Critical to advancing future directions of research capitalizing on the information provided.	
This effort should more strongly drive the 1+3 and other programs to achieve alignment	
Too much information on slides; not possible to read in time displayed; should concentrate slides on important points	

Fungal Genomics

Technology Area: Biomass Program
Project Number: 2.4.1.2
Performing Organization: Pacific Northwest National Laboratory
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.57	0.53
Approach	4.43	0.53
Technical Progress	4.57	0.53
Success Factors	3.86	0.69
Future Research	3.86	1.07

Overall Principal Investigator Response(s)

We thank the reviewers for their comments on our research program. We are pleased that the reviewers recognized the value of the research to date and potential of fungal biotechnology to make important contributions to the emerging biorefinery.

The reviewers highlighted some of the complexities of metabolite data that we routinely generate. We agree data is complex, and in part we deal with this by focusing on those metabolites of more immediate interest to us. To avoid missing other key metabolites, we have started to work with a specialist in NMR at PNNL and we are always looking to improve our analytical chemistry protocols. Another complexity issue is associated with our genomics focus. Indeed, with new sequencing technologies, the amount of data generated is immense. We are developing our own tools and through collaboration with others (for example the DOE Joint Genome Institute) we are working to analyze these large datasets.

The reviewers also highlighted the importance and difficulties of moving microbial strains and processes from the lab into the biorefinery. As noted in the review, we are working with key

industry partners who will be responsible for commercialization of new technologies.

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
Strong industrial partners.	Value may be longer term or indirect.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Relevant work that needs to be addressed. Broad-based, highly industry relevant goals. Focus on translation is significant and important. Thoughtful about how to think about DOE and industrial goals.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Understanding the fundamental processes used by commercially relevant fungi could provide useful tools and targets to improve biomass	

conversion strategies.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Strong analysis of the potential for fungal based systems. There are many potential advantages of fungal systems that need to be analyzed and further developed. Good use of extensive systems biology data.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
important to consider new fungal systems and the topics presented are relevant—the methods used are important as examples for other groups targeting slightly different topics	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Excellent basic R&D for improving cellulosic enzyme production	Low ethanol tolerance and low temperature fermentations not good for ethanol production in biorefinery.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Accumulating basic understanding of metabolic potential that partners can use in real life using the rapidly advancing fund of sequence information.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few *areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.*

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Good use of DOE OS facilities (JGI) to develop rational approaches. Using systems approach.	Not clear if rationale scientific approach will add significant value to industrial efforts in the short term
PI Response: No response to this comment has been provided by the Principal Investigator.	
Fungi are clearly important organisms to be understood. Approach is broad across industrial needs and is relevant to program and industrial goals.	The metabolic diversity of this class of organisms is, as recognized by the presenter, immense. It would be useful to understand how to collect the plethora of data and synthesize it into conclusions. It is recognized, however, that each project is limited in scope to small number of organisms, such that this kind of questions may be challenging to answer. Understanding the implications of a filamentous organism into process development would be valuable.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Employs genomics based tools to further exploit fungal production of hydrolytic enzymes. Retrospective analysis of mutations in T. reesei is an interesting to approach to indentifying targets for improvement. Identification of metabolic targets to improve fermentative	While filamentous fungi have been biocatalysts of choice for enzyme and organic acid production, it seems unlikely that they will replace yeasts or bacteria for production of fuel ethanol

capacity of these organisms is also warranted..	
PI Response: No response to this comment has been provided by the Principal Investigator.	
good ideas, using modern tools, good systems biology approach	
PI Response: No response to this comment has been provided by the Principal Investigator.	
work with filamentous fungi which of high relevance to biofuel production good to study basic fungal biology cores are protomics and genomics multiple tasks related to biorefinery foci are enzyme production (cellulase) and carbon flow (itaconic acid)	not clear how will pinpoint the genes that actually dictate the behavior targeted
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good use of genomics for basic research	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Much discovery available since early stage for "systems biology" approaches.	Need good assays. "approach" slide was sort of a pep talk
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives

and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	**Weaknesses**
Good progress in identifying legacy changes in fungal strain associated with previous selection for increased productivity and in identifying pathway for organic acid production.	Will need additional tools to identify rate limiting steps for both enzyme production and carbon flow. Didn't seem to have a clear plan for identifying bottlenecks and priorities.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Re-sequencing effort provides a strong retrospective tool to understand the successes already achieved. Strong collaborative approach has generated significant data and compelling early data. Early carbon flow studies have validated EST and genomic approach. Rapid and significant progress to date.	How this information can be generalized would be a valuable add, especially as every process and every organism has specific requirements for optimization. Including meta-genomic analyses may help to provide this information (or whether it is not a conclusion that is achievable).
PI Response: No response to this comment has been provided by the Principal Investigator.	
The research program has made good progress in identifying underlying basis for superior enzyme producing mutants and metabolic events associated with a value added chemical product	
PI Response: No response to this comment has been provided by the Principal Investigator.	
tied many global techniques for new insight, comparative genomics of industrial vs. native, good use of genomics to identify itaconic acid pathway, very high potential for broad impact of these techniques	
PI Response: No response to this comment has been provided by the Principal Investigator.	
using state-of-the-art methods for assessing	may be difficult to go from identifying groups

enzyme production	of genes to actual commercial improvements
PI Response: No response to this comment has been provided by the Principal Investigator.	
Excellent approach and work on enzyme production and itaconic acid production	Did not make a strong case for use of filamentous fungi in a ethanol fermentation
PI Response: No response to this comment has been provided by the Principal Investigator.	
Examples overproduction, metabolic engineering. Classical strain improvement has been done in the past for catabolite repression relief. JGI is sequencing the parent and intermediates. Take the result to a new host? Sequencing result? Which mutation is responsible? 235 point mutations 63 genes mutated; 2 del. in final from great grand parent, also grandparent to be seq missing one of four. Partners JGI, Novosyme, TU Vienna, IFP looking at. What happens during classical strain improvement. Metabolic engineering for new efforts? Understanding Carbon flow; Aspergillus terreus; output itaconic acid for model; production genes unknown; 40-50 g/L made JGI collab; Est sequencing for three stages inh bioprocess--preproduction, production initiation (phosphate depletion); post initiation, where production is tailing off. Cluster of genes up, transporters, cis-aconitatse decarboxylase, tc factor, p450.	Have they shown the id'd operon is related to production?
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and

strong strategies to overcome possible showstoppers are identified.

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3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Strong team with relevant capabilities at PNNL.	More to understanding productivity than control of gene expression. Need to understand stability, activity and degradation.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Has identified some key factors	Aims of manipulation of organisms and acceptance of GMOs by public have not been addressed by this plan, with the latter not really a key limiting factor of the research thus far. Understanding plans on manipulating organisms would be valuable. Would be useful to understand the potential limiting technical factors, though none have been seen yet.
PI Response: No response to this comment has been provided by the Principal Investigator.	
The project identifies events that will constitute success and challenges to acceptance of GMOs. Controlling genetic and metabolic activity will likely be challenging.	While continued research will most certainly uncover much valuable information on the fundamental processes in these organisms, translating these to practice using rational approaches will be challenging.
PI Response: No response to this comment has been provided by the Principal Investigator.	

taking a good fundamental approach applied at a scale that can be applied to practical problems	
PI Response: No response to this comment has been provided by the Principal Investigator.	
are using good approach to look at improvements in enzyme production	may be difficult to interpret genomics analyses to determine high cellulase producing enzymes
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Did not outline show stoppers for a biorefinery.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Control of expression--what tools are already available? Metabolic engineering has been done with some of these. Novozymes is working with this. Partner review board keeps result relevant.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Using analysis to understand how past changes led to improvements.	Most "random" mutations may be silent or even modestly deleterious. Did not give a thoughtful description of future approaches and priorities.
PI Response: The reviewer notes that a detailed plan for future research on the strain resequencing part of the project was not clearly articulated. We are currently in the process of developing that detailed plan and are considering both forward and reverse genetic approaches to deconvolute the data. We appreciate this input and will include it in our planning.	
Future work is well planned and builds on prior accomplishment. Scope is well focused. Commercial alignment is clear in future planned work.	Understanding how the process limitations will be explored would be useful.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Future research towards translation of findings on gene expression and metabolism to improved strain development is warranted.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
focused but looking more broadly	
PI Response: No response to this comment has been provided by the Principal Investigator.	
future work will be clear extension of what they are doing presently, have good collaborations so should be productive group	very complex biosynthetic/regulatory pathways that may be difficult to understand in the short term
PI Response: No response to this comment has been provided by the Principal Investigator.	
Will work on genes in itaconic acid path. Genetics can help evaluate the result. How do they decide on ethanol relevance--the previous analysis talk?	How to identify the rate limiting steps for production of ethanol from cellulose? Chaperones. Mapping to identify critical. Other strains? Resequencing.
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	**PI Response**
Relevant partners have been selected that can speed the work, and potentially facilitate commercialization.	
Excellent commercial partners. Partnership review board has members that will be helpful further development of discoveries.	
well tied to many fundamental and applied areas and research groups	
plan a joint publication	
Appears to be excellent collaboration/technology transfer on enzyme production and itaconic acid production.	
Publications will be made. Utility may be enzyme discovery for C5 to transplant to yeast.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Future metagenomics work could speak to the potential to generalize conclusions to unexplored fungi and potentially beyond.	
The work is important, it provides a representative rational way to evaluate fungal systems	

Lignin as a Facilitator, not a Barrier, during Saccharification by Brown Rot Fungi

Technology Area: Biomass Program
Project Number: 2.4.1.3
Performing Organization: University of Minnesota
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	4.43	0.79
Approach	4.43	0.79
Technical Progress	3.57	0.53
Success Factors	4.00	0.82
Future Research	3.71	0.76

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	Weaknesses
Good commercial partners.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Project is in line with OBP goals. Offers a synergistic approach to better understand lignin breakdown Unique viewpoint of how lignin can be approached, supporting value of this program.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Addresses biomass recalcitrance issues through examining the action of brown rot fungi on lignocellulose. A novel approach that combines pretreatment with saccharification. Does its own hydroxyl radical pretreatment then hydrolyzes and converts carbohydrate polymers without exocellulase. How do they do this??? Could provide some interesting insights into how to deconstruct biomass.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
A different perspective—a route to CBP?	
PI Response: No response to this comment has been provided by the Principal Investigator.	
understanding biochemistry of brown rot fungi lignocellulose utilization; potential application of this knowledge to industrial pretreatment	

schemes
PI Response: No response to this comment has been provided by the Principal Investigator.
Novel approach could provide new information
PI Response: No response to this comment has been provided by the Principal Investigator.
Addresses one of the key barriers to economical pretreatment. Novel approach to developing treatment
PI Response: No response to this comment has been provided by the Principal Investigator.

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	Weaknesses
Characterize (spatial, sequential, synergy) and then use the approach of brown rot fungi to extract polysaccharides from lignocellulose with removing lignin. Objective is to combine pretreatment and deconstruction as a system. Innovative approach.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Logical approach to understand biological approach set to define a position for results within industrial standard approach.	Unclear how synergies will be explored and understood. Could benefit from exploring how well this system relates to other lignin digesters
PI Response: No response to this comment has been provided by the Principal Investigator.	
Characterize spruce and other biomass feedstocks before, during and after brown rot fungal conversion to study the timing and extent of BR fungal induced lignin modification and enzymatic activity. Include commercial enzymes in a comparative approach.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
looking for insight into how brown rot treats wood to extract sugars is important -- it is an effective process	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- uses approaches related to the study of strength-loss in wood - evaluate the system with similar rationale as those looking at industrial processing - using techniques to allow timing/spatial investigation of when/where things are occurring - use spruce, poplar, stover as substrates	

- focusing on early time points of lignin modification	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well-conceived focus on spatial distribution of reaction targets. Weight loss by harvest week, looking at various sugars.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Started in October 2008. Characterizing spruce, poplar and stover. Spruce to post-saccharification accomplished; poplar and stover in progress. Strong publication record.	

PI Response: No response to this comment has been provided by the Principal Investigator.	
Good early data to validate approach.	No emergent synergy. More work will need to be done to clarify potential industrial relevance.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Early in project: Have demonstrated increase in enzymatic sugar release over time with biological pretreatment with BR fungi	
PI Response: No response to this comment has been provided by the Principal Investigator.	
on target	
PI Response: No response to this comment has been provided by the Principal Investigator.	
project has just started - have started working out methods to be used in future studies - have done weight loss studies to demonstrate components responsible for weight loss in different stages - have done saccharificaiton studies of brown-rot treated samples	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Just started	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Interesting results given not much time yet	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and

strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	**Weaknesses**
Well prioritized set of factors to explore. Many questions are present—lack of synergy between pretreatment and digestion in this natural, successful system.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Good understanding of success factors Good recognition of challenges Plan to overcome challenges has been built preliminarily into approach	No clarity on window of opportunity. No consideration for a need to diversify approach
PI Response: No response to this comment has been provided by the Principal Investigator.	
Focuses on how to translate these phenomena to practice and potential to mimic fungal pretreatment. Would like to determine the actual role of lignin in the process [scaffold?]. Can the process be selectively applied?	
PI Response: No response to this comment has been provided by the Principal Investigator.	
identifying the right issues	
PI Response: No response to this comment has been provided by the Principal Investigator.	

- being able to mimic brown-rot pretreatment - understanding the role of lignin in brown-rot saccharification	- must be able to understand the chemistry of the brown-rots -rate of pretreatment and cost of pretreatment seem to be limiting
PI Response: No response to this comment has been provided by the Principal Investigator.	
Well identified	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

Strengths	**Weaknesses**
Clear plan for future work	Additional granularity on timing and goals would benefit the project Would benefit from description of clear goals.
PI Response: No response to this comment has been provided by the Principal Investigator.	
Continuing the characterization of materials	

though the process should yield interesting data on the timing of events and changing characteristics of the biomass materials. Use of labeled proteins to monitor their interaction with materials with time will be very interesting.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
the weighting of work elements will await developments -- issue is the role of lignin in the process	
PI Response: No response to this comment has been provided by the Principal Investigator.	
do proposed studies using brown-rot as pretreatment of chosen substrates and fundamental studies of underlying chemistry	
PI Response: No response to this comment has been provided by the Principal Investigator.	

1) Technology Transfer/Collaborations
Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
ADM as a partner facilitates tech transfer Good collaboration base and PI with good publication record	
Industrial partnership will be beneficially to potential commercial application of discoveries form this research.	
Good interactions with industry partners	
Has industry partners, also plans to publish work	

Has and will publish; works with others.	
Good collaborations with industry planned.	

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	**PI Response**
Well focused project. Future work could explore commonalities with other species of lignin degraders	

Arkansas State University Ethanol Fuel Development

Technology Area: Biomass Program
Project Number: 7.2.4.1
Performing Organization: Arkansas State University
Number of Reviewers: 7

Evaluation Criteria	Average Score	Standard Deviation
Relevance	3.71	0.49
Approach	3.86	0.38
Technical Progress	3.86	0.69
Success Factors	3.29	0.76
Future Research	3.57	0.53

Overall Principal Investigator Response(s)
No Overall PI Response

1. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.

4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.

3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.

1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.

Strengths	**Weaknesses**
University researcher with biotech partner.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Goals are generally in line with OBP	Goals are limited in scope and breadth
PI Response: We are focused on the issues that we believe are the most critical for increasing protein accumulation in corn seed compartments--promoters, germplasm and accessory activities.	
Using corn as the production vehicle, reduce cellulase loading and costs by incorporating these in transgenic corn seed. A novel approach to production of industrial enzymes.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
A potentially important source of enzymes and insights into degradation.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
characterization of cellulases from transgenic maize seed - aims to lower cellulase enzyme costs - aims to lower cellulase enzyme loads	not clear how exogenous cellulases cloned into corn will impact plants
PI Response: The plants with cellulases in seed have been bred for 6 generations with no apparent affect on plant health. Because they are in seed, the most likely effect would be on germination. The germination rate of these seeds is no different from the wild type without the	

exogenous gene.	
High productivity platform explored	GMO apparently doesn't have support of the region
PI Response: I'm not sure what region is suggested here, but we work diligently to show "no harm" from the transgenic corn while still following compliance rules for the USDA field trials. We have modeled how to grow the regulated corn cost effectively while working toward non-regulated status (Howard and Hood, 2007).	

2. Approach to performing the Research, Development and Demonstration (RD&&D)

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.

4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.

3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.

2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.

1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.

Strengths	**Weaknesses**
Using genetic and molecular techniques to improve the activity and recovery of cellulase enzyme functionality from plant production	

system. Target is 3x or better.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Approach is clearly aimed at meeting the goals. Good recognition of association of approach to meet research and industrial needs. Good connection back to core research opportunities. Important recognition of the relative economic implications for various factors	Approach appears to apply an existing system to a problem of interest,
PI Response: No response to this comment has been provided by the Principal Investigator.	
Building on prior work using E1 and CBH1 as the enzymes, target expression of these in corn grain. Evaluate new promoters to direct localization and improve enzyme activity. Would like to elucidate underlying genetic controls of protein accumulation.	
PI Response: No response to this comment has been provided by the Principal Investigator.	
in early stages	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- corn transformation (group appears to have considerable experience in this field) - use several germ plasms - use genetic and molecular genetic techniques - develop processing techniques for enzyme recover - looking for enzyme expression in germ of kernel	
PI Response: No response to this comment has been provided by the Principal Investigator.	
	Needs proof-of-concept early on that can produce sufficient active enzyme to be cost effective
PI Response: Our enzyme trials and models of expression levels combined with processing have shown cost-effective production. These estimates are confidential to the company who is likely to commercialize the products.	

Has background to understand the corn genetics. Planning more molecular studies to understand. Economic analysis included Understands regulatory issues
PI Response: No response to this comment has been provided by the Principal Investigator.

3. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.

4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.

3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.

2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.

1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.

Strengths	Weaknesses
Project funded July, 2008. Largest space for improvement is expression. CBH1 constructs with several new, strong promoters made for transferring into corn. Also adding transcription factor.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

Core approach and methodology validated Fair amount of work providing improved systems Initial data provides a good baseline Assay development is an important contribution	Unclear how advances impact economics Progress slower than expected
PI Response: Higher protein (enzyme) accumulation directly impacts cost--inversely proportional. Progress is on track after hiring was completed.	
Making good progress on molecular biology, but still early in evaluation of this as a strategy for production of high activity enzymes.	Analysis of enzyme activity was able to detect activity but it is hard to understand what these relative activities mean. A more standard assay of enzyme activities may be helpful.
PI Response: We are working on standardizing assays and comparing different methods of analysis. We will present these data at the next review.	
early	slow start
PI Response: No response to this comment has been provided by the Principal Investigator.	
nine months into project - have looked at range of germplasm - have tried different promoters at different site - have worked out methods/assays	
PI Response: No response to this comment has been provided by the Principal Investigator.	
Expression at present all in germplasm; need to move to new tissues such as endosperm	
PI Response: No response to this comment has been provided by the Principal Investigator.	

4. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.

4-Good. Key critical success factors and showstoppers are identified and there are clear

strategies developed to overcome showstoppers.

3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.

2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.

1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.

Strengths	Weaknesses
Show enzymes extracted from corn flour will be competitive in price with those derived from fungal culture, or will enhance the activity of the fungalderived enzymes. Use dry milling to fractionate and recover germ plasm. Scaleup has little capital cost.	Plan for scale up and need for regulatory licensing. No data on relative improvement in yield.
PI Response: The commercialization partner is working on scale up and non-regulated status. Improvements in yield with breeding are at the end of the inbred cycle when the two sides of the hybrid are crossed. We will have data on the hybrid performance with the cellulase after the summer 2009 growing season.	
Basis for doing this work is clear.	Challenges are more field related than project specific. Project would benefit from analysis of its specific risks. Unclear what is essential to make this project a success
PI Response: We have modeled many times the cost of recovery of enzymes from germ flour. We have predicted the cost-sensitive points and are working toward solving those--higher expression, de-regulation and good germplasm.	
The big question is can cellulases from corn flour or germ be cost effective and competitive with fungal culture based enzymes or complement these.	
PI Response: No response to this comment has been provided by the Principal Investigator.	

a good list	
PI Response: No response to this comment has been provided by the Principal Investigator.	
- produce enzyme preparations at lower costs than those currently available - produce sufficient quantities of enzyme with demonstrate activity	- impact of introduced genes on overall plant performance and viability of seeds for future growth - regulatory issues
PI Response: The seeds show no adverse affects from the transgenes. Regulations are being addressed by our commercialization partner.	
	Better definition of show stoppers needed
PI Response: We will think about these in more detail.	
Competent list of issues	
PI Response: No response to this comment has been provided by the Principal Investigator.	

5. Proposed Future Research approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.

4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.

3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.

2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.

1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.

<table>
<tr><th>Strengths</th><th>Weaknesses</th></tr>
<tr><td>Plan for continuing current work and perhaps extending to other enzymes.</td><td>What are rate limiting steps in enzyme accumulation? Is glycosylation different in corn and fungi and does this effect recovery or activity.</td></tr>
<tr><td colspan="2">PI Response: The enzyme activity from corn appears to be equal to the fungal enzyme. Glycosylation does not appear to affect enzyme activity to any great degree. We are trying to understand what any rate-limiting steps are in enzyme accumulation. So far, nothing we have tried has seemed to cap accumulation.</td></tr>
<tr><td>Well defined goals to achieve the programmatic goals High level milestones well defined</td><td>Could use timeline with discrete quantitative milestones. As project is behind, plan to catch up would be beneficial.</td></tr>
<tr><td colspan="2">PI Response: We have made some critical decisions on how to speed progress on the project. We have hired some talented undergraduates to help with the rate-limiting experiments.</td></tr>
<tr><td>Finish vector development, transformation of corn, and assess enzyme production and activity.</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>This work is targeted sharply on determining the viability of the plant production of enzymes.</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>complete project as proposed</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
<tr><td>Plans for research are well conceived including in licensing vectors. Good understanding of decision points</td><td></td></tr>
<tr><td colspan="2">PI Response: No response to this comment has been provided by the Principal Investigator.</td></tr>
</table>

1) Technology Transfer/Collaborations

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

Reviewer Comment	PI Response
Well defined collaborations to support publication, information distribution, and tech transfer Very significant discussion of proprietary factors, which is surprising for academic work.	The PIs have had a lot of industry experience.
not discussed, presumably this technology would be licensed	
Needs an industrial partner.	We have several industry partners lined up who are working on the critical commercialization factors.

2) Recommendations for Additions/Deletions to Project Scope

Reviewer Comment	PI Response
A more detailed economic breakdown and the component wise implications would be useful	We will work on the economics of the project for the next review.

Attachment One: Conversion Project Review Form

Project Evaluation Form

Session: **R&D**

Reviewer Name: ____

Title of Project: ____

Presenter Name: ____

Reviewer Self Assessment of Subject Knowledge (Circle One): None Novice Intermediate Expert

1. **Project Stage of Development** as Identified by PI ______________________

2. **Project Stage of Development** as Recommended by Reviewer ______________________

3. Relevance to overall Program objectives and market need.

The degree to which the project continues to be relevant to the goals and objectives of the Biomass Program Multi-Year Program Plan. Market application of the expected project outputs have been considered.

Project Relevance to OBP Objectives and Market		
5-Excellent. The project is critical to and fully supports Multi-Year Program Plan objectives. The project is critical to and fully supports the needs of target customer(s) and market(s); customers and markets are fully identified.		Specific Comments:
4-Good. Most aspects of the project align with the plan objectives. Most aspects of the project align with the needs of customers and markets; customers/markets are identified and important.		
3- Satisfactory. Many aspects of the project align with plan objectives. Many aspects of the project align with the needs of customers and markets; customers/markets are identified.		

2-Fair. The project partially supports the plan objectives. The project partially supports the needs of customers and markets identified.		
1-Poor. The project provides little support to the plan objectives. The project does not meet the needs of customers and markets; customers/markets not identified.		

4. <u>Approach</u> to performing the Research, Development and Demonstration (RD&D).

The degree to which the project uses a sound, well-designed RD&D approach and clear project management plan, which incorporates well-defined milestones for monitoring the progress of the project and methods for addressing potential risks.

5-Excellent. The project has a sound, well-designed approach and has developed and implemented effective project management practices. Difficult for the approach to be improved significantly.		Specific Comments:
4-Good. The approach is generally well thought out and effective but could be improved in a few areas. The project has developed adequate milestones and potential risks have been identified but management approaches may not be fully developed.		
3-Satisfactory. The approach is satisfactory to meet project objectives and some milestones are developed. Improvements in approach would improve project quality.		
2-Fair. Some aspects of the project may lead to progress, but the approach has significant weaknesses.		
1-Poor. The approach is not responsive to project objectives and unlikely to make significant contributions progress.		

5. Technical Progress and Accomplishments

The degree to which the project has made progress in its stated objectives, achieving milestones as planned and contributing to OBP goals and objectives as outlined in the OBP MYPP and overcoming technical barriers outlined in the MYPP.

5-Excellent. The project has made excellent progress towards project objectives, OBP goals and objectives and overcoming one or more key technical barriers. Progress to date suggests that the barrier(s) will be overcome.		Specific Comments:
4-Good. The project has shown significant progress toward project objectives, OBP goals and objectives and to overcoming one or more technical barriers.		
3-Satisfactory. The project has shown satisfactory progress toward project objectives, OBP goals and objectives and contributes to overcoming technical barriers.		
2-Fair. The project has shown modest progress towards stated project goals and OBP objectives and may contribute to overcoming technical barriers.		
1-Poor. The project has demonstrated little or no progress towards stated project goals, or OBP objectives and technical barriers.		

6. Critical Success Factors and Showstoppers

The degree to which the project has identified critical success factors (technical, business, and market factors) which will impact technical and commercial viability of the project; and the degree to which the project has identified potential show stoppers (technical, market, regulatory, legal) which will impact technical and commercial viability.

5-Excellent. A comprehensive list of critical success factors and showstoppers are identified and strong strategies to overcome possible showstoppers are identified.		Specific Comments:
4-Good. Key critical success factors and showstoppers are identified and there are clear strategies developed to overcome showstoppers.		
3-Satisfactory. Many critical success factors and showstoppers are identified and strategies to overcome showstoppers have been proposed.		
2-Fair. Some critical success factors and showstoppers are identified. Strategies to overcome showstoppers are not well developed.		
1-Poor. Little to no identification of critical success factors or showstoppers. Little to no recognition of relative importance or prioritization of activities.		

7. **<u>Proposed Future Research</u>** approach and relevance (as defined in the project).

The degree to which the project has effectively planned its future, considered contingencies, understands resource or schedule requirements, built in optional paths or off ramps, or identified other opportunities to build upon current research to further meet OBP goals and objectives.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.		Specific Comments:
4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.		
3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.		
2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.		
1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.		

8. **<u>Technology Transfer/Collaborations</u>**

Does the project adequately interact, interface, or coordinate with other institutions and projects, providing additional benefits to the Program? Have Project Performers Presented or Published on the Progress or Results of the Project?

9. **<u>Recommendations for Additions/Deletions to Project Scope</u>**

1) **Proposed Future Analysis**

The degree to which the analysis activity has highlighted areas of future analysis or research or further developments that can facilitate the growth of the biofuels industry.

5-Excellent. The future work plan clearly builds on past progress and is sharply focused to address one or more key technical barriers in the OBP MYPP in a timely manner.		Specific Comments:
4-Good. Future work plans build on past progress and generally address removing or diminishing OBP MYPP barriers in a reasonable period.		
3-Satisfactory. Future work plans are loosely built on past progress and could address OBP MYPP barriers in a reasonable period.		
2-Fair. The future work plan may lead to improvements, but should be better focused on removing/diminishing key OBP MYPP barriers in a reasonable timeframe.		
1-Poor. Future work plans have little relevance or benefit toward eliminating OBP MYPP barriers or advancing the program.		

6. Provide Comments on Overall Strengths and Weaknesses

Strengths:

Weaknesses:

7. Recommendations for Additions/Deletions to Project Scope:

Attachment Two: Conversion Platform Review Form

Platform Review Form

Reviewer Name:____

Platform:____

Reviewer Self Assessment of Subject Knowledge (Circle One)**:** None Novice Intermediate Expert

1) Are platform goals, technical targets and barriers clearly articulated? Are platform goals realistic and logical? Do the platform goals and planned activities support the goals and objectives of the Biomass Program as outlined in the MYPP? How could the platform change to better support the Biomass Program's goals?

Platform Goals		
5-Excellent. The platform goals are critical and fully support achieving OBP goals. The platform goals are clear, realistic and logical.		Specific Comments:
4-Good. The platform goals are important and support achieving almost all OBP goals. The platform goals are clear and logical.		
3-Satisfactory. The platform goals support achieving the majority of OBP goals. The platform goals are defined, but could be improved.		
2-Fair. The platform goals support achieving some OBP goals. The platform goals need better definition.		
1-Poor. The platform goals support achieving few OBP goals. The platform goals are not well defined.		

2) How well does the platform approach (platform milestones and organization, RD&D portfolio, strategic direction) facilitate reaching the Program Performance Goals for each platform as outlined in the MYPP? What changes would increase the effectiveness of the Platform?

Platform Approach		
5-Excellent. The quality of this platform approach is exceptional and fully supports achieving Program Performance Goals.		Specific Comments:
4-Good. The quality of this platform approach is above average and supports achieving almost all Program Performance Goals		
3-Satisfactory. The quality of this platform approach is sufficient to support achieving the majority of Program Performance Goals		
2-Fair. The quality of this platform approach supports achieving some Program Performance Goals		
1-Poor. The quality of this platform approach supports achieving few Program Performance Goals		

3) The degree to which the Platform RD&D is focused and balanced to achieve Biomass Program and Platform goals? (WBS, unit operations, pathway prioritization)

Platform R&D Portfolio		
5-Excellent. The platform R&D is focused and balanced and fully supports achieving OBP and Platform goals.		Specific Comments:
4-Good. The platform R&D is focused and balanced and supports achieving almost all OBP and Platform goals.		
3-Satisfactory. The platform R&D is balanced and supports achieving the majority of OBP and Platform goals.		
2-Fair. The platform R&D supports achieving some OBP and Platform goals.		
1-Poor. The platform R&D supports achieving few OBP and Platform goals.		

4) Based on the presentations given, how well is the platform progressing towards achieving Biomass Program and Platform goals? Are we meeting our performance targets? Is it on track to meet the goals presented? Please provide recommendations on improvements for tracking progress in the future.

Platform Progress		
5-Excellent. The platform is making exceptional progress towards achieving OBP and Platform goals.		Specific Comments:
4-Good. The platform is making above average progress towards achieving almost all OBP and platform goals.		
3-Satisfactory. The platform is making sufficient progress towards achieving the majority of OBP and platform goals.		
2-Fair. The platform is making progress towards achieving some OBP and platform goals.		
1-Poor. The platform is making little progress towards achieving OBP and platform goals.		

5) Please note any specific platform strengths.

6) Please note any specific platform weaknesses.

7) Are there any gaps in the Platform RD&D Portfolio? Do you agree with the RD&D gaps presented by the Platform Manager?

8) Additional Recommendations, Comments and Observations

Attachment Three: Biochemical Conversion Platform Review Agenda

U.S. Department of Energy
Energy Efficiency and Renewable Energy
Bringing you a prosperous future where energy is clean, abundant, reliable, and affordable

Office of the Biomass Program
Biochemical Platform Review
April 14-16, 2009 in Denver, Colorado

April 14, 2009		
Biochemical Platform Review		**Time**
Welcoming Remarks and Overview of Peer Review Structure and Biochemical Platform	**Leslie Pezzullo** Office of the Biomass Program	9:30 a.m. – 10:00 a.m.
Biochemical Platform Portfolio Overview and Overview of Analysis Projects	**Gene Petersen** Golden Field Office	10:00 a.m. – 10:30 a.m.
Analysis		
Biochemical Platform Analysis 2.6.1.1	**David Hsu, Ph.D.** National Renewable Energy Laboratory	10:30 a.m. – 10:55 a.m.
Analysis for Production-Technical and Market Analysis 2.6.1.2	**Sue Jones** Pacific Northwest National Laboratory	10:55 a.m. – 11:20 a.m.
New Concepts and Fundamentals		
Fungal Genomics 2.4.1.2	**Scott Baker, Ph.D.** Pacific Northwest National Laboratory	11:20 a.m. – 11:50 a.m.
Lunch		11:50 a.m. – 1:00 p.m.
Feedstock Interface		
Overview of Feedstock Interface	**Gene Petersen** Golden Field Office	1:00 p.m. – 1:10 p.m.
Storage Systems, Feedstock Supply, etc. 2.1.1.1 and 2.1.1.3	**Gary Gresham**, INL **Nick Nagle**, NREL	1:10 p.m. – 1:40 p.m.
Extremophilic Microalgae: Advanced Lipid and Biomass Production for Biofuels and Bioproducts 2.1.1.6	**Brent M. Peyton, Ph.D.** Montana State University	1:40 p.m. – 2:05 p.m.
Improving Cost Effectiveness of Algae-Lipid and Biomass Production for Biofuels and Bioproducts 2.1.1.7	**KC Das, Ph.D., P.E.** University of Georgia Research Foundation, Incorporated	2:05 p.m. – 2:30 p.m.
Bioenergy Demonstration Project: Value-Added Products from Renewable Fuels 7.2.1.1	**Paul Blum, Ph.D.** University of Nebraska-Lincoln	2:30 p.m. – 2:55 p.m.

Afternoon Break	2:55 p.m. – 3:10 p.m.

Core Research and Development: Pretreatment and Hydrolysis	
Overview of Core Research and Development: Pretreatment and Hydrolysis **Christy Sterner** Golden Field Office	3:10 p.m. – 3:20 p.m.
Pretreatment and Enzymatic Hydrolysis 2.2.1.1 **Rick Elander** National Renewable Energy Laboratory	3:20 p.m. – 4:05 p.m.
Value Prior to Pulping 2.2.1.2 **Masood Akhtar, Ph.D. and Carl Miller, Ph.D.** CleanTech Partners, Incorporated	4:05 p.m. – 4:30 p.m.
Energy Corn Consortium 2.2.2.2 **Michael J. Blaylock, Ph.D.** Edenspace Systems Corporation	4:30 p.m. – 4:55 p.m.
Integration of Leading Biomass Pretreatment Technologies with Enzymatic Digestion and Hydrolyzate Fermentation 2.3.1.4 **Charles E. Wyman, Ph.D.** CAFI	4:55 p.m. – 5:20 p.m.

Day One Close	5:20 p.m.

April 15, 2009	
Biochemical Platform Review	Time
Core Research and Development: Pretreatment and Hydrolysis Continued	
Enzyme Solicitation Support and Validation 2.2.2.3 **James D. McMillan, Ph.D.** National Renewable Energy Laboratory	8:00 a.m. – 8:20 a.m.
Enhancing Cellulase Commercial Performance for the Lignocellulosic Biomass Industry 2.2.2.5 **Mike Arbige, Ph.D.** Danisco USA, Incorporated	8:20 a.m. –8:50 a.m.
Development of a Commercial Enzyme System for Lignocellulosic Biomass Saccharification 2.2.2.6 **Manoj Kumar, Ph.D.** DSM Innovation, Incorporated	8:50 a.m. – 9:20 a.m.
Project Decrease: Development of a Commercial-Ready Enzyme Application System for Ethanol 2.2.2.7 **Paul Harris, Ph.D** Novozymes, Incorporated	9:20 a.m. –9:50 a.m.

Morning Break	9:50 a.m. – 10:10 a.m.

Commercialization of Customized Cellulase Solutions for Biomass Saccharification 2.2.2.8 **Justin Stege, Ph.D.** Verenium Corporation	10:10 a.m. – 10:40 a.m.
Addressing the Recalcitrance of Cellulose Degredation through Cellulase Discovery, Nano-scale Elucidation of Molecular Mechanisms, and Kinetic Modeling 2.2.2.9 **Larry Walker, Ph.D.** Cornell University	10:40 a.m. – 11:05 a.m.

April 16, 2009		
Biochemical Platform Review		**Time**
Core Integrated Research and Development: Processing and Fermentation Continued		
A Novel Simultaneous-Saccharification-Fermentation Strategy for Efficient Cofermentation of C5 and C6 Sugars Using Native, non-GMO Yeasts 2.3.2.8	**Sasidhar Varanasi, Ph.D.** The University of Toledo	8:00 a.m. – 8:25 a.m.
Production of Higher Alcohol Liquid Biofuels via Acidogenic Digestion and Chemical Upgrading of Organic Industrial Wastes 2.3.3.1	**Peter van Walsum, Ph.D., P.E.** University of Maine	8:25a.m. – 8:50a.m.
BioEthanol Collaborative (SC) 7.2.3.1	**Mike Henson, Ph.D.** Clemson University	8:50 a.m. – 9:15 a.m.
New Concepts and Fundamentals		
Overview of New Concepts and Fundamentals	**Gene Petersen** Golden Field Office	9:15 a.m. – 9:25 a.m.
Targeted Conversion Research 2.4.1.1	**Mike Himmel, Ph.D.** National Renewable Energy Laboratory	9:25 a.m. – 10:10 a.m.
Lignin as a Facilitator, not a Barrier, during Saccharification by Brown Rot Fungi 2.4.1.3	**Jonathan Schilling, Ph.D.** University of Minnesota	10:10 a.m. – 10:35 a.m.
Ethanol Fuel Development 7.2.4.1	**Elizabeth Hood, Ph.D.** Arkansas State University	10:35 a.m. – 11:00 a.m.
Morning Break		11:00 a.m. – 11:10 a.m.
Co-Products		
Overview of Co-Products	**Christy Sterner** Golden Field Office	11:10 a.m. – 11:20 a.m.
Biofuel Production Initiative (SC) 7.4.1.2	**Dan Page** Claflin University	11:20 a.m. – 11:45 a.m.
Sustainable Energy Center Biodiesel from Algae (MI) 7.4.1.4	**John B. Miller, Ph.D.** Western Michigan University	11:45 a.m. – 12:10 p.m.
Lunch		12:10 p.m. – 1:15 p.m.
Bioeconomy Initiative 7.4.2.4	**Susanne Kleff, Ph.D.** MBI International	1:15 p.m. – 1:30 p.m.
Intermediary BioChemicals (MI) 7.4.2.6	**Dr. Doug Burdette** Intermediary BioChemicals (MI)	1:30 p.m. – 1:55 p.m.

Development of Applied Membrane Technology for Processing Ethanol from Biomass **7.4.5.2**	**Sudip Majumdar, Ph.D.** Compact Membrane Systems	**1:55 p.m. – 2:20 p.m.**
Snohomish County Biodiesel Project (WA) **7.4.1.6**	**Deanna Carveth** Snohomish County	**2:20 p.m. – 2:45 p.m.**
Connecticut Biodiesel Power Generator **7.4.3.7**	**Carla York and Robert Schmitz** The Greater New Haven Clean Cities Coalition, Inc.	**2:45 p.m. – 3:05 p.m.**

Platform Review Close	**3:30 p.m.**

Reviewer Discussion – CLOSED SESSION	**3:30 p.m. – 5:30 p.m.**

Attachment Four: Conversion Platform Review Attendees

Conversion Platform Review Attendees

First Name	Last Name	Organization
Andy	Aden	National Renewable Energy Laboratory
Pradeep	Agrawal	Georgia Tech Research Corporation
Carl	Anderson	Brookhaven National Laboratory
Michael	Arbige	Genencor, A Danisco Division
Suzanne	Atkinson	Navarro Research and Engineering, DOE Golden Field Office
Richard	Bain	National Renewable Energy Laboratory
Scott	Baker	Pacific Northwest National Laboratory
Robert	Bartek	KiOR Inc
William	Batchelor	Mississippi State University
Linda	Belte	Weyerhaeuser
Bryna	Berendzen	U.S. DOE
David	Berry	Flagship Ventures
Lindsay	Bixby	BCS, Incoprorated
Michael	Blaylock	Edenspace Systems Corporation
Peter	Bluford	Consultant to the Life Science Industry
Paul	Blum	University of Nebraska
Jim	Brainard	National Renewable Energy Laboratory
Tim	Brandvold	UOP LLC A Honeywell Company
Adam	Bratis	National Renewable Energy Laboratory
Craig	Brown	Catchlight Energy, LLC
Robert	Brown	Iowa State University
Alexander	Brown	Sandia National Labs
Daniel	Burciaga	ThermoChem Recovery Int'l, Inc.
Doug	Burdette	IBC Tech

Tom	Butcher	Brookhaven National Laboratory
Stewart	Campbell	Canadian Bioenergy Corporatoin
Cole	Carveth	Colorado School of Mines
Deanna	Carveth	Snohomish County Public Works
Chris	Cassidy	USDA
Jean-Marie	Chauvet	USDA Office of Energy Policy & New Uses
Singfoong	Cheah	National Renewable Energy Laboratory
Shulin	Chen	Washington State Univeristy
Senthil	Chinnasamy	University of Georgia
Mike	Cleary	National Renewable Energy Laboratory
Eric	Connor	ThermoChem Recovery Int'l, Inc.
Mike	Cotta	USDA-ARS
Kurt	Creamer	Novozymes
Stefan	Czernik	National Renewable Energy Laboratory
Keshav	Das	University of Georgia
Ryan	Davis	National Renewable Energy Laboratory
Mark	Davis	National Renewable Energy Laboratory
David	Dayton	RTI International
Roxanne	Dempsey	U.S. DOE
Neville	Dolan	Raceland Raw Sugar Corporation
Nancy	Dowe-Farmer	National Renewable Energy Laboratory
Steve	Duke	Auburn University
Abhijit	Dutta	National Renewable Energy Laboratory
David	Eakin	Pacific Northwest National Laboratory
Jane	Earley	Consulting
Rick	Elander	National Renewable Energy Laboratory

Douglas	Elliott	Pacific Northwest National Laboratory
Noureen	Faizee	Red Lion Bio-Energy
Calvin	Feik	National Renewable Energy Laboratory
Larry	Felix	Gas Technology Institute
Robert	Fireovid	USDA/Agricultural Research Service
Daniel	Fishman	BCS, Incoprorated
Gretchen	Fitzgerald	Navarro Research and Engineering, DOE Golden Field Office
Gary	Folkert	Cargill
Thomas	Foust	National Renewable Energy Laboratory
Nick	Frasier	Navarro Research and Engineering, DOE Golden Field Office
Jim	Frederick	National Renewable Energy Laboratory
Hiroyuki	Fukui	Toyota
Stephen	Gatto	BioEnergy International, LLC
Mark	Gerber	Pacific Northwest National Laboratory
Cindy	Gerk	National Renewable Energy Laboratory
Dr. Douglas	Goodale	SUNY Cobleskill
John	Gordon	Los Alamos National Laboratory
Johan Willem	Gosselink	Shell Global Solutions International
Paul	Grabowski	U.S. DOE
Garold	Gresham	Idaho National Laboratory
Raghubir	Gupta	RTI International
Neal	Gutterson	Mendel Biotechnology
Bonnie	Hames	Ceres, Inc
Molly	Hames	Navarro Research and Engineering, DOE Golden Field Office
Paul	Harris	Novozymes, Incorporated
J. Michael	Henson	Clemson University

Richard	Hess	Idaho National Laboratory
Mike	Himmel	National Renewable Energy Laboratory
William	Hitz	DuPont Company
Nancy	Ho	Purdue University
David	Hogsett	Mascoma
Elizabeth	Hood	Arkansas State University
John D.	Howard, III	Coronal, LLC
David	Hsu	National Renewable Energy Laboratory
Ryan	Hubbart	Power Ecalene Fuels, Inc.
George	Huber	University of Massachusetts at Amherst
Kristiina	Iisa	National Renewable Energy Laboratory
Whitney	Jablonski	National Renewable Energy Laboratory
Gene	Jackson	Power Ecalene Fuels, Inc.
Gene	Jackson	Power Ecalene Fuels, Inc.
Alisha	Jarnagin	Genencor, A Danisco Division
Edward	Jennings	National Renewable Energy Laboratory
Samuel	Jones	Iowa State University
Bruce	Jones	Minnesota State University
Susanne	Jones	Pacific Northwest National Laboratory
Mark	Jones	The Dow Chemical Company
Jay	Keller	Sandia National Laboratories
Ellyn	Kerr	Industrial Biotechnology / Mary Ann Liebert
George	Kervitsky	BCS, Incoprorated
Charles	Kinoshita	University of Hawaii
Susanne	Kleff	MBI International
Rick	Kleiner	PALL

Brian	Kneale	BP
Michael	Knotek	Knotek Scientific Consulting
Stephen	Korstad	Coronal, LLC
Curt	Krause	Chevron
Manoj	Kumar	DSM White Biotechnology
Mike	Lanahan	Agrivida Inc
Paul	Larsen	Power Ecalene Fuels, Inc.
Adeniyi	Lawal	Stevens Institute of Technology
Dennis	Leppin	Gas Technology Institute
Victor	Lin	Iowa State University
Ke	Liu	GE Global Research
Lance	Lobban	University of Oklahoma
Kim	Magrini	National Renewable Energy Laboratory
S	Majumdar	Compact Membrane Systems
Jonathan	Male	U.S. DOE
Richard	Mallinson	University of Oklahoma
Kyriakos	Maniatis	European Commission, DG TREN
John	McDermott	GE Global Research
Scott	McDonald	Archer Daniels Midland
Jim	McMillan	National Renewable Energy Laboratory
Josh	Messner	Navarro Research and Engineering, DOE Golden Field Office
John	Miller	Western Michigan University
John	Monks	DSM
Liz	Moore	Navarro Research and Engineering, DOE Golden Field Office
Jose	Moran-Mirabal	Cornell University
Nathan	Mosier	Purdue University

Nick	Nagle	National Renewable Energy Laboratory
Paul	Nikitovich	Bioenergy Investments, LLC
Mark	Nimlos	National Renewable Energy Laboratory
David	Nunn	Verenium
Nicole	Oester	Colorado School of Mines
Judy	Partin	Idaho National Laboratory
Michael	Penner	Oregon State University
Janice	Pero	BioEnergy International, LLC
Gene	Petersen	U.S. DOE
Brent	Peyton	Montana State University
Leslie	Pezzullo	U.S. DOE
Benjamin	Phillips	Emery Energy
Steve	Piccot	Southern Research
Frans	Plantenga	Albemarle
Larry	Prado	Innovation Drive/The Greater New Haven Clean Cities Coalition, Inc.
Jessica	Price	Navarro Research and Engineering, DOE Golden Field Office
Roger	Prince	ExxonMobil Biomedical Sciences, Inc
Alan	Propp	Merrick & Company
Elisabeth	Raleigh	New England Biolabs
Richard	Range	PALL
Valerie	Reed	U.S. DOE
Kinkead	Reiling	Amyris
Ronald	Reinsfelder	Shell Global Solutions (US) Inc.
Patricia	Relue	University of Toledo
John	Rezaiyan	Southern Research
Fabio	Ribeiro	Purdue University

Debbie	Sandor	National Renewable Energy Laboratory
John	Sawyer	PALL
Daniel	Schell	National Renewable Energy Laboratory
Jonathan	Schilling	University of Minnesota
Robert	Schmitz	Sabre Engineering, Inc.
Susan	Schoenung	Longitude 122 West, Inc.
Robert	Schuetzle	PRF
Amy	Schwab	National Renewable Energy Laboratory
Joaquim	Seabra	National Renewable Energy Laboratory
Miroslav	Sedlak	Purdue University
Chris	Shaddix	Sandia National Labs
Rishi	Shukla	Archer Daniels Midland Company
Joseph	Smith	Idaho National Laboratory
John	Smyth	King County, WA
Seth	Snyder	Argonnne National Laboratory
Michael	Sparby	Agricultural Utilization Research Institute
Don	Stafford	Lafarge Cement
Philip	Steele	Dept. of Forest Products, Mississippi State Unviersity
Bernie	Steele	MBI International
Justin	Stege	Verenium Corporation
Christy	Sterner	U.S. DOE
Don	Stevens	Pacific Northwest National Laboratory
Greg	Tamblyn	REII
Ling	Tao	National Renewable Energy Laboratory
Gene	Taylor	West Biofuels, LLC
Steve	Thomas	Ceres, Inc.

Brian	Trewyn	Iowa State University
Clifford	Unkefer	Los Alamos National Laboratory
G. Peter	van Walsum	University of Maine
Sasidhar	Varanasi	The University of Toledo
Sridhar	Viamajala	Utah State University
Larry	Walker	Cornell University
Wayne	Walker	Power Ecalene Fuels, Inc.
James	White	Battelle PNWD – PNNL
Mark	White	Mississippi State University
Tom	White	U.S. DOE
Kevin	Whitty	University of Utah
Robert	Wimmer	Toyota
Carl	Wolf	BCS, Incoprorated
Ed	Wolfrum	National Renewable Energy Laboratory
Mark	Wong	Agrivida Inc
Charles	Wyman	University of California Riverside
Joyce	Yang	U.S. DOE
Steve	Yanik	Mountain Climbing Consultants Co.(MCCC)
Bryan	Yeh	SAIC
Carla	York	Innovation Drive, Inc.
Matthew	Yung	National Renewable Energy Laboratory
Alan	Zacher	Pacific Northwest National Laboratory
Dustin	Zastrow	Mainstream Engineering
Ralph	Zee	Auburn University
Conrad	Zhang	KiOR Inc

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DECEMBER 2009

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