2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

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

2.6.1.2 Analysis for Production - Technical & Market

May 20, 2013

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Technology Area Review: Biochemical Conversion

Organization: Pacific Northwest National Laboratory

PNNL-SA-95160

Goal Statement

- Support the DOE Bioenergy Technologies Office's goal to **reduce the estimated mature technology processing cost** for converting cellulosic feedstocks to hydrocarbon fuels via biochemical and catalytic means by developing **analysis tools** and **techno-economics** to identify pathways with the potential to meet the $3/gallon gasoline equivalent target

- Perform analysis as requested by the Biochemical and Catalytic Conversion Technology Area

Quad Chart Overview

Timeline

- Project start date: FY03
- Project end date: FY15
- FY11-FY12 100% complete, FY13 50% complete

Budget

- Funding for FY11: $250k
- Funding for FY12: $270k
- Funding for FY13: $190k
- 11 years funding at an average of $328k/year

Barriers

- At-A. Lack of Comparable, Transparent, and Reproducible Analysis
- Bt-J. Catalyst Development
- Bt-K. Biochemical Conversion Process Integration

Partners

- INL for modeled feedstock costs
- NREL for consistent modeling assumptions and work on Tech Memos
- Project management – communication as needed with other labs, and DOE

Project Overview

History

- FY03-07: Products focus -“Top 10” products from biomass report, Products from lignin report, Co-products analysis in a biorefinery
- FY08-11: Economics of ethanol production via fungal organisms
- FY10-12: Economics of co-products, economics of microbial production of hydrocarbon compatible fuels
- FY13: Preliminary metabolic modeling of oleaginous organisms to support understanding of hydrocarbon production

Context

- BETO’s portfolio has expanded to include hydrocarbon fuels
- Need for preliminary analysis of candidate technology pathways

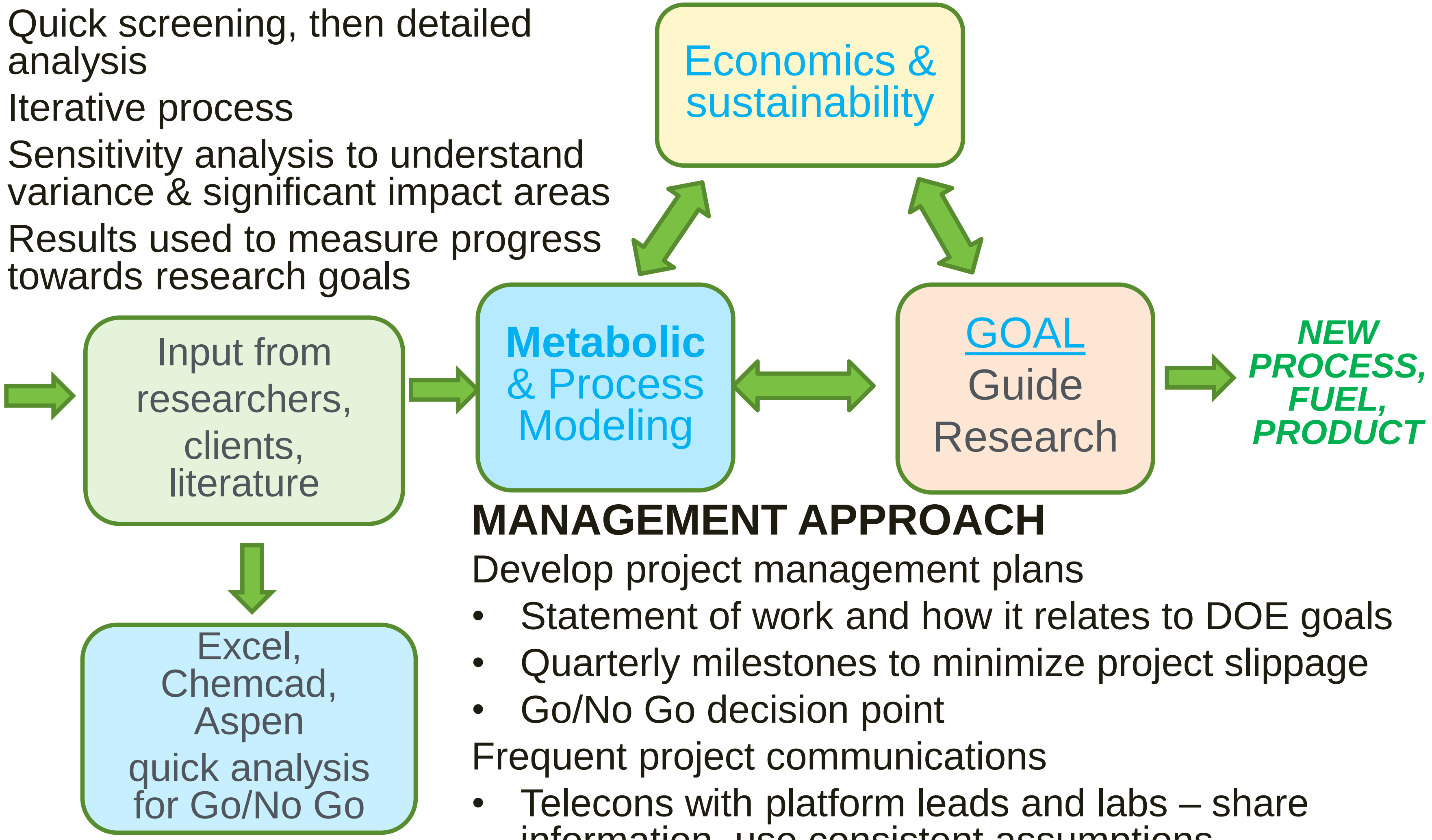
Objective

Focusing on fungal processing, the objective is to provide economics and gap analysis in support of the BETO goals to enable the production of renewable transportation fuels, particularly, gasoline, diesel and jet blendstocks

Approach

TECHNICAL APPROACH

- Quick screening, then detailed analysis
- Iterative process
- Sensitivity analysis to understand variance & significant impact areas
- Results used to measure progress towards research goals



Economics & sustainability

Input from researchers, clients, literature

Metabolic & Process Modeling

GOAL Guide Research

NEW PROCESS, FUEL, PRODUCT

Excel, Chemcad, Aspen quick analysis for Go/No Go

MANAGEMENT APPROACH

Develop project management plans

- Statement of work and how it relates to DOE goals
- Quarterly milestones to minimize project slippage
- Go/No Go decision point

Frequent project communications

- Telecons with platform leads and labs – share information, use consistent assumptions
- Quarterly formal reporting to HQ

Technical Accomplishments / Progress / Results

This project has three separate analysis tasks:

- **Task 1: Screening economics for production of co-products in an ethanol biorefinery (FY11&12)**
  - Potential chemical candidates
  - Avoidance of chemical market saturation

- **Task 2: Screening economics for hydrocarbon production through an oleaginous organism and determination of maximum practical yield (FY12 &13)**
  - Preliminary economic model
  - Metabolic modeling coupled with experimental work (from a separate project) to inform the economic model

- **Task 3: This project also partially funded the analysis and document preparation for the BETO pathways analysis for sugars conversion (FY13)**

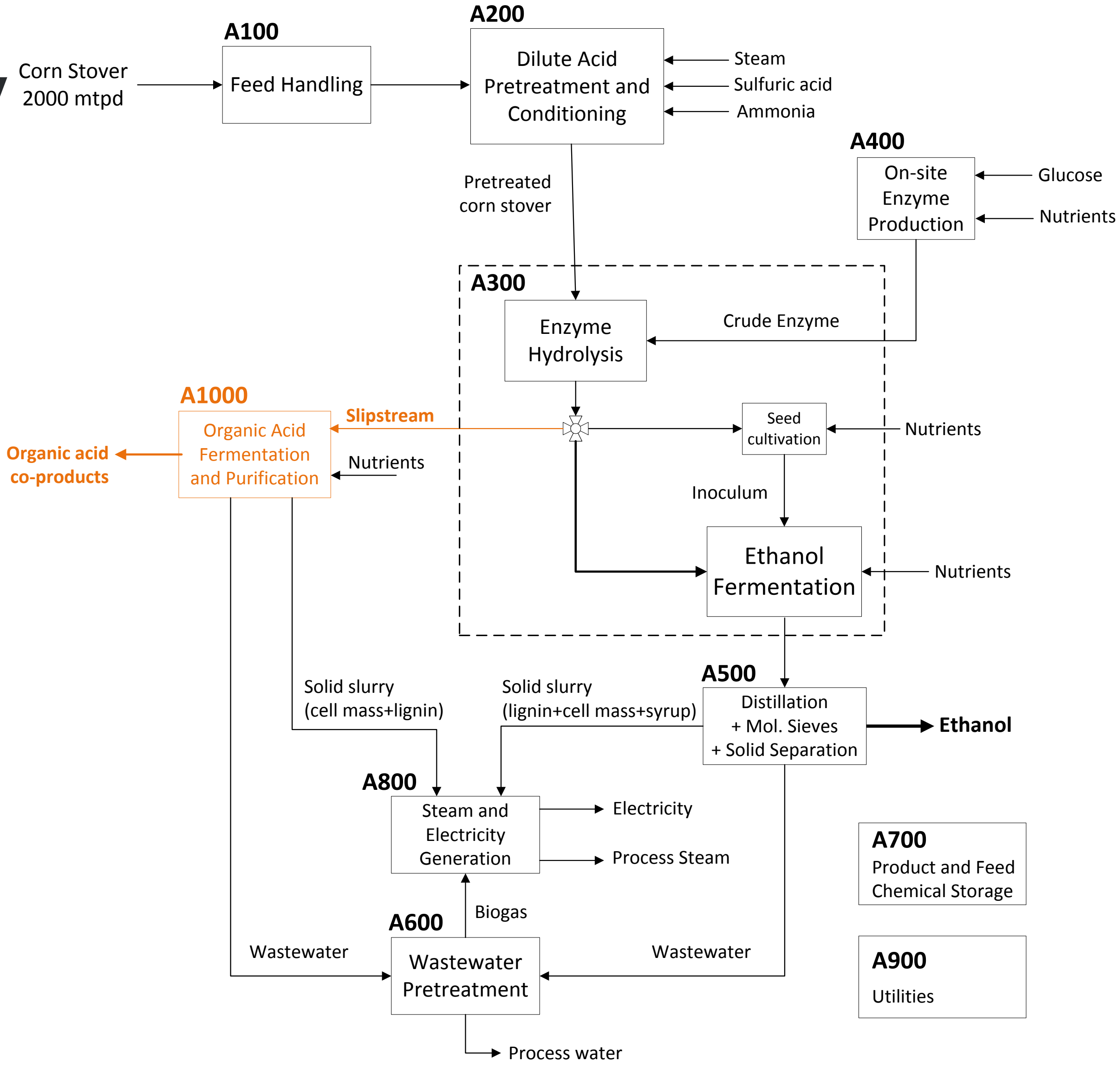
Technical Accomplishments
Task 1

TASK 1: Co-Products in an Ethanol Refinery

Enzymatic hydrolyzate slipstream (10-20%) used for co-product production. Four non-food, non-pharmaceutical organic acids produced.

Acids produced in blocked continuous fashion. Equipment added for chemical production and storage.

Basis: 2011 Biochemical Ethanol Report (Humbird et al) and organic acid conversion data from literature. Analysis in 2007$.



Technical Accomplishments
Task 1 - Results

Acids chosen as examples of good match between market size and productivities

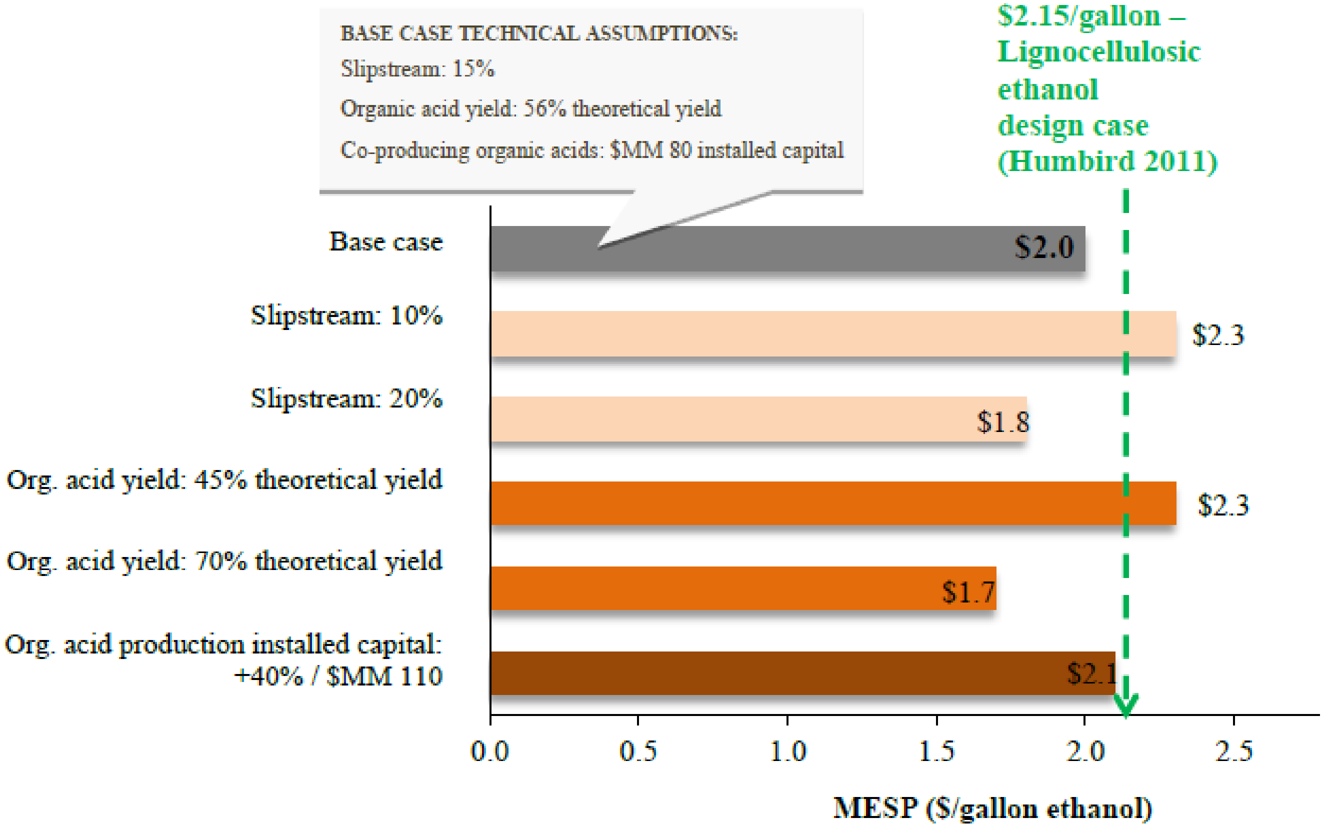
Ethanol production	52 mm gal/yr		
Ethanol Yield per feedstock	67 gal/dry ton		
Total co-product production	77 mm lb/year		
Citric acid:	20.1 10^6 lb/yr	Itaconic acid:	13.5 x 10^6 lb/yr
Lactic acid:	18.8 10^6 lb/yr	Succinic acid:	24.6 x 10^6 lb/yr

Key Outcomes:

10-20% hydrolysate slipstream diverted to chemicals production has potential to reduce ethanol costs by $0.15-0.45/gal

Additional capital offset by co-product value

Multiple co-products prevent market saturation



BASE CASE TECHNICAL ASSUMPTIONS:

Slipstream: 15%

Organic acid yield: 56% theoretical yield

Co-producing organic acids: $MM 80 installed capital

$2.15/gallon – Lignocellulosic ethanol design case (Humbird 2011)

Technical Accomplishments
Task 2

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Task 2: Hydrocarbons from Oleaginous Yeast

Basis: 2011 NREL Biochem Ethanol Report. 2011$

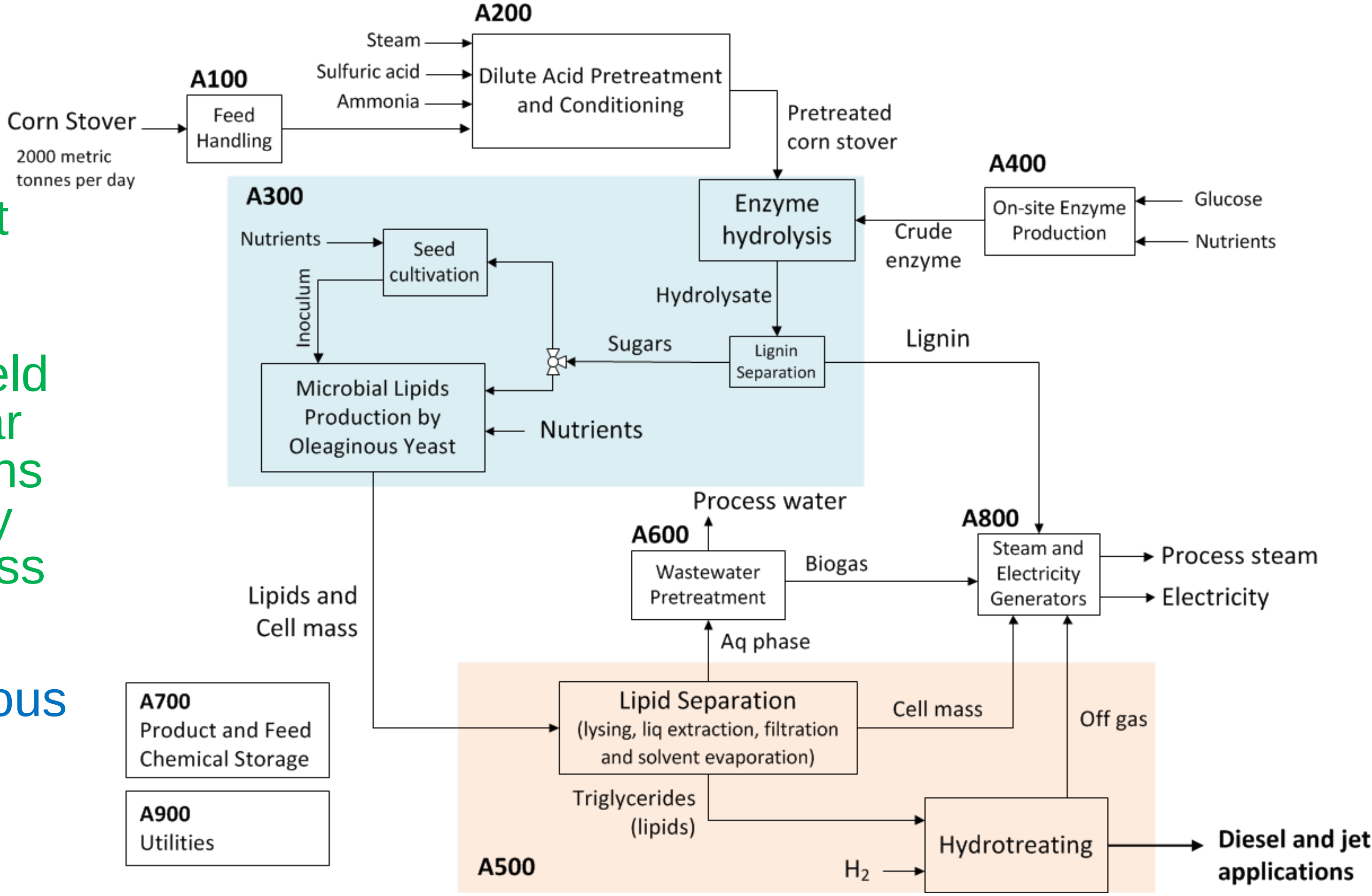
Yields and product recovery from literature:

Base case lipid yield = 0.17 g oil/g sugar produces 25 gallons of hydrocarbon/dry short ton of biomass

Relevance:

1st look at oleaginous yeast pathway

Model will be updated in FY13 with experimental results



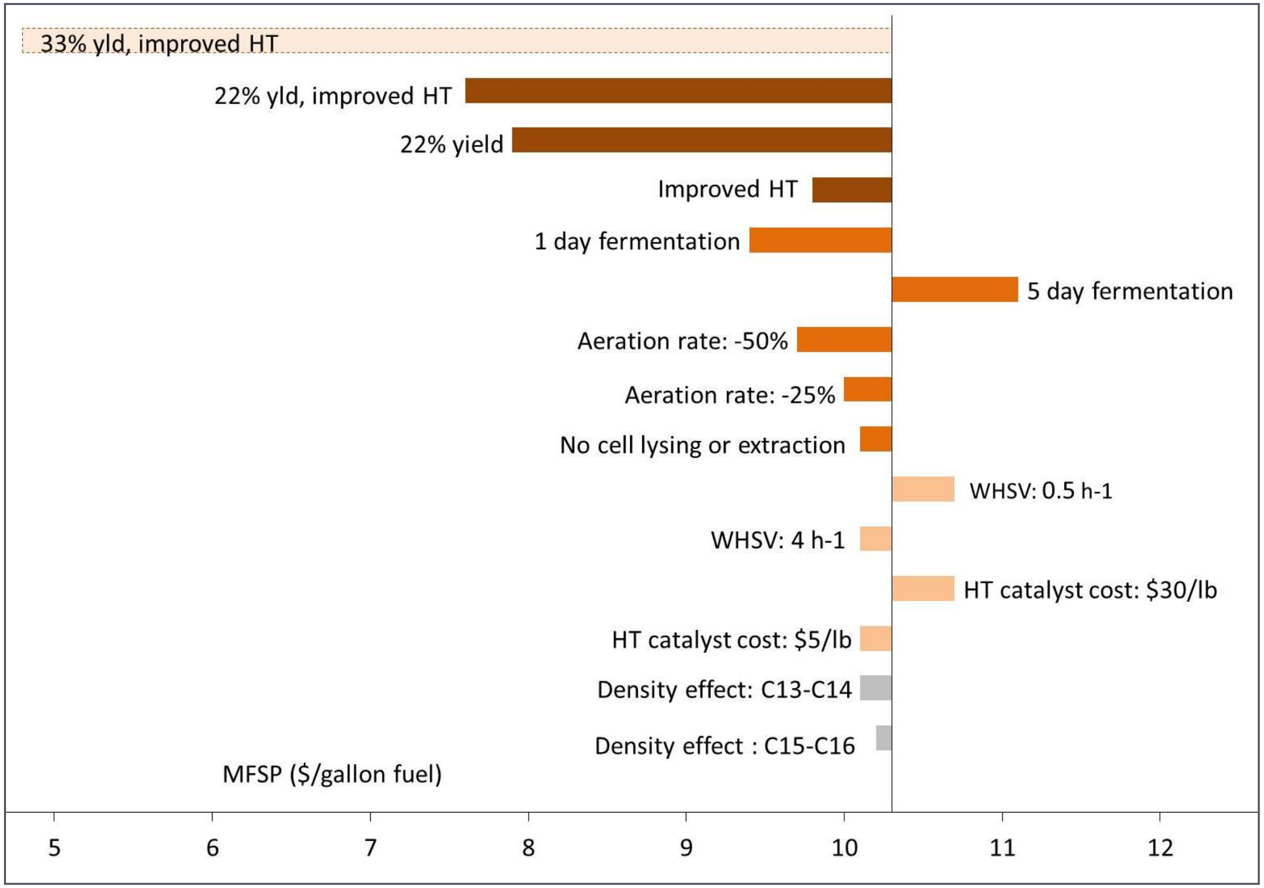
Technical Accomplishments
Task 2 - Results

Improvements needed to reach $5/gal:

- Improve lipid yield
- Increase tolerance to inhibitors (such as substrates, intermediates and products)
- Reduce aeration & nutrients
- Reduce fermentation time
- Auto-release triglyceride
- Produce products in middle distillate range, e.g. C_{14}

Lignin usage for fuel or products needed to reach $3/gal

Literature Based Screening Economics



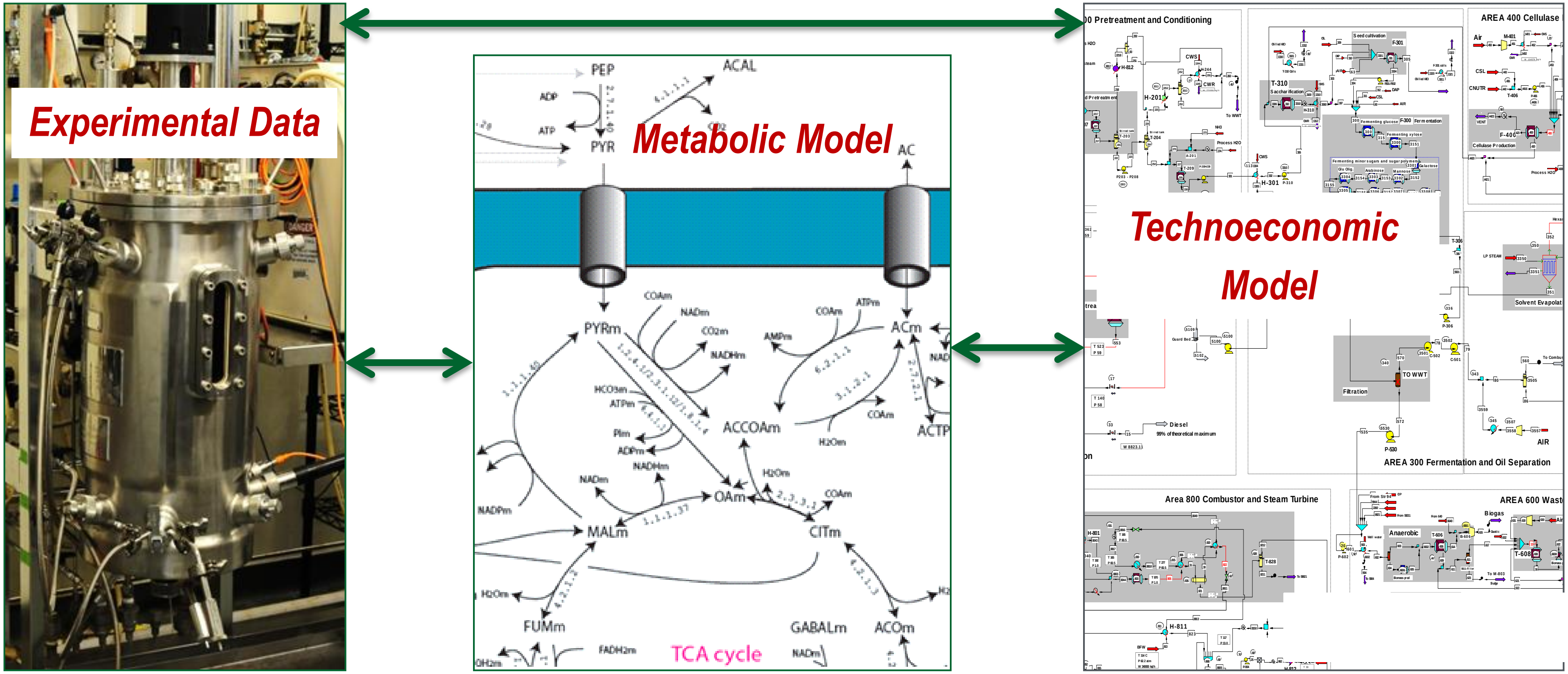
HT = hydrotreating, WHSV=HT weight hourly space velocity

Conclusions: yield and carbon usage dominate costs – FY13 modeling and experimental work will provide directions for improvement

Technical Accomplishments Task 2 (FY13)

Task 2 – Oleaginous Yeast Metabolic Model *Lipomyces starkeyi*

- Goal: improve modeled economics – what are the practical yield limits?
- Approach: combine process modeling & economics, metabolic engineering with experimental work
- Leverages published yeast models to reconstruct *L. starkeyi* metabolism
- 30-Liter bioreactor data from 2.4.1.2 informs both metabolics and economics

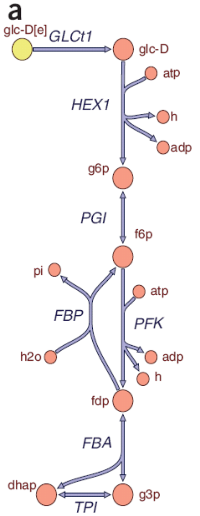


Technical Accomplishments
Task 2 (FY13)

Metabolic Network Modeling Methods

- Constraint-based reconstruction and analysis (COBRA) approach is being used.
- COBRA integrates metabolic network stoichiometry into a system of linear equations.
- The COBRA Toolbox provides a MATLAB-based simulation environment for predicting optimal distributions of mass fluxes within the metabolic network that maximize an objective function (typically, cell growth and/or specific metabolite production).
- The stoichiometric matrix and constraints on nutrients and excreted metabolites (from experimental data) determine the flux predictions (such as for lipid accumulation).

Network → Reactions and Genes → Stoichiometric matrix

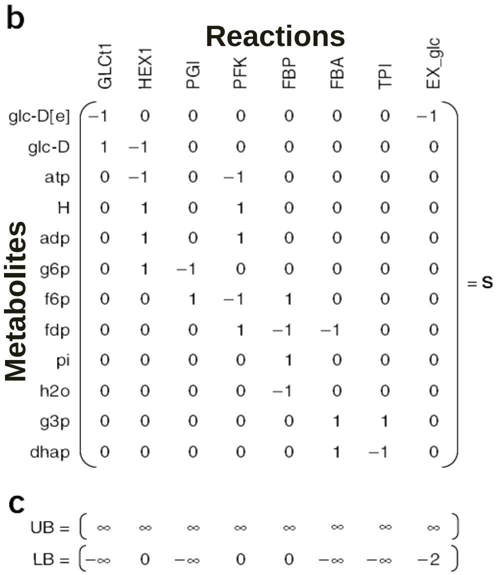


atp[c] + D-glucose[c] -> h[c] + g6p[c] + adp[c]
(YALI0B22308g or YALI0E15488g or YALI0E20207g)

g6p[c] <=> f6p[c]
YALI0F07711g

atp[c] + f6p[c] -> h[c] + fdp[c] + adp[c]
YALI0D16357g

fdp[c] <=> g3p[c] + dhap[c]
YALI0E26004g



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Reactions

Metabolites

$$\begin{array}{c|cccccccc} & \text{GLCt1} & \text{HEX1} & \text{PGI} & \text{PFK} & \text{FBP} & \text{FBA} & \text{TPI} & \text{EX\_glc} \\ \hline \text{glc-D[e]} & -1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ \text{glc-D} & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ \text{atp} & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 0 \\ \text{H} & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ \text{adp} & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 \\ \text{g6p} & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 \\ \text{f6p} & 0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 \\ \text{fdp} & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 \\ \text{pi} & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ \text{h2o} & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ \text{g3p} & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 \\ \text{dhap} & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 \end{array} = \mathbf{S}$$

c

$$\text{UB} = \begin{pmatrix} \infty & \infty & \infty & \infty & \infty & \infty & \infty & \infty \end{pmatrix}$$

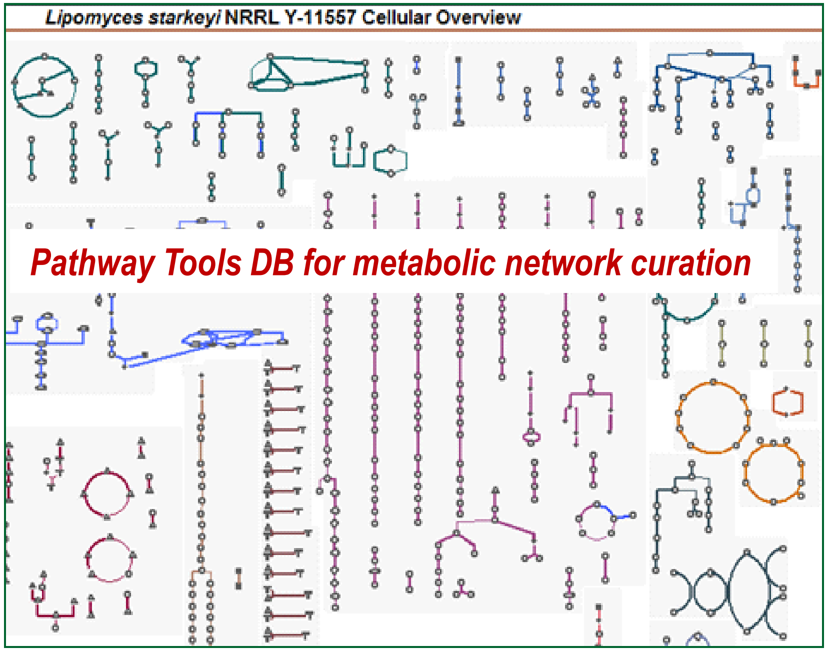
$$\text{LB} = \begin{pmatrix} -\infty & 0 & -\infty & 0 & 0 & -\infty & -\infty & -2 \end{pmatrix}$$

- Becker SA, Feist AM, Mo ML, Hannum G, Palsson BØ, Herrgard MJ. Nature Protocols 2007;2(3):727-38.
- Schellenberger J, Que R, *et al.*, Nature Protocols. 2011 Aug 4;6(9):1290-307.

Technical Accomplishments Task 2 (FY13)

L. starkeyi Metabolic Modeling Progress to Date

- **Developed Pathway Tools database:** organizes *L. starkeyi* genes, enzymes, reactions, and metabolites for metabolic network curation
- **Leveraged published models**: Orthologous Markov Clustering matched *L. starkeyi* enzymes with enzymatic reactions within previously published COBRA models for the yeasts: *Y. lipolytica, S. cerevisiae, S. stipitis,* and *P. pastoris*



Pathway Tools DB for metabolic network curation

Next Steps: Finish integration of preliminary metabolic network and new experimental into full COBRA model for *L. starkeyi* to

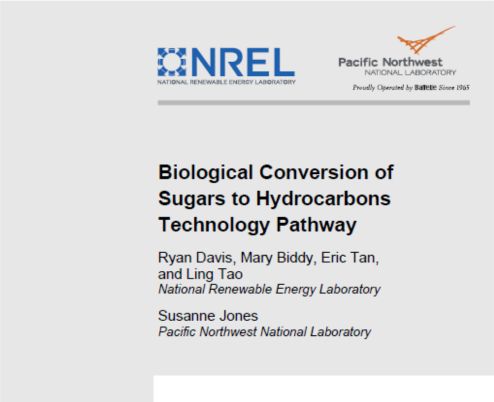
- Predict optimal carbon yield
- Guide metabolic engineering

Key Project Outcome: Enable timely progress towards solving the complex problem of maximizing carbon yield to fuels

Technical Accomplishments Task 3

Task 3 Pathways Analysis

- FY12 NREL and PNNL collaboration to perform analysis for hydrocarbon biofuels pathways
- Integrated efforts with INL for feedstock interface and costs
- Vetted assumptions and basis with researchers and scientist for pathways
- DOE BETO selected 2 sugars based pathways for further development; published as Tech Memos



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Biological Conversion of Sugars to Hydrocarbons Technology Pathway

Ryan Davis, Mary Biddy, Eric Tan, and Ling Tao
National Renewable Energy Laboratory

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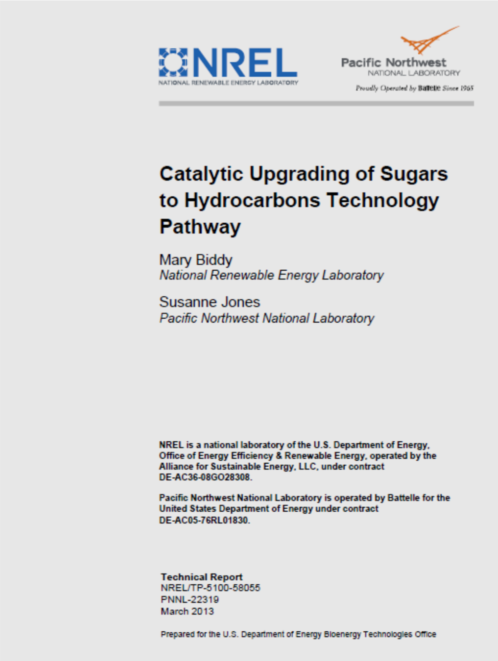

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Catalytic Upgrading of Sugars to Hydrocarbons Technology Pathway

Mary Biddy
National Renewable Energy Laboratory

Susanne Jones
Pacific Northwest National Laboratory

NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency & Renewable Energy, operated by the Alliance for Sustainable Energy, LLC, under contract DE-AC36-08GO28308.

Pacific Northwest National Laboratory is operated by Battelle for the United States Department of Energy under contract DE-AC05-76RL01830.

Technical Report
NREL/TP-5100-58055
PNNL-22319
March 2013

Prepared for the U.S. Department of Energy Bioenergy Technologies Office

Milestones/Metrics and Progress

Title/Description	Due Date	Completed
Complete Screening for co-product routes that meet the 2012 Reference Case target of $2/gal of Ethanol	31 Dec 2011	✓
Completed model for fungal base co-products production with goals to meet the 2012 target of $2/gal Ethanol	31 Mar 2012	✓
Complete model for hydrocarbon production via biochemical route to assess potential for meeting the 2017 target of $3/gal gasoline	30 Jun 2012	✓
Complete sensitivity analysis for both cases to identify research needs which meet reference case targets	30 Sep 2012	✓
Complete the generation of a pathway-genome database for *L. starkeyi*, comparative analysis with other members of the *Saccharomycotina* fungi, and draft metabolic network reconstruction	31 Dec 2012	✓
Complete refinement of *L. starkeyi* central metabolism pathways	31 Mar 2013	✓
Complete refinement of *L. starkeyi* fatty acid synthesis pathways	30 Jun 2013	On schedule
Complete preliminary TEA for *L. starkeyi* based process and improvements leading to $3/gal gasoline equivalent	30 Sep 2013	On schedule
Complete analysis reports and models, create draft manuscript	30 Sep 2013	On schedule

Relevance

- Directly supports the Bioenergy Technologies Office development of new pathways and technical targets for the 2017 to 2022 timeframe

- Helps DOE and industry understand critical issues related to hydrocarbon production

- Enables the Biochemical Conversion Area’s strategic goal “*to develop commercially viable technologies for converting biomass feedstocks via biochemical routes into energy dense, fungible liquid transportation fuels*, *as well as bioproducts or chemical intermediates, and bioenergy.*”

Critical Success Factors

Hydrocarbon fuel production from biological processes requires a combination of tools to **successfully direct** and **accelerate future research** and **ultimately reduce costs**

To be successful, our metabolic model for the oleaginous yeast *L. starkeyi* must be able to:

- Integrate the **best available data** to predict the **maximum** metabolically feasible **lipid yield** from the wild-type strain
- **Identify targets** for modifying the metabolism of the wild type strain that **raise the lipid yield** nearer to the theoretical maximum of 33%, thus **reducing the cost** of cellulosic diesel towards the BETO goal of $3/gallon gasoline equivalent

Future Work

ML, DL or Go/No Go	Description	FY13 Q3	FY13 Q4	FY14 Q1	FY14 Q2	FY14 Q3	FY14 Q4
M.1.ML.3	Theoretical Yield Estimates & Economics: Refine fatty acid synthesis pathways	🟩					
M.1.ML.4	Theoretical Yield Estimates & Economics: Complete updated TEA for *L starkeyi*		🟩				
M.1.DL.1	Theoretical Yield Estimates & Economics: Final Report		🟩				
N.1.ML.1	Theoretical Yield Estimates & Economics: exercise models to direct next research steps			🟩			
N.1.ML.2	Theoretical Yield Estimates & Economics: assess experimental results for model use				🟩		
N.1.ML.3	Theoretical Yield Estimates & Economics: refine models					🟩	
N.1.DL.4	Theoretical Yield Estimates & Economics: Final Report						🟩
N.1.GN.1	Go-no go decision						🟩

Overall:

- Continue collaboration with experimentalists to refine metabolic and economic models and model outcomes
- Develop understanding of yield limits on glucose and eventually, complex substrates
- Develop strategies to control unwanted side reactions (e.g. secreted exopolysaccharides that increase broth viscosity)

Summary

Approach: Combine multiple strengths: economics, metabolics, experimental

Technical accomplishments:

- Completed economic analysis of a co-product scheme
- Completed literature based process model for hydrocarbon production
- Developing metabolic model for *L. starkeyi*
- Published (with NREL) 2 Tech Memos for sugars conversion

Relevance: Supports BETO goal of economic hydrocarbon fuels from sugar

Critical Success factors and challenges:

- Obtaining sufficient experimental data for metabolic & economic models
- Deployment of metabolic & economic model to influence research directions

Future Work:

- Leverage *L. starkeyi* experimental work to improve models
- Exercise metabolic and process models to suggest research directions

Technology transfer: Publication of results

Acknowledgements

Bioenergy Technologies Office – Leslie Pezzullo, Joyce Yang, Bryna Berendzen, Katy Christiansen

PNNL Team – Aye Meyer, Iva Tews, Jim Collett

NREL – Mary Biddy

Additional Slides

Responses to Previous Reviewers' Comments

2011 Reviewer Comments

- The analysis provides a useful decision making and analysis tool to assist researchers. For the long term planning [2012-2017] the assumptions the models are based upon a most likely highly speculative since little experimental information exists on many of these. Rigid adherence to these could lead to the adoption of wrong research paths to pursue as well as elimination of potentially promising avenues of investigation.
- This type of project reminds me of the chicken or egg controversy - of which came first. The project seems necessary in order to determine which avenues are feasible and worth further consideration, i.e. funding to generate more data. On the other hand, it is difficult to make realistic determinations of "feasibility" when the data available on a given topic is sparse. Hence, I see the project as important but the focus must be on systems/processes for which there is considerable data upon which to make judgments
- The researcher may want to avail them self of work done in other institutions such as Stanford Research Institute which may be very beneficial. With this I believe the bioinformatics would be more firmly based.

Response to review comments

The reviewers generally recognize the need to establish some criteria for determining research goals and measuring progress towards goals, but regretted the lack of experimental data and reliance upon sparse literature information. To remedy this, the economic analysis for hydrocarbon production is beginning to be supplemented with the development of metabolic modeling and experimental results aimed at informing both the economic and the metabolic models.

As per the reviewers' suggestion, we have employed the Pathway Tools bioinformatics software package from SRI (Stanford Research Institute) to integrate genomic sequence data and biochemical literature information into highly curated metabolic pathways. We are assembling these pathways into a process model of the stoichiometric reaction network within the oleaginous yeast *Lipomyces starkeyi* to inform techno-economic analysis and to identify metabolic engineering strategies that will reduce the cost of cellulosic hydrocarbon fuels toward the BETO goal of $3/gallon.

Publications, Presentations, and Commercialization

Publications:

FY13: PA Meyer, IJ Tews, JK Magnuson, SA Karagiosis and SB Jones, “Techno-economic analysis of corn stover fungal fermentation to ethanol”. Accepted for publication by Applied Energy

FY13: Biological Conversion of Sugars to Hydrocarbons Technology Pathway
http://www.nrel.gov/docs/fy13osti/58054.pdf
http://www.pnl.gov/main/publications/external/technical_reports/PNNL-22318.pdf

FY13: Catalytic Upgrading of Sugars to Hydrocarbons Technology Pathway
http://www.nrel.gov/docs/fy13osti/58055.pdf
http://www.pnl.gov/main/publications/external/technical_reports/PNNL-22319.pdf

Presentations:

FY11: A poster entitled “Novel Microbial Conversion of Biomass to Hydrocarbon-Compatible Fuels: A Survey” was presented at the 33rd Symposium on Biotechnology for Fuels and Chemicals in early May.

FY12: Iva Tews presented a summary of the PNNL analysis work on Biochemical Pathways to Fuels at the Recent Advances in Fermentation Technology (RAFT IX) Conference entitled “A Techno-economic Assessment of Cellulosic Bio-Conversion Routes: Ethanol and Beyond.” November 2011

FY12: Pimphan (Aye) Meyer ACS National Spring Meeting March 2012, San Diego CA. "A Techno-economic Analysis for Ethanol Production from Corn Stover by Fungal Fermentation".