(3)

Gene Peterson

PMC-EF2a
(2.06.02)

U.S. DEPARTMENT OF ENERGY
EERE PROJECT MANAGEMENT CENTER
NEPA DETERMINATION

RECIPIENT: Clemson University **STATE:** SC

PROJECT TITLE : BioEthanol Collaborative

Funding Opportunity Announcement Number	Procurement Instrument Number	NEPA Control Number	CID Number
Not applicable	DE-FG36-08GO88071	GFO-08-140a	GO0

Based on my review of the information concerning the proposed action, as NEPA Compliance Officer (authorized under DOE Order 451.1A), I have made the following determination:

CX, EA, EIS APPENDIX AND NUMBER:
Description:

B3.6 Siting, construction (or modification), operation, and decommissioning of facilities for indoor bench-scale research projects and conventional laboratory operations (for example, preparation of chemical standards and sample analysis); small-scale research and development projects; and small-scale pilot projects (generally less than two years) conducted to verify a concept before demonstration actions. Construction (or modification) will be within or contiguous to an already developed area (where active utilities and currently used roads are readily accessible).

Rational for determination:

Clemson University (Clemson) would demonstrate the feasibility in developing switchgrass and sorghum varieties in South Carolina and the Southeast as a biofuel. This project was previously funded with GFO-08-140 on June 13, 2008 with a CX3.6. Laboratory work would take place at the existing Clemson Biosystems Research Complex in Clemson, South Carolina, and the Aiken Country Technology Laboratory (ACTL) in the Savannah River Research Campus, in Aiken, South Carolina. The project is divided into two tasks for Part A and two tasks for Part B:

PART A: APPROACH/METHODOLOGY
Task 1.0: Evaluate selected biomass pretreatment and hydrolysis technologies for sweet sorghum. (Only stalks of the plants would be tested.)
Utilizing an ammonia pretreatment and recovery technology optimized in the past year, Clemson and SRNL would advance the applied research from switchgrass to sweet sorghum stalks. This technology pretreats the cellulosic material remaining after C12 sugars are extracted, removing the lignin and converting the remaining celluloses and hemicelluloses into a form suitable for effective hydrolysis. Advanced ammonia pretreatment and recovery techniques show considerable promise to opening the lignocellulosic structure without creation of enzymatic or fermentation inhibitors. The remaining polysaccharide structure is reduced into convertible C5 and C6 monomers. Collaborative efforts with Dyadic International would utilize a suite of fungal expressed enzymes to convert the polysaccharide structures. After rinsing of pretreated samples to remove lignin, the mixtures would be tested for hydrolysis potential with novel enzyme mixtures obtained from Dyadic, Inc and compared to existing commercial enzyme mixtures to assess their potential. This is a technology that has potential applications in a variety of cellulosic conversions including woody biomass and other renewable sources.
Genetically modified organisms (GMOs) used are exempt from NIH Guidelines and registration with the Institutional Biosafety Committee under Sections III-F-3, III-F-5, and III-F-6 of Section III-F Exempt Experiments, NIH Guidelines for Research Involving Recombinant DNA Molecules, April 2002. The GMOs used in this work would be kept in the laboratory or greenhouse.
Task 2.0 Develop fermentation process for pretreated and hydrolyzed switchgrass feedstock(s)
Following on the work from last year's project, fermentation cultures would be developed using switchgrass pretreated and hydrolyzed feedstocks as the carbon source. These cultures would include traits for fermenting both C5 and C6 sugars either in mixed culture, separate culture or within a single organism (e.g. E. coli KO11, K. marxianus) and yeast (Saccharomyces bayanus) organisms. Fermentations would be preformed using a wide variety of target organisms. The high performing organisms would then be tested at 5.0 L bench-scale to characterize initial performance. The final milestone would include the fermentation organisms and schemes that are best suited for the specific feedstock varieties.

Task number: 2.1 Select or develop fermentative cultures for switchgrass pretreated and hydrolyzed feed stocks.

Determine the most effective approach to ferment C5 and C6 sugars simultaneously.

1. Planned Activities: Select or develop fermentative cultures for switchgrass pretreated and hydrolyzed feed stocks. Determine the most effective approach to convert C5 and C6 sugars simultaneously to ethanol.

Initial ethanol production studies from C6 and C5 sugars would be conducted on the selected organisms. Direct comparisons would be made with regards to productivity. Analytical equipment required for these studies is available to quantify glucose, xylose, acetate, and ethanol concentrations. Methods conforming to NREL standards are being used. Experiments have been designed to obtain time series concentration levels, such the consumption and production levels can be determined and compared.

Bioreactor configuration and general operational parameters were established based on existing literature. Preliminary assessment directs the use of a 2-stage bioprocess. This configuration would use a first-stage that enzymatically converts xylose to xylulose with a second-stage that mesophilically converts both glucose and xylulose to ethanol (Enzyme/Fermentation ethanol production). The very high ethanol concentrations are expected in second stage.

Studies with the organism S. bayanus would be completed to determine the kinetics for glucose (C6) and xylulose (C5) conversion to ethanol in batch and fed-batch reactors.

Initially, S. bayanus would be cultured in a minimal synthetic media containing glucose at different concentrations to obtain baseline kinetic data. Data to be obtained would include growth rates on the single sugars for the exponential growth phase; substrate levels with time; and ethanol production with time. Biomass levels would be correlated to dry cell mass per liter. Yields and final conditions would be determined. The lag phase times would be recorded to assess adaption to new growth conditions. Any major side products observed would be assessed if determined to impact productivity. Ethanol inhibition on growth and yields would be investigated by spiking ethanol into the media.

The same experimental plan used for the single sugars would be used for mixed sugar studies. Glucose and xylulose would be the mixed sugars, and potentially glucose/xylose would be examined depending on some preliminary xylose growth studies that are on-going. Ratios of the sugars (glucose and xylulose) would be similar to what is expected from the enzymatically prepared pre-treated switchgrass (40:25). Kinetics and yield on the mix sugar substrates would be compared to the single sugar data. Additionally, pre-treated switchgrass would be investigated as the sugar substrate source. Kinetics and yields would be determined and compared to the single sugar and mixed sugar studies to determine the effects of the non-quantified material in the pre-treated switchgrass.

Data formats to be obtained would include: Yields [Biomass (g dcw / g substrate) and Ethanol (g ethanol / g substrate)]; Final Concentrations [Biomass (g dcw/L), Substrates (g/L), Other side products (g/L), and Ethanol (g/L)]; and Kinetics [Biomass kinetics (growth rates for exponential phase, □ (h-1)), Substrate consumption rates (g/L□h), and Ethanol production (g/L□h)].

PART B: PROJECT RESEARCH STAGE
EXPECTED OUTCOMES:
Task 1.0: Optimization of ammonia pretreatment and recovery techniques to advance enzymatic hydrolysis with conversion rates of total available C5 and C6 of greater that 70% for switchgrass and sweet sorghum.
Task 2.0: Optimization of C5 and C6 fermentation to ethanol achieving greater than 80% total conversion rate for switchgrass.

Clemson and ACTL claim no additional permits are needed and there would be no generation of air emissions associated with this work. Vented gas cabinets and fume hoods are used with scrubbers to prevent release of air pollutants. Clemson and ACTL claim that all hazardous/toxic/effluent waste is disposed of according to university, local, state, and federals regulations as appropriate. According to Clemson, a Chemical Hygiene Plan, waste disposal, and safety protocols are in place monitored by the university's Environmental Health and Safety office. According to ACTL, an Integrated Safety Management System and Residue Management and guidance are in place that meets DOE and OSHA regulations.

Based on the information above, this project's impacts to the human and natural environment can be deemed less than significant and this project would qualify for Categorical Exclusions B3.6.

NEPA PROVISION

DOE has made a final NEPA determination for this award

Insert the following language in the award:

Note to Specialist :

none

SIGNATURE OF THIS MEMORANDUM CONSTITUTES A RECORD OF THIS DECISION.

NEPA Compliance Officer Signature: _____ Date: 11/23/09
NEPA Compliance Officer

FIELD OFFICE MANAGER DETERMINATION

☐ Field Office Manager review required

NCO REQUESTS THE FIELD OFFICE MANAGER REVIEW FOR THE FOLLOWING REASON:

☐ Proposed action fits within a categorical exclusion but involves a high profile or controversial issue that warrants Field Office Manager's attention.
☐ Proposed action falls within an EA or EIS category and therefore requires Field Office Manager's review and determination.

BASED ON MY REVIEW I CONCUR WITH THE DETERMINATION OF THE NCO :

Field Office Manager's Signature: _____ Date: _____
Field Office Manager