Final Scientific Report

Project Title: Value Added Products from Renewable Biofuels

Award Number: DE-FG36-08GO88055 / A000

Recipient: University of Nebraska – Lincoln

Project Location: Rooms E223/E156/E138 in Beadle Center, 19th and Vine St. Lincoln, NE 68588-0666.

Date of Report: July 31, 2014

Project PI: Paul Blum

Confidentiality: No intelletual property disclosed in the report

Excecutive Summary

Cellulosic ethanol is an emerging biofuel that will make strong contributions to American domestic energy needs. In the US midwest the standard method for pretreatment of biomass uses hot acid to deconstruct lignocellulose. While other methods work, they are not in common use. Therefore it is necessary to work within this context to achieve process improvements and reductions in biofuel cost. Technology underlying this process could supplement and even replace commodity enzymes with engineered microbes to convert biomass-derived lignocellulose feedstocks into biofuels and value-added chemicals. The approach that was used here was based on consolidated bioprocessing. Thermoacidophilic microbes belonging to the Domain Archaea were evaluated and modfied to promote deconvolution and saccharification of lignocellulose. Biomass pretreatment (hot acid) was combined with fermentation using an extremely thermoacidophilic microbial platform. The identity and fate of released sugars was controlled using metabolic blocks combined with added biochemical traits where needed. LC/MS analysis supported through the newly established Nebraska Bioenergy Facility provided general support for bioenergy researchers at the University of Nebraska. The primary project strategy was to use microbes that naturally flourish in hot acid (thermoacidophiles) with conventional biomass pretreatment that uses hot acid. The specific objectives were: to screen thermoacidophilic taxa for the ability to deconvolute lignocellulose and depolymerize associated carbohydrates; evaluate and respond to formation of “inhibitors” that arose during incubation of lignocellulose under heated acidic conditions; identify and engineer “sugar flux channeling and catabolic blocks” that redirect metabolic pathways to maximize sugar concentrations; expand the hydrolytic capacity of extremely thermoacidophilic microbes through the addition of deconvolution traits; and establish the Nebraska Bioenergy Facility (NBF) at the University of Nebraska-Lincoln.

Comparison of actual accomplishments with goals and objectives of the project

Original project abstract: The University of Nebraska-Lincoln requests $2 million to establish a Bioenergy Core Facility (BCF) at the University of Nebraska-Lincoln that will support bioenergy researchers in their efforts to develop new and renewable energy sources and to create new sources and types of value-added chemicals. It also will support development of new technology using extremophiles to accelerate lignocellulose processing from switch grass (corn and wheat) and to maximize sugar release for bioethanol production. Establishment of a research facility and program will help ensure economic viability of the rapidly expanding biofuel industry during periods of commodity price uncertainty for grain and ethanol. This demonstration project and research program also will work toward achieving the U.S. Department of Energy goal of displacing 30 percent of 2004 gasoline demand with biofuels, primarily ethanol, by 2030. Achieving this ambitious goal requires a rapid expansion of the fuel ethanol industry and research on the most efficient and cost-effective means of producing ethanol and of utilizing the by-products of that process.

Technical Project Accomplishments.

The project funds were divided at the outset into two components. 90% of the funds were dedicated to the establishment of a bioenergy facility (Nebraska Bioenergy Facility) acting at a bench scale to support research and applications at the University of Nebraska. The remaining 10% of the funds were used to develop consolidated bioprocessing technology through research efforts. The research project demonstrated that thermoacidophiles utilize lignocellulose as sole carbon and energy source and that this trait can be modified to conduct saccarification in a bioprocess that is integrated with hot acid pretreatment. This met the primary objective the original project proposal. To do this the following individual accomplishments were needed. To prevent the consumption by thermoacidophiles of sugars from cellulose and hemicellulose, this project engineered cell lines that were blocked in sugar catabolism. This was done in three distinct ways in order to identify the best method that had the least impact on microbial metabolism. This was done by inactivating the bottom half of glycolysis (glycerol 3-phosphate dehydrogenase gene disruption), by identifying and then inactivating a unique cellodextrin membrane protein transporter, and, by using mutant cell lines that had a transcriptional regulatory defect in the expression of the upper half of glycolysis called car mutants. Dehydration toxins produced from sugar exposure to hot acid were also detected in this project and cell lines were constructed that were resistant to such toxins. This was accomplished by inactivating one of several alcohol dehydrogenase genes. It was also shown that overexpression of this same gene increased toxin sensitivity. Enzymes that degrade cellulose were also identified and characterized that were naturally produced by thermoacidophiles. These were secreted endoglucanases that are lodged in the cytoplasmic membrane facing outwards to enable their abililty to act on large polymeric substrates. Overproducing host cell lines have been constructed that make these cellulases and hemicellulases to support future efforts between the PI and US National Labs and commercial entitites.

This project benefited Nebraskans.

Development of new lignocellulose to bioethanol technology, other biorenewable technologies and provision of Nebraska Bioenergy Facilities is critical for regional and national needs. They will help solve the energy crisis, improve the economy, protect national security and the environment, expand regional employment opportunities and achieve goals outlined in the 2007 Energy Independence and Security Act. This project was and remains a critical component in the establishment and growth of the University of Nebraska's new Innovation Campus. Established chemical/energy companies and new business ventures are likely partners that will benefit from access to the expertise and technology available through the Nebraska Bioenergy Facility (NBF) and its associated research laboratories. The project PI also provided support to the Nebraska Dept Labor's project SyNErgy for workforce development and advised prospective Innovation campus clients focusing on algal value added products.

This project expanded the university's research, education, service capability and capacity.

Creation of the Nebraska Bioenergy Facility (BNF at nuenergy.unl.edu) continues to provide a recuitment-based asset for faculty hiring including benefits in 2009, 2010 and 2012. The BNF provided unique training opportunities for a continuing National Science Foundation REU project entitled Bioenergy Systems (2009-2014). This project promoted recruitment and training of talented undergraduates and science training to Nebraska high school teachers. This project has trained over 60 students from across the US in Bioenergy Systems Science.

This funding was leveraged to continue the project and related research.

Four new federal funded projects were leveraged with the funds received from DOE. These included: i) 2008-2011 Department of Energy (DOD) Office of Science, DE-PS02-08ER08-12 "Biohydrogenogenesis in the Thermotogales" $525,000 to P. Blum (co-PI); ii) 2008-2011 National Science Foundation (NSF) and Department of Defense (DOD) "REU Site: Integrated Development of Bioenergy Systems". Blum PI, $269,592; iii) 2009-2011 DOD/DTRA HDTRA1-09-1-0030 Uranium Mobilization By Extremely Thermoacidophilic Archaea, $512,000 to P. Blum (co-PI); iv) 2009-2011 NIGMS 1R01GM090209-01 Metabolic Engineering Studies of Extreme Thermoacidophily $450,000 to P. Blum (co-PI).

Products developed under the award and technology transfer.

Patents: none

Publications:

Tevatia, R., J. Allen, P. Blum, Y. Demirel, and P. Black. 2014. Modeling of rhythmic behavior in neutral lipid production due to continuous supply of limited nitrogen: Mutual growth and lipid accumulation in microalgae. Bioresource Technol. (In Press).

Tevatia, R., Y. Demirel and P. Blum. 2014. Influence of sub-environmental conditions and thermodynamic coupling on a simple reaction-transport process in biochemical systems. Industrial & Engineering Chemistry Research. DOI: 10.1021/ie500941w , Publication Date (Web): April 9, 2014.

Renewable Energy Global Innovations selected our recent publication Tevatia 2012 (see below) for summary presentation in their next series on the Energy Sector.

Lalithambika S, Peterson L, Dana K, Blum P. 2012. Carbohydrate Hydrolysis and Transport in the Extreme Thermoacidophile Sulfolobus solfataricus. Appl. Environ. Microbiol. 78(22):7931-8.

Maezato Y., Blum P. 2012. Survival of the Fittest: Overcoming Oxidative Stress at the Extremes of Acid, Heat and Metal. Life. 2(3):229-242.

Tevatia, R., Y. Demirel, and P. Blum. 2012. Kinetic modeling of photoautotropic growth and neutral lipid accumulation in terms of ammonium concentration in Chlamydomonas reinhardtii. Bioresource Technology 119:419-424.

Maezato, Y., K. Dana and P. Blum. 2011. Engineering Thermoacidophilic Archaea Using Linear DNA Recombination. Methods Mol Biol. 765:435-45.

Friest. J. A., Y. Maezato, S. Broussy, P. Blum and D. B. Berkowitz. 2010. Use of a Robust Dehydrogenase from an Archael Hyperthermophile in Asymmetric Catalysis-Dynamic Reductive Kinetic Resolution Entry into (S)-Profens. J. Am. Chem. Soc. Apr 8. [Epub ahead of print].

Chemical & Engineering News, 88(16), April 19, 2010 summarizes the process chemistry implications of Friest et al., 2010, arising from the use of hyperthermophilic enzymes from thermoacidophilic microbes for asymmetric synthesis.

Miller PS, Blum PH. 2010. Extremophile-inspired strategies for enzymatic biomass saccharification. Environ Technol. 31(8-9):1005-15.

Presentations:
(by the PI)
Apr 2014 University of California Santa Cruz, Dept. Chemical and Biomolecular Engineering. "The Synthetic and Omic Biology of Extremophiles", Santa Cruz, CA
Apr 2014 University of California Davis, Dept. Biochemical Engineering & Genome Center. "The Synthetic and Omic Biology of Extremophiles", Davis, CA
Oct 2013 Sandia National Laboratory, " Microbial Biotechnology and Bioengineering", Livermore CA.
Sep 2013 NASA-Ames, "Astrobiology", Moffit Field, CA.
July 2013 Argonne National Laboratory, "Evolving Extremophiles for Mechanism and Applications", Chicago, IL.
May 2013 Sandia National Laboratory, " Extremophiles in Environmental Microbiology", Livermore CA.
May 2013 DOE EERE BETO Panel Review, Washington DC

(by other personnel on the project)
Lalithambika, S., S. McCarthy, M. Walter, L. Peterson, B. Bira and P. Blum. 2012. Consolidated bioprocessing using hyperthermoacidophiles for coupled pretreatment and saccharification. International Biomass Conference, Denver CO.

Madhusoodhanan, S. 2011 Extremophile Enzymes for Biomass Processing: Characterization of a Novel endoglucanase from Sulfolobus solfataricus, University of Nebraska-Lincoln. ASM MV Branch Meeting, Lincoln NE.

Yukari Maezato, Mary Walter, Karl Dana, Paul Blum. 2011. Extremophile-based Consolidated Bioprocessing for Third Generation Bioethanol: Engineering Tolerance Towards Pentose Dehydration Products. Society for Industrial Microbiology Annual Meeting, New Orleans, LA.

Mary Walter, Yukari Maezato, and Paul Blum. 2011. Molecular Basis for Engineering Resistance to Biomass-derived Toxins using a Purified Hyperthermophilic Alcohol Dehydrogenase. University of Nebraska Annual Research Fair, Lincolln NE.

Blum, P. October, 2011. Applications of Thermophiles in Energy Research. Green Chemistry Symposium, cosponsors UNL Department of Chemistry and American Chemical Society.

Blum, P., D. White, and R. Singh. September, 2011. The Genetics of Hyperthermophiles. Thermophiles Meeting, Bozeman MT.

Maezato, Y., M. Walter, K. Dana, and P. Blum. July 2011. Extremophile-based Consolidated Bioprocessing for Third Generation Bioethanol: Engineering Tolerance Towards Pentose Dehydration Products. Society for Industrial Microbiology Annual meeting San Francisco, CA.

Dana, K., S. Madhusoodhanan, K. Starostka, P. Blum. 2010. Extremophile endoglucanases, cellodextrin catabolism and cellulosic ethanol using the thermoacidophile Sulfolobus solfataricus. American Society for Microbiology Annual Meeting, San Diego, CA.

Blum, P. 2010. Life at high temperatures: Genetics and engineering of microbes from geothermal environments. Invited talk, American Society for Microbiology Missouri Valley Branch Annual Meeting, Kansas State University, Manhattan, KS.

Tevatia R., Y. Demirel, and P. Blum. 2010. Growth kinetics of wild type strain (cc124) of Chalmydomonas reinhardtii grown under controlled conditions of carbon and nitrogen source. University of Nebraska, Research Fair, Lincoln NE.

Maezato, Y., and P. Blum. 2010. Genome resequencing of thermoacidophilic archaeal carbon flux mutants. DOE Genomic Sciences Annual Meeting, Crystal City, VA.

Blum, P. 2011. “Life at temperature extremes: mechanisms and applications”. Department of Biology, Wichita State University, KS.