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

Synthetic Biology and BETO

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Technology Area Review: Biochemical Conversion

G.R. Petersen & L. Pezzullo

Technology Manager and Lead Technology Manager

Goal Statement

BETO

Goal of program effort: Bring to bear the power of synthetic biology on key barriers within biochemical routes to fuels and chemicals

First needed to have a definition of Synthetic Biology:

"Synthetic biology" was generally defined as follows although other variations of this definition were acceptable. This definition is cited from Wikipedia: Synthetic biology is a new area of biological research and technology that combines science and engineering. It encompasses a variety of different approaches, methodologies, and disciplines with a variety of definitions. The common goal is the design and construction of new biological functions and systems not found in nature. An additional definition was supplied from the recently published National Bioeconomy Blueprint: "… the design and wholesale construction of new biological parts and systems, and the re-design of existing, natural biological systems for tailored purposes, integrates engineering and computer-assisted design approaches with biological research."

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Barriers were addressed by two topic areas:

Topic Area 1- Intermediate Production: Innovative synthetic biological approaches to the cost-effective fractionation of lignocellulosic biomass, both terrestrial and aquatic, into processable components such as fermentable sugars, modified lignin suitable for conversion to higher value materials, and oligomeric sugar fractions or biopolymers that are more easily converted to monomers for further processing.

Topic Area 2 – Intermediate Transformations: Innovative synthetic biological approaches to the cost-effective and high yield conversion of processable component fractions into advanced biofuels and high-energy impact bioproducts. (*Even synthesis gas was included)*

R&D at TRL Levels 2-4 ONLY

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Merit Review Process

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Letters of Intent

Full Applications Received

Initial Compliance Review

Evaluate to determine:

- Applicant eligibility
- Required information submitted
- Mandatory requirements
- Responsive to objectives

Applications Forwarded for Merit Review

Individual and Committee Merit Reviews (Sequential)

Evaluate based on criteria:

- Criterion 1: **Impact of the Proposed Technology Relative to State- of- the- Art (30%)**
- Criterion 2: **Overall Scientific and Technical Merit (50%)**
- Criterion 3: **Applicant Roles and Capabilities (20%)**

Determined a Program Policy Review was unnecessary (2 TA1, 3 TA2)

All possessed clear diversity in technology areas, geographic diversity was adequate, cost-share waiver was used by 4 of 5 (5th one was large business). Hence congruence to program technology was the only factor remaining and all five applications are different and cover a broad range of program needs.

Selection Recommendations to Selection Official (2 in TA1, 3 in TA2)

Management Briefings

Announcement

Key Criteria

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(1) Impact of the Proposed Technology Relative to State- of- the- Art (30%) - This criterion involves consideration of the following factors (selected from complete list):

- The extent to which the proposed effort clearly shows a definitive integration of the proposed technology improvements between lignocellulosic feedstock sources and production of advanced biofuels or high impact biobased products;
- The extent to which the proposed quantitative material and/or technology metrics demonstrate the potential for **a transformational and disruptive (not incremental)** advancement in the topic area chosen through the use of synthetic biology;

(2) Overall Scientific and Technical Merit (50%) - This criterion involves consideration of the following factors:

- The extent to which the proposed approach is **unique and employs innovative uses** of synthetic biology;
- The extent to which the applicant proposes a sound and feasible technical approach to accomplish the proposed R&D objectives;

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Key Criteria

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Table B-ex: Enzyme Performance & Cost Contribution Example			Benchmark	Intermediate Target	Final Target
		% Improvement in cost contribution from benchmark	-----	133%	444%
C_E	Enzyme Cost	$/gal Biofuel or lb bioproduct	$0.42	$0.32	$0.10
E_P	Enzyme Price	$/L or lb-product	$0.50		
E_L	Enzyme Loading (7 day residence time)	g protein/ (g cellulose + g xylan entering enzymatic hydrolysis)	0.020	0.015	0.005
B_N	Enzyme Concentration	g protein/ L or lb-product	150		
Y	Biofuel or bioproduct Process Yield	gal Biofuel or lb bioproduct/ (g cellulose + g xylan in raw feedstock)	1.579E-04		
	Biofuel or bioproduct Process Yield	gal Biofuel or lb bioproduct/ ton biomass	83.8		
F_C	*Cellulose Fraction in Raw Feedstock*	wt fraction	0.374		
F_H	*Xylan Fraction in Raw Feedstock*	wt fraction	0.211		
C_C	*Cellulose Conversion to Glucose (including pretreatment conversion)*	g cellulose converted/ g cellulose total	0.90		
C_H	*Xylan Conversion to Xylose (including pretreatment conversion)*	g xylan converted/ g xylan total	0.90		
C_G	*Glucose Conversion to Biofuel or bioproduct*	g glucose converted/ g glucose total	0.95		
C_X	*Xylose Conversion to Biofuel or bioproduct*	g xylose converted/ g xylose total	0.85		
R_C	*Theoretical Cellulose per gallon Biofuel or bioproduct*	g cellulose/ gallon Biofuel or lb of bioproduct	5250		
R_H	*Theoretical Xylan per gallon Biofuel or bioproduct*	g xylan/gallon Biofuel or lb bioproduct	5135		

Required performance tables differentiate BETO and OS FOAs

* Provide best data based on current level of your technology development or your partners or known processing technology (please provide citations)

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Key Criteria

Process Details and Costs			PROPRIETARY/BUSINESS SENSITIVE		
Feedstock Type		Benchmark or Current Process*	Intermediate Target (~6-8 months)	Final Target (18-24 months)	Anticipated cost improvements are due to:
Feed Rate (dry ton/day)					
Biofuel or Bioproduct Yield (gallons or lbs/dry ton)					
Line 1: Annual Biofuel or Bioproduct Production (MM gallons					
Equipment Costs (2005$) as applicable	**Description**	**Installed Capital Cost (MM$)**		**Installed Capital Cost (MM$)**	
Feedstock Handling					
Pretreatment					
Neutralization/Conditioning					
Fermentation Organism Production (here or in operating costs)					
Saccharification & Co-fermentation					
Distillation & Solids Recovery					
Wastewater Treatment					
Storage					
Utilities (include steam/electricity here or in operating costs)					
Line 2: Total Installed Capital					
Total Installed Capital per Annual Gallon or lb (line 2 divided by line 1)					
Operating Costs (2005$) as applicable	**Description**	**MM$/yr**		**MM$/yr**	
Feedstock					
Organism Production Nutrients					
Fermentation Nutrients					
Enzymes (Cellulase)					
Fermentation Organism (include licensing fees)					
Other Raw Materials					
Waste Disposal					
Steam					
Electricity					
Labor and Maintenance					
Line 3: Total Operating Costs					
Line 4: Co-product Credits					
Line 5: Net Operating Costs (line 3 minus line 4)					
Net Biofuel or Bioproduct Production Costs ($/gal) (line 5 divided by line 1)					

* Provide best data based on current level of your technology development

Required Economics Tables Differentiate BETO and Office of Science FOAs

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Key Criteria

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Validation of the applicant's data is required

Minimum of two validations by independent parties

1. Validation of baseline experiments that yielded data in Techfin tables will be required at the outset of project
2. Validation of progress against original baseline data near the end of the project will be conducted

Mirrors the validations required under previous FOAs covering ethanologen development, improved enzymes, and biomass integration. However, the requirements were modified to fit the TRL levels of these selectees.

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Quad Chart Overview

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Timeline

- Submitted July 21, 2012
- Selections Fall, 2012
- Announced Winter, 2012/2013
- 24 month projects
- Most start April – July, 2013

Barriers: Bt-A; C-G; I-K

A. Biomass Fractionation
C. Biomass Recalcitrance
D. Pretreatment Chemistry
E. Pretreatment Costs
F. Cellulase Enzyme Production Cost
G. Cellulase Enzyme Loading
I. Cleanup/Separation
J. Catalyst (bio & chem) Development
K. Biological Process Integration

Budget

- Original: $12M
- Actual: $10.2M selected

Partners

- Office of Science in reviewing FOA
- Selections include the following
  - J. Craig Venter Institute
  - Novozymes, Inc.
  - Pacific Northwest National Laboratory
  - Texas AgriLife
  - Lygos, Inc.

J Craig Venter Institute

Project Title: Maximizing Multi-Enzyme Synergy in Biomass Degradation in Yeast

Objectives: Although biomass-active enzymes are known to act synergistically, the interactions among these enzymes are insufficiently explored. Using synthetic biology and genome engineering in yeast, the team will rapidly identify combinations of enzymes that catalyze synergistic processes of lignocelluloses and develop yeast strains with optimal enzyme sets for cost-effectively converting biomass to sugars.

Innovation: *Generate yeast strains containing many combinations of enzymes*: Starting with 51 enzymes with demonstrated activity on cellulosic materials, the team will generate roughly 100,000 (= 10^5) yeast strains containing various combinations of enzymes. *Identify combinations that maximize biomass degradation*: To identify strains that have synergistic effects, the team will screen a great number of generated strains using a viability-based assay. *Scale up processing of lignocellulose biomass*: Industrially relevant strains can be developed by combining generated materials with currently existing tools. The team will test these strains in a bench-scale reactor system.

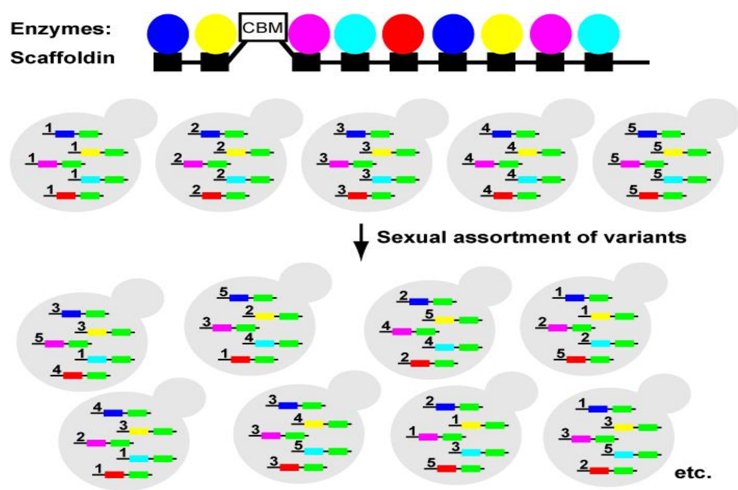
Budget:

- DOE Share $1,266,227
- Cost Share $140,692

Partner:

- NREL

Principal Investigator: Yo Suzuki



Project Title: SynTec: Synthetic Biology for Tailored enzyme cocktails

Objectives: The goal is to create a chassis for rapid assessment of unexplored natural diversity which can be targeted to deliver cost effective enzyme solutions that are tailored for processes employed by specific industrial biorefineries**.** The expected outcome is an enzyme cocktail that enables production of mixed sugars at a cost competitive with starch-based sugars.

Innovation:. Developing a synthetic biological screening system which streamlines and standardizes the process whereby diverse glycoside hydrolase, esterase, oxidoreductase, carbohydrate binding and other protein modules are screened in combinatorial fashion. The purpose of the proposed work is to develop a biological system for screening output synergistic cocktails from combinatorial modular libraries.

Budget:

- DOE Share $ 2,500,000
- Cost Share $1,455,870

Partners:

- Novozymes, Davis, CA
- Novozymes, Franklinton, NC
- MBI, Lansing, MI

Principal Investigator: Sarah Teeter

Project Title: Design and Optimization of Biofuel Production with Biosensor-Guided Evolution

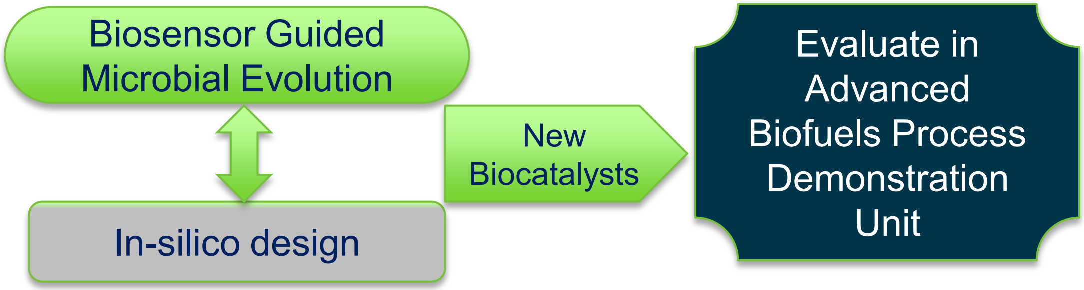
Objectives: The goal of this proposal is to develop rapid, inexpensive methods to generate biocatalysts that employ non-food biomass feedstocks to produce chemicals traditionally produced from non-renewable petroleum. A platform chemical will be the target molecule which can be chemically converted into additional, downstream chemical and fuel molecules.

Innovation: Lygos is developing high-yielding microbial catalysts that will be economically and environmentally advantaged relative to existing petrochemical routes. The methods proposed include building genetic tools to rapidly alter multiple areas of microbial metabolism. These modifications are coupled to direct measurement of small-molecule production using a novel biosensor-based detection method; the biosensor is capable of "seeing" more variation with higher resolution than traditional detection methods. Finally, the data produced from this effort will be used to refine existing models of metabolism, resulting in an increased understanding and ability to design biocatalysts *de novo*.

Budget:

- DOE Share $1,858,487
- Cost Share $206,499

Principal Investigator: Eric Steen



LYGOS

Pacific Northwest National Laboratory

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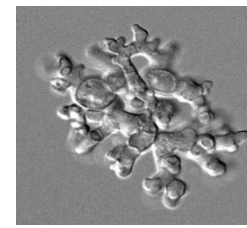
Project Title: Design, Construction, and Implementation of Novel Biofuel Production Capabilities in Filamentous Fungi – PI: Kenneth Bruno

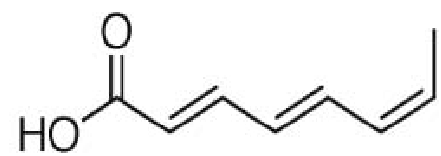
Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle Since 1965

Objectives: The goal of this proposed work is to increase the production of fuel precursor molecules in *Aspergillus niger* growing on lignocellulosic hydrolysate. This will be achieved by employing a metabolic model of *A. niger* to direct the use of standardized functional components in order to engineer new capabilities within this commonly used industrial species. Standardized components will be developed to alter metabolic pathways that will optimize growth on lignocellulosic sugars as well as enhance production of target molecules. These tools will be used in a synthetic biology approach for the generation of a strain with production capabilities not currently found in any known organism.



Budget:

- DOE Share $: 2,402,611
- Cost Share $ 276,298



pkiA-octatrienoic acid

Target precursor molecule (C8) suitable for conversion to advanced fuels

Partners:

- University of Kansas
- University of Southern California
- State University of New York at Buffalo

Cost Share provided By:

- Bend Research
- Technical University of Denmark
- Novozymes of North America, Inc.

Texas AgriLife Research

Project Title: Synthetic Design of Microorganisms for Lignin Fuel

Objectives: (1) Synthetic design of secretion systems and functional modules in RHA1 to enable effective lignin depolymerization; **(2)** Modification and integration of functional modules to improve carbon flux from aromatic compound catabolism to lipid production, as well as the design of genetic circuits to balance lignin depolymerization and derivative conversion for higher conversion efficiency; & **(3)** Optimize the fermentation of lignin to lipids using synthetic and wild type strains.

Innovation: A potentially viable, biological platform for conversion of lignin into advanced biofuels. *R. opacus* grows on kraft lignin as sole carbon source, accumulating lipid to up to 15% of its biomass. This unique organism would be the basis for developing the proposed platforms that depolymerize lignin and produce precursors (a lignin CBP approach).

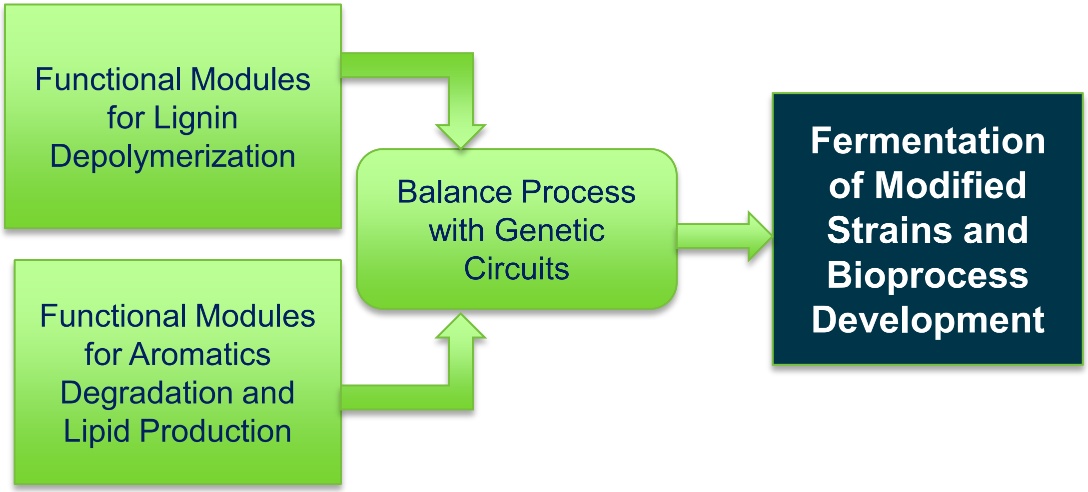
Budget:

- DOE Share $2,399,977
- Cost Share $ 468,118

Partners:

- Texas A&M University
- Georgia Tech University
- University of British Columbia
- Washington State University

Principal Investigator: Joshua Yuan



Critical Success Factors

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Major success factors are technical and business related

Examples:

- New enzyme cocktails that have the potential to reduce cost per gallon fuel (via higher efficiency or reduction in cost of enzyme cocktails)
- Validation of new or unique microbial systems that generate advanced biofuels or bioproducts at the bench scale
- Improved tools for assessing and managing biomass deconstruction or microbial conversions

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Relevance and Future Work

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Relevance: By definition, outcomes of these R&D projects impact barriers to biomass conversions to fuels and chemicals

Future Work: BETO intends on continuing to solicit work in the areas of Biochemical Integration to which these technologies could be included

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Summary

New paradigm for BETO. Ventured into TRL levels of R&D typically covered by Office of Science but bridged into BETO

Results: Applications from DOE Bioenergy Research Centers and ARPA E projects were submitted. New applicants were involved. Commercial entities ventured into basic R&D areas that could have long term impact on their long term business objectives

Cost Share: Employed reduced and blended cost share to encourage participation by labs, small businesses, nonprofits and partnering

Results: Relatively few single party applicants. Most applications were Industry/ Academia; Industry/Labs; Labs/Academia or even all three including non-profits

Acknowledgements

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Questions?

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