DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review
Protein Fermentation
March 24, 2015
Algal Feedstocks

This presentation does not contain any proprietary, confidential, or otherwise restricted information
Goal Statement

• Optimize bioconversion of microalgal proteins to mixed alcohol liquid fuels
• Increase the yield of algae biofuel intermediates by integrated conversion of all of the major algal biochemical pools to achieve BETO’s Algae Biofuel Program MYPP milestones and ultimate goal of affordably achieving 5,200 gal/acre/year by 2022
• Provide means for domestic production of renewable liquid fuels from algal biomass source that does not compete with agriculture
Quad Chart Overview

Timeline
• Project start date: 1/30/2013
• Project end date: 9/30/2015
• Percent complete: ~70% (based on scope of work)

Barriers
• Barriers addressed
  – Ft-N: Algal feedstock processing
  – Ft-B: Sustainable production
  – Ft-J: Biomass material properties

Budget

<table>
<thead>
<tr>
<th></th>
<th>Total Costs FY 10 – FY 12</th>
<th>FY 13 Costs</th>
<th>FY 14 Costs</th>
<th>Total Planned Funding (FY 15-Project End Date)</th>
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<tbody>
<tr>
<td>DOE Funded</td>
<td>$215,770</td>
<td>$173,397</td>
<td>$1,105,026</td>
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<tr>
<td>Project Cost Share (Comp.)*</td>
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Partners
• Partners
  o FY12-14 NREL (15%): optimize hydrolysis and recovery of algal carbohydrates and lipids
  o FY14 LBL-ABPDU (10%): scale-up of integrated carbohydrate and protein bioconversion process
• Collaborator: James Liao (UCLA) for fermentation strain refinement
1 - Project Overview

- High productivity cultivation correlates to high protein content (e.g. ATP3); half of biomass is lost using current processing strategies.

- Algae biomass has limited utility for feeds, not sustainable because of nutrient use.

- High nitrogen (protein) feedstock present significant hurdles for alternative processing methods (AD, HTL).

- Protein conversion generates stoichiometric yield of ammonium and alcohol products: utility for coupling generation of fuels with sustainability via N-recycling.
2 – Approach (Technical)

• Major technical success factors:
  1) *yield! yield! yield!*
  2) *high product titers*
  3) *fast conversion kinetics*

• Technical challenges:
  1) *need low cost & high efficiency pretreatment regimen that minimizes inhibitors*
  2) *need substantial substrate loading to maximize titers*
  3) *process mush facilitate low cost product recovery*

• Approach:
  1) *identify major process inhibitors & feedstock variation impacts*
  2) *apply corrective measures independently and in combination for process refinement using designed experiments at bench scale*
  3) *scale-up to ~100L using most promising conditions at ABPDU*
2 – Approach (Management)

- Critical market success factors that will define technical and commercial viability:
  - identify feedstock specificity/flexibility
  - means for interfacing bioconversion with other processing options (e.g. HTL) for obtaining optimal yield & fuel quality
  - minimize unit operations for bioconversion process scale-up

- Project Milestones & Go/No-Go criteria heavily focused on maximizing yield of protein conversion
  - 2012 baseline ~42% of theoretical yield
  - 2014 Go/No-Go ≥70% of theoretical yield
3 – Technical Accomplishments/Progress/Results

**Solubilization and hydrolysis of microalgal biomass for biochemical conversion**

- Partner NREL successfully achieved 90% carbohydrate hydrolysis and lipid recovery
- Dilute acid (2-4% H₂SO₄) pretreatment is effective for solubilizing >80% of the proteins & carbohydrates, and hydrolyzing ~90% of the carbohydrates
- Proteins are only partially hydrolyzed to amino acids (~40%) via dilute acid
- Combination of dilute acid hydrolysis and enzymatic digestion using pronase cocktail yields ~80% protein hydrolysis to amino acids
Fermentation inhibitors & enhancers

Conversion products

Biomass components
We have developed an effective route to protein conversion

- *Saccharomyces/Zymomonas* fermentation of carbs $\rightarrow$ ethanol (NREL)
- YH83 E. coli fermentation proteins $\rightarrow$ isobutanol/isopentanol (Sandia)
- Highest total fuels yield was obtained from following process:
  1. Dilute acid pretreatment $\rightarrow$
  2. Ethanolic fermentation $\rightarrow$
  3. Distillation (retain heavy fraction) $\rightarrow$
  4. Protease digestion (55ºC) $\rightarrow$
  5. Protein fermentation

- IL pretreatment also produced high yields, without need for dilute acid & ethanolic fermentation/distillation

- Acetoin intermediate detected in samples not exposed to pronase; 2,3-butane-diol detected in samples exposed to protease
Consolidation of lipid and alcohol recovery increases yield & reduces cost

- Retaining lipids in protein fermentation broth reduces alcohol toxicity
- Lipid microparticles are released from algae during pretreatment
- Microparticle size distribution shifts in the presence of mixed alcohols
- Co-extraction / in situ transesterification of lipids + alcohols

Origin of size dependent ‘ruffling’?

Retaining lipids in protein fermentation broth reduces alcohol toxicity

Manuscript in preparation
Redox cofactor engineering provides 60% yield improvement

- NADPH depletion observed during time course studies
- IlvC and ADH keto-acid pathway enzymes modified for NADH specificity
- 5 new *E. coli* strains showed increased conversion yield relative to YH83
Energy balance based on ATP3 biomass

- Hypothetical yield of ~2000 gal/acre/yr based on ATP3 13 g/m²/day (75% of MYPP goal)
- ~1500 gal/acre/yr as lipids and mixed alcohols
- Protein fermentation combined with increased productivity will lead to achieving 2500 gal/acre/yr
3 – Technical Accomplishments/Progress/Results (cont’d)

- Describe progress made in meeting project objectives and following the project management plan
- Describe the most important technical accomplishments achieved (from the last review to the present for existing projects, or progress to date for new projects)
- Summarize key milestones and status
- Include sufficient slides to explain what tasks were executed leading to the technical accomplishments
- Include data, results, new knowledge, and tie results to applications
- Benchmark the progress versus previously reported results (if applicable)
4 – Relevance

- Describe how project accomplishments contribute to meeting the platform goals and objectives of the BETO Multi-Year Program Plan.

- Demonstrate how the project considers applications of the expected outputs in the emerging bioenergy industry.

- Your objectives should be clear regarding the relevance of your project to the Bioenergy Technologies Office, MYPP goals, and relevance for the overall bioenergy industry.

- Demonstrate that the successful project will advance the state of technology and positively impact the commercial viability of biomass and/or biofuels.

- Tech transfer/marketability should be discussed here (not separately).

(View the PowerPoint “Notes” page for additional information)
5 – Future Work

• Explain what it is you plan to do through the end of the project with emphasis on the next 18 months (through September 30, 2016)

• Highlight upcoming key milestones

• Address how you will deal with any decision points during that time (Go/No-Go Points) and any remaining issues with proposed abatement actions

(View the PowerPoint “Notes” page for additional information)
Summary

• Summarize the key points you wish the audience and reviewers to take away from your presentation in the following categories:
  1. Overview
  2. Approach
  3. Technical Accomplishments/Progress/Results
  4. Relevance
  5. Future work.

• For projects presented at the 2013 Project Review, please provide a status of your current progress in reaching specific technical targets versus the status shown in 2013.
Additional Slides
The following slides are to be included in your submission for Peer Evaluation purposes, but will not be part of your oral presentation –

You may refer to them during the Q&A period if they are helpful to you in explaining certain points.
Responses to Previous Reviewers’ Comments

• If your project is an on-going project that was reviewed previously, address 1-3 significant questions/criticisms from the previous reviewers’ comments (refer to the 2013 Peer Review Report, see notes section below)

• Also provide highlights from any Go/No-Go Reviews

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.
Publications, Patents, Presentations, Awards, and Commercialization

• List any publications, patents, awards, and presentations that have resulted from work on this project
• Use at least 12 point font
• Describe the status of any technology transfer or commercialization efforts

Note: This slide is for the use of the Peer Reviewers only – it is not to be presented as part of your oral presentation. These Additional Slides will be included in the copy of your presentation that will be made available to the Reviewers.