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

# DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

## Protein Fermentation

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Algal Feedstocks

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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)

## Budget

| | Total Costs FY 10 – FY 12 | FY 13 Costs | FY 14 Costs | Total Planned Funding (FY 15-Project End Date |
|---|---|---|---|---|
| DOE Funded | | $215,770 | $173,397 | $1,105,026 |
| Project Cost Share (Comp.)* | | | | |

## Barriers

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

## Partners

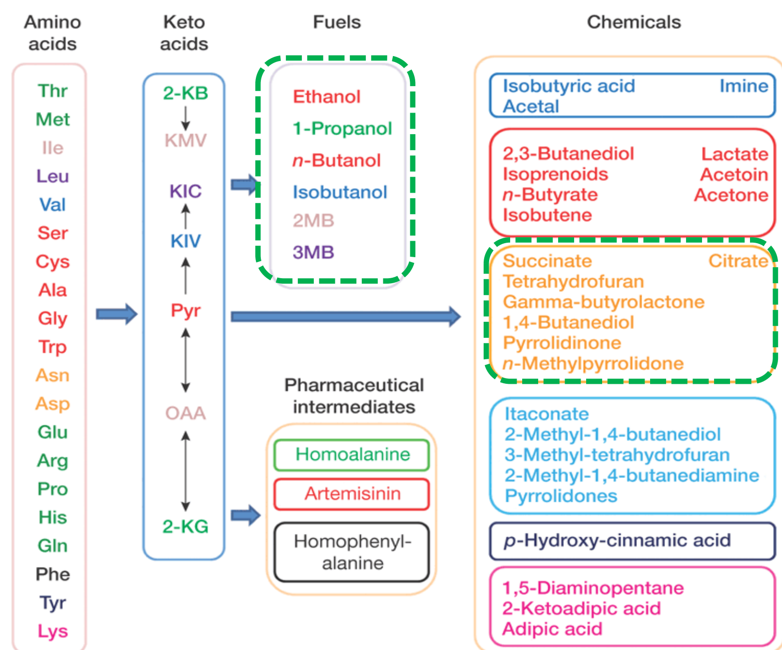
- Partners
  - FY12-14 NREL (15%): optimize hydrolysis and recovery of algal carbohydrates and lipids
  - FY14 LBL-ABPDU (10%): scale-up of integrated carbohydrate and protein bioconversion process
- Collaborator: James Liao (UCLA) for fermentation strain refinement

# 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

Chemical composition of alga

| Strain | Protein | Carbohydrates | Lipids |
|---|---|---|---|
| Scenedesmus obliquus | 50-56 | 10-17 | 12-14 |
| *Scenedesmus quadricauda* | 47 | - | 1.9 |
| *Scenedesmus dimorphus* | 8-18 | 21-52 | 16-40 |
| *Chlamydomonas rheinhardii* | 48 | 17 | 21 |
| Chlorella vulgaris | 51-58 | 12-17 | 14-22 |




Huo, et al *Nat. Biotech.* 2011

# 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*

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# 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*



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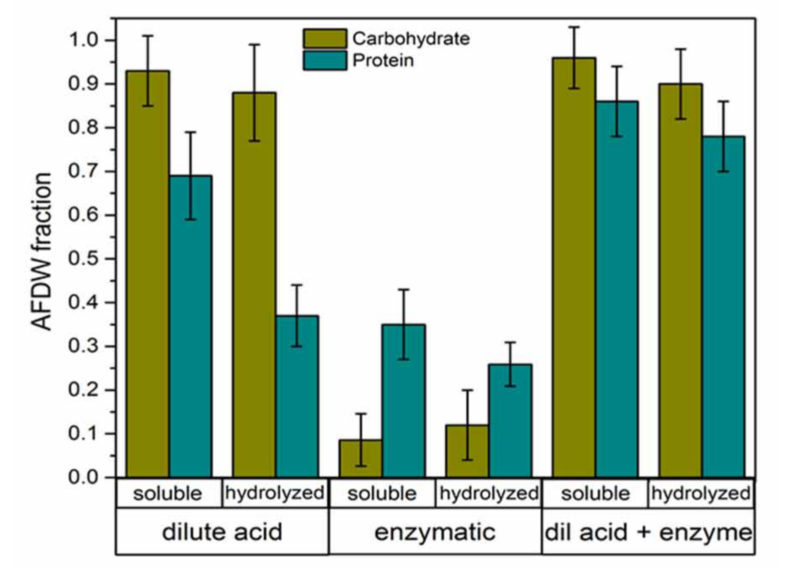
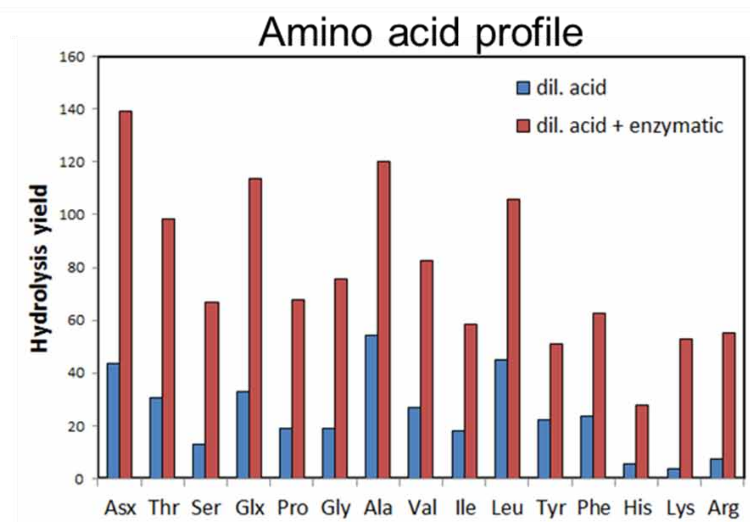
# 3 – Technical Accomplishments/ Progress/Results

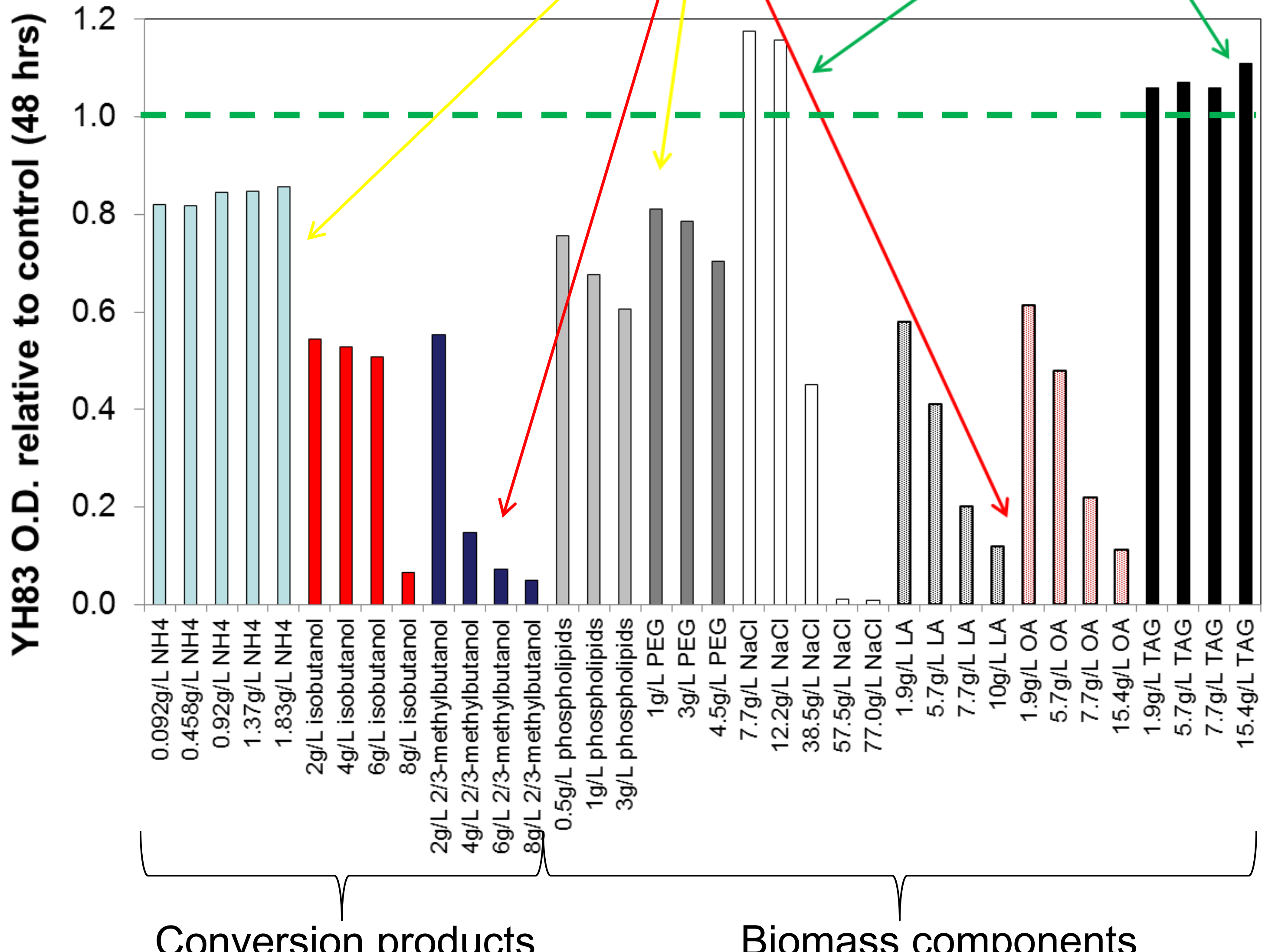
## Solubilization and hydrolysis of microalgal biomass for biochemical conversion

- Partner NREL successfully achieved 90% carbohydrate hydrolysis and lipid recovery
- Dilute acid (2-4% $H_2SO_4$) 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



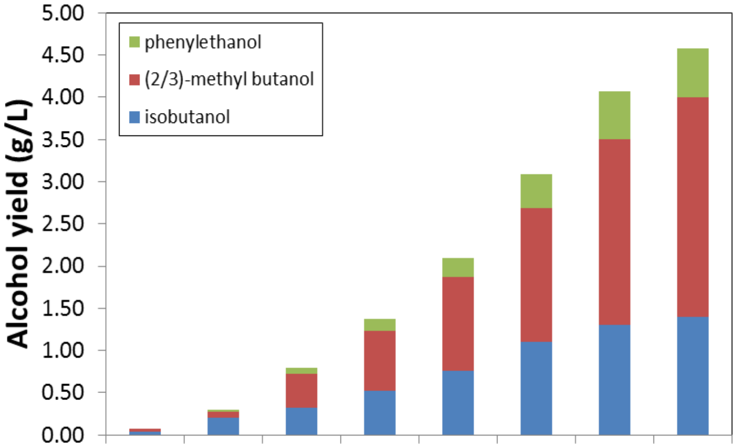



# Fermentation inhibitors *& enhancers*

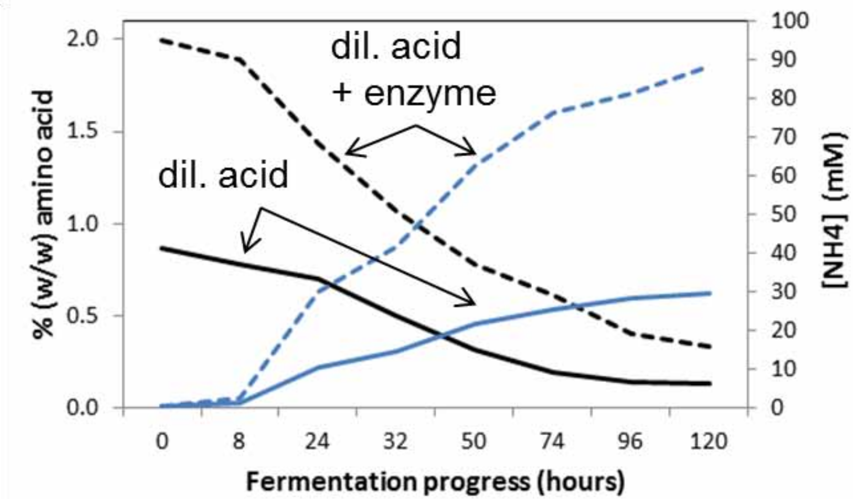


# We have developed an effective route to protein conversion

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

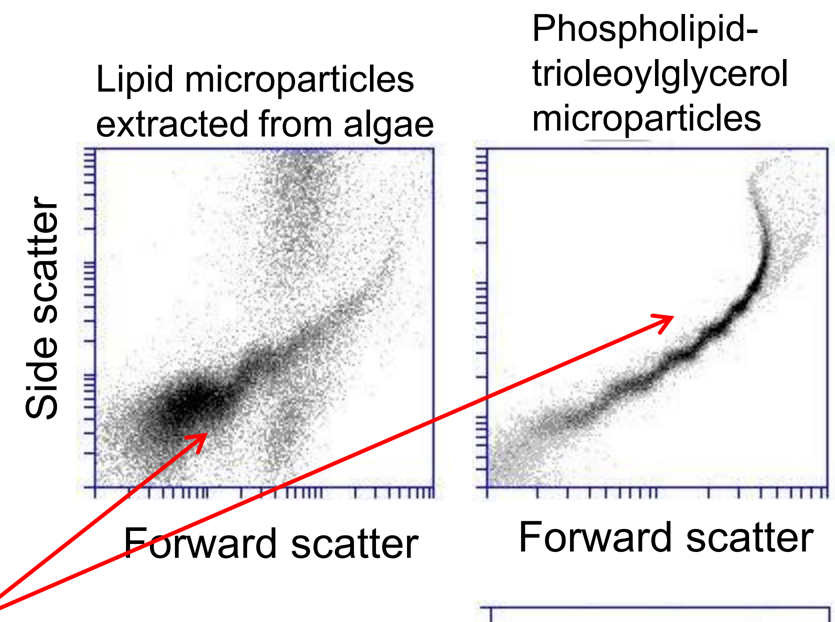



- IL pretreatment also produced high yields, without need for dilute acid & ethanolic fermentation/distillation
- Acetoin intermediate detected in samples not exposed to pronase; 2,3-butane-diol detected in samples exposed to protease

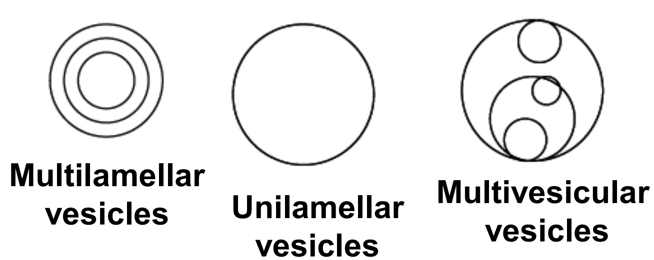


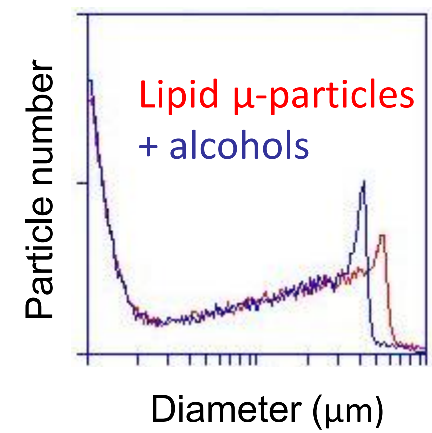
# Consolidation of lipid and alcohol recovery increases yield & reduces cost

- 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'?

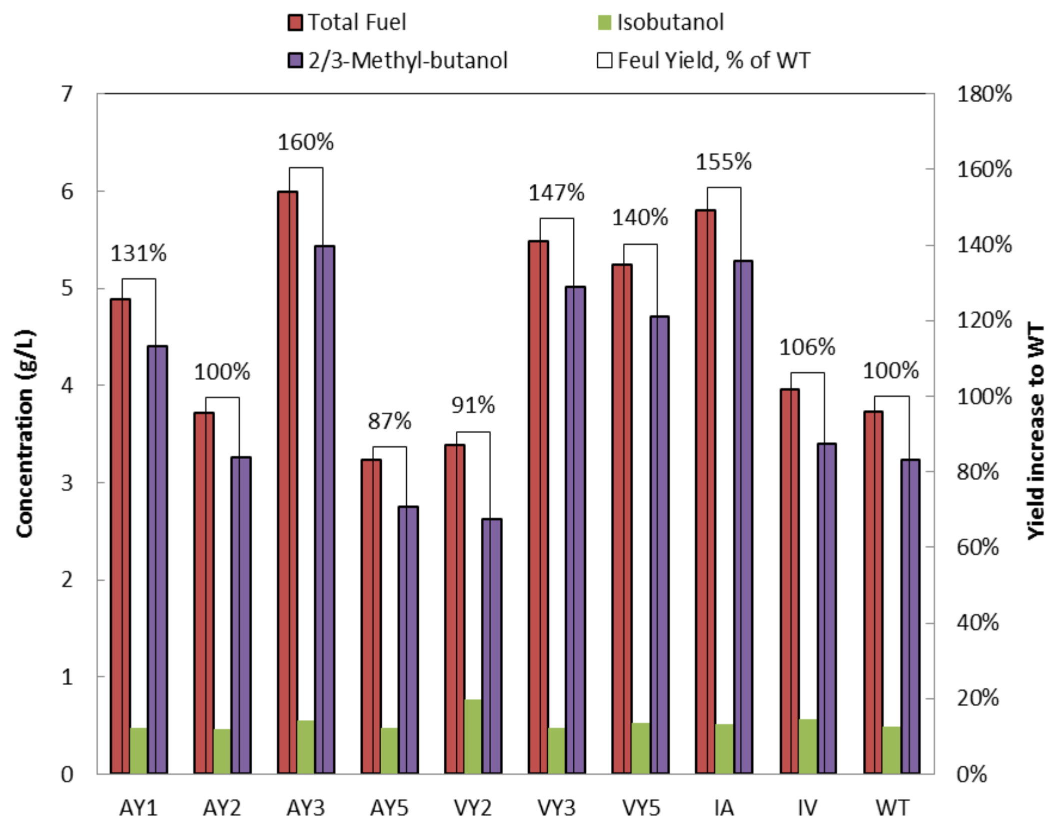



*Manuscript in preparation*

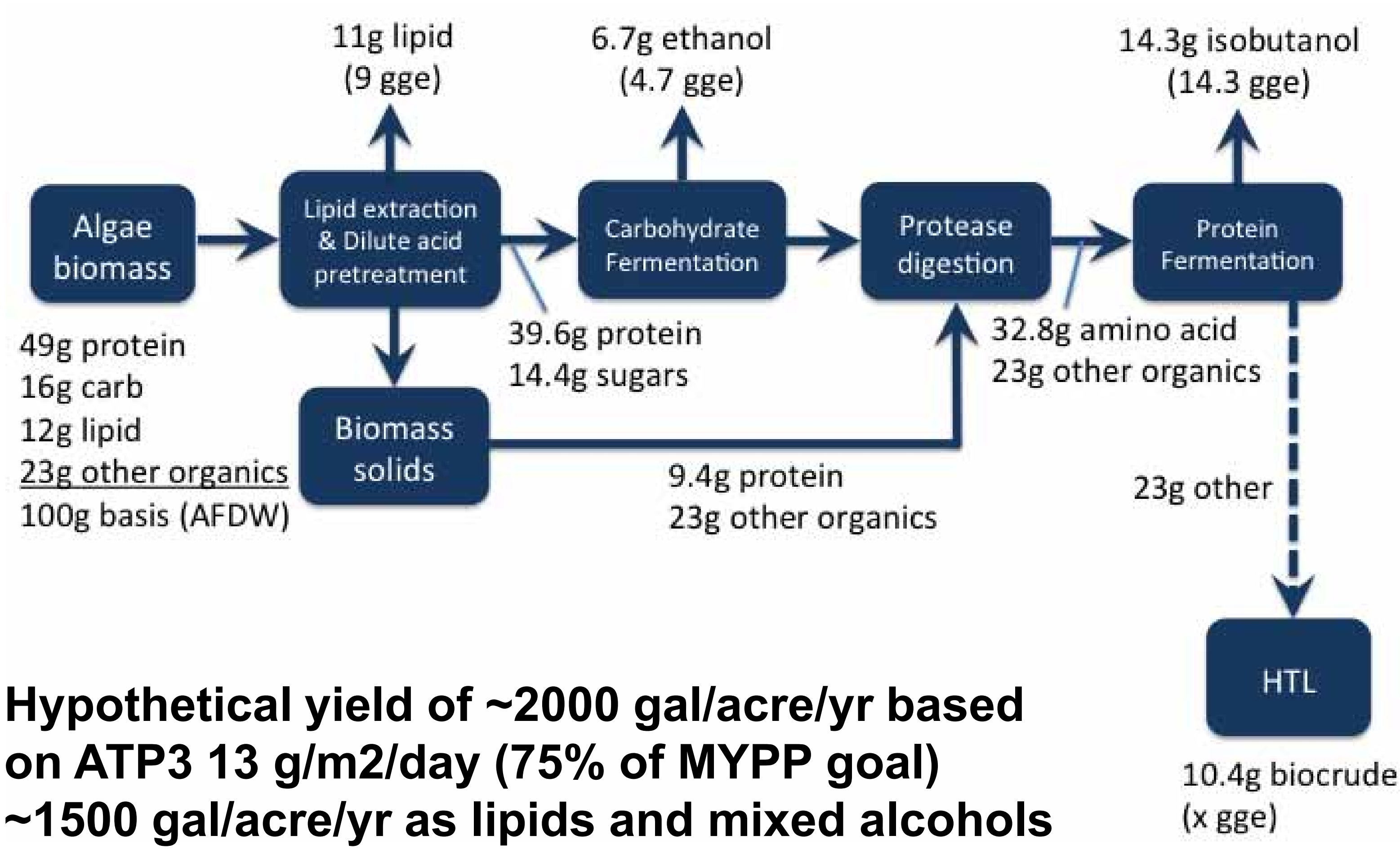
# Redox cofactor engineering provides 60% yield improvement

- 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




Redox mutants screening

# 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

**(Not a template slide – for information purposes only)**

- *The following slides are to be included in your submission for Peer Evaluation purposes, but <u>will **not** be part of your oral presentation</u> –*
- *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.