

## Bioconversion of Algae Biomass for Fuels and Bioproducts

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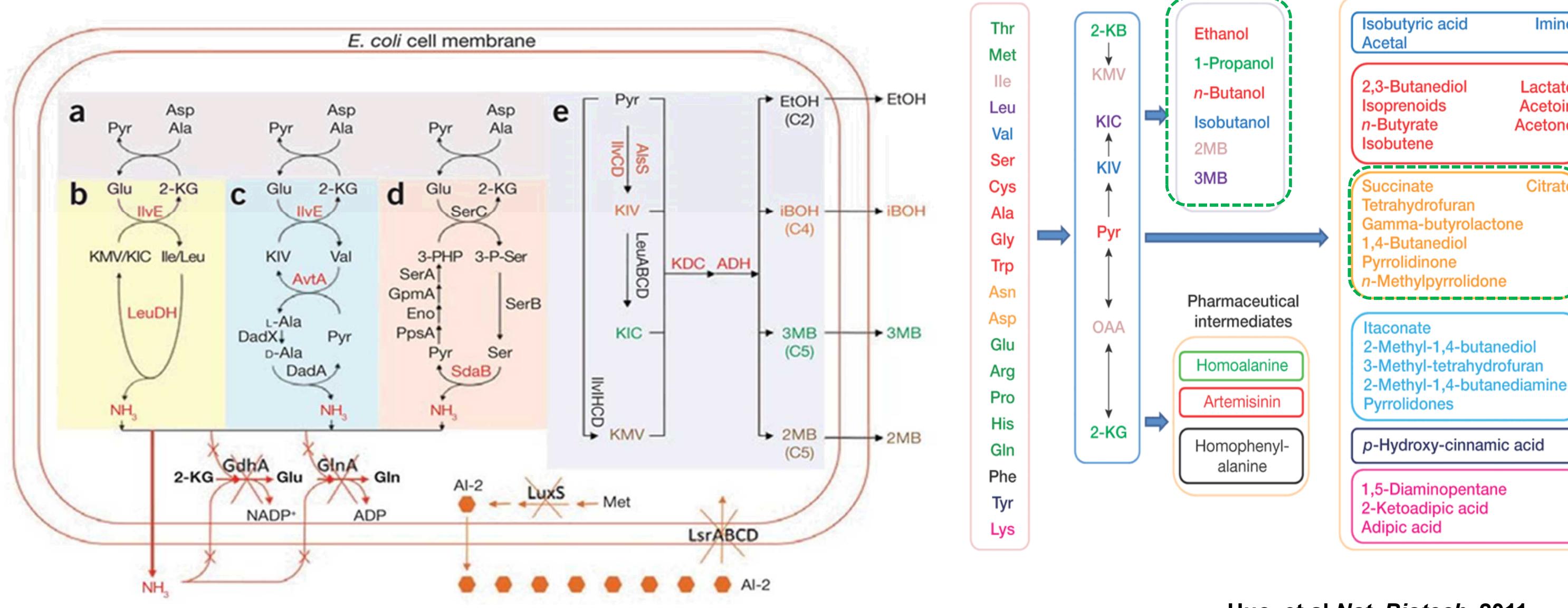
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### Motivation and Strategy

The goal of this effort is to enable the production of algae biofuels at 5,200 gallons/acre/year. To achieve this goal, maximal conversion of all of the algae biomass components to liquid fuels and biobased products is required. Proteins and carbohydrates comprise ~80% of the ash-free dry weight biomass at maximum growth conditions. We are applying bioconversion strategies for generation of mixed ( $\geq C2$ ) alcohols, terpenes, and ammonium by comprehensive utilization of the biomass to increase the total yield of biobased fuels and products and recycle the major nutrients, nitrogen and phosphorus. Algae feedstocks under investigation include monoculture microalgae from outdoor raceways and biomass from naturally occurring benthic algae assemblages from ATS™ wastewater treatment. Through our efforts, we have identified pretreatment and biochemical conversion processes that integrate bioconversion of algal carbohydrates and proteins to produce mixed alcohols or terpenes with co-separation of the algal lipids and fermentation products. Maximum bioconversion yields of ~80% of theoretical were obtained at bench scale by minimizing product inhibition and biochemical redox imbalance.

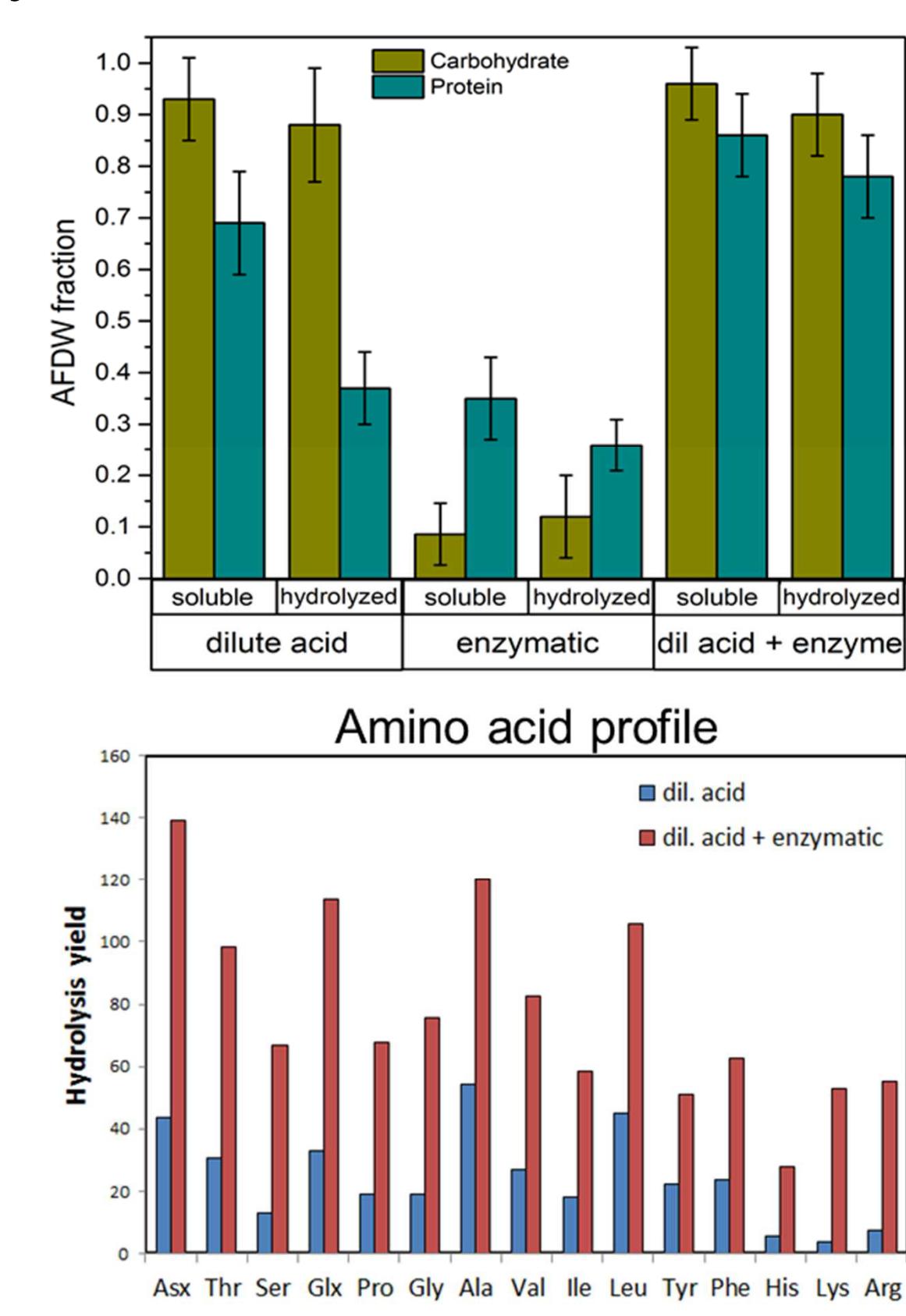
### Protein Conversion Process Overview

- High productivity and semi-continuous cultivation correlate to high protein content (e.g. ATP3); lose half of biomass using current processing strategies
- Algae biomass have limited utility for feeds, not sustainable because of nutrient use
- High nitrogen feedstock present major hurdles for alternative processing methods (AD, HTL)
- Protein conversion generates stoichiometric yield of ammonium and alcohols products



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

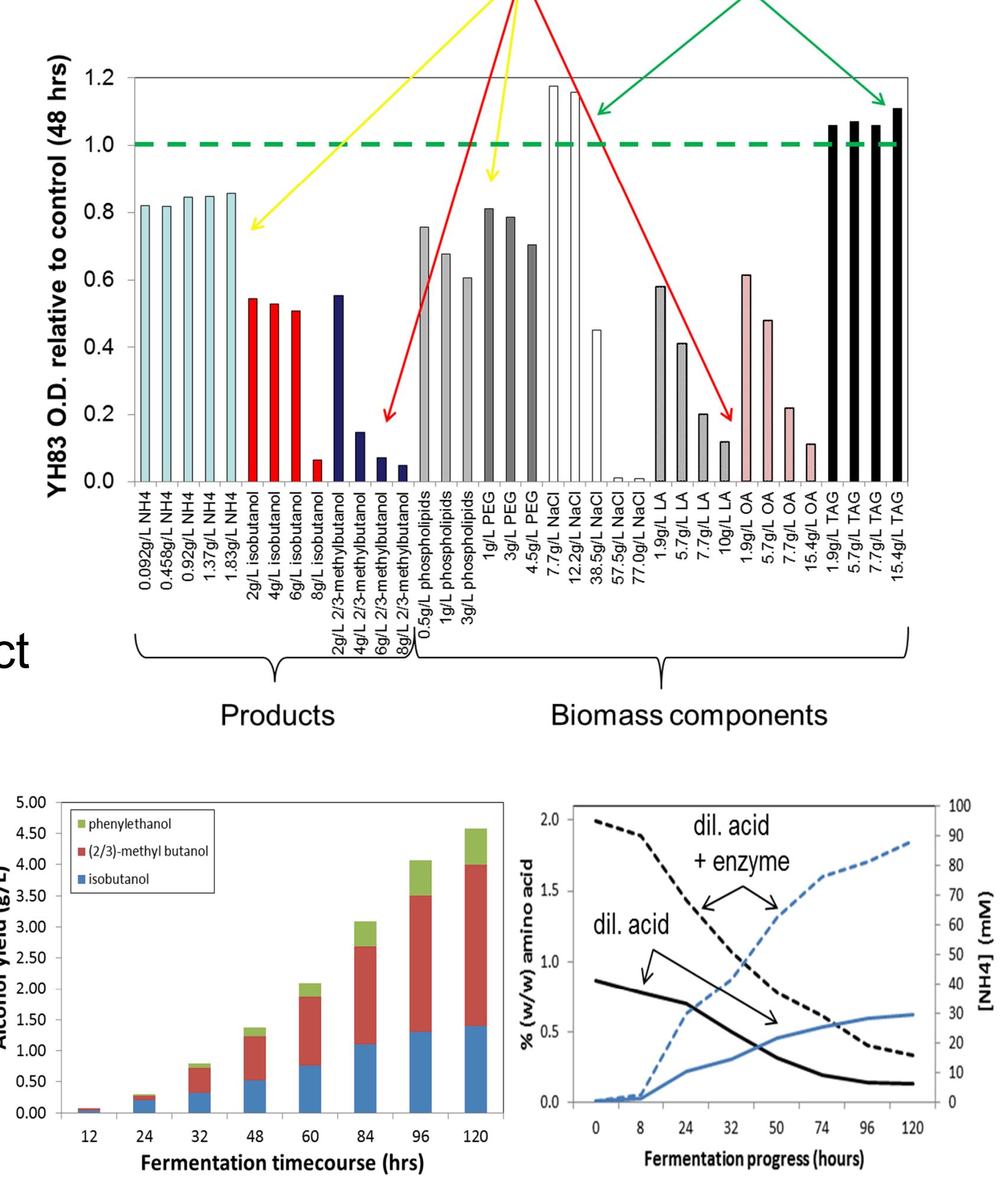
- Dilute acid ( $2\text{-}4\% H_2SO_4$ ) pretreatment is effective for solubilizing >80% of the proteins & carbohydrates, and hydrolyzing ~90% of the carbohydrates
- Proteins are only partially hydrolyzed to amino acids (~40%) via dilute acid
- Combination of dilute acid hydrolysis and enzymatic digestion using pronase cocktail yields ~80% protein hydrolysis to amino acids
- High quantity of non-natural amino acids in pretreated residual solids, including:
  - $\gamma$ -aminobutyric acid (GABA)
  - $\beta$ -aminoisobutyric acid
  - $\beta$ -alanine



### Yield Optimization

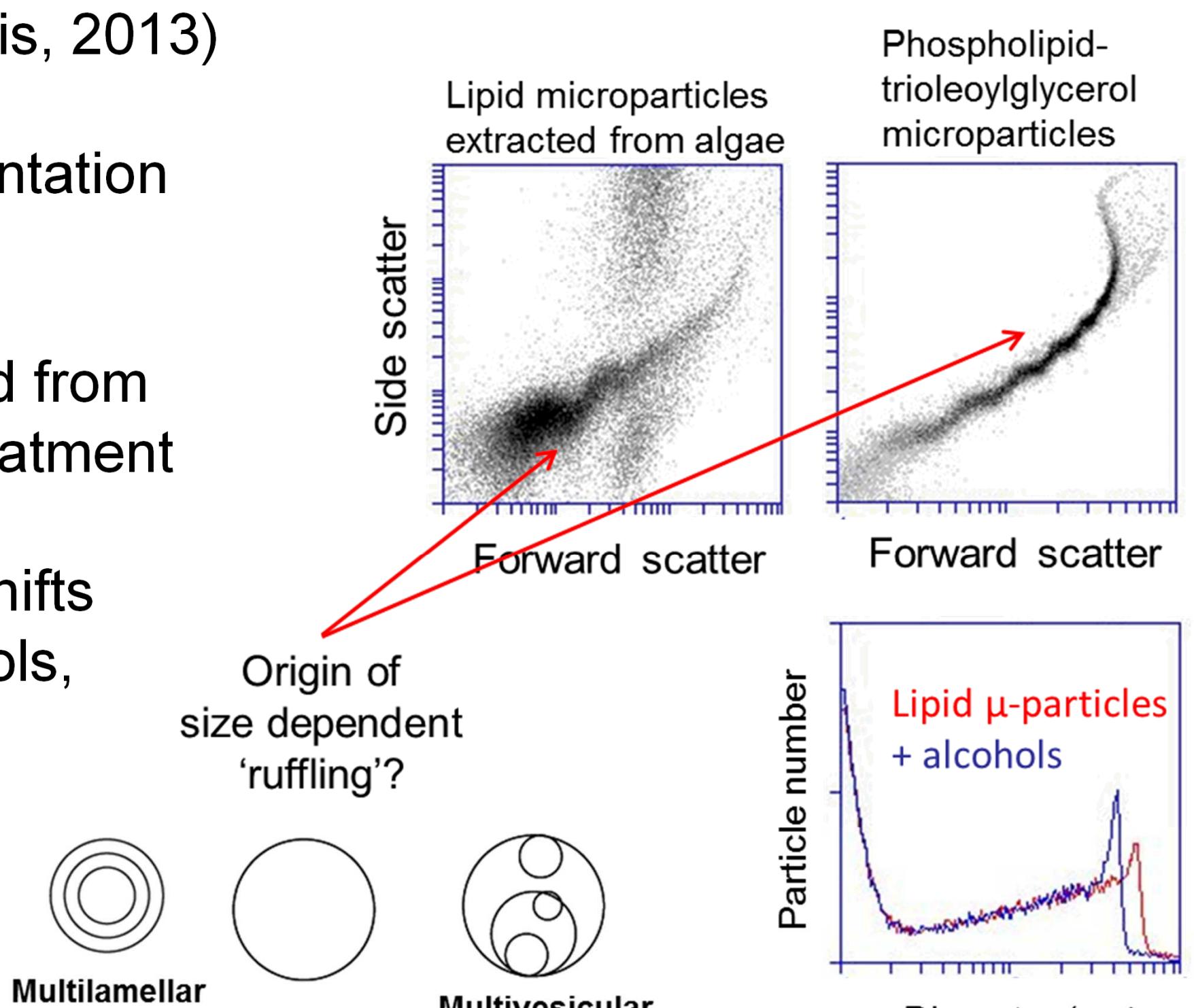
- Major fermentation inhibitors:
  - C5 alcohol, terpene products
  - FFA
  - Furfural/HMF
- High tolerance for salt,  $NH_4$
- Yield increased to ~80% of net theoretical by 3 separate process refinements:
  - retention of lipids removes product inhibition
  - Redox balancing by protein engineering:  $NADPH \leftrightarrow NADH$
  - Anaerobic fermentation
- Strains, substrates, products:
  - KO11: Carbs  $\rightarrow$  EtOH
  - AY3: Proteins  $\rightarrow$  isopentanol > isobutanol > propanol > dioxo-C4's
  - YH40-TS/DH1-TS: Proteins + carbs  $\rightarrow$  monoterpenes + sesquiterpenes

#### Fermentation inhibitors & enhancers



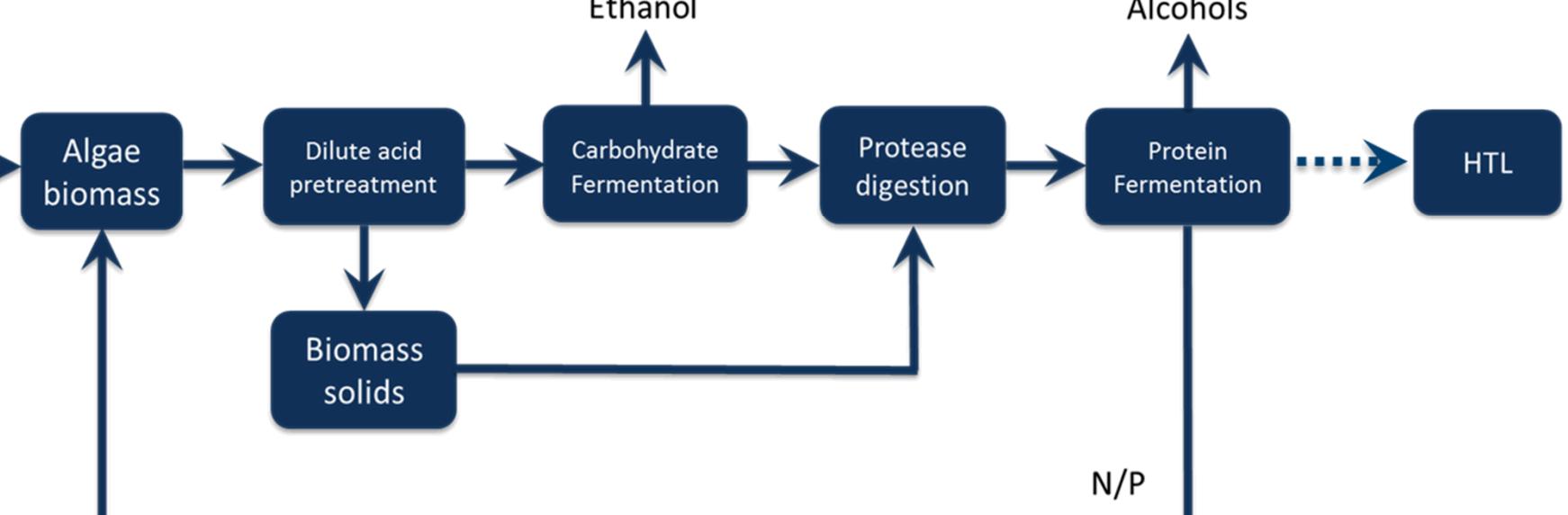
### Recovery of Lipids & Fermentation Products

- Azeotropic distillation of mixed alcohols was identified as the dominant energy cost for protein conversion to fuels (Davis, 2013)
- Retaining lipids in protein fermentation broth reduces alcohol toxicity
- Lipid microparticles are released from the algae biomass during pretreatment
- Microparticle size distribution shifts in the presence of mixed alcohols, indicating phase separation
- Co-extraction of lipids + alcohols demonstrated feasible using established biphasic methods



### Integration & Future Directions

- Supports Sandia's Biomass Program through funding from DOE's BioEnergy Technologies Office
- FY16: 1-pot co-production of intermediate value commodities and renewable fuels from algae feedstocks
- Techno-economic extrapolation to high productivity, high protein cultures indicates that proteins could account for ~37% of the total fuels from algae biomass



### Acknowledgments

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