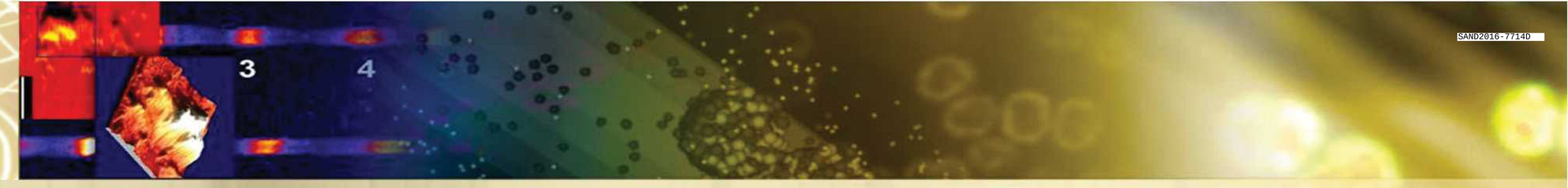
SANDIA BIOSCIENCE



SAND2016-7714D

Comprehensive bioconversion of algae biomass to liquid fuels and intermediate value products

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Motivation and Strategy

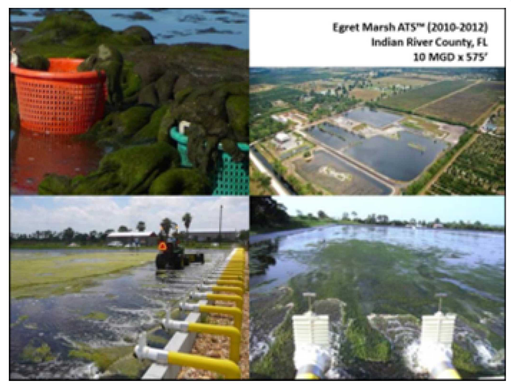
The goal of this effort is to enable to the production of cost competitive algae petroleum replacements at 5,000 gallons/acre/year. To achieve this goal, maximal conversion of all of the algae biomass components – not just lipids – to liquid fuels and biobased products is required. We are applying bioconversion strategies for generation of mixed fusel (≥C2) alcohols, terpenes, and ammonium by comprehensive utilization of the biomass to increase the total yield of 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 algae turf wastewater treatment from a variety of water sources and environments. 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. Future work will focus on fermentation strain development, process intensification, and scale-up through sponsorship from the DOE and collaboration with industry and academia.

Feedstock Flexibility

- High genetic and biochemical diversity among alga – conservatively >100,000 species worldwide
- Diversity allows wide tunability range for various environments & water sources
- Co-culture and cultivation of natural assemblages provide resilience to seasonable variation*
- Challenges for processing: ash content, flowability, and biomass recalcitrance
- Biomass production partners:

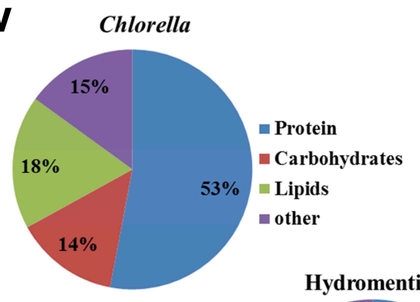
HydroMentia

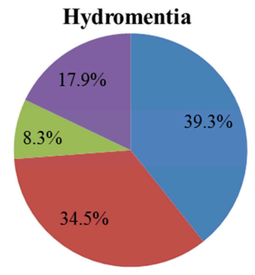
AzCATI Arizona Center for Algae Technology and Innovation

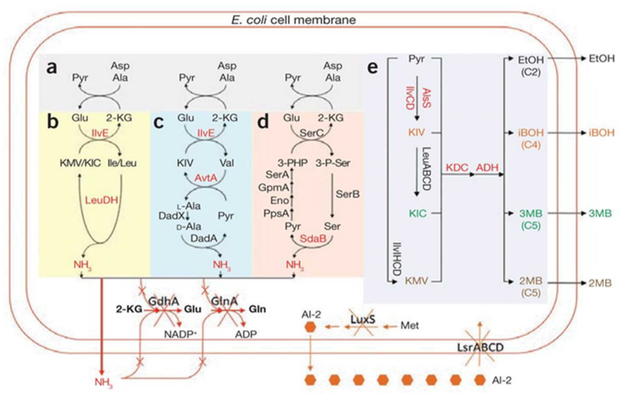


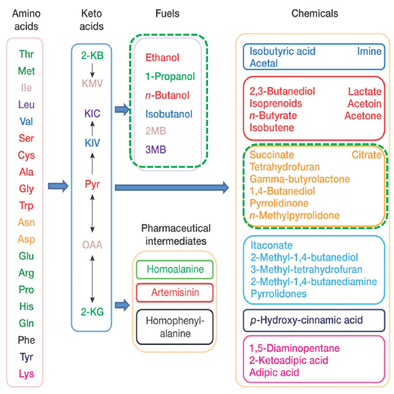
Conversion Process Overview

- High productivity and semi-continuous cultivation correlate to high protein content; half of biomass is unutilized using current lipid processing strategies
- Algae biomass have limited utility for feed, 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





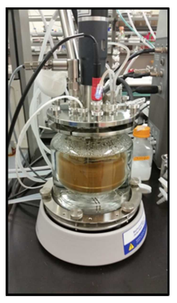


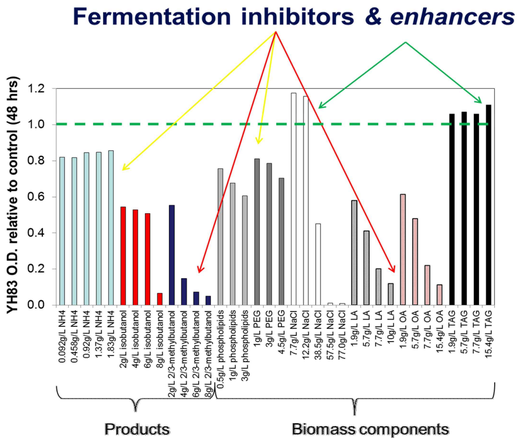


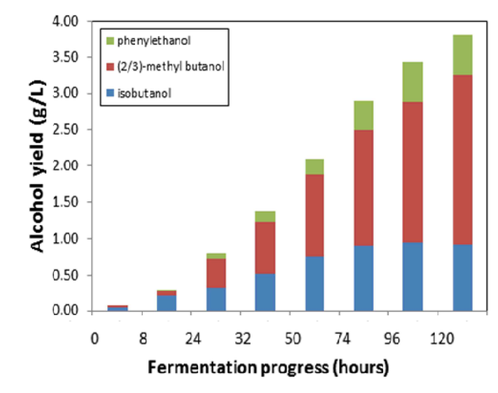
Huo, et al *Nat. Biotech.* 2011

Fermentation Optimization & Strain Development

- Major fermentation inhibitors:
  - C5 alcohol, terpene products
  - Furfural/HMF
  - Free fatty acids
- 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: substitute NADPH with NADH
  - Anaerobic fermentation
- *E. coli* is current bioconversion host, additional strains are being tested for reduced sensitivity to inhibitors, increased product titers

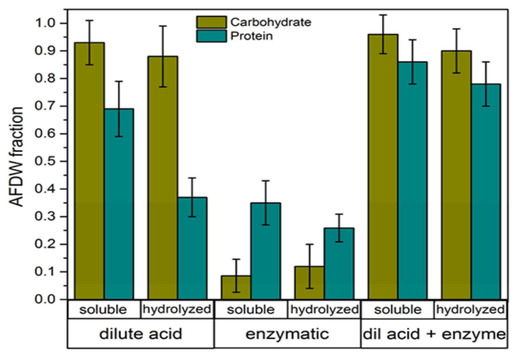


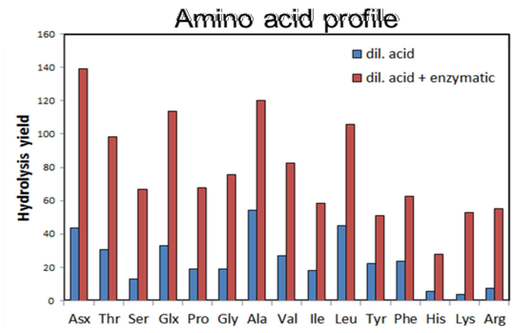




Wu and Davis, *Algal Res.* 2016(a)
Wu, et al *Algal Res.* 2016(b)

Biomass Solubilization and Hydrolysis

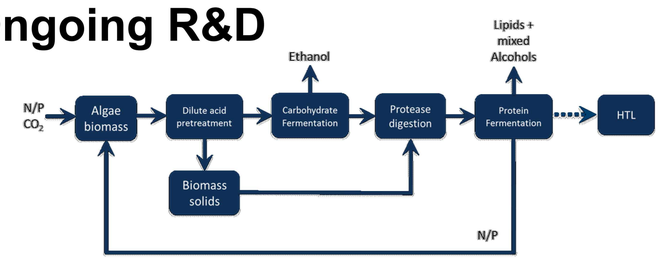


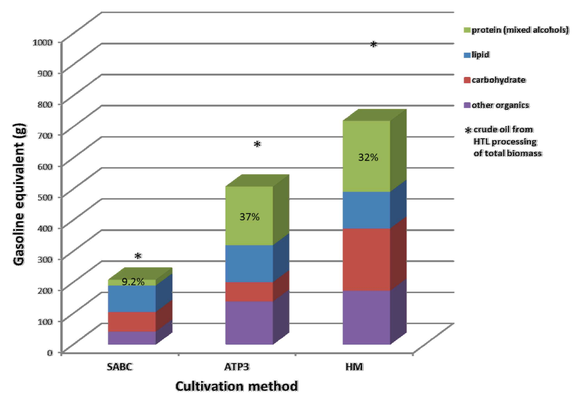


- Dilute acid (2-10% H_2SO_4) pretreatment is effective for solubilizing >80% of the proteins & carbohydrates, and hydrolyzing ~90% of the carbohydrates
- Proteins are only partially hydrolyzed to amino acids (~40%) via dilute acid
- Combination of dilute acid hydrolysis and enzymatic digestion using pronase cocktail yields ~80% protein hydrolysis to amino acids
- Technoeconomic analysis suggests that pretreatment, esp. enzyme costs are the dominant cost driver for process

Process Integration & Ongoing R&D

- Supports DOE-EERE's Biomass Program through the BioEnergy Technologies Office
- FY16/17 Milestones: 1-pot co-production of intermediate value commodities and renewable fuels from algae feedstocks
- Bioconversion yield indicates >2000 gal/acre/year fuel intermediate at current biomass productivity, ~13 g/m^2/day





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