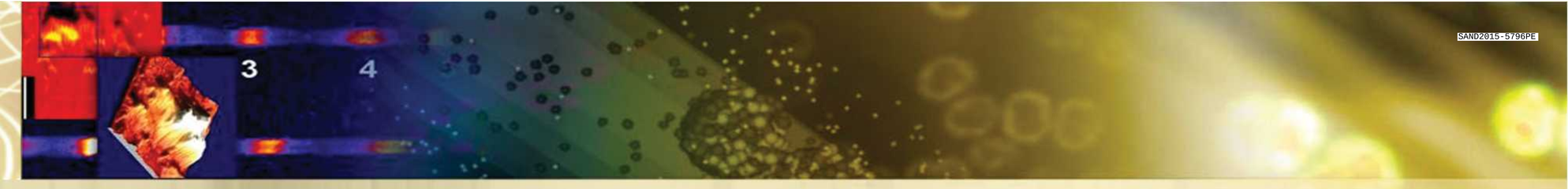
SANDIA BIOSCIENCE



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Bioconversion of Algae Biomass for Fuels and Bioproducts

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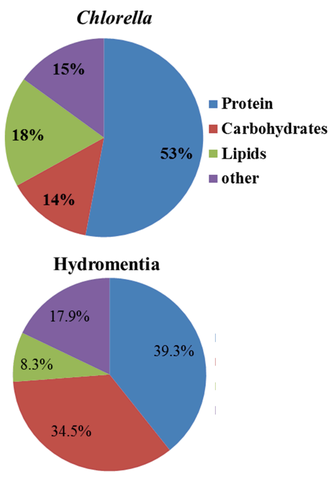
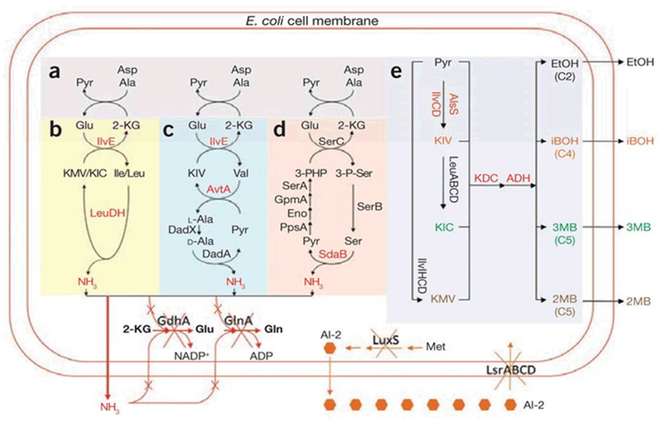
Motivation and Strategy

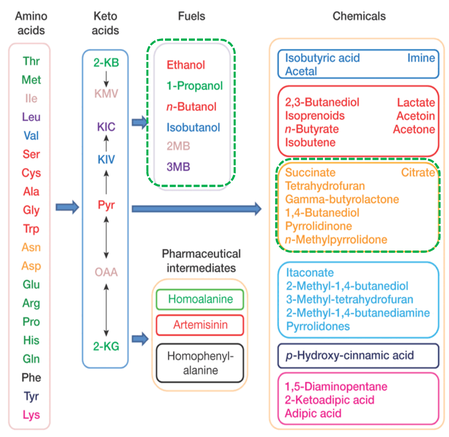
The goal of this effort is to enable to the production of algae biofuels at 5,200 gallons/acre/year. To achieve this goal, maximal conversion of all of the algae biomass components to liquid fuels and biobased products is required. Proteins and carbohydrates comprise ~80% of the ash-free dry weight biomass at maximum growth conditions. We are applying bioconversion strategies for generation of mixed (≥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 occuring 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



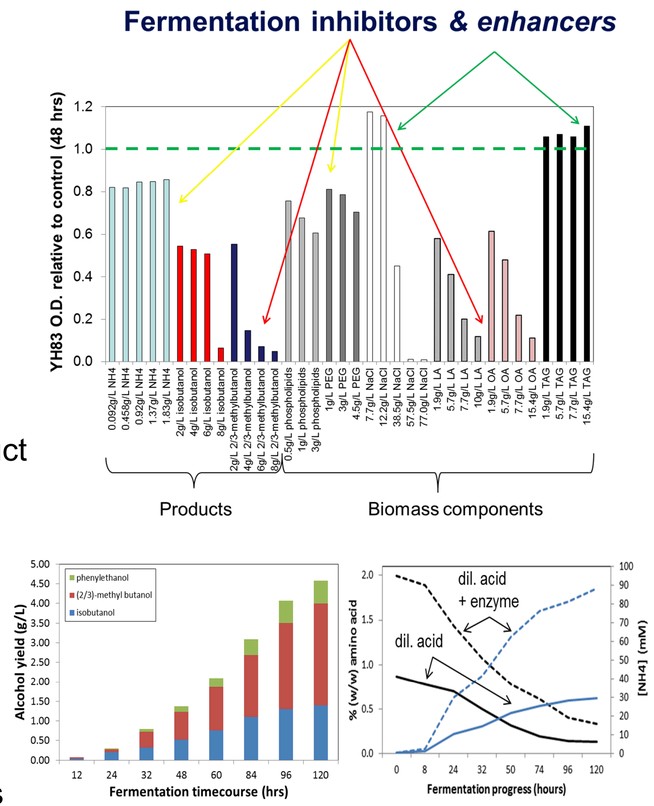




Huo, et al *Nat. Biotech*. 2011

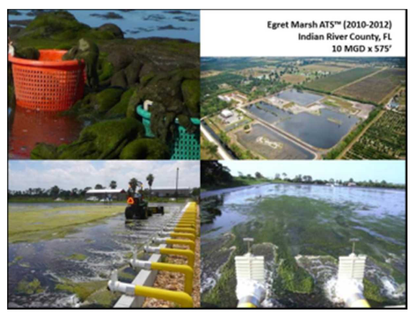
Yield Optimization

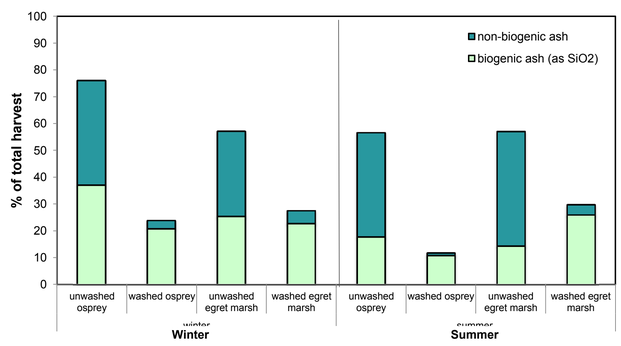
- Major fermentation inhibitors:
  - C5 alcohol, terpene products
  - FFA
  - Furfural/HMF
- High tolerance for salt, NH_4
- Yield increased to ~80% of net theoretical by 3 separate process refinements:
  - retention of lipids removes product inhibition
  - Redox balancing by protein engineering: NADPH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



Ash content: Seasonal variation and removal

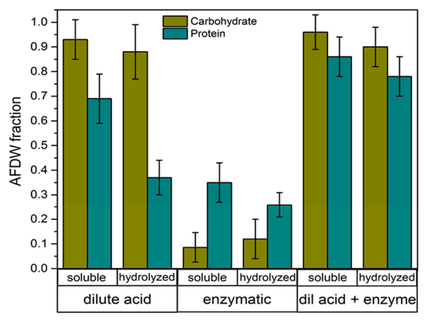
- Ash content in benthic algal assemblages is a major hurdle for optimizing biomass loading for efficient upgrading
- Field washing of harvested biomass was found to be effective for removal of non-biogenic ash
- Winter harvested biomass had higher biogenic (entrained) ash content
- These algae samples are being further investigated to identify seasonal differences in chemical composition

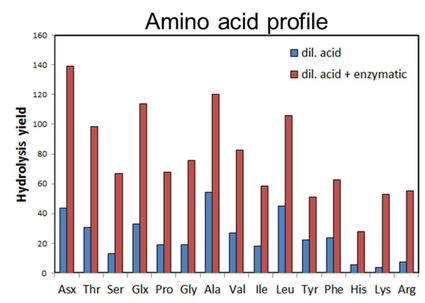




Solubilization and hydrolysis of algal biomass for biochemical conversion

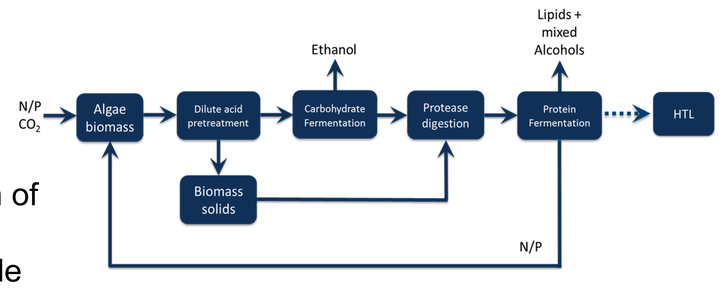
- Dilute acid (2-10% H_2SO_4) pretreatment is effective for solubilizing >80% of the proteins & carbohydrates, and hydrolyzing ~90% of the carbohydrates
- Proteins are only partially hydrolyzed to amino acids (~40%) via dilute acid
- Combination of dilute acid hydrolysis and enzymatic digestion using pronase cocktail yields ~80% protein hydrolysis to amino acids
- High quantity of non-natural amino acids in pretreated residual solids, including:
  - γ-aminobutyric acid (GABA)
  - β-aminoisobutyric acid
  - β-alanine

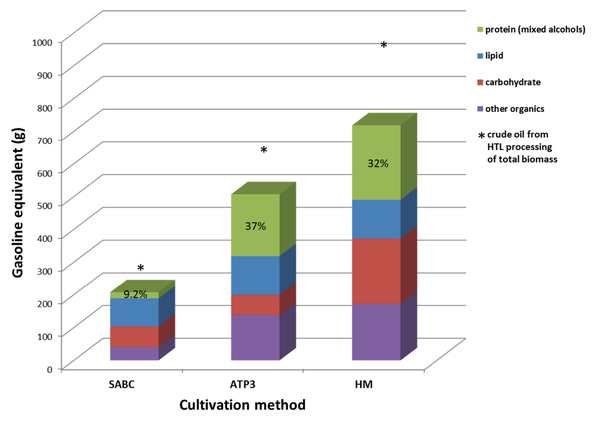




Integration & Future Directions

- Supports DOE's Biomass Program through the 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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