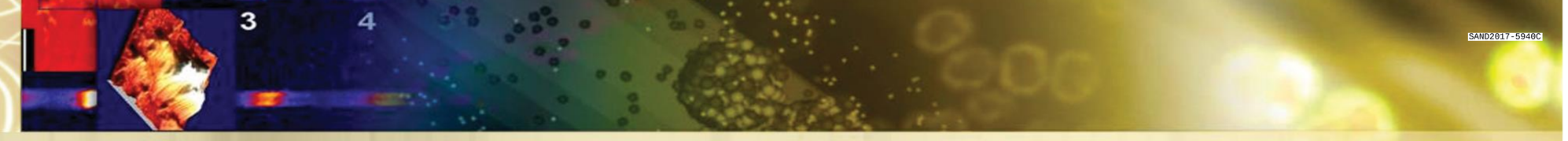
SANDIA BIOSCIENCE




# Integrated bioconversion of algal carbohydrates and proteins to liquid fuels and intermediate value products

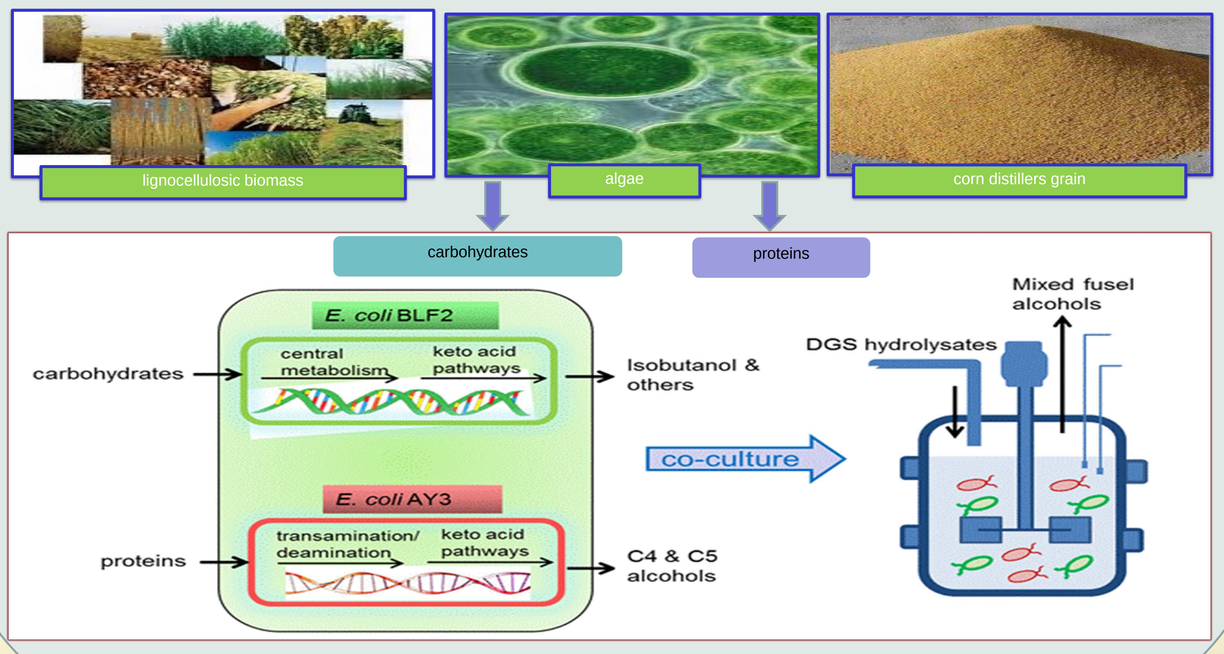
**Mary Tran-Gyamfi, Weihua Wu, James Jaryenneh, Eric Monroe, Fang Liu, Ryan W. Davis**

Sandia National Laboratory - Livermore CA – Biomass Sci & Conversion Tech.

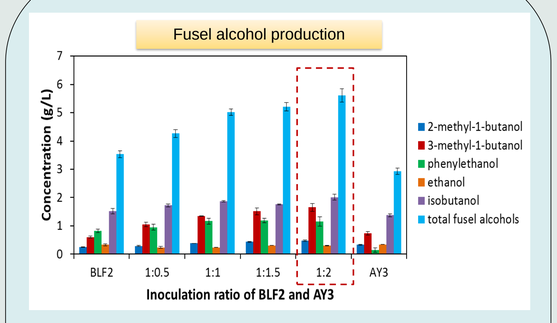
## Overview

With an increase in worldwide demand on energy coupled with the consumption of finite fossil fuels resources, the demand for sustainable alternatives from renewable resources has shown growing interest. Algae-based biofuels are a promising energy source because of their mitigation of $CO_2$ and their potential grow at high productivities.

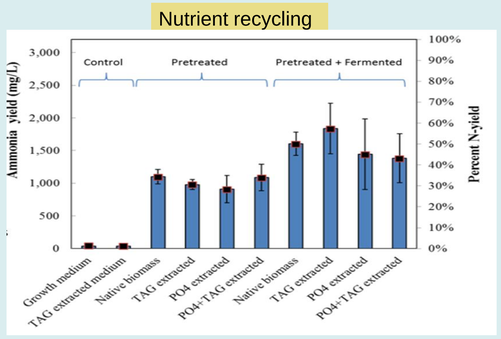
A primary challenge for achieving economically viable large-scale algae biomass production lies in the ability to efficiently convert the bulk of the biomass into sustainable commodities and recycle the major nutrients. At high algal productivities, the dominant biochemical components of algae biomass are proteins and carbohydrates. Employing efforts in metabolic engineering of microorganisms has shown promise in the bioconversion of high protein biomass to fuels and chemicals. Our work has demonstrated a process that integrates dilute acid and enzymatic pretreatment of microalgae with serial microaerobic fermentations for high yield bioconversion of algae biomass to C2-C8 alcohols, and remineralized N/P nutrients. This bioconversion process has been shown to be applicable on algal hydrolysates as well as on distiller's dried grains with solubles (DDGS).



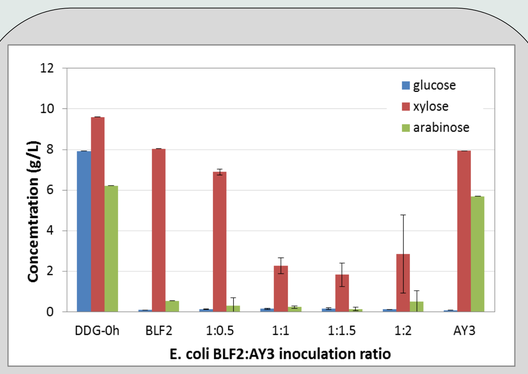

## Bioconversion of hydrolysates

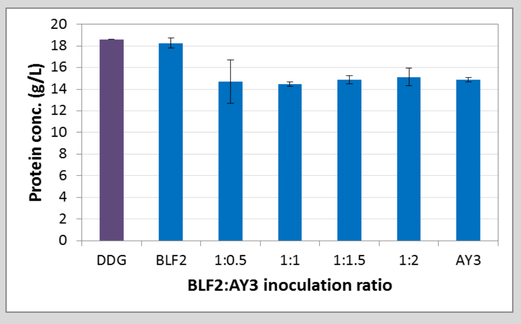


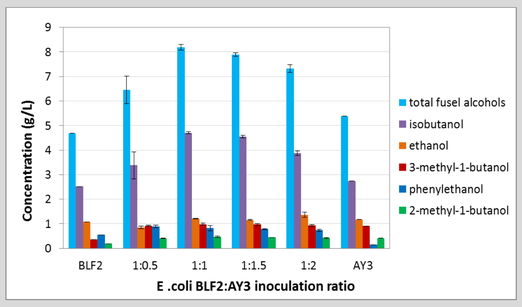

- *Nannochloropsis oculata* was pretreated with 10% dilute acid and pronase protease
- The consortium with the 1:2 inoculation ratio of *E. coli* BLF2 and AY3 achieved the highest fuel yield.
- Up to 5.6 g/L of total fusel alcohols were produced with isobutanol and isopentanol as the major products.




- Dilute acid and protease pretreatment converts ~35% of the total N to ammonia
- Protein fermentation conversion provides an additional ~27% of total N to ammonia
- Extraction of TAG or PO4 did not significantly alter the ability to remineralize biological N to ammonia
- The N-remineralization yield was 57% (±14%) of theoretical



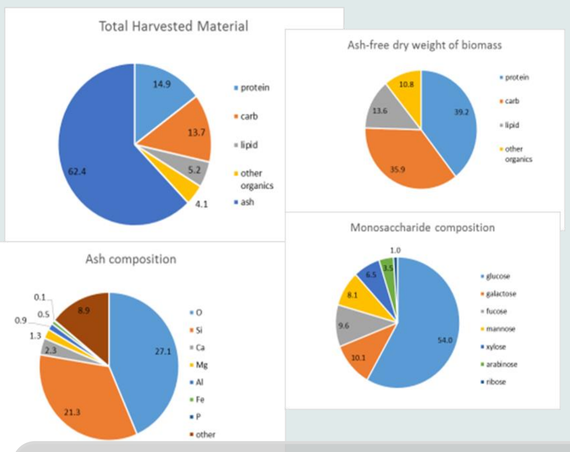




One-pot conversions of DDGs carbohydrate and protein into fusel alcohols using a synthetic microbial consortium with different inoculation ratios. A: sugar utilization, B: protein consumption, C: fusel alcohol yields.

## Methods

- Characterization of microalgal monocultures and mixed benthic biomass hydrolysates
- Engineer E.coli strains to biologically produce >10g/L fusel alcohols by redox engineering of key proteins, which provide bioenergetics efficiency gains in the fusel alcohol pathway
- Optimize dilute acid and enzymatic pretreatment protocols
- Demonstrate a "one-pot" bioconversion of protein and carbohydrate fractions of the hydrolysates into fusel alcohols by co-culturing two strains of E.coli BLF2 and AY3



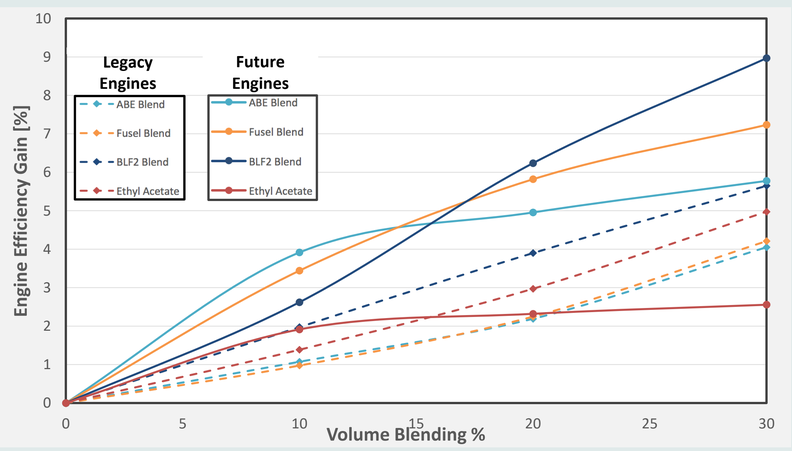

- Hydrolysates were characterized for AFDW, ash content, & fermentation substrates
- Monoculture (*Nannochloropsis*) hydrolysates contain ~95% glucose; benthic biomass contain ~50% of sugar alcohols, esp. mannitol




Figure1 The keto-acid pathways for fusel alcohol production in *E. coli* and the construction of the *E. coli* strain BLF2. AlsS (acetolactase synthase), IlvC (acetohydroxy acid isomeroreductase), IlvD (dihydroxy-acid dehydratase), Kdc (2-ketoacid decarboxylase), Adh (alcohol dehydrogenase), Ldh (lactate dehydrogenase), Pta (phosphotransacetylase), Ack (acetate kinase), KIV (2-ketoisocaproate), KIC (2-ketoisocaproate), KV (2-ketovalerate), KB (2-ketobutyrate), KMV (2-keto-3-methyl-valerate)

## Future Applications

### Bioconversion Products as Blending Agents with Gasoline for Spark Ignition Engines



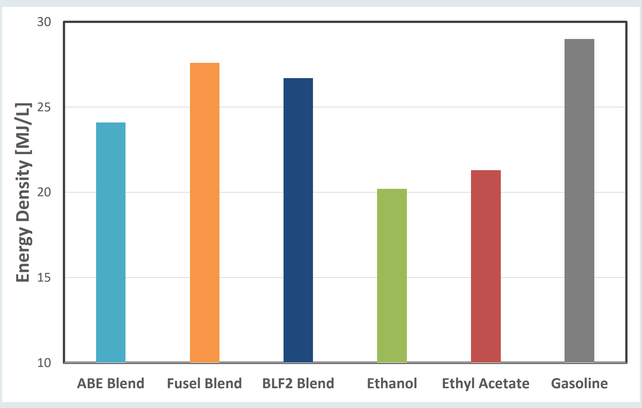

Energy Efficiency gain vs. volume % for BLF2 blend and Fusel blend (two possible blends from products we see in different fermentations)

Comparing our blend to competing mixtures, such as ABE (acetone-butanol-ethanol), and ethyl acetate

From initial tests, our mixtures have similar energy boosts as ethanol and have a much higher energy density than ethanol and other biofuels/fermentation mixtures

| Blend Name | Compound | Volume % |
|---|---|---|
| ABE Blend | Acetone | 60 |
| | n-butanol | 30 |
| | Ethanol | 10 |
| Fusel Blend (Protein Fermentation) | Isobutanol | 33.3 |
| | 3-methyl-1-butanol | 33.3 |
| | 2-methyl-1-butanol | 33.3 |
| BLF2 Blend (Carbohydrate Fermentation) | Isobutanol | 57 |
| | 2-methyl-1-butanol | 6 |
| | 3-methyl-1-butanol | 12 |
| | Ethanol | 10 |
| | Phenyl Ethanol | 15 |

Composition of blends




Energy density for our blends

## Acknowledgements

- This project was supported by the DOE Bioenergy Technology Office