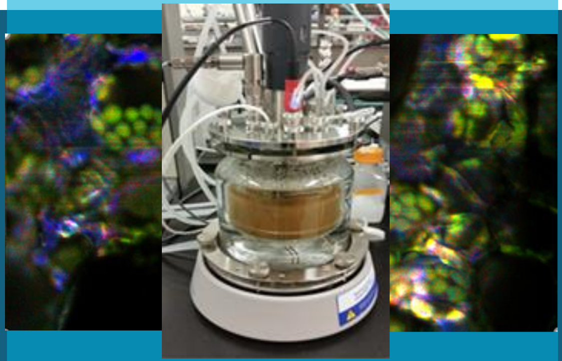
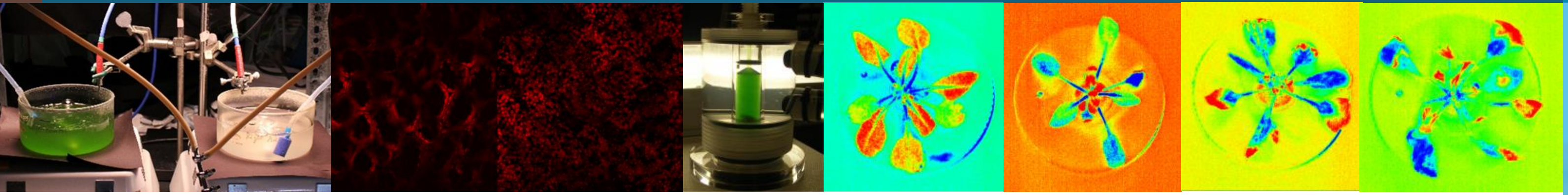
DOE Bioenergy Technologies Office (BETO) 2021
Project Peer Review

Bioconversion of Algae Proteins and Carbohydrates





March 24, 2021
Advanced Algae Systems

PRESENTED BY

Ryan W Davis

Sandia National Laboratories

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Project Overview

- The Bioconversion of Algal Carbohydrates and Proteins to Fuels project is meant to provide the technical means to surmount current limitations for conversion of algae biomass to fuels and bioproducts by efficient utilization of proteins and carbohydrates, which are the dominant components of algae biomass at high growth rate
- We are testing processing of diverse algae sources, including microalgae biomass from Open Raceway Ponds (via AzCATI and MicroBio Engineering, Inc) and periphytic algae from attached algae /Algae Turf Scrubber cultivations
- We are applying state-of-the-art biomass pretreatment and fractionation processes, and developing novel biocatalyst strains and bioconversion consortia to maximize titers, rates, and yields from algae biomass to commodity-scale fuels and bioproducts
- We are generating data to support TEA and LCA for scale-up and pond-side processing, and identifying opportunities for co-product and major nutrient recovery with joint tasks with NREL
- Project Objective: The current AAS-Conversion interface project goal is to demonstrate bioconversion of whole algae to maximize yield of petroleum displacing products with value at parity to the combined biomass production and conversion costs (~$900 /ton)

1 – Project Management

Task Name	FY21 Budget Authority
Task 1: Biocatalyst strain development	$150,000
Task 2: Fermentation process development	$85,000
Task 3: Joint TEA/LCA for proteinaceous algae biomass processing	$15,000
Project Total	$250,000

Go/No-Go Decisions

Name	Description	Criteria	Date
Aminocaprotoate production from proteinaceous algae biomass	Bioconversion of algae carbohydrates and proteins to aminocaproate.	Engineer bioconversion chassis to produce 0.4 g/g aminocaproate at 0.1 g/L/h.	6/30/21

1 – Project Management

Sandia National Laboratories [Ryan W Davis] – Dilute Acid pretreatment, biomass compositional and fermentation metabolite analysis, biocatalyst development, bioprocess testing, and retrosynthetic analysis

Old Dominion University [Sandeep Kumar] – Development of Flash Hydrolysis process and biomass pretreatment testing

Colorado State University [Jason Quinn] – TEA/LCA, co-products evaluation

Arizona State University [Arul Varman] – Enzyme engineering and in vitro assays

NREL [Ryan E Davis] – Joint tasks for comparing sustainability metrics between this project and NREL's CAP project

Imperial College London [Paul Fennel] – No fee collaboration for Anaerobic Digestion testing and N/P/metals reclamation

Industry Partners:

ZIVO Biosciences [Bill Pfund] – Strategic Partnership for utilization of functional carbohydrates and proteins from algae biomass

Johnson-Matthey [Ronan Bellabara] – Process engineering for application of proteinaceous biomass processing technology

Living Ink [Scott Fulbright] – Evaluation of bioprocessing residuals for algae ink application

Bimonthly meetings with SNL, CSU, ODU, and ICL project teams. Frequent communication with NREL, ZIVO, Johnson-Matthey, and Living Ink for delivery of samples and data

Key Partnerships & Industry Engagement

OLD DOMINION UNIVERSITY

ASU
Arizona State University

LIVING INK®

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Imperial College London

JM
Johnson Matthey

ThermoFisher SCIENTIFIC

μBIO
MicroBio Engineering

ZIVO BIOSCIENCE

2 – Approach

- **Biomass procurement & pretreatment:**

  - Open raceway pond algae biomass obtained from AzCATI, MicroBio Engineering, Inc., and Turf Algae from new and pre-existing deployments

  - Flash Hydrolysis and Dilute Acid Hydrolysis pretreatment evaluation. Challenges: obtaining sufficient biomass loadings for high reactor titers, minimizing chemical inputs and reaction times

- **Compositional determination:**

  - AFDW and proximate analysis with detailed amino acid and sugar profiling from pretreated biomass. Challenges: biomass logistics and storage, high biochemical diversity

- **Biocatalyst strain development:**

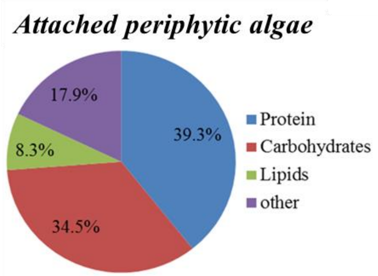
  - Computational Flux Balance and RetroSynthetic Analyses, biosynthetic pathway installation and metabolic flux engineering in *E. coli* and *Corynebacterium glutamicum* strains and co-cultures. Challenges: optimizing heterologous pathways for bioprocessing conditions, minimizing by-products

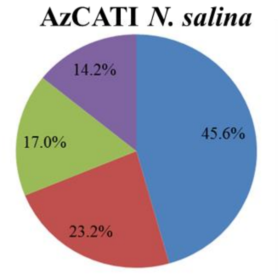
- **Bioprocess development:**

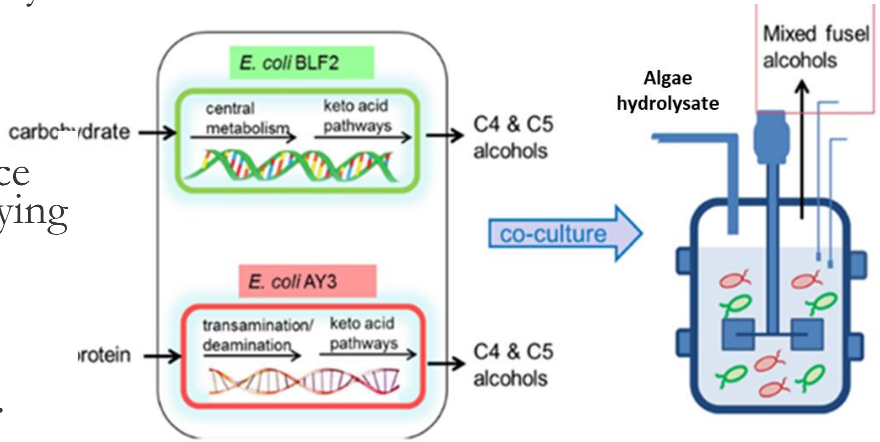
  - Process-controlled bioreactor studies for determining titer, rate, and yield performance metrics. Challenges: maximizing utilization of diverse substrates simultaneously, identifying generally applicable processes for diverse feedstocks

- **Sustainability and economic modeling:**

  - TEA, LCA, coproducts - Colorado State U (Quinn), NREL (Davis), Quiroz (Sandia). Challenges: comparing conversion technologies is complicated by varying levels of end product specification, co-product opportunities







3 - Impact

Provides technologies for valorization of low-value bulk algae biomass - fills data gap for efficient processing of high protein (>35%) low-lipid (<25%) biomass associated with high productivity cultivation. Process integration efforts identify process intensification and multi-product (biorefining) opportunities.

Achieved highest net yield of fit-for-purpose fuel production – 0.47 g/g fatty acid fusel ester (high performance biodiesel) from whole algae biomass, @ 25% lipid, 40% protein, and 35% carbohydrate composition biomass

Demonstrated compatibility with co-products recovery - applications testing using LEA for pigments and high value lipids; as well as process specific outputs, including high value amino acids, and functional sugars

Identified net positive ROI bioproduct scenario from low quality (low-lipid, high ash) algae – integrated TEA/LCA shows biomass cultivation and processing for fusel alcohol ester product at $11/GGE (with no co-products), $2.50 /GGE with $800/ton co-product value on biomass residuals basis

Valorization of nitrogenous organic content of high-protein biomass - Unique proposition for biobased commodities from algae via amine-bearing monomer, caprolactam, which is the required cross-linker for biobased Nylon-6,6 (with adipic acid). Current year Go/NoGo production milestone.

Reduces major N/P nutrient costs & sustainability concerns - Amino acid catabolism approach provides N/P nutrient recycle via struvite precipitation in bioprocess, accounting for >75% P recycle from recovered biomass

See associated publications and IP in supplementary material.

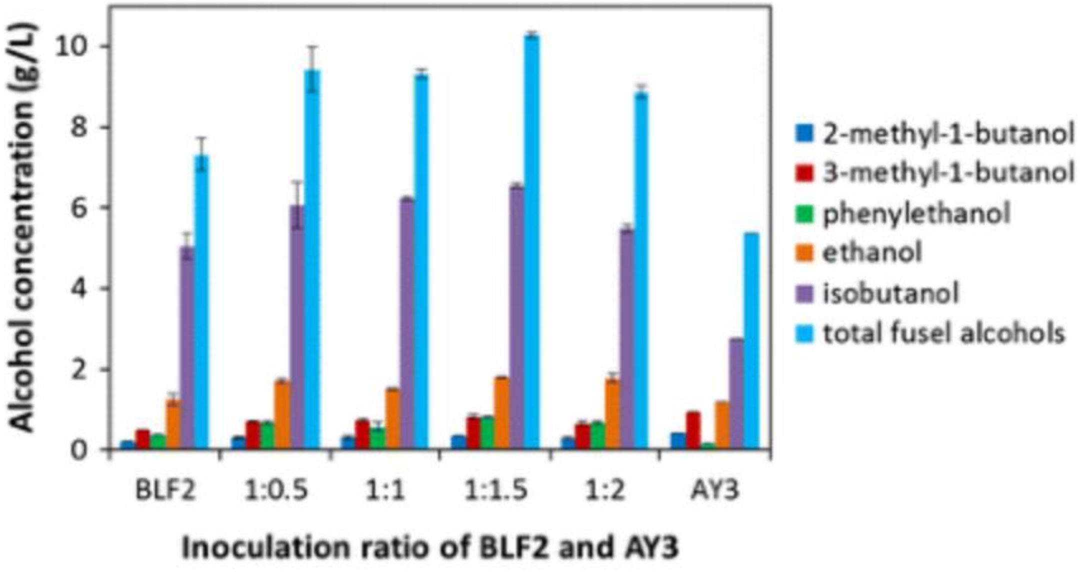
4 – Progress and Outcomes

- Fusel alcohol production process maximizes utilization of protein and carbohydrate substrates commonly found in whole algae biomass and lipid extracted algae

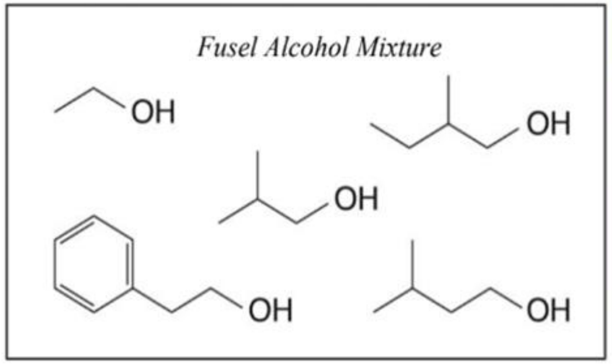
- Production methods compatible with existing bioethanol production and distribution infrastructure, potential for biorefinery diversification

- Co-product opportunities for high value amino acid enrichment from proteinaceous biomass

- Extensive fuel properties evaluation obtained by engine testing activities in BETO-CoOptima initiative, included as “Top 10 bioblendstock for spark ignition fuels application”



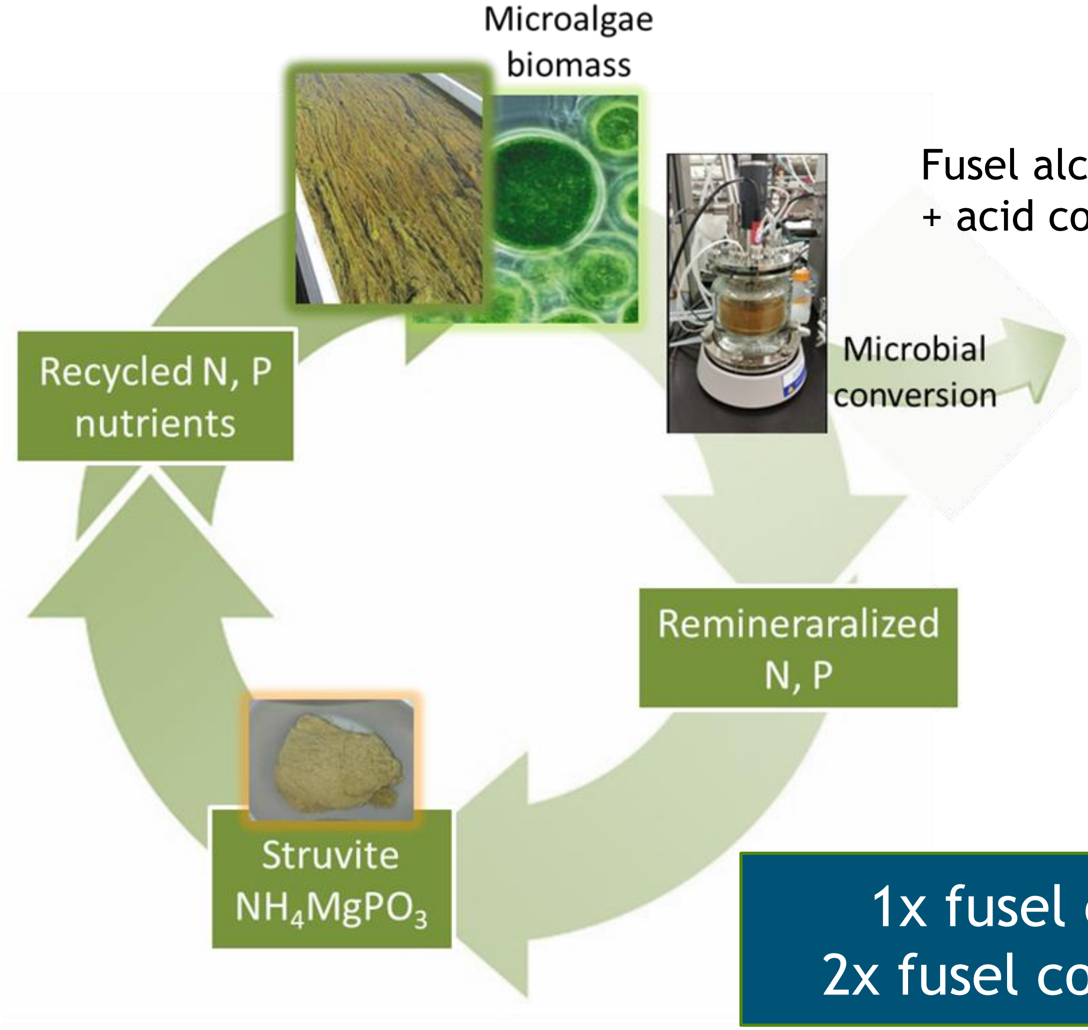
Yield from hydrolyzed protein: 0.26 g/g
Yield from hydrolyzed carbs: 0.40 g/g



Property	Fusel Alcohols
RON/MON	110/98
Sensitivity	12
bRON at 20%	115
HOV (kJ/kg)	637
LHV (MJ/L)	26.8

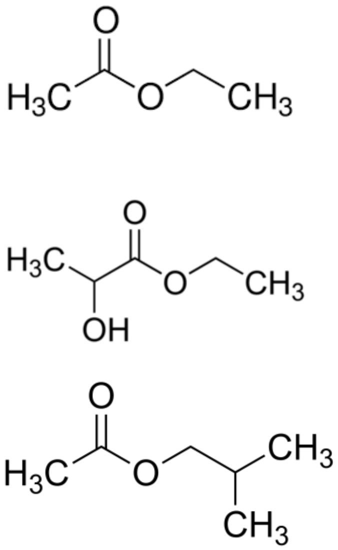
Liu et al *Biores Tech* 2019
Gaspar et al OSTI 2020
DeRose et al *Environ Sci Tech* 2020

4 – Progress and Outcomes



Fusel alcohol
+ acid coupling

Oxygenate / product titers



2.2 g/L

3.2 g/L

8.1 g/L

Fusel-esters: maximizing yield from central metabolism-derived intermediates

1x fusel conjugate: high octane gasoline blendstock
2x fusel conjugate: high performance diesel blendstock

<u>Yield potential ~50% higher than ethanol:</u>

acid from protein/carbohydrates: 0.81 – 0.93 g/g
alcohol from carbohydrates: 0.44 g/g
**alcohol from protein: 0.25 g/g

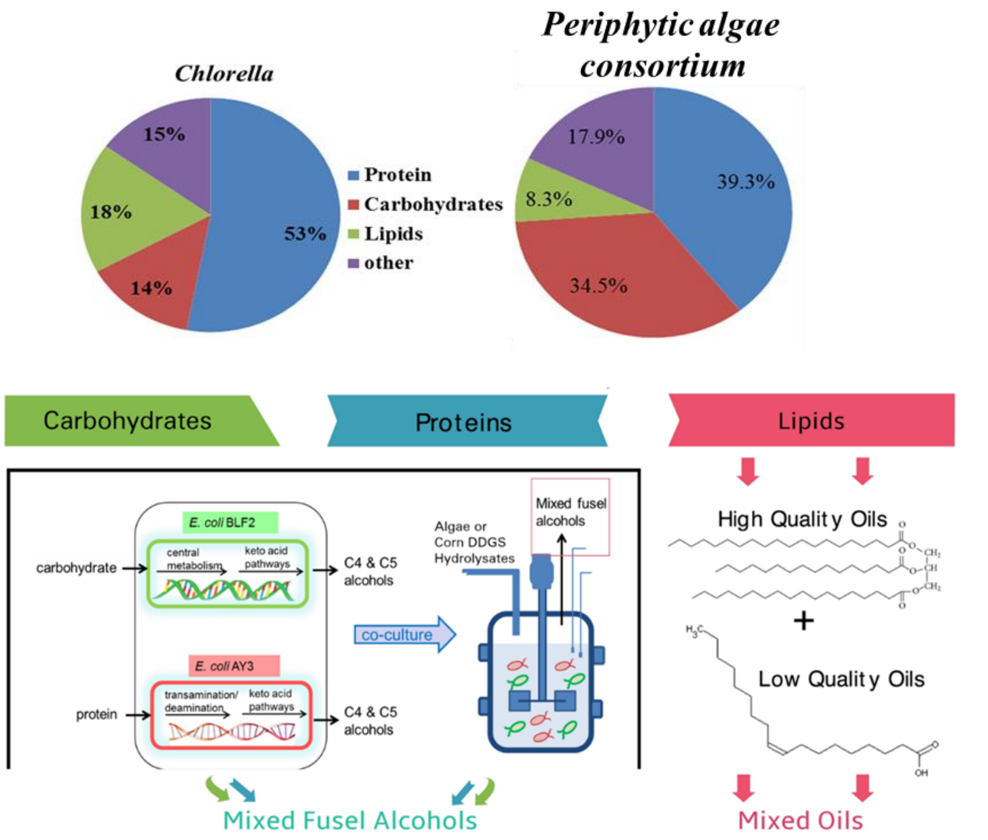
4 – Progress and Outcomes

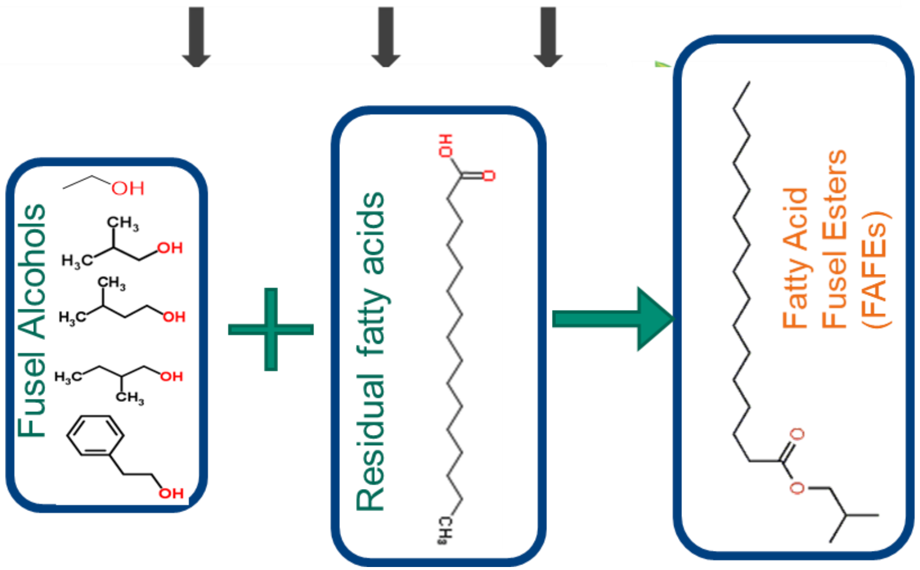
- Production process maximizes utilization of mixed substrates commonly found in algae: roughly equal fractions of lipids, carbs, and proteins

- FAFEs provide *improved LHV (+15%), cetane (+21%), and cold flow (-7°C cloud point) performance* compared to FAME, without sacrificing viscosity, lubricity, oxidative stability, or sooting metrics

- Production methods compatible with existing biodiesel production and distribution infrastructure

- Further chemical processing and fuel performance testing being performed through hand-off to BETO-CoOptima initiative





0.47 g/g fuel demonstrated

Monroe et al *Fuel* 2020
Carlson et al *Energy & Fuel* 2020

4 – Progress and Outcomes

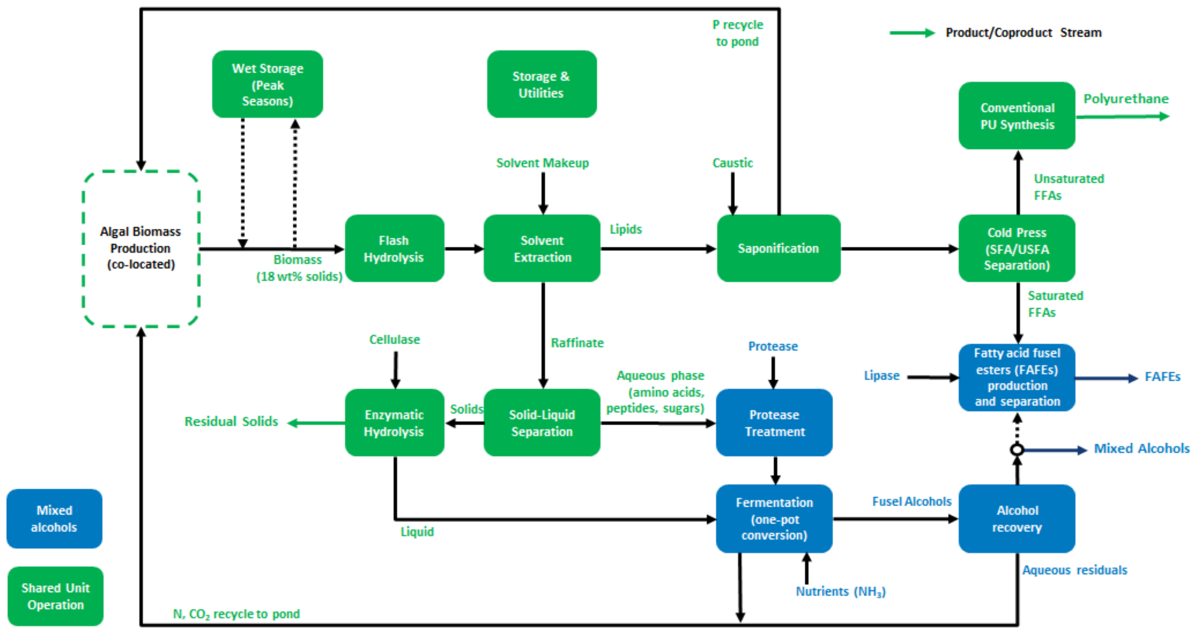
- Joint milestone between SNL and NREL for evaluation of algae fractionation processes, based on likelihood of highest productivity biomass having high protein content

- Common framework TEA/LCA based on fuel and co-product biorefining scenarios to establish MFSPs and relative GHG emissions

- Co-product assumed to be algae-derived polymer, functional substitute for polyurethane

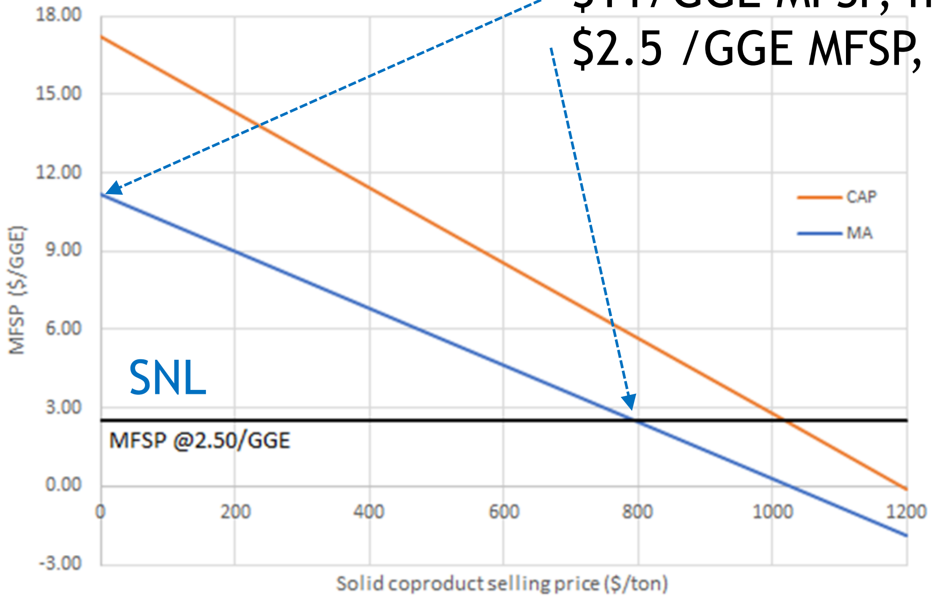
- Sensitivity analysis indicates strong dependence on maximizing utilization of all fractions of biomass (proteins, carbohydrates, lipids)

- Co-product value is within current algae market offerings ($1100/ton)



Integrated TEA/LCA for BETO algae fractionation pathways

$11/GGE MFSP, no co-products
$2.5 /GGE MFSP, $800/ton co-products



4 – Progress and Outcomes

Slurry (1% w/w)
18.4 g FD Algae
7.72 g Carbon
1.26 g Nitrogen

Flash Hydrolysis
(280 ℃, 9 s)
Sample #1 Tribonema
Microalgae

Hydrolysate
14.4 g FD Hydrolysate
6.10g Carbon
1.05g Nitrogen

Solids (lipidic char)
1.85 g Solids collected
1.06g Carbon
0.11g Nitrogen

Slurry (7.5%w/w)
20.4 g FD Algae
4g H_2SO_4/5.0g $Ca(OH)_2$
410mg protease
8.55g Carbon
1.39g Nitrogen

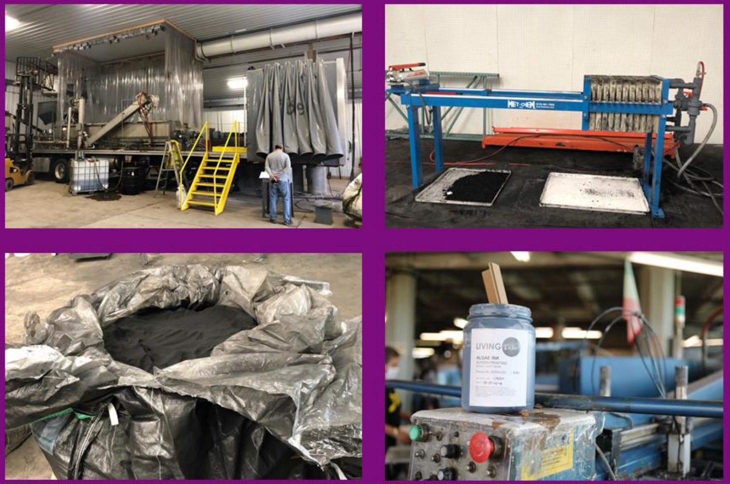
Dilute Acid Hydrolysis
(140 ℃, 1.5%, 1hr)
Sample #1 Tribonema
Microalgae

Hydrolysate
16.1 g FD Hydrolysate
6.61g Carbon
1.20g Nitrogen

Solids (Unspecified Coproduct)
6.83 g $CaSO_4$
1.94g Carbon
0.19g Nitrogen

Pretreatment mass balances

Investigating algae bioprocessing by-product opportunities



Living Ink
Pigment Production, Purification, and Formulation

Multiple combinations of fuel + coproduct (ink, foam, feed) Show high probability for profitability at $800/ton dry biomass value

BLOOM

BLOOM Foam™ Algae Shoe

LIVING INK®

4 – Progress and Outcomes

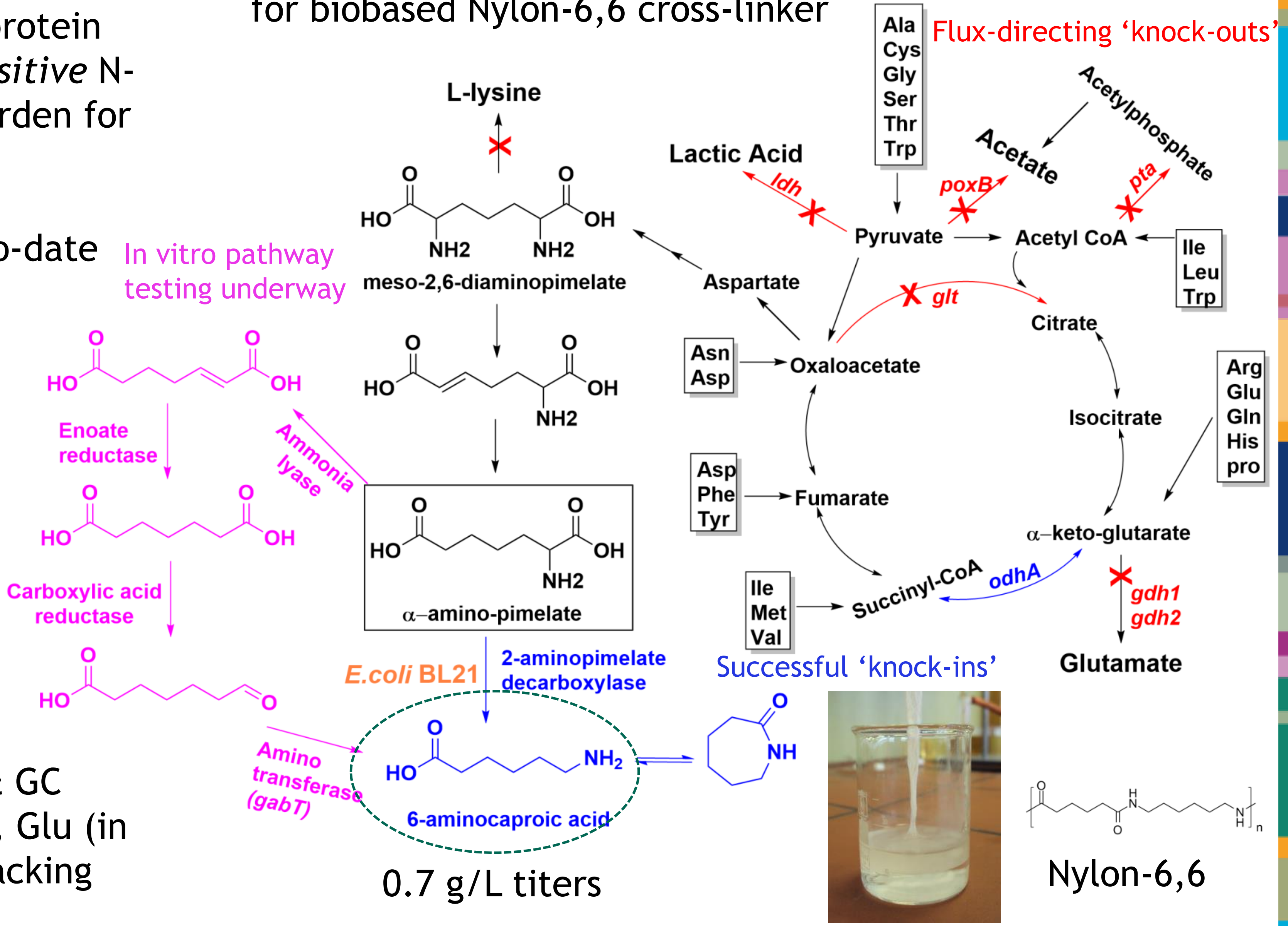
- **N-flux optimization:** conversion of protein hydrolysate to ACA result in a net *positive* N-efflux, +2.7% (removes metabolic burden for N-reuptake inhibition, etc)

- **Retrosynthetic analysis:** progress to-date provides titers >10mg/L based on ‘established’ 2KG intermediate, enzymes using this intermediate are not suitable for production

  New RSA-results indicated improved titers from Asp-mediated (lysine) biosynthetic pathway which, which utilizes only well characterized microbial enzymes

- **Analytical methods ‘pivot’:** HPLC & GC methods developed for Asp, AAP, Lys, Glu (in addition to 6-ACA) for metabolite tracking

Biosynthetic pathway development for utilization of low-value algal amino acids for biobased Nylon-6,6 cross-linker



5 – Summary

- Bioconversion of Algal Carbohydrates and Proteins provides means to surmount current limitations for utilization of low value algae biomass and lipid extracted algae for production of biobased commodities and co-products
- Demonstrated effective algae biomass pretreatment using dilute acid and flash hydrolysis pre-processing (7.5 - 10% solids loading), comparative analysis, including algae-ink coproduct underway
- Successfully achieved >10 g/L, >70% yield of algae carbohydrates and proteins to suite of fusel alcohol products and derivatives thereof (fusel lactates, FAFE) with major nutrient recycling via struvite.
- New TEA/LCA for proteinaceous biomass shows path for fusel alcohols and high value amino acids production (Quinn, CSU) and fusel esters and bioplastic (SNL/NREL).
- New bioprocess being developed for maximizing value of nitrogen which is copious in proteinaceous algae biomass for production of fully biobased Nylon-6,6.
- Technology transfer activities underway with Johnson-Matthey and ZIVO Biosciences.

Quad Chart Overview

Timeline
- Project Start Date: 10/1/2019
- Project End Date: 9/30/2021

	FY20	Active Project
DOE Funding	(10/01/2019 – 9/30/2020)	*$700,000*

Project Partners
- Colorado State University
- Old Dominion University
- Arizona State University

Barriers Addressed
Algal Biomass Characterization, Quality, and Monitoring (Aft-E)

Algal Feedstock Material Properties (Aft-G)

Algal Feedstock Processing (AFt-I)

Project Goal
The current year goal is to demonstrate the ability for bioconversion of whole algae biomass to provide bioproduct at parity with the biomass production and conversion costs (~$900 /ton).

End of Project Milestone
Demonstrate titers, rates, and yields of bioconversion of whole algae biomass to caprolactam of 1 g/L, 0.1g/hr, 0.4 g w/w algae protein

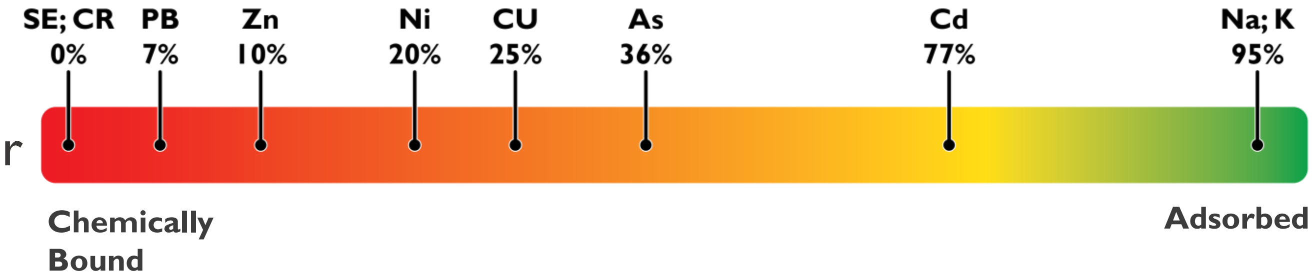
Funding Mechanism
BETO-AAS-AOP

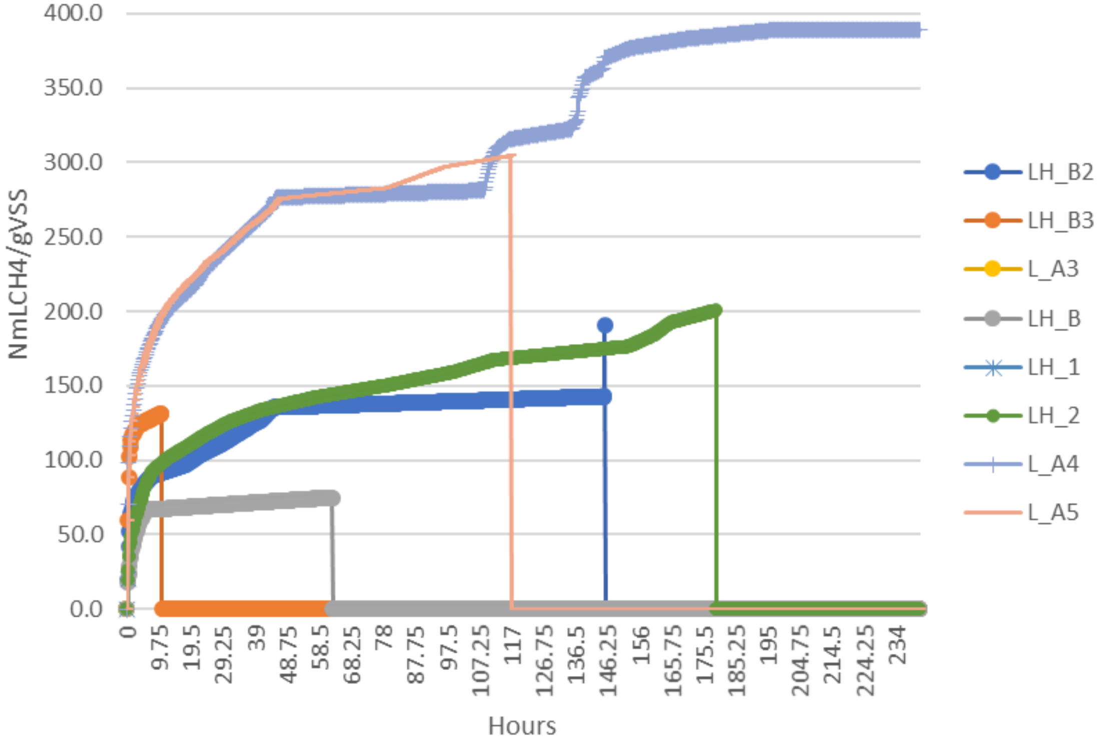
Anaerobic digestion 'baseline' for utilization of low-value, waste-water cultivated algae

- Chemical titration of biomass using EDTA to evaluate whether metals chemically or physically bound, i.e. can we 'clean' the biomass?

- Preliminary data on bio- and thermochemical conversion for fuels applications, utilization as a blendstock in thermopolymers, aquaculture feeds, and biostimulants, however, contaminants may limit adoption for these applications

- 'Off-the-shelf' means for coupling metals concentration & disposal via **anaerobic digestion (AD)**, if scales can be matched. Bench-scale yields up to 46% C-basis (0.39 g/g), approx. one week retention time. TEA/LCA underway with project partner, Prof Quinn (ColoStateU)





FY20, AAS-Conversion Q4 Milestone (NREL/Sandia/Algix):

Submitted to BETO 10/2020: Joint comparison of NREL CAP & SNL Proteinaceous Algae Processing pathways for SOT. ***Results indicate favorable TEA for Algae Fuels + Bioplastics biorefinery scenarios***

NATIONAL RENEWABLE ENERGY LABORATORY

Comparative Techno-Economic Assessment for Technologies to Convert High-Protein Microalgae to Fuels and Value-Added Products

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[b] Biomass Science and Conversion Technologies, Sandia National Laboratories, Livermore, CA 94550, USA

[c] Algix, 5168 Water Tower Rd, Meridian, MS 39301, USA

Abstract

Microalgae has become established as a novel feedstock possessing numerous advantages for use in producing renewable fuels and products, with techno-economic analysis (TEA) modeling frequently used to highlight the economic potential and technical challenges for utilizing microalgae for this purpose. However, many historical TEA studies have focused on conversion pathways reflective of high-quality biomass compositions, generally representing elevated levels of carbohydrates and lipids with lower levels of protein (corresponding with a nutrient-deplete state upon harvesting algal biomass from cultivation), which incurs substantial burdens in the ability to achieve high cultivation productivity rates relative to nutrient-replete, high-protein biomass. Given a strong dependence of algal biomass production costs on cultivation productivity, further TEA assessment is needed to understand the economic potential for utilizing generally lower-cost but lower-quality, high-protein microalgae for biorefinery conversion.

Supplementary info – recent publications and IP

Improve algae biomass utilization opportunities for biobased commodities and nutrient recycling to support production scale-up based on variable composition and co-product opportunities

Recent peer-reviewed Publications:

1) Monroe, Shinde, Liu, Varman, Carlson, George, Davis "Superior performance biodiesel form biomass-derived fusel alcohols and low grade oils: Fatty acid fusel esters" *Fuel* (2020) https://doi.org/10.1016/j.fuel.2020.117408
2) Aseidu, Davis, Kumar "Catalytic transfer hydrogenation of flash hydrolyzed microalgae into hydrocarbon fuels production (jet fuel)" Fuel (2020) https://doi.org/10.1016/j.fuel.2019.116440
3) DeRose, DeMill, Davis, Quinn "Integrated techno economic and life cycle assessment of the conversion of high productivity, low lipid algae to renewable fuels" *Algal Research* (2019) 38 https://doi.org/10.1016/j.algal.2019.101412
4) Liu, Lane, Hewson, Stavila, Tran-Gyamfi, Hamel, Lane, Davis "Development of a closed-loop process for fusel alcohol production and nutrient recycling from microalgae biomass" *Bioresource Technology* (2019) https://doi.org/10.1016/j.biortech.2019.03.006

Patents:

1) Davis, Simmons, Tran-Gyamfi, Wu "Biochemical upgrading of high protein biomass and grain products" US Patent No. 10,683,519B1
2) Davis, Varman "Production of fusel lactates from biocatalysis" US Patent Appl No. 17/003,061
3) Davis, Monroe, Carlson "Coupling high yield biochemical intermediates for fuel production" US Patent Appl No. 16/929,439

Tech Transfer: process adaptation and licensing agreements underway with Johson-Matthey through DOE-OTT

Responses to Previous Reviewers' Comments

The goal of this project is to extract additional value from algal biomass, which is currently expensive to produce. The basic idea is to use algal biomass as a substrate for a microbial fermentation process. Initial work was focused on the production of fusel alcohols. Current work is focused on the production of ethyl lactate using an engineered microbial consortium. Overall, the project is very innovative. The team is making reasonable progress. It is not clear how the metabolic engineering work fits into this project—the details are somewhat vague. The project would benefit from TEA and more focus on product recovery.

>> The metabolic engineering details were deemed to be beyond the scope of this presentation, but are provided in full detail in the primary literature generated from this work, building on the nitrogen flux optimization work from Prof. Liao (UCLA). Since the previous review, we have redoubled our efforts to gain insights from TEA/LCA for processing cost drivers. These include peer reviewed manuscripts from the lab of Prof. Quinn (Colo State U) for co-production of fusel alcohols and high value protein, and a joint TEA with NREL/SNL for co-production of fusel alcohol esters and bioplastics (polyurethane assumed).

• This team has demonstrated important improvements in the production of alkanoates and fusel alcohols from microalgal biomass. So far, they have achieved near-theoretical yields in pretreatment, demonstrated the first in vivo production of ethyl lactate, and are well on the road toward demonstrating a two-times yield improvement over their previous target. • On the overall, this project is technically sound and aims to add value from the refining of algal biomass from non-oil streams. Identifying the right product mix and markets for these products will be challenging. This project, the way it is presented, seems like a complicated one with stretch goals/targets. Better project management, clear go-no-go decision making, and a better explanation of current progress is needed to make it clear what the project status is, and how the team is planning to meet milestones.

>> We are seeking to better define the most profitable product spectrum using rigorous TEA analyses, which are updated here. The concerns over clearly explaining the project management and better go/no-go decision making have been taken seriously, and are more clearly described in this iteration, as well as stating the specific challenges being addressed in the approach.

• The goal of this project is to utilize algal biomass as a feedstock for producing chemicals. After extraction of lipid products from algal cultures, an abundance of protein and carbohydrates remains that can be used as a substrate. The team has focused on making hydroxyalkanoate esters from algal protein and carbohydrate. This is a complicated process, requiring organisms to take up a complex mixture of substrates and make two different compounds and then condense them by esterification. The team has made very commendable progress given the modest budget and is in a position to make a very high impact if the progress continues. The team has a good vision for future work, but specific tasks associated with improving product titer are not described. In the future, they should focus on some of the metabolic challenges in getting all the pathways to work in coordination. Growing multiple strains in coculture at scale is high risk and has no commercial validation

>> Since the last peer review, we have begun employing computational flux balance and biochemical retrosynthetic analyses to identify and quantify product titer improvement potential, and enzyme performance regimes based on the fermentation conditions (pH, co-factor balancing, etc). We acknowledge that co-culture fermentation strategies are not currently implemented at scale, however, growing interest in engineered community based catalytic systems, as well as our relatively simple implementation strategy, based on dual inoccula (at ratios corresponding to the relative concentration of protein and sugar substrates) should compete very favorably over pots-in-series bioprocessing for both CAPEX and OPEX.