Lipids from High-Protein Algae Biomass

Eric P. Knoshaug, Robert S. Nelson, Tobias Hull, Stefanie Van Wychen, Nick Nagle, and Jake Kruger

Abstract

Biofuels from renewable and sustainable sources will be a critical component of reducing greenhouse gasses. To this end, in achieving maximum biomass productivity in outdoor ponds at a reasonable cost, algae must be grown at a maximal rate. This current algae cultivation strategy results in high-protein biomass because there is insufficient time for deplete-hold steps that increase lipids but also cost. Thus, methods to convert high-protein need to be developed for biofuels. The Combined Algae Processing (CAP) strategy has previously shown utility on high-carbohydrate algae biomass and has now been expanded to include methods aimed at converting the protein fraction to lipids. After acid-pretreatment, hydrolysate liquor is oxidatively treated to deaminate and convert soluble proteins and amino acids into four main carboxylate acids; formic, acetic, succinic, and propionic. These acids are readily and completely fermented into intracellular lipids using oleaginous yeast. Using a fed-batch fermentation strategy and no added nutrients, we achieved nearly 30% intracellular lipids and 1.3 g/L lipids demonstrating the potential of our expanded CAP process to generate additional lipids for conversion to biofuels as part of an algae-focused biorefinery.

Reference: Hull, T., et. al. Tuning algal hydrolysate composition through wet oxidation and ion exchange for enhanced fermentation by oleaginous yeast. Manuscript in preparation.

Materials and Methods

- High-protein, open raceway grown *Nannochloropsis salina* biomass.
- Pretreatment was performed in a 4 L (1 L working volume) batch ZipperClave reactor. 300 g of wet algal paste was loaded into the reaction chamber along with H_2SO_4 and water to achieve a final solids loading of 20% (w/w) at an acid concentration of 2.0% (w/w). Pretreatment was performed at 175º C for 15 minutes. Centrifugation was used to separate the solids.
- Mild Oxidative Treatment was conducted in a Parr-4552 2-gallon reactor loaded with 2.5 kg hydrolysate pressurized with 12 bar zero air at room temperature, to give 35 bar total pressure at the reaction temperature. The reactor was heated to 200 °C, held for 60 min, and cooled with an internal cooling coil carrying chilled water. Heat up and cool down times were each approximately 1 h. When cool, the reactor was depressurized and opened, and the contents were pumped into a receiving vessel using a peristaltic pump.
- Cation exchange was conducted by loading the post-MOT hydrolysate and ion exchange resin (Amberlite IRC-120) in a vessel at a ratio of 3 mL of solution to 1 g resin. The slurry was stirred at room temperature with a magnetic stir bar for 30 minutes, after which the resin was filtered out of solution using Buchner funnel vacuum filtration. Half of the ion-exchanged post-MOT hydrolysate (IX1) underwent an additional ion exchange step following the same procedure (IX2).
- *Cutaneotrichosporon oleaginosus* (ATCC 20509) was used to convert post-MOT-IX liquors and was followed for cell growth and FAME production using fermenters. Operating conditions were: 30 °C, 225 rpm, pH 6.5, 300 mL working volume (1 L total volume). Inoculum cultures were grown aerobically at 30° C at 225 rpm in shake flasks for 18 hours and used to inoculate the fermenter at a starting OD 600 nm of 0.2.

Compositional Analysis of *Nannochloropsis* High-Protein Algae Biomass

Algae Species	FAME Lipids	Protein	Carbohydrates	Ash	Mass Balance	Glucose	Mannose	Galactose	Other
N. salina	8.5	32.2	9.2	25.6	75.5	2.7	0.5	1.6	4.4

Reported as percent of total dry mass, percent mass balance closure, and sugars as percent of total biomass

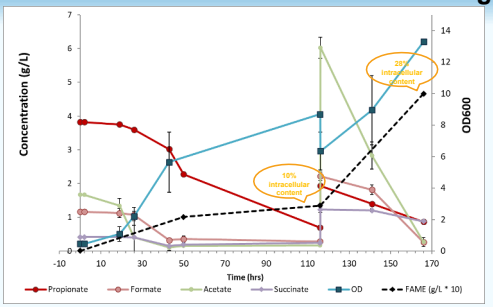
Acid Pretreatment / Mild Oxidative Treatment / Ion-Exchange

	Carbon Retention	TOC [g/L]	Organic acids [g/L]	Nitrogen Release	Ammonia [g/L]
Large Scale MOT (L)	59.3 %	10.06	2.69	105 %	5.92

	Compound [g/L]				
Solution	Ammonia	Formic acid	Acetic acid	Propanoic acid	Succinic acid
Post-MOT Hydrolysate	5.92 (~333 mM)	0.50	1.6	0.29	0.30
IX-1	1.05 (~56 mM)	0.47	1.10	0.10	0.27
IX-2	0.53 (~27 mM)	0.45	1.09	0.08	0.24

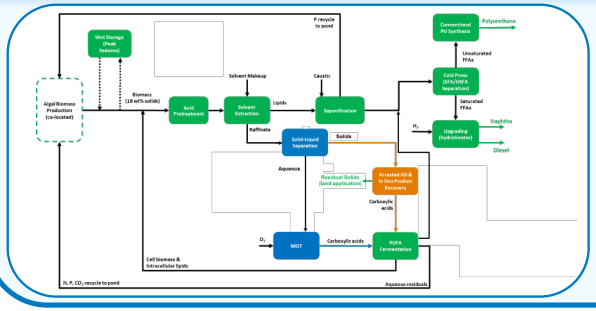
- Fermentation of post-MOT without IX was toxic/inhibitory and nothing grew
- Must remove high concentrations of NH4 >10-fold above typical fermentation media
- Removed 92% of the NH4 to ~27 mM
- Pure recovered stream of NH4 available back to pond for sustainability and cost savings

Fermentation Profile in DAP-MOT-IX2 high-protein hydrolysate



- Acids utilized rapidly and concurrently initially then slow down
- Feed of concentrated MOT-IX2 gave major boost allowing accumulation of intracellular lipids
- Increase in initial lipid titer >590-fold with a yield of 0.52
- Concentration of MOT materials after IX maybe required to provide adequate feedstock for lipid accumulation
- Fed-batch may provide path forward for economic lipids production from high-protein algae biomass

Multi-product High-Protein Algae Biorefinery



- High-protein biomass has potential for multiple products; polyurethane, naphtha, diesel, biochar
- Acid pretreatment and MOT-IX allow for several facile recycle streams
- MOT-IX material from high-protein algae biomass was non-toxic and easily fermentable
- *C. oleaginosus* provides protein-based additional lipids that can be integrated as a product into an algal biorefinery

Conclusion

High-protein algae biomass was successfully demonstrated to be a readily convertible material and expands upon the suite of algae feedstocks suitable for conversion in CAP. After standard acid pretreatment, an oxidative treatment was used to convert proteins/amino acids/carbohydrates to a range of carboxylic acids for further biological funneling to intracellular lipids. This treatment also led to quantitative release of NH4 from the amino acids causing ammonium toxicity and was removed to create a pure and easy to recycle back to the pond N stream. Fermentation of the MOT-IX material was initially rapid producing a large increase in intracellular lipids. A fed-batch strategy was successful in further increasing lipid titers though further concentration may be necessary to reach economic titer/yield/productivity targets.

This work was authored by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office . The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.

International Conference on Algal Biomass, Biofuels, and Bioproducts
Waikoloa Beach, HI
June 12-14, 2023
NREL/PO-2700-85700