Growing the World's Fuels

Sapphire Energy®

BETO 2017 Project Peer Review

March 2017

Goal Statement

- In response to the BETO's ABY funding opportunity (DE-FOA-0000811), SEI proposed to advance our current biomass production technologies across three priority areas to meet the target 2,500 gallons of oil per acre per year by 2018.
    - **Priority Area 1: Improve Algal Biomass Productivity**
    - **Priority Area 2: Improve Pre-Processing Technologies**
    - **Priority Area 3: Integration of Algal Biomass Unit Operations**

- *Project outcome: Achieved annual average productivity of 12 g/m2/day, producing equivalent of 3031 gal/acre/year of HTT product, over one year in 127m2 raceways in Las Cruces, NM*

- *Productivity is directly related to the economics of algal biofuels production. By improving average productivity, cost of fuel production is greatly decreased.*

Quad Chart Overview

Timeline

- Project started 1Q2014
- Project ended (algae work) 3Q2016
- Essentially complete (NREL work being completed under no-cost ext.

Budget

	Total Costs FY 12 –FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	$1,298k	$2,539k	$862k	0
Project Cost Share (Comp.)*	$355k	$749k	$585k	0

Barriers

- Technical barriers– pond stability issues net low average productivity
- Directly addresses 2,500 gallons of biofuel intermediate per acre per year MYPP target
- Changing markets, algae strains, and deployable technologies

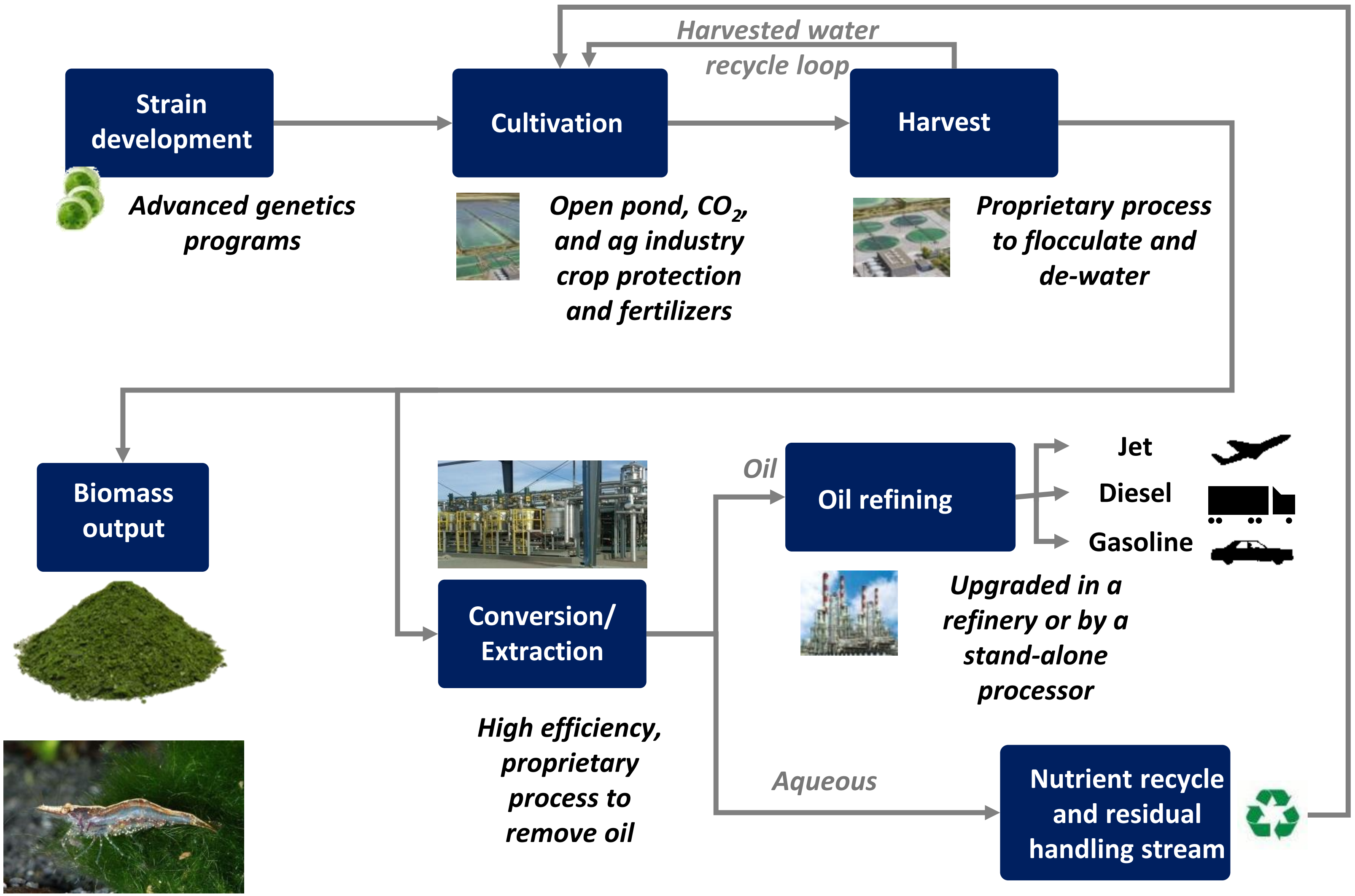
Partners

- Systems biology work partnered with Institute for Systems Biology
- NREL for analytical chemistry, TEA and LCA analysis
- Phil Savage lab, University of Pennsylvania, for biofuels work

Agenda

8:30-9:00	Introductions
9:00-10:00	Project overview
10:00-10:30	Project management
10:30-10:45	Break
10:45-12:15	Technical Performance I
12:15-13:00	Working Lunch
13:00-14:30	Technical Performance II
14:30-15:00	Updated TEA & LCA; Review of commercialization
15:00-15:30	Break
15:30-16:00	Open discussion; project team departs
16:00-17:00	Panel discussion & summary

Overview of Sapphire Energy's process



Priority Area 1: Improve Algal Biomass Productivity

Focus on improving areal productivity at lab and field scale

- Improvements in basal productivity
- Improvements in pond uptime

Objectives

- **Objective 1:** Improve biomass productivity of *Tetraselmis* strain, SE50416, in lab scale simulators using Evolutionary Engineering (EE) approaches.
- **Objective 2:** Improve predicted biomass yield through rational strain design by (1) doing a complete functional annotation and comparative genomic analysis of the model algae *Chlamydomonas reinhardtii* and our fuel production strains, (2) building an EGRIN to understand and predict transcriptional responses to environmental perturbations and integrate with a metabolic FB model, and (3) using the integrated model to identify and rank target genes for optimization of fuel production
- **Objective 3:** Develop a novel, high throughput system for assaying algal communities under varying conditions of temperature and nitrogen source to develop consortia which demonstrate that increasing diversity can decrease the variation of productivity across different environmental conditions.
- **Objective 4:** Improve biomass productivity of *Nannochloropsis* production strains to over 9 g/m2/day annual average by (1) refinement of production strain options, and (2) agronomic practice optimization, coupled with (3) improved yield loss strategies.

EGRIN-FB Modeling Preliminary Results

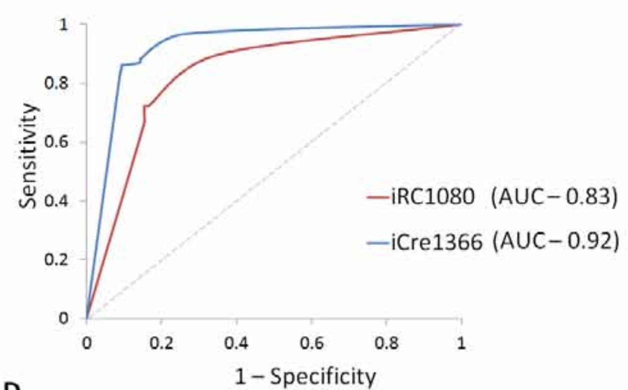
http://networks.systemsbiology.net/chlamy-portal/

- **Metabolic Network Analysis**: Using information from genomic and metabolic databases (ChlamyCyc2.0, KEGG, NCBI and Phytosome 10), ISB constructed an updated, manually curated genome-scale metabolic model for *C. reinhardtii* named *i*Cre1366
- Accounts for a significantly larger portion of the *C. reinhardtii* genome
- Shows significantly improved predictive capabilities relative to its predecessor *i*RC1080
- *i*Cre1366 proved to be a useful model for prediction of growth rate, as the predicted growth rates were very close to those observed from chemostat grown cells

A.

	iRC1080	**iCre1366**	Increase
Genes (Transcripts)	**1073 (1086)**	**1366 (1474)**	**29%**
Nucleus	1073	1325	
Chloroplast	0	34	
Mitochondria	0	7	
Metabolites	**1068**	**1132**	**6%**
Reactions	**2190**	**2400**	**10%**
Lit. evidence	1101	1174	

B.



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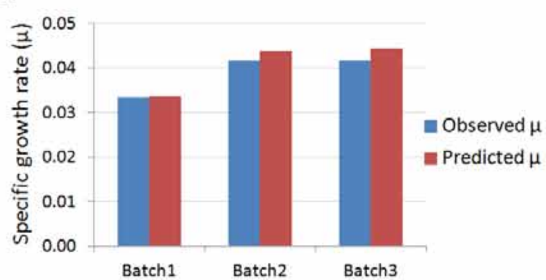

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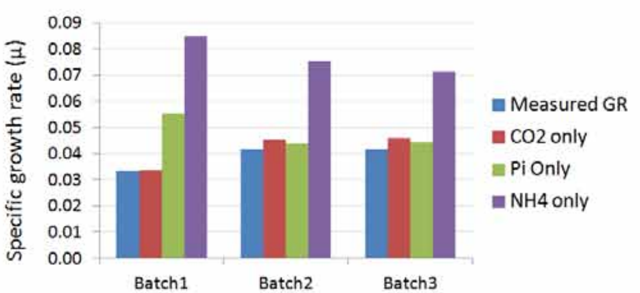
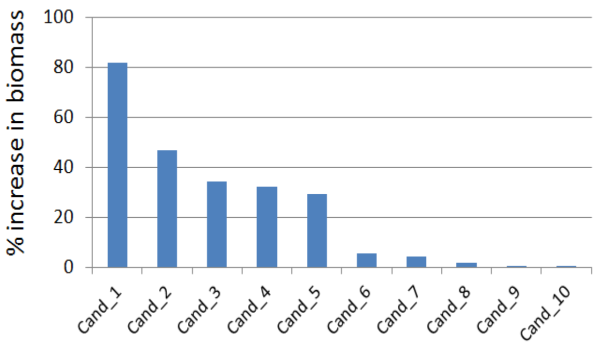


Defined Potential Targets for TAG accumulation and Biomass Production

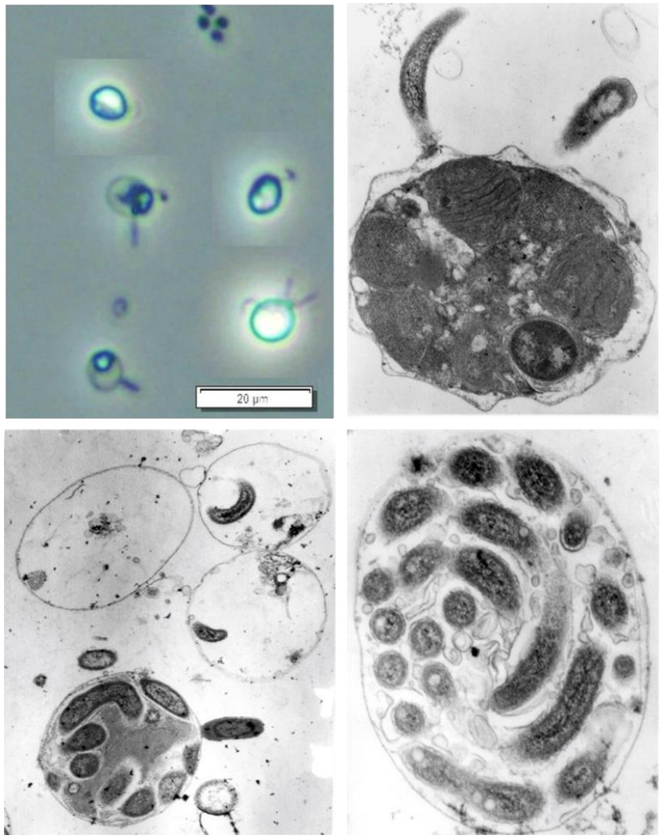
- Publicly available data and transcriptional wave analysis of GRN model were used for metabolic network analysis-complement analysis from a metabolic perspective
- Utilization of constrain – based modeling techniques to analyze genome-scale model (iRC10801, Chang et al 2011)
- Flux-balance analysis –central method for analysis
- Development of new EGRIN-FB model ***i*Cre1366**
- **Ranked list of targets from model for increased biomass production**



ID	Enzyme name	Gene association
Cand_1	Complex III (ETC)	Multi-enzyme complex
Cand_2	Na+/H+ antiporter, chloroplast	(Cre16.g671250 OR Cre09.g395288)
Cand_3	Rubisco	(ChreCp049 AND (Cre02.g120100 OR Cre02.g120150))
Cand_4	Acetyl-CoA:CoA antiporter	Cre07.g339554
Cand_5	Complex IV (ETC)	Multi-enzyme complex
Cand_6	Carbon-monoxide:acceptor oxidoreductase	(Cre09.g391450 AND Cre09.g391650)
Cand_7	NADH dehydrogenase (Complex I ETC)	Multi-enzyme complex
Cand_8	Malonyl-CoA decarboxylase	Cre02.g145200
Cand_9	Malate dehydrogenase (NADP+)	(Cre14.g629700 OR Cre14.g629750...)
Cand_10	Alcohol dehydrogenase (ethanol, NADP)	Cre09.g394658

FD111 Identification

- Microscope observation revealed the presence of both hook and rod shaped bacteria attached to the surface of the algal cell. (It is unclear if either one or both of these organisms are pests.)
- Isolation efforts have so far been unsuccessful, however complete isolation is not necessary for study of treatments and may not be possible since many pathogenic organisms are host-dependent.
- Sequencing of 16S region of the bacterial genome identified a number of potential leads on the identification of the pest. The most commonly identified sequence, and the only one consistently identified in different sources was similar to *Pseudobacteriovorax antillogorgiicola* strain RKEM611 (Accession KJ685394), a bacterium that infects other bacteria with a life cycle that includes a stage called a bdelloplast which is an elongated cell found within the host that later divides into the daughter cells (1)(McCauley et al., 2015).



Electron microscopy by Peter Letcher at the University of Alabama

Annualized Productivity for *Nannochloropsis Sp.*

- **Technical Target:** Generation of a set of algal strains with demonstrated algal productivity value of over 9 $g/m^2/day$ in the field in our 30,000 L pilot ponds
- **Measurable outcome:** Algal biomass productivity measured by dry weight (or optical densities converted to dry weights via strain specific calibration curves) in laboratory-based pond simulators and measured by harvested biomass productivity in SEI's Las Cruces, NM open pond facility.
- **Relevance to FOA Goals:** The proposed work will develop optimized agronomic and crop protection practices for *Nannochloropsis sp.* algal strains to produce maximal biomass yields and thus achieve the goal of Priority Area 1.
- **Relevance to downstream efforts:** Optimized agronomic and yield protection practices will increase yield and stability, and provide a steady stream of biomass for downstream processes ultimately driving down costs.

	Average In-situ Productivity (g/m2/day)
January	3.86
February	3.90
March	3.88
April	11.80
May	17.00
June	20.53
July	18.71
August	18.35
September	17.00
October	11.80
November	3.90
December	3.86
Annual	11.22

Priority Area 2: Improve Pre-Processing Technologies

Focus on improving process from harvest through oil production

- Improve harvesting performance
- Improve oil quality

Objectives

- **Objective 1:** Optimize methods to harvest algae from open ponds at low cost based on existing, well-characterized sewage sludge treatment technology.
- **Objective 2:** Improve yield and quality from hydrothermal treatment (HTT) through (1) conducting detailed characterization of process oils and product streams and (2) improve overall profitability through the introduction of a catalyst into hydrothermal treatment to improve oil quality, specifically reduced metal content in the oil.
- **Objective 3:** Establishment of a detailed molecular understanding of the composition of high temperature and high pressure generated components in green crude oils from algae.

Priority Area 2: Improve Pre-Processing Technologies

Objective 2: Improve yield and quality from hydrothermal treatment (HTT)

- **Approach:** Catalyst Screening for Hydrothermal Catalytic Treatment of Microalgae: We will test different catalysts under different conditions in batch mini-reactors, with the precise conditions to be determined after completing the literature review. We will recover the biocrude, aqueous, and solids product fractions after each HTT experiment and determine the yields of each gravimetrically. We will determine the metals content in each product fraction, the metals loading on the catalyst, and also get data for the elemental composition of the biocrude.

- **Technical Target:** Increased algal oil yield, as well as informing efforts in Priority Area 1
- **Measurable outcome:** Increase in the gross margin from an algae bio-crude facility through oil yield increases.
- **Relevance to FOA Goals:** Optimization of Green Crude yield from algal biomass increases per acre yields of algal-based biofuels and revenue per acre through increased sale price. Improved oil yields and a model that accurately predicts HTT output will be the basis for the performance period 2 go/no-go decision.
- **Relevance to downstream efforts:** A catalytic process to reduce metals and improve yield may enable improved revenue through higher oil sales price with minimal additional capital required. In addition, an improved understanding of oil quality, oil characterization and oil yield will lead to process design improvements, the potential for higher revenue and easier acceptance of HTT produced oil by refinery customers.

Demetallation of Algal Biocrude from Hydrothermal Treatment: Effect of CoMo Catalyst and MTBE

- Methyl Tertiary Butyl Ether (MTBE) was used in place of dichloromethane (DCM), to recover biocrude after hydrothermal treatment of algal biomass (provided by SEI) at 400 °C for 60 min.
- Using MTBE resulted in a lower biocrude yield (22 wt%) compared with using DCM (38 wt%).
- However, the concentrations of both Fe (1900 ppm) and Na in the biocrude also decreased (to 500 ppm for Fe).
- Additionally, use of a commercial CoMo catalyst (25 wt% loading relative to biomass) reduced the concentration of Fe in the biocrude to 35 ppm.
- This combination of MTBE and CoMo/Al_2O_3 leads to just 1% of the Fe in the original biomass appearing in the biocrude.

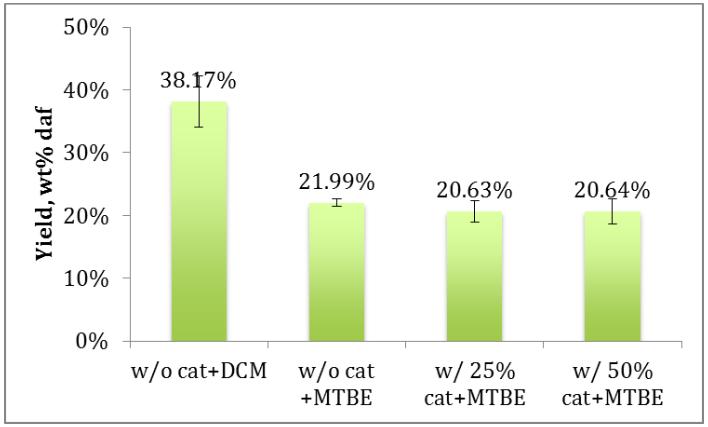


Figure 1. Biocrude yields from HTT at 400 °C for 60 min

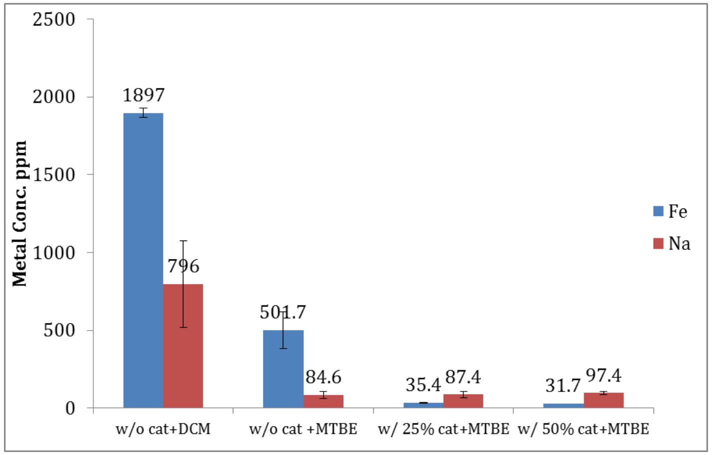


Figure 2. Fe (blue) and Na (red) concentrations (ppm) in biocrude from HTT at 400 °C, 60 min

Al	**B**	**Ca**	**Cr**	**Cu**	**Fe**	**K**	**Mg**
323	12.5	9447	1.7	17	1245	11391	3991
Mn	Na	Ni	P	S	Si	Zn	
179	38575	1.0	10988	7732	123	93	

Table 1. Metal Concentrations (ppm) in Dried Algae Biomass

Demetallation of Algae Crude Oil

Elemental analysis was performed at Atlantic Microlab to compare the elemental compositions of the crude oil obtained from catalytic HTL.

The compositions of the biocrude fractions are about the same for catalytic and noncatalytic reactions. After using CoMo/Alumina during the demetallation process, there was an increase in the carbon and hydrogen contents in biocrude oil, and a decrease in nitrogen and oxygen content.

Thus, the presence of catalyst will lead to some extent of deoxygenation, which helps improve the quality of biocrude oil.

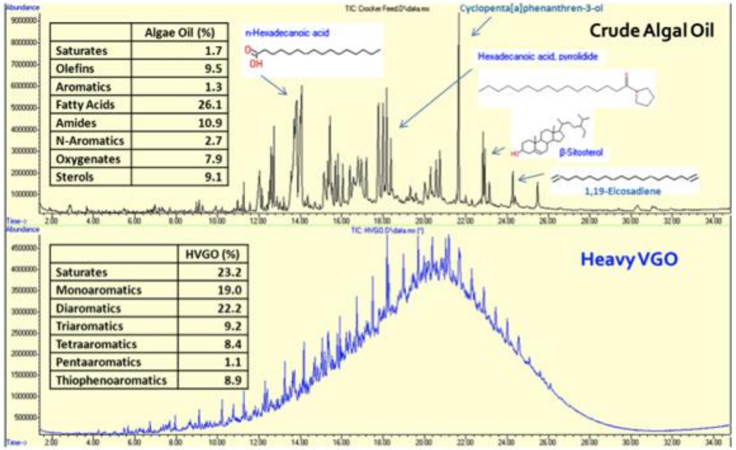
Table 1. Elemental Compositions (wt%) of algal crude oil for 7 samples. (Reactions conditions were listed)

Temperature	Catalyst	Solvent	C	H	N	O	H/C	N/C
350ºC	N/A	MTBE	77.7	10.45	4.61	7.24	1.61	0.83
350ºC	Activated Carbon	MTBE	77.69	10.73	4.25	7.33	1.66	0.77
350ºC	CoMo/ Alumina	MTBE	78.36	10.47	4.34	6.83	1.60	0.78
400ºC	N/A	DCM	78.94	10.24	4.18	6.64	1.56	0.74
400ºC	N/A	MTBE	78.92	10.41	3.74	6.93	1.58	0.66
400ºC	Activated Carbon	MTBE	78.62	10.92	3.24	7.22	1.67	0.58
400ºC	CoMo/Alumina	MTBE	79.27	10.68	3.85	6.2	1.62	0.68

- **Other:**
  - **A two stage demetallation process was attempted with unsuccessful results**
    - Two-stage reactors that have a frit in the middle to prevent solids flowing through each end were tried. Also post-HTL demetallation by using some existingmethods in petroleum crude oil area.
  - **Hemin was studied as a model compound for algae crude oil**
    - 5% of hemin model compound was loaded into the mini-batch reactors at 400°C and 60min. The biocrude product was characterized with GC-MS in a chemistry lab at Penn State
    - The major components of the crude bio-oil include organic acids, long-chain saturated hydrocarbons, indoles, and other N-containing compounds. In comparison, algae derived crude oil includes more components such as organic acids, long-chain hydrocarbons (saturated and unsaturated), indoles, piperidine derivatives, cholestane, cholestene, amides, and other N-containing compounds.

2B – Technical Targets

Priority Area 2: Improve Pre-Processing Technologies



2.3 Oil Analysis

- **Objective 3:** Oil Analysis
    - **Approach 1:** Conducting detailed characterization of process oils and product streams: Initial work will apply approaches to reduce the complexity of molecular composition of crude oils. At the completion of year 1 we will have an analytical pipeline based on high-resolution mass spectroscopy for Green Crude characterization that will allow information feedback to the HTT process development team and allow for optimization and improvement of process yield and oil quality. In Year 2 we will focus on developing a high efficiency fractionation scheme to isolate and characterize heteroatom-containing compounds from Green Crude to provide additional information for HTT reaction modeling and information on product quality to inform strain development. This information can, for example, be used in the SB model from Objective 1 as an influence on gene selection for creating GE strains.

- **Technical Target:** Improved product quality, as well as informing efforts in Priority Area 1

High-level Objectives

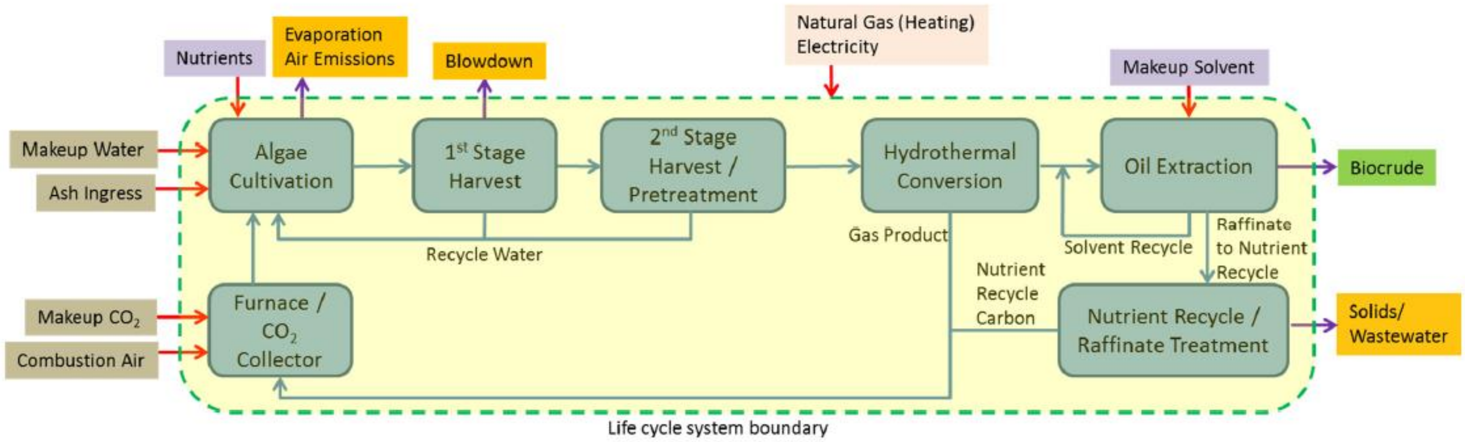
Priority Area 3 – Technical advances that enable integration of algal biomass unit operations

Objective 6: Using strains in Objective 1 studies, maximize integration and techno-economics of the biocrude process by achieving a high percentage replacement of nutrient chemical needs through nutrient recycling from non-oil streams (e.g. raffinate, solids).

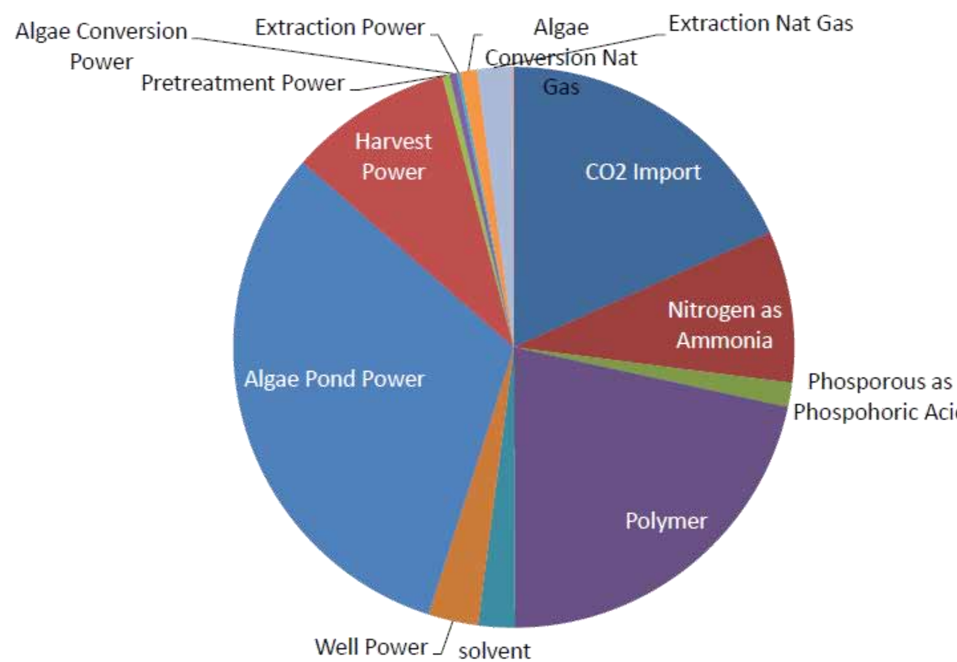
Objective 7: Expand the current techno-economic model—which incorporates cultivation, harvest, conversion, and extraction processes from end-to-end based on single sets of parameters—to allow process modeling over extended periods and with changing parameters.

Objective 8: Integration of life cycle assessment in process development and decision-making process for research options to ensure a maximum energy return on investment (EROI) and minimum life cycle greenhouse gas (GHG) emissions.

LCA system boundaries & TEA analysis



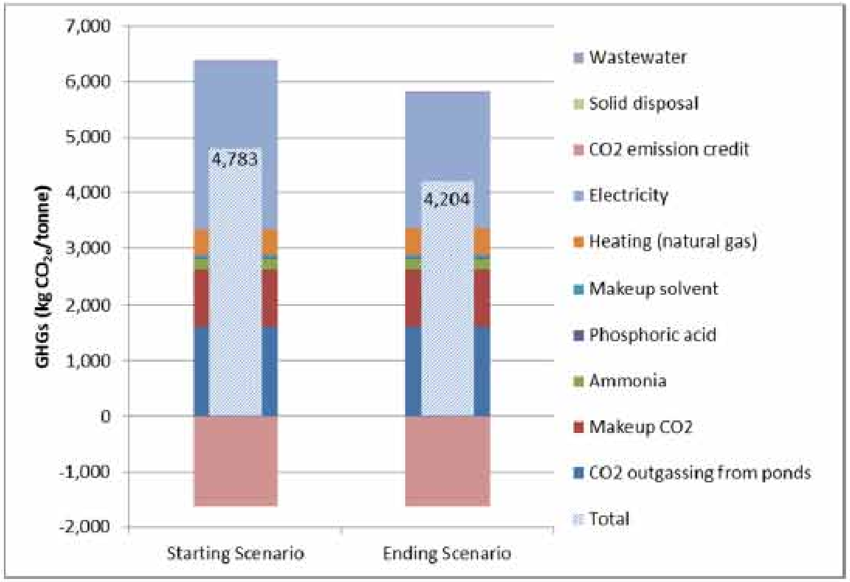
Pilot scale summary results

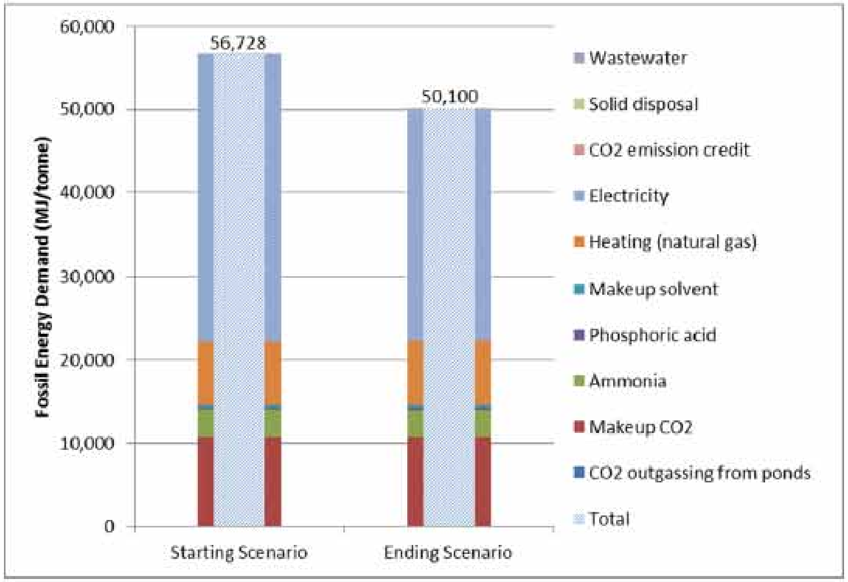


- Current TEA model doesn't include full likely discount on crude due to metal content; $/GGE calculated on 37MJ/kg
- Does not include capital cost of plant
- Baseline at $281/bbl ($7.59/gge)
- Final at $193/bbl ($5.21/gge)

LCA Results Summary

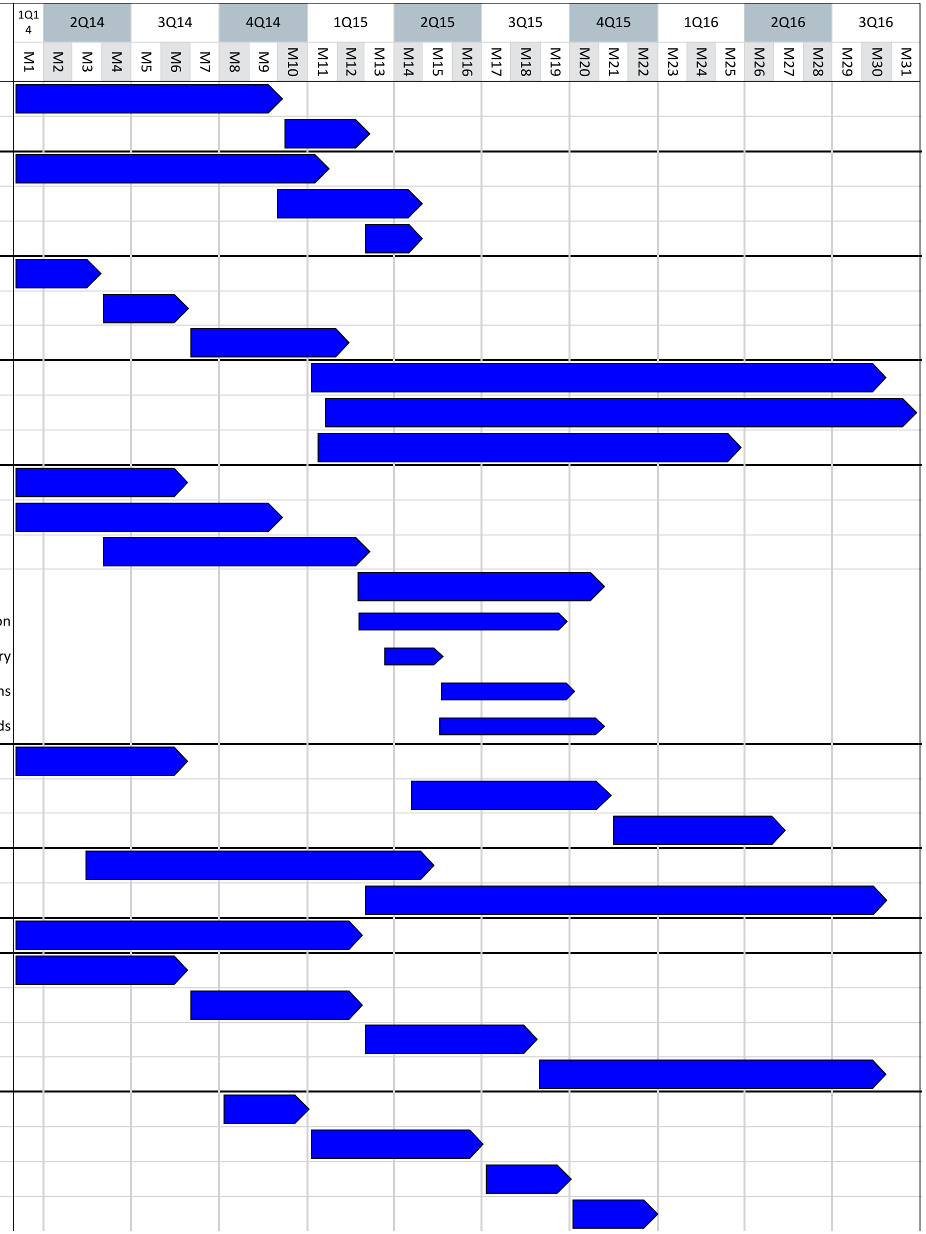
	GHG Emissions		Fossil Energy Consumption	
	Starting Scenario kg CO_{2e}/tonne	Ending Scenario kg CO_{2e}/tonne	Starting Scenario MJ/tonne	Ending Scenario MJ/tonne
CO_2 outgassing from ponds	1,621	1,622	--	--
Makeup CO_2	1,018	1,018	10,738	10,742
Ammonia (nutrient)	174	173	3,331	3,313
Phosphoric acid (nutrient)	22	22	260	260
Makeup solvent (dichloromethane)	38	43	341	384
Heating (natural gas)	477	477	7,644	7,647
Electricity	3,003	2,419	34,262	27,601
CO_2 emission credit	-1,621	-1,622	--	--
Solid disposal	1	1	9	9
Wastewater	52	52	143	143
Total	**4,783**	**4,204**	**56,728**	**50,100**





Technical progress - Overview

WBS	Activity Group
1.1 Evolution-based strain engineering	1.1.1 Strain generation and isolation
	1.1.2 Candidate strain lab evaluation
1.2 Systems biology	1.2.1 Data collection and curation
	1.2.2 Network modeling
	1.2.3 Candidate gene identification
1.3 Pond Ecology	1.3.1 Pond metagenomics
	1.3.2 Paired strain lab evaluation
	1.3.3 Designed consortia lab evaluation
1.4 Agronomic Practices	1.4.1 Lab Strain Evaluation
	1.4.2 Crop Protection
	1.4.3 Field Testing
2.1 Harvest	2.1.1 Particle size
	2.1.2 Settling rate
	2.1.3 Harvestability
	2.1.4 Omega Harvest
	Strain Selection
	Optimizing harvest process chemistry
	Optimizing DAF operations
	Continued cultivation process improvement via harvest methods
2.2 Hydrothermal treatment (HTT)	2.2.1 Decreased water in HTT feed
	2.2.2 Catalyst screening for hydrothermal catalytic treatment of microalgae
	2.2.3 Process improvement appraisal
2.3 Oil analysis	2.3.1 Basic analysis method
	2.3.2 Detailed characterization
3.1 Nutrient recycling	3.1.1 Raffinate characterization and lab testing
3.2 Process modeling	3.2.1 Advanced process model
	3.2.2 Expanded and validated model
	3.2.3 Energy and mass balance analysis
	3.2.4 Reduced energy optimized processes
3.3 Life cycle analysis	3.3.1 Process variable identification
	3.3.2 Baseline LCA
	3.3.3 High-impact process change identification
	3.3.4 LCA impact of grant work



Relevance

Meeting the platform goals of BETO MYPP

The described project directly fed into the Bioenergy Technologies Office Mission and Goals as the objective of the project was to increase the fuel productivity of an integrated process not simply in a laboratory atmosphere. Results will help in laying the foundation of a sustainable, nationwide production of biofuels that is commercially viable.

Application in the emerging bioenergy industry

Performance period 1 is dedicated in developing and piloting the technologies required for improving biomass productivity. All efforts converge into the anticipated performance period 2 where the entire process is to be piloted at a 1 acre scale for an entire year.

Impact on commercial viability

Demonstrating at lab and pilot scale in performance period 1 the necessary performance metrics (e.g., extractable oil fraction, biomass productivity, etc.)

Future work

Next 18 months

All work is complete except remaining portion of FFRDC work

Highlight key future milestones

Completion of biocrude characterization at NREL