

**U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review**

PACE: Producing Algae for Coproducts and Energy



March 2017
Advanced Algal Systems

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Quad Chart Overview

Timeline

- Research and development start date: April 1, 2016
- Project end date: March 31, 2019
- Percent complete: 25%

Barriers

- Increase areal algal biomass productivity three-fold
- Reduce the cost of producing fuels 30-50% by producing high-value and high market demand co-products
- Integrate algal harvesting and cultivation systems to reduce parasitic energy costs to <10% of algal biomass energy content



PACE | PRODUCING ALGAE FOR COPRODUCTS AND ENERGY

Partners

- Colorado School Mines
- LANL
- Reliance Industries Ltd
- Arizona St. Univ.
- Colorado St. Univ.
- Genifuel
- Sonosep
- Washington St. Univ.
- Pan Pacific
- PNNL
- New Mexico Consortium

Quad Chart Overview

PACE Budget

Budget Period	BP1		BP2		BP3		Total			
	Start Date	End Date	Budget	Costs	Budget	Costs*			Budget	Costs
	10/1/2015	3/31/2016	\$455K	\$455K	\$4,509K	\$1,632K	\$4,036K	n/a	\$9,000K	\$2,087K
			\$0	\$0	\$102K	\$10K	\$93K	n/a	\$195K	\$10K
			\$0	\$0	\$102K	\$35K	\$58K	n/a	\$160K	\$35K
			\$0	\$0	\$39K	\$0	\$20K	n/a	\$59K	\$0
			\$4K	\$4K	\$500	\$99	\$239	n/a	\$5K	\$4K
			\$10K	\$10K	\$136K	\$48K	\$117K	n/a	\$263K	\$58K
			\$9K	\$9K	\$135K	\$48K	\$149K	n/a	\$293K	\$57K
			\$70K	\$70K	\$1,382K	\$141K	\$682K	n/a	\$2,134K	\$210K
			\$9K	\$9K	\$57K	\$31K	\$107K	n/a	\$173K	\$40K
			\$13K	\$13K	\$38K	\$23K	\$39K	n/a	\$90K	\$37K
Total			\$570K	\$570K	\$6,501K	\$1,967K	\$5,301K	n/a	\$12,372K	\$2,537K

*as of 12.31.16

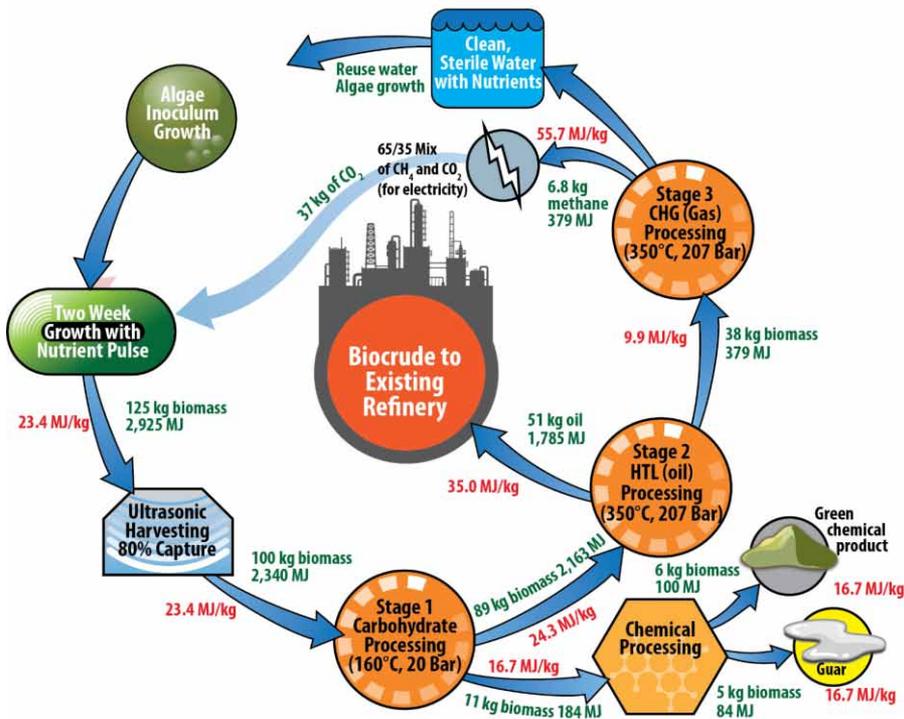
Current expenditures are on target

PACE Project Goals

Energy Flow Diagram

For 100 kg dry weight of harvested algae

Energy Density Total Energy



Objectives:

- Produce algal biofuel at < \$5 gge
- Achieve an EROI > 8
- Achieve a carbon index < 40 g CO₂/mJ
- Recover and recycle > 80% nutrients and CO₂ while reducing water-use 50%

Strategy:

- Increase areal algal biomass productivity three-fold
- Reduce the cost of producing fuels 30-50% by producing high-value and high market demand co-products
- Integrate algal harvesting and cultivation systems to reduce parasitic energy costs to < 10% of algal biomass energy content
- Enhance overall sustainability by maximizing CO₂, nutrient, and water recovery and recycling, and bio-power co-generation
- Carry out continuous life cycle and techno-economic performance assessments to enhance process improvement and integration

Project Overview

Improve Biomass Yield: Increase algal biomass productivity to >32 gdw/m²/day using robust engineered algal strains to reduce costs $>2X$.

Produce Coproducts to Offset Fuel Costs: Produce and harvest high-value, high market demand co-products compatible with continuous flow, sequential HTP technology to reduce biofuel costs an additional 30-50%.

Improve and Optimize Integrated Process: Develop and improve process/engineering systems to achieve overall EROI > 8 , carbon index < 40 g CO₂/mJ, and a nearly 2-fold reduction in fuel cost to $\leq \$5.00$ gge.

Technical Scope Summary

• **Improve Biomass Yield (completed by end of BP2):**

- Improve growth 2X by phototropin knock outs
- Improve CO₂ utilization to increase yield 30%
- Increase sink strength to increase yield 20%
- Optimize lipid/carbohydrate accumulation to maximize profitability
- Develop crop-protection technologies to reduce possible pond crashes
- Develop bio-containment of GM algae to reduce GM escape likelihood
- Stack value-added and GM biocontainment traits to give 3X yield increase

Project Overview; continued

Technical Scope Summary

Produce Coproducts (completed by mid BP3): Produce and harvest high-value, high market demand co-products compatible with continuous flow, sequential HTP technology to reduce biofuel costs an additional 30-50%.

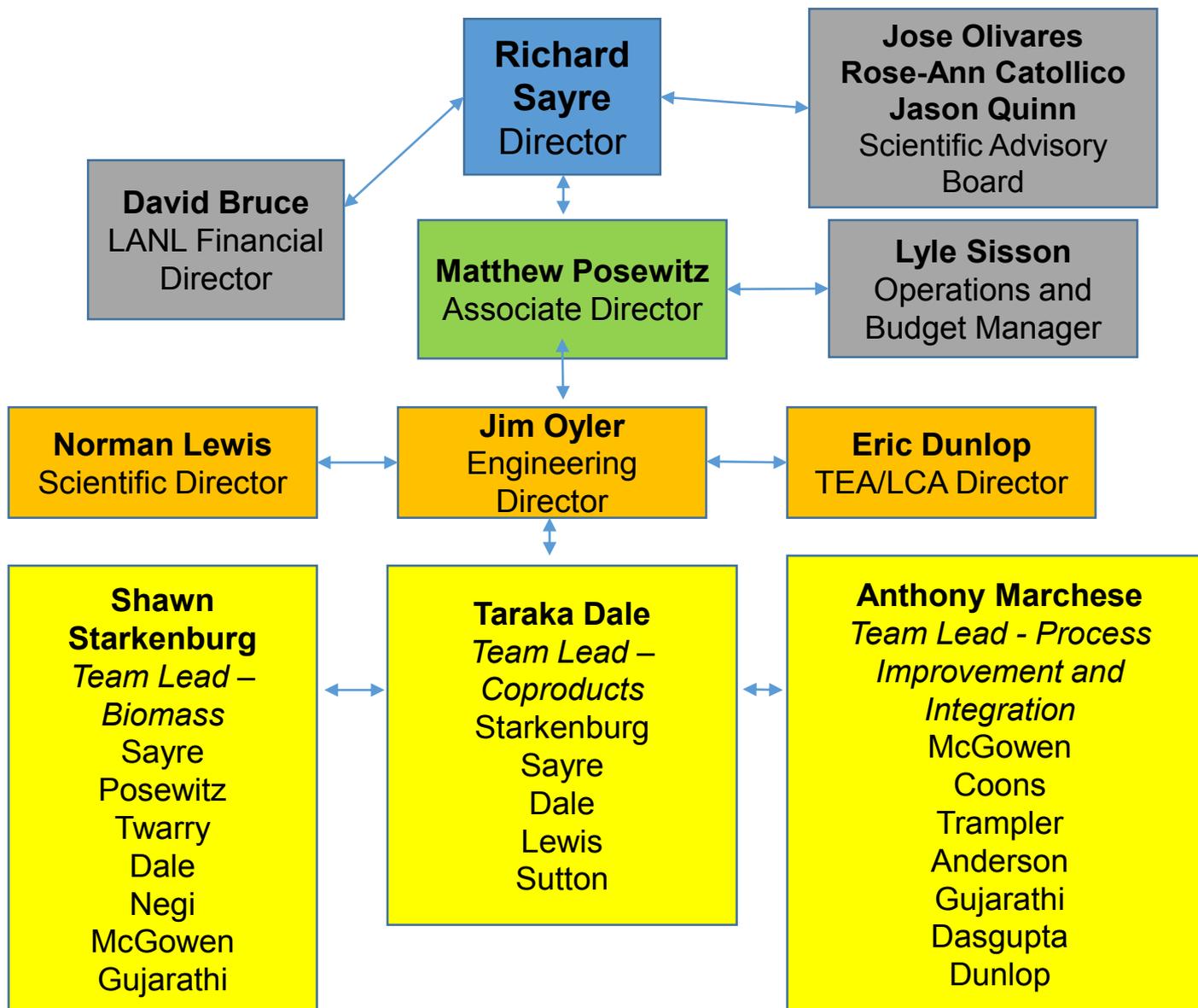
- Develop GM algae to produce guar and/or 2-phenylethanol (PEA) glucoside (PEA Glc)
- Chemically convert starch into hydrocarbons (fuel) and coproducts

Improve and Optimize Integrated Process (completed by end BP3):

Develop and improve process integration and engineering systems to achieve overall EROI >8, carbon index <40 g CO₂/mJ, and a 2-fold reduction in fuel cost to ≤ \$5.00 gge.

- Compare and contrast large-scale outdoor cultivation systems at AzCATI and Gagwa.
- Develop nutrient-pulse inoculation systems to control weedy algae
- Develop and evaluate ultrasonic harvester with EROI >8
- Develop and evaluate sequential, continuous flow HTL and CHG
- Co-product, biocrude, feedstock and fuel evaluation
- Perform LCA/TEA

Management Structure



2 – Approach (Management)

- Monthly PACE Webex meeting on research highlights and progress, and management issues
- As needed specialized meetings to address unique challenges
 - Algae transformation group meetings
- Quarterly survey and reporting on progress towards meeting all milestones and deliverables
- Annual all hands meeting with review and feedback from Scientific Advisory Board
- Management Team Oversight

2 – Approach (Technical)

- **Major Challenges:**

- Developing robust genetic transformation systems and CRISPR/Cas9 genome editing tools for *Chlorella sorokiniana*
- Producing and sequentially extracting coproducts (guar and phenylethanol) from biocrude to offset the cost of biofuel production
- Developing an energy efficient algal harvesting system that is scalable to full operational size

- **Major Success Indicators:**

- Overall EROI > 8
- Carbon index <40 g CO₂/mJ
- 2-fold reduction in fuel cost to ≤ \$5.00 gge

Project Overview and Accomplishments

Improving Biomass Yield

Task: Crop Protection (Richard Sayre)

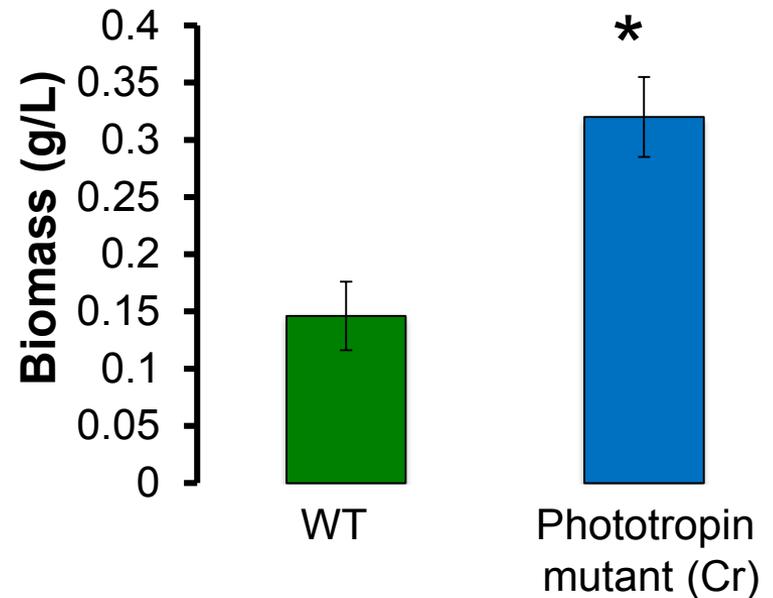
- Express AHL-lactonase in *C. sorokiniana* to reduce harmful bacterial biofilm formation. **AHL-lactonase transgenics confirmed by DNA sequence analysis**
- Express pyrroline-5-carboxylate synthase (P5CS) in *C. sorokiniana* to improve its thermal tolerance. **P5CS transgenics confirmed. Provide heat tolerance up to 55 °C.**
- Express antimicrobial peptides (AMP) to protect against bacteria and rotifers. **AMPs identified that kill both *Vampirovibrio* and rotifers. Genes being introduced in *C. sorokiniana***
- Develop algal suicide gene system for biocontainment of genetically-modified algae. **Caffeine-controlled RNase being transformed into *Chlorella* after proof-of-concept in *Chlamydomonas***



Task: Increasing photosynthetic efficiency and biomass yield

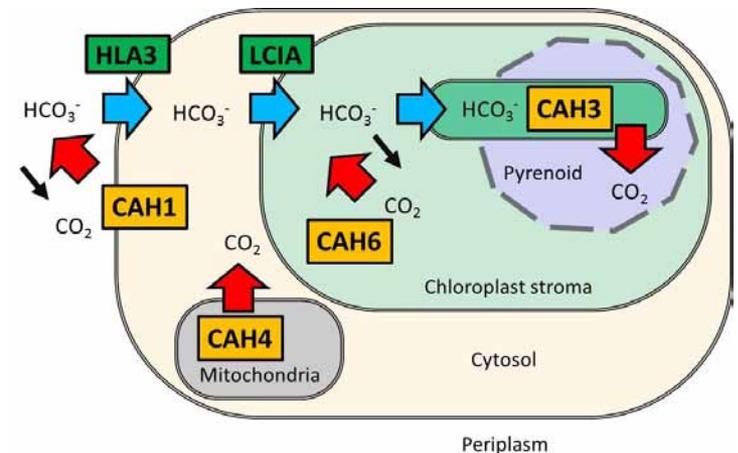
(Sangeeta Negi, NMC)

- Knock out phototropin gene in *Chlorella sorokiniana* using CRISPR Cas9 genome editing to enhance growth 2X. Potential transformants being verified via PCR for transgene insertion.



(Scott Twary, LANL)

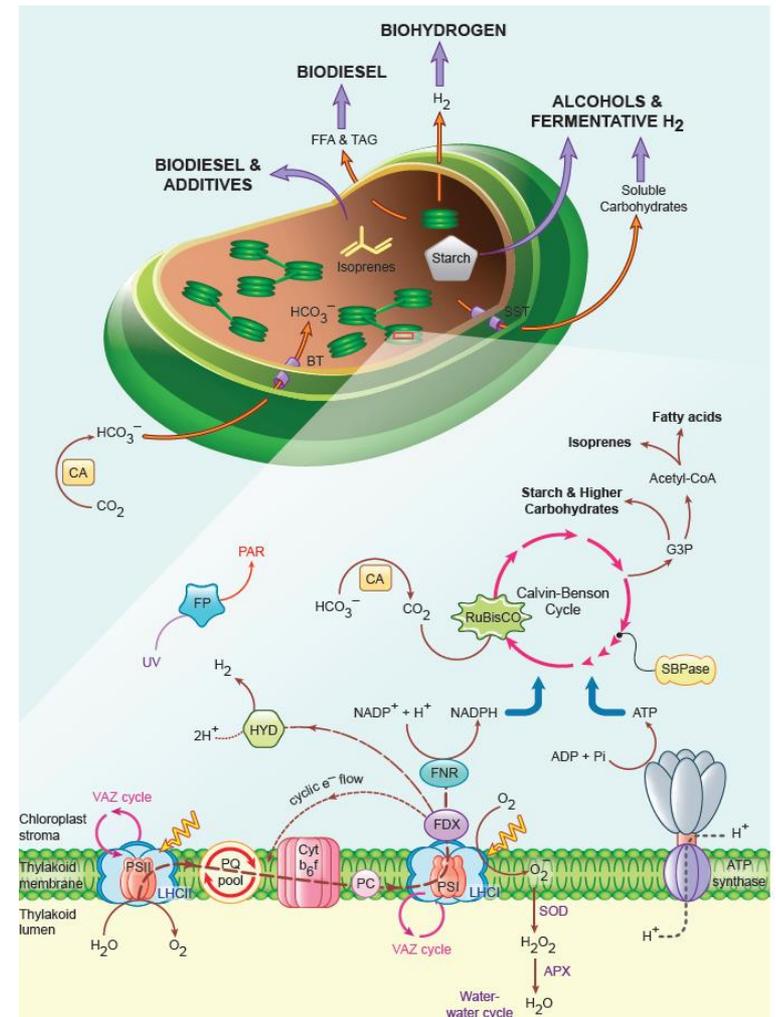
- Expression of targeted carbonic anhydrase genes to enhance CO₂ fixation. Transgenic *C. sorokiniana* expressing carbonic anhydrase 4 (CAH4) have been generated. Other CA transformants in progress.



Task: Increase Biomass Sink Strength to Enhance Yield (Matthew Posewitz, CSM)

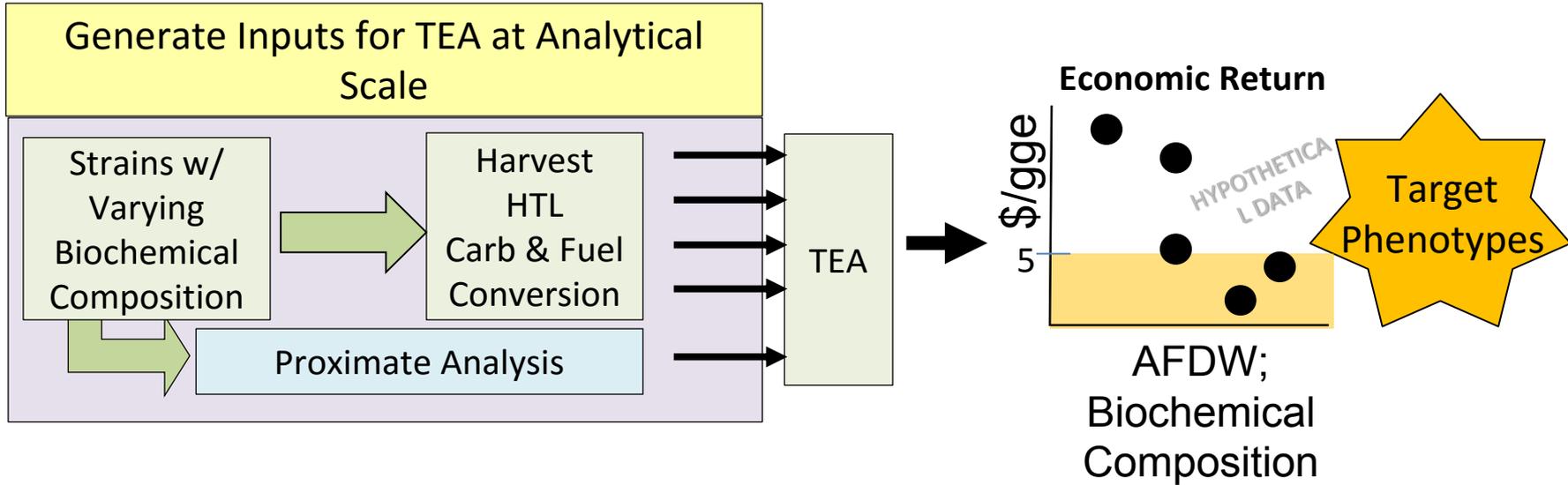
Overexpress isoamylase, ADP-glucose pyrophosphorylase, FBPase and SBPase to enhance photosynthetic sink strength with an emphasis on starch accumulation

Antibiotic resistant transformants are being tested for transgene integration and expression.



Task: Empirical Determination of Economic Return for Varied Phenotypes (Taraka Dale, LANL)

- Depending on growth conditions and genetic modifications, we expect to get varied carbon partitioning, in addition to changes in productivity
 - Established BETO “gold standard” proximate analysis assays at LANL
 - Validated results w/ ATP³ & NREL
 - Surveys for target wild-type phenotype in progress

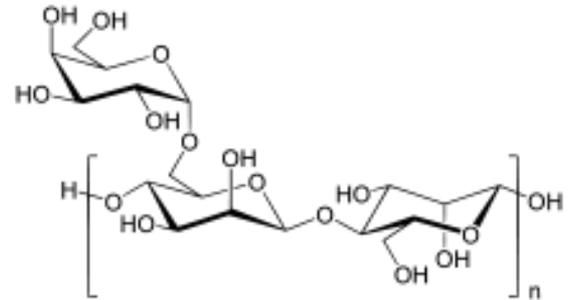


Project Overview and Accomplishments

Coproduct Production

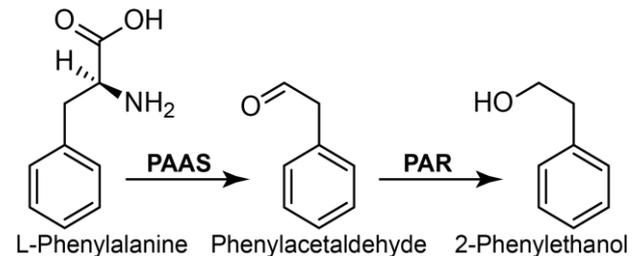
- **Task: Guar (large market, intermediate value; Shawn Starkenburg, LANL)**

Demonstrate guar synthesis transgene expression in transgenic *Chlorella*. **Four vectors constructed with 1 or 2 Guar transgenes; Antibiotic resistant transformants being tested for transgene integration**



- **Task: 2-phenylethanol (small market, high value; Norman Lewis, WSU)**

Develop GM algae to produce 2-phenylethanol (PEA) glucoside (PEA Glc). Express phenylacetaldehyde synthase (PAAS) and phenylacetaldehyde reductase (PAR). **PAAS and PAR engineered into PACE vectors. Genes are being transformed into *C. sorokiniana*.** Express phenylacetaldehyde synthase (PAAS), phenylacetaldehyde reductase (PAR) and UDP-glucosyltransferase (UGT) in *C. sorokiniana* to produce PEA-Glc. **UGT engineered with PAAS and PAR into PACE vectors. Genes are being transformed into *C. sorokiniana*.**



Project Overview and Accomplishments

Improve and Optimize Integrated Processes

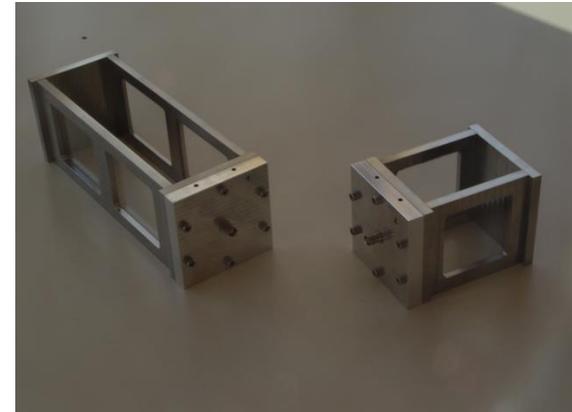
Task: Outdoor Cultivation, (John McGowen, AzCATI)

- Cultivate WT at scales of 4.2 m² (1000 L) to 60 m² (15,000 L) at AzCATI using optimized growth conditions (e.g., media, dilution rate, etc.) determined from ePBR experiments **Adapted *C. sorokiniana* to grow on 80% of full seawater with no growth impairment, additional adaptation ongoing.**
- Produce biomass for downstream testing and implement nutrient pulse technology and evaluated for ability to reduce contamination **Biomass generated for QA/QC stock for proximate analysis and supplied to downstream teams in kg quantity.**
- Obtain EPA approval for TSCA Environmental Release Application (TERA) for open release of GM algae field-trial **Submission on track to allow for late spring/summer 2017 first trial of a GM strain at AzCATI**



Task: Develop Energy Efficient Ultrasonic Algae Harvester (Jim Coons, LANL and Felix Trampler, Sonosep)

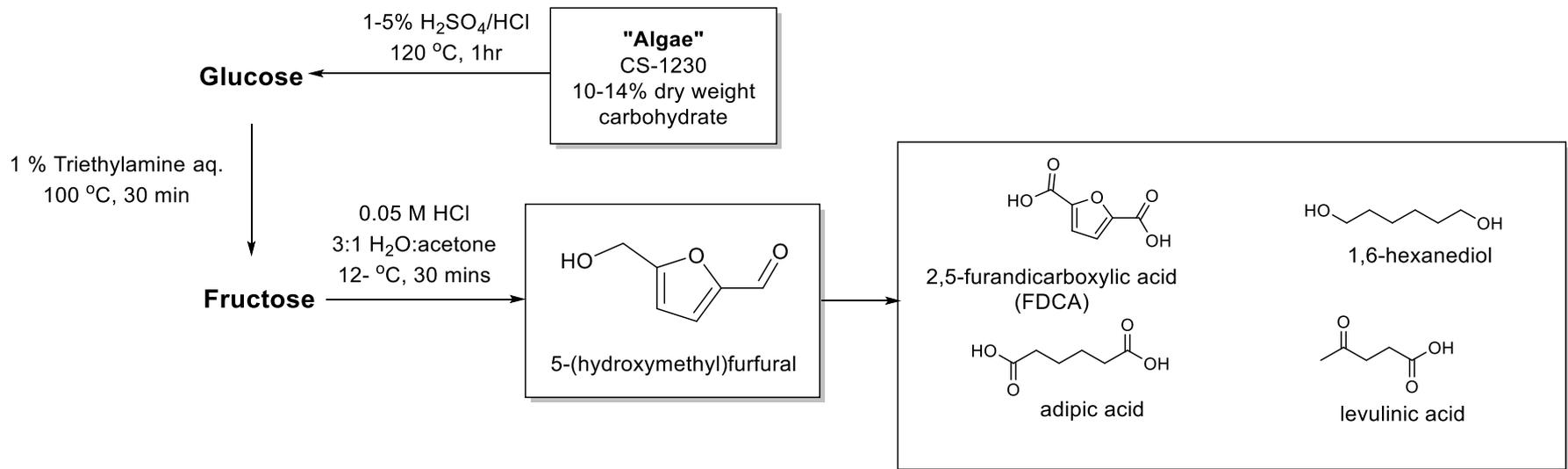
- Manufacture/Delivery of Sonosep experimental equipment to LANL for frequency optimization. **Sonosep manufactured and delivered 1, 1.4, 1.7, and 2.1 MHz vessels.**
- *C. sorokiniana* (WT) property measurements and frequency optimization completed. ***C. sorokiniana* 1230 harvesting properties favorable for alga with high starch density. Frequencies between 1.4 and 1.7 MHz chosen for scale-up.**
- Manufacture/Delivery of Sonosep experimental equipment to LANL for acoustic length optimization. **Two batch vessels (10 and 25 cm) were made and delivered to LANL. Two additional optimized vessels (5 and 15 cm) will be supplied, based on preliminary tests**



Acoustic length optimization vessels

Task: Convert Carbohydrate Fraction into Hydrocarbons and Coproducts (Andy Sutton, LANL)

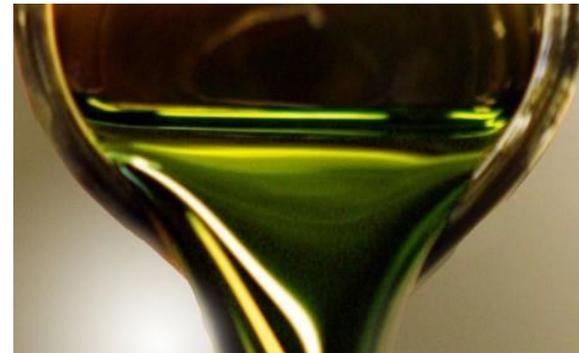
- Demonstrated carbon-chain extension and alkane conversion chemistry using algal starch derived from HTL
- Determined product and process that reduces costs based on LCA/TEA of product, conversion efficiency, capital and operating costs to < \$3 gge



Task: Biomass to Fuel Conversion and Coproduct Harvesting Using 2-Stage HTL. (Dan Anderson, PNNL)

Optimize value from algal biomass by first extracting a high-value product, then converting the residual biomass (hydrophobic fraction) to fuels by continuous flow HTL

- Developed batch methods for extraction and biomass fractionation achieving >50% recovery of carbohydrates, and >70% of residual biomass for conversion to fuel
- Developed mini-scale continuous HTL process for extraction of carbohydrates and validation of batch results
- Completed initial design for commercial-scale process to convert residual biomass to fuels, and provided data for TEA based on design
- Next step is to test extraction of guar by “spiking” wild-type algae with guar, and incorporating polysaccharide extraction into two-stage continuous system



Task: Product Evaluation and Fuel Performance (Anthony Marchese, CSU)

Milestones (Year 2 and 3)

- Complete characterization of biocrude oil. **Instrumentation calibrated in Fuel Characterization Laboratory and work is ready to proceed.**
- Complete fuel property testing of upgraded fuels **Instrumentation calibrated in Fuel Characterization Laboratory. Initial experiments performed on upgraded HTL (from another source).**
- Complete engine testing of upgraded fuels. **Performed initial engine calibration testing on John Deere PowerTech Plus engine comparing emissions and performance between Certification Diesel and upgraded HTL (from another source).**



Task: LCA/TEA Analysis of Integrated Process (Eric Dunlop, Pan Pacific)

- **1st Order Model of Process and Economics (TEA):** Excel model based on previous modelling and updated with current information. **Completed and circulated for review.**
- **Product Value Chain:** Analysis of where, and by how much, value is added by each coproduct. **Completed.**
- **Flowsheet Development:** CO₂ /NH₃ absorption. Intermediate drying/storage to minimize HTL size/cost. **In progress.**
- **Energy Analysis (EROI):** Pinch model developed for existing HTL and some concepts developed for further energy minimization. **First stage completed**
- **Aspen Model:** New model for Ultrasonic Harvesting (unit op) **Developed.** Aspen baseline model **Mostly completed.**
- **LCA:** Data gathering. **In progress.**

3 – Technical Accomplishments/ Progress/Results

Major Accomplishments:

1. Development of a potentially “universal” genetic transformation system for recalcitrant algae based on enhanced transgene integration into chromosomes with induced double-stranded DNA breaks.
2. Application of CRISPR/cas9 to knock out a target gene (phototropin). Replacement insert being tested.
3. *C. sorokiniana* adapted to 80% seawater with no loss in productivity.
4. Development of world’s first full-scale, fully integrated algal biofuel system at Reliance Industries Ltd in Gagwa, India.
5. Utilization of data generated from Gagwa facility to begin first full-scale LCA/TEA analysis.

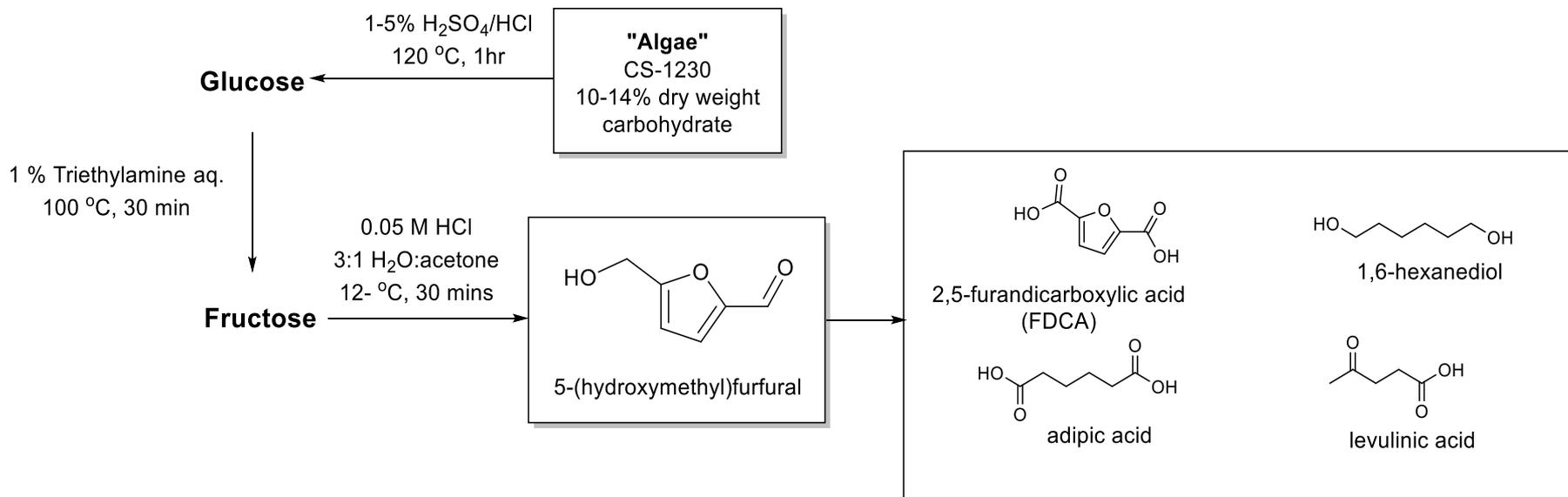


Image of RIL Gagwa facility. Longer ponds on left are over 1 km long. Includes harvesting, HTL, media mixing, central control room. Refinery nearby.

3 – Technical Accomplishments/ Progress/Results

Major Accomplishments :

- Developed mini-scale continuous flow HTL process for efficient first-stage extraction of algal carbohydrates and validation of batch results.
- Demonstrated conversion of HTL-derived algal starch to alkanes with carbon-chain extension; algal starch to fuels achieved!



3 – Technical Accomplishments

Major Accomplishments

- First order TEA analysis of the PACE Aspen model completed for a 1000-acre pond, including mass and energy balances, capex and opex, cash flow, and cost summaries with sensitivity analysis
- The purpose is to identify gaps in data and process design to guide experimental work. Co-products will be modeled together in full process model.

Biomass productivity	Co-Product		
(g/m ² /d)	<i>Guar gum</i>	<i>HC from starch</i>	<i>PEA-Glc</i>
13.7 (AzCATI, Sept 2016)	11.65	13.72	10.76

Biocrude cost (\$/gge) with 13.7 gdw/m²/day biomass productivity:
Each co-product modeled separately

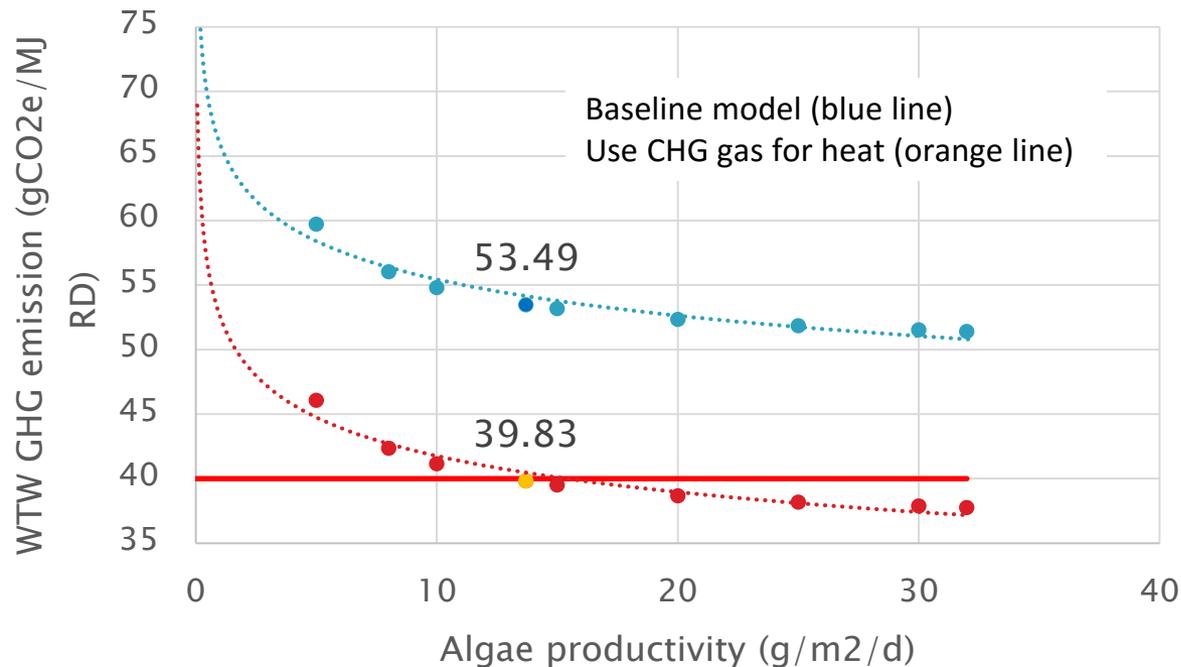
Production scenarios modelled and identified
to approach PACE target of \$4.75/gge

- Improve biomass productivity (> 25 g/m²/d)
- Energy integration of co-product separation and HTL-CHG
- Reduce pond circulation energy cost
- Use CHG gas for heat to reduce utility cost
- Cheaper pathway for PEA-Glc separation
- Integrate all three co-products
- Need more separations research

3 – Technical Accomplishments

Major Accomplishments

Preliminary GHG emission analysis indicates that target objective of $< 40 \text{ gCO}_2/\text{MJ}$ is achievable with addition of CHG to HTL process



Relevance

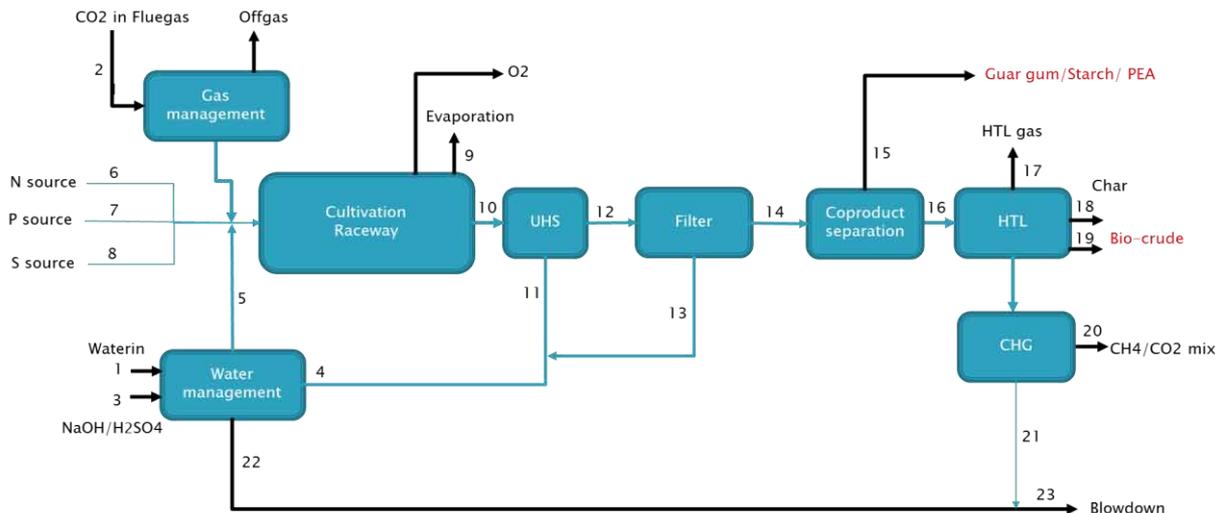
Objectives:

- Produce algal biofuel at < \$5 gge
- Achieve an EROI > 8
- Achieve a carbon index < 40 g CO₂/mJ
- Recover and recycle > 80% nutrients and CO₂ while reducing water-use 50%

Importance: To enhance energy security and reduce the impacts of fuel combustion on climate change, we will develop sustainably-produced hydrocarbon-based transportation fuels from algae that reduce carbon emissions, have high energy return on investment, and which are affordable.

Addressing BETOs Multi –Year Program Plan Objectives for Algal Biofuels: Biomass Availability and Cost; Sustainable Algae Production; Biomass Genetics and Development; Algal Feedstocks Logistics; Sustainable Harvesting; Algal Biomass Characterization, Quality, and Monitoring; Algal Feedstock Material Properties; Overall Integration and Scale-Up; Algal Feedstock On-Farm Preprocessing; Resource Recapture and Recycle

Advancing the Technology Through Continuous Integrated LCA/TEA Feasibility Analyses



Model basis:

- Pond size: 5000 acres
- Harvesting: ultrasonic harvester and filter press *
- Co-product separation:
 - Guar gum: low temperature HTL, 5% yield, \$6.5/kg guar gum**
 - Starch: low temperature HTL, 10% yield, then convert to HC with 50% yield, \$3/kg HC ***
 - PEA-Glc: SC-CO₂ extraction, 1% yield, \$100/kg PEA-Glc****
- Conversion: HTL-CHG (Genifuel flowsheet), 40% yield

Relevance



PACE addresses BETOs algal biofuels target for sustainably producing fuels at < \$5 gge.

- Project addresses improvements in algal biofuels production systems including strain improvement, cultivation and harvesting systems, coproduct production to offset fuel costs, biomass conversion to fuels, fuel performance, and LCA/TEA studies

Our Strategies Include:

- Increasing areal algal biomass productivity three-fold
- Reducing the cost of producing fuels 30-50% by producing high-value plus high market demand co-products
- Integrating algal harvesting and cultivation systems to reduce parasitic energy costs to < 10% of algal biomass energy content
- Enhancing overall sustainability by maximizing CO₂, nutrient, and water recovery and recycling, and bio-power co-generation
- Project metrics and targets driven by continuous life cycle and techno-economic performance assessments to enhance process improvement and integration

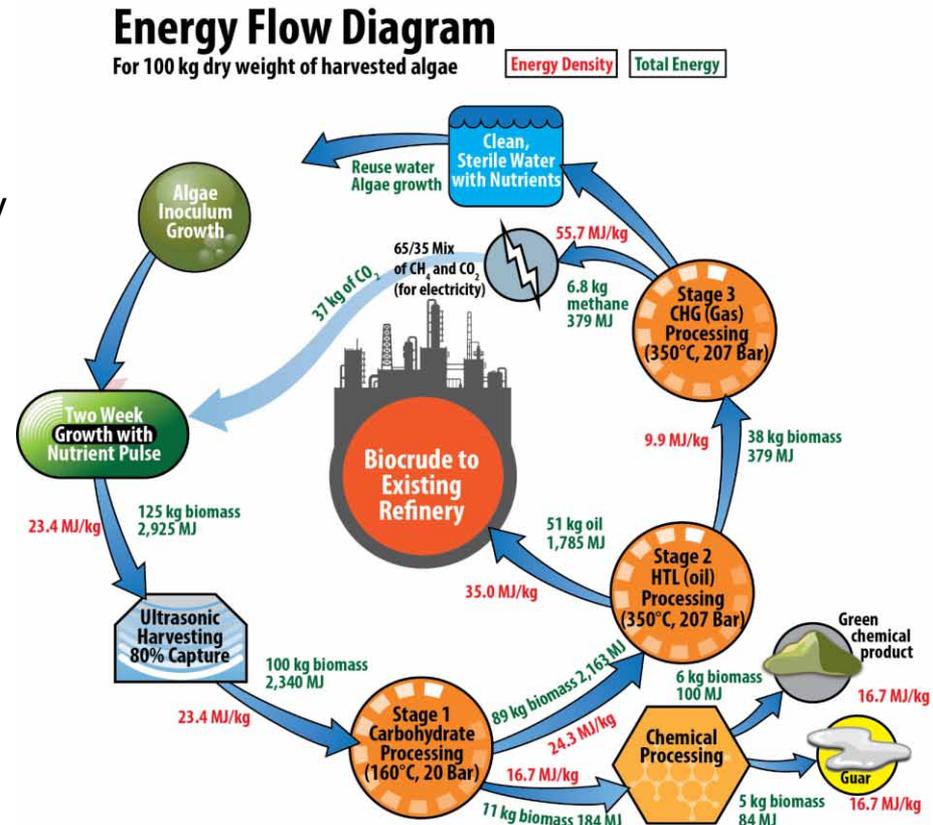
5 – Future Work

Objectives:

- Evaluate phenotypic performance characteristics of genetically modified algae in the field towards achieving a targeted 3-fold increase in yield and sufficient coproduct yields to reduce fuel costs to <\$5 gge
- Optimize acoustic focusing harvester energy efficiency
- Demonstrate efficient harvesting of coproducts, carbohydrates, and bio-oil from continuous flow, two-stage HTL system to meet LCA/TEA benchmarks
- Demonstrate scalable chemical conversion of carbohydrates to fuels and coproducts
- Model full-scale production at the Gagwa facility for LCA/TEA. Incorporate the contributions of GM value-added traits in model.

Management:

- Major Go/No-Go Decision: EPA approval of GM algae field trials. Application on schedule.
- Budget: On schedule



Summary

1. Overview:

- Improve sustainable biomass yield by >2X
- Produce coproducts to offset fuel costs an additional 30-50%.
- Improve and optimize integrated process to achieve overall EROI > 8, carbon index < 40 g CO₂/mJ, and a nearly 2-fold reduction in fuel cost to ≤ \$5.00 gge.

2. Approach:

- Increase areal algal biomass productivity three-fold
- Reduce the cost of producing fuels 30-50% by producing high-value and high market demand co-products
- Integrate algal harvesting and cultivation systems to reduce parasitic energy costs
- Enhance overall sustainability by maximizing CO₂, nutrient, and water recovery and recycling, and bio-power co-generation
- Carry out continuous life cycle and techno-economic performance assessments to enhance process improvement and integration

3. Technical Accomplishments/Progress/Results

- Developed robust genetic engineering and genome editing protocols in *C. sorokiniana* to express targeted genes
- Cultivation of *C. sorokiniana* at scale in 80% seawater
- Substantial progress on improving acoustic harvester efficiency
- Successful chemical conversion of algal carbohydrates to fuel and coproducts
- Continuous flow 2-stage HTL to separate carbohydrates from oil fraction
- RIL Gagwa facility is first fully integrated algal biofuels system operating at scale. Emerging LCA/TEA model predicts remaining constraints to meet target objectives

4. Relevance:

- Addressing BETOs Multi –Year Program Plan Objectives for Algal Biofuels

5. Future work:

- Evaluate phenotypic performance characteristics of genetically modified algae
- Further optimize acoustic focusing harvester energy efficiency
- Demonstrate efficient harvesting of coproducts, carbohydrates, and bio-oil from continuous flow, two-stage HTL system
- Demonstrate scalable chemical conversion of carbohydrates to fuels and coproducts
- Model full-scale production at the Gagwa facility for LCA/TEA. Incorporate the contributions of GM value-added traits in model.

Publications, Patents, Presentations, Awards, and Commercialization

Publications:

- Negi S, Perrine Z, Friedland N, Kumar A, Minagawa J, Govindjee and Richard Sayre (2017) Dynamic modulation of light harvesting antenna size substantially enhances photosynthetic efficiency and biomass yield in algae. (in review)

Patents:

- PCT/US16/36077 For: *Improved Productivity and Bioproduct Formation In Phototropin Knock/Out Mutants in Microalgae*. Inventors: Sangeeta Negi, Richard Sayre, Shawn Starkenburg
- Provisional patent in progress for acoustic harvester technology. Jim Coons

Presentations:

- Dunlop, E. H. and Zhao Y.,(2016) Advanced Bioreactor Design for Micro-Algae: Problems and Some Solutions, Chemeca Conference, Adelaide, Australia,
- Zhao Y. and Dunlop, E. H. (2016) Application of pinch analysis for energy optimization for hydrothermal liquefaction in algal biofuels processes, Chemeca Conference, Adelaide, Australia
- Lewis, N.G., Marques, J.V., Dalisay, D.A., Costa, M.A., Herman, B., and Davin, L.B. Integrated advanced multi-omics strategies: A new paradigm for agricultural and medicinal plant biotechnology with metabolomics/metabolite imaging *in situ*. PacifiChem 2015, Honolulu, HI, December 15-20, 2015
- Lewis, N.G. Some favorite medicinal plant and biotechnology discoveries: Reflections and future potential. 2016 International Symposium on Agricultural Biotechnology: From Systems Biology to Translation Agriculture, Academia Sinica, Taipei, Taiwan, May 16, 2016.
- Lewis, N.G. Reflecting on some favorite agricultural and medicinal plant biotechnological discoveries. 55th Annual Meeting, Phytochemical Society of North America, University of California, Davis, CA; August 6-10, 2016.
- Lewis, N.G. Some favorite agricultural and medicinal plant biotechnological discoveries since 2000: Reflections and future potential. The International Symposium on Natural Products for the Future 2016 (ISNPF2016) Tokushima, Japan, September 1-4, 2016.
- Negi S (2016) Phototropin: A master regulatory control gene that controls photosynthesis and growth processes in *Chlamydomonas*. 5th Pan Pacific Conference Plants and Bioenergy, Santa Fe, NM
- Sayre RT (2016) Improving photosynthetic efficiency in plants and algae. Dept. Chemistry New Mexico Technology Institute.
- Sayre RT (2016) Improving photosynthetic efficiency in plants and algae. Dept. Biology, Colorado State University
- Sayre, RT (2016) Molecular modulation of bacterial quorum sensing by the green alga, *Chlamydomonas reinhardtii*. A Symposium on Cell Signaling. Santa Fe, NM.
- Sayre RT (2016) Improving photosynthetic efficiency in plants and algae. 5th Pan American Congress on Plants and Bioenergy. Santa Fe, NM.
- Sayre RT (2016) Producing Algae for Coproducts and Energy; the PACE algal biofuels program. 6th International Algal Biomass, Biofuels and Bioproducts Conference, San Diego, CA.

Publications, Patents, Presentations, Awards, and Commercialization

Awards:

- Norman Lewis, Pioneer Award, Phytochemical Society of North America, August 2016.

Commercialization:

- A pilot-scale HTP system is in operation at RIL for algae, and a system 10X larger is in the design phase for wastewater solids