DOE Bioenergy Technologies Office
(BETO) 2017 Project Peer Review

Advancing Commercialization of Algal Biofuels through Increased Biomass Productivity and Technical Integration DE-EE0006637

March 9, 2017
Advanced Algal Systems Session

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(Presented by Babetta Marrone, Ph.D., Los Alamos National Laboratory)

ADVANCEMENTS IN ALGAL BIOMASS YIELD-1 (DE-FOA-0000811)

This presentation does not contain any proprietary, confidential, or otherwise restricted information

Goal Statement

Cellana DOE ABY1 Project Goal

- Achieve improvements in algal biomass productivity and lipid yield in a fully-integrated Algae Feedstock operation, using advanced strain improvement, cultivation, and processing technologies at the commercially relevant scale available at Cellana's Kona Demonstration Facility (KDF)

Quad Chart Overview

Timeline

- Award Start Date: 09/01/14
- Validation & Go Decision: 12/05/14
- 100% complete
- Project End Date: 9/30/16

Budget

	FY 10 - 13 Costs	FY 14 Costs	Total Planned Funding (FY 15-Project End Date)
DOE Funded	$0	$0	$3,500,000
Project Cost Share (Comp.)*	$0	$0	$ 892,110

Barriers Addressed

- *Improve strain productivity and robustness*: Aft-A. Biomass Availability and Cost, Aft-B. Sustainable Algae Production, Aft-C. Biomass Genetics and Development
- *Evaluate energy efficient harvesting and fractionation technologies:* Aft-D. Sustainable Algae Harvesting, Aft-I. Algae Feedstock Preprocessing
- *Assess "integratability" of unit operations*: Aft-H. Overall Integration and Scale-up

Partners

- Los Alamos National Lab (15%)
- Sandia National Lab (8%)

Project Overview (1 of 3)

- The focus was to demonstrate sustainability of a biorefinery producing advanced biofuels at levels consistent with meeting the BETO target goals in the MYPP. To do this, improvements in biomass productivity, lipid yield, and integrated operations at commercially relevant scale were required.

- Results were also required for validation of the techno-economic viability for commercialization. This has been successfully achieved for an algal multi-product model that includes feed and Omega-3 nutritionals in addition to fuels.

- The team has had experience on many prior DOE & USDA funded projects, including NAABB, Cornell's Marine Algal Biofuels Consortium, ATP^3, and the BETO AOP program; effectively leveraging prior support and key learnings to drive work on this project.

Project Overview (2 of 3)

Project Objectives

- Priority Area 1: Improve algal biomass productivity
- Priority Area 2: Improve pre-processing technologies
- Priority Area 3: Integration of algal biomass unit operations

We capitalized on Cellana's experience producing algae biomass at demonstration scale and LANL's prior experience with strain improvement to identify strains with potential to reach 2500 gal per acre annually of biofuel intermediate. Strains selected were suitable for Cellana's commercial product streams:

Biofuels
Animal Feeds
Nutraceuticals
and more

Project Overview (3 of 3)

- Priority Area 1 (Productivity): Improved strains were generated in the laboratory using cell sorting techniques and tested indoors at lab-scale and outdoors at mid-, and demonstration-scale for increased biomass and lipid productivity.

- Multiple cultivation approaches were tested at demonstration scale to improve productivity and lower operational costs.
  - Flue gas utilization
  - Pond microbial community characterization
  - CO_2 delivery and utilization, Increased light utilization

- Priority Area 2 (Pre-processing): Dewatering technologies were tested at demonstration scale and optimized for improved energy efficiency; an extraction method for high value lipid extraction was evaluated.

- Priority Area 3 (Integration): Selected strains and the integrated process at demonstration scale resulted in successful progress towards meeting the 2500 gallon/acre/year biofuel intermediate goal.

Approach (Management)

Communication and Data Sharing

- Centralized communications with Cellana's project manager
- Face-to-face meetings between project and task managers and DOE, often leveraging attendance at other meetings, were very important
  - 2015: ABLC; ABBB; ABS; ABFC; BETO Harmonization Workshop
  - 2016: ATP^3 Workshop; BETO Toolbox Workshop
- Monthly status calls with PIs (Cellana, LANL, Sandia) and DOE
- Technical calls, informal calls/emails as needed
- Quarterly Technical and Financial Reports filed
- DOE Project Management Plan format was used to track tasks and team responsibilities
- Smartsheet, a collaborative web based application used across team for
  - Pre- and post-award DOE document storage; Data sharing; Literature results storage; DOE report collaboration and compilation
  - SOP development and experimental planning

Approach (Technical-Priority Area 1)

Priority Area 1: Improve Algal Biomass Productivity

Goals

- Increase in lipid and biomass productivity
- Increase in culture stability
- Improve transfer of carbon dioxide into growth media
- Increase efficiency of CO_2 usage to reduce cost

Tasks

- Identify & Improve Algal Strains (Cellana/LANL)
  - A *Nannochloropsis* subpopulation with increased lipid content isolated using fluorescence-activated cell sorting (FACS)
- Pond Microbial and Contaminant Characterization (Cellana/Sandia)
  - Bacterial probiotic effect tested on *Nannochloropsis* culture
- CO_2 Utilization (Cellana/LANL)
  - Flue gas utilization as a sole CO_2 source tested
  - CO_2 delivery and pH control examined for impact on productivity
- Increased Light Utilization (Cellana)
  - Split pond approach examined

Approach (Technical-Priority Areas 2 & 3)

<u>Priority Area 2: Improve pre-processing technologies</u>

Goals

- Improve Harvest Efficiency and Biomass Quality
- Reduction in Capital Cost and Energy Use

Tasks

- Low-Shear Centrifugation (Cellana)
- Ultrasonic Harvesting (LANL/Cellana)
- Supercritical CO_2 Fluid Extraction (Cellana)

<u>Priority Area 3: Integration of algal biomass unit operations</u>

Goals

- Verify projected cost reduction and process economics in light of the 2,500 gallon/acre/year goal

Tasks

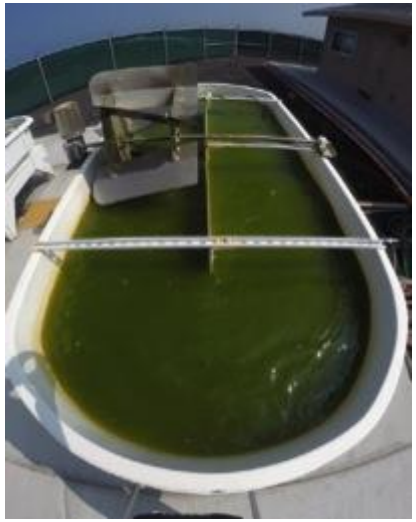
- Demonstrate unit operations at scale for improvements in lipid and biomass productivity

Technical Accomplishments: Introduction

Integrated Outdoor Cultivation at Mid- and Demonstration Scale at KDF

Indoor Carboys → Outdoor Hanging Bags → 24,000L Tubular PBRs → 60,000L Open Ponds

Mid-scale Open Ponds

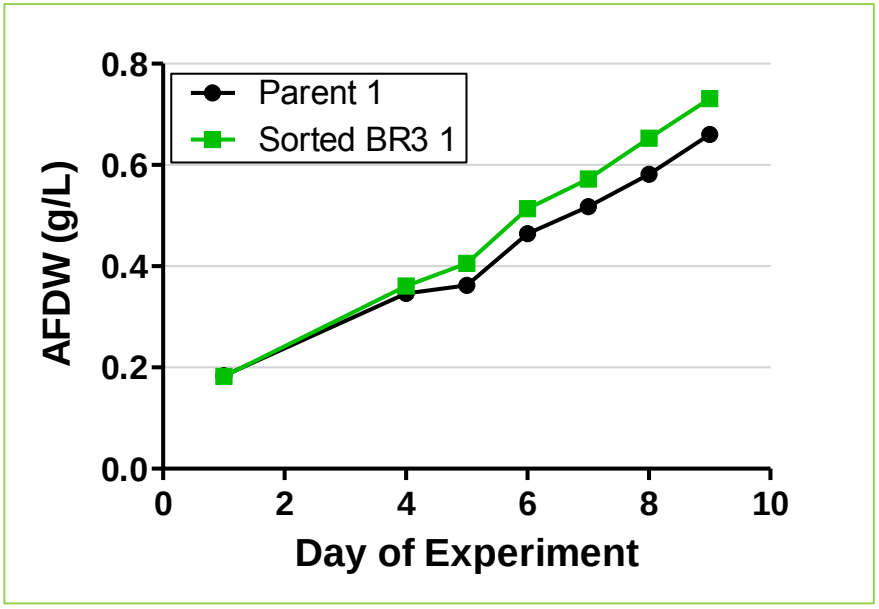


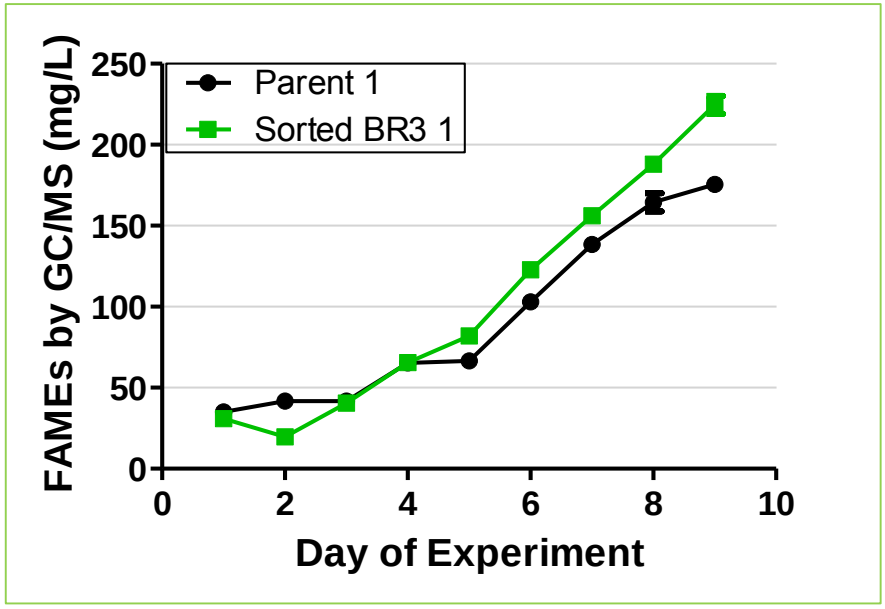
Photos by Gary Meddock, Cellana

Technical Accomplishments Highlights

Priority Area 1: Improve algal biomass productivity (Cellana/LANL/Sandia)

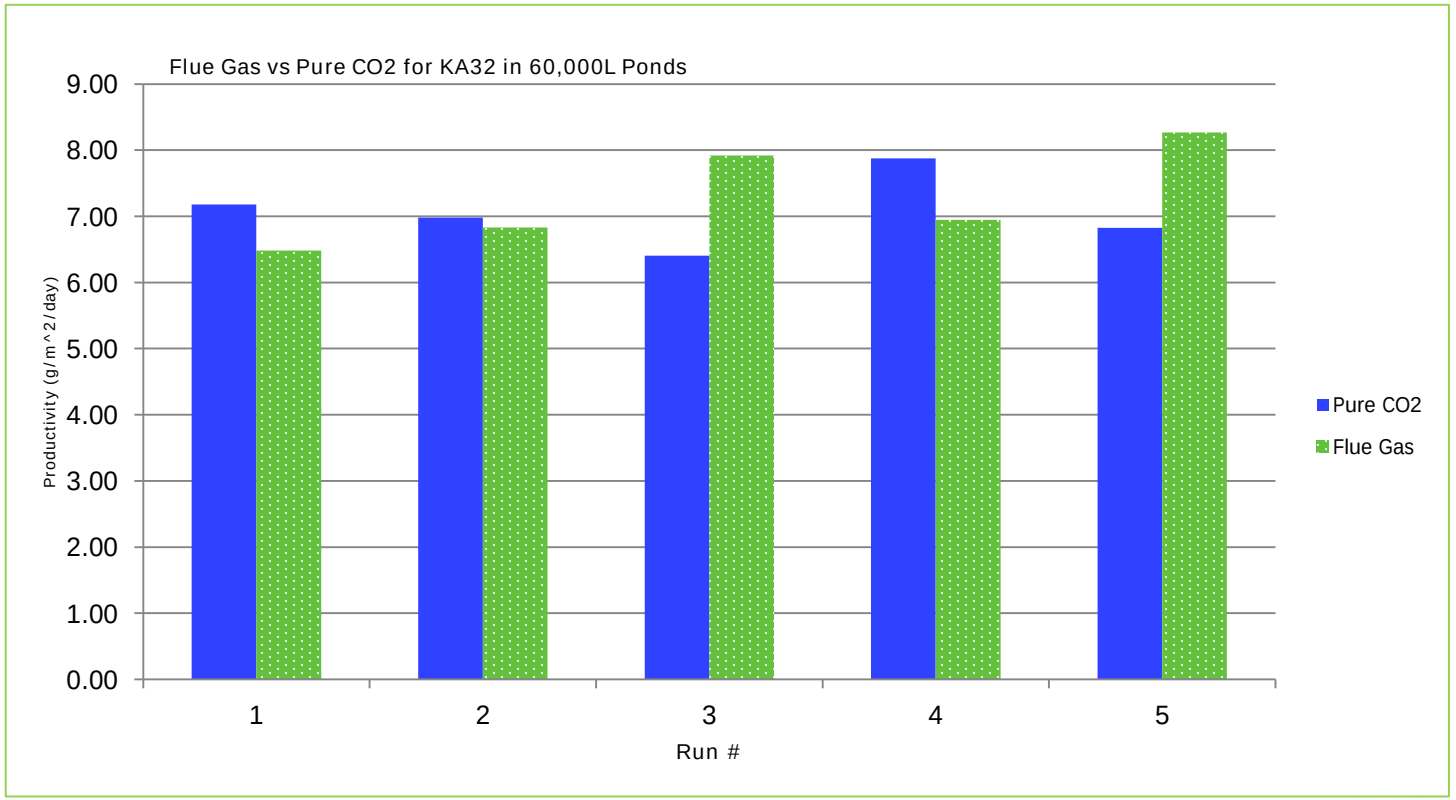
N. salina FACS sorted/unsorted populations showed up to 48% total lipid content when grown at demonstration scale





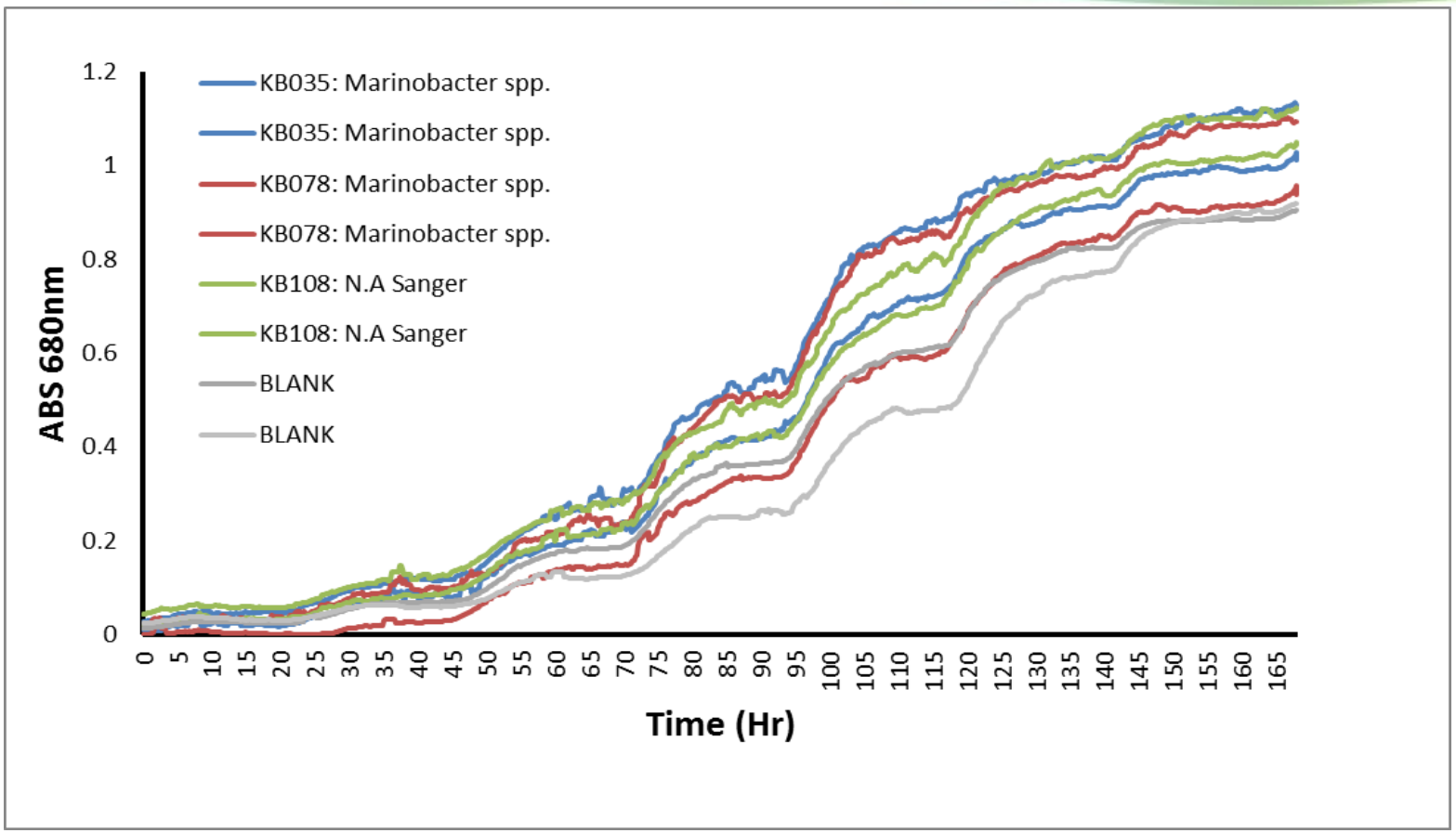
Priority Area 1: Improve algal biomass productivity (Cellana/LANL/Sandia)

- Recycled Flue Gas as sole CO_2 source maintained biomass productivity



Priority Area 1: Improve algal biomass productivity (Cellana/LANL/Sandia)

Identified bacteria that promoted *Nannochloropsis* growth



Duplicated KB035 and KB108 both promoted KA32 growth compared to blank control; KB078 results are mixed

Technical Accomplishments
Project Goals Successfully Achieved

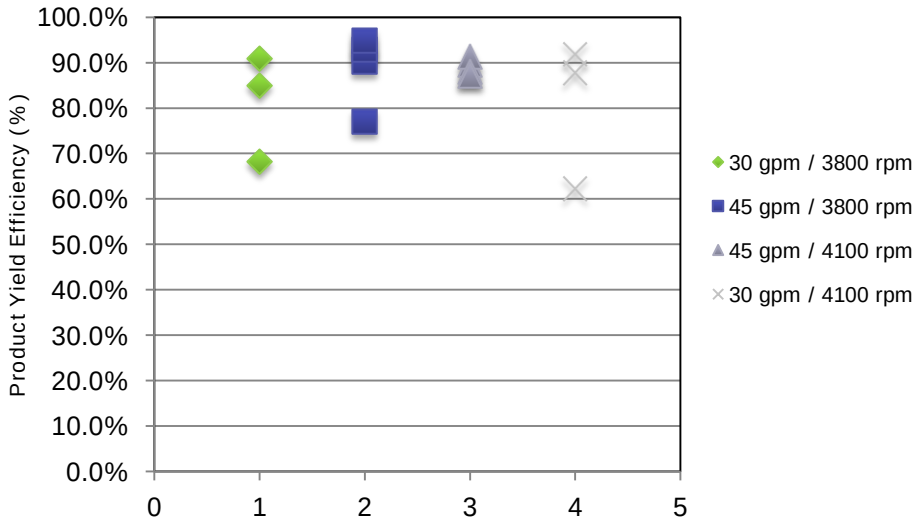
Priority Area 1: Improve Algal Biomass Productivity

Cultivation Task Areas	Baseline	Accomplishment	Target
Strain modification & selection	KA19, KA32 *Nannochloropsis oceanica, Nannochloropsis maritima*	• KA32 grew more robustly than KA19 • *Nannochloropsis salina,* 1776 and 1776-BR3, selected for improved lipid content, & tested later in ABY, grew robustly with high productivity as well	N/A
Increased Biomass Productivity	9.6 $g/m^2/day$	14.8 $g/m^2/day$ "Harvest" productivity	15 $g/m^2/day$
Improved Lipid Content	32%	~50% on nutrient depletion with *N. salina*	37%
[EPA]	2.98%	3.12% (high of 3.7%) KA32 ATP3 studies	>2.98%
Reduced CO_2 usage	N/A	• 21% reduction on biomass DW basis (low pH studies) • 100% reduction of pure CO_2 (flue gas studies)	>10%

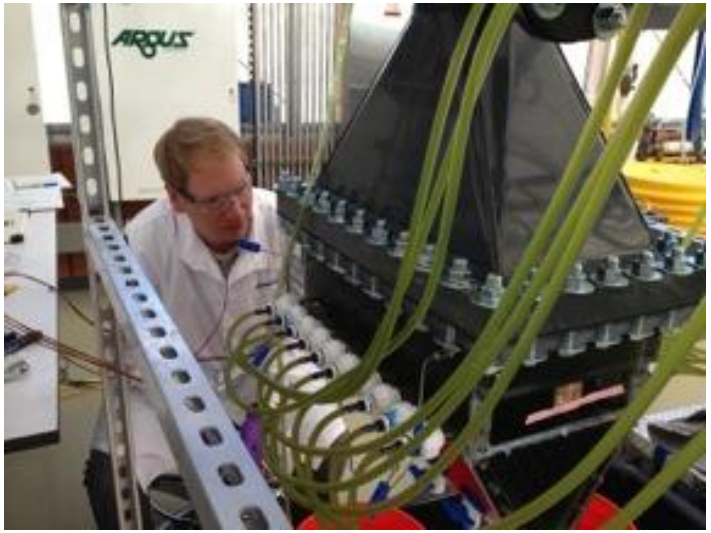
Technical Accomplishments Highlights

Priority Area 2: Improve pre-processing technologies

- Harvesting

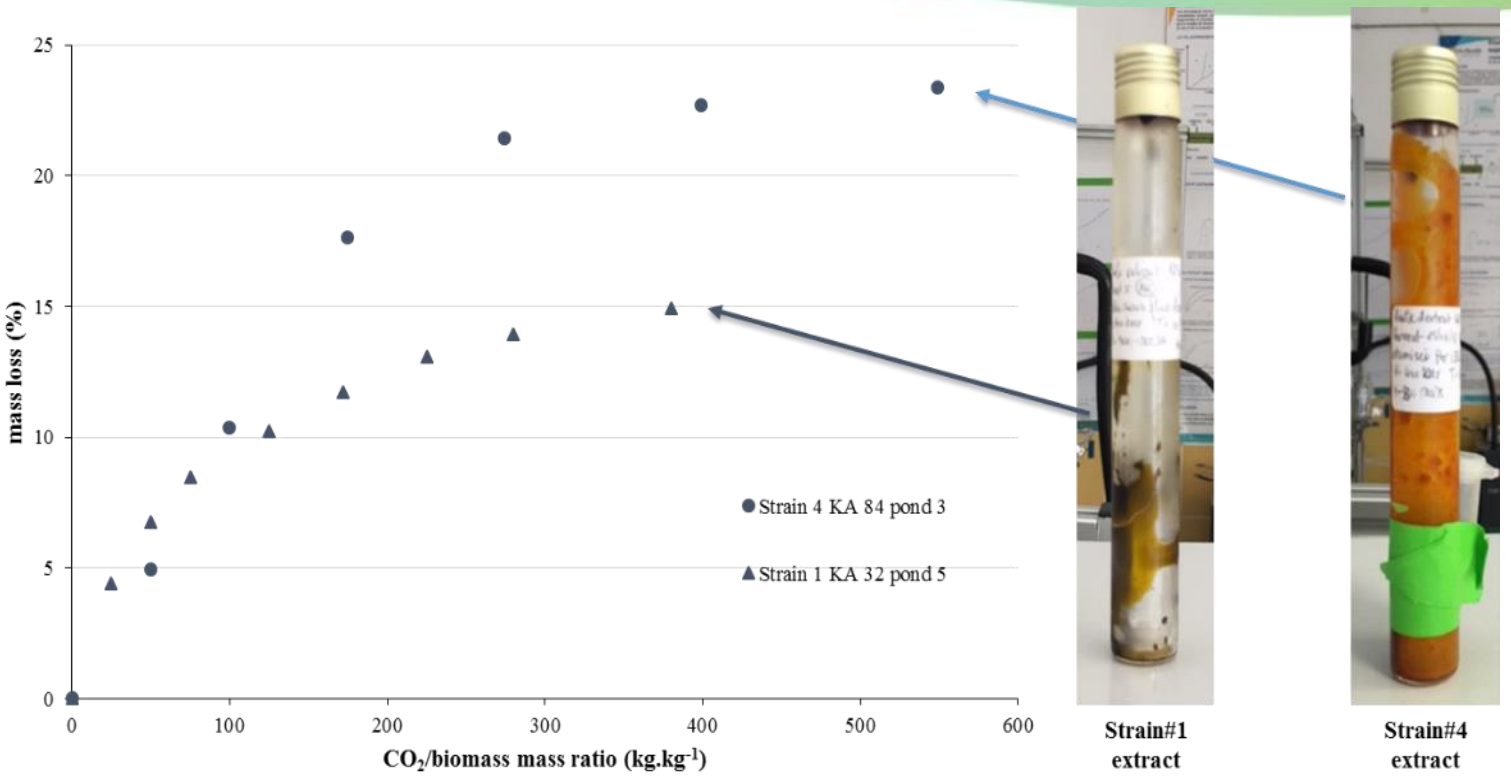


>80% yield from primary dewatering step (centrifugation); operational improvements made



- Largest ultrasonic harvesting vessel in the world assembled and tested.
- Spent 2 weeks testing modules at KDF

- SuperCO$_2$ Lipid Extraction



*Comparison of extraction curves obtained from Nannochloropsis maritima (KA32) and Nannochloropsis salina (BR3) at 400 bar, 333 K and 0.5 kg*h^{-1}* . >90% of neutral lipids and up to 80% of Omega-3 extracted using sCO$_2$.

Technical Accomplishments
Project Goals Successfully Achieved

Priority Area 2: Improve pre-processing technologies

Key Performance Parameter	Baseline Value	Demonstrated Value	Target Value
Harvest and Dewatering Mass Efficiency (wt.%)	N/A	85% recovery post-centrifugation	N/A
Harvesting and Dewatering Energy usage	0.012 kW hr/gal	0.010 kW hr/gal	0.011 kW hr/gal
Biomass Conversion Yield (g oil / g biomass)	22% via solvent based extraction	25% via sCO2 extraction (includes pigments)	>22%

Please discuss the scale and duration associated with tests used to generate the harvesting:

- Harvest efficiency and energy usage based on running commercial scale GEA Westfalia centrifuge for processing of 10, 60,000L ponds and ~250kg biomass in low shear centrifugation testing
- Biomass conversion yield based on lipid extraction using sCO2 with approximately 10kg biomass

Technical Accomplishments
Project Goals Successfully Achieved

Priority Area 3: Integration of algal biomass unit operations

Key Performance Parameter	Baseline Value	Demonstrated Value	Target Value
Intermediate yield, gal/acre/yr	1912	2511	2500 (by 2018, harvest pond basis, no processing yield loss)
Total Lipid Content (ash free Dry wt.%)	32.38	~50% *(N. salina)*	37
Average Annual Productivity, g/m2/day	9.6	14.8	15
Amount of Biomass Process	Total amount during project ~2000kg		
Please discuss the scale and duration associated with the productivity value Scale: 60,000L ponds, 3 replicates, ~1 mo., low pH condition			
Please discuss the Level-of-Process Integration associated with results Biomass grown and harvested via centrifuge in fully integrated demonstration facility. High lipid strains leveraged from laboratory work at LANL. Extraction of lipids performed in France. Analytical testing done at Cellana, LANL, ASU/AzCATI & in France.			

Relevance

BETO MYPP

- All aspects of this project were designed to help the algae industry move forward and achieve its goal: 2018 demonstrating algal 'biofuel intermediate' productivity of 2,500 gallons per acre per year, on a trajectory to demonstrating 5,000 gallons per acre per year by 2022
- Project metrics were driven by TEA

Global/USA Impact

- Reduce reliance on fossil-based fuels through the commercialization and worldwide expansion of algal-based biofuels
- Reduce carbon footprint for co-products and other bioproducts by using algal feedstocks

Cellana/LANL/Sandia

- DOE's funding for algal projects is essential for the industry
- Key strain and process improvements from ABY-1 have been incorporated into Cellana's operations
- Since this project, Cellana has initiated 2 new off-take agreements

Cellana's Multi-Product Business Model: up to 4 Products From Each Strain Via ALDUO™ + **"Conventional"** Upstream/Downstream Processes

- "Off-the-shelf" ag inputs + sunlight + CO_2 + ALDUO™ = ReNew Algae
- Existing or new & improved separation/extraction techniques = + ReNew Feed + ReNew Omega-3 + ReNew Fuel

ReNew Algae
the planet's most sustainable™

- Whole algae for high-value product extraction
- Aquaculture hatchery feed

PIVEG

GalilAlgae

LIVING INK TECHNOLOGIES

~70% tot. vol. (post-extraction)

ReNew Feed

ReNew Omega-3

ReNew Fuel

NESTE

cellana

Overall Impressions and Lessons Learned

- Achieving *sustained* high lipid productivity and high biomass yield remains a challenge
- Opportunities for cost savings in cultivation and preprocessing still need to be explored, and new technologies advanced; but even operational changes can make a big difference to energy efficiency and resource usage
- There is still a need for further investment to improve prediction of performance for *a)* indoor to outdoor transition, and *b)* between 1000L to 60kL scales
- A strong advanced algae feedstock R&D network has developed through BETO support, and was leveraged in this project

Summary

Technical Approach/Accomplishments/Progress/Results

- Project was initiated on 12/05/14, and concluded 09/30/16; Post-validation completed and Final Report submitted
- Approaches in strain improvement, demonstration-scale cultivation, dewatering technologies, lipid extraction, and process integration drove technical results sufficient to meet or exceed project target metrics
- Successful demonstration of improved biomass and lipid productivity was sufficient to meet intermediate goals consistent with the BETO MYPP and ABY-1 FOA targets
- Techno-economic viability has been validated using a three-product business model and the "best of the best" demonstrated project results

Relevance

- Achievement of objectives are aligned with BETO's 2018 goals and on the path to the 2022 goals; and demonstrate that algae-derived products provide a platform for economically sustainable businesses

Future Work: N/A as project is sunsetting

- *The team thanks DOE for its continued support and looks forward to continuing work needed to drive the industry to success in establishing a bio-based economy*

cellana™

algae-based products
for a sustainable future™

Mahalo!

Cellana: David Anton, Johanna Anton, Avery Kramer, Selena Ellis-Vizcarra, Martin Sabarsky

LANL: Jim Coons, Taraka Dale, Babs Marrone, Scott Twary

SNL: Todd Lane

Additional Back Up Slides

Publications, Presentations, Patents

Complete List of Conference Presentations, Peer Reviewed Publications and Patent Filings

Patents:

J.E. Coons, et al., Acoustic Manipulation of Fluids Based on Eigen Frequency, S133320, January 2016.

Publications / Presentations:

Coons, J.E., and Dale, T. Property evolution of *Nannochloropsis* species in nitrogen-deplete conditions and its ramifications on overall process energetics, presented at the 6th International Conference on Algal Biomass, Biofuels, and Bioproducts held in San Diego, CA, June 2016. Oral presentation.

Dale, T., Twary, S.N., Marrone, B.L., Anton, J., and Saracco, M. Cultivation of parent and cell sorted populations of *Nannochloropsis salina* in laboratory and outdoor environments. 6th International Conference on Algal Biomass, Biofuels, and Bioproducts held in San Diego, CA, June 2016. Oral presentation.

Coons, J.E., Kalb, D., Dale, T., Kramer, A. and Anton, J. Ultrasonic harvester test results at Cellana's Kona Demonstration Facility. Algal Biomass Summit, Phoenix, AZ, October 2016. Poster presentation.

Dale, T., Twary, S.N., Marrone, B.L., Anton, J., and Saracco, M. Cultivation of parent and cell sorted populations of *Nannochloropsis salina* in laboratory and outdoor environments. Algal Biomass Summit, Phoenix, AZ, October 2016. Oral presentation.

Coons, J.E., and Kramer, A. Meeting BETO's Harvesting Cost Target by Coupling Ultrasound and Centrifugation. Algal Biomass Summit, Phoenix, AZ in October 2016. Oral presentation.

Anton, J., Saracco, M., Yamane, K., McGowen, and Harmon, V. A Comparison of Outdoor Semi-continuous and Batch Production Modes at Mid and Large Scale. Algal Biomass Summit, Phoenix, AZ, October 2016. Oral Presentation.

Publications, Presentations, Patents *(cont.)*

Publications / Presentations (continued):

Kramer, A., *et al* Optimizing neutral lipid extraction from *Nannochloropsis sp.* with supercritical carbon dioxide. Algal Biomass Summit, Phoenix, AZ in October 2016. Oral presentation.

Anton, D.L. Carbon Capture Instigated by Synthetic Biology. World Congress on Industrial Biotechnology, April 2016, San Diego, CA. Panel Discussion.

Anton, D.L. Algae as the New Feedstock for the Advanced Bioeconomy. Advanced Bioeconomy Feedstocks Conference, June 2016, Miami, FL. Oral Presentation.

Biofuels Digest, June 30, 2016, "Feedstock for the Advanced Bioeconomy: The Digest's 2016 Multi-Slide Guide to Cellana"

Sabarsky, M. Creating Commercial Success in the Bioeconomy…with Algae. Bioenergy 2016 Conference, July 2016, Washington, DC. Oral Presentation.

Barr, W. J., Landis, A. E. Environmental Sustainability Assessment of a Commercial Microalgal Multiproduct Biorefinery, *manuscript in preparation* (Cellana provided information from ABY-1)

Barr, W. J., Stirling, R., Landis, A. E. Economic Potential of a Commercial-Scale Algae Multiproduct Biorefinery Model for the Production of High-Value Products and Biofuel, *manuscript in preparation* (Cellana provided information from ABY-1)

Barr, W. J., Stirling, R., Landis, A. E. Sustainability Assessment of a Commercial Algae Multiproduct Biorefinery for the Simultaneous Production of Biofuel and High-Value Products, *manuscript in preparation* (Cellana provided information from ABY-1)