ADVANCED ALGAL SYSTEMS

TECHNOLOGY AREA

CONTENTS

INTRODUCTION

The Advanced Algal Systems (AAS) Program is one of 12 technology areas that were reviewed during the 2023 Bioenergy Technologies Office (BETO) Project Peer Review, which took place April 3–7, 2023, in Denver, Colorado. A total of 32 active projects were reviewed in the AAS sessions by two panels of four external reviewers. Of the 32 projects reviewed, 14 were reviewed by the Cultivation and Strain Development panel and 18 were reviewed by the Integration panel. For information about the structure, strategy, and implementation of the program and its relation to BETO's overall mission, please refer to the corresponding Program and Technology Area Overview presentation slide decks, which can be accessed at the Peer Review website: www.energy.gov/eere/bioenergy/2023-project-peer-review.

This review addressed a total U.S. Department of Energy (DOE) investment value of approximately $61.7 million, which represents approximately 11% of the BETO portfolio reviewed during the 2023 Peer Review. During the Project Peer Review meeting, the presenter for each project was given 30 minutes to deliver a presentation and respond to questions from the review panel.

Projects were evaluated and scored for their approach, impact, and progress and outcomes. This section of the report contains the Review Panel Summary Report, the Technology Area Programmatic Response, and the full results of the Project Review, including scoring information for each project, comments from each reviewer, and the response provided by the project team.

BETO designated Daniel Fishman as the Advanced Algal Systems Technology Area review lead, with contractor support from Jamie Meadows of Boston Government Services and Phillip Lee of Allegheny Science and Technology. In this capacity, Daniel Fishman was responsible for all aspects of review planning and implementation.

CULTIVATION AND STRAIN DEVELOPMENT PANEL

Name	Title and Affiliation
Dr. Tyler Johannes*	Professor at University of Tulsa
Dr. Laura Carney	Former Director of Ag Science at Heliae
Dr. Ify Iwuchukwu	Director, Pilot Plant at Oxford Biomedica Solutions LLC
Dr. Kalyani Maitra	Associate Professor at Fresno State University

* Lead Reviewer

INTEGRATION PANEL

Name	Title and Affiliation
Dr. Lora Cameron-Landis*	Associate Director of Upstream Process Development and Manufacturing at Eli Lilly
Valerie Harmon	President and CEO at Harmon Consulting Inc.
Dr. Mark Jones	Dow (retired)
Dr. Paul Roessler	Algenol (retired)

* Lead Reviewer

CULTIVATION AND STRAIN DEVELOPMENT REVIEW SUMMARY REPORTS

Prepared by the Advanced Algal Systems Review Panel – Cultivation and Strain Development

INTRODUCTION

The cultivation and strain development of algae have emerged as a promising avenue for addressing the challenges of sustainable energy and resource production. As the world seeks alternative sources of fuel and raw materials, BETO has taken a proactive role in supporting and funding research in this field. The 2023 BETO Peer Review of the AAS – Cultivation and Strain Development serves as a testament to DOE's commitment to driving innovation and remaining a global leader in this domain.

The AAS Program focuses on advancing the cultivation techniques and genetic engineering of algae to optimize biomass production and improve the efficiency of biofuel production. Through strategic partnerships with academia, national laboratories, and industry stakeholders, BETO aims to accelerate the development and deployment of sustainable and cost-effective algal production technologies. This Peer Review offers an opportunity to assess the progress made by the AAS Program, evaluate the effectiveness of the funded projects, and identify areas for further improvement.

The findings of the 2023 BETO Peer Review reflect BETO's unwavering dedication to advancing cultivation and strain development technologies. The review highlights the successful outcomes achieved by funded projects, such as the development of robust genetic tools, engineering systems for improved cultivation, and real-time monitoring toolkits for algal culture. These accomplishments demonstrate the efficacy of DOE's funding approach in driving meaningful advancements and bridging the gap between research and commercialization.

By reaffirming DOE's position as a global leader in funding cultivation and strain development technologies, the 2023 BETO Peer Review underscores the importance of ongoing research and development efforts in the algae cultivation sector. As the world transitions toward a greener and more sustainable future, BETO's continued support in this field will play a vital role in unlocking the full potential of algae as a renewable resource for biofuel production and other applications.

STRATEGY

The AAS Program's strategic goal is to develop technologies that enable the production of environmentally sustainable algal feedstocks that perform reliably in conversion processes to yield renewable fuel blendstocks, as well as bioproducts and chemical intermediates. The program structure encompasses cultivation and strain development, as well as integration, covering a wide range of research areas to achieve the goal using different approaches. The program has reflected on learnings, addressed gaps, and adjusted investments accordingly. It has successfully funded critical path technologies and filled known gaps. The funding mechanism, including innovative and high-risk seed projects, has been appropriate.

The recent lab calls align with the barriers and challenges to achieving BETO's goals. The program should ensure that successful approaches have potential for commercial deployment and consider closer collaboration with industry partners to harmonize efforts and attract continuous funding. The previous and ongoing funding announcements have been well designed and managed. Efforts to monitor portfolios for overlaps and consolidate efforts for better outcomes should continue. Encouraging and funding new principal investigators (PIs) with fresh ideas and approaches is recommended. The strategic objective of focusing on algae as a renewable resource for biofuels and byproducts is well defined, and the funding mechanisms have facilitated collaboration and progress. While the academic and national lab efforts seem to focus on pond crashes, industry perspectives should be considered to ensure alignment. Targeted funding for bioprospecting or genetic modification of extremophiles may be worth exploring. Productivity improvements are still needed, and research on strains tolerant to higher temperatures caused by climate change could be beneficial.

STRATEGY IMPLEMENTATION AND PROGRESS

The implementation of the strategy within the AAS Program has shown significant progress and successful management of funded projects. A broad range of disciplines, including strain development, carbon utilization technologies, wastewater integration, engineering systems, logistics, techno-economic analysis (TEA) and life cycle analysis (LCA) models, and system integration, have been funded, demonstrating a comprehensive approach toward achieving the goals and targets set forth by the AAS Program. Key accomplishments such as the EcoRecover™ process for wastewater treatment, the real-time monitoring toolkit for algal culture, genetic tool development, and productivity improvement highlight the advancements made in addressing the barriers and technical challenges identified in the program.

The AAS Program has effectively used BETO funding to address challenges related to the successful deployment of algal production technologies. The program's transparent platform has facilitated stakeholder engagement and provided a means to track progress for funded projects. The diverse range of institutions and locations represented in the funded projects demonstrates the program's ability to bring together national labs, industrial partners, and academic institutions to contribute to the development of renewable carbon resources using algae as feedstock.

While significant progress has been made in various aspects, including biomass forecast systems, harmonization of analysis data, and the production of resilient algal strains and toolkits, there is a need for further advancements in low-cost, sustainable biofuel production from algal oil. The AAS Program should continue to fund projects focusing on strain improvement and encourage diversification in strain selection criteria during bioprospecting. Additionally, combining projects on bioprospecting, strain evolution, and engineering with existing technologies and toolkits could lead to breakthroughs in biomass productivity. Consideration should also be given to the exploration of extremophiles and thermophiles, as they can contribute to contamination control, carbon utilization efficiency, and summer production.

Overall, the AAS Program has made substantial progress in implementing its strategy, managing projects effectively, and promoting collaboration among diverse stakeholders. Continued funding for critical areas such as strain development, engineering, and bioprospecting, along with a focus on commercialization and industry adoption, will contribute to the achievement of BETO's goals in biomass production and low-cost sustainable biofuel production from algal sources.

RECOMMENDATIONS

Based on the comments provided, the three most important recommendations to strengthen the portfolio in the near to medium term are:

1. **Address pond crashes and stability**: During the review, it was noted that two industrial teams expressed no concerns regarding pond contamination. Interestingly, both of these teams use extremophile algae cultivated at high pH levels. While there has been significant attention from academic and national lab efforts in addressing this issue, it is important for AAS to investigate the validity of these claims, considering that the current funding opportunity announcement (FOA) focuses on algae crop protection. If pond contamination is predominantly a challenge encountered in smaller-scale academic and national lab settings rather than in industry, allocating AAS funds towards pest management may not provide significant value. However, if there is indeed a genuine discrepancy, it highlights an inherent disconnect between academic/national lab efforts and industry, which BETO should actively work to resolve.

2. **Target efforts for high-pH- and heat-tolerant algae strains**: BETO should focus on identifying and/or engineering algae strains that are tolerant to high pH levels and heat. These traits are likely to be

important for improving biomass productivity and resilience in outdoor pond conditions. By prioritizing research in this area, the program might be able to address the recent slowdown in biomass productivity gains and work toward achieving higher biomass productivity targets.

3. **Engage industry partners and address scale-up challenges**: BETO should actively engage industry partners to identify gaps and barriers in scale-up efforts. Collaborating with industry stakeholders will provide valuable insights and help address challenges related to commercial deployment. Industry involvement should be encouraged to ensure the technologies developed through the program have a higher likelihood of adoption. Input from industry partners will also contribute to the TEA and LCA processes, improving the program's ability to assess the cost and energy effectiveness of biofuel production.

By implementing these suggestions, the AAS Program can strengthen its portfolio, accelerate progress toward achieving BETO's goals, and foster greater industry engagement in the algae sector.

INTEGRATION REVIEW PANEL SUMMARY REPORT

Prepared by the Integration Review Panel

INTRODUCTION

Integration projects—those focused on the technology development required to bring biofuel cultivation from lab-scale, proof-of-concept work to demonstration- and commercial-scale operations—are clearly necessary for the creation of an algal biofuels industry. Integration projects include pilot-scale operations, process analytics, dewatering, fractionation, and development of the algae fractions into fuel and valuable materials. The majority of the portfolio relies on coproducts to improve biofuel production economics. Some of the projects target only algal products with no fuel production. While the initial proof-of-concept work is centered around bioprospecting, strain development, genetic modifications, metabolic engineering, carbon capture and delivery, cultivation, and refinement of algae fractions into petrochemical substitutes, integration aims to bring these disparate projects together as a unified whole, resolve any incongruities, and promote access to the energy market for algae-based biofuels by creating fully developed drop-in solutions. The bottom-up approach supports the maximum amount of creativity in solving problems unique to scalability, results in more informed research directions, and allows for a deep understanding of the complexities of algae production and processing, while general top-down guidance is provided by the strategic requirements of each FOA.

Additionally, the integration projects are intended to provide collaboration between industry and academic research. By working with academic labs, customers for algae products and operators of commercial algae operations are connected to potential supplies of raw materials and opportunities for new product distribution and new market value propositions. Researchers in turn receive additional funding, collaboration opportunities, facility and material resources, and access to economic and operational expertise.

STRATEGY

Due to the nature of the integration work, spanning several fields and specialties, the integration technology area covers a wide range of technologies. The intent of the projects is to move technologies closer to commercialization, taking lab-scale discoveries and showing they are viable in the field at larger scale. Gaps are filled between lab projects to bring together technologies to create a whole, functional production operation capable of meeting BETO fuel production targets. The goal, regardless of the individual project, is to enable the creation of an economically viable set of products from algal raw materials. The department identified and implemented several strategies toward this end:

- Projects must be guided by an agreed-upon model of TEA to illustrate the relevance and cost effectiveness of the project plan relative to BETO goals.

- Projects must collaborate with at least one industry partner to better assess the commercial feasibility of the work.
- Projects must meet the applicable productivity and carbon capture requirements established by the FOA to achieve economic targets that enable sustainable production and compete on the world stage with petroleum-based products.
- Projects in the integration category must meet a minimum technology readiness level of 3, with the proof of concept already established at small scale.

Historically, moving algae cultivation from the lab into open ponds has encountered significant challenges: contamination, predation, transgenic organism control requirements, weather variability and climate disruption, the available hiring pool for staff, land costs, water availability, cost of goods, and the need for specialized process monitoring. All create meaningful barriers to entry for algae-based fuels and coproducts. The technical challenges of mixing, carbon capture, facility energy use, mass transfer, and strain development at large scale all must be overcome to create an algal biofuels industry. In order to better address these gaps, integration projects contribute to the overall goal of the program to create an economically viable path toward production of algae-based fuels and coproducts, which are not associated with any particular unit operation or specific technological advancement. They focus only on successful integration of largely known and developed technologies into an overall, workable system.

STRATEGY IMPLEMENTATION AND PROGRESS

The strategy execution addressed the following categories:

Algae batch growth and direct air capture (DAC) technologies: Due to the nature of integration, many of the projects had overlap with algae growth projects to demonstrate feasibility in the field and scale-up. There were several strategies for DAC of CO_2 at pilot or larger scales.

Montana State University used high-pH and high-alkalinity capture in raceway systems (1.3.4.002) to shift the equilibrium reaction toward bicarbonate generation rather than carbonic acid and demonstrated a new mixing method that used a moving belt with cleats rather than a paddlewheel to improve kLa in the raceway and thereby improve CO_2 transfer to the media. A proof-of-concept prototype was created to demonstrate the mixing concept overall. The energy balance, use in algae cultivation, and scale-up proof of concept to develop this mixing method further will be performed later in the project.

A collaboration between the National Renewable Energy Laboratory (NREL); University of California, San Diego; Algix; and Gross-Wen Technologies (1.3.4.004) leveraged *in silico* modeling of different carbon capture proteins to create a new carbonic anhydrase (CA) overexpression system, characterizing metabolic networks and signal peptides for extracellular secretion in the process and resulting in significantly improved biomass generation in a Revolving Algal Biofilm (RAB™) cultivation system. Supplementation of the RAB system with exogenous CA as a CO_2 capture method increased yields by approximately 25% over sparging, and doubled yields from the RAB system alone. The biomass produced was found to be suitable for plastics manufacturing. Large-scale testing of the carbonic-anhydrase-secreting strains will be completed in the future.

A MicroBio Engineering project (1.3.4.006) using high-pH algal "weed" strains from Cyanotech investigated the use of the pond itself as a carbon sink via high alkalinity, added CA, and improved buffering capacity to spread the CO_2 transfer to the algae over the course of the day more efficiently compared to limited peak daytime transfer. One of the strains achieved the baseline productivity requirements and is moving forward with further evaluation. Carbon flux modeling was used to identify critical parameters for pond depth; however, the DAC skid is currently under development by Global Thermostat (GT) and will be implemented later in the project.

Duke University is also working on a DAC skid (1.3.4.010) to deliver CO_2-enriched air to algae ponds and storing it in the pond as bicarbonate. The benefit of the DAC module being produced by MoleculeWorks was the ability to use thinner membranes that could be stacked tightly together, allowing for a small footprint. Additionally, they are also investigating marine strains that may have higher productivity, especially as it relates to protein-based products with increased economic value. Optimal inoculation density was modeled for batch cultivation and will be tested for various strains from the Marine Algae Industrialization Consortium (MAGIC) project in the future.

Arizona State University's AUDACity project (1.3.4.003) developed a new ion-exchange polymer capable of absorbing CO_2 from the air and delivering it to algae directly, and identified the operational parameters required for efficient CO_2 capture and delivery. Scale factors of the polymer were identified, and optimal parameters were characterized. Drying time required to reenable CO_2 absorption, nitrate competition for adsorption sites on the polymer, and an initial washing period to remove growth-inhibiting leachables were studied, and implementation strategies were developed. The novel polymer system performed similarly to an existing commercially available polymer, discharging the captured CO_2 efficiently to the algae pond, enabling a 2.2-fold increase in methyl laurate yield, and eliminating the losses normally observed during CO_2 sparging.

Los Alamos National Laboratory's (LANL's) work on media optimization and recycling of media components (1.3.4.205) using Bayesian machine-learning methods identified the most efficient way to use nitrogen and phosphate dosing and recycling at scale, lowering cultivation costs. The initial modeling and data set is promising, and it will be interesting to see how the model is validated against empirical testing; dosing methods and forms will need to be characterized, along with water quality and optimal temperature conditions.

Global Algae Innovations (GAI) (1.3.4.001) presented the challenge of growing algae outdoors during unusual rain conditions, responding to the events in a way that maintained high-productivity and high-value products. A large number of patents are in progress for downstream product development and unit operations, though step yields and product quality data for the various product streams remain to be seen. There were insufficient data from the rain event to sufficiently develop a mitigation strategy. Due to the patent strategy, the results will not be disseminated to industry until patent applications have been submitted or published.

An Arizona Center for Algae Technology and Innovation (AzCATI) project (1.3.5.287) provided further insight into culture failures and how they can be modeled, discovered early, treated if possible, and optimally managed in a facility to maintain productivity. The team encountered a challenging parasitoid infection during their work that necessitated a change in approach and risk mitigation measures to better understand and manage the pond dynamics. Resistance to fungicide was observed to develop quickly, which demonstrated one of the practical issues associated with open cultivation. Quantitative polymerase chain reaction (qPCR) identification of microbial contaminants and creation of a gene-based library for rapid diagnostics will be useful for moving the field forward, particularly with respect to developing best practices for cohesive contamination control.

University of Illinois at Urbana-Champaign's wastewater-grown algae demonstrated impressive 73% increases in oil productivity, a method for reducing ash content, and increased oil conversion (1.3.5.286); they also tested the benefit of media supplements and a method for recovering organics from hydrothermal treatment of wastewater. The use of wastewater-based rotating biofilm cultivation improves the economics of production. Demonstration-scale projects are planned for the future to show scalability of the improvements.

Finally, Colorado School of Mines developed a novel high-biomass, high-lipid-productivity strain using a novel mutagenesis technique and fluorescence-activated cell sorting to select the most productive clones, which demonstrated continued increased productivity in outdoor ponds (1.3.5.282) after enrichment for tolerance of stressful events. It will be important to see how it compares directly to control strains at scale, and how well the strains will maintain their productivity over time, especially *Nitzschia.*

Downstream algae fractionation and fraction refinement: The Combined Algal Processing for the Synthesis of Liquid Oleofuels and Products (CAPSLOC) project (1.3.4.204) developed a downstream biorefinery process (i.e., mild oxidative treatment [MOT] of acid-hydrolyzed biomass) that could accept starting materials from multiple feedstocks, yielding lipid, solid, and hydrolysate fractions to be further processed into fuels, fine chemicals, polymers, graphitic carbon, and protein. The development of a feedstock-agnostic biorefinery with multiple product lines is a critical contribution toward both renewable energy production and reduction of petrochemical use, and provides a pathway for supply chain debottlenecking of many of the products.

A Pacific Northwest National Laboratory (PNNL) project (1.3.4.102) to develop hydrothermal processing for multiple feedstocks identified several cost-advantaged feedstocks that could meet BETO's price target for algal biofuels, reducing the cost of fuel significantly, and used the method to process a worst-case scenario of high-ash wastewater-grown nuisance algae successfully into struvite fertilizer and fuel fractions. The flexibility of feedstocks is impressive and seems likely to be economically advantageous compared to dedicated single-source facilities.

Lumen Bioscience's Access Carbon project (1.3.4.008) expanded the downstream product offering into biotherapeutics, leveraging a recombinant spirulina platform to provide recombinant antibodies for intranasal delivery. Media optimization, pH, and alkalinity parameters were optimized by design of experiment and machine learning to minimize the range-finding experiments typically necessary for platform development and characterization.

Process analytics and diagnostics: Strain generational stability and trait drift, as well as contamination diagnostics, were characterized in the Optimizing Selection Pressures and Pest Management to Maximize Algal Biomass Yield (OSPREY) project (1.3.5.280), led by the New Mexico Consortium. Based on the observation that many lab strains fail catastrophically in field trials due to contamination and possible trait drift, the study developed field-ready pest detection techniques that provided results rapidly enough for pond management to respond to the infection and rescue the algae productivity. Loss of genetic stability in the field was significantly higher than observed in the laboratory strains due to outdoor selection pressures, and trait drift of pond cultures was identified as potentially having site-dependent phenotypes rather than being linked to genetic instability. The field-deployable qPCR method for diagnostics and monitoring may be helpful, especially over several seasons and when compared across different facilities, seasons, and scales.

A project from GAI (1.3.5.284) presented the use of rapid offline spectroscopy for protein, carbohydrate, and lipid content prediction using machine learning and neural networks to optimize the quantification of specific fractions. These methods allow for standardized materials handling and processing to improve the comparability of strain improvements and assess the efficacy of media supplementation, as well as the impact of environmental effects. Improvements to productivity were reported using both biotic and abiotic methods, which will be described in upcoming patent applications.

Techno-economic models: The major development in the techno-economic model field was the highly detailed Aspen Plus model developed by NREL (1.3.5.200), with an Excel version available to the public. The model was iteratively validated by 8 years of real-world data from the Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR) project, integrating polyurethane (PU) and protein coproducts into the final analysis. This revised model demonstrated the criticality of coproducts toward the goal of making biofuels economically feasible. Adoption of the model framework by many other laboratories validates its usefulness and impact on the field as a whole, and the team has made excellent efforts to make the work publicly available. Keeping the model updated with current data, including product quality metrics, will provide even more value to the industry and the field.

This model was integrated with a PNNL model of the high-throughput hydrothermal liquefaction (HTL) unit operation (1.3.5.202) and downstream processing for hydrogen generation, naphtha and diesel production, sustainable aviation fuel (SAF) production, and a struvite fertilizer coproduct generated by PNNL from a mix of biomass sources. The critical result of the PNNL model demonstrated the importance of leveraging wastewater-grown algae and other waste materials as a source material to achieve a price of $2.61 per gasoline gallon equivalent (GGE). Further empirical testing of product quality will be required for validation; initial testing of oil products for jet fuel has supported the model.

Several projects incorporated updates to the harmonized TEA in their work, especially as it relates to increased productivity, reduced system input requirements, and additional coproducts with higher market prices than commodities.

Training: The Algae Technology Educational Consortium (ATEC) remains the centerpiece of training the current workforce for not only biofuels production, but also biotechnology jobs more generally (1.3.5.201). ATEC's outreach, especially during the COVID-19 pandemic, has expanded to 41 schools and served more than 190,000 students via their course offerings, which include in-person coursework at universities and community colleges, internships, K–12 STEM education, internships, and massive open online courses (MOOCs). Their outreach to historically Black colleges and universities, Hispanic-serving institutions, Asian American and Native American Pacific Islander-serving institutions, and Alaska Native and Native Hawaiian-serving institutions has made biotech education accessible even in regions without algae cultivation operations, enabling workforce development and thereby providing a scientifically trained labor pool for employers as well as job opportunities for historically underserved groups.

RECOMMENDATIONS

Overall, the reviewers feel that the projects reviewed were well aligned with BETO goals and are being well managed. In general, projects are making excellent progress in advancing the state of algae technology development. Projects are moving cultivation and processing technology forward toward contribution to the displacement of petroleum-based products in a cost-effective, economically competitive manner. Projects are monitored actively, and the interaction of staff with investigators appears to be working well. Projects were adjusted appropriately in response to new data. We commend the good work and see no major corrections to the program needed. That said, we do have some minor corrections and comments on individual projects:

1. The focus on DAC of CO_2 to minimize reliance on transported CO_2 and/or collocation with flue gas production will be critical for making algae production for commodities and fuel economically feasible, enabling the use of inexpensive land and reducing logistics and process piping requirements for commercial-scale facilities.

2. There remain a few critical gaps between academic research and industrial implementation: contamination response and culture rescue, downstream processing of the various algae components for commercial sale, and a clear scale-up characterization that would bring a shorter path between the $n = 1$ facility design and the n^{th}-plant optimized version.

3. With respect to the contamination response, we would like to see increased understanding of contamination and the microbial flora associated with algae cultivation generally, standardized contamination response methods (which are common in industry), and more nuanced definitions of what constitutes acceptable versus unacceptable contamination levels, "crashing," and conditions that more subtly limit productivity in an outdoor pond environment.

4. Downstream processing of the algae components should also be given more in-depth consideration: While high-priced coproducts contribute toward a more economically feasible TEA, the volumes of those coproducts relative to their market size, in comparison to the demand for fuel, is unbalanced.

Further, downstream product TEA standardization could benefit the field by providing a true comparison of the different methods and their feasibility. In order to meet economic requirements, oil fractions were often diverted to other products rather than fuel, reducing renewable fuel production. Product quality and comparability to the current market products needs to be characterized to validate the economic viability of the processing steps.

5. At the very large scales representative of the ponds needed for commodity chemical and fuel production, we feel that more work around characterizing mixing and kLa could inform the scale-down and pilot models being used to represent at-scale processes. While modular systems such as the rotating biofilm reactors should be relatively straightforward to scale up, novel DAC methods and large pond mixing systems are unlikely to scale linearly and require testing at scale. Industry could support better scale-down work by releasing monitoring data or allowing sensors to collect data during both successful and unsuccessful cultivation in order to better understand what parameters constitute successful batches as opposed to the effects of normal environmental flora and algae stress events.

6. Another concern is the number of process patents generated being used as a metric of success. Process patents are notoriously challenging to enforce, and the addition of multiple unit operations for material processing seems likely to add significant expense and risk of process failure. Due to the lack of enforceability and the existing prior art associated with seed oil processing and fermentation product fractionation and recovery, the value of process patent development may not be a significant outcome of the research.

BETO's clear, comprehensive, and industry-focused goal setting overall has been highly successful in guiding industrial and academic labs to focus on critical roadblocks toward at-scale commercial production. The stepwise advancement of the program has created impressive advancements in the implementation of real-world economics to hypothetical models, validation of the models with field trials and empirical evidence, and viable products that can be used as drop-in replacements for petroleum-based chemistries. Scalability has progressed significantly over the past several years, and the new goals for SAF will continue to address practical real-world challenges.

ADVANCED ALGAL SYSTEMS PROGRAMMATIC RESPONSE

The 2023 review of the AAS Program owes its success in large part to the excellence and dedication of the diverse group of eight individuals who agreed to serve as independent peer reviewers. The AAS Program, as it continuously strives to hone its strategy and improve on the outcomes of its investments, is deeply indebted to their service and values the highly relevant and actionable recommendations that resulted from their efforts. From all at the AAS Program and BETO: Thank you reviewers!

The AAS Program is deeply grateful to the reviewers for their acknowledgment of DOE's "unwavering dedication" to advancing the state of the art for advanced algal systems. It is very heartening to hear that the efforts of the AAS Program to craft relevant, aggressive strategies to accelerate the commercialization of "sustainable and cost-effective algal production technologies" are recognized by the reviewers as a globally leading approach. In the AAS Program, because of our strong mission focus, we constantly seek to improve through disciplined and continuous evaluation of strategic direction and execution. Thus, we cannot thank the reviewers enough for holding the AAS Program up as a "testament to DOE's commitment to driving innovation."

This review cycle is particularly valuable to BETO as it undergoes a realignment of how it manages its algae and feedstocks work. Beginning in fiscal year (FY) 2023, BETO embarked on changes to move the AAS Program into a new program area entitled Renewable Carbon Resources and to integrate the management of the formerly separate AAS Program activities with the former Feedstock Technologies Program. This was done in recognition of the suite of available carbon resources that BETO is focusing on to meet decarbonization targets. Continuing the realignment of its strategy, BETO shifted a significant amount of FY 2023 AAS budget resources to its Scale-Up Program (Systems Development and Integration) for pre-pilot work on algal systems.

In 2023, the AAS Program presented its portfolio of projects in two parallel tracks, split roughly between the development of individual technologies or approaches to strain and cultivation improvement and the integration of multiple systems to improve productivity and cultivation performance. These two tracks, “Cultivation and Strain Development” and “Integration,” were reviewed by separate review panels even though the projects and strategy remain unified. This achieved the desired outcome of lessening the burden of the review on reviewers, staff, and presenters by shortening the in-person commitment from 5 days in 2019 to 2. Steps were taken to ensure that the separate panels of reviewers nonetheless received a complete picture of the AAS Program, its strategy, and management.

Although there are two separate Review Panel Summary Reports, the response here focuses on the top recommendations from each report in a unified response. The recommendations made by the panels serve as important guidance as BETO moves forward in its mission. Neither panel noted the need for major corrections to the AAS Program strategy or execution, but both did offer valuable recommendations for consideration going forward. The program offers a detailed response to the top recommendations here.

Recommendation 1: Further advancements in low-cost sustainable biofuel production from algal oil, including efforts on extremophile strains and increased focus on culture stability and contamination response

The recommendation to pursue further advancements in low-cost and sustainable algal biofuel production is one that the AAS Program is glad to take. While the AAS Program is pleased that the reviewers recognize the significant advancements in algal biofuel technology and progress in algae productivity made possible through the AAS Program’s efforts, we recognize that further development must take place to accelerate the commercialization of algal biofuel technologies. We agree with this recommendation and feel that it validates our program strategy in that further advancements are not only needed but are attainable through the continued execution of the AAS Program strategy. An initial step in this direction was taken by issuing a call for national laboratory projects focused on “Carbon-Negative Algal Biofuels” using FY 2024 requested funding that seeks to understand analytically and experimentally the feasibility of pathways that can leverage low-carbon-intensity products and/or environmental services to provide low-cost and low-global-warming-potential algal biomass for conversion to SAF. The recommendations to focus on strain improvement and diversification of strain selection while combining efforts on bioprospecting, strain evolution, and engineering with existing technologies is a valuable one and can be considered as the program continues to pursue increases in productivity.

The suggestion to consider a more targeted investigation of extremophile strains aligns well with the direction that the DISCOVR consortium is taking to rescreen strain libraries at higher pH and to continue to implement efforts to grow strains at high pH, salinity, and temperature. To further this area of development, the AAS Program released an FY 2023 funding opportunity for algal crop protection technologies and looks forward to announcing the selections by the end of FY 2023.

Recommendation 2: More consideration to downstream processing and conversion of algae components

While the AAS Program has historically dedicated the bulk of its focus to algae strain development, biomass production, and cultivation improvements, there has been a dedicated focus area on the interface between algae biomass and conversion. These efforts—directed primarily through engagement with relevant centers of core capabilities at NREL and PNNL for the low-temperature fractionation of biomass into constituents for conversion and upgrading and the high-temperature deconstruction and upgrading of whole biomass—have informed critical developments in the state of technology (SOT) for converting algal biomass and often serve as the basis for scale-up considerations by industrial partners. However, this area has not been a primary focus of the AAS Program's efforts, largely due to the challenges of matching the conversion process requirements for both continuity and fidelity of biomass production to the downstream process. Consequently, the challenge in providing consistent and adequate supplies of representative algae biomass for conversion process development has been great. However, as both the pathways to algae-based products and the ability to produce biomass in the desired composition and formats become more attainable due to the steady progress achieved in strain development and cultivation improvement, we are excited to explore more opportunities in this space, particularly in ways that demonstrate the potential for low-carbon-intensity fuels enabled by advantaged products and/or services.

Recommendation 3: Engage industry partners and address scale-up challenges

This recommendation is especially valuable to the AAS Program, and we are glad that the reviewers see the need for continued engagement with industry stakeholders. The suggestion to incorporate industry inputs into TEA and LCA development is especially valuable and is something the program has long considered. Another consideration that the program made in FY 2023 that was not reviewed by this set of reviewers was the allocation of $15 million in the FY 2022 and FY 2023 budgets away from the Research and Development (R&D) Program and toward the Systems Development and Integration Program. This allowed the selection of five pre-pilot algae biofuel projects through the FY 2022 Scale-Up FOA. Scale-up projects, even at the pre-pilot scale, are an important progression from R&D activities because they allow for the construction and operation of unit operations that can begin to provide the necessary engineering data to de-risk further scale-up and integration of technologies. This suggestion is very aligned with BETO's commitment to continue seeking partners for pre-pilot, pilot, and demonstration projects for algal biofuels.

In conclusion, the AAS Program is grateful for the opportunity to work with the 2023 independent peer reviewers and appreciates the overall sentiment that the continued funding of the AAS Program strategy will contribute to BETO's overall goals for biomass production and conversion. With the recommendations summarized above in hand, BETO will continue to pursue strategies to move the state of the art forward for algal systems. BETO looks forward to presenting the results of its efforts to accelerate the advancement of algal system technology development and deployment to the public once again in the 2025 Peer Review.

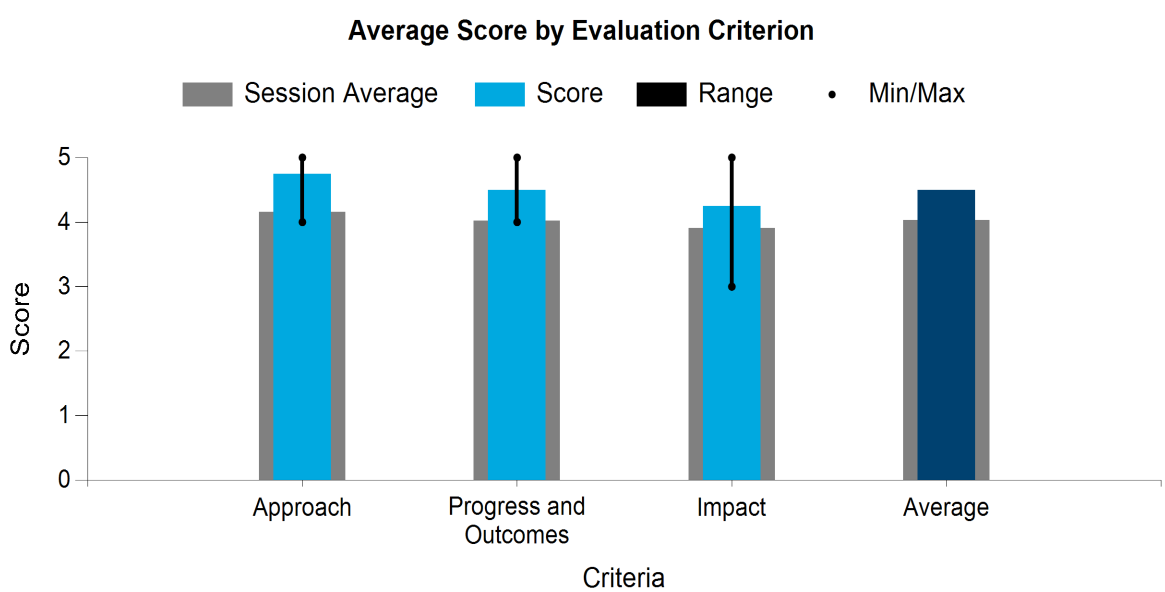
ALGAE BIOTECHNOLOGY PARTNERSHIP

National Renewable Energy Laboratory, Los Alamos National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.1.131
Presenter(s):	Michael Guarnieri
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$2,550,000

Development of advanced genetic and phenotyping tools will be integral to achieving BETO algal biomass productivity, composition, and cost targets. However, despite recent advances, algal genetic engineering pursuits remain largely limited to "one gene at a time" targeting and analysis, and broad host-range tools are generally lacking, hindering the development timeline for newly emerging strains. Establishment of genome-scale, high-throughput genetic and phenotyping tools represents a key technical hurdle that must be overcome to realize the full potential of microalgae as an economically viable feedstock. To this end, the Algae Biotechnology Partnership proposes to develop high-throughput tools to generate and phenotype genome-scale libraries of an industrially relevant microalgae. The resultant pipeline will enable full characterization of the genetic basis of photosynthesis, carbon storage, and nitrogen metabolism to improve algal biomass composition and productivity. Key challenges currently being targeted include high-efficiency transformation and genome editing and high-throughput and automated adaptation of established phenotyping methodologies. To date, we have demonstrated tool transferability across multiple top-candidate deployment strains and initiated generation of a genome-scale *Picochlorum renovo* knockout library, laying the foundation for first-in-class tools for basic and applied research in deployment-relevant algae.



COMMENTS

- This project focuses on developing a high-throughput and automated pipeline for screening and genetic engineering tools to accelerate improvement in industrial strains of algae and cyanobacteria. The project is of clear relevance to BETO's Multi-Year Program Plan goals. An additional goal was to generate genome-scale libraries for *P. renovo* that will be publicly accessible.

- The project performers seem to have clear management plan while leveraging expertise from the different investigators. The team provided a project management, communication, and collaboration plan. Risk and challenges involved in the execution of this project were identified, and mitigation strategies were outlined as major milestones. The team also outlined an approach to addressing diversity, equity, and inclusion (DEI) in their project plan.
- The team has developed an optimized transformation and cryopreservation procedure for 96 well plate, next-gen barcode sequencing to enable library validation, as well as an established CRISPR system for targeted genome editing. These tools have also been used to enable platform expansion across multiple DOE offices. The team has made significant progress in 18 months and seems to be on track to achieving their goals. One area to be considered is ensuring that phenotyping and characterization methodologies are performed under simulated outdoor conditions to increase the chance of success. It's also unclear if these strategies will result in strains with improved biomass productivity that can be commercially deployed. Overall, the project seeks to address key research needs stated in BETO's Multi-Year Program Plan with the goal of developing strain improvement toolkits and technologies for improved biomass productivity.
- The project has made very good progress on strain engineering via high-throughput-capable genetic tool development, and it integrates well into BETO's goal for achieving strains with high mass productivity that are cost efficient and have carbon storage capacity. The team has established a partnership with three other national labs and an academic institution, all working together in a very cohesive environment with a high level of productivity. A major milestone has been reached for FY 2022 by achieving >5x transformation efficiency enhancement in *P. renovo*, exceeding 100 colony forming units (CFU)/µg DNA, as stated in the report. Another task focuses on delivering a publicly accessible, genome-scale mutagenic library of *Picochlorum* spp., and it has made significant progress despite some obstacles. The next task on phenotyping and characterization has been initiated by the team for *P. renovo* genome-scale mutant libraries. The team has addressed the DEI component in their project and remained on task, and one can only predict that the project will be successful to meet BETO's goal on producing sustainable, cost-effective algal biofuel. Data on how the engineered *P. renovo* is faring on open raceway ponds were not presented. Has any effort been made to design genetically engineered strains with resistance to predation? The team has effectively dispersed their research findings through several publications, patents, and presentations.
- This project appears to be managed well and involves teams with appropriate expertise and backgrounds to achieve the project goals. They have a DEI component even though it was not required at the time of funding. The project aims to develop/deliver a toolkit that can transform any algae strain. The work started with a top production strain (*Picochlorum* sp.), chosen due to its high productivity and doubling time. They have so far demonstrated the transformation toolkit on 12 strains, most of which are BETO relevant, but appear confident that it will work for any strain going forward (within 1–4-week turnaround time). Mutants are screened based on lipid and carbohydrate production but could include other targets like pigment production, supporting both fuel and coproduct pathways. Several mutants have already been grown outside at larger scale. The team appears to have met, and in some cases exceeded, their project goals.
- The project team is focused on delivering universal, high-throughput-capable genetic engineering tools. The approach uses the latest genetic engineering techniques to improve strains, and the project aligns well with helping BETO achieve biomass targets. The project appears to be on schedule, and appropriate risk mitigation strategies have been employed. Mutant libraries and high-throughput phenotyping methodologies for two *Picochlorum* strains have been successfully developed to date; thus, the team has made appropriate progress toward project goals. The wider impact of the work is difficult to assess given the focus on only *Picochlorum* strains; thus, it is recommended that the project team test the developed

tools on other industrially relevant microalgae species before the end of the project. The project team has made the strains and DNA sequences publicly available, and the developed tools should aid microalgae commercialization efforts.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their positive and constructive feedback. The project team shares the reviewers' enthusiasm regarding progress to date; we have made substantial progress in strain engineering and phenotyping, achieving all project milestones to date, and we are on track to achieve end-project goals. Regarding performance under simulated outdoor conditions to increase the chance of success, we agree with the reviewer's suggestion; all strain validation will be performed under diel light and temperature cycling to reflect outdoor deployment at the AzCATI test bed. Regarding the likelihood that our proposed strategies will result in strains with improved biomass productivity that can be commercially deployed, we note that the overarching goal of the project is to establish enabling capabilities to rapidly generate and characterize strains with an array of phenotypes, including, but not limited to, altered carbon storage flux to lipids and/or carbohydrate, growth rate, and pigment content. It is our expectation that the resultant mutant library generated from our work scope will result in a subset of mutants with enhanced deployment properties. Importantly, the resultant tools emerging from this project will provide an invaluable resource to the algal R&D community, providing a blueprint for high-throughput strain engineering and phenotyping, as well as a *Picochlorum* genome-scale library, representing a first-in-class tool for a non-model, deployment-relevant microalga. Regarding strain performance outdoors, to date we have deployed our wild-type cultivar, which achieved >20-g/m^2/day outdoor productivity at the AzCATI test bed. Though deployment is outside the scope of the current project, we have a series of active complementary projects focused on strain deployment, including development of phosphite-mediated cultivation, which present a promising crop protection and predation resistance strategy. Regarding the wider impact of the work beyond *Picochlorum* strains, we note that we have demonstrated our genetic toolbox efficacy in a series of top-candidate BETO DISCOVR deployment strains. It is our expectation that the tools developed herein will be readily transferable to other industrially relevant cultivars as they come online. To this end, parallel efforts are already underway to incorporate *Scenedesmus* sp. 46B-D3, a representative industrial high-storage carbon strain, into our development pipeline. Efforts to date have established genetic transformation capabilities therein, using the genetic toolbox developed in our initial period of performance, and phenotyping during diel cycling, including ash-free fry weight, carbohydrate, lipid, and proteins.

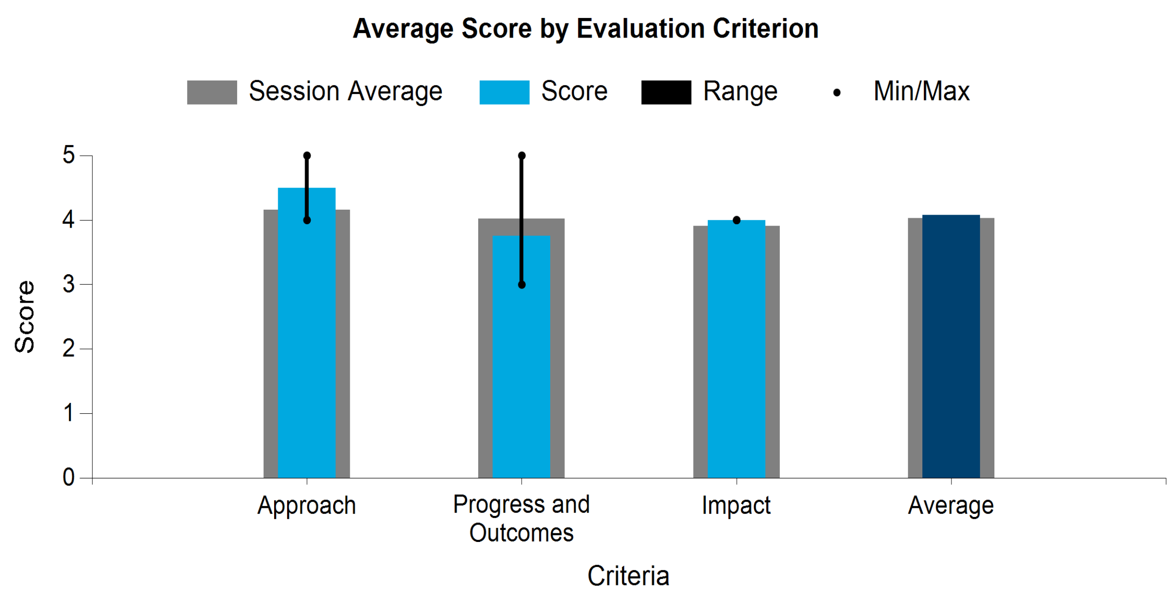
ALGAE DISCOVR

Pacific Northwest National Laboratory, Los Alamos National Laboratory, Sandia National Laboratories, National Renewable Energy Laboratory, Arizona State University, Colorado School of Mines

PROJECT DESCRIPTION

WBS:	1.3.1.501
Presenter(s):	Michael Huesemann
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2022
Total Funding:	$8,768,025

To meet the DOE 2030 annual productivity target of 25 g/m^2/day, the DISCOVR consortium—a collaboration between PNNL, LANL, NREL, Sandia National Laboratories, Colorado School of Mines, and the AzCATI test bed at Arizona State University—was tasked with identifying and testing new high-productivity algae strains and finding ways to further improve biomass productivity during cultivation, enhance biomass value, and increase crop protection and culture stability. Major accomplishments include successful utilization of the DISCOVR strain downselection pipeline, culminating in the top seven strains being deployed in outdoor growth trials at AzCATI. These were selected from a comparative study of temperature and salinity tolerance of 42 strains, followed by productivity assessment of 25 strains under climate-simulated conditions, leading to 13 strains cultivated in outdoor ponds. Adding growth-promoting molecules, reducing oxygen inhibition, and using luminostat control each increased productivity by >20% relative to baseline productivity. New methods were developed to simulate outdoor ponds in photobioreactors (PBRs) and to predict and prevent pond crashes using spectroradiometric monitoring and integrated pest management. A concerted effort in year-round cultivation trials led to a 60% increase in annual biomass productivity since 2018 (11.7 to 18.5 g/m^2/day) when strains from the DISCOVR pipeline started to be deployed in SOT trials, equivalent to a reduction in biomass selling price of 27%, from $824/ton to $602/ton.



COMMENTS

- This project focuses on reducing total microalgae biofuels production cost by applying an integrated screening platform to identify highly productive strains, testing new concepts to improve productivity and culture stability, and using TEA to identify economically feasible concepts to test out on ponds. The expected outcome is to reach the BETO 2030 target of $488/ton years ahead of schedule. Their goals and approach are reasonable.

- The team had a clear management plan with a well-defined task structure and leads. They identified risks and implemented mitigation strategies, as well as establishing channels of communication and collaboration among team members. The also outlined go/no-go decision points and milestones that were all met. The team is composed of six national labs and two institutions.
- The team was successful in selecting seven top strains for SOT trials that resulted in a 60% increase in the SOT productivity to 18.5 g/m^2/day and a 27% decrease in minimum biomass selling price (MBSP) to $600/ton. The project made significant progress toward increasing productivity and stability in outdoor ponds. The project had a good balance between lab-scale and outdoor testing, which is crucial to demonstrate the feasibility of the proposed technology and commercial deployment of the technology. From the strain selection pipeline, there seems to be no improvement in productivity since 2020. The project performers should consider diversifying their strain selection criteria and integrating strain improvement technologies and toolkits. For instance, the team should consider integrating previous projects that have demonstrated glycogen knockdown and deletion on a sucrose phosphate synthase enzyme that showed improved photosynthetic oxygen evolution and biomass productivity.
- The team demonstrated improved growth in flask and outdoor simulated environmental PBRs using indole-3-acetic acid (IAA) and suggest reduction in MBSP with IAA. It's unclear how stable IAA will be in outdoor conditions and the impact of contaminant and ecological diversity on the performance of IAA. If a higher concentration and multiple feeds of IAA are required for outdoor cultivation, the cost impact may be detrimental.
- OptiLum operations showed a significant improvement in biomass productivity and a reduction in cost of about 22%–38%. It's unclear if the hydraulic load and cost of pumping associated with a semicontinuous process was considered and if the project performers are considering recycling the water. Significant progress has also been made in the area of crop protection using fungicides and saline strains that have shown more than 60% improvement in productivity. The use of algal bacterial co-cultures showed promise, but concerns of bacteria overtaking the pond and competing with nutrients should be considered, as well as the overall impact on biomass productivity/quality and downstream processing.
- Plans for the next 3-year cycle include demonstrating 70% CO_2 utilization efficiency, achieving mean time before failure target of 20 days, and attaining annual biomass productivity of 20 g/m^2/day. It's not clear what approach the team intends to use to achieve the goals.
- Overall, significant progress has been made in selecting high-producing strains for the different seasons, deploying a biomass improvement and crop protection strategy. The team has demonstrated productivity improvement in the AzCATI outdoor algae cultivation and leveraged a TEA model to identify key cost drivers. One area the team can focus on is commercial deployment of these strategies and collaboration with industrial partners.
- The team has made significant progress in their effort to achieve the goals outlined in the DISCOVR project. Through uninterrupted cohesive interactions, communications, synergy, and productive coordination by employing a structured, tiered assessment process, the team has been successful in identifying seven algal strains that are currently under pond SOT trails at the AzCATI test bed. The DISCOVR project advanced through inputs and knowledge sharing with the community, as well as researchers in other BETO projects. The consortium uses their core capabilities to actively collaborate with algae industries, academia, and a culture collection center. They have successfully delivered their outputs in several areas involving data collection, SOT, and pest resilience of cultures, as well as to

downstream projects related to genome sequencing, multi-omics, molecular toolboxes, biomass, and nutrient cycling. The stream of continuous feedback data facilitates DISCOVR team researchers adding in new strains to the pipeline as the project progresses. DISCOVR's productivity data seem to be on track and successful in keeping up with BETO's target 25-g/m^2/day annual average by 2030. The impact of the DISCOVR project is displayed by the product that the team has to offer to the community from their last 3-year effort. The team has made a remarkable contribution to addressing some of the major challenges and issues related to algae-derived biofuel cost, productivity, culture resilience, and biochemical composition. DISCOVR has been active in sharing their research findings through extensive publications in peer-reviewed journals and partnerships with industries and academia. The team has made a significant effort to diversify and democratize their gained knowledge about algae cultivation in every possible way that will benefit society and provide solutions to challenges. One aspect that seemed to be missing was the data for any specifically engineered pest repellent strain, which would be an important area to explore and consider for crop protection considering the vastness of expertise in the team. If one has been employed, the emphasis on it was not clearly mentioned in the report.

- As one of BETO's flagship projects, this consortium has generated many technologies and a huge amount of data useful to inform algal production. The management team seems well put together, and no communications issues are obvious. The work also benefits from a third-party advisory board that was recently reshuffled to be more scale-up focused. Importantly, DISCOVR has so many entities involved in the effort with a high degree of outreach to the academic and national lab sectors that there is high potential for future algae leaders to emerge from this effort. They also appear to have a DEI component even though this was not a requirement of the project. However, after 9 years of work, it is disappointing that more formal connections with industry have not been made or spun off from the project. The leveling off of MBSP over the last several years is also concerning, as there was a steady decrease over the first 5–6 years. The presenter stated that the initial decreases in MBSP were due to new strains coming on board that were highly robust and productive (namely *Picochlorum*). However, the conclusions were that they had exhausted the available strains and instead would address the leveling off with OptiLum technology and growth-promoting molecules. Neither of these have been validated enough to be confident of the economic feasibility or the magnitude of the effect on productivity. Growth-promoting molecules could be promising because they are used so widely in traditional agriculture, but work still needs to be done to make sure they remain stable in outside pond conditions and, if needed, multiple doses are still economically feasible. This poses a lackluster end to an otherwise strong effort. From the 2021 report, there seemed to be discussion of opening up the DISCOVR pipeline and outdoor testing to engineered strains, which could be an important tool to target MBSP, but that was not mentioned this year to my knowledge. Perhaps a stronger industry tie would uncover more strains (improved or otherwise) to test. Lastly, handoff of the technologies developed during this consortium would be more impactful to a future industry if the project included an outdoor validation step at multiple geographies, although this would be a much larger undertaking and likely outside the scope of BETO funding budgets.

- DISCOVR serves a valuable role in validating and testing collected strains in their strain pipeline for BETO. DISCOVR made impressive productivity and MBSP gains in 2019 and 2020; however, minimal gains have been realized over the last 2 years. The collection of additional *Picochlorum* strains from the Gulf of Mexico is encouraging, but increased efforts should be made to identify new strains to introduce into the DISCOVR pipeline. The management plan is clear, and the project team seems to communicate effectively, even given its large size. Future work might be directed to the isolation of high-lipid, alkali-tolerant strains for outdoor cultivation given the success of other projects in the BETO portfolio in regard to pond crashes.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewers' comments and the opportunity to present a brief response to the major points raised. Regarding the need to identify new strains for introduction into the DISCOVR pipeline: As the DISCOVR project is now shifting its focus on improving CO_2 utilization efficiency at a higher pH, we will "reopen" the strain screening pipeline to identify strains that meet the new requirements. Building on the success of previous BETO-funded projects, PNNL established a collection of strains that are promising for high-pH cultivation. All the strains will be evaluated and downselected following the original DISCOVR pipeline concept but with new criteria (e.g., high pH). Furthermore, we are evaluating additional novel unique species from bioprospecting efforts beyond the focus on *Picochlorum* species. Since February 2023, additional cultivars have been obtained from bioprospecting in the Gulf of Mexico. *Picochlorum* persistently dominates most grow-outs from this area, but several diatoms and an *Ostreococcus*-like alga have also been isolated, which may be added to the DISCOVR pipeline if warranted after additional productivity testing. Two separate sampling trips have also been conducted at high-pH and saline lakes across the Western United States, as well as on the Pacific Coast. We hypothesize that sampling from a geographically diverse set of locations with high-pH waters will yield alternative species or strains with higher pH tolerance for competitive carbon use efficiencies. In addition to improved growth at higher pH, we are targeting new strains with different biomass compositions relative to *Picochlorum* species, specifically strains with a higher lipid content, which will improve MBSP. These newly identified strains will be tested in parts or all of the DISCOVR pipeline. Finally, we are continuing to identify new strains from culture collections or from prior successful cultivation efforts in other projects. One genus that stands out is *Tetraselmis*, which is currently one of the marine SOT test bed strains. *Tetraselmis* is the only genus to have exceeded, on an extended cultivation basis, the outdoor pond productivity values obtained by *Picochlorum*. Importantly, many strains in this group are significantly easier to harvest than *Picochlorum*, with associated reductions in MBSP. Regarding commercial deployment of DISCOVR strains and concepts and collaboration with industrial partners: We agree with the reviewers that collaboration with industrial partners is highly beneficial and should be pursued to the maximum extent possible. In the past, we collaborated with ExxonMobil (they provided the top summer season strain *Picochlorum celeri*) and Algenol. As part of our DISCOVR call for collaboration, we evaluated several crop protection compounds from Aequor. Given that DISCOVR team members already have connections to other companies, such as Heliae, Qualitas, Viridos, GAI, and Chevron, we will build on these to create potential collaborations with the DISCOVR project. We are also leveraging the technical advisory board in quarterly all-hands review meetings of planned research. This is an established framework that we can build on to specifically solicit feedback on challenges experienced by industry that the R&D tasks could better align with. Although our technical advisory board has numerous members from industry (Lumen, Qualitas, Umaro Foods, and MicroBio Engineering) that we can reach out to for collaborative opportunities, we will consider adding several new industry members (e.g., Chevron) next year to facilitate the offtake of microalgal biomass to SAF. Finally, we will keep the "call for collaboration" open to engage with industry. Regarding the stability and cost of the growth-promoting molecule IAA: We agree with the reviewer's comment that we have not yet analyzed the stability of IAA in our experiments. However, IAA has been used successfully at scale under outdoor agriculture conditions and in our experiments with outdoor mimicking scripts (high light intensity). IAA is effective at very low concentrations (1 µM) and cost-effective (inexpensive) at bulk price. Also, to address the issue of cost-effective supply of IAA, we are developing an algae:bacteria co-culture system to supply bacteria-produced IAA and make IAA supplementation sustainable and cost-effective. Regarding the cost of pumping associated with OptiLum operations: Changes in water movement due to lower harvested densities using OptiLum-based cultivations are effectively captured by the NREL Algae Farm Model, which translates them to compatible variations in the costs of pumping and dewatering operations. Recycling the spent medium from dewatering operations back to cultivation ponds is a longstanding assumption of SOT assessments in the BETO portfolio and, while not actively researched in the DISCOVR consortium, this consideration could be revisited whenever further experimental data are available from partner BETO projects. Regarding the concern that bacteria could overtake the pond and reduce productivity when using algal bacterial co-cultures for crop protection: It should be noted that bacteria are already present in any and all open-pond production systems. Algae and bacteria have coexisted and influenced ecosystems with all modes of interactions—from mutualism to parasitism. The bacteria used in this project are derived from ponds where they were already present and were determined to have no negative impact on productivity prior to our use. Because outdoor ponds are being operated under photoautotrophic conditions, as opposed to mixotrophic conditions, there is very low risk of the bacteria overtaking production ponds or significantly competing for nutrients. Regarding the approach to meeting the next 3-year cycle goals for CO_2 utilization efficiency, mean time before failure, and annual biomass productivity: Although per BETO guidance, presenting plans for future research was not required, we provided 19 slides in the supplemental section outlining the objective, approach, expected outcomes, and impacts of 19 different subtasks that will be carried out during this 3-year cycle. Here is a short overview of the six different tasks that will be executed during the current 3-year cycle: Task 1 focuses on improving biomass productivities via (a) OptiLum and Turbidostat culture control, (b) addition of growth-promoting bacteria, and (c) evaluation of seaweed pond culture. Task 2 is aimed at improving CO_2 utilization efficiency by (a) minimizing CO_2 outgassing via cultivation at air/water equilibrium pH; (b) optimizing the medium water chemistry; (c) isolating high-lipid, alkali-tolerant strains; and (d) optimizing CO_2 utilization efficiency in large outdoor ponds. Task 3 centers around biomass quality tracking and optimization and addresses the development of (a) high-throughput biomass compositional analysis, (b) valorization of protein, and (c) preservation of biomass intrinsic value. Task 4 focuses on increasing crop protection and culture stability by evaluating (a) companion cultures, (b) radiospectrometric monitoring, (c) biomarker discovery and assay deployment, and (c) crowd-sourced agent characterization. Task 5 involves performing outdoor cultivation trials at the BETO SOT test bed at AzCATI. Task 6 focuses on carrying out techno-economic and life cycle analyses in support of DISCOVR tasks. Regarding missing data on specifically engineered pest-repellent strains for crop protection: Engineering of pest-resistant strains is dependent on *a priori* knowledge of mechanisms of resistance, including the genetic basis for those mechanisms. To date, very few resistance mechanisms have been characterized to the degree needed for precise engineering of algal strains of interest. At this point in the development of crop protection strategies, it is far more important to add to our knowledge base of resistance mechanisms than to focus our efforts on generation of new genetically modified strains. However, armed with sufficient understanding of pathogen resistance mechanisms, we do foresee our results providing fundamental knowledge that will guide informed biodesign of resistance engineering targets for algal production strains. Regarding the lack of validation of OptiLum operations and use of growth-promoting molecules: Because of limitations in budgets and resources, we had to carry out initial proof-of-concept OptiLum research in bench-scale indoor PBRs. We fully agree that further validation is necessary. Therefore, we have plans to evaluate the performance of OptiLum operations in indoor and outdoor raceway ponds, including at the SOT test bed at AzCATI. Regarding the validation of growth-promoting molecules, we are shifting toward algae:bacteria co-culture methods wherein the bacteria produce the growth-promoting molecules. We believe this will be a more sustainable and cost-effective approach than applying growth-promoting molecules directly. We plan to test this algae:bacteria co-culture approach in outdoor ponds in Year 3. While we may not expect the step change in productivity that we observed in early years, the focus of this current 3-year cycle is to establish a strong foundation in operational conditions (OptiLum, Turbidostat, growth-promoting molecules) to position us to help scale improved strains in a future effort. Regarding the lack of testing of genetically engineered strains: The evaluation of genetically modified strains was repeatedly discussed over the years. It was decided to evaluate only wild-type cultivars to simplify outdoor cultivation trials—i.e., avoid regulatory permitting for many different genetically modified strains. Instead of considering genetically modified strains as a solution to achieve higher productivity and stability, we are using a pond ecology and companion culture approach to achieve these goals in an environmentally compatible manner with extremely low or zero risk. Regarding outdoor testing at multiple geographies: We currently have two test bed sites (i.e., the PNNL Algae Testbed and the SOT Testbed at AzCATI) at different geographies, although both are located in Arizona due to favorable climatic conditions for outdoor pond cultivation. We agree that outdoor cultivation at multiple geographies, particularly in different climates, would be beneficial. Consequently, several years ago, we identified a potential second SOT test bed site at Texas AgriLife in Corpus Christi, Texas, located at the Gulf of Mexico. We also considered a potential test bed location in Miami, Florida. However, due to limited BETO funding, we have been unable so far to proceed with plans to conduct outdoor pond trials at additional geographies. We are investigating opportunities to bring additional test beds online in the future to cover a more diverse climate spectrum, provided funding is available for training and deployment.

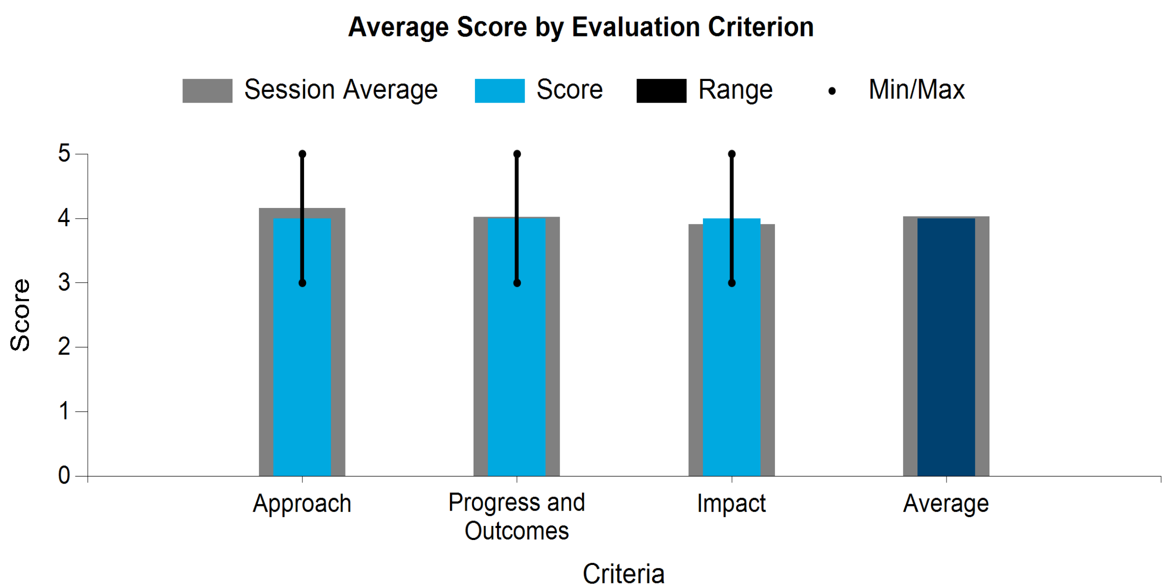
DIRECT AIR CAPTURE INTEGRATION WITH ALGAE CARBON BIOCATALYSIS

Arizona State University

PROJECT DESCRIPTION

WBS:	1.3.1.670
Presenter(s):	John McGowen
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$4,000,000

This project will deliver a full experimental integration of DAC technologies with novel algae strains—derived from the genetic engineering of *Scenedesmus* (or *Acutodesmus*) *obliquus* (UTEX393). Both of these are more resilient than the wild-type strain and increase annual productivity by at least 20% while maintaining the quality of the biomass for conversion to fuels and products. This novel project aims to open the field for DAC integration with increased photosynthetic carbon capture and storage biocatalysis by addressing key barriers that currently limit overall process efficiencies, accompanied by a cost and sustainability assessment. The collaborative team of scientists at Arizona State University and NREL, supported by DAC partner Silicon Kingdom Holdings, will build on years of research in both algae cultivation and central carbon metabolic engineering that have pushed the boundaries of increasing and controlling biomass productivity and quality. This team is uniquely positioned to both push the frontier of algae engineering and directly align new technologies with ongoing multiyear SOT outdoor production trials. This project presents opportunities for techno-economic and sustainability analysis for DAC technologies, supported by Silicon Kingdom Holdings partners. A critical contribution of this project is the proposed installation of a MechanicalTree™ at AzCATI, allowing for the unique and seamless integration of biological and chemical carbon capture technologies.



COMMENTS:

- This project proposes a technology that uncouples algae production from collocation with point sources of waste CO_2, opening options for siting of algae cultivation. The project performers allowed three approaches: (1) integration of passive DAC into algae cultivation using innovative carbon delivery and enzyme catalysis, (2) engineering of the UTEX393 strain for carbon flux control, and (3) pond design for improved time to failure and overall productivity. This goal is of clear relevance to BETO's mission and Multi-Year Program Plan goals. Successful execution will reduce the cost of production and eliminate the constraints of siting algal production systems close to industrial CO_2 supply.

- The team did not outline a management plan but had a well-defined task structure and leads. They identified risks and outlined mitigation strategies. The clearly defined their baselines for cultivation performance targets with go/no-go decision points.

- The team proposes to use a commercial-scale demo unit for passive DAC that will be integrated with the AzCATI test bed and novel membrane-based CO_2 delivery in combination with an enzymatic biocatalysis unit. It's unclear how the cost of this deployment will impact biomass production cost and whether the benefits outweigh the cost. The impact of environmental conditions such as wind and dust on passive DAC performance should be considered for cost analysis. The approach the project performers plan to use to engineer UTEX393 for carbon flux control is also unclear. Overall, not a lot of progress has been made toward the goals outlined due to delays in contracting.

- This proposal aims to design a modeled, integrated, passive DAC for outdoor algae cultivation that will encompass innovative carbon delivery and enzyme biocatalysis application by strain engineering to provide a sustainable and economical pond system. The team had the initial success of setting up the commercial-scale MechanicalTree, which is being tested to optimize its operation and shows promising results; it has achieved 50% carbon utilization efficiency with the coupling of the membrane technology. The operation of the membrane technology was not very well explained and was difficult to understand. The continuous monitoring of the pond health and the microbial profiling work have provided significant data for crop management, which is a challenge for open-pond production. The task of metabolic engineering of strain UTEX393 showed improved photosynthetic carbon assimilation. The AzCATI team has made good progress in all the defined areas as stated in their project goals and is currently on schedule.

- This project is led by a comprehensive team and plan. The management structure/communication appears to be clear and includes industry partner(s) involved in developing/deploying the technology. There is a high risk that the engineered strain will be more susceptible to pathogens, and in that case, the team will rely on mitigation strategies already in place or switch to a different strain in order to meet their goals. The technology being developed is very interesting and potentially highly valuable in utilizing DAC to grow algae. The tree technology raises concerns about being too complicated to operate long term, and it is hard to imagine it will be economically feasible at the scale needed for algae biofuel and in the environments where scale-up will be feasible (i.e., dusty non-arable land). Feasibility would be increased if the team had an algae producer interested in partnering, or at least demonstrating the technology at a small scale. It will be important to have a well-worked-out TEA for this technology that can be validated.

- The passive DAC technology seems innovative and avoids having to collocate the algae cultivation near a waste CO_2 source. Appropriate risk mitigation strategies have been identified. The project passed initial verification in January 2022, but the project wasn't awarded until October 2022. Some preliminary results have been achieved, and progress does seem to be accelerating. Some technical detail was provided for the mechanical tree that inspires more confidence in the project. Uncoupling algae farming from point source CO_2 with the novel DAC-sourced carbon would have a significant impact on large-scale algae cultivation.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their time, thoughtful comments, and constructive criticism. With respect to the perceived technology complexity for the mechanical tree, we feel the opposite is true, and this passive DAC has the potential for massive scaling. In fact, the targets for our partner Carbon Collect are scales of CO_2 removal necessary for sequestration, not just reuse. The main goal with respect to the modeled integration of this DAC technology with algae cultivation is to perform a thorough TEA and LCA at different farm scales to assess feasibility for algae cultivation and allow Carbon Collect to assess the commercial potential for algae cultivation as an early target market for their technology. To ensure maximum impact, we are talking with algae cultivation companies and are actively pursuing additional funding opportunities that would allow for the next stage of actual integration into an algae farm. Having access to a commercial-scale, technology readiness level 6 demonstrator of the passive DAC technology, operating outdoors in an arid, dusty environment, is to gain the necessary data and understand the actual performance. Different sorbents and operation modes are being developed to allow for multiple deployment environments, and Carbon Collect, outside the scope of this project, is working to understand how those factors affect performance.

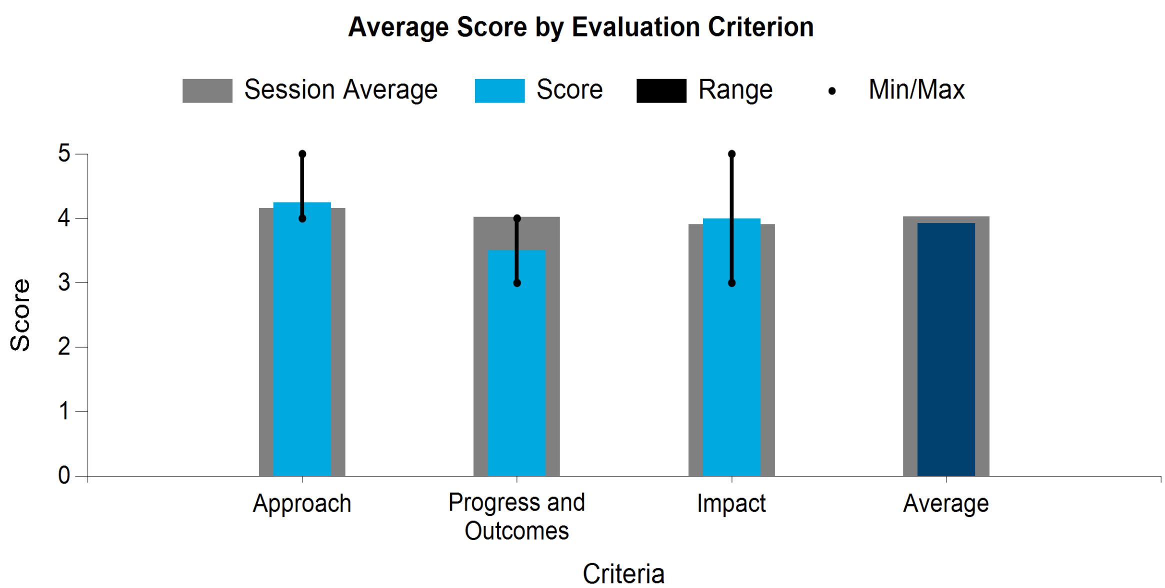
ENHANCED PRODUCTION OF ALGAE LIPIDS AND CARBOHYDRATES FOR FUEL AND POLYURETHANE PRECURSORS

University of California, San Diego

PROJECT DESCRIPTION

WBS:	1.3.1.672
Presenter(s):	Stephen Mayfield
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$4,000,000

In this project, we are combining genetic engineering, traditional breeding, and high-throughput screening to generate high-quality biomass for the production of fuels and high-value PU coproducts. We first identified extremophile strains of one green algae and one cyanobacteria and characterized these strains for the accumulation of naturally occurring polyurethane precursors (PUPs). We have translated an entire suite of previously developed genetic tools to these commercial strains and used *in vitro* evolution to evolve strains for improved biomass productivity in high-salt and high-pH media, as well as increased PUP production. We are now using synthetic biology to engineer metabolic pathways to optimize the production of fuels and PUPs in these strains. We have already started developing the chemical methods to convert these PUPs into fuels and PU monomers, including novel PUs containing aromatic polyols. In collaboration with our key industrial partner, Algenesis, we have used these novel polyols to make commercially relevant PU products and determined their physical specifications, including an ability to biodegrade. Finally, with the Kendall lab at the University of California, Davis, we have started TEA and LCA to model the impacts of producing high-value PU coproducts on the economics of fuel production in algae.



COMMENTS

- The goal of this project is to increase productivity by 20% over their baseline productivity using strain and/or cultivation improvement approaches under environmentally simulated and outdoor conditions. The proposed production of high-value PUP as a coproduct will drive biomass production cost down. This is in line with BETO's goals of increasing productivity, reducing biomass production cost, and developing strain improvement toolkits. The use of highly productive extremophiles will enable cultivation under high pH and high salt concentration.

- The team seems to be a good mix of research and industry partners. The team had a well-defined task structure that will ensure success of the proposed work; however, they did not address any risk or mitigation strategies. The team management did not demonstrate a well-established channel of communication and collaboration among team members.
- Progress has been made in the team's multipronged approach to strain improvement and culturing in high salinity to reduce contamination. They have demonstrated their ability to cultivate and genetically modify extremophilic cyanobacteria and green algae, which is incredibly valuable across the industry. It's unclear whether there are impacts of genetic modification on biomass productivity and what the project performers are doing to address this. The project performers should also consider outdoor performance of PCC 11901 in the spring and fall, when temperature and light are not optimal for growth and productivity.
- The project has explored in depth the usefulness of extremophile algae with strong reasoning and has been successful in identifying a halotolerant species that was genetically transformed, bred, and produced a robust strain with improved characteristics. The project has delivered PUPs from this extremophile algae via chemical means that have been used to produce algae-based novel PU foam products. This is highly commendable. The PI mentioned very abruptly that the rest of the biomass left after PUP extraction will contribute to biofuel production, and not much information was given in this regard, indicating a probable shift of focus for the project. There are two project partners, and how often the team meets to plan and discuss progress was not mentioned. How the concept of DEI will be addressed in the project was also not mentioned.
- This project aims to demonstrate the use of an engineered extremophile alga (*Chlamydomonas*) for fuel and coproducts. The major focus presented was for PUPs, which can be converted to fuels and/or bioplastics. This project has an industry partnership with Algenesis, which specializes in biodegradable plastics. Although this is an exciting and promising space for algae, this does not appear to be geared toward fuel production, and no algae grow-out demonstrations are involved in the project. The presenter does make a compelling case for growing extremophile algae for coproducts in terms of the media facilitating reduced pests and increased DAC potential. This begs the question of why extremophiles are not more prevalent in the industry, another reason why a grow-out component would be highly beneficial for this project. The team's genetic engineering work is also very exciting. However, this project does not appear to include an effort to advance scale-up/cultivation technologies, and it is unclear how it advances the BETO goals for this funding cycle. Otherwise, the technologies described here are poised to add considerable value to commercializing algae.
- The approach of focusing on high-pH extremophiles and high salt tolerance seems appropriate. Furthermore, the production of high-value coproducts such as PUP is also worthwhile; however, the current project plan seems to lack a credible effort to produce some type of fuel. The project team is making good progress according to their project workplan and seems to be on track to complete project objectives. The genetic tool development in this project is strong and should be useful for engineering other extremophiles. The impact of the high-value PU coproduct would be significant; however, it is not clear if the remaining biomass could convert into useful fuels to meet BETO targets.

PI RESPONSE TO REVIEWER COMMENTS

- Response to reviewer comments (response after reviewer comment): The project performers should also consider outdoor performance of PCC 11901 in the spring and fall, when temperature and light are not optimal for growth and productivity. We are downselecting to the extremophile algae strain for continued optimization, and for that strain, we will examine growth rates in the fall and spring.

- The PI mentioned very abruptly that the rest of the biomass left after PUP extraction will contribute to biofuel production, and not much information was given in this regard, indicating a probable shift of focus for the project. After PUP extraction (starch and 3HP), we will do a complete lipid extraction and use that material to make SAF.
- There are two project partners, and how often the team meets to plan and discuss progress was not mentioned. We meet every week with Algenesis, and once a month with BASF.
- Although this is an exciting and promising space for algae, this does not appear to be geared toward fuel production, and no algae grow-out demonstrations are involved in the project. We will have algae grow-out this spring and summer at our Biological Field Station; we will use three 300-liter indoor ponds to produce sufficient algae biomass to extract at least 1 kg of PUP. We also have sixteen 800-liter small ponds and two 15,000-liter larger ponds, but these are outdoors, so are not allowed for transgenic algae grow-outs.
- This begs the question of why extremophiles are not more prevalent in the industry, another reason why a grow-out component would be highly beneficial for this project. As stated previously, we will have a grow-out to obtain the data required for the LCA and TEA. As far as extremophile algae under industrial use, extremophiles are grown at Cyanotech in Hawaii and EarthRise in Imperial Valley under continuous growth. All other large-scale algae production is short batch mode, like at Qualitas in New Mexico. So extremophiles are used by industry, they are just not used by university and national lab researchers—which are not part of the algae industry!
- However, this project does not appear to include an effort to advance scale-up/cultivation technologies, and it is unclear how it advances the BETO goals for this funding cycle. We did not propose to develop new cultivation technologies in this project; we leave that to projects with the appropriate chemical engineering approaches that we do not have. We will, however, most certainly test our genetically engineered strain in a grow-out to provide the proof of concept that our engineered strain is biologically superior to the baseline native strains we started with.
- It is not clear if the remaining biomass could be converted into useful fuels to meet BETO targets. In Phase II, we will extract the residual lipids and demonstrate that they are suitable for conversion into SAF, per the BETO requirement.

ADVANCING ALGAL PRODUCTIVITY THROUGH INNOVATION IN CULTIVATION OPERATION AND STRAIN TRAITS (ADAPT-COST)

Colorado State University

PROJECT DESCRIPTION

WBS:	1.3.1.673
Presenter(s):	Ken Reardon
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$4,001,133

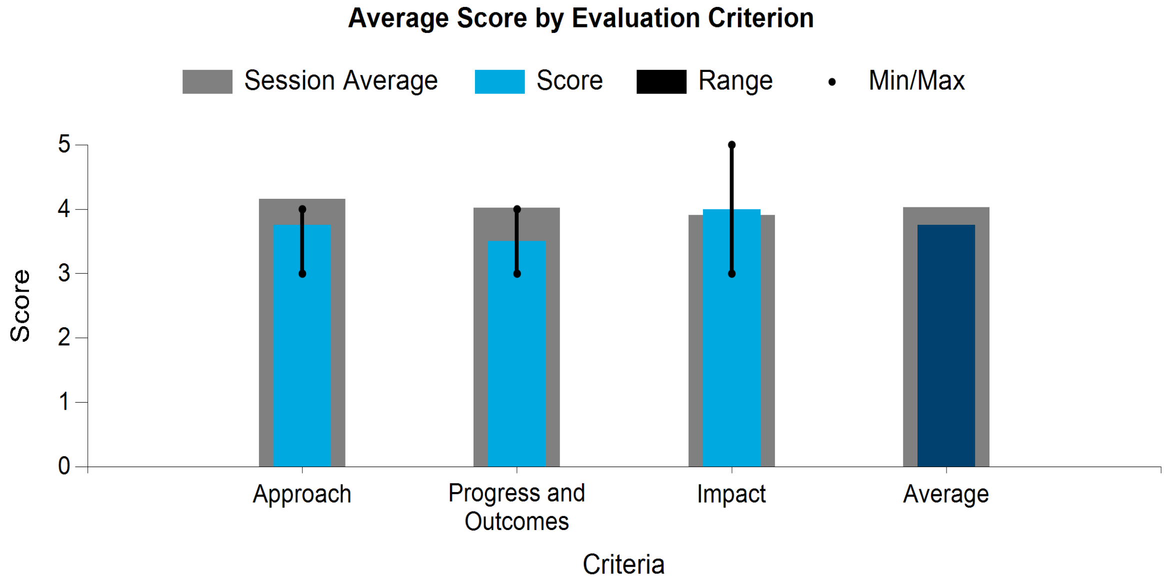
Current algal productivity levels remain insufficient to achieve BETO's goal of $2.50/GGE for algal biofuels. In this project, a set of strain improvement strategies will be linked with novel approaches to improve cultivation operations based on traditional CO_2 supply (Subtopic 2a). The overall result will be a 20% increase in areal productivity, with biomass quality improved to achieve a fuel yield of more than 85 GGE per ton of biomass.

The project uses the microalga *Nannochloropsis oceanica* CCAP84910, closely related to the strain used by Qualitas Health in their Imperial, Texas, facility. *N. oceanica* was chosen because Qualitas has a substantial track record with this organism at industrial scale and because Colorado State University, LANL, and Qualitas have experience with *N. oceanica*, including genetic modification, genome assembly/annotation, and cultivation in an industrially relevant saline medium. *N. oceanica* is stable under real environmental conditions, and Qualitas has developed solutions to minimize pest pressures. This project focuses on cultivation in spring, with outdoor tests at Qualitas and AzCATI.

The strain improvements to be performed are chosen to improve the ability of cells to use both photons and carbon. Photosynthetic carbon assimilation and biomass productivity are limited by ribulose-bisphosphate carboxylase-oxygenase (RuBisCO) inefficiencies. Algae have developed RuBisCO isoforms with modestly enhanced CO_2 affinity or a variety of carbon-concentrating mechanisms. We will engineer an advanced carbon-concentrating mechanism into *N. oceanica* to increase CO_2 assimilation efficiency, resulting in improved biomass yields and productivity.

The cultivation improvements are designed to overcome the limitations of traditional sequencing batch operation, manual offline measurements of biomass and nutrients, and poor prediction of large-scale cultivation performance from pilot cultivations. In this project, we will develop a strategy for continuous/near-continuous cultivation. This advanced cultivation mode will be supported by novel sensors of biomass and nutrients. A computational fluid dynamics model will translate results from mini- to full-scale ponds and guide the advanced cultivation operation, as well as the location of sensors. The cost of supplied CO_2 will be reduced by delivering it to ponds without sparging, either using the CA membrane technology currently being developed in a BETO project or a simpler abiotic membrane.

The combination of strain and cultivation improvement technologies will lead to a 20% increase, indoors and outdoors, in areal biomass productivity and biomass quality, yielding more than 85 GGE/ton of biomass. These technologies can be translated to other algae and other seasons, supporting the BETO productivity goals throughout the year and across the industry.



COMMENTS

- This project focuses on improving the productivity and biomass quality of *N. oceanica* in the spring through genetic modifications to enhance photon and carbon use efficiencies, developing sensors and strategies for effective cultivation operation, and integrating both strategies for deployment. This is of clear relevance to the BETO Multi-Year Program Plan goals. The project's goals are innovative and ambitious, but reasonable.

- The project performers seem to have a clear approach while leveraging expertise from the different investigators. Task structure was proposed with quantifiable go/no-go decision points. However, the team needs to provide more clarity on channels of communication and collaboration. Some risks involved in the execution of this project were identified and mitigation strategies outlined. Other risks, like fouling sensors and saturation of biomass probes, were not addressed.

- Overall, not a lot of progress has been made toward the goals outlined due to the late start of the project, but successful deployment of an improved strain and implementation of continuous cultivation with biomass and nutrient sensors will improve understanding and increase biomass productivity. It's unclear what impact hydraulic load will have on cultivation due to semicontinuous operations, and the project team should consider media recycle for the proposed strategy.

- The project focuses on implementing molecular biology techniques for genetic modification of *N. oceanica* to form pyrenoids that will potentially increase the efficiency of CO_2 fixation by the RuBisCO enzyme. In this realm, the project also emphasizes the deployment of sensors to monitor effective cultivation operations addressing nutrients and environmental factors for pilot-scale outdoor raceway pond systems. Despite having subcontracting issues, the project has made significant progress in specific areas defined in the project. A low-cost nutrient sensor, cQUBE, for ammonia and iron has been developed and will be in operation shortly. The particular need or reason for determining only iron and ammonia (and not other dissolved ions) is not articulated in the project. It would be helpful to know if it is a marker for any event prediction in the pond. The team has multipronged approaches for robust strain development and good mitigation strategies laid out for different challenges. The question comes up for the strain engineering effort—did the team explore the idea underlying the role of carbonic anhydrase CAH1 and RuBisCO's activity relationship in carbon fixation for these mutated strains? Similar work is reported on the *N. oceanica* CCMP1779 strain from Lawrence Berkeley National Laboratory in the literature. It would be good to know if some work was conducted on *Nannochloropsis oceanica* CCAP84910. How the project partners coordinate the workflow and how the DEI component will be incorporated into the project has not been discussed.

- This project aims to improve the productivity of *Nannochloropsis* by genetically engineering pyrenoid formation, improve carbon fixation, and improve biomass sensor technology during cultivation. Multiple entities are involved in this project, and communication between them was not made clear. The technology approach is very comprehensive, focusing on one strain, and could deliver valuable toolkits for future biofuel production. All permits are already in place for grow-out demonstration at AzCATI and at Qualitas in Texas. Although stated as stretch goals, having two different locations for outdoor validation and having an industry partner (Qualitas) is an advantage for this project, making it stand out in the BETO funding portfolio. Outdoor growth would be done in continuous culture, using contamination mitigation practices already in place; however, contamination during continuous cultivation poses a significant risk. The presenter did not think switching to batch culture, if continuous fails, would hinder them achieving their goals. The project did have a delayed start but appears to be on track to complete in time.

- The strain improvement approach of modifying *N. oceanica* to form pyrenoids is high risk, high reward, but if successful might have a significant impact by increasing carbon fixation at ambient CO_2 levels. The risk mitigation strategy identified if the pyrenoid strain improvement strategy is unsuccessful is vague and needs to be more clearly articulated. The project was delayed due to subcontracting issues and started in January 2023. Appropriate go/no-go milestones have been identified. The impact of the work is difficult to assess based on the delayed start of the project.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their thoughtful comments, and we appreciate that they have recognized the innovation of our project. In the paragraphs below, we provide responses to these reviewer comments. Team collaboration and communication: The project is designed to be very collaborative, with data, materials, technology, and samples being shared across tasks and team members. Examples include the testing at AzCATI of strains developed at Colorado State University and LANL, the application of sensors developed at Colorado State University and QBI at AzCATI and Qualitas Health, and the use of data from all experimental work in the TEA and LCA modeling. The main channels of communication are team meetings (currently monthly but transitioning soon to biweekly) and a shared data site (Box). This has proven successful in other similar projects. Sensor development risks: We are aware of the potential for fouling and for optical signals beyond the linear range, and have mitigation plans to address these should they be observed in our testing. Chemical sensor selection: A reviewer asks why sensors are being developed only for iron and urea. The answer is that QBI has already developed sensors for nitrate, nitrite, ammonium, and phosphate. The two new sensors will complete the platform for the major nutrients in microalgal cultivation. Strain engineering and CA: A reviewer asks whether we have explored the "underlying role of carbonic anhydrase CAH1 and RuBisCO's activity relationship in carbon fixation." We are aware of the publication mentioned by the reviewer and appreciate the suggestion to include the role of CAH1 in our research. Our hypothesis remains that including a functional pyrenoid in *N. oceanica* will have benefits for cell growth and carbon efficiency. As noted in the cited publication, the CAH1 system appears to result in leakage of CO_2 to the surroundings. With a functional pyrenoid, that leakage could be reduced or eliminated. DEI: We appreciate the suggestions for enhancing the diversity and inclusion aspects of our project. While the FOA under which our project was funded did not have a DEI requirement, our team is committed to the principles of DEI. We make efforts to recruit a diverse set of students, especially undergraduates, to participate in this project, and plan to conduct outreach events at Colorado State University's Spur campus in Denver, with the goal of reaching students from communities underrepresented in STEM fields. Contamination during continuous cultivations: A reviewer notes a concern that contamination during continuous culture could pose a risk. While we are not aware of data for continuous cultivation, there is a report on the effects of semicontinuous harvesting in which more frequent harvesting resulted in *lower* contamination rates. This was only one study, of course, and we will soon learn from our research whether the same trend is observed.

METABOLIC CARBON FLUXOMICS DURING COMPOSITIONAL SHIFTS

National Renewable Energy Laboratory

PROJECT DESCRIPTION

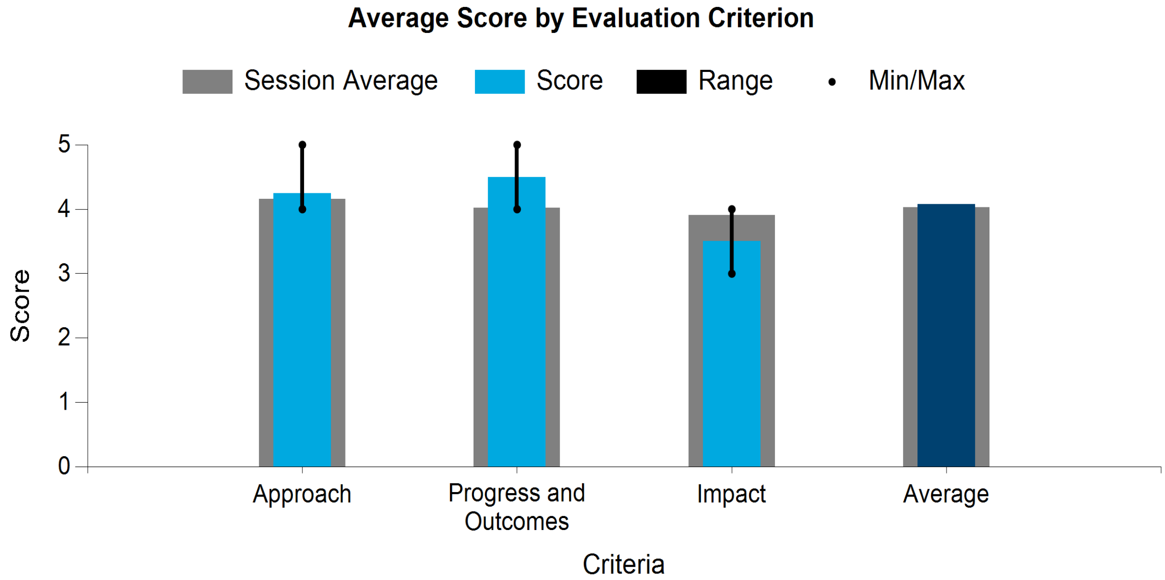
WBS:	1.3.2.005
Presenter(s):	Lieve Laurens
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$750,000

This project aims to pursue the identification of biomass compositional shift metabolic markers for multiple DISCOVR-relevant species of algae through the utilization of quantitative targeted and untargeted metabolomics, coupled with machine learning. This will allow for mapping and manipulating the shifts when algae cells are exposed to a wide array of environmental stressors. The goal is to identify parameters that are under the control of the future algae farmer to tune algal biomass composition toward selective applications, at minimal cost to the production and/or fuel cost.

Sharing this project's findings with industry and collaborative test bed operations to identify scenarios to implement to achieve compositional improvements, this project alone will not directly become commercialization-ready with an open-source dissemination strategy of the approach, algorithms, and methods associated with this work toward collaborators and partner projects.

This in-depth metabolomics approach to understand the respective metabolic signatures and dynamics has largely been missing from the quest to understand the primary drivers behind shifting biomass composition toward more conversion-friendly carbon sources (lipids and carbohydrates). To specifically understand the primary carbon storage rates in different biochemical sinks, it is necessary to present a more holistic metabolomics profile for all metabolites along the carbon assimilation pathways, including the Calvin–Benson–Basham cycle, known to be at the basis of the careful orchestration of the fate of carbon in photosynthetic cells.

We have collected a physiologically diverse set of metabolomics data sets for multiple strains by varying the light, temperature, nutrients, and diel light conditions, each leading to a distinct compositional profile of the biomass. The application of an unsupervised learning approach to reduce the dimensionality of the data has already indicated a set of metabolites that are correlated with compositional shifts for *Scenedesmus* biomass and will be extended to achieve a more quantitative prediction by this project's interim decision point in FY 2023.



COMMENTS

- The project focuses on identifying metabolic predictors of compositional shifts in algal outdoor production. Their approach is to identify nutrient conditions that enable a shift in composition without impacting biomass productivity and then propose metabolic engineering targets/pathways that are implicated in the composition shift. This is an innovative approach, and if successful, can provide information that will improve the productivity of algal strains by metabolic engineering.

- The team had a clear management plan with a well-defined task structure and leads. They identified risks and implemented mitigation strategies, as well as established channels of communication and collaboration among team members. The team also outlined an approach to addressing DEI in their project plan.

- The project performers have made significant progress in developing a high-throughput physiological testing environment, as well as optimizing a workflow for high-performance liquid chromatography–mass spectrometry. They have developed a data set for two strains under 28 different growth and media conditions. This could be a useful tool for metabolic engineering of high-producing strains if heatmaps for metabolite upregulation at different time points can be correlated to the different growth conditions, biomass productivity, and composition shifts. The project performers should also consider the risk of doing all the work indoors, where contaminants and outdoor conditions can change the metabolic profile. The end goal of the project is the selection of implementable physiological strategies to shift biomass compositional quality and value with minimal impact on biomass productivity. It's unclear from the presentation how the project performers intend to achieve the end goal.

- The project aims to increase the opportunity for algal biomass productivity to meet BETO's SAF targets. The main objectives for the project are to identify metabolic predictors for composition shift of outdoor algae pond cultures based on nutrient conditions (phosphate and ammonium) without affecting the biomass production and advancing the knowledge for metabolic engineering targeting this shift. The team envisions that this metabolomics predictor toolkit coupled to machine learning will enable them to contribute to BETO's goal of producing high-SAF feedstock for biofuel production. This is a very ambitious goal, and the project has made significant progress by building synergy between different national labs to conduct several simulated experiments while addressing several risk factors and subsequent mitigation strategies. The project has demonstrated some unique relationships between metabolites/pathways and compositional profiles and claims to have implemented a workflow for strain-agnostic metabolomics fingerprinting. The team has a well-defined workflow structure, and the DEI component has been incorporated in the project. There is no mention of patents/publications, except about presenting at one of the upcoming international conferences.

- This project aims to develop a toolkit to predict the composition dynamics of algae biomass in order to steer a crop toward specific coproducts, thereby increasing the value of algae biomass in general. This project has a clear management structure and benefits from being tied into other efforts at the national labs. The team appears to have made a strong effort toward the BETO DEI targets, even though this particular project was not required to do so. The team has valuable expertise and access to sophisticated equipment and has generated some very nice data using PBRs. There is no targeted outdoor component to this project specifically; however, the learnings will have to be validated in outdoor reactors before any strategies become readily implementable by industry, etc. Contamination pressure and an unpredictable outdoor environment could make key learnings in PBRs irrelevant. The presentation given by the DISCOVR consortium described how the compositional analysis will be used by them, and it seems there is some validation planned in outdoor ponds that will be beneficial. This project approach and impact would have been stronger if the validation was directly built into their goal structure.

- This project is focused on unraveling the metabolic predictors to control the fate of carbon allocation, has sustainable merit, and is an important part of aiding BETO in achieving more carbon-efficient SAF production. The management plan seems appropriate, and risk mitigation strategies are in place. Collaborations with DISCOVR and the Algae Biomass Organization are appropriate. The project is halfway through the funding period and seems to be making good progress. The project team has developed and implemented a workflow for strain-agnostic metabolomics fingerprinting, built a data set with 28 different physiological conditions, and identified some unique relationships between metabolites and compositional profiles. The impact of the work is clear and has identified correlations that indicate a strong relation to high carbohydrate and lipid content in biomass as value drivers.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewers' thoughtful and complimentary comments on the project presentation. A couple of the points raised focused on the connection between indoor and outdoor relevance of the approach and ultimately the application space tested. This work is a focused effort on controlled-environment probing of predictive factors for shifting biomass composition to become more amenable to bioconversion. Specifically, the implementable strategies that could achieve the goal of shifting the biomass quality can be nutrient formulation and cultivation operations (e.g., N:P ratios, pH, and adding signaling metabolites to alter carbon allocation in the cells), as well as metabolic engineering targets for synthetic biologists manipulating particular critical metabolic pathways. For all the work presented, we use an indoor bioreactor system that has been validated as climate-simulating outdoor cultivation (of the algae test bed at AzCATI), both in terms of growth and biomass composition (work that was briefly presented as part of the DISCOVR consortium presentation), which gives us confidence that some of the identified metabolic and physiological responses to abiotic perturbations are translatable to at least the outdoor environments. One important factor that is not included in these simulations is the biotic impact of microbial ecology on biomass composition. This is a nascent research area that makes developing and simulating relevant conditions much more complex, and is outside the scope of this work. Outdoor biotic contaminants are likely to be variable parameters, and replicating these indoors can convolute metabolomics results and make it impossible to dial in on predictors of composition shift. With the successful validation of the predictive metabolic signatures, a future trajectory of this work may include the translation to outdoor test bed confirmation, which would include biotic and abiotic influences. The interaction between metabolic predictors under pressure from contaminants is unclear and will need to be tested outdoors. However, if successful, the implementable strategies will still improve the valuable biomass component (carbohydrate/lipid) productivity of the strain. In this initial project, we want to first identify (and deconvolute) the metabolomics response of composition shift for the strain in axenic cultures to guide metabolic engineering. We are currently focused on quantifying the carbon flux parameters under selected physiological conditions for a select set of species, and we are preparing a manuscript that describes the unique contribution of the unbiased carbon allocation study that we have completed. The overarching deliverable of this work is to not only understand but also make carbon allocation metabolomics more broadly accessible and comparable, and we will make methodologies and reports openly available.

BROAD SPECTRUM ANTIFUNGAL POND PROTECTION

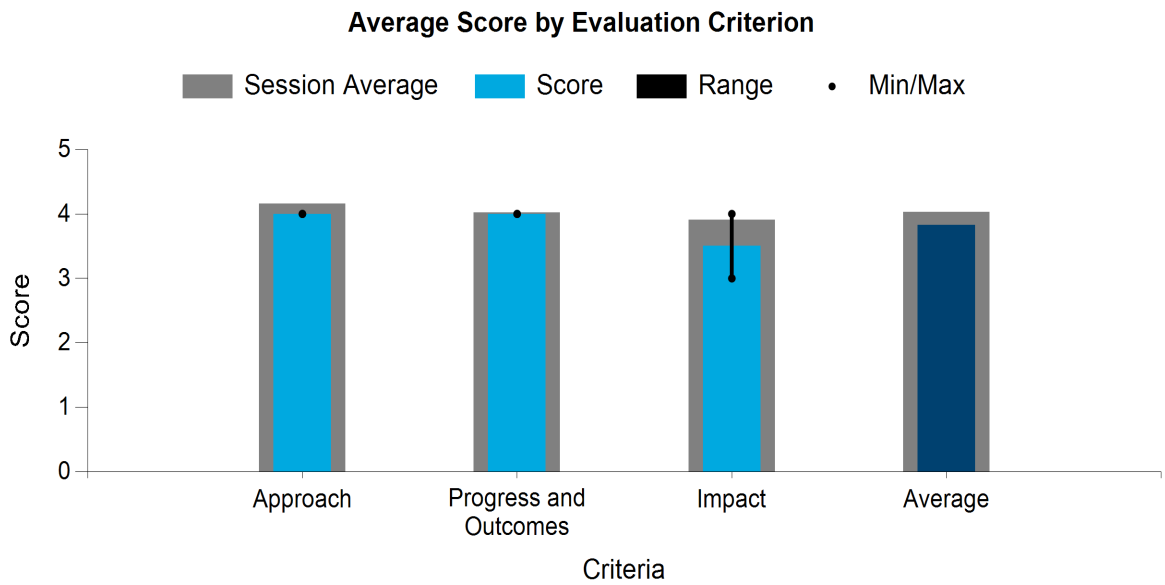
Sandia National Laboratories

Project Description

WBS:	1.3.2.044
Presenter(s):	Todd Lane
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$1,000,000

The economic production of algal biomass for biofuels and coproducts is dependent on the development of crop protection strategies and technologies that are both cost effective and broad spectrum—offering projection from the greatest diversity of deleterious species. In previous work at Sandia National Laboratories, we isolated multiple independent microbial consortia that are capable of protecting *Michrochloropsis salina* from grazing by the rotifer *Brachionus plicatilis.* These consortia were shown to be persistent, with protective effects enduring for more than 30 days in outdoor cultivation trials. They also have no deleterious effect on algal production and, in fact, appear to enhance the growth rate of the algae. They can be readily maintained in co-culture with algae, not requiring a separate cultivation and inoculation system. Thus, crop protection systems based on biological control by selected bacterial consortia add essentially no additional cost to algal production.

In the current project, we are extending such consortia-based systems to the protection of a diversity of algal strains against a variety of fungal parasites. We have screened and selected multiple independently derived microbial consortia that protect algal strains from a diversity of fungal species. These consortia have been shown to increase the time to failure in standard fungal infection assays by 70%–100%. Each independent consortia is being cross-evaluated with a range of algae and fungal combinations to identify the isolates or consortia that display the greatest breadth of protection. The microbial community structures of these consortia are being determined via second-generation sequencing. We are identifying the organisms that are most strongly correlated with the protective activity and specificity of each consortia. In future work, we will combine consortia to create novel consortia with expanded protective capabilities. These second-generation consortia will be tested for persistence and range of protection (both algae and fungal pathogen) and stability in outdoor culture systems. This project is designed to develop cost-effective crop protection strategies and technologies that are broadly applicable to a variety of production algae and deleterious species. By reducing the frequency of pond crashes due to biocontamination, this technology will increase annualized yields and thus reduce the overall cost of algal biomass production.



COMMENTS

- The project proposes to develop a microbial consortium that will reduce pond crashes from fungal infection. Their approach is to co-cultivate these protective strains or consortia with algae, allowing for a cost-effective method of prophylaxis. If successful, the project will advance BETO goals of minimizing pond crashes that will increase annualized yields and thus reduce the overall cost of algal biomass production.

- The team had a clear management plan. They identified risks and outlined mitigation strategies, as well as established channels of communication and collaboration among team members. The team also outlined an approach to addressing DEI in their project plan.

- Overall, the team has made significant progress toward their goal. Successful demonstration of this pond protection approach on an outdoor trial will enable commercial deployment. The team developed standard crash assays for all their agents. The project performers should consider nutrient availability, stability of consortia during the run, competition when increasing consortia concentrations to extend the life of the production pond, and overall impact on biomass productivity. The impact of revived biomass from rotifer on biomass quality and downstream purification should also be considered. It is also unclear how this strategy will be commercially deployed considering the robustness of consortia pond control for different algal strains and environmental conditions.

- The goal of the project focuses on selection of microbial consortia that can be co-cultivated with algae, thus preventing fungal infection leading to pond crash. This will provide a zero-cost method for prophylactic treatment by avoiding the use of biocides. This project at Sandia National Laboratories has been able to establish a panel of six fungal pathogens for algae and six different sources of bacteria with prophylactic effect that assisted in 36 indoor laboratory-scale experiments. Sandia National Laboratories has been successful in demonstrating through several experiments that multiple protective strains or consortia can prevent fungal pathogenesis and hence effectively prevent pond crashes. The risks and mitigations have been addressed, which seems to be adequate considering the broadness of this area, especially when considering open-pond crashes. Algae test bed demonstration is in progress to check the stability of both pond and protective consortia over a greater period of time. Although the progress has been decent, there is no mention of the biomass productivity, which is an important aspect for BETO's mission. The major question is: How will this bacterial protection affect biofuel and bioproduct quality of the algal mass downstream? This project has embraced the DEI component, and the PI is enthusiastic about communication and collaboration with other labs and stakeholders to increase the breadth of the project.

- The specific management/communication structure was not made clear in the presentation. The approach to fungal pest management is novel and poses a low-cost (to no-cost) strategy using biological control from a beneficial consortium. The approach was validated with rotifers and had good results. Consortia data for fungal pests look promising so far, and it will be interesting to see these validated in outside cultures. For the 9/30/22 milestone data shown (Slide 14), sampling intervals were not consistent, and details may have been obscured. Any future testing, especially outdoors, should include daily sampling so as not to miss important inflection points of these complicated interactions. For the same milestone, Slide 15 shows the consortia only being effective after a reinfection event and not for the first 20 days of cultivation. This lag may not be feasible at scale unless it is built into the preproduction phase. The presenter reported that the consortia can be freeze-dried for storage/transport, which will be important for adoption by industry. No industry partner was identified, and although this was not a requirement of the BETO funds, it is unknown how open industry would be to using a pest control method like this versus chemical, unless the consortia are proven to be stable and/or maintain efficacy after the preproduction phase.

- The approach focuses on developing a low- to zero-cost prophylactic treatment to prevent pond crashes due to fungal infections. Risks and appropriate mitigation strategies have been addressed. The project appears to be on schedule, and project milestones are being successfully achieved. Current industrial efforts seem to be focusing on microalgae strains that tolerate high pH (>10). Most fungi seem to grow well only in the pH range of 4.0–8.0; thus, it's not clear how impactful this work will be if current industrial efforts continue to focus on extremophilic microalgae. The project team noted that temperature seems to be a driver of fungal infections; thus, it would be interesting to know what effect pH has on the rates of fungal infections in growing microalgae cultures.

PI RESPONSE TO REVIEWER COMMENTS

- Comment: "The project performers should consider nutrient availability, stability of consortia during the run, competition when increasing consortia concentrations to extend the life of the production pond, and overall impact on biomass productivity. It is also unclear how this strategy will be commercially deployed considering the robustness of consortia pond control for different algal strains and environmental conditions." Comment: "It is unknown how open industry would be to using a pest control method like this versus chemical, unless the consortia are proven to be stable and/or maintain efficacy after the preproduction phase." Response: Determining the stability of the consortia under production conditions is the focus of the second half of this project. We have already demonstrated that the consortia are stable for at least 30 days and retain full activity under laboratory culture conditions. As part of the current work, we are already evaluating the robustness of each consortium in the presence of multiple algal species. It is currently in the work plan for the remaining half of the project to test the consortia at pilot scale at both simulated outdoor conditions and in outdoor production systems with an appropriate partner. This testing will allow us to evaluate the robustness of the consortia under differing environmental conditions and with different strains. Our current evidence indicates that it is not necessary to increase the consortia concentration to increase stability and activity, as the consortia are stable and active at the concentrations that occur "naturally" in co-culture with each algal species.

- Comment: "Although the progress has been decent, there is no mention of the biomass productivity, which is an important aspect for BETO's mission. The major question is: How will this bacterial protection affect biofuel and bioproduct quality of the algal mass downstream?" Response: It is important to keep in mind that all open production systems already contain bacterial/algal co-cultures and that many of the consortia that we are testing are derived from outdoor algal cultivation systems. All consortia were initially screened for their impact on algal growth rate and biomass yield. Consortia that demonstrated a negative impact on either parameter were removed from consideration. We will be further investigating the impact of the consortia on biomass quality as part of the work plan for the second half of the project.

- Comment: “The team had a clear management plan.” Comment: “The specific management/communication structure was not made clear in the presentation.” Response: This is currently a relatively simple project from a management perspective, as it currently is a single-PI project at a single national lab, and all workers on the project report directly to the PI. In the event that we decide to collaborate with an outside institution for field trials, this effort will be mediated through a procurement agreement with that institution and Sandia National Labs, and will include a defined statement of work and reporting requirements.
- Comment: “For the same milestone, Slide 15 shows the consortia only being effective after a reinfection event and not for the first 20 days of cultivation. This lag may not be feasible at scale unless it is built into the preproduction phase.” Response: This lag in protection was due to the nature of the assay, where we infected at a very high multiplicity of infection. Infection with an initial high concentration of agents would not be the case in a natural infection. We have evidence that under a lower multiplicity of infection, such that is present in the early phases of a natural infection, the consortia will be able to interdict the infection and prevent biomass loss before the agent overruns the culture. In addition, we believe that the experiments, carried out at high multiplicity of infection, have resulted in a more protective consortium that that will be protective throughout cultivation and not just after the first round. Such evolution of the protective consortia is an intentional and integral component of our consortia development process, and we are currently evaluating the resulting consortia from these high-multiplicity-of-infection trials.
- Comment: “Current industrial efforts seem to be focusing on microalgae strains that tolerate high pH (>10). Most fungi seem to grow well only in the pH range of 4.0–8.0; thus, it’s not clear how impactful this work will be if current industrial efforts continue to focus on extremophilic microalgae.” Response: The overall strategy of development of microbial consortia for protection against deleterious species has been demonstrated to be effective against both grazer species and parasitoid fungi. There is every reason to believe that the strategy would be effective against other biocontaminants.

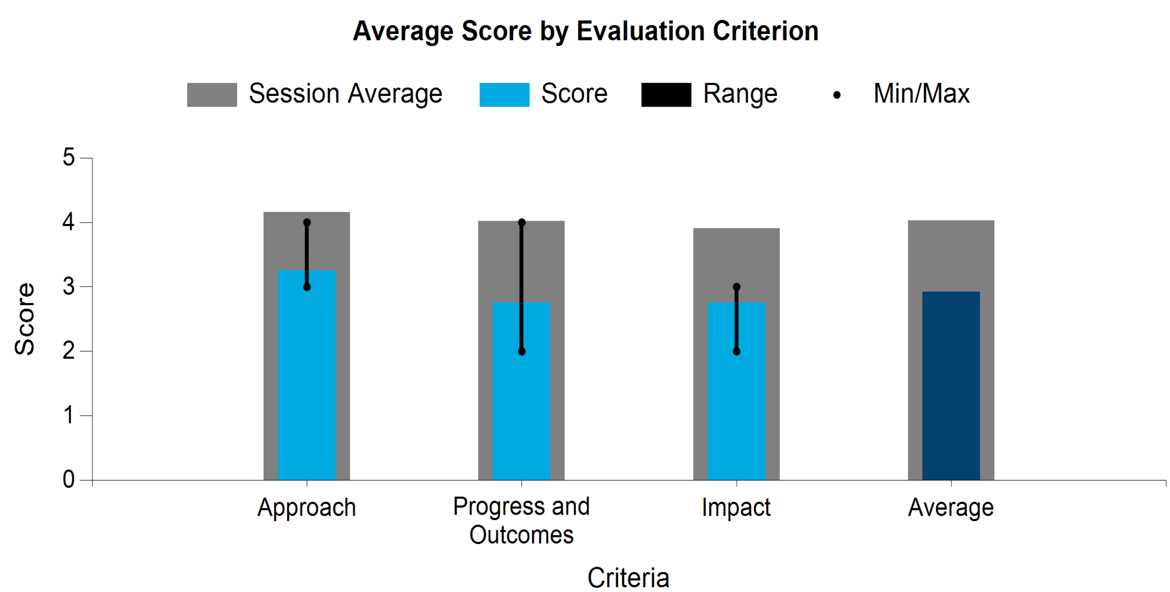
CARBON UTILIZATION EFFICIENCY IN MARINE ALGAE BIOFUEL PRODUCTION SYSTEMS THROUGH LOSS MINIMIZATION AND CARBONATE CHEMISTRY MODIFICATION

Duke University

PROJECT DESCRIPTION

WBS:	1.3.2.440
Presenter(s):	Zackary Johnson
Project Start Date:	10/01/2018
Planned Project End Date:	03/31/2023
Total Funding:	$1,928,295

High productivity and yields of microalgae grown in open-air raceway ponds for biofuel production require active inorganic carbon delivery to the water medium. In most research (and commercial) operations, this carbon is supplied in excess from external, often limiting, CO_2 sources. However, TEA/LCAs show that this approach can dramatically limit the broader application of algae-based biofuels. To address this gap, our team is using top biofuel algae strain candidates to (1) identify the minimum concentration of dissolved inorganic carbon (DIC) that can support baseline and target productivities and yields to improve CO_2 use efficiency, (2) test the enhancement of productivity and yields of candidate algae by supplying "CO_2" in the form of bicarbonate, (3) test a patented CO_2-based conversion technology as an improved carbon source on open raceway ponds at an established algae facility, and (4) test this technology on scalable sources (waste streams) of CO_2 using open raceway ponds. To date, we have made substantial progress on all of these goals, including transitioning to larger-scale trials and integration with industry.



COMMENTS

- The goal of this project is to demonstrate enhanced algal growth with overall reduced CO_2 requirement at an industrially relevant scale. If successful, the proposed work will have benefits to the algal industry and is well aligned with BETO's mission and Multi-Year Program Plan goals of reducing the cost of production.

- The management plan was not outlined, although team expertise and previous experiences are leveraged in this project. Risk and mitigation strategies were identified. The task structure was proposed, but quantifiable go/no-go decision points were not clearly outlined.

- Progress has been made toward the goals outlined. The project performers did not highlight outcomes of the strain assessment. It would have been helpful to see how strains with reduced pCO_2 threshold for growth and strains with growth enhancement on converted DIC waters compare to their baseline or current SOT. The team has done tremendous work testing the proposed approach in outdoor race ponds and showed improvement with DIC, but they did not outline the baseline and show improvement over the baseline. Progress has been made with TEA/LCA to assess commercial deployment of the technology, but the discussion would benefit from more specifics on how the strain's use of calcium carbonate ($CaCO_3$) and integration with the brewery will drive the production cost down, given that the use of limestone is not sustainable. Additional work is likely needed to achieve the end-of-project milestone of enhanced algal growth on high-DIC water at an industrially relevant scale.

- The team mentions the use of DAC coupled with DIC to produce high-performance outdoor algae cultivation for biofuels on developing approaches to minimize CO_2 use and losses to enhance overall algae productivity. It will deliver TEA data generated from the R&D to demonstrate lower potential costs for algal biomass and increased potential revenue from the incorporation of DAC with production of valuable algae products. The main mission of BETO is to generate substantial algal biomass, and this project aims to modulate the carbon input into the algal pond to maximize the production of algae biomass and bioproducts by avoiding undue excess use of the carbon source. Numerous strains have been assessed, and a few have been identified that show a growth stimulation in converted DIC water. The team mentions demonstrating the reduced capital and operating expenses for algae-derived biofuels being more economically feasible, but more data collection may be necessary to establish a higher confidence level and robustness of the system. The outdoor 1-liter ponds with the calcium carbonate/bicarbonate chemistry have shown promising results. More outdoor trails need to be counted to provide a consistent data set. It may still need some optimization to avoid precipitation of carbonate with the algae biomass. The plan for coupling the system with the local brewery for inorganic carbon has produced better results and reduced the use of carbon dioxide. The team has successfully published their research findings in peer-reviewed journals, making them available to the public. The PI of the project has received the Algae Biomass Organization Mid-Career Award, which is highly commended.

- This project targets increased productivity of algae using reduced CO_2 use and loss via DIC addition and incorporates a TEA/LCA. They planned to target two strains known to have good growth in benchtop bioreactors. It is certainly important and aligned with BETO goals to identify strains and conditions that allow for reduced CO_2 use, and lab-scale results looked promising. The presenter acknowledged that using a mined resource like limestone to increase DIC is not sustainable at larger scales; thus, this project appears largely academic in nature and may not help BETO get closer to their goal targets. The industry partner for this project is a commercial brewery, but due to the COVID-19 pandemic, they were not able to work with them, and instead simulated brewery inputs from a homebrew kit. The presenter listed various next steps, including doing replicate testing of their initial small-scale outdoor testing, doing outdoor testing at a larger industrially relevant scale, and working with the brewery directly. These are all critical steps to their objectives, which appear will not be completed, as the project officially ended last month. This level of completion is concerning, considering the project has been funded by BETO since 2018.

- This project seeks to demonstrate enhanced algal growth with an overall reduced CO_2 requirement at an industrially relevant scale. Risk mitigation strategies are identified; however, their implementation and effects weren't clearly communicated. Although the CO_2 conversion technology seemed intriguing, it seems likely that the work will not advance the state of the art. The high-carbon water seems to improve growth and yield at the cost of producing a precipitate, which could complicate downstream processing of fuels and coproducts. The project team prototyped a “femtoscale brewery” in an effort to satisfy Task 4, which focused on integration with industry. The principal outcomes from this small-scale prototype seem to be the quantification of CO_2 release and mass balance closure. The results from this prototype system do not give confidence that the technology could be scaled to a commercial brewery. The overall impact of this work seems minimal based on scalability issues and the current lack of industrial engagement.

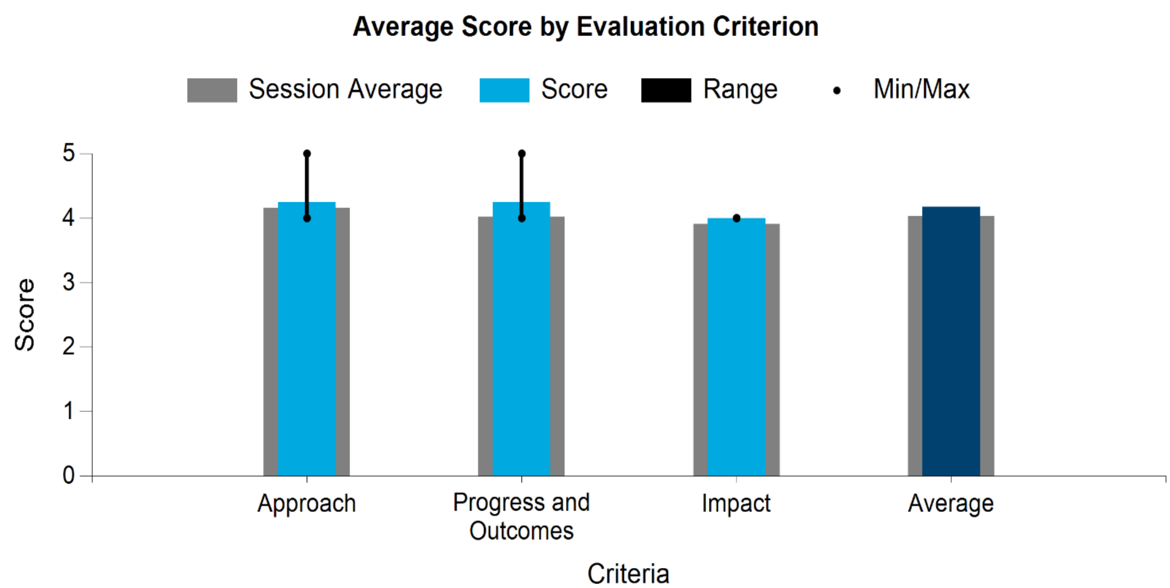
ECOLOGICAL MONITORING TECHNOLOGIES TO ENHANCE LARGE-SCALE MICROALGAE CULTIVATION, STABILITY, AND PRODUCTIVITY

Scripps Institution of Oceanography at the University of California, San Diego

PROJECT DESCRIPTION

WBS:	1.3.2.670
Presenter(s):	Lisa Zeigler
Project Start Date:	10/01/2021
Planned Project End Date:	08/31/2025
Total Funding:	$3,451,632

A critical component of any commercial-scale cultivation system is the challenge of ecological processes that can decimate or limit productivity of the target strain. Limitations may arise from interactions with contaminating negative biota (pests) or lack of algal adaptation to mixed communities—for example, axenic monocultures and closed-culture situations (engineered or natural strains) that result in reduced strain fitness among complex consortia. This project will bypass standard approaches of detecting and tracking single organisms using qPCR or Loop-mediated isothermal amplification (LAMP) assays to develop and employ affordable real-time monitoring using third-generation long-read sequencing. The Oxford Nanopore Technology is comparable to classical assay costs but has the advantage of providing real-time information about the physiological state of the algae and the associated microvirome, consisting of both microbes (bacteria, archaea, other algae, fungi, and protists) and viruses. Controlled laboratory-scale experiments will simulate diurnal raceway conditions and mimic current commercial-scale cultivation methods for pond transfer and scale-up, thus integrating across a lab-to-field model. In addition, prior and ongoing efforts on identifying positive ecological relationships now make it possible to engineer polymicrobial systems (designed ecosystems) aiming to promote optimal growth of algae pond ecosystems. Successful completion of tasks will result in the improvement of high-performance cultivation stability and reproducibility through advanced monitoring on an actionable time scale and biocontrol of field-scale campaigns.



COMMENTS

- This project focuses on developing and deploying real-time monitoring of algal cultures using third-generation long-read sequencing. The information generated will be used to build a database that will allow understanding of traits like stress and help drive mitigation strategy. This is in line with BETO's algae crop protection goals of developing crop protection methods and strategies to maintain robust productivity of algal cultivation systems and is part of Topic Area 2: APEX.

- The team seems to be a good mix of research and industry partners. The team had a well-defined task structure that will ensure the success of the proposed work and addressed any risk and mitigation strategies. The team management did not demonstrate a well-established channel of communication and collaboration among team members.

- The team has made significant progress in developing and implementing a method for real-time evaluation of a GAI pond-associated microbiome. A sample-to-sequence workflow with less than 12-hour turnaround time will accelerate understanding of microbiomes during periods of high performance and crash. It's unclear from this work how the project performers intend to use this information to correlate algal culture pond performance and stability. Also, more clarity is needed on how the use of this approach can inform the team running algal ponds about imminent problems with pond health so they can react accordingly given the technical expertise and hardware required for genome assembly and analysis of the resulting data.

- The project aims to develop a robust toolkit for outdoor algal cultivation that will facilitate and integrate lab-to-field easy transfer technology with the capacity to provide a curated algal microbiome database, perform systematic workflow analysis, detect ecological perturbation, evaluate stresses, and provide a collection of mitigation strategies. The team made good progress and transferred their lab-to-field trail in July 2022. There was no mention of how the initial ongoing stress and microbiome data are responding to the field protocol. Any projected information regarding the reduction of time and cost as an example would raise the level of confidence in the system. Some data or a mention of how successfully a simulated in-lab stress system was able to recover from the initial stress testing situation with application of any of the mitigating tools was absent. The team should plan to incorporate the DEI component into their project in the upcoming years.

- This project aims to deliver real-time pond composition monitoring, analysis workflows, and a decision tree for mitigation strategies for cultivation of elite algae strains. While still early in the project, the team has exceeded their real-time monitoring goals and reduced their sample-to-sequence workflow below target. The project appears well organized, but the management and communication structure was unclear. The technology will be validated at scale by an industry partner (GAI), which makes this project stand out among many in the portfolio. Standard operating procedure development for industry handoff is built into their goals, adding value to the project impact. The work is focused on the elite algae strain, *Nitzschia*, and it was unclear if/when the tools will be validated for application to additional strains.

- The approach of using real-time monitoring to analyze the ponds seems good. The project management plan is clear, and risk mitigation strategies are identified. The project is 1 year into the project timeline and seems to be on track. The technology will have a greater impact if the final version of the technology has an open framework such that anyone could use it.

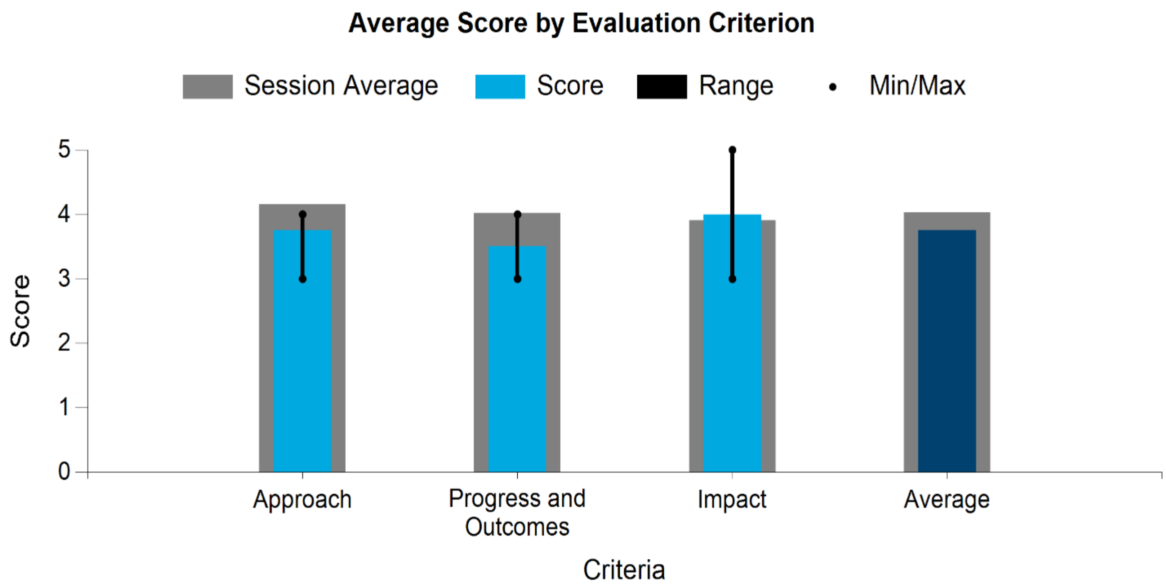
ENHANCED ALGAE PRODUCTIVITY IN CO_2 DIRECT AIR CAPTURE CULTIVATION

Global Algae Innovations

PROJECT DESCRIPTION

WBS:	1.3.2.671
Presenter(s):	David Hazlebeck
Project Start Date:	10/01/2021
Planned Project End Date:	03/31/2025
Total Funding:	$4,000,000

GAI was founded in 2013 to harness the unparalleled productivity of algae to provide food and fuel for the world, dramatically improving the environment, economy, and quality of life for all people. Our approach is to produce algae oil and algae protein meal coproducts that are economically competitive with current commodities. In this project, CO_2 is directly absorbed from the atmosphere into the open raceways so that no separate CO_2 concentrating or distribution system is needed. The main objective of the project is to increase productivity while maintaining a high lipid content. This objective will be achieved through application of a novel directed evolution approach to improve our elite biofuel strain of *Nitzschia inconspicua*, as well as a modification to the cultivation approach to leverage the characteristics of this strain to increase the overall productivity. The project has just started with facility improvements, and experimental work is anticipated to start in summer 2023.



COMMENTS

- This project focuses on developing improved strains and cultivation methods that increase productivity by at least 20% and lipid content by at least 35%, with all CO_2 supplied by DAC. This has clear relevance to BETO's mission and Multi-Year Program Plan goals. If successful, this will decrease the cost of CO_2 capture and delivery.

- The project performers did not map out a clear management plan or well-defined task structure. They identified technical challenges and risks and proposed some mitigation strategies.

- The team's approach to evolve three strains for high- and low-temperature weather will allow all-year cultivation. It's unclear how the project performers intend to improve the strain to tolerate a wide range of pH, temperature, salinity, and dissolved oxygen while still maintaining high productivity. This project will also benefit from the discussion of cultivation strategies proposed to improve productivity. Not a lot of progress has been made toward their goal.

- The focus of this project is on developing improved algal strains that will be cultivated in open raceway ponds with DAC and supplying CO_2 to the growing culture. GAI has worked on multiple similar projects with academic institutions and has expertise in indoor PBR productivity. GAI's project is on track and has made good progress, and production will be initiated soon. The company has about 20 patent applications filed from projects related to advances in algae cultivation. The system has been 90% installed, with the project currently in the equipment installation and checkout phase. Productivity of biomass and lipid content in Kauai was adjusted to 14.8 $g/m^2/d$. The PI should mention and provide some indoor bioreactor data/achievements where GAI has established high confidence levels. There is no mention of the DEI component in the project.

- The team structure is unclear but appears to be just GAI and Hamilton Robotics. The goal of this project is to increase algae productivity and lipid production for several improved *Nitzschia* strains while using DAC for CO_2, and a range of tolerances for pH, salinity, and dissolved O_2. The team uses a directed evolution approach and thinks they have a fix allowing engineered strains to perform better in open ponds than wild-type, a serious challenge for the industry historically. One major advantage of this project is the ability to do larger-scale outdoor testing at two GAI locations (in Hawaii and California). There were very few details given about the DAC piece to this project, but presumably it was presented previously or is non-disclosable due to intellectual property (IP) applications. In fact, the approach was not very detailed for most areas of the work, so it was hard to review. However, given that GAI is working toward commercial scale (presenter mentioned next 5 years), they do appear to be focused on their goals, self-constrained/guided by TEA, and have clearly made a lot of progress. Their efforts appear to fit well within the BETO portfolio.

- The project team seeks to develop improved strains through directed evolution and cultivation methods for open raceways with all CO_2 supplied via DAC. The method for supplying CO_2 via DAC does not seem to be clearly described in the presentation. The project team has passed initial verification and is currently working on installing equipment (90% installed and partially checked out). Some results were shown for a TEA, and the PI indicated that many production costs have recently increased significantly. The impact of the proposed work is difficult to assess given the content in the information provided. Future BETO updates should provide the reviewers with more technical information about the process and more detail about the proposed innovations.

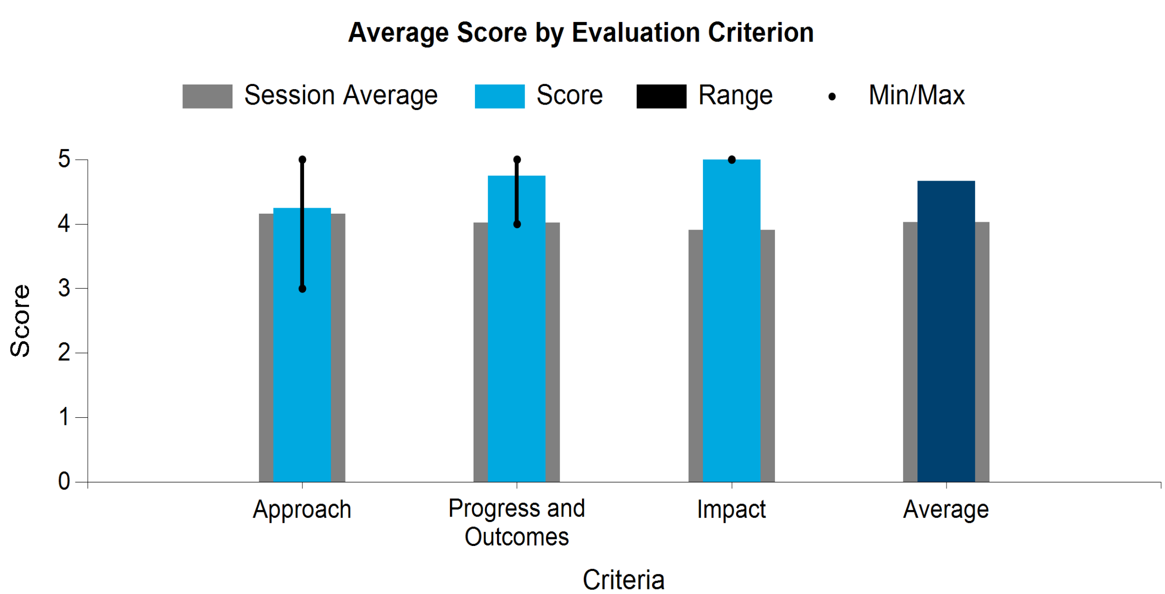
PROCESS OPTIMIZATION AND REAL-TIME CONTROL OF SYNERGISTIC MICROALGAE CULTIVATION AND WASTEWATER TREATMENT

University of Illinois at Urbana-Champaign

PROJECT DESCRIPTION

WBS:	1.3.3.001
Presenter(s):	Jeremy Guest
Project Start Date:	10/01/2020
Planned Project End Date:	12/31/2023
Total Funding:	$2,509,062

The goal of this work is to accelerate the commercialization of a high-productivity, mixed-community microalgal cultivation technology to synergistically recover phosphorus from wastewater and produce a biofuel feedstock. This project seeks to overcome challenges associated with the design and operation of high-rate processes that reliably achieve performance targets despite fluctuations in wastewater composition, climate, and microbial communities. The objectives of this work are to (1) develop a process simulator to predict process performance and sustainability, (2) develop a low-cost system for real-time monitoring of microbial community structure, and (3) leverage the simulator and monitoring system to advance the financial viability and environmental sustainability of algal cultivation on wastewater. These objectives are being accomplished through long-term characterization of a full-scale EcoRecover system installed at a wastewater treatment plant in Wisconsin, comprehensive characterization of microbial community structure and function, real-time microbial monitoring, process modeling, and sustainable design. This work has (1) identified indicators of stable system performance and impending process upset, (2) produced an open-source process simulator that predicts average effluent phosphorus within 0.01 mg P/L, and (3) yielded a novel, autonomous imaging technology achieving 86% accuracy for genus-level classification of industrially relevant microalgae.



COMMENTS

- The team proposes to integrate biomass technologies with municipal wastewater treatment to increase energy efficiency and reduce cost while still maintaining consistent biomass productivity. This is in line with Topic 2C's objective. The team approach to achieving the goals includes (1) advancing the current EcoRecover process, (2) deploying a low-cost microscope for real-time monitoring of cultivation ecology, and (3) validating an open-source process simulator for the cultivation of mixed communities. The current limitation of the technology is a lack of understanding of mixed community crashes, which will be addressed with the proposed work.

- The team had a clear management plan with a well-defined task structure and leads. They did not identify risks or proposed mitigation strategies.

- The project performers have made significant progress in demonstrating phosphorous removal using the EcoRecover process, using microscopy data to inform stable or variable performance and validate their process simulator. The impact of the wastewater treatment on biomass productivity and the consistency of yields given changes in feed stream for mixed communities are unclear. The team should consider the impact imbalance between ammonia-oxidizing bacteria/nitrite-oxidizing bacteria and the loss of *Scenedesmus* sp. on biomass productivity and the end use of the biomass. Overall, successful deployment of these technologies will not only be a huge benefit to wastewater treatment, but will also provide low-cost algal biomass.

- The project aligns with BETO's mission to support lowering the cost of biofuels through low-cost feedstocks while reducing the greenhouse gas (GHG) emissions of biofuels by offsetting the cost incurred during wastewater treatment for municipal water. The team aimed to develop a simulated open-source model following its calibration and validation, and well as assess the TEA and LCA success of cultivating several mixed microalgal communities. The second objective was real-life tracking of the structure of the microbial community via the use of low-cost microscopy, and finally, optimizing the EcoRecover process validation, locality-specific design optimization, and real-time monitoring of the system. The permanent EcoRecover installation was completed and is currently functional in the Village of Roberts, Wisconsin, and the construction of two more systems is in progress in the same state. The team has been successful in addressing their objectives in a very rational and efficient manner. The first system is in operation, and through organized teamwork and synergy, several areas—including online monitoring, on-site analyses, autonomous microscopy, biomass and omics characterization, TEA, and LCA for the cultivation of mixed microalgal communities—have been made operational systemwide. Through implementation of online sensors and analyzers, systemwide long-term monitoring and characterization have made this project greatly successful by achieving the goal of having effluent phosphorus.

- This project stands out from many in the portfolio in that there is a clear connection to an industry partner who is already using the base algae growth system to capture organic P from wastewater. The system is in operation at one small wastewater facility and under construction at two more in the same state. The project appears to have increased the reliability of this system with real-time monitoring of the community composition and environmental conditions. The team has gained knowledge on how to tweak the system if they see evidence of an imminent crash such that they can favor the target algae species. Project management was well laid out and is split between four teams, spanning a wide range of expertise. They appear well equipped to reach the project goals. The presenter explained that since the wastewater industry is highly risk averse, they have many industry and municipal advisors giving oversight to ensure they end up with a system that can be expanded nationally once it proves reliable. The project has exceeded its goals to date.

- The approach of developing real-time tracking of the microbial community and system control has substantial merit and significant potential to reduce phosphorus effluent in municipal wastewater. The project team has been working for 1.5 years on the project and seems to have made excellent progress in addressing project goals. The project team has met their go/no-go milestone for predicting effluent phosphorus, energy consumption, and biomass yield (i.e., amount of algae produced). The software developed in this project has allowed the ARTiMiS to approach 86% accuracy for species-level classification of industrially relevant microalgae, which is impressive. The overall potential impact of the project on other high-phosphorus-level areas seems significant, and it seems likely that the developed technology will be deployed to other wastewater treatment sites.

PI RESPONSE TO REVIEWER COMMENTS

- Response to Comment 1. We thank the reviewers for their supportive comments. With regard to risk management, two of the core risks are (1) if the system were to demonstrate completely stable performance with no variability or upset events (we cannot cause failure or intentionally undermine performance at an operating wastewater treatment plant) or (2) if we are unable to implement proposed process controls (due to perceived risk or inability to change at the Village of Roberts wastewater treatment facility). With regard to the first risk, the variability in performance of the upstream system has already resulted in adequate performance variability (and significant microbial community structure dynamics) that is enabling robust model calibration/validation, autonomous microscopy system development, and meaningful insight into full system functioning. With regard to the second risk, we have observed that executable process controls (e.g., increasing alkalinity, doubling the single-pass retention time in PBRs) have resulted in significant improvements in process performance and stability, as evidenced by the recent 3 months of winter performance with effluent total phosphorus below 0.03 mg P/L. Given these achievements to date, we are confident the existing system in the Village of Roberts provides adequate flexibility to mitigate these risks. Areal productivity over time is influenced by both the performance of the system and the influent phosphorus concentration. Although cultivation is phosphorus limited, phosphorus in significant excess can also undermine performance because it eliminates the selective pressure that drives stable system performance (i.e., P-available conditions in the mix tank and P-limited conditions in the PBRs). As recommended, we will more clearly link areal productivity to wastewater composition and two of the key drivers that have been identified—ratio of ammonia-oxidizing bacteria to nitrite-oxidizing bacteria and the loss of *Scenedesmus* sp. We will also link these events with the biochemical composition of the harvested biomass and its implications for downstream end uses.
- Response to Comment 2. We thank the reviewers for their positive comments. Moving forward, we will more clearly link operating data to BETO's goals for algal biofuel production. As a starting point, we will more clearly report areal productivities (e.g., we achieved high-performance periods with >40 $g/m^2/d$), and we will include conversion to biofuels and bioproducts in our TEA and LCA.
- Response to Comment 3. We thank the reviewers for their positive comments.
- Response to Comment 4. We thank the reviewers for their positive comments.

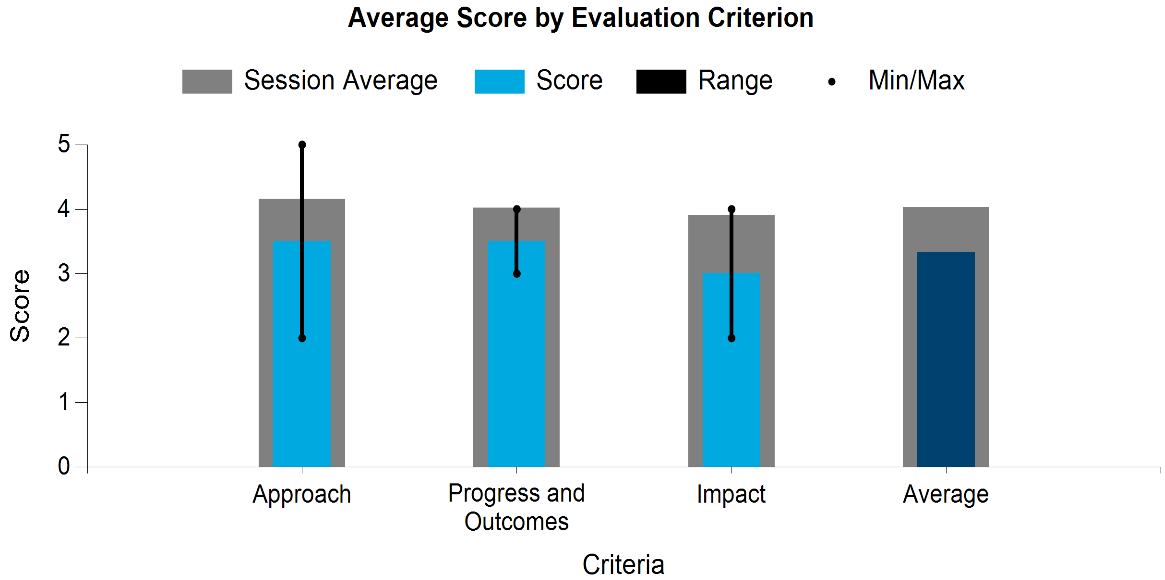
SYNERGISTIC MUNICIPAL WASTEWATER TREATMENT USING A ROTATING ALGAE BIOFILM REACTOR

Utah State University

PROJECT DESCRIPTION

WBS:	1.3.3.002
Presenter(s):	Ronald Sims
Project Start Date:	10/01/2020
Planned Project End Date:	05/31/2024
Total Funding:	$1,947,175

This project is a partnership among three industries, PNNL, and Utah State University to test a pilot-scale (1,100-gallon) algae biofilm platform—the Rotating Algae Biofilm Reactor (RABR)—at the largest municipal reclamation facility in the state of Utah (Central Valley Water Reclamation Facility [CVWRF]), treating anaerobic digester effluent to remove nutrients through cultivation of biofilm microalgae and to transform the harvested biofilm into biofuels and bioplastic. WesTech Inc. manufactured the RABR, and Algix will transform the feedstock biofilm into bioplastic. The six objectives of the project are to (1) increase the energy efficiency for total phosphorus and nitrogen removal, (2) improve the yield of biofilm indoor/outdoor tests, (3) improve the cost of total phosphorus and nitrogen removal, (4) meet the total phosphorus target after remediation, (5) produce bioplastic and TEA/LCA data, and (6) produce a TEA with three pathways for conversion of biofilm algae to bioproducts, including biocrude with HTL, bioplastic, and biofuels and bioplastic, and produce an LCA. The project approach includes four elements: (1) contribute to DOE's long-term vision of expanding the domestic resource potential of the bioeconomy by utilizing existing infrastructure to create a low-cost supply of algae biomass at water reclamation facilities nationwide; (2) produce renewable biofuels and bioproducts from biofilm algae using anaerobic digester effluent at CVWRF; (3) reduce the concentrations of phosphorus and nitrogen in the anaerobic digestate; and (4) apply the RABR technology for both cultivating and separating polyculture microalgae biomass from anaerobic digester effluent. Results from the first two budget periods that span from 10/1/2020 through 2/28/2023 include the 15 milestones in the statement of project objectives (Budget Period 3 is scheduled from 3/1/2023 through 5/31/2024 to address the remining 15 milestones). Results show that 14 milestones achieved target values that addressed biofilm yield and energy and cost reductions compared with baseline values for struvite formation and characterizations of the feedstock and RABR biofilms using microscopy and genetic testing. Milestone 5.1, producing 20 pounds of pilot RABR biomass (dry weight) has been delayed for 2 months (from February to April 2023) due to scheduling of the implementation of the mechanical infrastructure of the pilot RABR. This adjustment of the schedule for Milestone 5.1 does not impact the ability to achieve any of the other milestones of the project, including Budget Period 3. All of the targets identified for the go/no-go decision (Milestones 2.5, 3.1, 4.1, and 7.1) and the review meeting for the intermediate verification held on 1/26/2023 at the host site were achieved.



COMMENTS

- The goal of the project is to test a field-scale outdoor pilot RABR to remove total phosphorous and nitrogen from an effluent treating municipal wastewater and produce biofilm biomass for biofuels and bioplastics. The outcome of the project is to reduce phosphorus concentration by 70% and obtain a biomass yield of 2.4 tons per million gallons of treated water while lowering treatment costs.

- A management plan was outlined while leveraging the team's previous experiences in this project. Risk and mitigation strategies were identified. A task structure was proposed with quantifiable go/no-go decision points.

- The team has made progress in demonstrating productivity that exceeds their target of 7.5 g/m^2/day and proposes to implement TEA and LCA to help guide their work. The project performer should clarify the robustness of the harvest and seeding process, the impact on nutrient uptake, and how this technology can be scaled up. It is also unclear from this work what results in low nutrient uptake, given that contamination is not a challenge with biofilms. Overall, successful deployment of these types of technologies will not only be a huge benefit to wastewater treatment, but will also provide low-cost algal biomass if the cost of heating and raw materials are optimized.

- This project aims to utilize a RABR to remove nutrients, mainly phosphorus and nitrogen in the effluent water coming from the anaerobic digester of the municipal wastewater reclamation facility. The project aims to utilize the biomass from the algal biofilm for biofuel and bioplastic production in the downstream process, but not much information or data was provided related to how much initial progress has been made with the HTL process at Utah State University or Algix in the past 1.5-year time period that addresses BETO's goal for sustainable biofuel. The team has been quite successful on lowering the total phosphorus concentration in the effluent from the digestor by 47% and reducing the energy or power consumption cost for the RABR technology, as well as producing a significant amount of algal biomass. The optimization for biomass productivity needs to be closely addressed, as the current setup seems to be very susceptible to technical hiccups such as condensation and other related issues. A concern here is the use of plastic covers that can make the operational system more susceptible to microbial infestation and challenges as opposed to having a metal shed. This would bring up the cost for the setup but would provide sustenance through the different seasons. The genomic and taxonomic analyses for determining the composition and structure of the active algae-bacteria biofilm have made good progress for identifying the most dominant species at both lab-scale and outdoor experiments. Sharing and discussing data on establishing this co-relationship and parity between the outdoor and indoor RABR setup will be critical to understand the robustness of the system. There was information on how the team coordinates the work with student researchers, but mentioning how the work synergy is maintained with outside units and partners would provide the complete idea on workflow.

- This project demonstrates a RABR to capture nutrients from a wastewater facility in Utah. The idea behind the technology is very promising: a unique algal growth platform that, if done right, could bypass some of the risks associated with open raceways. The source algae for this project were collected on-site from a biofilm naturally forming on vent surfaces adjacent to the RABR. The algae community that populates the reactor appears to be quite robust to environmental and biotic contamination. Pest management was not an issue according to the presenter. Algae harvested could be used for various products, and an industry partner (Algix) is online for using it for bioplastics. It is unclear what the management structure is. The system does not appear to be scalable as is and would need to have harvest become automated, but it was unclear what stage of development this is. One limitation to this is that it has only been demonstrated in one location, but if it is expanded to other geographies, a local algae source would be used. The risk is that some of the knowledge gained in this project would not apply elsewhere. This project appears to be making good progress toward its quantifiable goals. Several risks were identified, mostly around engineering issues, and it is unclear how economic the proposed mitigation strategies are, especially if this system is scaled up.

- In its current form, the proposed RABR technology does not seem to advance the state of the art in municipal wastewater treatment. The project is on schedule and seems to have met targets on productivity, energy consumption, and removal of phosphorus. The risk mitigation strategies involving the plastic polycarbonate cover to control for temperature and wind effects and the use of electric heaters to control for condensation on the cover would seem to be impractical when scaling the RABR technology. The overall impact on the wastewater industry would seem to be minimal given the current method of scraping algae biomass off the rotating platforms. Other more scalable solutions should be explored for removing the biomass from the rotating platforms.

PI RESPONSE TO REVIEWER COMMENTS

- Our team would like to thank the reviewers for the engaged feedback regarding the project. As noted, the project met the interim go/no-go decision points for phosphorus removal and biomass productivity for the intermediate verification and will focus on robustness of harvest and the seeding process in the next phase of the project. Robustness of harvest is directly related to the sustainability of the cultivation surface, which is recycled post-consumer pop bottle plastic, and which has remained robust through the first 8 months of continuous pilot-scale operation using simple mechanical scraping of the surface layer of biofilm, leaving a residual layer of biofilm for continuous nutrient uptake. Robustness of seeding has been very good and was only required for the initial inoculum, which was sourced from the operating trickling filters at the host site, CVWRF. Because a residual biofilm remains on the cultivation material after harvest, nutrient uptake is also a continuous process. Regarding scale-up strategy, a full-scale RABR system for the treatment of a 0.6-million gallon per day side stream from CVWRF has been designed and was presented in a milestone report submitted to BETO with the diagram shown here. The design of the full-scale RABR system is based on discussion with project experts with CVWRF and WesTech. Project personnel also worked together to develop an economic analysis for this full-scale system, which was presented at the intermediate verification on-site meeting at CVWRF held on Jan. 26, 2023. While contamination was not a problem for the biofilm design, nutrient uptake observed and reported was low due to the low-temperature operations during late fall and winter in northern Utah, which is approximately 12°C–15°C. This temperature limitation will be eliminated at full scale because CVWRF will install temperature controls in 2024 as part of the upgrade to the entire facility that will maintain a minimum temperature of 25°C for optimizing other processes. The project staff will evaluate the anticipated improvement in operation and performance during the next phase of testing. The next phase (three) of the project aims to utilize the biomass to produce biofuel, through HTL, to address BETO's goal for sustainable biofuel and bioplastic production. The project team is addressing the technical hiccups, including temperature, through the upgrade to the full-scale CVWRF and condensation on the roof through construction of an improved greenhouse at full scale that will include a concrete base with improved ventilation and temperature control to provide sustainability through seasonal changes. The project will continue to evaluate genomic and taxonomic characteristics of the biofilm to understand the biofilm community structure and function. Work synergy is maintained through regularly scheduled weekly meetings, including a combination of Zoom for remote partners and in-person participation for local partners. Work synergy is also accomplished through both Zoom and on-site meetings among subgroups of partners; for example, coordination for genetic characterization between experts at PNNL and Utah State University; experts for HTL testing between PNNL and Utah State University; experts for wastewater treatment that include WesTech Inc., CVWRF, and Utah State University; and bioplastic production between Algix and Utah State University. This promising RABR technology project has demonstrated that pest management is not an issue due to the robust biofilm platform that can bypass some risks associated with open raceway contamination, as noted in the review comments, and limitations of light penetration through wastewater. Therefore, the scalability of the technology is the focus for the next phase of testing. The structural and functional components of bays, sections within bays, and cultivation shelves within sections, which were not discussed in detail, were developed as part of the initial RABR design with the end in mind for scalability and economy of operation. Determination of the full-scale size aspects, including physical components, automated harvesting, and cost of operation of a RABR system, is being undertaken during Phase 3, and is part of the TEA component. Based on the current pilot-scale unit, a full-scale RABR system has been designed based on discussion with project partner industrial experts, CVWRF, and WesTech Inc. Compared to the pilot-scale unit, the full-scale RABR system has a larger size for algae growth substratum, more frames per section, and supports for each rotating bay, which enables it to continuously treat 0.6-million gallon per day side stream water from CVWRF. Regarding applicability of the technology and RABR system to other sites, common characteristics of anaerobic digester effluent, which include high phosphorus concentration (50 mg/L) and high nitrogen concentration (500 mg/L), as well as warm temperatures (77°F), are typical and relatively independent of geographic location. The exception is the variable concentration of magnesium, which is necessary for struvite precipitation. Therefore, the host site (CVWRF) system is typical of municipal wastewater treatment facilities across the United Staes and provided the rationale for conducting the testing of the RABR system there. The economic aspects of a full-scale system are a focus of Phase 3, which includes a TEA. The current pilot-scale system treating 1,100 gallons of anaerobic digester effluent has provided insights with regard to temperature, wind, and harvesting challenges that are objectives for Phase 3 of the project, which is to develop solutions to these challenges. Temperature will be controlled at full scale with the plant for upgrade at CVWRF for other processes that will benefit the RABR technology, and therefore will not be an added cost. Wind will be addressed with greenhouse plans for the facility that will contain a RABR of 1.2 million gallons volume to treat a flow of 0.6 million gallons per day, which is considered viable for CVWRF and applicable to other water resource recovery facilities. Harvesting automatically remains the greatest challenge, and the project partner that manufactured the RABR, WesTech Inc., is working with Utah State University to identify solutions for testing and scale-up in terms of processes and costs. All of these factors potentially impacting scale-up are being incorporated into the TEA and LCA for a full-scale system. The comments of all four reviewers were very valuable and insightful and are currently being addressed and evaluated in Phase 3 of this project.

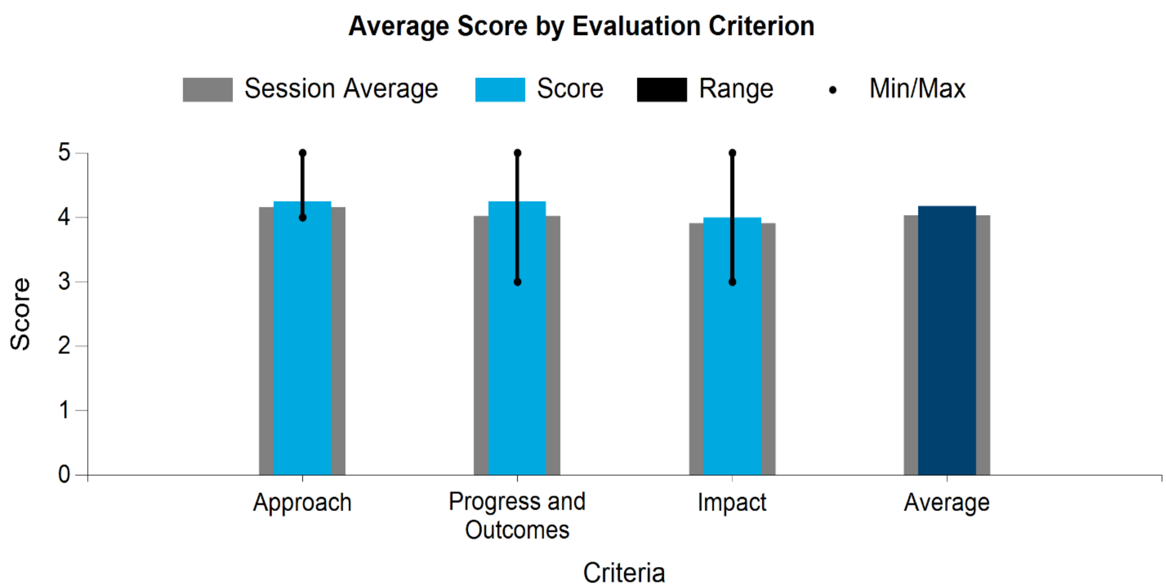
PRODUCTION OF ALGAE BIOFUEL WITH CO_2 DIRECT AIR CAPTURE

Global Algae Innovations

PROJECT DESCRIPTION

WBS:	1.3.4.001
Presenter(s):	David Hazlebeck
Project Start Date:	10/01/2020
Planned Project End Date:	12/31/2023
Total Funding:	$2,500,000

GAI has developed low-cost algae production technologies aimed at achieving commercially viable production of biofuel and high-protein meal. Radical advances have been designed and implemented throughout the entire process, resulting in many industry breakthroughs for large-scale algae cultivation, harvesting, and processing. In this project, CO_2 is directly absorbed from the atmosphere into the open raceways so that no separate CO_2 concentrating or distribution system is needed. The main objective of the project is to improve the economics of algal biofuel production by increasing the value of coproducts through improved separations and product development. A product spectrum was developed that doubles the value of the coproducts and has a market size commensurate with the biofuel market. The products are algae oil for fuel, PUs, and omega-3 oil; protein meal ingredients for food and feed; and nutrients recycled within the process. Many novel unit operations were developed for separation of the products. The focus of the remainder of the project will be on refinement of the downselected process flow and production of sample products for testing.



COMMENTS

- I agree with the author that there are a lot of unit operations to test and optimize, making the system quite complex. The complexity makes it more challenging to tech transfer the methods.
- There is excellent progress on the unit operation development.
- The outputs appear to include water in recycle nutrients. The mass balance does not sum up due to ash residues.

- The TEA looks very good at a net $226/metric tonne; the financial assumptions for cost of capital are reasonable. An unlevered internal rate of return of 20% is still quite good, depending on the underlying assumptions about land use (i.e., the land cannot otherwise be used for agricultural purposes, the land requires no utility from the local grid and can use solar power). Water rights may be quite complex to model depending on the location and could throw a real wrench into the TEA.
- More information on the new unit operations would be interesting.
- This project is aligned with the goals and is delivering against those goals. Concerns do exist, especially with product value. The claim that economics get better if higher-value products are made is hardly a revolutionary realization. Getting paid more for your products is always a plus.
- It is concerning that more products are the solution. Numbers of added unit operations add to complexity and capital cost. The fuel is the minor product by value. The data presented did not indicate careful examination of return on investment and thought about the complexity of marketing multiple products.
- The process patent count is big, but there must be a question about the true value created. The number of process patent applications was held up as a sign of progress. It does not come across as a compelling example of progress. One positive outcome of a patent is that it eventually becomes public information. It is concerning that these advances cannot be talked about for fear of wrecking a patent, making it impossible for us to review the novelty or impact. There are negatives. The biggest one is that process patents are difficult to police and frequently easy to circumvent. The patents were described as being in oil purification. This is a well-populated area due to the history of seed oil purification. It seems unlikely that there is an area of huge novelty remaining.
- Approach: The primary objective for this project is to develop a TEA-guided integrated process based on various novel unit operations that serve to lower the overall algal biofuel selling price by increasing the value of recoverable coproducts. This objective is in line with BETO goals. The project partners represent a good mix of companies for testing the coproducts for quality and marketability, but such testing has not yet commenced.
- Progress and Outcomes: The team indicated that an integrated process with 26 patentable steps has been developed that allows for the production of multiple coproducts (biofuel, plastic polymers, omega-3 oils plus glycerol, and protein [both concentrate and meal]), and which also claims credit for nutrient recycle. The combined value of these products was double the overall value of the original mix of biofuel plus protein meal. The presentation did not indicate a comparison of the combined cost of manufacture for the new suite of products versus that of the original products, so it's not really possible to say how much the net profit potential increased as a result of the new process, which is a more important metric than total selling price of the coproducts. In addition, it looks like the team is assuming that no substantial losses of material occur during this complex processing scheme, which is not realistic when the quality requirements for most of these products will dictate relative high purity levels.
- The coproduct suite shows that biofuel represents 17% of the biomass ash-free dry weight, and the mass balance indicates that the oil content of the harvested biomass is 50% of the ash-free dry weight (and fractionated oil was 40% of the ash-free dry weight after processing). Thus, the process as outlined does not appear to achieve the end-of-project milestone specifying that 50% of the oil should go to biofuel.
- Although favorable numbers were presented with respect to the enhanced economic value of the overall process, it is difficult to assess actual progress toward a favorable commercial outcome without a better understanding of the claimed unit operation enhancements and whether the higher-value products that contribute the most to the favorable economics have the characteristics, quality, and cost of manufacturing that would truly enable market entry. Although it is recognized that product testing can be

a long and expensive process and is planned in the remaining time of the project, it would have been highly desirable for the project team to present more information on the basic chemical nature of the multiple product streams and how those compare to existing products on the market.

- Information was not provided on one of the stated goals of the project, i.e., the mitigation of losses in DAC-maintained ponds after a rainfall event.
- Impact: The project team has developed a process that they feel would improve the economics for producing biofuels at large scale (reaching the selling price target of $2.50/GGE), primarily through coproduction of higher-value products. This will be an important outcome if the overall process is in fact economical and the markets for the resulting coproducts are robust. Currently, it is difficult to assess the real impact since the claimed improvements were not described due to patent applications that are expected to be filed.
- Approach: Good plan, good goals; not working with collaborators yet; risks were identified and the plan to address them is good. Outcomes: Lots of outcomes reported, but no data were shown, so it is difficult to know what has been accomplished. Impact: No plan for dissemination to industry weakens the impact.

HIGH-PH/HIGH-ALKALINITY CULTIVATION FOR DIRECT ATMOSPHERIC AIR CAPTURE AND ALGAE BIOPRODUCTS

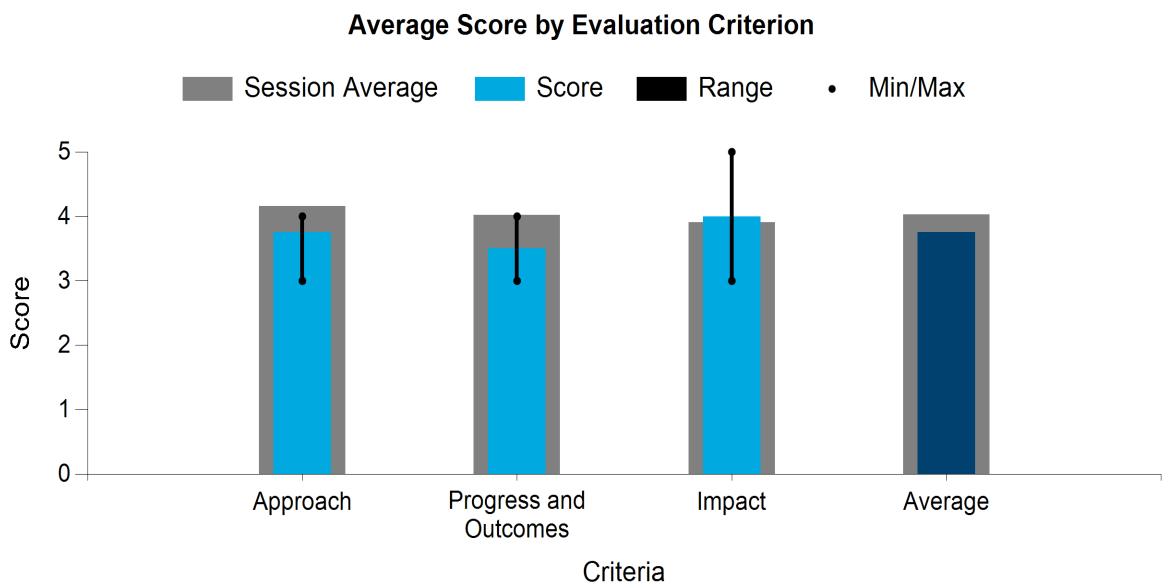
Montana State University

PROJECT DESCRIPTION

WBS:	1.3.4.002
Presenter(s):	Robin Gerlach
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2024
Total Funding:	$2,530,300

For algal biofuels to replace fossil fuels, it is imperative that cultivation systems are not constrained by the proximate availability of flue gas or other high-concentration CO_2 sources or the energy and infrastructure burden to deliver CO_2 over long distances. This project builds on our significant prior experience of cultivating algae in high-pH and high-alkalinity environments with enhanced DAC to produce fuels and high-value products. This project intends to test and improve a novel open-pond reactor design and facility operations to enhance CO_2 mass transfer from the atmosphere, as well as to improve cultivation-based strategies (nutrient supply, media recycling, and microbiome control) to optimize biomass productivities, biomass quality, and culture stability. The resulting biomass is being converted by pyrolytic fractionation into fuels and algae-based materials for the automotive and packaging industries, as well as for energy storage applications. Integrated TEAs provide feedback and forecast scenarios for different design and operation scenarios of prospective algal biorefineries.

This work contributes to eliminating the need for costly, external CO_2 supply, thus allowing for more flexible siting of algal production facilities capable of generating high-value coproducts using novel conversion pathways to improve the revenue potential of algal biorefineries.



COMMENTS

- Thank you for the org chart and communication slides; they are very helpful in understanding the management structure and function. The risk mitigation slides didn't clearly identify any risks or strategies.

- The bacterial oxidative stress hypothesis is interesting; it would be helpful to know iron levels in the media versus in bacterial transferrins to determine the feasibility of this hypothesis.
- The timeline was not provided for comparison.
- The engagement with Sonoco would be helpful to have more details on, as it is critical to the impact analysis.
- A useful experiment to demonstrate the actual contribution of the bacterial populations would be to individually add back isolates; even at shake flask scale, this would be a fairly simple factorial. Is it part of the plan?
- Power draw should be straightforward to measure and compare to the paddlewheel design.
- The team addressed all stated goals except the last presented, which is in the next phase.
- The power for the belt mixer and the advantages it creates were not well described. Just because something is different doesn't automatically mean it is better. The lack of clear metrics around why some changes were being made is a bit troubling. Additionally, the impact on capital cost and reliability was not even mentioned. It is different, but it is not clear what optimization variable drives adoption.
- Approach: The approach being taken to increase productivity while using DAC of CO_2 seems reasonable and is aligned with BETO goals. The multisite and multidisciplinary project team is well suited for conducting the different aspects of the project, although the interrelatedness of the subprojects is not always obvious. Communication and coordination between team members appears to be set up appropriately.
- Progress and Outcomes: The combination of a new pond mixing system and media carbonization/recarbonization with DAC shows promise, although the potentially higher energy requirements for the belt-and-cleat system need to be better understood because the increases in productivity and carbonization rate are not large.
- Very little information was provided regarding pyrolysis of algal biomass and PU foam production from algal biomass/oil. It would have been useful to show the chemical composition of the pyrolysis oil, as well as a comparison of the properties of foams made from polyols derived from algal oil, cottonseed oil (previous work from this lab), and petroleum.
- The TEA/LCA slide was not well explained; it was quite generic, and it wasn't clear whether it was showing past work based on typical growth systems or on the system under development in this project.
- Some academically interesting results regarding microbiome population dynamics were presented, although the connection of this work to the rest of the project was not obvious.
- Impact: It remains to be seen whether the belt-and-cleat system provides an advantage over typical paddlewheel-mixed ponds, but it is worth exploring in order to see if there are significant productivity increases and associated cost reductions.
- Although academically interesting and solid work, it is not obvious that significant value is being added to the overall project through the various analyses of the culture microbiome (e.g., metabolic modeling, population dynamics), especially because a clear benefit attributable to the microbiome has not been demonstrated in their system.

- Approach: Seems reasonable; however, I'm not sure how far this is moving the needle due to low technology readiness level. Outcomes: Goals have been met; it would be a good addition to the project to address the energy needed for mixing. Impact: The ponds are too small to have any relevance as far as the impact of a new design.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their comments and questions. Regarding the identification of risks, it became clear that the two major risks identified were not stated explicitly. The risks were (1) a loss of expertise in a key area, and (2) the possibility that the belt-and-cleat ponds will not result in increased recarbonization efficiency and/or biomass productivity. We agree that while the risks were not stated explicitly, the mitigation strategies were presented. The mitigation strategies are (1) "overlapping (and redundant) expertise and capabilities" and (2) "multiple strategies to achieve improvements in productivity" (which could each, separately, lead to the desired productivity improvements), including multiple possible recarbonization strategies (see below for details). The iron levels in the media are initially 0.018 mmol/L. The pH increases to 10.3 during cultivation, which will lower the solubility of Fe. Hence, we add chelated iron, and while we do not know whether the chelation holds at high pH, we have never seen an indication of iron limitation. Both Montana State University and the University of Toledo have, over the years, performed multiple iron add-back experiments with different iron sources but have not seen any growth enhancement, indicating that the cultures are not limited in iron. We have also tried to limit iron levels for work with microbiomes, but it has been difficult to achieve conditions in which iron is the limiting nutrient. The hypothesis regarding reduced oxidative stress was based on the observation that higher carbohydrate levels were detected in SLA-04 grown at high-pH and high-alkalinity conditions. Additionally, the metabolic modeling indicates that some of the bacteria in the microbiome might be involved in the mitigation of oxygen stress during the daytime and during glycolate degradation. Regarding the engagement with Sonoco: The team is in communication with Sonoco. We are still working toward generating appropriate size foam structures that Sonoco can test for thermal conductivity. However, we did test the small sample for density, and we are getting closer to the metrics provided by Sonoco. Regarding the noted usefulness of "add-back" experiments, the team has indeed conducted such experiments and has not observed any negative effects. We have had an occasional indication of a positive (i.e., growth-promoting) effect, but the effect has not been reproduced consistently. We are considering additional experiments to reproduce these results and scale up the size of cultivation systems as appropriate. Detailed results so far are available in the quarterly reports. Work is continuing, and a probiotics-based growth enhancement strategy is still being pursued for our SLA-04 cultures. We are planning to continue research in this area throughout the duration of this project and will report on this as appropriate in quarterly reports. The comments regarding the belt-and-cleat ponds are appreciated, and as indicated above and detailed below, we have multiple strategies outlined to potentially improve recarbonization efficiency in this project. The belt-and-cleat technology is just one of the approaches being evaluated as a promising alternative to the paddlewheel technology. The belt-and-cleat technology is new (project start: technology readiness level 3; expected project end: technology readiness level 5), and the principle is conceptually sound based on computational fluid dynamics modeling. Hence, the belt-and-cleat mixing technology has been a focus in this project so far. Distributed mixing with the angled cleats should increase surface renewal and thus CO_2 mass transfer from the atmosphere into CO_2-depleted cultivation medium. Our current comparisons are based on small-scale ~30-L raceways. While simply measuring power usage is indeed straightforward, the fact that we are using small (~30-L) raceways with oversized paddlewheels and cleats relative to what will be necessary in larger-scale production ponds complicates scalable comparisons between the belt-and-cleat design and the paddlewheel design. In general, the motors are also oversized for the raceway paddlewheels and belt-and-cleat system. We are discussing with the belt-and-cleat patent holder whether we can use his larger-scale prototypes (~10-m^2 surface area, ~2,500-L volume) for comparison tests, which would also include power consumption measurements. The metrics for the project are to compare the efficiency of belt-and-cleat versus paddlewheel ponds at equivalent power usage, and we are working toward a valid and defensible experimental comparison. Furthermore, as a measure of potential risk mitigation, the evaluation of alternative recarbonization strategies is built into the project if the belt-and-cleat technology does not prove to be an improvement for the high-pH, DAC algal cultivation approach. These alternative recarbonization strategies include designated recarbonization ponds (with paddlewheel, belt-and-cleat, or other mixing technology), membrane-based recarbonization, the use of absorber columns, and cascading flow. It is indeed a goal of the project to estimate the costs (both capital and operating costs) and compare them to paddlewheel-based pond designs. We appreciate the comment regarding the interrelatedness of the tasks and want to remark that each strategy we are pursuing has the potential to be implemented independently into the overall process of algal growth and post-processing of algal biomass. Each of the strategies pursued can result in an improvement in overall economics. Hence, the team we have assembled with its diverse backgrounds and areas of expertise is pursuing research independently, yet in a coordinated and complementary fashion, with the goal of providing more than an incremental step change as an overall outcome of this project. We are aware that only little information was provided regarding the details of the pyrolysis of algal biomass and PU foam production. Work on these tasks is ongoing. Information regarding the chemical composition of the pyrolysis oil and comparisons of the properties of foams made from polyols derived from different sources will be forthcoming. We have met our milestones and reported our progress in detail in the quarterly reports. More detailed information will be forthcoming in future quarterly reports, as well as during the next Peer Review meeting. Reviewers commented that the one TEA/LCA slide was perceived as generic, but we would suggest that this is a function of the need for brevity in these very short presentations. The LCA/TEA framework developed in this project is actually quite unique in that it involves a stochastic simulation that incorporates variability in weather conditions (temperature, solar irradiance, wind, and humidity) and market prices (using biodiesel prices as a proxy for algal biofuels) and does so at a daily time step. This allows for a more highly resolved characterization of variability in algal and algal biofuel production, as well as the corresponding impact on revenues, that then lead to an improved understanding of the financial risk that variable weather and market conditions can impose—an important criterion for investors and one that is garnering increased attention. Further research will involve the development of strategies for managing this financial risk via a combination of reserves and a novel index insurance product, with the latter being particularly interesting given the U.S. Department of Agriculture's recent designation of algae as a "crop" for the purposes of their subsidized crop insurance program. The stochastic nature of this LCA-TEA modeling platform and its focus on financial risk (both characterization and management) make it quite distinct within the biofuel space and should produce results that are both novel and of practical use to algae producers.

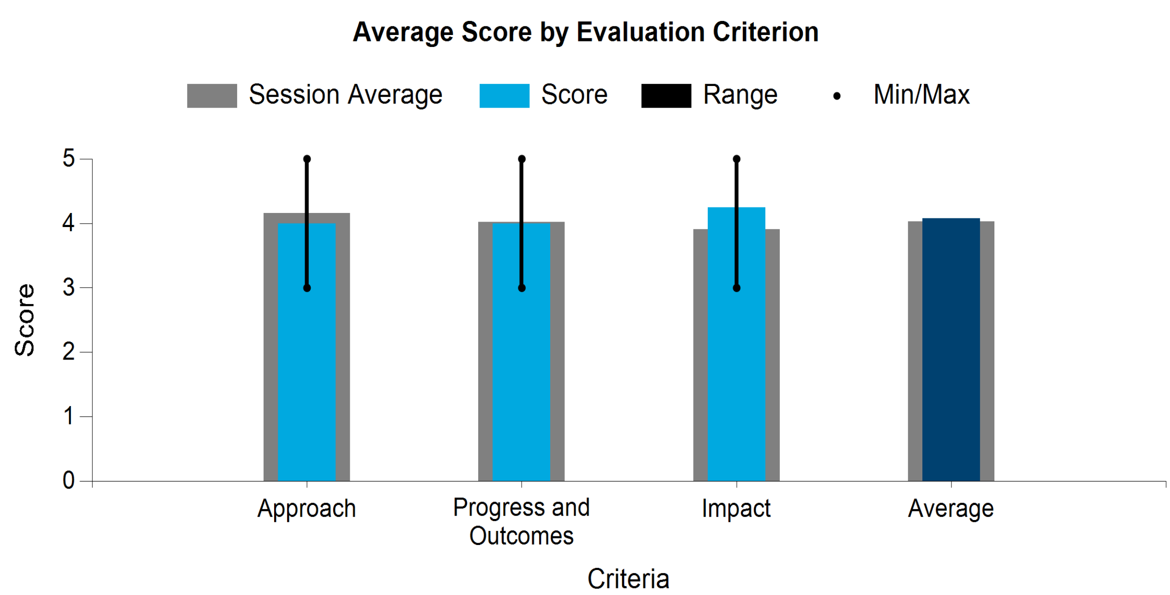
ARIZONA STATE UNIVERSITY'S DAC POLYMER-ENHANCED CYANOBACTERIAL BIOPRODUCTIVITY (AUDACITY)

Arizona State University

PROJECT DESCRIPTION

WBS:	1.3.4.003
Presenter(s):	Willem Vermaas
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2023
Total Funding:	$2,505,394

This project develops the AUDACity system, which efficiently delivers DAC CO_2 directly to cyanobacteria using moisture-driven sorbents and will demonstrate it in outdoor raceway ponds. AUDACity is used to cultivate *Synechocystis* sp. PCC 6803 strains developed at Arizona State University that have been engineered to excrete laurate or methyl laurate ($1/kg) and that contain phycocyanin (PC), a high-value natural dye for food and cosmetics ($1–$200/kg). The TEA of AUDACity shows that *in situ* methyl laurate harvesting can increase the fuel yield 2.2-fold over green algae, and fuel can be produced for $2.50/GGE when selling a coproduct valued at $6.30/kg, equivalent to whey protein. PC can be extracted in high yield and purity and was found to be stable at 100°C when dry. Synthesized and commercial DAC polymers have been shown to capture and rapidly deliver 90% of their CO_2 capacity in less than 1 hour. AUDACity was used to cultivate *Synechocystis* at lab scale using synthesized and commercial sorbents with productivities of 115–120 mg/L/d. Challenges include developing DAC polymers that rapidly capture and release CO_2 and that are biocompatible. Biocompatibility has been mitigated in part through adaptive laboratory evolution to select for strains that better tolerate the polymers. AUDACity impacts include reducing cost (projected <$50/tonne CO_2), reducing carbon intensity (from DAC CO_2), and eliminating ~70% CO_2 losses from sparging.



COMMENTS

- Communication with related projects or within the project is unclear.
- The scientific progress looks great. The team next needs to address extractables/leachables from the polymer due to the toxicity observed in the first immersion, as the fate of the extractable/leachable

material could have an impact on nutrient recycling and product adsorption of the material. For this reason, it may make more sense to stick with commercial anion exchange resins.

- There is no mention of whether the end product yields increased linearly with optical density in 96-h cultivation.
- The market size for blue dye should utilize current market size and make standard consumer product assumptions for market penetration unless there is an explicit reason to justify a different number.
- I have some reservations about PC carrying such huge value. That said, the project delivered against the stated goals, finding, perhaps, the proverbial "goose that lays the golden eggs," or at least an algae that lays blue eggs.
- PC is already a material of commerce. The presented economics are not overly compelling. A high-value coproduct will always improve economics. I remain skeptical simply because the disparity in cost and scale are so great between fuel and PC. I certainly believe an analysis with PC as the main product and fuel as a coproduct is warranted. This could be a good steppingstone to grow cultivation provided the market is truly underserved.
- Approach: The team is working to develop a CO_2 delivery system for algal ponds based on DAC that comprises polymer sorbents that capture CO_2 when dry and release the bound CO_2 when wet. A belt system that enters and exits the pond allows the polymer wetting and drying phases. This goal aligns with BETO goals to reduce the cost of algal biomass and biofuel production.
- Progress and Outcomes: A methacrylate-based polymer was synthesized and tested for CO_2 absorption and release kinetics. This was then tested (along with Excellion commercially available CO_2 sorbent sheets) to measure the growth of algae supplied with CO_2 in this manner. Although both materials supported growth of *Synechocystis*, growth proceeded for a longer time with the commercial sorbent.
- The methacrylate polymers were shown to be inhibitory to cyanobacterial growth, so effort was put into adapting the strains to the polymers using adaptive lab evolution. The mechanism of toxicity is not known; it would be useful to know if the polymers themselves were toxic or whether unpolymerized monomers or crosslinkers or other polymerization additives were the problem. For strains that appeared to develop a tolerance, it would be important to know whether the reported tolerance was a stable genetic change or simply a transient adaptation.
- Testing of the AUDACity system indicated that it is important for the sorbent belt to dry completely in order to reabsorb additional CO_2, requiring a longer belt. Problems were also encountered with nitrate in the medium binding to the sorbent instead of CO_2; it was not discussed whether other anions (e.g., chloride) would render this system unusable for saline or brackish waters, but this may be an important consideration. These issues would need to be resolved in an economically feasible manner before it could be deployed in commercial systems. (It is noted that a mitigation strategy is outlined to overcome DAC polymer fouling but has not been tested yet.)
- PC was examined as a higher-value coproduct to support biofuel production economics. Notably, the TEA and LCA indicate PC production of 40.5 tons/day, or >12,000 t/year. This amount greatly exceeds all forecasts for the PC market size over the coming decade, so the suitability of PC as a coproduct at the scale used in these calculations is questionable. (Note that the output values on the TEA/LCA slide also seem off, in that it reports 12,000,000 GGE/year of methyl laurate, but less than 10 GGE/year of biodiesel and naphtha.) Additionally, some of the energy or mass inputs are marked as 0, which also may be errors.

- Impact: Development of a low-cost CO_2 DAC system is certainly well aligned with BETO goals for reducing the cost of algal cultivation via lowering the cost of supplied CO_2. On the surface, however, the AUDACity system looks like it might be expensive to construct and implement, but the TEA slide presented didn't break out capital and operating costs for the system, so it's hard to assess. Considering the issues that have been seen in this project and the small amount of time remaining for the project, it will be difficult for the investigators to fully accomplish the most important goals of the project.

- Approach: Good project plan, good risk identification and mitigation plan, no management plan presented. It is not the correct approach to adapt an algae to a toxic substrate instead of switching to a nontoxic substrate, since one is available. Outcomes: The team is meeting goals, and research is going well. PC extraction is being done commercially, so this technology development is not a great addition to the project. Impact: If the technology works to catch CO_2 and supply it to the cultures, that is going to have a large impact. It seems like this technology would not be hard to implement. As part of project outcomes, I would like to see more on dissemination of information and how the CO_2 absorbent could be sourced (if it can). The PC work is not impactful as this is commercialized already; however, it is a great coproduct to increase value from the biomass.

PI RESPONSE TO REVIEWER COMMENTS

- We kindly thank the reviewers for their time and feedback on this project. We apologize for not covering team management due to time constraints, but we do have a very interactive and complementary team. Coproduct PC value and market size: The PC value and market (12,000 tonnes per year) used in the TEA was the value for whey protein, which has a much larger market ($6/kg; market size: 173,000 tonnes per year), so it is independent of PC as a coloring agent. Despite the disparity in cost and scale between fuel and PC, PC production is a great economic incentive for a pilot-scale facility or first-of-a-kind plant. However, the differentiator between AUDACity and commercial PC suppliers is that we have filed IP on a method to increase PC thermostability (100°C), which may greatly increase its food coloring market. Also, no PC cultivator is currently converting spent biomass to fuels. We have conducted an analysis with PC as the main product and fuel as the coproduct. We based the value and market size for PC on that for brilliant blue FCF (blue #1), which is ~5,000 tonnes per year, valued at $10/kg. PC largely scales linearly with biomass. Coproduct methyl laurate: There are typos in Slide 16. All fuel outputs should be in million GGE per year. AUDACity uses genetically modified strains that excrete laurate and/or methyl laurate into the cultivation medium during growth. Zero energy is required for methyl laurate extraction because it is a passive sedimentation and coalescence process. Once the biomass is harvested and high-value products are removed, residual biomass can be converted to fuels using HTL. Methyl laurate production is higher than that of HTL-generated fuels. Methacrylate polymer toxicity and strain adaptation: We initially were unsure of why the methacrylate polymers were toxic, so adaptive laboratory evolution was used to select for strains that grew well in their presence. Sequencing and continued growth without the polymer (followed by polymer exposure) are being performed to distinguish between stable genetic changes or a transient adaptation. Strains selected by adaptive laboratory evolution may have enhanced tolerance to quaternary ammonium compounds, which will benefit cell performance with all DAC polymers. Subsequent nuclear magnetic resonance analysis indicated that the polymers degraded under alkaline conditions due to Hofmann elimination and hydrolysis, which may release toxic oligomers into the medium. Suitability for AUDACity in brackish water or seawater: Cyanobacteria can be acclimated to grow well in brackish water or seawater. While our CO_2 capture principles rely on ions on the sorbent, which could exchange with inactive anions in high-salt medium, coatings or polymer materials that repel water and salt can be used to minimize ion exchange during brief (10–40-minute) exposures to the cultivation medium during CO_2 delivery, where CO_2(g) and H_2O(g) can still exchange during capture and release. Achieving goals with remaining time: The team continues to evaluate the top-performing sorbents for cultivation at the scale of 1 g CO_2/day and is gearing up for an outdoor cultivation trial (Aug.–Sept. 2023) at the scale of 100 g CO_2/day. We will apply for a no-cost extension to perform a second cultivation trial.

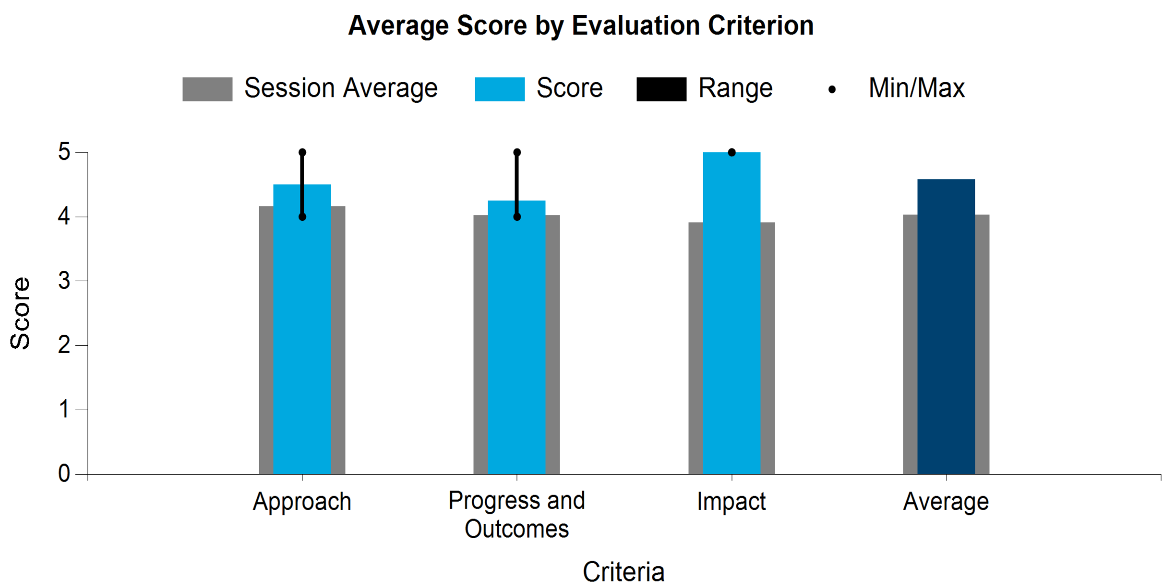
BIOMOLECULAR FILMS FOR DIRECT AIR CAPTURE OF CO_2

University of California, San Diego

PROJECT DESCRIPTION

WBS:	1.3.4.004
Presenter(s):	Michael Guarnieri
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2023
Total Funding:	$1,950,000

CO_2 delivery costs represent approximately 20% of the final biomass selling price in algal mass cultivation systems. Technologies that enable DAC of atmospheric CO_2 to decouple algae cultivation from CO_2 point sources thus present an opportunity to improve the economics and resource potential of algal biomass. Current DAC technologies typically employ amine- or caustic-based absorption, demanding significant water and/or energy inputs and incurring substantial capital expenditures. Conversely, bio-based approaches to DAC offer a means to bypass conventional techno-economic and sustainability hurdles. To this end, we propose to integrate recent advances in computational metabolic modeling, algal genetic engineering, algal cultivation, and algal biomass upgrading to enable secretion of CA for enhanced CO_2 capture and conversion in immobilized algal biofilms. Key challenges include genetic engineering of non-model microalgae for CA secretion and sustained, high biomass productivity in an immobilized biofilm. If successful, the resultant technology will present a first-in-class, no capital expenditure, renewable, bio-based approach to DAC and will ultimately increase the delivery and utilization of CO_2 by >20% relative to the current SOT. To date, we have achieved >50-g/m^2/day areal productivity from atmospheric CO_2, exceeding end-project goals and establishing a first-in-class, high-efficiency algal production system.



COMMENTS

- The approach using metabolic modeling to identify which proteins to target for redesign is good. *In silico* modeling is important.
- Densitometry is not a good quantitative measurement; it's better to use ELISA or an activity assay. The milestones are clearly identified and appropriate.

- Model first, then get data to validate the model. Task 6 should be earlier.
- Good job on the CA expression; the authors have a nice cassette system that is working well. I would have liked to see some molecular modeling to explain the decision of which CA to express and how to benchmark it against the bovine version they started with.
- There is no mention of risk mitigation strategies or whether they had to be deployed to stay on track. There was no mention of communication management.
- There is a clear connection to outcomes, particularly with polymer products and the DAC improvements and their financial impact.
- I wish the pond outcomes were more linearly scalable, but this is a known issue of transitioning to pond culture.
- What are you using for contamination control?
- A sensitivity analysis in the TEA to define the cost difference due to outdoor cultivation permitting processes would have been helpful to understand the applicability to other projects.
- Recent go/no-go review strongly indicates progress is being made against goals. Efforts are in line with stated goals and are showing progress. The project is early but has made commendable progress. Several positive results in different project areas show that the team is working well together across geographies.
- Approach: This project combines specific expertise and complementary knowledge and capabilities from several companies and institutions to address a number of important questions and objectives regarding algal biofuel production and DAC of CO_2. The activities of the different labs and companies seem to be well coordinated, and the interactions between the labs appear to be robust and productive. The TEA indicates that the team is on the right track to lower fuel production costs significantly via this approach, which is a key BETO goal.
- Progress and Outcomes: The metabolic model that was generated for *Picochlorum* has provided useful information on the presence of various biochemical pathways, and has also identified specific signal peptides that target native proteins to various locations in the cell, including extracellular secretion. This information will inform not only construct engineering for CA secretion, an immediate goal of this project, but also future metabolic engineering approaches for enhanced growth and product formation.
- Use of a common yeast expression system allowed rapid production and testing of numerous CAs from various sources under typical growth conditions associated with algal cultivation. This provided a shortcut for selecting and designing a heterologous gene for expression in *Picochlorum*. The summary slide indicates that the NREL researchers successfully engineered *Picochlorum* to secrete CA, but data were only presented for GFP secretion, so perhaps the CA expression results were still considered too preliminary to present.
- The team demonstrated an impressive increase in productivity in the RAB system when CA was added. This is an important outcome, assuming that the amount added was similar to what can reasonably be achieved via secretion of a heterologously expressed CA by an algal production strain. The activity and stability of the secreted CA in an open system will be critical information to understand, which will help to define the proper expression level needed (i.e., high enough for maximal ambient CO_2 use efficiency, but not so high that overall cell growth potential is limited).

- Finally, algal biomass from the RAB system was shown by Algix in preliminary testing to have promise as a feedstock for plastic composite production. Such coproduct formation will be important to lower the overall costs associated with algal biofuel production.
- Impact: The project team is making very good progress on enhancing atmospheric CO_2 update by algae in a commercially relevant production system. Successful completion of the project will advance the field and help BETO meet their current goals.
- Approach: Missing risk identification and mitigation. Are there any collaborations? Outcomes: Seem to be on or exceeding targets. The work on product quality is important, and increases impact. Impact: Seems relevant and impactful.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their time and constructive feedback. We agree with the reviewers' assessment that metabolic modeling presents a powerful approach to identify rational strain and protein engineering targets, and we will continue to refine and validate our models in the year ahead. Regarding protein quantitation, we agree that densitometric analyses are insufficient; we have completed activity assays and complementary mass spectral analyses in Budget Period 2 to quantify secreted protein concentration more accurately. Regarding CA downselection, we designed over 20 CA variants; top candidates demonstrating activity in seawater were transformed into a *Pichia pastoris* expression and secretion system to rapidly assess eukaryotic secretion capacity, followed by activity assays of culture supernatants. CA variants that retained activity in media and displayed active secretion capacity in *P. pastoris* were then incorporated into *P. renovo*. Growth assays under limiting CO_2 and CA activity assays on cell lysates served as a final downselection metric. Predictive modeling of protein secretion networks is currently being leveraged to further enhance the secretion capacity of three top-candidate variants. Regarding contamination control, this is outside the scope of the current proposal. However, we note that we have achieved biofilms comprising >90% of our target cultivar (as assessed via quantitative phylogenomic sequence analyses) over the course of >3-month cultivation trials. We hypothesize that this is, in part, due to the unique capacity of our engineered *P. renovo* to sequester and grow on atmospheric CO_2. Per the reviewers' suggestion, TEAs will evaluate additional costs associated with TERA permitting in outdoor cultivation configurations. As indicated by reviewers, all milestones and deliverables are on track for Budget Period 3. We have made substantial progress to date, including achieving >40-g/m^2/day productivity in RAB systems on atmospheric CO_2 (exceeding go/no-go target metrics). Initial efforts in Budget Period 3 have already indicated that our CA-secreting variants of *P. renovo* display a growth enhancement on RAB systems on atmospheric CO_2 relative to wild-type cultivars, indicating that we are achieving effective CA enzyme concentrations. Efforts moving forward will assess model-informed, secretion-optimized CA variants; fabrication of thermoplastic composites derived from RAB biomass; and assessment of protein fraction suitability for plasticization. These wet lab efforts will be complemented by refined TEAs/LCAs defining key cost and sustainability drivers en route to commercialization.

MICROALGAE COMMODITIES PRODUCTION WITH A DIRECT AIR CAPTURE PROCESS

MicroBio Engineering Inc.

PROJECT DESCRIPTION

WBS:	1.3.4.006
Presenter(s):	John Benemann
Project Start Date:	10/01/2020
Planned Project End Date:	12/31/2023
Total Funding:	$2,528,795

Purpose: The technology to be advanced in this project is the utilization of CO_2 from air to cultivate microalgae and produce biomass for higher-value nutritional products in the near term, and commodities including feeds, biofertilizers, bioplastics, and fuels in the longer term.

Relevance and Impacts: The two approaches to accomplish this objective are:

1. DAC by a physical-chemical process provided by GT that delivers a near-100% concentrated CO_2 stream to the algal cultures. The GT-DAC process could become commercial at the Cyanotech facility in the near term.

2. The use of the algal cultures and cultivation systems themselves to provide CO_2 absorption from air at a rate supporting production targets.

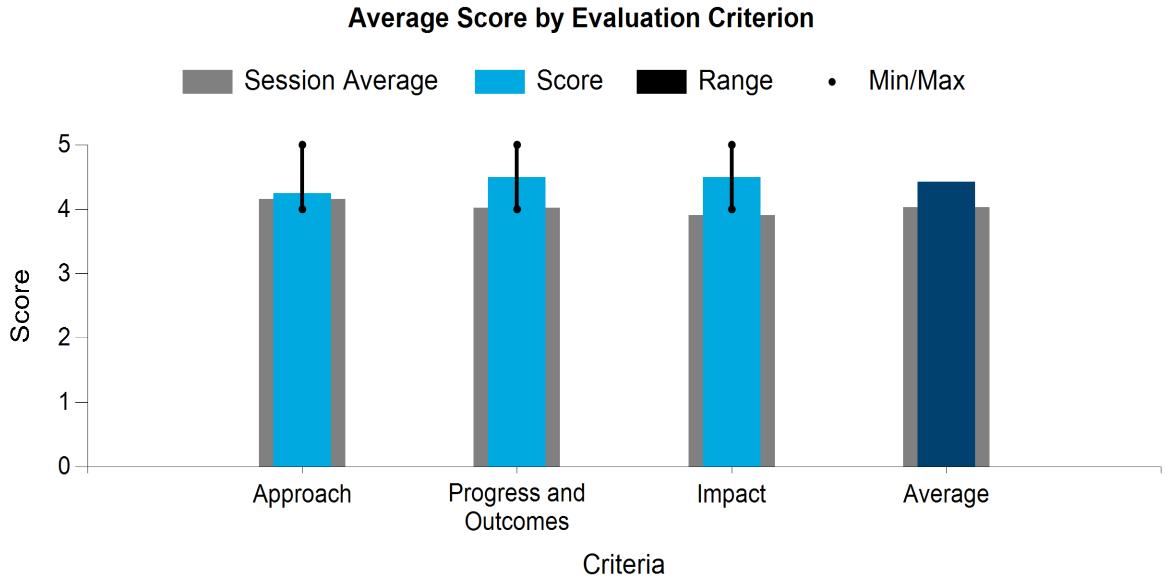
Challenges:

1. GT-DAC: Achieving similar productivities in raceway ponds with air CO_2, supplied by the GT-DAC process as with merchant CO_2, over several months of sustained cultivation.

2. For cultivation of algae directly on air CO_2: Achieving productivities in raceway ponds of >50% compared with use of enriched CO_2.

Outcomes and Technical Accomplishments:

- Completed design for GT-DAC pilot to provide 1–2 kg of air CO_2 per hour. Fabrication underway.
- Outdoor productivity met targets for commercial production of a high-value, whole-cell product.
- Validated an assay to identify presence/absence and relative levels of CA.
- TEA/LCA validated cost savings and reductions in GHG emissions by integrating DAC.



COMMENTS

- I love the graphics explanation of the approach demonstrating who is responsible for what portion of the work. Slide 9 showed a clear connection between the work and the department goals. The management Slide 17 with risks and Slide 18 with the schedule are particularly helpful for evaluation. Although pond depth will add storage capacity, what is the trade-off in productivity, as the lower levels of the pond won't be as well illuminated as the top layers?

- The progress is impressive; it will be interesting to see how it scales with Cyanotech. The impact is clearly identified and quantitative throughout. Graph legends would help to define the meanings of different colors, if these are different batches, different strains, or different conditions. Densitometry isn't a great way to do quantitation—an enzymatic activity assay would be much better. Optimization of pond depth for carbon sink function versus mixing and photosynthetically active algae will need to be done for the approach to be implemented.

- The project is early, awaiting equipment. Progress is being made consistent to the goals.

- Approach: The combined project team has well-established expertise and existing infrastructure in all the critical areas needed to conduct the research included in this project. Several approaches are being taken by the project team to enhance the prospects for using DAC for supply of CO_2 to algal mass cultures. The goals of the project are well aligned with the BETO goals of reducing cultivation costs in order to lower the cost of biofuel production. Pulling all the disparate parts of this project together in a cohesive and maximally informative manner will require significant communication, coordination of efforts, and appropriate timing of activities between the various teams.

- Progress and Outcomes: The carbon flux modeling provides good guidance on how to optimize carbon use efficiency under different cultivation conditions, which was used to shape parameters for supply of CO_2 via DAC.

- Cyanotech provided several eukaryotic algal strains (presumably that were isolated from their high-alkalinity ponds) to PNNL for alkalinity screening, medium development, and growth under various conditions, including outdoor pond evaluations. One of these strains (*Graesiella* CT3072, a member of the Chlorellaceae family) achieved the project's baseline productivity and had a carbon use efficiency >75%; this strain has been prioritized for further evaluation.

- The investigators also conducted research expanding earlier work on increasing CO_2 availability to algae by the addition of CA to cultures. Development of assays to measure the relative levels of exogenous CA in cultures and the CO_2 flux enhancement *in vitro* (without cells present) were reported, but data on the outcomes were not presented in any detail. Also, it was unclear which strain(s) will be looked at that may naturally secrete CA; see Slide 32. If it is the wall-less *Chlamydomonas* strain in the referenced paper, then the project team should bear in mind that this strain will likely have a difficult time surviving under outdoor cultivation conditions. Proof-of-concept lab studies with this strain may not translate well to outdoor conditions.

- A DAC skid is being manufactured by GT for delivery this summer to Cyanotech for testing with their ponds. The results from these studies will be interesting when the system becomes operable.

- Preliminary TEA modeling results suggest that in locations that are reliant on high-cost CO_2, the use of DAC can lead to a major reduction in biomass production costs. To put this in context, however, the project team needs to indicate what the existing baseline CO_2 cost used in the model was so that the results can be translated to locations where CO_2 is less expensive. It also doesn't look like capital costs (depreciation) or additional power for the GT-DAC system were added to the DAC model; this should be reconciled or explained.

- Impact: If the goals of this project are accomplished, it will provide useful data for determining the utility and practicality of the GT-DAC system. When combined with the additional topics covered (e.g., CA addition, use of high-alkalinity strains), the project results will provide relevant information about whether these combined strategies can reduce the cost of algal biofuel production by meaningful amounts.

- Approach: Project management was not discussed, but the project approach and methodology are good. Progress: The project is going well, as per the presentation; it looks like the carbon unit is not ready on time. Impact: The project will have an impact in expanding algae growth areas if successful; so far, results are not impactful, as they are not demonstrated at scale or with DAC technology.

PI RESPONSE TO REVIEWER COMMENTS

- Comments: I love the graphics explanation of the approach demonstrating who is responsible for what portion of the work. Slide 9 showed the clear connection between the work and the department goals. The management Slide 17 with risks and Slide 18 with the schedule are particularly helpful for evaluation. Although pond depth will add storage capacity, what is the trade-off in productivity, as the lower levels of the pond won't be as well illuminated as the top layers? The progress is impressive; it will be interesting to see how it scales with Cyanotech. The impact is clearly identified and quantitative throughout. Graph legends would help to define the meanings of different colors, if these are different batches, different strains, or different conditions. Densitometry isn't a great way to do quantitation—an enzymatic activity assay would be much better. Optimization of pond depth for carbon sink function versus mixing and photosynthetically active algae will need to be done for the approach to be implemented. RESPONSE: We thank the reviewer for their helpful comments. Regarding the question, "what is the trade-off in productivity, as the lower levels of the pond won't be as well illuminated as the top layers," we point to McGowen et al. 2023 (https://doi.org/10.1016/j.algal.2023.102995), who found no statistical difference in average harvest yield productivity in ponds operated at 10-cm versus 20-cm depth over two cultivation campaigns with *M. minutum* 26BAM in 2019 and 2020. Surely there are other literature studies pointing to the opposite conclusion, and others in the literature that argue that depth primarily impacts biomass productivity via differences in diel pond water temperature (i.e., larger daily temperature extremes in the shallower pond). Productivity trade-offs at different depths is a complicated question, and one that our carbon utilization model does not yet account for. However, from a fundamental photosynthesis perspective, there would be no difference in productivity for different depths, as algae density per unit area is not affected. In regard to the CA densitometry assay, we agree that results here have so far been inconclusive but see value in confirmation of the presence or absence of CA that enzymatic activity assays alone may not provide. We feel that multiple analytical approaches will be needed to conclusively attribute any increase in air-CO_2 flux to CA. Given the inconclusive protonography results thus far, we are moving to a Western blots assay to verify CA presence and are validating a dansylamide fluorescence CA assay previously demonstrated on complex matrices (Mustaffa 2017; https://doi.org/10.1002%2Flom3.10182). We agree that there are many optimization variables, in particular alkalinity, and anticipate that the Cyanotech field trials, PNNL lab screening, and MicroBio Engineering TEA/LCA will make progress in identifying cost/productivity/carbon use efficiency/GHG trade-offs.

- Comments: The project is early, awaiting equipment. Progress is being made consistent to the goals. RESPONSE: We thank the reviewer for their assessment.

- Comments: Approach: The combined project team has well-established expertise and existing infrastructure in all the critical areas needed to conduct the research included in this project. Several approaches are being taken by the project team to enhance the prospects for using DAC for supply of CO_2 to algal mass cultures. The goals of the project are well aligned with BETO goals of reducing cultivation costs in order to lower the cost of biofuel production. Pulling all the disparate parts of this project together in a cohesive and maximally informative manner will require significant communication, coordination of efforts, and appropriate timing of activities between the various teams. Progress and Outcomes: The carbon flux modeling provides good guidance on how to optimize carbon use efficiency under different cultivation conditions, which was used to shape parameters for supply of CO_2 via DAC. Cyanotech provided several eukaryotic algal strains (presumably that were isolated from their high-alkalinity ponds) to PNNL for alkalinity screening, medium development, and growth under various conditions, including outdoor pond evaluations. One of these strains (*Graesiella* CT3072, a member of the Chlorellaceae family) achieved the project's baseline productivity and had a carbon use efficiency >75%; this strain has been prioritized for further evaluation. The investigators also conducted research expanding earlier work on increasing CO_2 availability to algae by the addition of CA to cultures. Development of assays to measure the relative levels of exogenous CA in cultures and the CO_2 flux enhancement *in vitro* (without cells present) were reported, but data on the outcomes were not presented in any detail. Also, it was unclear which strain(s) will be looked at that may naturally secrete CA; see Slide 32. If it is the wall-less *Chlamydomonas* strain in the referenced paper, then the project team should bear in mind that this strain will likely have a difficult time surviving under outdoor cultivation conditions. Proof-of-concept lab studies with this strain may not translate well to outdoor conditions. A DAC skid is being manufactured by GT for delivery this summer to Cyanotech for testing with their ponds. The results from these studies will be interesting when the system becomes operable. Preliminary TEA modeling results suggest that in locations that are reliant on high-cost CO_2, the use of DAC can lead to a major reduction in biomass production costs. To put this in context, however, the project team needs to indicate what the existing baseline CO_2 cost used in the model was so that the results can be translated to locations where CO_2 is less expensive. It also doesn't look like capital costs (depreciation) or additional power for the GT-DAC system were added to the DAC model; this should be reconciled or explained. Impact: If the goals of this project are accomplished, it will provide useful data for determining the utility and practicality of the GT-DAC system. When combined with the additional topics covered (e.g., CA addition, use of high-alkalinity strains), the project results will provide relevant information about whether these combined strategies can reduce the cost of algal biofuel production by meaningful amounts. RESPONSE: The project scope, involving CA produced and released into the culture media, is explorative in nature. The walled and cell-wall-deficient *Chlamydomonas* strains are our first targets, chosen as likely to be positive (wall-deficient strain, excreting periplasmic CA into the media; Kimpel et al. 1983 https://doi.org/10.1093/pcp/24.2.255; Coleman et al. 1984 https://doi.org/10.1104/pp.76.2.472; Ynalvez et al. 2008 https://doi.org/10.1111/j.1399-3054.2007.01043.x) and negative (walled strain, minimal CA released into the media) controls to demonstrate proof of concept and assay validity. The assay would then be applied to strains previously characterized by the MicroBio Engineering teams to perform well under the alkaline conditions required for air-CO_2 ingassing to take place, as well as more immediate commercial viability. These include strains identified in the present and previous (MicroBio Engineering, PNNL, and Qualitas Health) DOE-supported project, and include *Chlorella* (potential initially for a nutraceutical market), SSL1 (relatively productive at high pH), or *Porphyridium* (polysaccharides, pigments). The baseline cost for CO_2 at this site is quite high, severalfold higher than that on the mainland, and supply interruptions have taken place, further increasing the value of a DAC process at this site. Thus, this is a very promising site for deploying a DAC unit. However, it must be recognized that DAC technology is still at an early stage of development, and that actual capital and operating costs (and energy consumption) are not yet well established. This project will provide important information on these topics that will be incorporated in the final report. As stated by the reviewer, the in-pond DAC and GT-DAC technologies could reduce the cost of algae production and expand algal biomass resource potential.

- Comments: Approach: Project management was not discussed, but the project approach and methodology are good. Progress: The project is going well, as per the presentation; it looks like the carbon unit will not be ready on time. Impact: The project will have an impact in expanding algae growth areas if successful; so far, results are not impactful, as they have not been demonstrated at scale or with DAC technology. MBE RESPONSE: We agree that deployment and demonstration of the DAC unit will be impactful, particularly when integrated with the cultivation scale and experience at Cyanotech.

ACCESS CARBON – ALKALINE CARBON CAPTURE AND EXPRESSION-STREAMLINED SPIRULINA CULTIVATED IN AIR FOR RELIABLE BIOPRODUCTS, OIL, AND NUTRITION

Lumen Bioscience Inc.

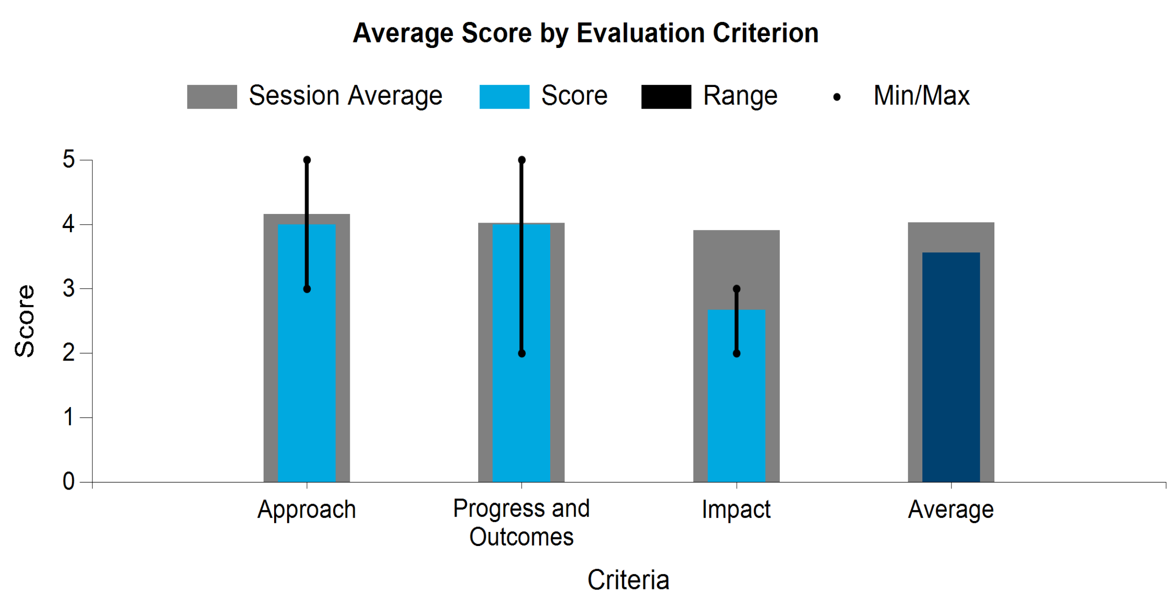
PROJECT DESCRIPTION

WBS:	1.3.4.008
Presenter(s):	Mark Heinnickel
Project Start Date:	10/01/2020
Planned Project End Date:	06/30/2023
Total Funding:	$2,500,000

Lumen Bioscience has developed a novel current good manufacturing practice (cGMP) biomanufacturing platform that enormously decreases the cost of developing and manufacturing new biologic drugs for certain illnesses. The platform builds on recently discovered methods that enable the bioengineering of the commercially important cyanobacterium *Arthrospira platensis*, also known as the common food algae spirulina.

We advanced the platform by developing strains to demonstrate the effectiveness of spirulina for mucosal delivery of protein biotherapeutics, including demonstrating intranasal administration of a spirulina-manufactured neutralizing antibody that can prevent disease by a respiratory virus SARS-CoV-2 in a hamster model. We describe production of this strain in photosynthetic culture using DAC of atmospheric carbon in alkaline pH. Optimal pH and alkalinity values were determined through machine learning. Conveniently, the modified media significantly increases (>100%) molecules convertible into biofuels (especially carbohydrates). Lumen also used a statistical design of experiments approach to increase therapeutic production (59%) with minimal replicates, significantly improving the economics of optimization.

LCAs were carried out with NREL to show that these adaptations lowered the carbon footprint of therapeutic production. In addition, computational flow dynamics are being employed to construct models that can lower this value in outdoor settings.



COMMENTS

- Making therapies from spirulina is a really interesting approach going for the maximum value type of products. This is a really compelling story because the value is so high. The missing part is fuel. The environmental drivers presented were a bit vague, quoting all U.S. health care, not the impact of the pharma industry. There are many reports detailing the impacts of the pharma industry. The data are available. I suspect the algae routes will be quite positive in an industry where the emissions are high. I think it will be a good story, it just needs to be told. The tie to DOE emissions and/or fuel goals is the only thing I found wanting. Really well presented, aligned with the project goals, and clearly showing progress.

- Approach: The project team is looking at ways to produce therapeutic proteins (monoclonal antibodies) in cyanobacteria (*Arthrospira*) at lower cost when compared to standard cell culture methods for monoclonal antibody production, and that have a lower GHG footprint relative to those current methods. Residual biomass would be available for conversion to biofuel products.

- Progress and Outcomes: The team reported that they had found conditions that resulted in a 59% increase in protein expression, but no information was provided on what those conditions were (Cultivation changes? Expression induction method? Changes to constructs?) or even what protein was being expressed. This makes it hard to assess the relevance of the outcome to BETO goals and the field in general.

- It would have been helpful to know what type of carbohydrate was elevated in the phosphate limitation experiments (e.g., glycogen versus small soluble carbohydrate versus cell wall carbohydrate); this can determine whether the increased carbohydrate content was relevant for fuel production from residual biomass.

- On Slide 14, the authors indicate that raising the pH of *Arthrospira* cultivation led to a large increase in CO_2 use efficiency. This is an expected result because of the higher capacity for carbon absorption into the higher-pH medium. *Arthrospira* is well known to be an alkaliphilic strain, so commercial production always occurs at high pH. Because no information was provided on the actual pH used, it is not clear whether the project team did anything different in their approach than what has been done in the spirulina industry for several decades.

- It is not appropriate to make side-by-side comparisons of carbon footprints for conventional monoclonal antibody production versus *Arthrospira*-produced monoclonal antibodies if boundary conditions for the analyses are not the same. The referenced paper for conventional monoclonal antibody production includes CO_2 emissions associated with the manufacture of steel fermenters and plastic bioreactors (and associated waste management, among other things), whereas this report appears to consider mainly CO_2 emissions associated with operating costs. In other words, to truly compare carbon footprints, the project team would also need to include CO_2 emissions associated with PBR manufacturing, all processing steps, etc. More clarity on the specific boundaries used in the reported LCA would be needed to make a true comparison possible.

- Impact: The amount of residual biomass from monoclonal antibody production would be very low relative to the biomass volumes necessary for meaningful fuel or commodity chemical production. As a consequence, although the research conducted in this project may very well have significant ramifications for the pharmaceutical industry, it's not clear that the work will have a significant impact on technology development for large-scale algal-based fuel and/or commodity chemical manufacturing. Having said this, it is recognized that the successful commercial use of cyanobacteria to produce therapeutic proteins could boost interest in algae for other beneficial purposes, including fuels and related products.

- Approach: Good planning and good collaboration, clear management plan for the project, and risks and mitigations have been identified. Outcomes: The project is on target and on time, and the results exceed expectations/predictions/goals. Impact: It is not clear how this information will be shared and will drive the industry forward. Are publications planned? This is not a large enough industry for this research to have a large impact on carbon emissions or advance large-scale production of biomass for energy.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for the time they took with our presentation and their feedback. Some of the comments focused on the broad applicability of the work presented. We view this work not only as a means to improve photosynthetic therapeutic production but also as a proof of concept that sets the stage for biomass, protein optimization, and increased carbon utilization efficiency, all of which extend beyond the Lumen algae systems and applications. These parameters are identified as critical components for the success of future algae biorefineries. While our volumes for COVID therapeutics would be small, the technology can be applied to protein products that apply to larger markets. If this occurs, it could have a more significant impact on the biofuel industry. Beyond publications (planned and in preparation), this project will have optimized toolkits whose applicability extends further than pharmaceutical production. These toolkits will be publicized in peer-reviewed publications and patents and could lead to further collaborations with commercial partners (e.g., the carbon utilization improvements can have impact across the value chain). The reviewers also highlighted the lack of experimental details underpinning some of the presented improvements. We are unfortunately constrained by disclosure limits in information that is deemed business-sensitive; we do plan to disseminate the methodologies applied in the public domain. The major accomplishments of this work revolve around the improved production of VHHs (Slide 4) and biofuel intermediates (carbohydrates) by *Arthrospira* in DAC conditions. The protein expression was increased by 59%; however, details of the conditions and protein identity are commercially sensitive and cannot be disclosed publicly. The carbohydrates (which also increased significantly) that were produced in this experiment were mainly glycogen, which can be fermented to ethanol. NREL confirmed that the glycogen content increased specifically by measuring the monosaccharide composition of the carbohydrate fraction as fucose, rhamnose, glucosamine, galactose, mannose, xylose, ribose, and glucose, and observed a significant increase in glucose content at the expense of a reduced contribution of the other monosaccharides. The DAC conditions were achieved by altering our standard media. Lumen's standard growth conditions are similar to those used in commercial spirulina production. As part of this work, Lumen raised the pH significantly above standard outdoor cultivation pH values. Details of the conditions are commercially sensitive and cannot be disclosed publicly. However, the methodology behind this optimization (machine learning and computational fluid dynamics) will be the subject of several forthcoming peer-reviewed publications. A last point was raised on the boundaries of the LCA of this project. This work was carried out at NREL and used standard emissions-burden approaches that have been well documented across BETO projects. The LCA showed that Lumen and NREL's technology could produce VHHs for a much smaller carbon cost in our facility. The inclusion of the facility footprint in the LCA, when amortized across decades of operating life, contributes minimally to the product GHG. This is particularly true for biorefineries like Lumen's that are similar to conventional chemical plants. For this reason, we believe the boundaries selected for this project are appropriate. While decades of DOE-funded research allow us to understand the emissions profile of our process quite clearly, as with many high-value specialty products, the LCA data on most pharmaceuticals are quite sparse. For this reason, a comparison of all of our projects to their companion products in the pharmaceutical industry remains challenging.

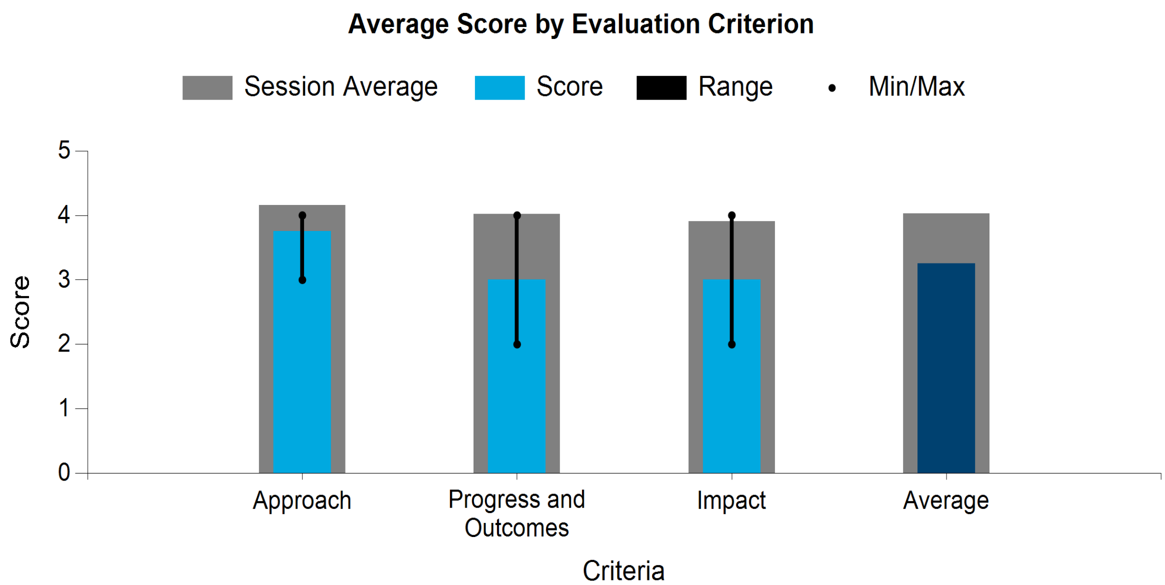
DEVELOPMENT OF HIGH-VALUE BIOPRODUCTS AND ENHANCEMENT OF DIRECT AIR CAPTURE EFFICIENCY WITH A MARINE ALGAE BIOFUEL PRODUCTION SYSTEM

Duke University

PROJECT DESCRIPTION

WBS:	1.3.4.010
Presenter(s):	Zackary Johnson
Project Start Date:	10/01/2020
Planned Project End Date:	12/31/2023
Total Funding:	$2,513,524

DOE FOA DE-FOA-0002203 Topic 3 has three major objectives: (1) increasing the revenue of the algae biomass while ensuring that the fuel specifications are met, (2) increasing productivity over baseline levels while using carbon supplied by DAC, and (3) increasing the percentage of carbon supplied by DAC while still reducing the costs associated with supplying CO_2. Our project's overarching goals are to (1) enhance the growth and productivity of marine microalgae through cultivation system design and operation improvements, (2) increase the market value of post-fuel algae biomass residuals by assessing alternative high-value products (i.e., collagen, whey protein substitute, and eicosapentaenoic acid/docosahexaenoic acid), and (3) demonstrate DAC as a source of CO_2 for algae cultivation. Although only approximately 1 year into the 3-year project, here we show substantial progress toward each of these goals, including development of a quantitative algae production model, screening of top biofuel candidate marine algae, and piloting of a novel DAC approach. Our project is on track with respect to its milestones, and our team continues to make substantial progress in marine microalgae commercialization.



COMMENTS

- An energy balance on the AHX module would have been helpful.

- Dewatering and further purification steps (HTL?) and other downstream operations are not included in the TEA/LCA, although they are not trivial in impact. The TEA should include throughput assumptions and wintertime mitigation strategies, particularly with respect to siting.
- There was no DEI approach included.
- The project appears to be doing interesting science largely unrelated to the goals. Reports on actual yields to high-value products, a centerpiece of the agreed efforts, appear completely lacking. The DAC component really has nothing to do with algae. Partners are developing a machine for air capture that will produce an enriched CO_2 stream, quoted to be at about 20% enrichment. The machine doesn't require algae, and integration, from what was presented, is nonexistent. It is a DAC machine capable of supplying any user of dilute CO_2, next to an algae facility capable of accepting any enriched CO_2.
- The description of why the DAC is advantaged failed to be convincing. The slides fail to paint a compelling case on where an advantage derives from and compared with what.
- Further, the discussion of bicarbonate was confusing, even when explicit questions were asked about it. The presentation certainly does not amplify the benefits of bicarbonate for storage.
- Product values appear exceptionally high, creating favorable TEA calculations. This is, of course, to be expected. Sensitivity to those product values is needed.
- Approach: The goals of this project—namely lowering the overall cost of biofuel production via a combination of increasing the coproduct value coupled to reducing CO_2 costs and availability through a DAC process—are fully aligned with the BETO goals related to algal biofuel production. The general approaches as stated are reasonable and similar to some other projects funded via this FOA. The management plan, including how the main collaborators interact, was not described. The role of the University of California, Santa Cruz, was not discussed.
- Progress and Outcomes: Fifteen strains, half of which were identified in the previous Marine Algae Industrialization Consortium (MAGIC) project and half of which were considered strains worthy of SOT status, were examined for high coproduct value potential. It was a bit confusing why collagen and whey (proteins) were included on this list, as collagen is known to be uniquely produced by animals and whey is a dairy (i.e., animal-produced) byproduct; these exact products simply cannot be expected to be made by naturally occurring algae. If the research team is looking for collagen or whey substitutes, they didn't indicate the desirable properties being screened for or how the screening is taking place. In any case, no substantive results were presented regarding coproduct development.
- The work presented on stocking density (inoculum level) and light field-related productivity was a large part of the presentation, but it was unclear what these somewhat narrowly focused studies had to do with the stated goals of the project involving coproducts, DIC control, or DAC-based CO_2 supply; this seems to be major disconnect. It was not disclosed what strains were analyzed and whether they produced high-value coproducts; if so, the impacts of growth mode on coproduct content were not mentioned. No results were presented on CO_2 use optimization or, in general, on CO_2 supply to cultures.
- Although behind schedule, information was presented on the MoleculeWorks AHX DAC module that will be tested outdoors at the Duke algal growth facility. If economical, this system has potential to provide CO_2 captured from the atmosphere to mass algal cultures, but since an operable system does not appear to be available yet, it is premature to assess the value of the system. The final TEA and LCA models will rely heavily on the costs and efficiency of the AHX-based system, and how the system integrates with algal cultivation.

- According to the quad chart, the project was scheduled for completion in December 2022, so a no-cost extension was apparently granted. It seems like there is still quite a bit of work to do to achieve the project objectives.
- Impact: If more progress is made on the various components of the project and they become better aligned and integrated, this project has potential to have a positive impact on the economics of algal biomass (and biofuel) production. It is not clear at this point whether the necessary progress will be made in the remaining time of the project, however.
- The publications listed in the supplemental slides don't seem to have much to do with this project.
- Approach: The overall approach is good, and risk mitigation is in place. The plan is reasonable. Feedback to other DOE portfolios is low, but there is collaboration within the group.
- Outcomes: It's very early on in the project, so strain screening is just initiating and outcomes are not completed yet. The work with DAC is very promising.
- Impact: Building on existing data increases the potential for higher impact, but it's too early to evaluate the impact of the overall project.

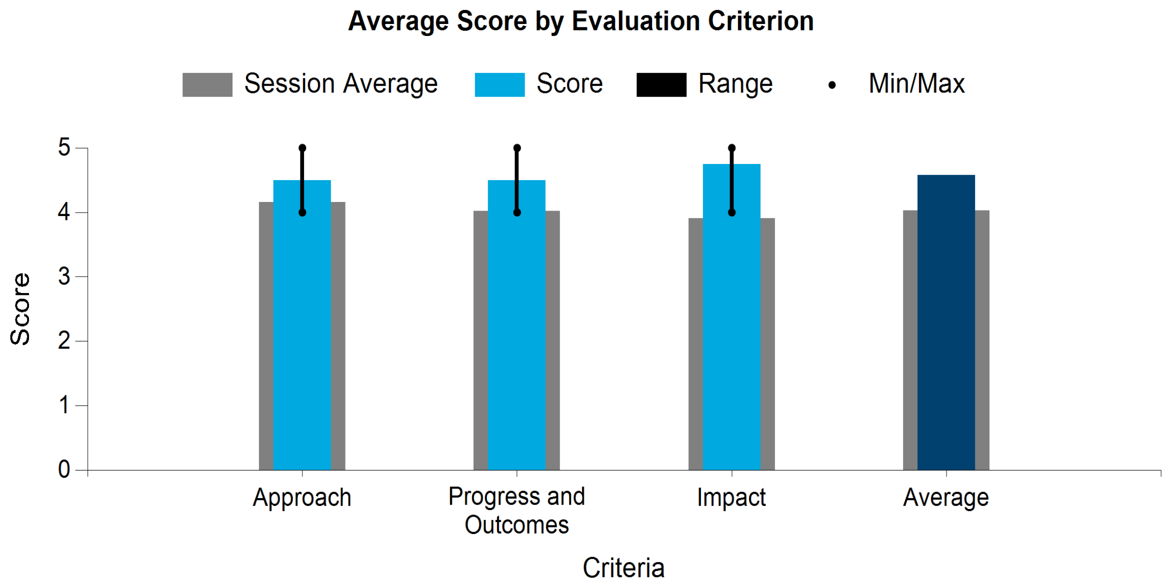
HTL DEVELOPMENT

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.4.102
Presenter(s):	Peter Valdez
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2022
Total Funding:	$1,575,000

The Hydrothermal Processing for Algal-Based Biofuels and Coproducts project is focused on developing advanced HTL methods to improve process efficiency and reduce capital and operating costs to produce carbon-negative biofuels and coproducts from algae. Cost-advantaged algal biomass is being investigated to enable cost-effective fuels and coproducts. Examples of cost-advantaged algae include residuals from algal-based products, algae used for remediation services such as wastewater treatment or pollution management, and harmful algal blooms collected and disposed in environmental services. Cost-advantaged sources, such as wastewater-grown algae, are available but underutilized (e.g., landfilled). New strategies are being developed to address high ash content, less energetic algal composition, and high-viscosity slurries. The project leverages experience with single-pass and sequential HTL to maximize the conversion of biomass. Coproducts such as extracted polysaccharides, fertilizer substitutes, and building materials are also being investigated. All data from these efforts directly support the algal HTL process model, economic and environmental impact models, and annual SOT reports. The SOT reports highlight key research and development targets to achieve DOE cost and production volume targets.



COMMENTS

- The collaboration slide and supplemental slides were very helpful to understand information sharing and project management. The DEI inclusion was also helpful. The success of the risk mitigation plan is great. Excellent job making the model and then iteratively validating it as more information came in. I also like the flexibility of using the available feedstocks and adjusting to seasonal changes, as this represents a more realistic scenario for different sites and weather patterns. This method is a critical unit operation for the feasibility of many biofuel projects and has broad applications. I would suggest looking into the anaerobic methods for material storage, as they are considerably less expensive than freezing; freezing will wreak havoc on your TEA. The Wendt lab in Idaho has done some work on this. Of course, the great interest of the moment is how to deal with sargassum nuisance blooms.

- This is that rare project trying to do something for everyone and with any feedstock. The analysis and work to date is impressive, showing more flexibility than I would have thought possible. That said, making a device capable of consuming anything and making products fit for established markets is an almost impossible task. The results obtained so far are commendable and in line with the description of the project goals.
- The DEI component is consistent with the established goals, but is more focused on outreach than aimed at increasing diversity. Reinforcing, they did what they said they would do.
- Approach: The project objectives and plans to achieve them are well described in the presentation. The project team members are well suited for the planned research, and the means that were established to communicate and collaborate are appropriate.
- An important objective that was not clearly stated would be to determine whether there really is a cost advantage to certain waste algal biomass sources relative to farmed algae. This would take into account the consistency of availability (impacting scale and uptime of processing facilities), feedstock stability, transportation costs, costs for making the biomass suitable for handling and HTL, etc. The assumption that waste biomass will be free (or at a negative cost), which is an important driver of the reduced costs, needs to be verified if the companies involved in water treatment, nuisance bloom harvesting, or other sources come to believe that there may be more value in the biomass than a straightforward savings on disposal fees. Different collection and transportation costs for a variety of scenarios should be examined in this regard.
- Developing methods to reduce ash content and improve the flowability of waste biomass is an important goal for this year. Identifying and verifying coproduct opportunities is also a worthwhile objective.
- Progress and Outcomes: Seven samples of low-cost algal biomass were acquired from collaborators; so far, one of these (algae from wastewater treatment) has been treated and processed via HTL with promising initial results. Even with the reduced yield, the TEA indicated a reduction in GGE cost to $2.61, which is near the BETO target. According to the related 2021 SOT, the costs associated with cultivation, harvesting, dewatering, and transportation of the wastewater-grown algae were not included when deriving this GGE cost, presumably because of counter savings by the treatment facilities for water treatment and sludge disposal. It will be important to determine the actual full total cost of the biomass when availability and processing requirements are better understood. Results from HTL of the other cost-advantaged biomass received will hopefully be conducted soon.
- Results of HTL using *Picochlorum* biomass (i.e., not a cost-advantaged algae as defined for this project) were reported, including a favorable comparison of the upgraded HTL oil properties with SAF requirements. Unless this information was included simply for future comparative purposes, it's not clear why these studies were conducted (or at least reported) within this project. The HTL results will likely differ when processing waste algae biomass due to the differing chemical compositions and potentially variable heteroatom levels present prior to oil upgrading. It will be highly important to conduct such quality tests with actual biomass generated from wastewater treatment or other cost-advantaged biomass.
- Impact: The results of this project should shed light on whether algal biomass provided from waste sources (e.g., algae from wastewater treatment, harvested micro- and macroalgal blooms) can be collected, transported, and converted to fuels and coproducts in an economically viable manner.

- Information was not presented on the total volume of cost-advantaged algae feedstock that is expected to be available, which is important to know in order to assess the impact of the proposed technology. It is worth considering whether such low-cost algae could be coprocessed with farmed algae or stored and processed in farmed algae conversion processing plants during slow growth periods as opposed to making dedicated, distributed, lower-volume processing facilities.
- Approach: Safety considerations are industrially relevant and important; good work plan.
- Progress and Outcomes: On schedule with the project and good execution.
- Impact: Large impact from the work; it's good that it is agnostic to feed source.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their thoughtful and constructive comments and questions. We will address key questions and areas that need further clarification. For storage topics, we have collaborated with Idaho National Laboratory to investigate the anaerobic storage of algae/wood blends prior to hydrothermal processing. Our published results (https://doi.org/10.1016/j.algal.2021.102622) of an algae/wood feedstock show that there is minimal impact to the quality of fuel products after 123 days of storage. Freezing algae samples is generally used to preserve experimental feedstocks for lab-scale processing. Current investigations in this project and other HTL projects at PNNL (3.4.2.301 and 2.2.2.302) are planned to assess the viability of any potential feedstock for HTL. When pilot- and commercial-scale operations commence, it will be the decision of the process operator to define a strategy for selecting a specific operating envelope for feedstocks and products. The objectives of the project are to produce experimental results to enable a broad selection for both feedstocks and products. We acknowledge that our estimates for feedstock costs and availability are still speculative. The current objectives of this project are to examine the technical feasibility and development of cost-advantaged algal feedstocks. We will work with the partners that provide the algal feedstocks and the project team leading the analysis work (1.3.5.202) to investigate current assumptions for cost and availability and determine the realistic potential of each feedstock. HTL of farmed monocultures of algal feedstocks to produce SAF was a previous objective examined during the project. It was included to demonstrate the national-scale potential for producing SAF from farm-cultivated microalgae and will be used in the future for comparative analyses against new results. The project team is focusing on outreach at K–12 programs as the first step in increasing diversity in STEM careers. The team will continue to think creatively to develop effective methods to improve DEI at PNNL and within STEM fields in general.

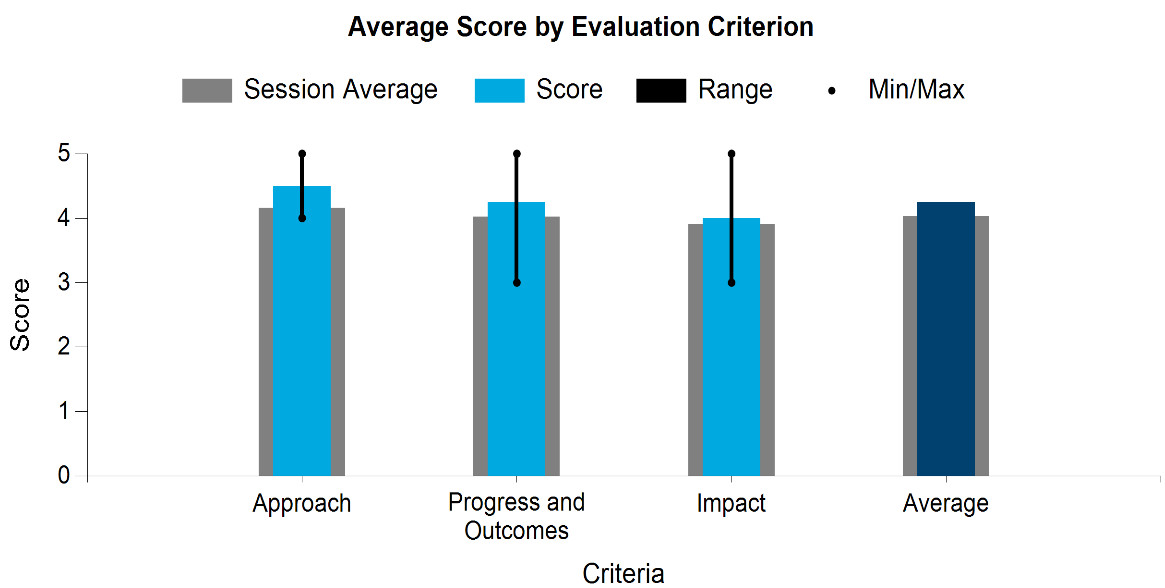
COMBINED ALGAL PROCESSING FOR THE SYNTHESIS OF LIQUID OLEOFUELS AND PRODUCTS (CAPSLOC)

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.4.204
Presenter(s):	Tao Dong
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$2,100,000

CAPSLOC aims to develop a biorefinery concept that is composition-agnostic and enables economically, environmentally, and socially viable biofuel production by maximizing the value from each fraction. To achieve this, we have employed an iterative approach between R&D and TEA/LCA to establish process targets and quantify improvements in the minimum fuel selling price, reduce carbon intensity, and support the SOT. We have surveyed various methods for biomass pretreatment and successfully demonstrated pre-pilot-scale pretreatment (100 kg) in batch mode. Using techniques such as lipid modification, fermentation, MOT, and carbonization, we have developed biofuel precursors and a range of value-added coproducts. Our project has demonstrated significant improvements in key aspects of algae processing, particularly in pretreatment technologies suitable for variable composition, coproducts available from lipid and extracted solid streams, conditioning and fermenting of high-protein hydrolysates, and recovery of nitrogen and phosphorus nutrients for recycling to cultivation ponds. We have demonstrated a pathway to generate drop-in algal biofuels at modeled cost of <$2.50/GGE and >50% GHG emissions reduction versus fossil incumbents. The success of this project will support the commercialization of a sustainable microalgal biorefinery industry.



COMMENTS

- Thank you for the slide explaining the management approach and communications strategy. The DEI approach is good, as it points to concrete actions taken. Downstream operations are critical to the success of commercialization, so there is clear impact and a strong connection to the economic case, especially of platform development as opposed to specialty chemicals. The use of machine learning to improve nitrogen availability is innovative. The connection to the Polaris licensing of non-isocyanate PU, Qualitas, Clearas, and GWT is excellent, but I would have liked to see a lipid fuels connection as well. There needs to be more understanding of the PU market in order to have more solid data for the TEA—how to get better market penetration. One hundred kilograms is a reasonable pilot scale for downstream operations. Ammonium sulfate can be quite expensive for the quantity needed; I would recommend looking at other options such as carbonic acid.

- This is one of the most wide-ranging projects reviewed; it has many moving parts and somewhat ambiguous goals. The umbrella goal is certainly good, and all projects are directed at that goal. There is a lot of quality exploration being done. Perhaps it was the short time allowed for the presentation, but there was a lack of coherence.
- Approach: Identifying and optimizing a downstream process that could be applied to multiple types of algal biomass feedstock to achieve a suite of products is a worthwhile goal that is in line with BETO's objectives. A good multidisciplinary team has been assembled for this project, and the communication and DEI plans are appropriate. Interim milestones and specific objectives were not provided in the presentation, however, so it's not really possible to know whether the approach being taken will meet the objectives in the project's time frame.
- Progress and Outcomes: Milestones and objectives for the subprojects were not specified, so measuring progress is not straightforward.
- Based on the presentation, MOT of acid-hydrolyzed algal biomass appears to be a good process for making such biomass a more viable feedstock for the fermentation step of the CAPSLOC process. An added benefit is the potential to recycle more nutrients back into algal cultures, which would be expected to reduce cultivation operating costs. However, although the MOT process was described in the 2019 and 2020 CAP SOT reports, it was dropped from the 2021 CAP SOT report, which would lead one to assume that it is no longer being considered a viable process option (perhaps due to the inability of the process to work at commercially relevant solids concentrations?). This disconnect should have been addressed in the presentation.
- A substantial amount of economic credit is being given in the TEA to the coproduct PU, but information on the assumed market size and sales price are not provided, again making it hard to understand whether the assumptions are reasonable or not. It is recommended that the research group obtain input from existing large-scale PU producers to validate the assumptions being made on product requirements, comparative manufacturing costs, potential market penetration, and bulk pricing of the algal-based isocyanate PU and non-isocyanate PU end products.
- Impact: A one-size-fits-all process to convert algal biomass from different sources to useful and profitable products would be quite valuable to the field. It would have been helpful for the researchers to report on the use of the CAPSLOC process for different types of algal biomass in order to know whether it is truly a universal process. It will also be important to know whether past issues with processing high-salinity strains have been overcome.
- More information in the presentation on the assumptions made in the TEA models as to which improvements are required in the existing CAPSLOC process to achieve the $2.50/GGE minimum fuel selling price target would have been useful. Without reading the current SOT in detail, which is beyond the scope of this review, it is hard to determine whether the TEA forecasts are reasonable, and thus the overall impact is difficult to assess.
- Approach: Good plan and working collaboratively with other BETO portfolio projects, adapting as needed to other project results to maximize output; risks and mitigation have been defined. Outcomes:

Met all project goals early and continuing to improve on targets. Impact: Large, as this is very practical due to wide variety of biomass streams; it may not work for all coproducts desired.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their positive and constructive comments. While the overall project goal is broad, we also have more specific goals delineated in quarterly milestones. We have achieved all seven of these milestones in the last 2 years, including comprehensively evaluating six pretreatment methods on nine different algal biomasses (Slide 9), scaling up pretreatment to the 100-kg scale (Slide 9), optimizing MOT regarding carbon and nitrogen yields to enhance lipid fermentation and nutrient recycling (Slides 11 and 12), demonstrating non-isocyanate PU production (Slide 13) and carbon products from posted extracted solids (Slide 14), and hosting one MEISPP intern (Slide 7). In the context of biomass conversion, we started at the pretreatment step and established that dilute acid is a universal approach to fractionate algal biomass with a broad range of composition. However, different algae give very different compositions of hydrolysate and solids, and establishing biomass-agnostic downstream conversion is an ongoing focus. MOT has been used as a tool to funnel carbons into biofuel precursors. Besides MOT of the post-extracted residuals, we will explore arrested anaerobic digestion for organic carbon funneling to produce volatile fatty acids as precursors for SAF production via sequential chemical and biological approaches. Though we have made significant progress, there's still a lot of work that needs to be explored and validated to establish a robust and universal algal biorefinery. While the TEA team did refer to MOT in prior SOT reports, it has never been a formal SOT pathway, and thus has not been the subject of focus in the SOTs to the same degree as the biochemical fermentation/lipid extraction pathways. We believe that MOT still exhibits potential for certain applications as a means of producing intermediates for subsequent upgrading while recovering nutrients, though challenges in conversion efficiency of insoluble solids have hindered its application as a stand-alone biomass conversion technology. After MOT, we extracted ammonium by ion-exchange resin, though regeneration of the resins has not been a focus of the research thus far. Regeneration with sulfuric acid to produce ammonium sulfate is a well-known baseline process, but we appreciate the reviewer's suggestion to use sustainable carbonic acid instead and will explore this option in our future work. In the last 2 years, we have been focusing on low-cost algal biomass (usually high in protein and low in lipid contents), but we do have a plan to recover lipid from the biomass and test its suitability for SAF production via the ASTM-approved hydroprocessed esters and fatty acids approach. This effort will be explored and benchmarked by the end of FY 2023. The PU foam market size is approximately 2.1 million tonnes/year (U.S.) or 14 million tonnes/year (global). These market sizes are significant and can support many algal biorefineries before reaching saturation. In addition, the price of PU has increased significantly to around \$3.5/lb. This will be incorporated into future TEA models along with adjusting the prices of other raw materials and capital costs using price indices. We have realized the importance of working with existing large-scale PU producers to validate the assumptions being made on product requirements. The separate NREL algae TEA/LCA team has recently initiated a subcontract with an engineering contractor to assist in refining our understanding of non-isocyanate PU manufacturing costs in comparison to traditional PU production. Besides licensing this technology to Polaris Renewables, we have been working with several world-renowned companies, all delivering project cost share, as part of two consecutive DOE Technology Commercialization Fund-supported projects. These projects focus on developing non-isocyanate PU technology for market penetration and have yielded valuable insights that advanced commercialization. The CAPSLOC project works closely with NREL's TEA/LCA project (WBS.1.3.5.200) to better understand the improvements required to achieve minimum fuel selling price and decarbonization goals. To summarize recent SOT and design reports, further improving pretreatment efficacy to boost yields to fermentable intermediates, increasing catalyst performance (activity and stability) during upgrading of intermediates to finished fuels, and producing high-value, scalable coproducts from all non-fuel portions of algae feedstocks are areas with significant potential within the CAPSLOC scope.

MEDIUM OPTIMIZATION WITH RECYCLED ELEMENTS (MORE) FOR BETTER BIOMASS

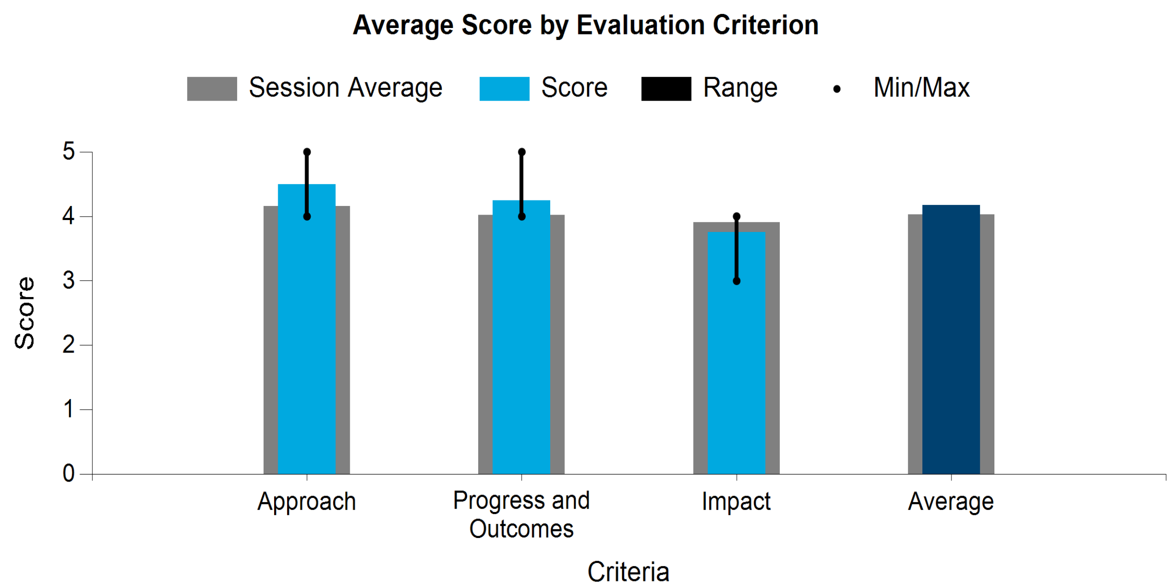
Los Alamos National Laboratory, National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.4.205
Presenter(s):	Claire Sanders
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2024
Total Funding:	$1,500,000

Microalgae offer great potential as a renewable source of biofuels and high-value bioproducts. However, the cost of algal biomass still remains a primary impediment to using algae for the production of biofuels and bioproducts. Past efforts to reduce biomass cost have included altering media components; however, the composition of primary nutrients has varied little from a standard starting nitrogen concentration [N] of 40–70 ppm and a nitrogen:phosphorus (N:P) ratio of 16:1 (Redfield's), with the aim of maintaining a nutrient-replete status during BETO SOT field trials. Nutrient inputs that are too low could adversely affect productivity, but conversely, the implications of overloaded nutrients are not understood.

In this project, we have leveraged multiple years of SOT data for *Picochlorum celeri* and *Tetraselmis striata* as inputs for machine learning to identify areas of improvement for N and P application and pond management. Machine learning has been combined with laboratory and outdoor testing using varying nutrient inputs, with which we have shown robust growth maintenance for both *P. celeri* and *T. striata*. TEA has been implemented to determine the MBSP cost savings of changing nutrient management strategies. Additionally, we are testing the use of recycled nutrients from biomass to support economically viable pond growth. Using these approaches, we have shown that data-directed nutrient input management will reduce the costs of biomass production.



COMMENTS

- The connections between all the different workflows are great. Very encouraging, especially the direct comparison of indoor PBRs to outdoor ponds. This has historically been a massive issue that is very challenging to address properly and is absolutely critical to resolve. Risk mitigation and connection of the approach to the impact is clear. I like that you are doing the model first and then validating against the data instead of leaving the model for last; it demonstrates that the authors know the value of *in silico* work and understand the point of the TEA. Thank you for the DEI slide; this is also helpful. I would have liked to see more of an industry connection. The Bayesian strategy for machine learning is solid, given the limited amount of data available to feed into the initial model and seasonal variability. I would like to know how the code versions are being tracked; versioning should be performed through GitHub or some similar validated method, which can also facilitate wider use of the code. The composition results and real-life media recycling data should be interesting when you have them.

- I will defer to the program about the approach, since this seems like very much a first step, almost a proof of concept. The presentation certainly did not leave the impression that the model is robust and useful across a range of conditions. It is curious that uptake rather than concentration is in the input and gives some pause. It would be best if the model relied only on controllable settings.

- It seems that progress has been made, but there is much left to be done. Good inclusion of DEI. Well considered and well presented.

- This project is trying something new, but it is still wanting. It is commonly said as a joke, but the adage "all models are wrong, but some are useful" is actually a statement carrying wisdom. It is insufficient to develop a model; it must be tested sufficiently to be proven useful. Perhaps those tests will come, but it is not clear they are being done or being planned based on what was presented.

- Approach: The project team members take advantage of existing data sets and use machine learning to gain insights into the impacts of nitrogen and phosphorus levels on algal growth (primarily *Picochlorum* and *Tetraselmis*). Additional lab testing is used to verify or refine the machine-learning results. Appropriate connections between team members at LANL, NREL, and AzCATI appear to have been made. The project goals are aligned with those of BETO, although it must be recognized that there will be limits on the applicability of the results to strains, cultivation conditions, and media that were not directly examined in this study.

- Progress and Outcomes: In the machine-learning correlation (Slide 11), NH_3 uptake is listed as the largest contributor to *P. celeri* productivity. This is most likely a result of high productivity rather than a cause. As shown on supplemental Slide 31, the actual NH_3 concentration in the medium didn't seem to have a large effect.

- It's important to recognize that, in addition to the impact that starting nutrient concentrations can have on productivity, the manner in which nutrients are added (e.g., once and done feeding, steady feeding over the duration of a cultivation, periodic feeding based on culture density) and the form of the nutrient (e.g., NH_3 versus NO_3 versus urea) can have large effects on productivity and nutrient utilization efficiencies.

- The analysis involving nutrient addition into environmental PBRs in which medium is recycled is interesting and shows the importance of balancing nutrient inputs with growth needs.

- Experiments indicating the possibility of utilizing MOT-extracted nutrients for growth are important because the premise has been that this process can reduce costs through nutrient recovery. Hopefully the inhibitory substances can be identified and easily removed.

- Impact: It would be more broadly impactful to the algae industry and research community if approaches similar to the ones described in this project could be used to understand nutrient use dynamics for a variety of available water types and for different types of algae, rather than a very limited scope (i.e., *P. celeri* or *T. striata* in f/2 medium). (Note that it is recognized that these were the most comprehensive data sets that were available for analysis.) Due to the effects of water chemistry on nutrient availability (especially metals and other micronutrients, which were beyond the scope of this study), as well as differing nutrient uptake systems associated with different types of algae, it will likely prove necessary to obtain empirical growth and nutrient use data for each unique cultivation system and strain. Therefore, a desirable outcome for this project would be the development of a general analysis tool and testing protocols that could be used by labs and companies for their particular situation.

- Approach: Data analysis is not necessarily novel, but the amount of data used is; good team interactions, good plan, and mention of equity and inclusion. What about media recycle? Is that being done? Not clear. Outcome: Good outcomes, with data analysis defining what is important and controllable with today's technologies. Impact: The impact is large if the industry listens to it. The take-home message is to feed your algae and control temperature if you can. Can salinity be considered as a variable, as it would most likely also be on the list of high-productivity impact metrics?

PI RESPONSE TO REVIEWER COMMENTS

- Thank you to the reviewers for committing their time to assessing the progress and outcomes of this project and for the insightful comments. We have integrated experimentation (both indoor and outdoor), machine learning, and TEA with investigators from LANL, NREL, and AzCATI to achieve the goals of this project, and we appreciate the reviewers recognizing our well-integrated team. We agree that there are many factors with nutrient inputs that need further study. Different macronutrient sources, micronutrients, types of media (e.g., fresh, marine), and timing of feeding have the potential to change the algae behavior, including biomass accumulation, and a valuable output of this work will be a framework that can be utilized in further studies. We are glad that it is recognized that the environmental PBR cultivation system is invaluable in predicting the behavior of outdoor cultures. Our ability to utilize this array has made the realistic study of a variety of media compositions possible. Recycled nutrients can be derived from two sources: media and biomass. In this project we are focused on the recovery of nutrients from extracted biomass; this has been little studied and is an important consideration as we work toward a reduction in waste from the production of algae. Media recycling is another important factor in waste reduction, and one that is currently out of the scope of this project but will be important in future research. Our work on macronutrient compositions in media and the resultant optimization will reduce loss of ammonia and phosphate (as N and P sources) during non-perfect media recycling, which improves MBSP, as shown by TEA. Development of a new machine-learning model takes considerable time and inputs. We have worked to ensure that the data inputs from multiyear outdoor growth trials are robust and have been screened for erroneous/missing data. The model was originally built to encompass all data, including deltas, directly available or directly calculatable, from the data sets. Now that the model is functioning and we have demonstrated its applicability on data sets from multiple species, we are working to identify parameters that are dependent and should not be considered in our analysis. Parameter input optimization will increase the robustness of the model and give further insight into pond performance. During development, software goes through many iterations, and we appreciate the reviewers recognizing the importance of version tracking. Currently, our machine-learning software is being tracked internally; when the software is ready for release, we will use a version control system, such as GitHub, where it will be publicly available. We do understand that dissemination of all our work to industry is imperative to realizing its significance in the field. We will continue to communicate our results at relevant conferences and publications, as well as by seeking opportunities for discussion. We appreciate the recognition of our progress in the first 18 months of this project and agree there is still much to do. We look forward to continuing our close collaborations and work to realize the impact of controllable factors, such as nutrient input, on biomass accumulation.

ALGAL BIOFUELS TECHNO-ECONOMIC ANALYSIS

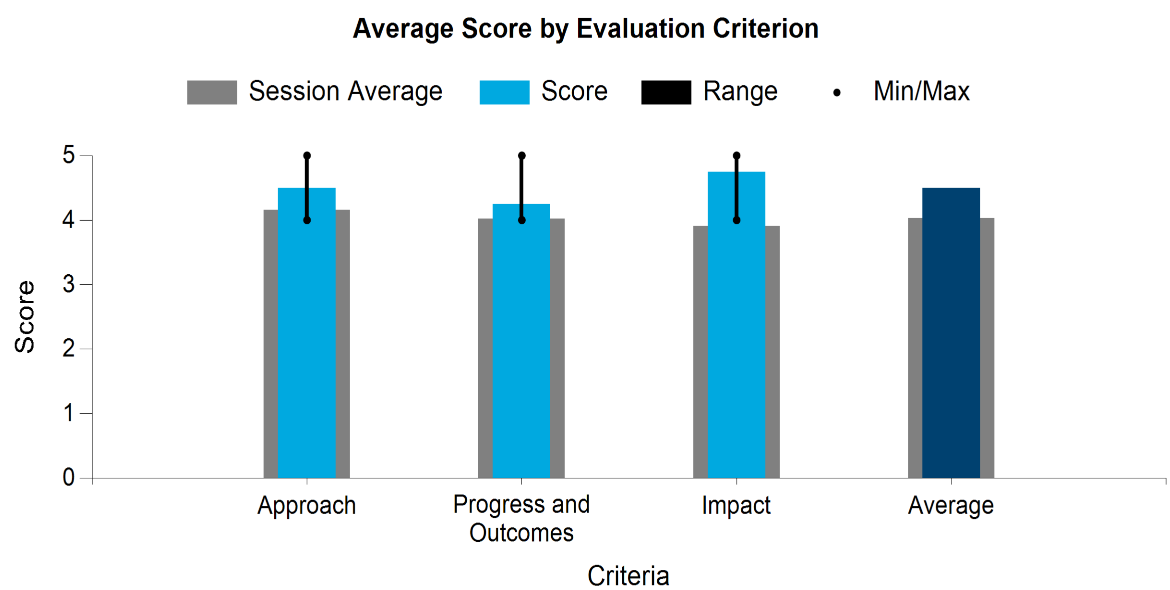
National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.200
Presenter(s):	Ryan Davis
Project Start Date:	10/01/2022
Planned Project End Date:	09/30/2024
Total Funding:	$1,050,000

The objective of NREL's Algal Biofuels TEA project is to provide process modeling and analysis to support algae program activities, utilizing TEA models to relate key process parameters with overall economics for cultivation, processing, and conversion of algal biomass to fuels and coproducts. By quantifying the economic implications of key process metrics, TEA models highlight the technical requirements to achieve future program goals for economic viability and sustainability, while identifying drivers and technology barriers that must be addressed through research prioritization.

This project provides high impact and relevance though the generation of critical cost data tied to funded research, with our analyses subsequently exercised by BETO to guide program goals and planning documents. This includes costs for both algal biomass production and downstream conversion, while working with lab partners to support LCA, ultimately to demonstrate paths to meeting the latest program goals for maximizing decarbonization potential at 70% or more GHG reduction while maintaining economic viability. This project has made numerous achievements since the 2021 Peer Review, including continued improvements to SOT cost benchmarks, publication of a high-visibility report documenting near-term opportunities for utilizing waste algae resources, and ongoing collaboration with other lab partners on an updated algae harmonization analysis.



COMMENTS

- The validation of the model with ongoing projects and industry experts is absolutely critical to the success of the model; this is great.
- It's also good to see multiple algae sources; this makes modeling more complex as the inputs shift, but it is also more practical.

- Good finding about the wastewater treatment and harmful bloom containment aspects.
- It's a very nice model, very detailed; I would like to see how it tech transfers to industrial organizations.
- The estimated time to build long term is 3 years, but that is with a fully developed facility that can be copy/pasted; realistically, after permitting, facility buildout, and modifications to optimize, you're looking at 7 years. The material storage assumptions are unclear. Is there a user interface in the future that would enable more sophisticated modeling than Excel can provide? I would also like to see a gap analysis between the current state and n^{th}-plant model to give a clearer idea of the path forward.
- TEA was certainly front and center of every talk. I am heartened to see that TEA is as important as it is in the program. I certainly hope there is some harmonization of assumptions used in those TEAs. Based on a line of questioning, tracking the database of assumptions used in any particular TEA would seem to be useful. What is disconcerting is that project-to-project comparison requires understanding what the assumptions are, or at least whether they are based on the same assumptions.
- Concerns are directed at the program. For the program, it would be useful for some indication of whether projects are using the same assumptions. Assumptions will be the main drivers. Some version of being able to track assumptions to allow for updating and harmonizing results from different times would be useful.
- Approach: The general approach for collecting input and conducting TEAs for algal biomass production and conversion is robust and has been formulated and shaped over years of interactions with multiple research groups and industrial partners. The feedback from knowledgeable consultants and stakeholders is an important element of this effort in order to enhance the accuracy of modeling outcomes. The harmonization efforts with other groups in this field are very important in order to enable meaningful comparison of results and enhance the reliability of the ensuing research guidance.
- Progress and Outcomes: The new effort to examine waste sources of algae from wastewater treatment, algal bloom collection, extracted biomass associated with nutraceutical manufacturing, etc. is interesting in that it can provide information on smaller-scale, distributed conversion processes. This could help with gaining operational knowledge for potential larger-scale, purpose-grown algae facilities. Regarding the collection of bloom algae, the investigators need to ensure that collection and dewatering costs and energy requirements for such dilute algal biomass streams are fully accounted for, especially considering the rather large water remediation credit needed for a positive outcome.
- Impact: The results of this ongoing project help to provide economics-driven research guidance in order to focus on the most impactful areas, as well as to provide credibility (or not) to new ideas on cultivation, processing, and commercialization of algal-based technologies.
- The fact that there are many downloads of publications and reports from this analysis/modeling group attests to the breadth of impact for the project.
- If not already doing so, the primary investigators should make sure that input on coproduct market prices and costs for certain services (e.g., wastewater treatment costs to municipalities, including biomass disposal, bulk prices for PUs and precursors, and fusel alcohols) are vetted by companies that are actually producing these products or providing services with conventional processes (i.e., don't rely strictly on prices/costs provided by small aspirational companies).
- Approach: Nothing novel here, but good to keep this updated each year and available for updating. Progress and Outcomes: It's critical to keep updating the model with new information. It would be

interesting to see if the models work if systems are not scaled in size as much—but are scaled in modules. Impact: Impactful to the community due to open access to the work and Excel sheet.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their positive comments and recognition of the importance of this project. We agree with the second reviewer's comments regarding the importance of understanding and harmonizing assumptions made between different projects who use our (and others') TEA models to make economic projections, though recognizing that comment is directed more toward the BETO algae program than to this project specifically (although we are happy to assist in that capacity as we can). Regarding the comments pertaining to specific inputs/assumptions made in the models—e.g., facility scale, construction time, storage assumptions—the parameters attributed to our "base case" scenarios (as documented in the pertinent report links shown in the slides) are reflective of n^{th}-plant conditions with sufficient economic and technical maturity to enable 5,000-acre algae farms modularized for construction to be completed in a 3-year period. Variances to those base case assumptions can be readily investigated, and in many cases are already reflected in sensitivity analyses published in the accompanying reports. Interested users can also vary such parameters directly using our public TEA algae farm model tool (https://www.nrel.gov/extranet/biorefinery/aspen-models/). While our TEA modeling efforts to date have historically focused on such an n^{th}-plant framework per BETO work scope authorization, there has been more interest recently in better understanding n^{th}-plant versus pioneer plant comparisons, and we will investigate options for expanding into such a comparison (in this project or elsewhere) moving forward. We also agree with the comments regarding the need to use well-validated inputs for both processing costs (e.g., for harmful algal bloom/wastewater treatment biomass collection and processing) and coproduct credits/market values. To this end, we have worked closely with industry collaborators such as AECOM on the harmful algal bloom study and Gross-Wen Technologies on the wastewater treatment study to understand key processing details for collection and dewatering of such biomass sources, and resultant water treatment remediation credits. Market values for algal-derived chemical coproducts are typically sourced from industry databases reflecting macro-industry pricing. The TEA sensitivities to both of those considerations are documented in the pertinent reports as sensitivity analyses; time constraints did not allow for presenting such sensitivities in the slides.

ALGAE TECHNOLOGY EDUCATIONAL CONSORTIUM

National Renewable Energy Laboratory

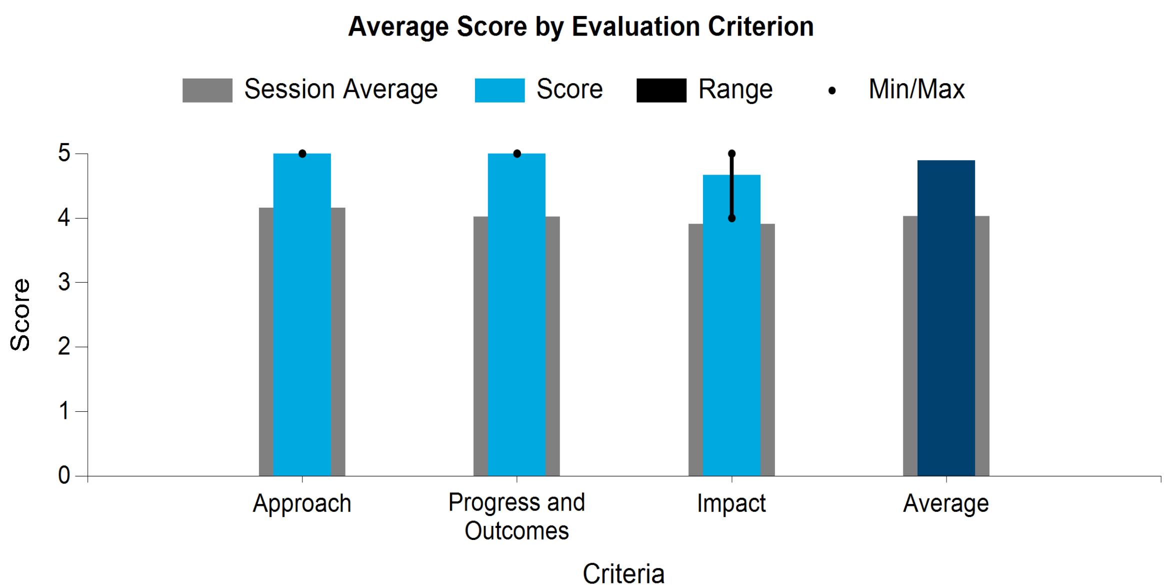
PROJECT DESCRIPTION

WBS:	1.3.5.201
Presenter(s):	Cindy Gerk
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2025
Total Funding:	$1,650,000

The ATEC project is a collaboration of academics, national laboratory researchers, and commercial algal experts to develop state-of-the-art workforce development, education, and training programs serving ages 5–80, in all 50 U.S. states and 100 countries. ATEC has a 7-year history of developing two separate collegiate curricula in algal cultivation and algal biotechnology. Additional efforts include three algal MOOCs, Algae Academy (a K–12 STEM education program), Algae Cultivation Extension Short-courses (ACES), and a digital badging program, supporting the development of the next generation of algal professionals. Future efforts include formalizing relationships with minority-serving institutions, including historically Black colleges and universities and Hispanic-, Native American-, and Native Hawaiian-serving institutions.

ATEC initiated the DOE-supported AlgaePrize 2022–2023, a national student-based 18-month research competition enhancing the algal-based bioeconomy. The culmination of the inaugural AlgaePrize is scheduled for April 14–16, 2023, at NREL in Golden, Colorado.

ATEC entered into a formal collaboration with Prairie View A&M University focused on the development of the Algae Center of Excellence for Climate Resilient Food-Energy-Water Systems, intended to be the nation's ultimate algae-based research, workforce development, education, and training center.



COMMENTS

- The risk mitigation slide is helpful, especially the COVID disruption. The collaborations are impressive, especially the industrial advisory board. As DEI is absolutely critical to this particular project, it's wonderful to see so much outreach. The impact on student learning and careers is wonderful; the quantitation and clear success of the program are fantastic.

- The curriculum is frequently updated based on feedback from industry and networking events to ensure the relevance of the material. Many students choose entrepreneurship and work in related fields rather than directly in algae cultivation, as the jobs anticipated have not materialized; however, the transferrable skills are strong and readily used. For example, Austin Community College is part of the program, yet there is no algae cultivation ongoing in Texas. The program specifically provides more in-depth experience than many kit-based labs can provide, as there is more hands-on work, and a richer understanding of the course materials is required in order to perform the work.
- Most impressive was the way in which the course was able to transition to online education with materials shipped from various facilities. The program leadership was able to anticipate a potential shutdown with an earlier experience of a bird flu epidemic and had already preadapted their programming by leveraging online education connections at Santa Fe Community College and some other collaborators who were already online.
- This was a very enthusiastic presentation and had a clear commitment to education about algae. In general, very positive with two very minor concerns: goal alignment and metrics. The move to heterotrophic growth is one example. I find it hard to relate this to the stated program goals. It may well benefit the students but seems unaligned with BETO goals. The second is about metrics for success. I've been involved with education programs in the past. The data on student surveys as a measure of success are certainly wanting. If the intent of the program is a prepared workforce, some more meaningful metric such as post-training employment would be a more reasonable metric. For secondary education, a post-class survey is not compelling as a measure of success for classroom learning but may be a bit more acceptable, as more rigorous data collection is likely difficult. Lots of excitement and engagement; my only question is about the measure of impact relative to programs attempting to provide certifications for employment.
- Approach: The approach taken by ATEC is comprehensive and addresses some key general BETO goals regarding education and workforce development in the bioenergy field, as well as specific program goals related to algal systems for energy production.
- ATEC has developed and rolled out training curricula at levels everywhere from elementary school through colleges and universities. Multiple disciplines are covered, including algal biotechnology and cultivation science. Both microalgae and macroalgae are covered, which adds to the depth of the training offerings.
- Progress and Outcomes: The number of students who have participated in training modules developed through ATEC over the years is really quite impressive. This has certainly had a positive impact on getting the word out about the potential for algae in the bioeconomy, both in the United States and abroad.
- The addition of new modules to the MOOC series and the expansion of schools that participate in ATEC offerings will continue to further the reach of ATEC and ensure that the curricula are kept fresh, comprehensive, and up to date.
- Impact: The various ATEC programs continue to be impactful for generating interest in algae for students of all ages and for providing skills and knowledge that should enhance the job prospects for college-level program participants who choose to pursue jobs in algal-based businesses and research labs. This impact is confirmed by the large and growing number of students who have participated in ATEC programs and products, including MOOCs, the Algae Academy, community college courses (including earned certifications), AlgaePrize applicants, etc.

- As the algae industry expands in the future, there will be numerous qualified job applicants for open positions that will already have many of the skills required for successful integration into the industry.

PI RESPONSE TO REVIEWER COMMENTS

- The ATEC team is very grateful to the BETO reviewers for their recognition of accomplishments and to BETO for continued support of bioeconomy workforce development, algal education, and training. The ATEC curriculum and the Algae Academy have reached all 50 states and more than 100 countries. We continue to expand the ATEC partnering collegiate network through our collaborations with historically Black colleges and universities, InnovATEBIO, and Future Farmers of America. It is deeply gratifying to receive such a strong positive review. It sends us a clear message that we are on the right track and encourages us to continue to focus our energies on expanding the program to bring algae awareness to more and more students. We acknowledge the reviewer's comments concerning analytical metrics measuring impact and have scheduled this as a major area of effort over the next 2-year period. Regarding the comment about aligning with BETO goals, the example given was the development and inclusion of heterotrophic cultivation of algae within our cultivation curricula. Currently, the United States' microalgae market is split between the phototrophic and heterotrophic, with the corporate giants DSM and Corbian relying on heterotrophic cultivation for omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid), along with the development of Thrive, an algal-based cooking oil. With the inclusion of bioplastics, biofeeds, and biofoods along with algal-based fuels, heterotrophic cultivation techniques are a valuable arrow in the quiver of current cultivation expertise. Our industry relevance is reflected in NREL including ATEC experience as a preferred qualification for job applicants and Corbian lowering the degree requirements from a 4-year degree to a 2-year degree for graduates with an ATEC algal cultivation certificate.

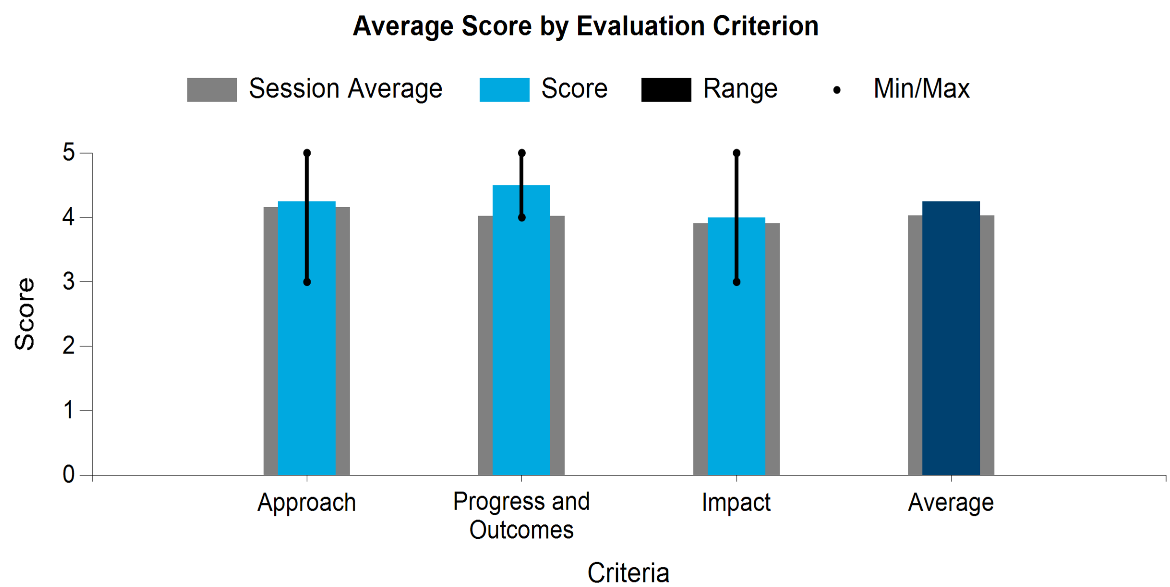
HTL MODEL DEVELOPMENT

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.202
Presenter(s):	Peter Valdez
Project Start Date:	10/01/2017
Planned Project End Date:	09/30/2023
Total Funding:	$600,000

The HTL Model Development project provides TEA and sustainability inventory for algae conversion via HTL and upgrading to fuels and coproducts. A conceptual biorefinery model was first developed in 2014, and since then, SOT assessments have been reported annually. The purpose of the SOT assessment is to identify barriers, cost reduction strategies, and sustainability impacts to track research progress toward BETO's 2030 cost target of producing fuels at $2.50/GGE. The annual SOT report documents the modeled costs and the associated research incorporated into the modeling. Synergistic work between the analyst and experimentalist teams directs research toward high-impact results, driving conversion costs toward the cost target. In the 2022 SOT report, the cost of HTL conversion of *Picochlorum celeri* was $0.35/GGE, compared to $0.88/GGE reported in 2019. The reduction is achieved through the value of recycled nutrient credits. The conversion cost is nominally affected by the inclusion of a denitrogenation step to produce high-quality distillates for SAF. However, the cost of the farm-cultivated feedstock results in a minimum fuel selling price of $5.42/GGE. A no-cost algal feedstock, as reported in the 2021 SOT report, resulted in increased conversion costs but achieved the lowest reported minimum fuel selling price of $2.61/GGE.



COMMENTS

- The slides are telling me that you are communicating and that you have some industry collaborations, but how are you communicating? Is it with meetings, online, Slack, or what? Who in the industry are you collaborating with specifically? On which aspects of the project? Slide 11 reads like a decrease in production after optimization; is this correct? Slide 14 states that 23% of the biocrude is within range of jet fuel, but this doesn't seem to be what the graph is showing. The mixed materials with wood waste blend will be helpful. I would have liked to see a gap analysis of how long it will take to get to the *n*[th]-plant scenario.

- There is still considerable ambiguity. Within that ambiguity, the project has done a great job of attempting to get to actionable conclusions. I am particularly impressed with the ability to accept changing feedstocks, including even non-algae feedstocks like wood. My only negative comment is that the results still show that considerable improvement is needed to be viable.

- Approach: The project team includes different groups that are able to provide good input for the various analysis parameters. The team helps to identify processing pathways that increase the economic value of the overall HTL process.

- There appears to be quite a bit of overlap between this project and the other HTL project reported by the same presenter (Hydrothermal Processing for Algal-Based Biofuels and Coproducts). Comments for this presentation should be considered in tandem with the comments made for that presentation.

- Progress and Outcomes: Modeling results have been completed in the past 2 years for HTL of purpose-grown algae, indicating a minimum fuel selling price of $5.42. These results, including a higher credit for nutrient recycle, were provided as input for the 2022 SOT report. Information from this study was also used for the upcoming version of the algae harmonization report.

- Emphasis was placed on protein coproduction in order to lower the achievable fuel selling price and to reduce GHG emissions from farmed algae (*Picochlorum*). Because of the significant modeled cost reduction, it will be important to validate that the protein quality is suitable for use as a feed or food product, as well as to ensure that the GHG displacement credits are accurately calculated.

- Considering the large credit being given to struvite as a coproduct of HTL of algal biomass from wastewater treatment, it will be important to actually test the struvite coproduct for use as a fertilizer for crop plants (and possibly algae) to make sure that there are no growth-inhibiting substances present to validate the assigned economic value.

- It is important to understand whether the products from the HTL process have characteristics that are acceptable for their intended purposes, and thus whether the assigned values are appropriate. The small-scale testing of the HTL oil products from *Picochlorum* suggests that it would be suitable for use as a jet fuel; this is a good outcome. Similar studies will need to be conducted for HTL products form other algal biomass sources.

- Impact: The team has published SOTs and other reports the last couple of years that provide research guidance for further areas for HTL process improvement. In combination with SOTs for alternative downstream conversion processes, funding decisions on the most promising paths forward can be made.

- Approach: Good; good collaboration on the project. Outcomes: Meeting goals, advancing the SOT. Impact: High due to lots of publications and patents.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their thoughtful and constructive comments and questions. We will address key questions and areas that need further clarification. With respect to our collaborative activities, we interact via emails and meetings (in person and virtual) with providers of the algal feedstock, equipment vendors, and others implementing HTL technology. We typically engage with our industrial collaborators to learn about current trends that impact the development of algae HTL, such as feedstock availability and costs, market potential for HTL products, and equipment capabilities. An abbreviated list of collaborators is included in the presentation for project 1.3.4.101. In the presentation materials, the draft results for the optimized scenario show that there is a reduced production volume of fuel. In the optimized scenario, the production volume of protein concentrate is unconstrained, resulting in a higher production of protein products, which are favored over fuel products because of their higher value. However, please refer to the final, published version of the harmonization report for full details of the analysis and results. In upcoming work, to produce the design case study report, we will investigate scenarios related to the first-of-a-kind plant and the predicted costs and anticipated design challenges. We will continue investigations in the experimental project (1.3.4.101), working with collaborators and relevant product experts to validate the process assumptions that are used in the analyses for this project. Additional analysis work in this project and with support from Argonne National Laboratory (4.1.1.10) will ensure the accuracy of our process and environmental model calculations to assess the project's economic and environmental impact.

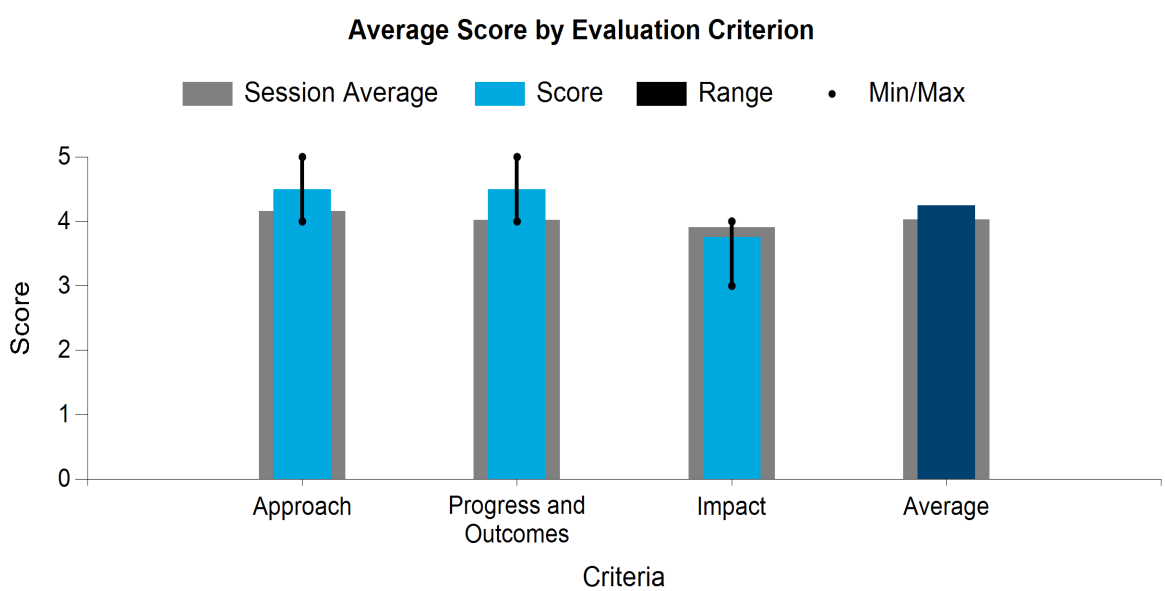
MICROALGAE ANALYSIS

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.203
Presenter(s):	Andre Coleman
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2023
Total Funding:	$1,725,000

The Biomass Assessment Tool (BAT) provides a core capability in the BETO program, enabling a linked geospatial, biophysics, and partial techno-economics model and analysis tool for linking key BETO and industry research activities to achieve high-impact objectives. To date, project technology transfer has been successfully achieved through 31 peer-reviewed publications, the technical assistance program, workshops and conferences, direct collaboration with academia and industry, and integration with the Bioenergy Knowledge Discovery Framework. Outcomes from the past annual operating plan (AOP) cycles have continually addressed priority issues for the emerging algal biofuels industry. Under the current AOP, the project objectives are to (1) continue participation in the BETO SOT for experimental design and scale-up of field studies in time and space; (2) parameterize and model two high-performing saline strains from DISCOVR; (3) provide resource assessment and limited techno-economics for the multi-lab Microalgae Model Harmonization effort that includes water and nutrient collocation opportunities; (4) perform resource assessment and techno-economics for offshore macroalgae cultivation; (5) develop microalgae and macroalgae chapters for Oak Ridge National Laboratory's Billion-Ton report; (6) complete the development, validation, and documentation of the enhanced high-rate algae pond model; and (7) develop, mature, and demonstrate the microalgae biomass forecasting system.



COMMENTS

- This project focuses on modeling the sustainable supply of cultivated biomass by employing open-pond PBRs, saline water, wastewater, and CO_2 collocation. Their approach includes development, enhancement, and application of available tools and resources to understand opportunities and trade-offs and realize new pathways.

- The team appears to be well managed with well-defined task structure and leveraging team expertise and previous experiences. The team highlighted some challenges with the various approaches and identified mitigation strategies.
- The team has made significant progress in using a microalgae biomass forecast system to demonstrate improvement in biomass productivity by over 40%. They have identified collocation opportunities and offshore macroalgae biomass potential. The team should consider the impact of water quality from the different location on biomass productivity for the selected strains. The algae industry will benefit from the work done here and may provide inputs and new technologies that are currently missing from this analysis.
- The goal of this project was to provide an algal biomass assessment tool to BETO and industries as a harmonized model for building a systematic assessment of site-scale capability considering all environmental factors related to land, water resources, and other such parameters. Several partners have contributed to this project, improving and updating the model. It now involves parameterized assessment for both macro- and microalgae, salinity, drought, and other such variables. The project has been well coordinated between the partners and employed in several experimental operations. It would be helpful to know how many industries have adopted this BAT model to their benefit. The project has resulted in several publications in peer-reviewed journals.
- This project has very clear management and communication, and they appear to have exceeded their goals/milestones. The project goal is to deliver a national assessment tool that can answer where, who, and how for algae cultivation. The team appears motivated to get the tool into the hands of industry but has had little success so far. It is concerning that no industry link has been made to date, as this was a topic of discussion during the 2021 review. It is unclear if this is due to the lack of companies that could utilize the model or a shortfall of the project. Once it is in the hands of industry, the model should become a highly valuable tool, particularly informing technology trade-offs for various geographies. One of the project's biggest limitations is the quality and quantity of data inputs, but it is understandable that these data are either IP protected or just do not exist. It was refreshing to see a project in the BETO portfolio taking a national view on the algae industry's potential and not just for the limited locations where most algae work has been done. Hopefully the data will catch up with the model capabilities. The recent inclusion of macroalgae is also good foresight.
- This project approach focuses on a national assessment of where algae production can occur, how much nutrients are required, land and water resources required, how much biomass/energy is produced, and what interactions/trade-offs exist between technologies. The development of this BAT is an important part of the BETO algae cultivation program. The project is on schedule, and the project team has accomplished most project tasks. It is not clear what would be displaced (houses, farmland, etc.) in the sites identified in California, Texas, Louisiana, and Florida.

PI RESPONSE TO REVIEWER COMMENTS

- Reviewer #1 Response: We are grateful to the reviewer for providing an accurate summary of the research topics and including constructive comments. The work elements included in this project provide insights on BETO research priorities while being informed on needs, gaps, and questions in the community (BETO SOT, DISCOVR's industry advisory board, Advanced Research Projects Agency – Energy [ARPA-E] Macroalgae Research Inspiring Novel Energy Resources [MARINER] industry partners). Our focus has been to provide high-resolution, site-scale, national analysis that can help inform industry on a range of location and configuration trade-offs for use in their own strategic operations. The same analysis is also informative for helping to set future production and sustainability targets that can ultimately provide input into policy. The reviewer noted the consideration of water quality into the BAT analysis. In some aspects, water quality has been considered, specifically with respect to avoiding potential algal cultivation sites that would use groundwater with constituents (e.g., arsenic) that exceed standards established by the Environmental Protection Agency, and establishing waste resource budget models to utilize wastewater and associated nutrients from municipal wastewater treatment plants where algae cultivation sites can be potentially collocated. For our open-pond microalgae model, we have generally assumed this to be a closed-loop system for freshwater, where harvested or centrifuged water is recycled back into production ponds. The new BAT-enhanced pond model will also track pH and nutrient balance, and a future expansion of the model could include water quality measures if we assume water is being routed elsewhere after harvest. On another point, for the saline water open-pond model, blowdown volumes are processed through a forward osmosis system, where the freshwater fraction (~85% of the processed blowdown) is recycled to the pond for salinity management, and the remaining hypersaline fraction (~15%) is disposed of via deep-well injection. Alternative forms of hypersaline water disposal have also been investigated, including evaporation ponds. Our team will further consider how water quality can be better incorporated and represented.

- Reviewer #2 Response: We thank the reviewer for their favorable comments. A key pillar in the BAT modeling work is to link the resource assessment (i.e., what is required and consumed and how much biomass is produced under varying locations and operational configurations) with the expertise and capabilities of our lab partners. This has been exercised under three separate BETO model harmonization efforts and provides a unique coupling of resource assessment, techno-economic modeling (NREL), LCA (Argonne), and modeled biomass-to-energy conversion pathways. In addition, the BAT team has contributed to the BETO SOT efforts with the microalgae biomass forecast system; informed on DISCOVR experiments; and brought microalgae, and now macroalgae, into the Billion-Ton studies. Our recent addition of macroalgae modeling is possible through partnerships with the ARPA-E MARINER program (and industry partners) and the University of California, Irvine. The BAT team has long participated in and contributed to various consortia efforts, directly supporting and informing industry partners and contributing analysis data to support research at other national labs outside of the BETO model harmonization effort, as well as to numerous individual universities. With respect to the reviewer's comment about industry adoption, we've had strong industry collaboration in the past, directly providing analysis for informed decision-making. We also recently submitted a new project proposal with an industry partner focused on scaling up SAF and renewable diesel production. Unfortunately, our team has not kept a tally of users, but the primary use of BAT is through its data products, which have been published as open-source data sets through Oak Ridge National Laboratory's Knowledge Discovery Framework, and the methods and analysis results have been documented in 25 peer-reviewed publications and several reports. We regularly field requests for data and analysis products and are happy to provide these to the community.
- Reviewer #3 Response: We thank the reviewer for the positive comments, but also for sharing concerns. As noted to Reviewer #2, the BAT team is a regular collaborator and partner on many fronts, including the BETO SOT efforts, the Algae DISCOVR experiments, and multi-lab efforts such as the BETO model harmonization study and the Billion-Ton studies. Our recent addition of macroalgae modeling is possible through partnerships with the ARPA-E MARINER program, the program's industry partners, and the University of California, Irvine. Additionally, the BAT team has long participated and contributed to various consortia efforts that included private industry, as well as directly supporting and informing industry partners and numerous individual universities. Our methods and data analysis products have been regularly peer-reviewed and improved upon by those in industry. We would like to clarify some points, as these likely weren't made clear in the Q&A session of the presentation. First, some of our team's closest industry partnerships are fragmented due to the volatility of a still nascent industry. Having said that, there is more our team can do to continue outreach and build relationships so industry can benefit from tailoring BAT's data and analysis capabilities. In addition, with the recent publications of the microalgae forecast system model, we feel this will be of interest to industry, and near-term efforts will focus on establishing industry collaborations. Second, our team has continued to publish our

methods, analysis, and data to make them publicly accessible or accessible by request. In fact, our team regularly fields data requests from various entities, including academia, industry, government organizations, and national laboratory partners, though admittedly, it is hard to quantify what the specific impact has been for industry. Third, the BAT is a complex collection of big data, numerical models, interaction mediums, high-performance computing components, and Linux OS implementations that currently does not lend itself well to a downloadable and easy-to-use model for industry. Admittedly, if there were industry interest, a future project effort could be established to move the modeling platform to a higher technology readiness level and make this a deliverable product. As a note, a version of BAT was instituted in a web-based interactive platform, though it was later taken down due to emerging security vulnerability risks in the underlying web technology used. Additionally, there hasn't been significant sponsor interest to revive or establish a goal toward a transferable or web-interactive model. As a final response, the reviewer noted that "one of the project's biggest limitations is the quality and quantity of data inputs." The BAT team continually strives to use the highest-quality, most resolute, and latest publicly available data to inform the various models. We believe that the reviewer's comment here was specifically referring to open-pond observation data that we use for model validation; this ties to a question posed and a time-limited answer provided in the Q&A session. In our experience, the open-pond observation data have not been abundantly available, not appropriate (i.e., 100-L raised ponds), and/or not ready for release, but what we have received through industry and academic partners reveals very good open-pond modeling performance. We will continue to work with collaborators to acquire data so we can assess the modeling performance of the open-pond model, PBR model, biomass growth model, and nutrient and pH models.

- Reviewer #4 Response: We thank the reviewer for their comments and recognition of BAT as "an important part of the BETO algae cultivation program." Regarding the reviewer's comment on displacement by potential algal cultivation sites, it is worth briefly explaining BAT's multicriteria land suitability model. The land suitability model provides the fundamental basis for all other models in BAT, specifically identifying potential cultivation locations that meet a user-specified minimum area requirement (i.e., 1,000 acres). A key building block in the land suitability model lies at the currency and quality of spatial data used to inform the scenarios, and thus efforts are taken to process and use the latest high-resolution (10–30-m) national (contiguous and outside contiguous United States) data available. The model first identifies areas with slopes <2%, and then from these areas proceeds to eliminate open water bodies; wetland and riparian areas; forested lands; productive agricultural and pasture lands; urban area boundaries; various density developed areas; roadways and airport runways; military lands; protected lands, including local, state, and national parks, wilderness areas, fish and wildlife lands, and environmentally sensitive lands; and lands with significant levels of net primary productivity (high carbon storage). Brownfield sites, 25% of U.S. Department of Agriculture-defined marginal croplands, and defined croplands that have been idle for >5 years are added back into the screening pool. Finally, from the remaining screened lands, all contiguous areas less than the user-specified area threshold value are eliminated. Further site screening is implemented in additional models with regard to sustainable freshwater or saline water availability, minimum annual average biomass productivity of 25 g/m^2/day, access to existing infrastructure, access to collocated waste resources, and so on. This rigorous screening process is intended to provide the locations that have the greatest potential for success, while minimizing displacement impacts. Because this screening is occurring nationally, it is still necessary to understand site-level conditions and nuances that cannot be captured with available mapping data.

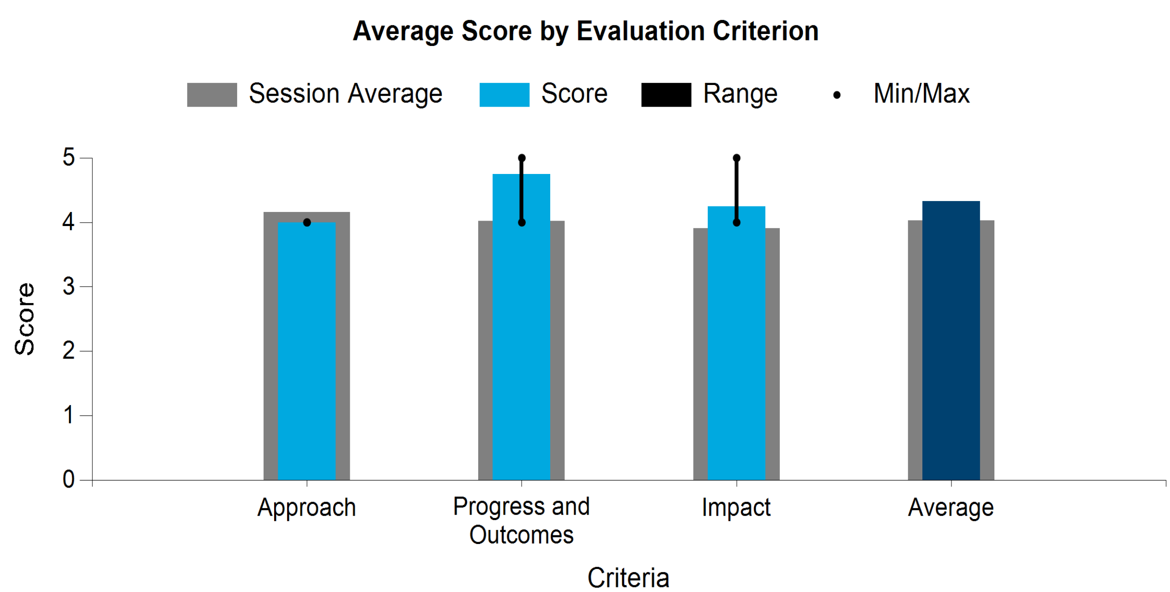
LIFE CYCLE ANALYSIS

Argonne National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.204
Presenter(s):	Troy Hawkins
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2023
Total Funding:	$500,000

This project provides energy and environmental LCA of advanced algal systems to support BETO R&D decisions and to provide reliable benchmarks for algae production and fuel/product pathways and estimates of their life cycle energy and environmental metrics for BETO stakeholders. It is a continuation of a task that has been active at Argonne for 10 years. In the first year of this new project cycle, the project team is analyzing the life cycle energy and environmental benefits and trade-offs of alternative carbon dioxide sources for algae production, considering CO_2 from DAC, electricity generation, industrial sources, and high-purity sources. We are collaborating with the National Energy Technology Laboratory (T. Skone) for this effort and will leverage LCA data sets for DAC and capture from fossil energy sources. During the first year, the team is also analyzing algae production in saline water, continuing a collaborative effort with PNNL that began in FY 2020 focusing on understanding the energy and environmental implications of salinity maintenance and brine management. In the second and third years of the project, we will expand the focus to LCA, examining production of higher-value products from algae and integration of algae production with wastewater/manure management systems.



COMMENTS

- The goal of the project is LCA for algal systems that include CO_2 sources, saline algae, algal bioproduct, and integration with wastewater. This has clear relevance to BETO's goals of increasing the supply of sustainable algae and reducing the resource intensity, as well as system integration and resource recycling.

- The team appears to be well managed with well-defined task structures and leveraging team expertise and previous experiences. They identified risk and outlined mitigation strategies. The project performers are constantly refining and harmonizing data across models to make them more relevant to the field and have good collaboration with research partners. The team has delivered several milestones and is well on its way to achieving the goals outlined for this project.

- The project analysis demonstrated that fossil fuel CO_2 will result in the highest GHG emissions when compared to biogenic or atmospheric CO_2. It is unclear if this analysis includes the use of CAs and other improved CO_2 capture methods like operating at high pH and the iteration process for integrating data and technologies into the model. The project performers should consider integrating industry feedback into the model, as most of the inputs are based on national lab data. Overall, the team has made significant progress in delivering on goals.

- The project, focusing on LCA of advanced algal systems, runs out in September of this year but is in a situation where it can extend to another 3-year period by filing another application. The team talks about its success on being able to align their tasks with BETO's goals as projected on five areas by (1) providing LCA data for algae production, salinity management, and pond operations; (2) evaluating and comparing carbon dioxide sources affecting algae production; (3) integrating algae cultivation with wastewater and manure management; and (4) harmonizing TEA and LCA within BETO's AAS Program. It is a little difficult in certain parts to understand how the harmonization analysis data flow was achieved across the models between the other national labs and Argonne's Greenhouse Gases, Regulated Emissions, and Energy Use in Technologies (GREET) model. It would be very affirmative if the team could share some success stories that users are benefiting by implementing these models (like an example from the GREET users), which is BETO's mission. There is not much mention about the task on algae cultivation with wastewater and with manure management, which currently falls under the last 5 months of the grant's lifetime. The team has disseminated their findings to the community and stakeholders by publications in peer-reviewed journals, conference presentations, and reports.

- Team management and communication are well outlined, and this appears to be a well-organized project. The goal of this project is to deliver LCA for key production/conversion pathways. They have had a few key findings, including identifying PU from algae as a highly valuable and influential coproduct. The concern with this project is that it is only sourcing data from the national labs, which may be a bit insular and limit the applicability of the findings as the industry grows, although it is unclear if the scope of data required to input is available from other sources. The team does appear to be working with two industry partners directly involved in algae. The focus areas of this project are well aligned with BETO goals. The timeline on the last slide was very helpful. Hopefully the topic areas of focus remain live after each stage such that they can continue to be updated and remain current as the industry emerges.

- The approach of analyzing algal systems using a life cycle approach is important and crucial for BETO to meet its targets. The project team has made good progress in analyzing LCAs for saline algae production, cultivation with CO_2 from DAC, comparisons of CO_2 sources for CO_2 production, and algae bioproduct pathways. The project is on schedule, and the project team is on track to complete its last task involving LCA integration of algae cultivation with wastewater and manure treatment systems. The impact of the project is clear and should provide the value proposition for algal biofuel and bioproduct technologies.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewers' feedback on the project and support for this analysis to guide the development and scale-up of algal systems capable of delivering on BETO's strategic goals related to decarbonization of transportation, industry, and agriculture. We are dedicated to focusing on the key issues and driving consensus among stakeholders regarding the promising opportunities. The reviewers' comment regarding integration of industry data and perspectives in the project resonates with our plans. We have established relationships and data-sharing agreements with industry partners related to analysis of their technologies and will continue to expand these interactions. We have also leveraged the capabilities developed through this project in work on projects with the Office of Fossil Energy and Carbon Management and ARPA-E, as well as other BETO FOA projects involving engagement with industry partners. We regularly attend the Algae Biomass Organization Conference to present our progress and meet with industry stakeholders. We will continue to seek out opportunities for interaction with industry as we move forward in analyzing options for maximizing the economic and GHG benefits of algal systems through different product strategies and options for improving wastewater resource recovery through integration of algal systems. The reviewer's point about keeping the topic areas "live" as we move forward to explore new questions is well taken. We revisit the algae models in GREET with each annual release to consider updates to reflect the current state of the industry and our understanding. Moving forward, we hope to have the opportunity to renew this project so we can continue to update the GREET algae models, support the SOT assessments for algal biofuel systems, continue building deeper connections with industry stakeholders, and address new issues such as those suggested by the reviewers related to approaches for improved CO_2 utilization to enrich BETO and the community's understanding of the promising opportunities for algal systems. This is a rapidly evolving field with a strong need for analysis to guide research, development, and deployment efforts, and we are honored to have the opportunity to provide actionable results.

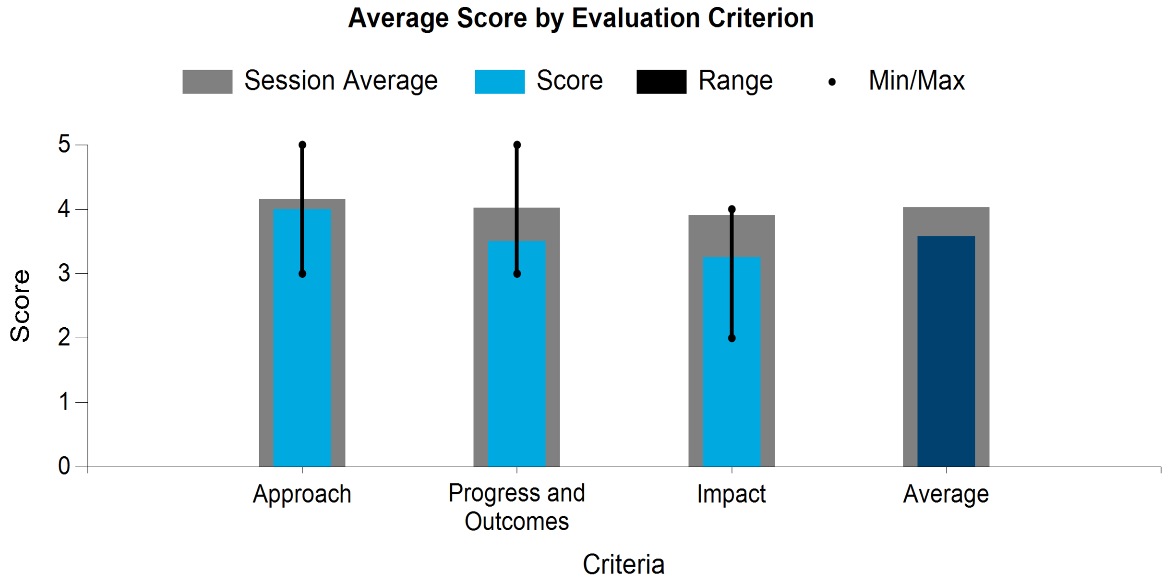
OPTIMIZING SELECTION PRESSURES AND PEST MANAGEMENT TO MAXIMIZE ALGAL BIOMASS YIELD

New Mexico Consortium

PROJECT DESCRIPTION

WBS:	1.3.5.280
Presenter(s):	Alina Corcoran
Project Start Date:	10/01/2019
Planned Project End Date:	02/01/2024
Total Funding:	$6,289,829

The Optimizing Selection Pressures and Pest Management to Maximize Algal Biomass Yield (OSPREY) project responds to a critical industry need to improve annualized productivity, stability, and quality of algal production strains for biofuels and bioproducts. We aim to generate process innovations rooted in outdoor cultivation for strain selection, maintenance, cultivation, improvement, and pest management that will result in a 50% improvement in harvest yield and robustness and a 20% improvement in conversion yield. Our project's components are built on a single foundation: the year-round cultivation of a field-adapted algal biofuel strain in outdoor systems at Qualitas Health (Imperial, Texas), Cyanotech Corporation (Kona, Hawaii), the California Center for Algae Biotechnology (San Diego, California), and the Fabian Garcia Science Center (Las Cruces, New Mexico). We envisioned that the unique environmental selection pressures of each outdoor system would allow us to naturally develop robust cultivars with different environmental tolerances. Project components include tracking trait drift and evolution in the field and lab, using metagenomic tools to identify and track pests/pathogens, using non-genetically modified approaches to improve the baseline field-adapted strain, implementing process improvements, and assessing the effects of improvements through sustainability modeling based on open-raceway pond growth architectures. To date, our technical accomplishments include (1) the cultivation of industrial field-adapted strains at three additional locations since summer/fall 2020, (2) phenotypic and genotypic trait tracking every 6 months, (3) metagenome characterization across sites, (4) detection of novel pests, (5) development of a fieldable qPCR tool, (6) application of selection and mutagenesis/selection to drive cold and hot tolerance, (7) hindcasting of productivities across sites, and (8) exploring the effects of shifts in biomass composition on the minimum fuel selling price. Selection pressures were not strong enough and/or the time was not long enough to capture cultivars in the field. This project responds directly to DE-FOA-00029 with an indoor/outdoor experimental framework and the development of tools to monitor cultivation health.



COMMENTS

- The risk mitigation, decision-making, and project management are all clearly laid out and easy to follow. It seems like the risk mitigation initial estimate wasn't as accurate as it could have been due to field selection pressures not being thoroughly understood; Qualitas and Cyanotech should be tracking the generations of field cultures and periodically sampling and should have shared these data to give you an initial working idea of how many generations are required to realize trait evolution. I love the overview of progress on Slide 16; this is perfect. There are no y-axis markings on the left side of Slide 17. It would be helpful to know if the field PCR analysis would be run in a time window sufficient to mitigate the infection/predation. Adding the data regarding ultraviolet kill of pests and its effect on the algae would have been helpful, as well as the data for clonal isolation from the mutagenized volumes that underwent clonal selection. The cryopreserved reference strain is a good benchmarking practice; it would also be good to select either single nucleotide polymorphisms (SNPs) or some critical gene to monitor for mutation as well.

- The project scope change complicates reviewing the goals against the objectives. It appears that the changes remained true to the goals of BETO, were made with the blessing of BETO staff, and were executed well. It shows the involvement of BETO staff in shaping and maintaining projects. Great example of active project management.

- The approach is thorough and sound. The team composition is complementary. qPCR in the field was promised and delivered. The outside mutagenesis and cryopreservation data being out in the open literature is a plus. In a program funded by public money, there is certainly a desire to have project learnings shared with the entire community. This is, of course, counter to the desires of a company seeking to develop and maintain a proprietary competitive advantage. This project is sharing some data, which is a good thing. I'm happy to see the team is thinking about how to strike a balance.

- Approach: This project is a collaboration between several well-qualified companies, universities, and government labs, each of which brings established strengths to the project. The general approach being taken is directly relevant to BETO and AAS goals, although the results may be applicable to a relatively small subset of strains (*Nannochloropsis*, in particular, if in fact that was the strain being evaluated throughout these studies, which was not entirely clear). However, the presentation did not reveal a well-integrated strategy; rather, it seemed that several fairly disparate research activities were undertaken that didn't have an obvious connection (e.g., strain preservation methods, qPCR for pest evaluation, in-field mutagenesis, SNP analysis to check on genetic drift).

- Progress and Outcomes: The mutagenesis experiments to develop a cold-tolerant strain were interesting, but more work would need to be done to see if this represents an industrially relevant advance (i.e., is the improvement great enough to actually enable profitable growth in cold seasons?). Due to the multigenic nature of cold adaptation, this reviewer feels that mutagenesis for enhanced growth under cold conditions is unlikely to yield strains that are significantly and stably improved such that the overall production economics are significantly improved. Efforts to find native strains that are naturally adapted to colder temperatures may be more successful, although even then the thermodynamic constraints of physiological and biochemical processes at low temperatures will likely limit potential enhancements.
- Fieldable qPCR is interesting from an R&D perspective, but it may not enable mitigation of pest strain contamination events unless the particular contaminant strains being tested for are amenable to specific or broadly applicable pest control efforts that can be initiated in a rapid and meaningful time frame.
- Higher levels of SNPs in strains that have gone through many generations is not altogether surprising, considering that many (or most) of these may represent silent or neutral mutations. It wasn't clear, at least to this reviewer, whether the SNPs were found in DNA isolated from the bulk population or from single cells that had been isolated from the cultures and grown up for DNA extraction as clonal cultures. This would help to understand if there are multiple SNPs occurring in single strains or across a whole population. Estimating the numbers of cell divisions (generations) for the different cultures being compared would be useful information.
- Impact: Determining the composition of contaminant populations and making correlations of particular species with periods of poor (and possibly good) productivity will help to build a database that informs culture maintenance strategies. For maximal value, this type of analysis should be conducted across multiple sites with several algal species and cultivation protocols in order to identify possible commonalities.
- It is well established in industrial microbiology (e.g., fermentation companies) that cryopreservation of strains and periodic reestablishment of inoculum seed trains with cryostocks is an important and necessary means to preserve strain integrity and maintain consistent production results. Although this work confirms this well-established principle for algae, it may not be worth continuing such studies beyond getting a better understanding of how long a semi-batch or continuous culture can be run, although even then, empirical productivity measurements will best define strain stability.
- Approach: Had to be changed in regard to trait drift and evolution. Unable to show trait drift in the field, which could relate to the amount of time permitted for drift. Progress and Outcomes: Had to change approach due to lack of progress; data output is here. Not clear that the data were particularly impactful. Better presentation of the data is needed to show the improvements claimed. Impact: The qPCR tool could have some impact; the data do not have a lot of impact other than to prove to industry that their strains should be stable in production. However, proving a lack of strain drift in the field is important, as consideration of trait drift in productivity decreases can prevent producers from finding the real root cause of production issues. The qPCR equipment work approach is good. This information is impactful.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for evaluating our project and welcome the opportunity to address key comments. We will start with the project approach. One reviewer noted that our project had a lot of components that were not strategically integrated. Our project's components—(1) balancing indoor and outdoor selection pressures, (2) optimizing pest management, and (3) improving field strain performance, resiliency, and composition, all to enhance biomass production and stability—are built on a single foundation: the long-term cultivation of *Nannochloropsis* at different field sites. We aimed to start with a field-adapted strain and use lab-field iterations to overcome some of the challenges associated with the lab-to-field pipeline commonly followed in R&D. We also want to clarify that the aim of the first component was not to confirm reestablishment of inoculum seed trains with cryostocks as a key practice. Rather, we planned to establish local cultivars that would show phenotypic advantages over the cryopreserved strain. As this result was not obtained, we shared the results we did have. With respect to qPCR, the analysis can be run in a few hours, such that if pond samples are taken in the morning, ponds can be treated in the afternoon. This approach has previously been used on specific pests at industrial scales. Regarding strain improvement, mutations were found in DNA isolated from the bulk populations. Generation number varied across sites, with the most at the University of California, San Diego, the least at New Mexico State University, and Cyanotech being intermediate. Additional work will focus on mutations that are linked to cell function. For our cold adaptation work, we agree that strains might not be improved enough to affect overall production economics. However, if successful, we will validate a pipeline that can be used in other seasons.

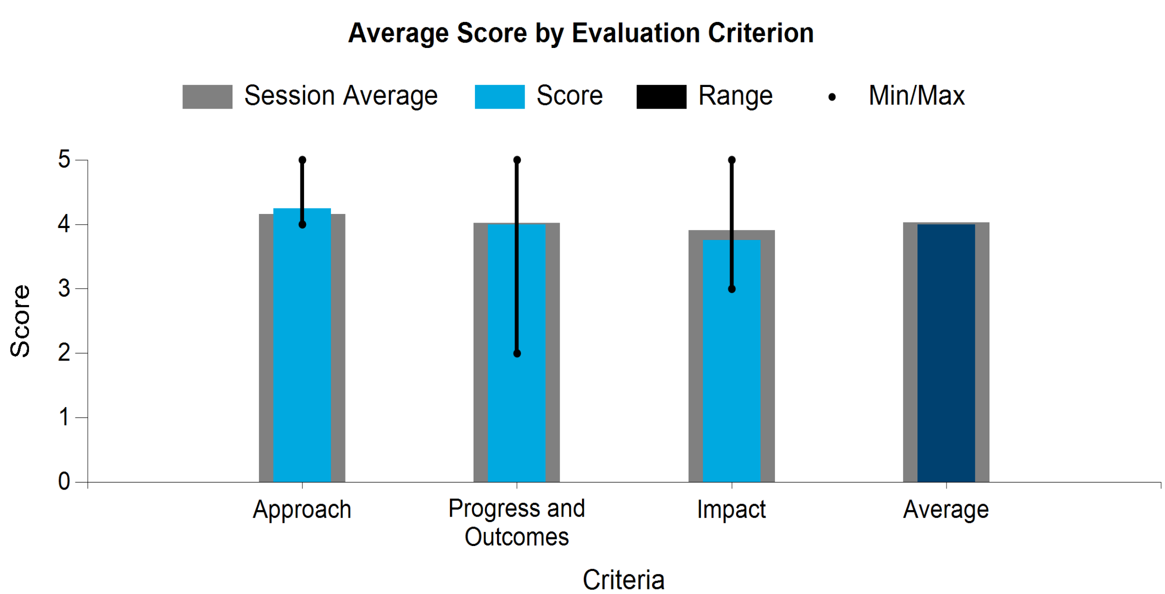
ALGAL PRODUCTIVITY ENHANCEMENTS BY RAPID SCREENING AND SELECTION OF IMPROVED BIOMASS AND LIPID PRODUCING PHOTOTROPHS (APEX)

Colorado School of Mines

PROJECT DESCRIPTION

WBS:	1.3.5.282
Presenter(s):	Matthew Posewitz
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2024
Total Funding:	$4,920,378

We are using random mutagenesis techniques (e.g., atmospheric and room temperature plasma mutagenesis) to generate genetic diversity in two oleaginous strains of algae (*Nitzschia inconspicua* and *Nannochloropsis granulata*). Mutant pools are grown in solar-simulating bioreactors to enrich for robust growth, and then high-lipid strains are sorted using fluorescence-activated cell sorting to isolate higher-oil-accumulating strains. After multiple rounds of enrichment and rapid growth campaigns, single cells are sorted from the highest-lipid cultivars during fluorescence-activated cell sorting. To date, we have downselected to four strains of *Nitzschia inconspicua* of interest. One strain (GAI-370) is able to attain diel yields of ~40 g/m^2/d in solar-simulating bioreactors and attained lipid yields of 60% in lipid phase growth. Initial outdoor growth campaigns yielded sustained productivities of ~22 g/m^2/d through lipid phase, with lipid yields >31% during lipid phase. Strain stability is being validated through sustained growth campaigns and after freeze/revive cycles. Diatom breeding is also being explored as a potentially powerful approach to generate biomass diversity for the screening and isolation of more oleaginous strains with phenotypes of interest. Initial libraries of *Nannochloropsis granulata* have been isolated and are being screened for high-growth/high-lipid isolates.



COMMENTS

- The risk mitigation, metrics, and decision points are clear. Fluorescence-activated cell sorting selection is good; it's a very proven method. The clone screening is excellent; thank you for picking enough clones. The screening criteria are clearly defined and relevant. It would be helpful to see how strain/clone 370 compared to other GAI strains. Other than GAI, it would have been good to see more industry engagement.

- Phenotypic screening and freeze/thaw behavior of the culture is not sufficient to ensure cell line stability: Genetic monitoring should also be used, particularly since another distribution of cells is observed after bioreactor cultivation and the observation of smaller cells present in the culture. The environmental enrichment for photobleaching events and oxygen tolerance is a good strategy.
- This project is well presented and well aligned with goals. Easily all aspects of the goals are comprehensively addressed. There are significant strengths.
- This is an example of a company being funded where information will remain proprietary. In a perfect world, information would be "free" once obtained on the public dime. This is, of course, directly opposed to company goals of protecting IP as a competitive advantage. I am impressed that the speaker indicated that attempts were being made to share widely, where appropriate. This is a very reasonable approach, and I commend it.
- Approach: The project personnel are attempting to use classical strain development strategies (e.g., mutagenesis and breeding, coupled with screening based on fluorescence-activated cell sorting) to increase at the same time both the lipid content and overall biomass productivity for *Nitzschia* and *Nannochloropsis*, and in a manner that is manifest in outdoor cultivation trials. This is clearly in line with AAS goals to lower the cost of algal biofuel production.
- The project team is well qualified to conduct the research, both with respect to strain development and testing in the lab and outdoors.
- Progress and Outcomes: Strains of *Nitzschia* were chosen after several screening rounds that demonstrated faster lipid accumulation upon induction; this is certainly a favorable outcome that can have a positive impact on the economic bottom line.
- Data were not provided as to whether the isolates selected using fluorescence-activated cell sorting with high BODIPY staining maintained that phenotype after numerous generations. Such data would help to assess whether the strains were truly stable mutants or whether the higher BODIPY staining was based more on the physiological state of particular individual cells or transient morphotypes (e.g., different stages of cell division).
- Without parallel, contemporaneous data for the control strain (GAI-337), it is hard to assess whether improvements seen for GAI-370 at lab scale translate into similar improvements in outdoor field trials. Retesting outdoors for GAI-370 alongside control ponds with GAI-337 will be critically important to answer this question.
- In the 2021 Peer Review, it was stated that strain collection/discovery efforts were underway, with one goal being to identify *Nitzschia* strains that could serve as mating partners for improving the existing *Nitzschia inconspicua* strain via sexual breeding. No updates were provided on this topic, including information on gamete formation of the existing strain or any new isolates. In addition, no information was provided regarding *Nannochloropsis granulata*, other than a statement that mutant libraries were being screened. Considering that the project has been up and running for 2.5 years, the overall progress seems somewhat behind; however, a timeline chart was not shown, so it's not possible to track actual progress in the different project elements against expected progress.
- Impact: The demonstration that improved *Nitzschia* strains were able to achieve high lipid levels faster than the parental strain would likely have positive financial implications because more commercial batch

cultures could potentially be completed in a given amount of time. It will be important to demonstrate that there is a statistically significant improvement of the new strains versus the control (parental) strain in outdoor cultivations.

- Progress and Outcomes: The project is on track with goals, perhaps slightly behind with the *Nannochloropsis* work, with just 1 year left to run the project. *Nannochloropsis* will not make it outdoors for much time at all, if at all. However, progress with *Nitzschia* is significant in terms of accomplishing established productivity goals. Impact: The impact and outcomes from publications and patents are good; the ability for industry at large to access these adapted strains does not seem to be possible. If the concept is that every company would have to go through this process to improve their own strain, then the impact is limited in scope.

PI RESPONSE TO REVIEWER COMMENTS

- Strain 370 is actively being compared to other GAI strains in use at the GAI farm site. To date, 370 has been among the best performers, but it is important to compare under more extensive and diverse conditions. We are in the process of filing initial IP claims on the strains selected, at which point it is expected that the strains can be utilized by other researchers. We agree that freeze/thaw is not sufficient for assessing strain stability. We are in the process of genome resequencing of the top-performing strains, determining whether genetic loci can be mapped, and then using DNA sequencing to verify strain maintenance and phenotype correlations. We thank the reviewer regarding their support for publishing our data. This is actively ongoing, and we agree that data sharing is important for the community. We are certainly monitoring phenotypes through generational grow-out campaigns. The reviewer is correct in identifying this critical issue, and experiments are underway to monitor growth/phenotype over a 1-year period. In the summers of 2023/2024, growth campaigns monitoring 370 relative to controls at the farm are planned to demonstrate comparative differences. To date, we have not identified any mating partners to *Nitzschia inconspicua*. These experiments are ongoing. The *Nannochloropsis* library has been generated. Screening efforts are planned for summer 2023. We agree that statistical significance of the improved strains is important to demonstrate at the pond, and efforts are underway to attain these data. It is hoped that *Nannochloropsis* will be run outdoors in summer 2024. The project still has ~1.5 years to run, and there should be time for outdoor *Nannochloropsis* work. It is our hope to make strains available to other interested parties as we publish our results.

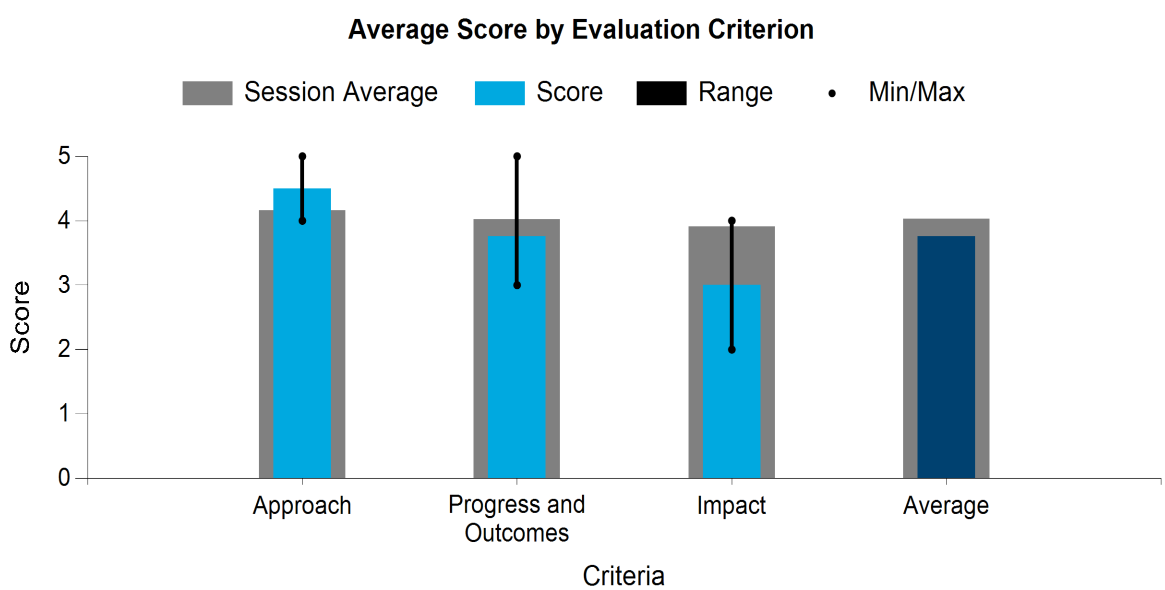
INNOVATIONS IN ALGAE CULTIVATIONS

Global Algae Innovations

PROJECT DESCRIPTION

WBS:	1.3.5.284
Presenter(s):	David Hazlebeck
Project Start Date:	10/01/2019
Planned Project End Date:	03/31/2024
Total Funding:	$5,625,000

GAI has developed low-cost algae production technologies aimed at achieving commercially viable production of biofuel and high-protein meal. Radical advances have been designed and implemented throughout the entire process, resulting in many industry breakthroughs for large-scale algae cultivation, harvesting, and processing. The goals of this project are to overcome the challenge in translating results between laboratory and mass cultures and to increase algal productivity by 50%, cultivation robustness by 50%, and lipids by 20% while achieving cost and LCA targets. A series of seven innovations in cultivation methods and three innovations in cultivation monitoring tools were developed, and a chain of test systems from laboratory-scale microplates through outdoor raceways producing kilograms of algae biomass was developed and tested to accelerate the indoor/outdoor cycle rate and improve the translation of laboratory results to mass culture. Through these advances, the lipid content at harvest was increased by 50% of the ash-free dry weight, which is a 90% improvement, and the productivity was increased by 15%–50% depending on the season, with greater improvement in the winter. During the next budget period, the lipid formation approach will be investigated in greater detail to provide greater understanding of the impact of abiotic and biotic conditions and to routinely achieve greater than 50% oil content in open outdoor cultivation. Additionally, a rapid compositional measurement tool was developed using near-infrared that accurately predicts the lipid and protein content of the algae. During the next budget period, the measurement tool will be optimized, used to support greater understanding of lipid formation, and moved toward a potential commercial product to make it widely available.



COMMENTS

- The goals are clear and quantitative. It wasn't clear how kLa and other scale factors are being compared across the methods, especially because mass transfer and mixing challenges are difficult to replicate at very small scales and the intermediate-scale systems leaked. It's great that the author is tightening up sample handling and processing, as this is the first step to process analytics with real-time controls, something that will be critical to some of the DAC implementations. The biotic versus abiotic information is really interesting; it would be nice if there was more explanation, even though many of the interventions are proprietary, particularly when it comes to impact and what the relative costs of those methods are. For the analytics, it would help to understand from an implementation perspective if this is something that could be done with an original equipment manufacturer Ocean Insight system or if it requires a custom build and peak validation.

- Several times it was mentioned that the project ran out of time during the budget period. Reasons were not given in detail. This is certainly a negative when asked whether the project is delivering appropriately against goals.

- The number of process patent applications was held up as a sign of progress. It does not come across as a compelling example of progress. One positive outcome of a patent is that it eventually becomes public information. Concerning is that these advances cannot be talked about for fear of wrecking a patent, making it impossible for us to review the novelty or impact. There are negatives. The biggest one is that process patents are difficult to police and frequently easy to circumvent. The patents were described as being in oil purification. This is a well-populated area due to the history of seed oil purification. It seems unlikely that there is an area of huge novelty remaining, and nothing said provided clear evidence that these were powerful aids to eventual success.

- In a program funded by public money, there is certainly a desire to have project learnings shared with the entire community. This is, of course, counter to the desires of a company seeking to develop and maintain a proprietary competitive advantage. It's good that equipment designs are being shared, great that tools are being used at multiple institutions, and great that there is a public toolkit for a new strain evolution method.

- Approach: This project is focused largely on the development of new tools, primarily small-scale growth systems along with equipment for determining the lipid, protein, and carbohydrate composition of algae. The tools are being used to assess new cultivation procedures to enhance the lipid content and productivity. Hamilton Robotics is listed as a project partner for certain aspects of the project, although their specific role was not defined in the presentation.

- Progress and Outcomes: Some of the tool development work reported in this project represents basically the same research topics that have been carried out and described by numerous groups (e.g., microplate and flask testing of strains, lab PBRs, automated monitoring for nutrient levels [and coupled feed systems], pH, DO, temperature). It wasn't clear in many cases what was actually novel, other than perhaps the small-scale sloped raceways.

- It appears that good progress is being made on biomass and/or lipid productivity, although details on results, methods, or procedures were not provided due to the desire to protect IP. Hopefully more clarity will be provided soon on the proprietary biotic and abiotic methods used to increase productivity. It would have been informative to know more details about how these experiments were conducted, including scale, whether the controls were run concomitantly or at different times, etc. More information on the lipid formation method (induction) and respiration control would also have been helpful to assess the progress and novel aspects of the project.

- Perhaps this is by design, but there seems to be overlap between the Colorado School of Mines project (1.3.5.282) and this one with respect to the results on improving the lipid production rate.
- Results were presented indicating that a spectroscopic tool had been developed that enabled measurement of lipid, protein, and carbohydrate levels in at least one strain. This would be a good outcome if it provides advantages over standard infrared measurement systems for these algal components and can be applied to multiple, disparate species.
- Impact: Assuming that the increases in lipid content and productivity that were reported are validated and that the methods that result in the enhancement are ultimately published or patented in a manner accessible to others, then the project results should be beneficial to the algae industry as a whole, especially if the methods are applicable to multiple strain types.
- It will be interesting to follow the commercialization and implementation of the spectroscopic compositional analysis equipment and procedures that were developed in this project. The specifics of the system and sample preparation methods weren’t disclosed, which makes it difficult to assess the novelty and utility of the system, but hopefully it becomes a useful and widely applicable tool for the algae research community.
- Approach: It isn’t clear why new cultivation testing tools were being developed (lab PBRs are not new). Why were on-the-market PBRs not viable as testing vessels? It is clear that this company has unique pond engineering and that mini-pond research specific to their ponds would require some modifications to most mini-ponds on the market. The spec methods for composition are a very nice new tool. There is only one collaborator for spectrophotometric work but no other lab, academic, or industry participants. The project seems to be managed well, but not much information on project management was presented. There was not enough forethought into difficulties with commissioning new systems, no mitigation plan in place for risk, and not enough resources to overcome issues during the project. Outcomes: Not all the areas that were planned to test to improve cultivation were done (nutrient addition and timing). Outcomes were not presented, as they were reported to be confidential. This makes determining outcomes and impact very difficult. The project greatly underestimated the effort to set up new systems, which limited outcomes significantly. Impact: Hard to evaluate—patents and confidential outputs, so impact is extremely limited as a result. Spectrophotometer methods are impactful.

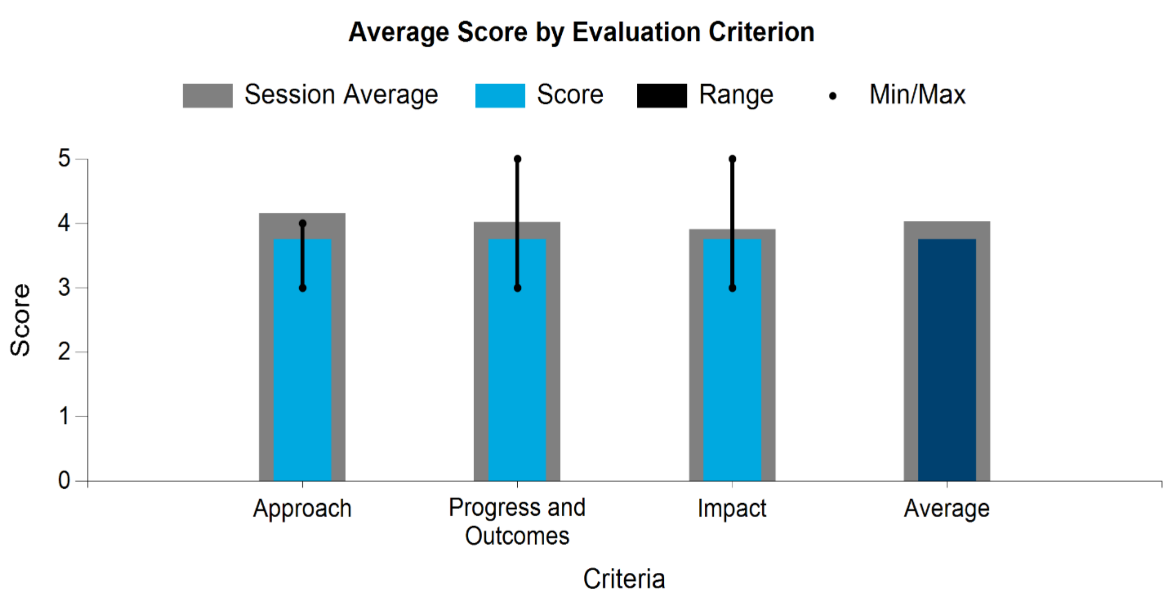
IMPROVING THE PRODUCTIVITY AND PERFORMANCE OF LARGE-SCALE INTEGRATED ALGAL SYSTEMS FOR WASTEWATER TREATMENT AND BIOFUEL PRODUCTION

University of Illinois at Urbana-Champaign

PROJECT DESCRIPTION

WBS:	1.3.5.286
Presenter(s):	Lance Schideman
Project Start Date:	10/01/2019
Planned Project End Date:	03/31/2024
Total Funding:	$3,764,553

Producing biofuels from algal biomass is currently one of the most promising approaches for meeting society's need for sustainable energy, because algae grows faster than other biofuel feedstocks while using marginal land and low-quality water resources that compete less with food production. However, high biomass production costs remain a significant challenge that limits the practical use of algal biofuels. Thus, the overarching goal of this project is to develop and demonstrate an integrated system for algal biofuel production and wastewater treatment that can reduce biofuel costs below DOE's minimum fuel selling price target ($2.50/GGE). This approach is compelling because of the significant coproduct value of treated wastewater and dual-use infrastructure, and because wastewater provides a low-cost source of major algae cultivation inputs (e.g., water, nutrients). In this project, we are developing novel biological and engineering approaches to enhance the biomass productivity of a commercially available algal wastewater treatment system, called Algaewheel, and conversion of the resulting mixed algal biomass to biofuels via HTL. The methods being studied to improve algae cultivation and biofuel conversion include bioaugmentation with bacteria that provide algal growth promoters, stress-induced endoreduplication to increase cell size, integration of adsorbents, dynamic control models, and nanofiltration of HTL aqueous products for improved carbon efficiency. The integration of these techniques at pilot scale has been shown to increase biomass productivity by more than 50% (>30 g/m^2/day) and has increased the efficiency of biofuel production by more than 20%. In the current budget period, these improvements will be validated in demonstration-scale testing at an operating Algaewheel plant used for domestic wastewater treatment.



COMMENTS

- The project timeline slide is good, though you have a lot of milestones all occurring at the same time, which could make risk management more challenging. The progress and outcomes look great, exceeding productivity goals, especially in the nanofiltration unit operation. More explanation of what happened to Lake 2 would be helpful. There is not much industry engagement in the impact section. The selection of two weather conditions (cold and warm) is good, as this has been problematic in past field work. Wild-type bacteria isolated from the environment would have been a more useful test than ATCC strains, which tend to be less robust and have a history of being problematic in the past (cross contamination, incorrectly identified, etc.). You will likely have to shift to a tangential flow filtration method of nanofiltration (whether hollow fiber or flat sheet), and this may add significant economic challenges.

- The definition of "pilot" was a bit ambiguous. Pilot is a word used with inadequate definition. This is a criticism that falls both on this project team and the algae program. I prefer defining a pilot as the smallest scale that can be operated to gain understanding sufficient to move to the next stage of scale-up. Ideally, a pilot allows the move to full production. For this particular technology, I don't know exactly what will define pilot. I believe this requires both the wheel and pond to be of sufficient size to be able to move with confidence to the next scale. If the thought has already been expended, it was not articulated. It appears the wheel is pilot scale. This may be OK, but it was not clearly stated. It is not clear how this is building to the future.

- The trends observed are all positive, whether productivity or ash content. The project appears consistent with the goals outlined for the project.

- Approach: The project has the primary goal to increase the productivity of the Algaewheel system for wastewater treatment, coupled to fuel production via HTL processes. A variety of treatments were applied to cultures to enhance productivity, and a process step was added to increase the yield of HTL-produced bio-oil. Field demonstrations at two sites in the United States are planned for the coming year. The approach and stated objectives are aligned with BETO and AAS goals to improve algal productivity and develop cost-effective biofuels and related coproducts.

- Progress and Outcomes: Data were presented indicating substantial increases in productivity and HTL-based bio-oil yield for Algaewheel-grown mixed algal/bacterial cultures.

- The premise that the formation of polyploid cells will improve productivity was not well explained or documented; in fact, the data indicating that genome duplication has occurred are not at all conclusive in this reviewer's perspective. It is not clear whether saline-induced polyploidy, if true, is a stable change or whether the increase in salinity simply transiently halted physical cell division. No data were presented indicating whether the larger cells reported to be polyploid cells actually led to enhanced productivity. Without corroborating data, it is not clear that continuing down this endoreduplication path is a worthwhile venture.

- It is also not clear from the data that the addition of *Azospirillum* cells made much of a difference in productivity, especially considering that there appeared to be a large (albeit delayed) increase in growth for both the control and treatment tanks upon increased carbon substrate addition (glycerol or tryptophan malate). The claimed 20% increase over the control was attributed to IAA production by the added *Azospirillum* cells, but the lack of error bars makes it hard to assess whether the increase was statistically significant or not. The presenter indicated during the Q&A session that experiments had been conducted that showed similarly large increases in productivity upon the direct addition of IAA, but the actual data weren't presented.

- The presenter showed a 73% improvement in HTL yields upon recycling the nanofiltration retentate, which is quite remarkable. In order to determine the fuel potential of the HTL oil produced in this manner, it would be helpful to know more about the organic composition of the retentate and the molecular weight range of HTL-derived oil products with and without recycling the retentate.
- It was not indicated in the presentation how harvesting would be accomplished with the Algaewheel system in a commercial-scale system, or how those costs were included in the interim TEA.
- Impact: If field trials scheduled for the coming year validate the data obtained to date, and if the assumptions in the TEA are verified by others in the field (e.g., NREL and PNNL analysis teams), then this research would align well with the BETO/AAS initiative to investigate low-cost algae with coproduct (i.e., wastewater treatment) potential for biofuel production.
- Approach: No input on the management plan was presented. Outcomes: Great work with presenting data and outcomes; I can see the progress from the project. Impact: Good impact with this work—if implemented by industry, it looks to be a game changer to a few issues. The next year will determine the amount of impact, depending how it goes in full deployment.

PI RESPONSE TO REVIEWER COMMENTS

- The timing of milestones, with many occurring simultaneously at the end of Budget Period 2, was designed to give the technologies that included bench-scale work more time to refine desirable operating conditions prior to upscaling to greenhouse pilot work with the Algaewheel rotating algal contactors. This did make the pilot testing for those technologies a bottleneck that contributed to the need for a no-cost extension, but ultimately, we were able to demonstrate a 50% increase in productivity and meet the Budget Period 2 go/no-go criteria. The HTL biomass conversion data for Lakes 1 and 2 show a range of results for different algal biomass sources when using nanofiltration to concentrate the HTL aqueous product. Both lake biomass samples were natural microalgal blooms collected by dissolved air flotation and further dewatered using a screw press. The recycling of the nanofiltered HTL aqueous product from Lake 1 did not improve oil yield, which we believe is primarily due to having a significantly lower biomass concentration and concentration of organics in the HTL aqueous product than the other samples. To achieve a higher oil yield when recycled, the Lake 1 HTL aqueous phase would likely require a more concentrated nanofiltration retentate. The recycling of the nanofiltered HTL aqueous product from Lake 2 did improve oil yield for some operating conditions (membrane type, chemical pretreatment, etc.), but other operating conditions did not increase oil yield. Overall, these supplementary data highlight that the nanofiltration process needs to be optimized for each source of biomass and HTL aqueous product. Since we wanted to test a variety of nanofiltration conditions, we decided to perform our experiments in a dead-end filtration system, which requires less volume of feed to be operated than other filtration systems. Tangential flow filtration systems offer enhanced scalability and will be a good option for the future scale-up of this process. Additional pretreatments to decrease fouling and flux decline, along with optimization of operational conditions, will help overcome the said economic challenges. It is worth mentioning that nanofiltration would still be less costly and uses less energy than the current state-of-the-art technology for aqueous-phase treatment (hydrothermal gasification), which can cost as much as HTL itself. Our commercial partner on this project is OneWater Inc., who is the commercial supplier of the Algaewheel system. They have more than 10 operating wastewater plants in the United States, which will provide a direct link for delivering the results of this project into the wastewater market. However, we agree with the need to do more general industry outreach in the future. Because the next phase of the project will incorporate the demonstrated productivity enhancements into two of the operating wastewater treatment plants, we feel that the resulting data from this upcoming effort will be more compelling for the wastewater industry. Some bench-scale testing was started with ATCC cultures so as to provide a more consistent starting point for our experiments that could potentially be replicated by others and compared with other literature data using these strains. Once in our lab, we struck out these strains to confirm purity and used matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) to confirm identification. Routine microbiological techniques such as single-colony plate streaking and MALDI-TOF were then used to prevent contamination and monitor culture purity over time. Ultimately, our work with pure cultures is a simplified experimental precursor to using the same productivity-enhancing techniques with the complex, mixed-species, algal bacterial cultures that are used in the Algaewheel system. Thus, the techniques must eventually have broad applicability beyond the single cultures used in our lab experiments. Each pilot-scale tank that we used is a six-wheel, 180-gallon system with an average flow of 60 gallons per day. We used the smallest of the commercially available Algaewheels that are currently used in most of their full-scale installations. Due to space and other location constraints, we opted for using a longer hydraulic retention time with a more concentrated simulated wastewater feed, but the daily loading of key wastewater contaminants (COD, NH_3) at the pilot scale was set to be within the range of long-term historical conditions for full-scale Algaewheel systems. Due to time constraints, we only briefly touched on our upcoming (Budget Period 3) full-scale demonstrations, as this experimental period has just recently begun. All the successful pilot treatments will be upscaled for use in an operating full-scale Algaewheel treatment plant. The first demonstration with full-scale Algaewheel tanks will have 50 wheels (same size as our pilot) and a volume of approximately 1,300 gallons and will receive an influent wastewater flow rate of 6,333 gallons per day. There are 12 Algaewheel tanks of this size at the first full-scale demonstration testing location in Gardner, Illinois, which recently began testing. The second full-scale testing location will be in Florida, which is being planned now and is expected to start demonstration testing in fall 2023. Due to time constraints, we did not provide as much information on the premise and potential for increased biomass production via endoreduplication. We provided some more information on these topics in the 2021 Peer Review and focused on other more recent pilot work in 2023. One of our collaborators has worked extensively on endoreduplication in terrestrial plants (https://doi.org/10.1007/s00442-019-04458-1) and has shown the potential for increasing biomass. More recent work has shown that endoreduplication can also be induced in algae to produce an increase in desirable biomass products (https://doi.org/10.1016/j.biortech.2019.121332). We subjected our productivity with and without the addition of *Azospirillum* cells to a pairwise Student T-test and found that it was statistically significant ($p < 0.05$). The average relative standard deviation of these data was 4.8% of the productivity value, which provides a general understanding of the size of the error bars. Experiments with the direct addition of IAA were conducted at bench scale with cultures of individual algae (e.g., *Chlorella vulgaris*) and showed biomass production that was 50% higher than controls. We did not conduct pilot tests with direct addition of IAA prior to the 2023 Peer Review meeting, but they are now being conducted. Our goal for the HTL experiments we presented was to identify the conditions that resulted in the highest oil yield increase. In our current and future experiments, we will perform gas chromatography–mass spectrometry for analysis of key chemical groups with thermogravimetric analysis to have a better understanding of the effects of recycling the aqueous phase on biocrude oil quality and fuel potential. Harvesting of biomass in our pilot system and in current full-scale Algaewheel systems occurs by natural sloughing and subsequent sedimentation in a clarifier. We have shown that increasing the aeration rate and spin rate of the algae wheels can enhance the rate of sloughing and amount of biomass harvested, while also reducing the ash content of the sloughed biomass. We believe the latter benefit is due to reduced time for predation on the surface of the wheels.

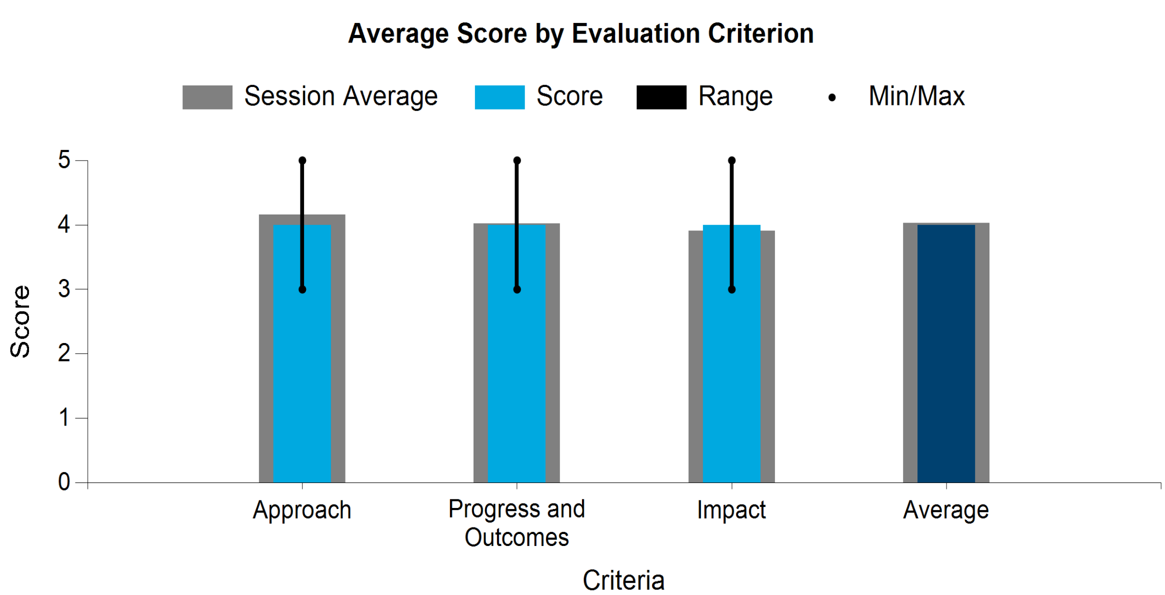
DECISION-MODEL-SUPPORTED ALGAL CULTIVATION PROCESS ENHANCEMENT

Arizona State University

PROJECT DESCRIPTION

WBS:	1.3.5.287
Presenter(s):	John McGowen
Project Start Date:	10/01/2019
Planned Project End Date:	06/30/2023
Total Funding:	$4,375,000

Current decision support models (TEA, LCA, and growth/productivity) for large-scale algae cultivation systems lack critically important quantitative, culture-failure risk data. At very large scales, semicontinuous versus full-batch cultivation strategies present very different risk profiles with respect to the consequences of culture failures from pathogens, grazers, and competitors. These uncertainties constitute a critical knowledge gap that must be closed to guide major investments in commercial algal biofuel systems and enable systems for crop insurance. We will quantify the economic and technical risks associated with different cultivation strategies through an integrated program of indoor lab studies, cultivation simulation and optimization, and multiscale "omics," including robust outdoor cultivation campaigns informed by more than 6 years of outdoor cultivation data generated at AzCATI. Through the development and deployment of a suite of novel real-time sensors for nutrient and water quality monitoring, we will gain better process control though novel insights, plus the ability to optimize productivity, robustness, and biomass quality of our selected high-performance strains. System optimization will include concurrent economic and life cycle modeling coupled with production process variability modeling. This work will directly integrate with experimental systems to understand the impact of the advancements and provide data feedback for focused investigations.



COMMENTS

- The explanation of communications and information management is helpful.
- Unfortunately, bacterial and fungal predation are very common and tough to address with lab-grown algae strains. Even if antibiotics and antifungals were cheap and plentiful, the costs and containment

especially are really difficult, and you can't use a good fraction of your products as they are too contaminated by the antibiotics and antifungals to be useful for fermentation media or fine chemicals starting materials. It also eliminates the possibility of using any of your products for human or animal consumption in any form, even as fertilizer for fields. You'll see a drop in culture health a day or two before you can detect the contaminant.

- The AzCATI site is a good robustness test due to the harsh environment. It appears that they actually went backwards in productivity based on the data in Slides 12, 15, and 17; is this correct? The fungicide used is slated to be banned based on its breakdown products. Although the fungicide itself isn't detected, its breakdown products are known to be problematic. Additionally, resistance tends to develop very quickly.
- Nice checking calculations with experiment.
- I'm not sure how to internalize the novel parasite impact on the project. Certainly, it looks like they failed to reach goals while taking very reasonable steps. The contamination issue is certainly daunting, yet it is hard to see that the project met the goals as defined.
- It's a little troubling that they are using a per- and polyfluoroalkyl substances (PFAS) fungicide. It seems like that should be on the sustainability slide.
- I believe staff decisions to alter scope and goals were good. The parasite issues, while preventing the attainment of the initial goals, warrant further exploration. Good example of active project management.
- Approach: The goal of this project was to identify and quantify factors that limit productivity and the length of time between culture crashes in outdoor ponds, and to develop mitigation strategies to overcome those negative factors. This is an ambitious project, but AzCATI and other project team members are well positioned to collect and analyze relevant data from outdoor cultivations. The goals of the project are well aligned with BETO objectives.
- Progress and Outcomes: Progress in increasing the mean time to failure of outdoor cultures was being made, based in part on the development of fungicidal treatment protocols to mitigate fungal parasitoid infection. Tolerance that developed to the fungicide used, along with new invasions by predatory bacteria, caused the mean time to failure to drop down again in 2022.
- The observations that contaminating fungi developed resistance to fungicides, along with the identification of new pests (i.e., predatory bacteria), are not surprising when considering the multitude of potential contaminants in nature and the diversity of ecological niches that they occupy. Although frustrating, this points out the realities of open mass cultivation systems that are generally not amenable to sterilization procedures.
- The identification of contaminating pests via genome analysis, followed by the development of qPCR assays, will be helpful for determining the prevalence of these (and some closely related) microbes at AzCATI and other outdoor sites.
- The TEA and LCA work done in this project is helpful for assessing the pros and cons of batch versus semicontinuous cultivation systems.
- Impact: Although undoubtedly frustrating for the research team, the reality is that they are subject to real-world challenges of outdoor mass cultivation of microalgae, especially the unpredictable problems related to contamination with destructive microbial pests. As such, AzCATI and collaborators are well positioned to try to find and report on solutions to these problems. It is anticipated that different locations

and different species of farmed algae will be impacted by different pests, so the value of this project is probably more in developing a framework for how to evaluate and establish robust culturing procedures and general contamination control strategies that other sites could leverage, as opposed to trying to identify mitigation procedures that might be specific to AzCATI's common contaminants but not universally applicable to all sites and algal types.

- Despite being academically interesting and based on solid science, the use of qPCR to identify a very limited number of known contaminants, and the use of elaborate real-time nutrient measurement systems, may be challenging to perform in an economically viable manner. Likewise, it's not clear if such data would be timely enough and specific enough to prevent culture failures in a commercial-scale production field. An indication of how these systems would be implemented in multi-acre facilities and the impacts on the capital and operating costs would be helpful to determine the value of continuing to pursue these particular lines of research.
- Approach: This project has a good plan, meeting goals, a good communication system, and good collaboration across the project. Outcomes: Good outcomes with probes; even a decrease in productivity over time (also could be looked at as site variability over time) is a good outcome, as it is a real-world variable that can teach industry what they need to be prepared for as their sites mature over time. Flow cytometry for higher throughput is good, and this reviewer disagrees that it is not cost-effective. However, this could become true as more cost-effective tools come to market. It's unfortunate not to be able to detect contaminants, etc., earlier with flow cytometry; however, perhaps current detection timelines are early enough. Could a look at the data from this perspective provide additional value? For example, we can detect pests with flow cytometry and pond monitoring by day *X* of them being present in the system, and this has proven to be early enough for mitigation tactics to be used successfully. In industrial settings, it is not just detection that needs to be fast; the trick is getting operators to act fast enough, so mitigation techniques need to be rapidly deployable. Impact: Far-reaching, as all data are open access. Development of tools and protocols for industry saves thousands. Protocols should also be open access and ready for deployment into a GMP document control program, as this is key development work for commercialization.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the time and effort put into the review process by the reviewers and the constructive and insightful comments. In response to the overall challenge of identifying and implementing effective integrated pest management for algae cultivation, we think while the overall results—in particular decreased time to failure and decreased productivity observed in the project primarily due to the novel predatory bacterial contaminants, which as of yet have not been reported in the literature—give a clear example of the iterative cycle of integrated pest management. In other words, one pest identified, mitigated, and managed, only to have a new pest appear, is valuable information to put out into the public domain. AzCATI remains relatively unique within the BETO portfolio, as we have years of seasonal cultivation data with the same strains, and thus we are able to observe trends and associated challenges, actually building a knowledge base for algal agronomy. We have pest models that have been developed and are in a good position to bring additional solutions to the table as part of the overall cycle of integrated pest management; in fact, we will be doing so as we wrap up this specific project in 2023. We clearly demonstrated the ability to mitigate contamination and keep productivity higher through operational shifts (e.g., batch versus semicontinuous) and clearly identified the trade-offs with respect to TEA and LCA based on those approaches, something that had not been delineated well in the literature and backed up by actual cultivation data. With respect to concerns about the fungicide used, this fungicide is in fact used in food production now and remains one of the few that, as of yet, is not banned in the United States. However, we acknowledge it's likely to be banned, and while technically *not* a PFAS compound, it is fluorinated and thus of concern. Resistance as well is of course always a possibility and an issue to be concerned with for any treatment methodology. Understanding and characterizing that while looking for ways to prevent it are key aspects in developing any effective integrated pest management program. The challenges faced in this project with respect to developing approaches to manage pests are the same ones any site will face in bringing algal cultivation forward. As described in the presentation, and to extend the impact of this BETO-funded work, our data are being made publicly available and also include any protocols as well as pest models we have or will develop. We have already transferred pests to collaborators at the national labs and other academic institutions.