ADVANCED ALGAL SYSTEMS

PROGRAM AREA

CONTENTS

INTRODUCTION

The Advanced Algal Systems (AAS) Program Area was reviewed during the 2019 Bioenergy Technologies Office (BETO) Project Peer Review. The review took place March 4–7, 2019, at the Hilton Denver City Center in Denver, Colorado. A total of 36 projects were reviewed in the AAS session by a review panel of five external experts from industry.

This review addressed a total federal share of approximately $108,000,000 (Fiscal Year [FY] 2016–FY 2019 obligations) in financial assistance awards and national laboratory direct funding, which represents approximately 14.1% of the dollar value of the overall BETO portfolio reviewed during the 2019 Project Peer Review. During the Project Peer Review meeting, the principal investigator (PI) for each project was given 30 minutes to deliver a presentation and respond to questions from the review panel.

Projects were independently evaluated and scored by the review panel plans using one of three standard review criteria and scoring rubrics (see introduction of report) depending on the age of the project—e.g., new start, ongoing, and sunsetting. Scoring criteria included the project approach, technical progress and accomplishments, relevance to BETO goals, and future work. This section of the report contains the results of the Project Peer Review, including average score by criteria for each project, summary comments from each reviewer, and any public response provided by the PI. Overview information on the AAS Program, scoring results and analysis, the Review Panel Summary Report, and the Technology Area Programmatic Response are also included in this section.

ADANCED ALGAL SYSTEMS OVERVIEW

The mission of the AAS Program is to reduce the cost of algal biofuels through public/private partnerships for early-stage research and development (R&D) on sustainable algae production, logistics, and conversion to biofuels. Projects presented within the AAS session address a diverse range of topics, including algal biology; algal cultivation; harvest and processing logistics; conversion interfaces and conversion technologies; and analyses of high-value coproducts, techno-economics, sustainability, and resource availability.

AAS R&D focuses on showing progress toward achieving high-yield, low-cost, environmentally sustainable algal biomass production and logistics systems that produce algal feedstocks well suited for conversion to fuels and other valuable products. Algal biomass includes micro- and macroalgae as well as cyanobacteria. Algal feedstocks include concentrated whole algal biomass, fermentable substrates, extractable lipids, secreted metabolites (alcohols or others), or biocrude resulting from hydrothermal liquefaction (HTL). These feedstocks must be upgraded, blended, and/or purified to produce a finished fuel or bioproduct. Developing algal feedstocks to achieve BETO's advanced biofuel price goals requires breakthroughs along the entire algal biomass supply chain.

ADVANCED ALGAL SYSTEMS SUPPORT OF OFFICE PERFORMANCE GOALS

The AAS Program performance goal is to deliver technologies that can enable the verification of technical performance of algae cultivation, harvesting, and conversion processes at the engineering scale capable of converting algal feedstocks to biofuels and bioproducts in support of BETO's goals for the mature modeled minimum fuel selling price (MFSP) of $2.5/gallons gasoline equivalent (GGE) for biofuels by 2030. For details on the program's technical goals and milestones, please review BETO's *Multi-Year Plan* (MYP).

ADVANCED ALGAL SYSTEMS APPROACH TO OVERCOMING CHALLENGES

The AAS Program approach to overcoming challenges and barriers is outlined in the work breakdown structure (WBS), organized around five key activities. Current activities are focused on:

- Algal strain improvement

- Cultivation system improvement
- Improving the capacity and efficiency of harvesting, preprocessing, storage, and handling
- Characterizing algae to interface appropriately with conversion methods
- Integrating algal R&D systems.

These activities are performed by national laboratories, universities, industry, and consortia teams.

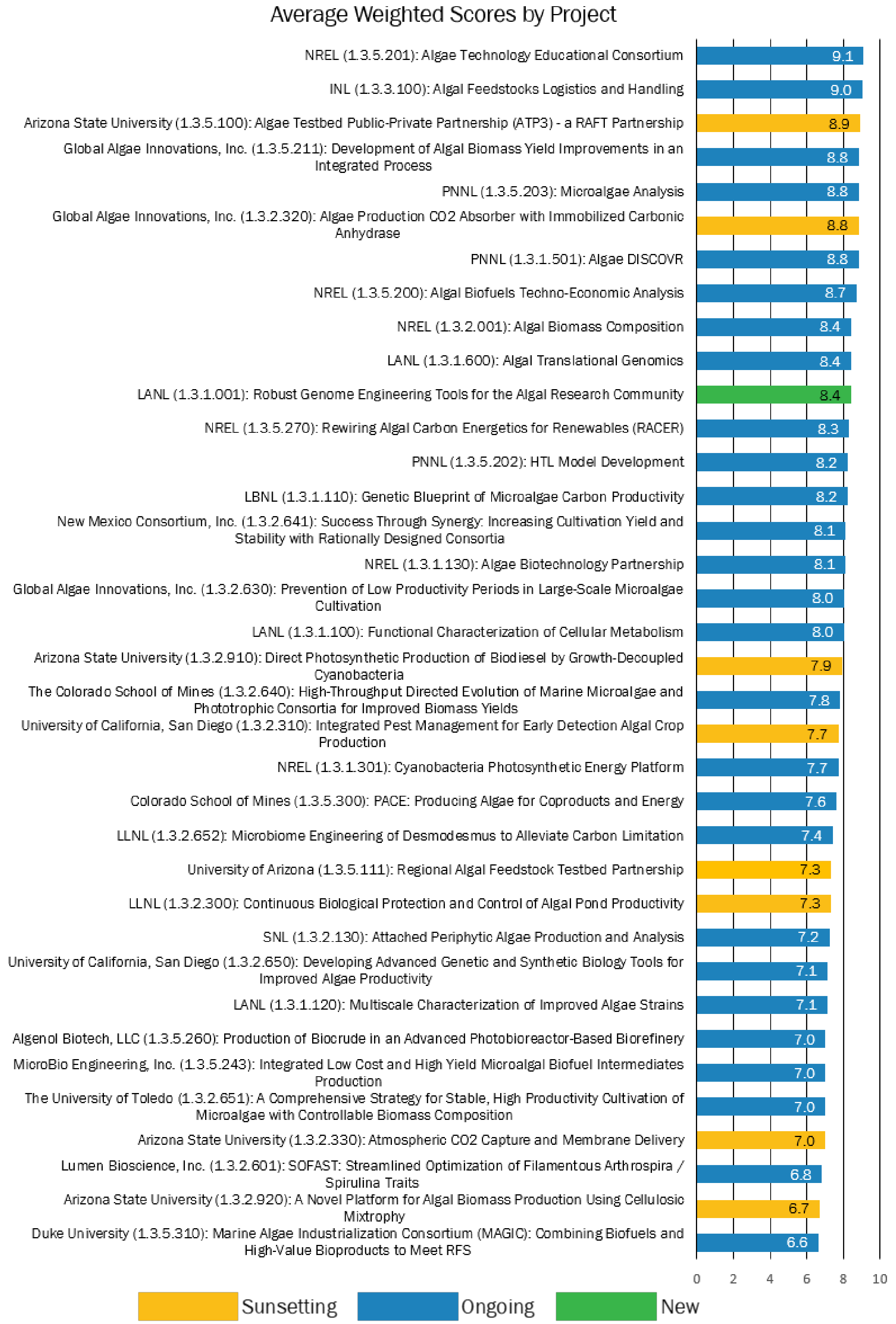
ADVANCED ALGAL SYSTEMS REVIEW PANEL

The following external experts served as reviewers for the AAS Program during the 2019 Project Peer Review.

Name	Affiliation
Toby Ahrens*	Larta Institute
Louis Brown	Synthetic Genomics
Michelle Legatt	Patagonia
Jose Olivares	Elsevier & Biologic Energy Partners
Becky Ryan	Indigo Agriculture

* Lead reviewer

TECHNOLOGY AREA SCORE RESULTS



ADVANCED ALGAL SYSTEMS REVIEW PANEL SUMMARY REPORT

Prepared by the Advanced Algal Systems Review Panel

BETO's AAS Program provides funding for a range of disciplines necessary for advancing energy production from algae at commercially meaningful scales. Project performance appears to be in good alignment with MYP goals, and state-of-technology (SOT) estimates of the cost of algal-based fuels ($/GGE) continue to trend toward meeting mid- and long-term BETO goals. Projects span a broad range of disciplines, including applied genetics, crop stability, engineered systems, logistics, and conversion, among others. Funded projects address individual bottlenecks as well as larger integrated efforts. Funding for continued incremental advances appears to be balanced with earlier stage high-risk/high-reward projects with transformative potential. Continued investment across the supply chain is still warranted.

The projects receiving the top four overall scores across the rating categories (i.e., approach, accomplishments, relevance, future work) reflected the institutional diversity and breadth of disciplines supported by the program. The top projects included two national laboratories, a university, and a private company. The highest ranked project targeted education and workforce development, followed by projects on logistics, public-private test beds, and large-scale integration.

- National Renewable Energy Laboratory (NREL), Algae Technology Educational Consortium (ATEC): This project reported broad-reaching impacts through curriculum development and coursework delivery for K–12 schools, community colleges, and continuing education.
- Idaho National Laboratory (INL), Algal Feedstocks Logistics and Handling: This well-managed project used a unique approach to address the stability of seasonal storage of algae. Improved storage could allow for processing in a lower cost facility designed for average annual production rather than a larger, more expensive facility designed for peak productivity but with spare capacity much of the rest of the year.
- Arizona State University, Algae Testbed Public-Private Partnership (ATP3) - a Regional Algal Feedstock Testbed (RAFT) Partnership: This public-private partnership provided access to a network of test beds and supported many studies that fed into BETO's SOT and other modeling efforts. Project participants included industry, academic, and laboratory partners. A wide range of efforts included education and training, high-quality publicly available cultivation data, method harmonization, and benchmarking/validation of many cultivation and conversion technologies.
- Global Algae Innovations, Inc. (GAI), Development of Algal Biomass Yield Improvements in an Integrated Process: This project integrated several innovations in an industrial setting, including improved contamination control, development of a low-energy harvester, and optimized drying and extraction. Reductions in cost across the supply chain resulted in a modeled selling price of $2.51/GGE.

Overall, the AAS Program is on track to address critical technology barriers and to meet MYP goals. Projects continue to report productivity improvements, and there appears to be good communication and coordination among national laboratories, public-private test beds, and industry partners. Interconnectivity among laboratory-led research projects was noted as a weakness in the prior program review but is one of the strengths and portfolio highlights in this current review. Continued advances in productivity improvements, culture stability, and coproduct development remain high priorities.

IMPACT

Progress is being made on many barriers and technical challenges identified in the MYP, and similar to conclusions from the previous peer review, government funding continues to play a critical role in de-risking technologies prior to significant private capital investment.

Several projects warrant specific mention. First, the effort on microalgae analysis led by Pacific Northwest National Laboratory (PNNL) continues to improve industry standards for biomass quantification and has provided recommendations that are widely disseminated and easily accessible. The project has improved confidence in data underpinning the SOT and techno-economic analysis (TEA), which are used to prioritize program funding. Method standardization across projects in the portfolio contributed to vast improvements in the clarity of methodologies used for productivity reporting in this current review, which had been noted as a point of confusion in previous reviews.

Notable progress has also been made on algal productivity, including understanding genetic traits for productivity (e.g., NREL's Rewiring Algal Carbon Energetics [RACER] project, Lawrence Berkeley National Laboratory's [LBNL's] Genetic Blueprint of Microalgae Carbon Productivity, the Colorado School of Mines project on High-Throughput Directed Evolution of Marine Microalgae and Phototrophic Consortia for Improved Biomass Yields) as well as demonstrated improvements in measured productivity in an outdoor, integrated industrial environment (e.g., GAI's Development of Algal Biomass Yield Improvements in an Integrated Process). The program's understanding of microbial diversity continues to expand (e.g., New Mexico Consortium's Success Through Synergy: Increasing Cultivation Yield and Stability with Rationally Designed Consortia project, Lawrence Livermore National Laboratory's [LLNL'] microbiome work), which will be critical for designing strategies for large-scale culture stability and integrated pest management strategies (e.g., University of California San Diego's project on Integrated Pest Management for Early Detection Algal Crop Production).

Other impactful projects have focused on specific bottlenecks in the supply chain, such as GAI's project on carbon dioxide (CO_2) capture and delivery (Algae Production CO_2 Absorber with Immobilized Carbonic Anhydrase) and INL's project focused on seasonal storage to enable consistent year-round biomass supplies for conversion facilities. INL's project feeds nicely into other downstream processing efforts, such as PNNL's work on HTL model development.

The NREL-led ATEC project was also notable in terms of the breadth of its impact on K–12 and continuing education students and programs.

INNOVATION

Advances are still needed across the supply chain, and BETO is supporting innovation through an appropriate mix of steady incremental advances and high-risk, high-reward opportunities. Funding for novel approaches is encouraged if kept at a small number of projects, as is the case in the current portfolio. Notable innovations included:

- Carbon capture and delivery: GAI, Algae Production CO_2 Absorber with Immobilized Carbonic Anhydrase
- Microbiome: New Mexico Consortium, Success Through Synergy: Increasing Cultivation Yield and Stability with Rationally Designed Consortia; and LLNL, Microbiome Engineering of Desmodesmus to Alleviate Carbon Limitation
- Diverse genetic improvement methods supported by strong justifications for target strains: NREL, RACER; LBNL, Genetic Blueprint of Microalgae Carbon Productivity; and Colorado School of Mines, High-Throughput Directed Evolution of Marine Microalgae and Phototrophic Consortia for Improved Biomass Yields
- Coproduct development: Arizona State University, Direct Photosynthetic Production of Biodiesel by Growth-Decoupled Cyanobacteria; and Duke University, Marine Algae Industrialization Consortium (MAGIC): Combining Biofuels and High-value Bioproducts to Meet Renewable Fuel Standards (RFS).

Several projects targeted innovative approaches to coproduct valorization, but some lacked techno-economic considerations comparable in quality to the SOT and TEA efforts for transportation fuels. For example, coproducts could require downstream processing steps such as separations, drying, purification, and/or formulation steps, and they might have additional regulatory considerations. Several projects reported exciting potential for novel coproducts, but one or more considerations in the preceding list were not included.

The proportion of risk in the portfolio appeared to be appropriate. In addition, projects should be encouraged to share challenges broadly to help accelerate the pace of the program's innovation, especially for high-risk efforts. Learning from these challenges could serve as an important foundation for future work.

SYNERGIES

In general, national laboratories provided clear articulation of multi-investigator and cross-laboratory collaboration, and these harmonization efforts contributed to the national laboratories receiving 7 of the highest 10 scoring projects. However, high ratings were not distributed evenly among all national laboratories. Interconnectivity among laboratory-led research projects associated with technology, life cycle assessment (LCA), and TEA appears to be strong, such as research feeding modeling efforts supported by PNNL, Argonne National Laboratory (ANL), and NREL.

Another improvement from the previous peer review was industry engagement through formal collaboration and industry advisory board (IAB) involvement. Two large national laboratory-led projects—the PNNL-led Development of Integrated Screening, Cultivar Optimization, and Verification Research [DISCOVR] project and the NREL-led RACER project—included IABs that appeared to convene on a regular basis and were engaged with project leadership. Industrial strains were also included in standardized productivity tests in laboratory-led projects (e.g., strains shared by Algenol).

There seem to be several opportunities for improved collaboration with other U.S. Department of Energy (DOE) Office of Energy Efficiency and Renewable Energy (EERE) programs. First, the AAS Program has funded innovative work in carbon capture and use for many years, and carbon use efficiency efforts in other EERE programs are encouraged to work openly with the AAS Program to build on these strengths. Second, if the AAS Program de-emphasizes large-scale demonstrations, the Advanced Development and Optimization Program appears to be a logical partner to fund critical pilot- and demonstration-scale activities in continuous, outdoor environments. Third, the portfolio includes many diverse approaches for genetic improvement of various strains, and there might be opportunities for collaboration on approaches used in other EERE programs focused on improvement of industrial microbes.

FOCUS

The peer review covered 36 projects, including 21 projects from national laboratories, 11 university-led projects, and 7 projects led by private companies. Many included multi-institutional collaborations. Funding was provided through a broad group of funding opportunity announcements (FOAs), ranging from opportunities to address specific bottlenecks, larger integrated multi-institutional efforts, and a call for innovative approaches that were not covered by previous FOAs. Reviewed projects included new efforts, projects in the middle of their funding period, as well as sunsetting projects. Funding covered the full supply chain—from genetic improvement of crops, crop stability, scale-up and production, harvesting, and logistics, through conversion and final products. The breadth of the portfolio was reflected in national laboratory-led projects, which included research on education, standards development for analyses, genetic improvement, culture stability, screening and scaling, logistics, conversion, and large-scale techno-economic modeling.

Funded projects appear to be well-aligned with the barriers and technical challenges outlined in the MYP. One gap noted in the previous peer review was a lack of interaction with end users of products and coproducts. This gap remains, and the program is encouraged to prioritize involvement with end users of all products that contribute significantly to financially viable approaches.

COMMERCIALIZATION AND RECOMMENDATIONS

The AAS Program has made consistent progress on MYP goals, and the quality of data underpinning the SOT and TEA continues to improve. After hearing progress from portfolio projects, the peer review panel has several recommendations that it feels will help the program continue its path to supporting cost-competitive energy production from a commercial algal industry.

Coproduct valorization appears to continue to be critical to support viable techno-economics for large-scale energy applications. Development efforts must include explicit product targets, including product specifications, downstream processing costs (separation, purification, formulation), supply chain logistics, and pricing assumptions. In addition, SOT models assume coproducts will play an important role in fuel techno-economics, but there is little consensus on whether early reliance on high-value coproducts can be easily transitioned to mid-value or commodity-scale coproducts. Future efforts will need to be explicit in identifying product targets, pricing, and processing assumptions to maintain relevance. Related TEA should be integrated throughout projects and used to prioritize effort when appropriate.

Another area for improvement includes further definition of what “outdoor conditions” mean to BETO and future commercialization efforts. If a future algal industry depends on production in large-scale open ponds, then testing in conditions that will be representative of environmental exposures is critical. Many projects acknowledged this challenge but defined outdoor conditions in different ways, with some addressing one specific environmental exposure and others addressing many variables. Program guidance for this stage of testing is suggested. Outdoor test bed facilities are critical, and the program currently relies heavily on a small number of test beds. The program is encouraged to maintain funding for current test beds and consider adding additional sites to capture additional variability in climate, source water, and pest pressures, which will be important for broad industry deployment. The scope of research in exposed environments should also include iterative outdoor-indoor testing, such as pond forensics, quality verification, ecology, and efforts to learn from stable outdoor cultures.

Promising results from early-stage genetic improvement work are being scaled up for continued testing in outdoor conditions, and use in test beds or industrial environments requires appropriate regulatory compliance. Several groups have worked hard to ensure current compliance, but these efforts will likely increase in number and scale in the future. The program is encouraged to support funding for risk assessment tools to inform future regulatory compliance.

Although the quality of data is improving overall, many projects could still benefit from improved experimental design. Objectives should be quantitative and defined prior to the experiment, the scale and duration of tests should match the question being asked, and data should be reported with appropriate statistics (*n* values, error bars, significance). Statistical standards should be encouraged for data integrity in modeling efforts such as the SOT and TEA.

The program is advised to encourage open discussion of lessons learned with funding recipients. Insights from challenges could be as valuable as filtered reporting of positive results.

Finally, the AAS Program has been a leader in carbon capture and use innovation, and the program is encouraged to have open and direct collaboration with carbon use efficiency efforts in other EERE programs.

ADVANCED ALGAL SYSTEMS PROGRAMMATIC RESPONSE

INTRODUCTION/OVERVIEW

The AAS Program staff thanks the review panel for commending the strategic direction of the program as well as the technical progress of AAS projects. The AAS Program strategy for prioritizing R&D gaps identified by the rigorous national laboratory-led analysis portfolio and working to close them with targeted calls for proposals has proven successful. We will continue to work along the supply chain to bring about foundational and impactful R&D to support BETO's goals. Although the AAS Program has a clear technology pathway of focus for benchmarking success, we make room in our portfolio for opportunities for innovation and novel technologies. BETO thanks the panel for acknowledging how critical government funding is in supporting this innovative technology area to reduce risks for private investors. We closely coordinate with industry and stakeholders through advisory groups, in-person meetings at DOE, workshops, Requests for Information, and conferences to ensure that the R&D we fund is relevant, necessary, and well planned. The review panel recognized the improvements we have made in national laboratory coordination and in industry engagement since the 2017 review. The AAS Program is well positioned to help accelerate the growth of the U.S. bioeconomy by filling a key role in the portfolio of domestic bioenergy feedstocks.

Recommendation 1: Coproduct valorization.

The AAS Program recognizes the key role that coproducts play in reducing the MFSP of algal biofuels. AAS continues to work toward better incorporation of technical standards for products within FOAs to ensure that selected projects understand product targets, pricing, size of market, adjacent markets, and processing assumptions to support commodity-scale production volumes and make appropriate contacts with downstream product users. AAS continuously improves the methodology for competitive project technical and financial verifications at the initiation, midpoint, and end of projects to ensure that projects are both disciplined and agile in responding to R&D learnings as projects progress.

Recommendation 2: Support outdoor testing.

The AAS Program agrees with the review panel that testing algal systems in conditions representative of commercial outdoor exposure remains a critical area of emphasis to continue making progress in overcoming challenges to algal fuel and products. AAS continues to develop strategies that are informed by outdoor testing requirements so that projects consider methodologies informed by minimum requirements to test outdoor deployment readiness. AAS also notes the panel's comment that publicly funded outdoor test bed facilities are a critical resource for high-impact R&D developments and data generation. AAS might continue to pursue test beds and could consider incorporating additional sites to add geographically diverse data points to support broad industry deployment in the United States. The FY 2019 AAS area of interest in the larger BETO-wide competitive FOA topic aligns with the panel's specific request to increase iterative indoor-outdoor-indoor testing of algal strain and cultivation technologies to identify and improve upon highly productive and robust mass cultures. Although the AAS Program's mission space does not include setting national regulatory policies and standards, the program remains informed of developments in this area through its involvement in the federal Biomass R&D Board Algae Interagency Working Group that includes representatives from federal agencies that are in this space.

Recommendation 3: Improve data quality.

With each competitive funding opportunity, AAS strives to improve upon the rigor of the technical verification process. These verifications help projects improve their experimental designs before work commences and understand progress toward projects' interim and final targets. AAS will continue to consider how projects are incorporating appropriate experimental designs, controls, and statistics as well. Within the national laboratory analysis portfolio, AAS can encourage all BETO-funded analysis projects to consider their statistical standards.

Recommendation 4: Share lessons learned.

The review panel suggestion to encourage open discussion of lessons learned, including insights from challenges and failures, is a broadly applicable suggestion. AAS will continue to request that project performers publish, present at conferences, and amplify success stories as well as lessons learned to ensure that government-funded work informs future technical efforts throughout the field. AAS has published scale-up challenges and lessons learned from the algal process development scale and integrated biorefineries within the *National Algal Biofuels Technology Review*.[1]

Recommendation 5: Carbon use efficiency.

AAS notes the review panel's recognition of the program's leadership in carbon use innovation. As we continue to execute our strategies for carbon use in algal systems, we will continue efforts to coordinate within BETO and with the DOE Office of Fossil Energy on CO_2 management R&D. We will work to investigate other potential collaborations within EERE and DOE to further algal systems potential to contribute to advancements in circular carbon economy strategies.

[1] U.S. Department of Energy. 2016. *National Algal Biofuels Technology Review*. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office, 66–71. https://www.energy.gov/sites/prod/files/2016/06/f33/national_algal_biofuels_technology_review.pdf.

ROBUST GENOME ENGINEERING TOOLS FOR THE ALGAL RESEARCH COMMUNITY

Los Alamos National Laboratory

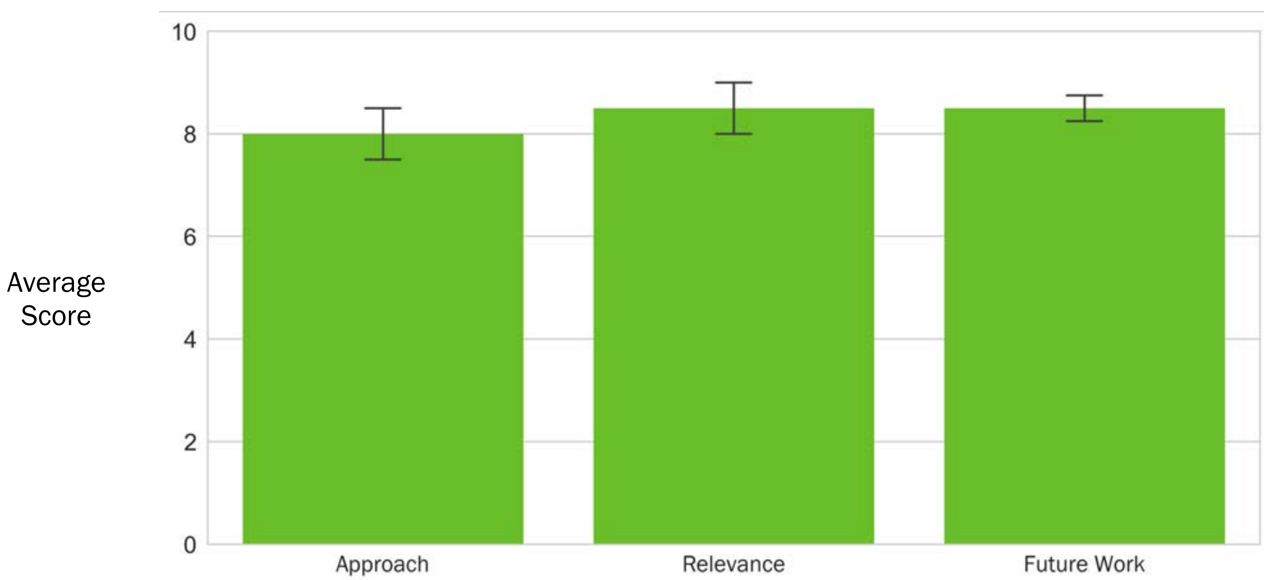
PROJECT DESCRIPTION

WBS:	1.3.1.001
CID:	NL0034646
Principal Investigator:	Dr. Blake Hovde
Period of Performance:	10/1/2018–9/30/2020
Total DOE Funding:	$200,000
DOE Funding FY16:	$0
DOE Funding FY17:	$0
DOE Funding FY18:	$0
DOE Funding FY19:	$200,000
Project Status:	New

To achieve target levels of biomass and biofuel production, it would be beneficial to greatly increase the number of genetically engineered algal strains under development to maximize algal biomass and improve biofuel and high-value bioproduct yields. This increase, however, requires changing algal genome editing and metabolic engineering experiments from a slow and arduous process, developing and testing only one or a few mutants at a time, to a more rapid "prototype"-focused (i.e., characterization of large numbers of genetically engineered strains) endeavor. The algal community needs a better set of molecular tools. This project aims to address two of the biggest problems hampering genome and metabolic engineering efforts across the algal research space. The first problem is the lack of available resources regarding promoter choice in genetic engineering expression cassettes for algal production strains. Currently, only a handful of extremely strong promoters are being used to drive gene overexpression for products of interest. Although these promoters will work to improve algal productivity in certain cases, researchers recognize that a wider variety of promoter sequences can be used to fine-tune expression with the potential to improve algae as a production platform, as demonstrated in current bacterial and yeast systems. The second problem is a lack of precise and rapid generation of new genetic mutants. Currently, most of the time spent on genome metabolic engineering

Weighted Project Score: 8.4

Weighting for New Projects: Approach-25%; Relevance-25%; Future Work-50%



One standard deviation of reviewers' scores

projects is simply generating the desired mutants. This leaves little time for characterizing mutant properties, let alone generating many mutants for characterization. To address these issues, we are performing two tasks during this seed project to model a rapid approach for generating engineering toolboxes for algal production strains:

- Task 1: Transcriptomic evaluation of *Nannochloropsis salina* in multiple environments for identification of variable-strength constitutive promoters and inducible promoters.
- Task 2: Validation and curation of a promoter library for inducible and variable-strength promoters for *N. salina*.

The output of this project will directly benefit BETO MYP barrier Aft-C: Biomass Genetics and Development, specifically regarding more control of synthetic biology and algal metabolic engineering efforts and significantly increasing the throughput of genetic engineering targets chosen as objectives for upcoming efforts to improve algal production. The investment in developing these tools now will pay dividends in upcoming years by removing large technical hurdles that currently hinder many projects. This work will provide access to an initial curated promoter library for *N. salina* that will be available to the algal genome engineering research community. This work will also provide a road map for the rapid development of molecular toolboxes for additional algal production strains.

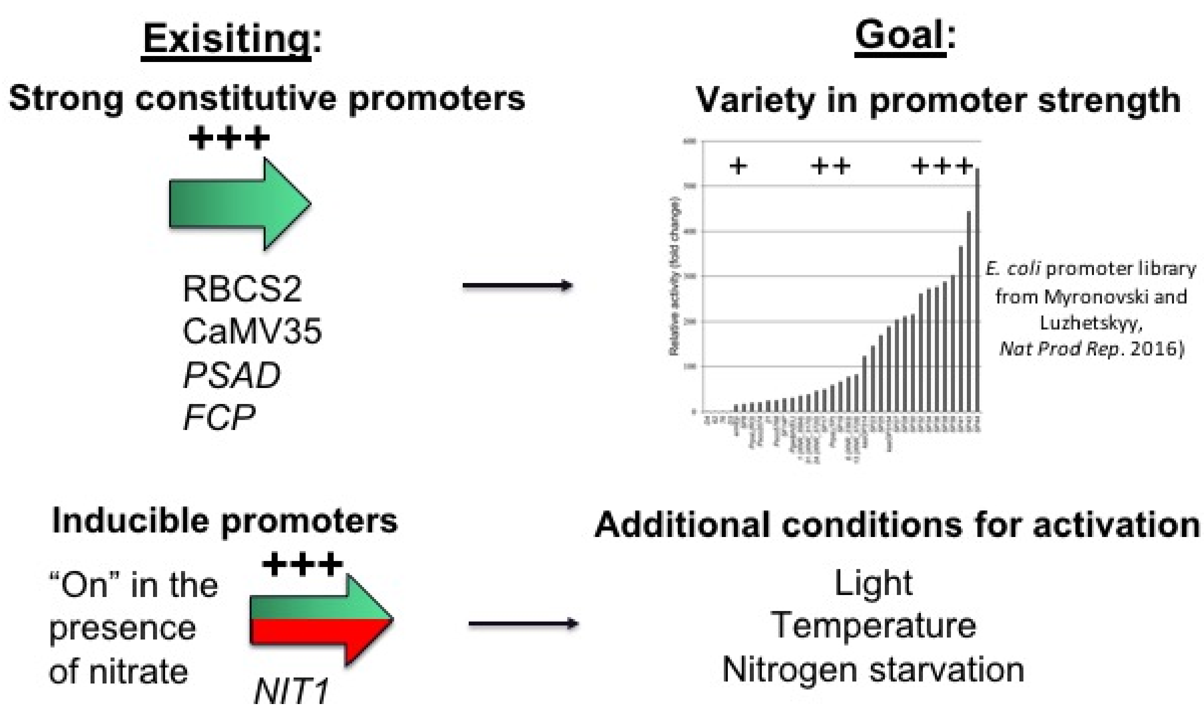


Photo courtesy of Los Alamos National Laboratory

OVERALL IMPRESSIONS

- This project's genetic engineering methodology strives to accelerate the time to identify and verify promotors and promotor strengths. If successful, the team will establish an important baseline for future applications.
- The project is clear and concise with reasonable goals for a 1-year project.

- This kick-starter project is a nice complement to the portfolio of BETO projects. The project team is working toward opportunities to make connections across the portfolio of projects. There is a clear focus on the rapid development of a genetic tool for use in an industrial relevant algal strain. Successful completion of the project will positively impact strain and tools development in alignment with MYP targets.
- This is a small, targeted project with clear direction and good relevance to the BETO mission and concurrent projects at national laboratories. The project is early in its merit review cycle, and initial accomplishments are on track. Future work tied to original project objectives appears to have a high chance of success.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Our team appreciates the positive feedback and the review committee's recognition that this work will improve the rate at which new algal genetic strains can be generated and improved. In addition, the reviewers recognized that the scope of work presented was appropriate for the project timeline and budget as a proof-of-concept task. Upon successful completion of this project, the applied model of using transcriptomic data to rapidly develop native promoter libraries for immediate use will be presented and expanded upon to (1) quickly generate useable promoter libraries for current and new BETO algal production strain candidates and (2) release a public-facing tool for users to generate candidate promoter libraries from their own transcriptomic data collections for new algal species of interest. These outcomes are synergistic with other BETO projects through leveraging existing or planned data collection as inputs. Expanding this project would likely provide beneficial outputs for other advanced algal system endeavors.

FUNCTIONAL CHARACTERIZATION OF CELLULAR METABOLISM

Los Alamos National Laboratory

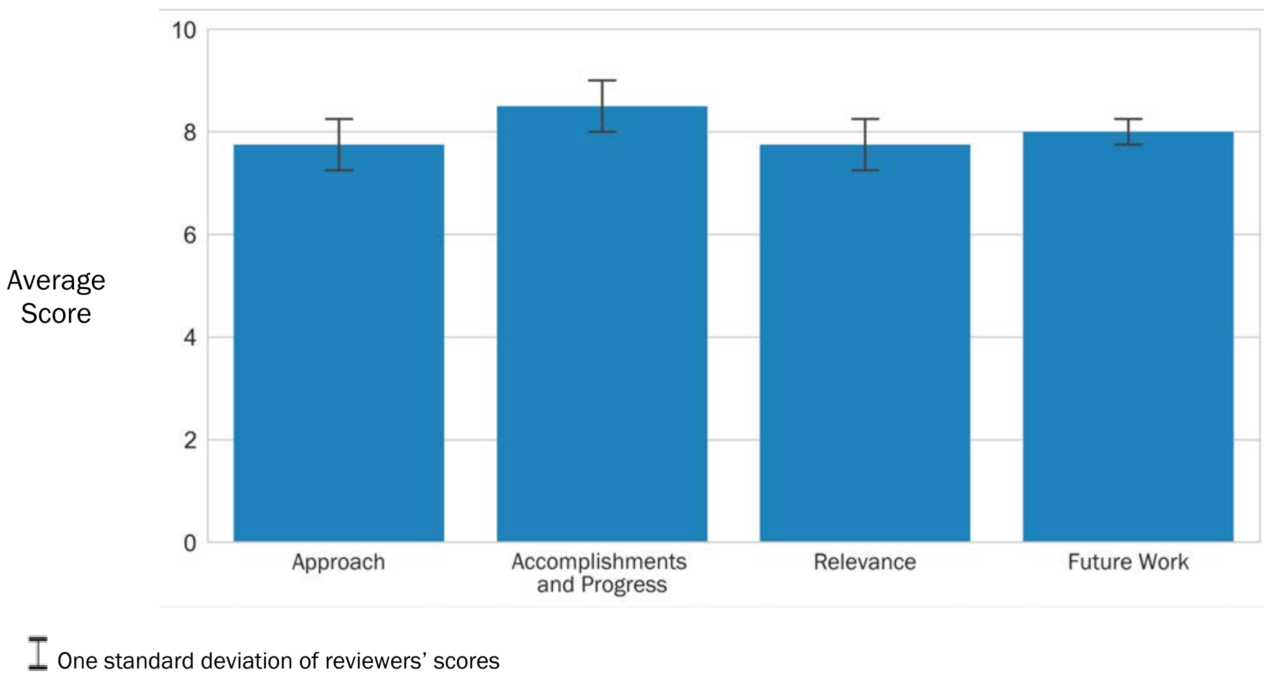
PROJECT DESCRIPTION

WBS:	1.3.1.100
CID:	NL0026328
Principal Investigator:	Dr. Scott Twary
Period of Performance:	10/1/2016–9/30/2020
Total DOE Funding:	$2,950,000
DOE Funding FY16:	$1,000,000
DOE Funding FY17:	$650,000
DOE Funding FY18:	$650,000
DOE Funding FY19:	$650,000
Project Status:	Ongoing

The objectives of this project are to integrate biotechnology and genomic developments for improved algal lines coordinating multiomic characterizations, genetic engineering, and flow cytometry phenotyping for universal applications to algal strain productivity and robustness improvements. We will directly focus on engineering nitrogen regulatory responses altering carbon dynamics to optimize biomass and product flux, developing flow cytometry tools for rapid physiological characterization and the development of robust strains, and developing novel epigenetic understanding of physiological regulation to develop techniques for accelerated strain improvement through an integrated improvement platform. This work will develop novel capabilities through an expanded suite of flow cytometry physiological assays, expanding the molecular engineering toolbox of *Nannochloropsis salina* to include Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas), and defining and regulating epigenome responses. These developments will identify key functional pathways and regulatory mechanisms as targets for advanced improvement strategies. We have currently adapted six physiological assays for multiple algal species characterizing intracellular pH, cellular actin structure, metabolic activity, reactive oxygen species, DNA ploidy, and lipid accumulation. These assays have also been applied toward population sorting to create more robust lines. We have characterized the methylation profile of

Weighted Project Score: 8.0

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



the epigenome in two algal species: *N. salina* and *Picochlorum soloecismus*. The percentage of the genome methylated under nitrogen replete (nonstress) conditions is less than 2%, significantly less than most species. The epigenome responds to both epieffector molecules and nitrogen stress with significant reductions in genome methylation. These changes result in increased lipid accumulation, suggesting that regulation of lipids can be controlled by these mechanisms. We developed constitutively expressing Cas9 and Cas12 lines in *N. salina* for targeted gene editing. Initial targets include regulatory genes involved in nitrate uptake and signaling. New targets will be derived from the metabolic responses of these knockout systems. All three capability advancements will be integrated to create a complementary approach for more powerful strain improvement.

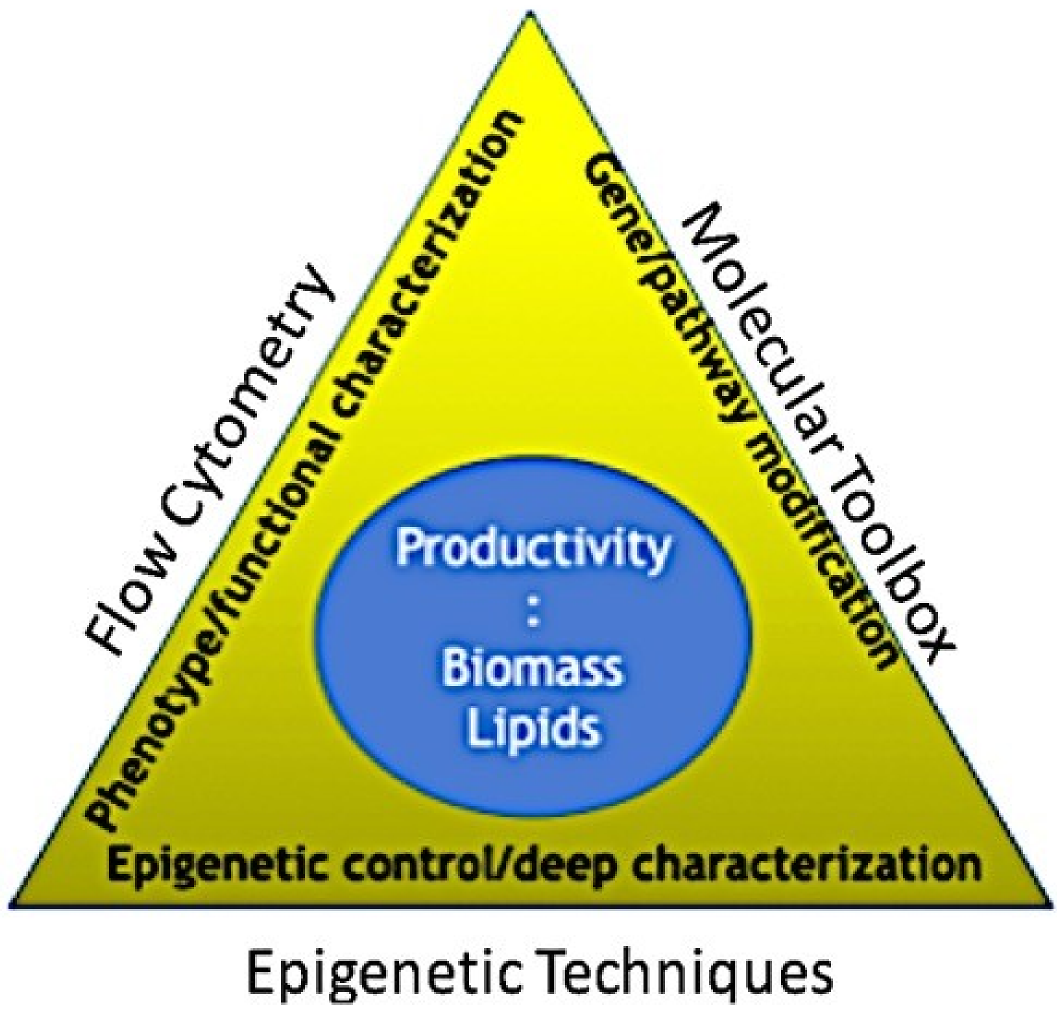


Photo courtesy of Los Alamos National Laboratory

OVERALL IMPRESSIONS

- The goals, technical, and management approaches of this project were clearly articulated. The team has made great progress on all project objectives, and objectives are related to the BETO mission. The team is encouraged to focus future efforts on the portions of the project with the greatest potential impact.

- This multifaceted project attempts to integrate different components into a single platform to establish a better understanding of genetic pathways involved in nitrogen's influence on algal growth—an ambitious goal with lots of coordination.
- This project is designing an integrated strain improvement platform using environmental, epigenetic, and genetic factors for targeted advances with rapid, comprehensive phenotyping leading to increased understanding of these modifications. The techniques being established and optimized for algal strains in this project will add significant value to future strain characterization projects. Large-scale testing will be needed to confirm the 50% increase in lipid formation.
- The project team is employing innovative approaches to improving strain characteristics targeted toward value drivers in algal production systems. The accomplishments to date and their connection to the BETO MYP targets were clearly communicated by the presenter. Of note, the improvements on methods to transform algae and modify methylation are demonstrating great potential value. There is an opportunity to expedite outdoor testing of the modified strains to validate the laboratory results.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Our team greatly appreciates the reviewers' critique of our technical approach and impacts. These inputs will be incorporated into improving our experimental plan. The primary focus is on nitrogen stress induction of lipid accumulation. Biomass productivity is defined by ash-free dry weight. Lipid productivity correlating to boron-dipyrromethene (BODIPY) staining is validated by gas chromatographic/mass spectrometry fatty acid methyl esters analysis. Increased strain productivity is assessed against parent lines grown under conditions ranging from the laboratory scale to greenhouse 50-L raceways. Potential collaborations are established for permitted outdoor genetically modified organism (GMO) trials to correlate laboratory results to outdoor production, if time and funding allow.
- The three-pronged approach integrating flow cytometry, epigenetics, and genetic engineering relies on critical achievements in assay optimization and dye viability effects, methylation assays and methylome sequencing analysis, and CRISPR-Cas editing stability and effectiveness.
- The initial component development focuses on identifying and advancing strengths that can be leveraged into the integrated system. The most effective and relevant results will be the focus for future investigations. For example, multiple epigenetic regulators will be screened for evaluating efficacy and phenotypic responses. The flow assay portfolio can be expanded as novel experimental developments are analyzed. We are reducing stable transformation selection cycles from weeks to days through coordinated flow cytometry sorting of fluorescent reporter genes linked to antibiotic selection. Population sorting into multiple or single-cell transformants can facilitate rapid transgene characterizations. This work demonstrates the value of integrating multiple tool kit developments into one enhanced strain improvement method. Manipulation and evaluation of phenotype using three different molecular approaches (phenotype, genotype, epigenome) increases the probability of generating a highly productive strain and generating a (more) holistic understanding of both physiological and epigenetic changes that occur from targeted genetic engineering strategies. This more comprehensive analysis allows greater elucidation of both primary and secondary responses, enriching the knowledge base to further advance the field.

GENETIC BLUEPRINT OF MICROALGAE CARBON PRODUCTIVITY

Lawrence Berkeley National Laboratory

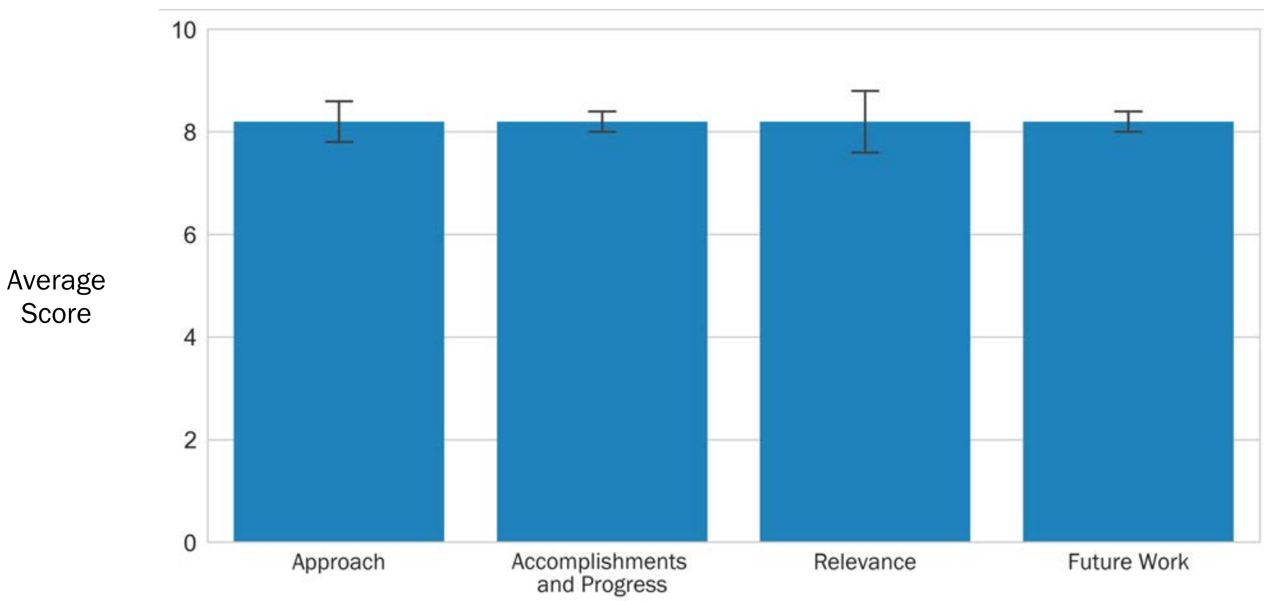
PROJECT DESCRIPTION

WBS:	1.3.1.110
CID:	NL0032266
Principal Investigator:	Dr. Igor Grigoriev
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$1,050,000
DOE Funding FY16:	$0
DOE Funding FY17:	$350,000
DOE Funding FY18:	$350,000
DOE Funding FY19:	$350,000
Project Status:	Ongoing

The potential of microalgae to emerge as major biofuel producers is limited by the fact that maximal internal carbon accumulation (lipids and/or carbohydrates) in algae occurs at the expense of cell growth. Further, different strains of algae have adapted and evolved in various environmental conditions, and thus rotation of specific "seasonal" strains is required to maximize stabilize biomass production throughout the year. A better understanding of algal biology—in particular, mechanisms (1) regulating carbon production and switches from rapid growth to stress-induced carbon storage and (2) growth responses at varied temperatures—is critical to overcoming these limitations. Improving the productivity and robustness of algal strains against perturbations will require extensive advanced genetic, genomic, and molecular biology tools, which are currently lacking for most algal species. This project directly addresses barriers to Aft-3: Genetic Modification and Development. Combining expertise in algal genomics, transcriptomics, metabolomics, and gene editing to characterize novel algal strains with the highest potential as third-generation biofuels will improve biomass production rates and decrease the lag time for genetic modification by 50%.

Weighted Project Score: 8.2

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

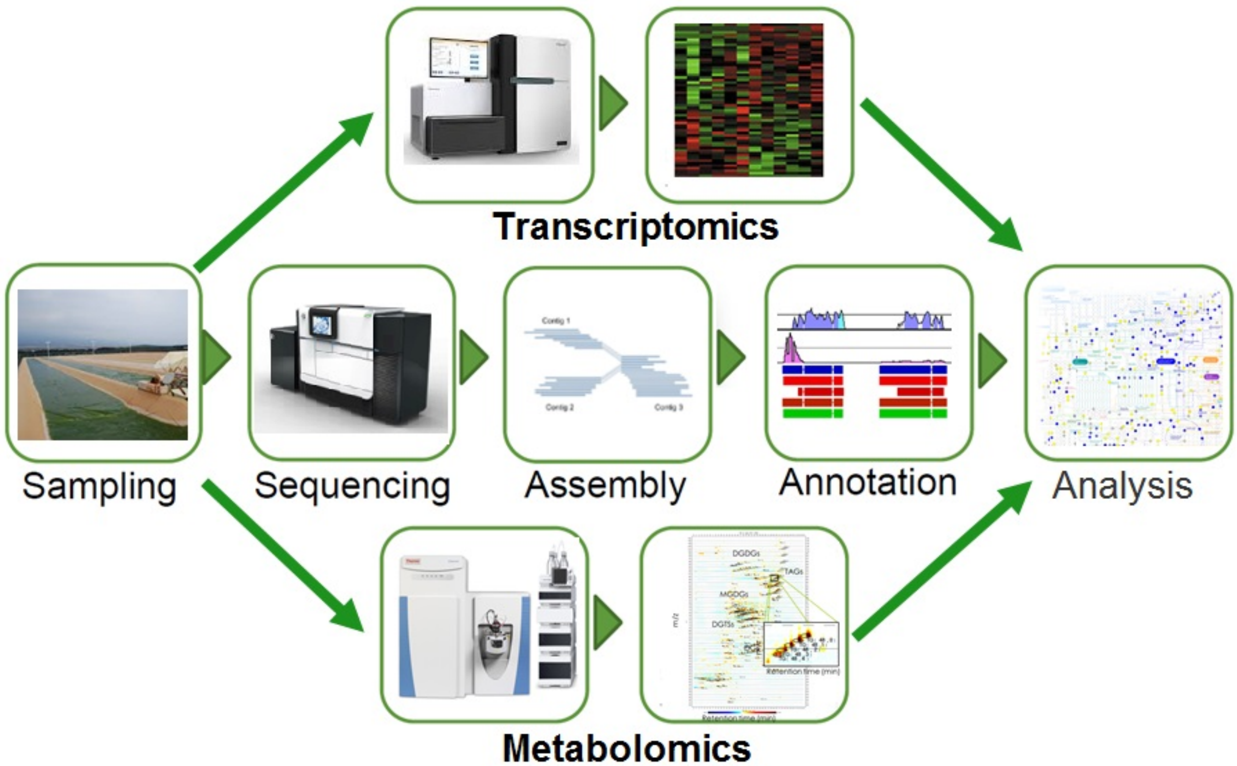


Photo courtesy of Lawrence Berkeley National Laboratory

OVERALL IMPRESSIONS

- Significant value has been realized through the development of a functional genomics pipeline designed to evaluate multiomic data relevant to algal production in real field stresses. Progress to date has identified drivers of cold stress tolerance and potential targets for modification. This project demonstrates the potential to use highly advanced genetic tools and machine learning in application toward commercial targets. Given the accomplishments to date, I expect the team to be successful in continuing to improve the efficiency and value of the omics toolbox.

- This annual operating plan (AOP) project is in the final year of its merit review cycle. The project appears to be managed well and is on track to meet the original project objectives. The project has clear relevance to the MYP goals, the BETO portfolio, and the broader algal industry.

- This project demonstrates the value of a functional genomics development pipeline to identify gene targets with the potential to increase algal productivity to achieve MYP goals. Accelerating the development pipeline during the next fiscal year will be incredibly beneficial.

- A 50% decrease in the time required for this level of carbon pathway mapping is an ambitious task but essential in propelling the overall field research forward. Several national laboratory teams, in addition to private companies, are working on this research path, and it will beneficial to harmonize efforts so no time is wasted or work is doubled.

- The team is developing an algal functional genomics pipeline for the production and interpretation of multiomic measurements from multistate perturbation experiments to identify gene targets for strain improvement and commercialization at an accelerated (50% reduced) development time. They will be using the newly developed pipeline to identify gene targets for strain improvement in *Scenedesmus sp.*

NREL 46B-D3 (NREL) within the first 2 years and then in *Monoraphidium minutum* 26B-AM (PNNL) within 1 (the third) year, achieving the target identification at 50% time reduction. The approach is reasonable and focused on two very relevant strains for the program. The team has sequenced and annotated the genome for *Scenedesmus sp.* and has constructed a metabolic model and curated it. They have also profiled the transcriptome and lipidome under cold and heat shock and done some gene network analysis to map genetic responses. The progress is deemed reasonable and impactful. Genome-based strain improvement is key to the MYP performance goal to increase seasonal areal productivity. These genetic improvements rely on well-sequenced, annotated, and understood genomes. This project helps advance the knowledge of individual key organisms associated with the BETO program. The team will move to sequencing the genome and transcriptome of *Monoraphidium minitum* next. After FY 2019, the team will optimize, enrich, and apply the Blueprint functional genomics pipeline to produce genomes, multiomes, and customized modeling/analysis tools for additional BETO-approved strains.

- No major weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Many thanks for your thoughtful review. Because algae have adapted and evolved in many environments, we hypothesize that the genetic basis of these adaptive traits can be identified and exploited to maximize biomass production throughout the year, particularly by improving productivity at the crossover points when "summer" strains are rotated in favor of "winter" strains. By measuring the systems-level responses with modern omics tools under heat and cold stress conditions, we were able to identify many genes that were differentially transcribed and verify the impact of these transcriptional responses by measuring the downstream impact within the lipidome and metabolome. In partnership with algal molecular biologists, we plan to validate our findings by modifying the expression of several differential expressed genes to preadapt the algal strains to a wider temperature range to improve productivity. Additional outdoor cultivation tests will be needed to fully validate the impact of any genetic modifications. Going forward, we plan to validate and refine our functional genomics pipeline on a diverse set of algae to build a solid understanding of algal physiology for each evolutionary distinct lineage and fold in additional omics measurements to improve the metabolic models and regulatory networks that control growth and biomass composition of industrially relevant production strains.

MULTISCALE CHARACTERIZATION OF IMPROVED ALGAE STRAINS

Los Alamos National Laboratory

PROJECT DESCRIPTION

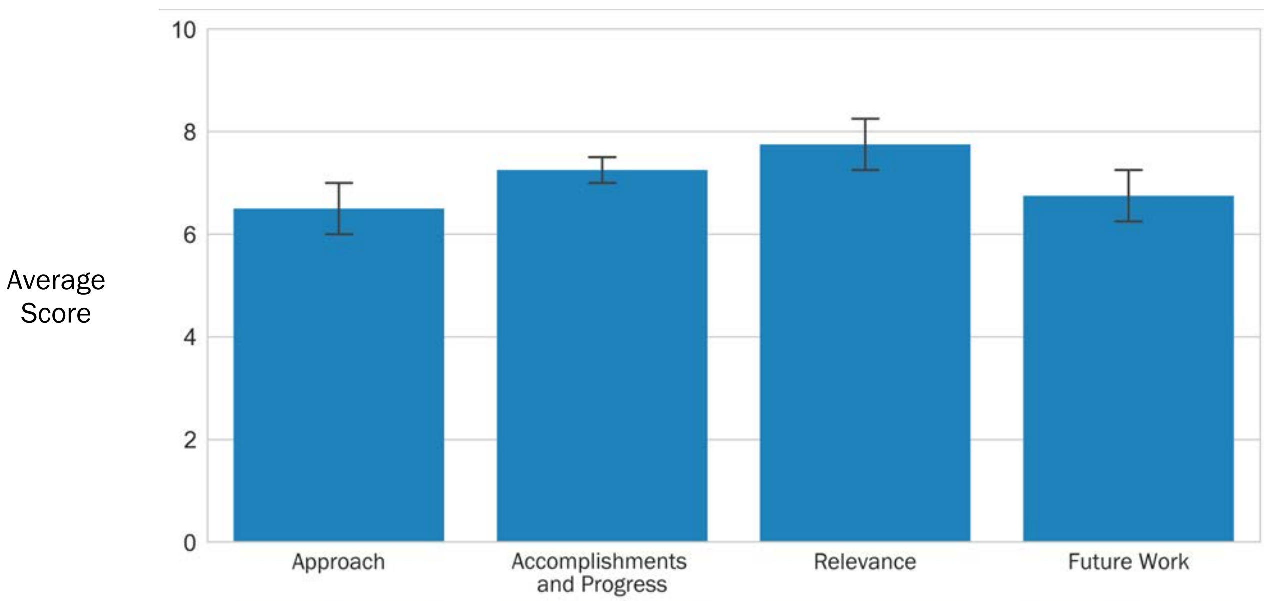
WBS:	1.3.1.120
CID:	NL0025841
Principal Investigator:	Dr. Taraka Dale
Period of Performance:	10/1/2015–9/30/2021
Total DOE Funding:	$2,300,000
DOE Funding FY16:	$500,000
DOE Funding FY17:	$600,000
DOE Funding FY18:	$600,000
DOE Funding FY19:	$600,000
Project Status:	Ongoing

Algae have the potential to be cost-effective and sustainable organisms for the production of renewable fuels and chemicals; however, several challenges must be overcome to realize the full potential of algal feedstocks. First, techno-economic analysis (TEA) of algal biofuels continues to point to algal productivity as a major contributor to biofuel costs. Second, although freshwater strains such as *Chlorella* and *Scenedesmus* routinely demonstrate high performance in laboratory screens, the use of large-scale freshwater systems for algal biofuels and bioproducts is not an acceptable approach from both a sustainability and a human agriculture competition perspective. Third, the relationship between indoor phenotypes of improved strains and outdoor phenotypes is still relatively poorly understood.

Our working hypothesis is that algal strains with improved outdoor productivities can be generated indoors by using a suite of algal strain improvement strategies. Thus, our goal is to develop this suite of improvement approaches using cell sorting, adaptation, and genetic modification techniques, and we focus specifically on tools for generating algal strains with increased biomass and carbon storage as well as environmental robustness (e.g., salinity tolerance). In this presentation, we share our progress on developing these tools and testing these strains across a range of scales, from indoor flasks to outdoor ponds.

Weighted Project Score: 7.1

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

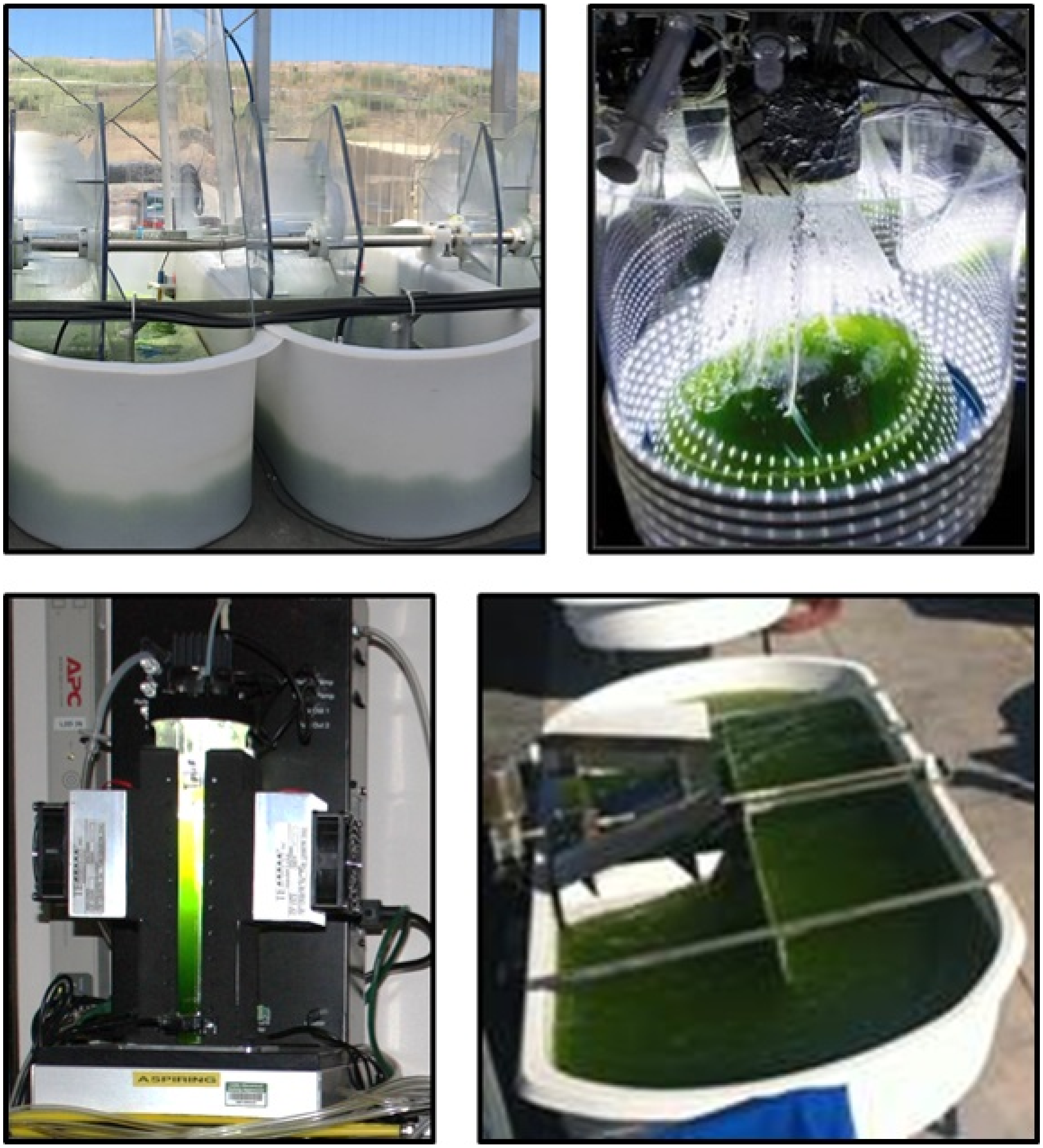


Photo courtesy of Los Alamos National Laboratory

OVERALL IMPRESSIONS

- This is a broad and ambitious project with clear relevance to MYP goals. Team members are encouraged to add clarity and specificity to the objectives and project management structure to ensure progress is made toward project goals.
- This project focuses on developing strain characterization and improvement techniques that are proven to successfully identify strains that translate desired performance from lab bench to outdoors. The unique indoor-outdoor-indoor approach will be incredibly valuable across the industry once it is optimized.

- The project has a great balance between lab-scale and outdoor testing, which is essential in the progress of the entire biofuel field. The project seems to be on two distinct paths: the non-genetically modified and genetically modified, with minimal synergy between the two. It could be beneficial to integrate both paths, resulting in a comprehensive improvement of strains that could be leveraged for outdoor scale-up.
- The multiscale characterization of the algal strains project clearly aims to connect the indoor assessment of strain performance with outdoor demonstration data. The project team has identified a promising strain with the potential to improve productivity and stress tolerance in the field. Improved methods to select for valuable-strain characteristics are also under development. Connecting these tools to realize the iterative feedback loop of lab to field and back to lab testing is critical to fully realizing the project value.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Our project management approach does have more detail than time would permit in the presentation. That said, we take your point and will review our objectives and project management structure and look for improvements to increase our chances of success.
- Thanks for the positive feedback.
- Our two strain improvement approaches are, in fact, currently distinct; however, we agree that in the future it would be valuable to integrate them. Of course, if each new strain is a genetically modified strain, that would entail getting regulatory approval every time we went outside, which would slow our ability to move outdoors. We have been involved in the successful preparation and approval of a Toxic Substance Control Act Environmental Release Application (TERA) for a different project, however, and we are actively considering writing a TERA for at least our most characterized genetically modified *Picochlorum* strain.
- We agree that the feedback from outdoor work is important. We do have specific examples of using outdoor data to inform our indoor work, but we opted to focus on other strain improvement successes in this particular presentation. We look forward to further tightening this interaction between the field and lab, and we will consider this comment as we move forward.

ALGAE BIOTECHNOLOGY PARTNERSHIP

National Renewable Energy Laboratory

PROJECT DESCRIPTION

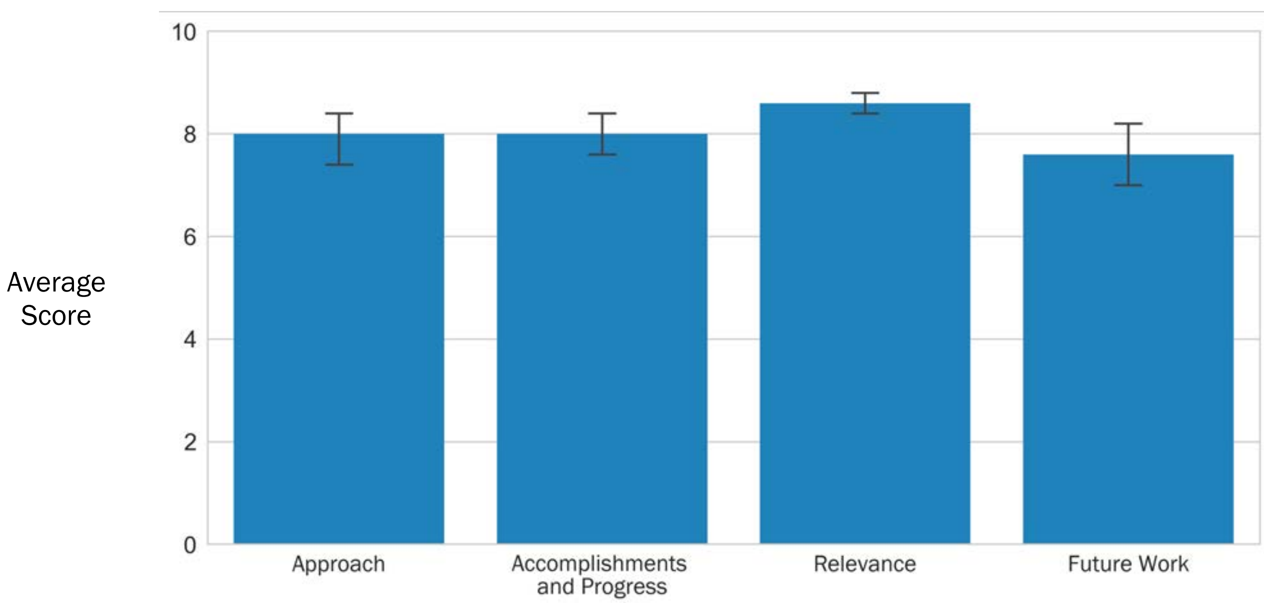
WBS:	1.3.1.130
CID:	NL0028812
Principal Investigator:	Dr. Michael Guarnieri
Period of Performance:	10/1/2015–9/30/2021
Total DOE Funding:	$1,560,236
DOE Funding FY16:	$200,000
DOE Funding FY17:	$500,000
DOE Funding FY18:	$430,236
DOE Funding FY19:	$430,000
Project Status:	Ongoing

The development of advanced genetic and genomic tools for targeted algal metabolic engineering pursuits will be integral to achieving target biomass productivity and, ultimately, the BETO goal of cost-competitive biofuels derived from algal biomass by 2022. At present, however, broad host-range tool development is currently hindered by strain-specific negative regulatory mechanisms. Indeed, the biological processes controlling algal transcription and translation are subject to complex host regulation, which often presents hurdles for targeted genetic engineering strategies. Advanced genetic approaches, such as CRISPR mediated genome editing offer a means to rewire these regulatory systems and/or introduce novel functionality into algal biocatalysts. Synthetic systems biology approaches also present a means to construct novel genetic regulatory networks and rewire natural biological systems to establish orthogonal networks wherein nonnative control elements are introduced into or evolved in host microbes for bypass of host control.

The Algae Biotechnology Partnership aims to develop advanced genetic editing tools, synthetic and orthogonal genetic regulatory systems, and functional genomic pipelines to enable universal metabolic engineering strategies in top-candidate deployment algal strains. Successful development will ultimately open the door for targeted strain-engineering strategies, aimed at maximizing algal outdoor biomass production, composition, and strain robustness. The project will initially develop orthogonal tools in *Picochlorum sp.* 39-A8, which is a

Weighted Project Score: 8.1

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



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top-candidate, halotolerant strain that was downselected and evaluated for outdoor production capacity. The strain displays a rapid maximum growth rate (less than 3-hour doubling time, among the fastest reported to date for eukaryotic algae) and high biomass accumulation capacity, superior to the current SOT. Our 3-year project objective is to demonstrate multistrain applicability of orthogonal tools in concert with genome editing capabilities.

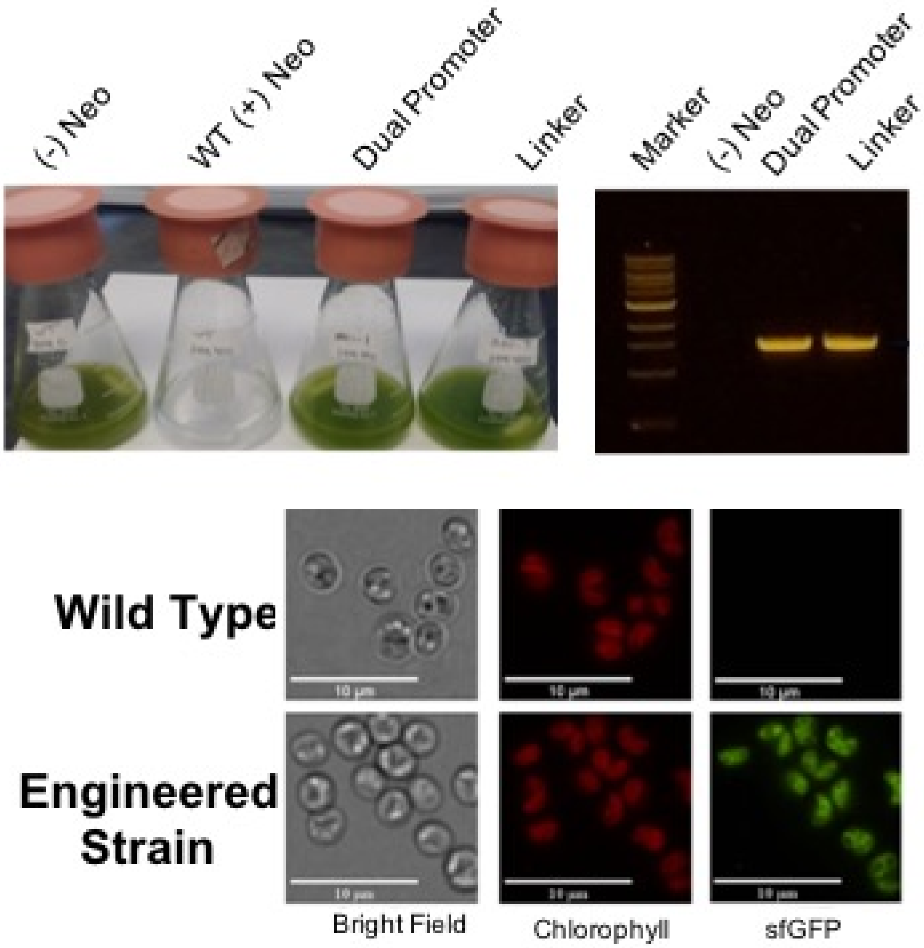


Photo courtesy of National Renewable Energy Laboratory

OVERALL IMPRESSIONS

- This is an ambitious project leveraging the core capabilities of participating organizations to develop a genetic engineering toolbox with broad applicability to improve strain productivity. This project's toolbox has the potential to drastically reduce the amount of time and resources to apply these techniques to different algal strains.

- The project is making a pivot from their previous work in strain development to focus on developing tools for most of the project timeline. Although it is imperative for the entire industry to have a robust genetic toolbox, by focusing only on developing genetic tools for the first 2 years and then deploying these tools to five different strains in year three seems potentially risky. Working toward alleviating issues regarding TERA permitting is essential for the entire biofuel program.

- The project team is transitioning focus from the identification of a highly productive halotolerant strain to the development of a universal toolbox for strain improvement. A significant accomplishment of the project was the completed screening of more than 300 strains under high temperature and salt, which led to the identification of a highly productive strain relevant to targeted field conditions. The current project goal to identify a broad host-range genetic tool kit is ambitious and potentially challenging to complete as defined. A universal system for improving strains has high potential value, especially given the increasing diversity in algal strains of commercial interest.

- This is an ongoing AOP project at the beginning of a new review cycle. The team provided an overview of previous work with a strong justification of the focus for future efforts. The project has clear relevance to BETO priorities, MYP goals, and the algal industry. The project appears to be managed well, and a clear technical approach is supported by quantitative objectives.

- The aim of the project is to develop advanced algal genome editing tools, including synthetic and orthogonal genetic regulatory systems with broad-host range applicability and functional genomic pipelines in top-candidate deployment algal strains. The goal is to demonstrate integrated system "universality" via targeted integration and orthogonally regulated gene expression of native and heterologous fatty acid biosynthetic pathway genes in five candidate deployment organisms. The approach is deemed to be reasonable. The accomplishments to date are deemed to be appropriate. The work addresses the industry-wide need for genetic tools in nonmodel systems—including tools, strains, and metadata—that will be publicly disseminated to enable rapid adoption by the algal industry for targeted enhancement of deployment strains. If successful in developing universal genetic manipulation tools, the project would have wide impact. The team will continue working on broad host-range genetic manipulation tools and will demonstrate tunable gene expression and CRISPR-Cas9 editing. The final step will be testing the "universality" of the tools on five representative organisms.

- No major weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the review panel for their encouraging and constructive critique as well as their recognition of the potential impact of our pursuits. We agree with the reviewers' assessment that although this work presents an ambitious and potentially challenging path forward, is also presents a potentially transformative route to address the industry-wide need to for broad host-range genetic tools in nonmodel, deployment-relevant systems. We are optimistic that the genetic tools pursued on this project have the potential to substantially increase the throughput of algal metabolic engineering pursuits targeting enhanced microalgal productivity, and thus we believe this work has clear relevance to BETO priorities and MYP goals. We also agree with the panel's assessment that working to alleviate issues regarding TERA permitting will be essential for the entire algal biofuel program; we are actively engaging on this front to ensure the work conducted here is compliant with regulatory guidelines and also hope our findings can help inform these guidelines moving forward. Related, we are actively deploying our top-candidate strains at various domestic test beds in coordination with other projects in the BETO algal portfolio (e.g., DISCOVR); and we will strive to deploy engineered variants of these, or other top-candidate engineered strains, within the current period of performance to evaluate outdoor performance and provide a proof of concept for the deployment potential of strains engineered with our team's novel genetic tools. We believe our progress to date to identify high-productivity, halotolerant algal strains, and to develop associated genetic and genomic tool kits therein, represents a critical advancement for the BETO algal portfolio and the larger algal research community. We look forward to continued efforts to enhance productivity in top-candidate strains via the further development of robust genetic and functional genomic tools amenable to phylogenetically diverse algal strains.

CYANOBACTERIA PHOTOSYNTHETIC ENERGY PLATFORM

National Renewable Energy Laboratory

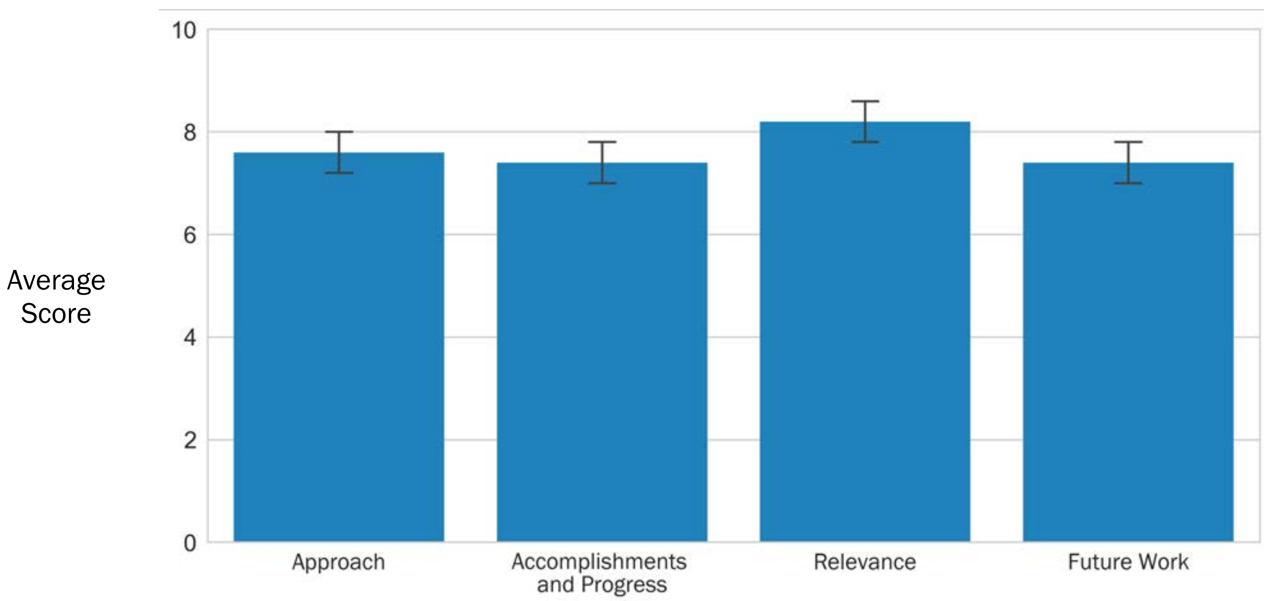
PROJECT DESCRIPTION

WBS:	1.3.1.301
CID:	NL0022533
Principal Investigator:	Dr. Jianping Yu
Period of Performance:	10/1/2015–9/30/2021
Total DOE Funding:	$1,600,000
DOE Funding FY16:	$400,000
DOE Funding FY17:	$400,000
DOE Funding FY18:	$400,000
DOE Funding FY19:	$400,000
Project Status:	Ongoing

The objective of this project for the new FY 2019–FY 2021 merit review cycle is to develop a cyanobacteria platform with the goals of exploiting photosynthetic energy conversion and carbon use for ultimate use in the fundamental biochemical targets to improve biomass productivity. This project leverages years of research and an excellent publication track record at NREL in photosynthesis and carbon and energy metabolism in the model strain *Synechocystis* and will translate and project this knowledge base to the cyanobacteria of industry interest, such as species from the *Synechococcus* and *Arthrospira* genera. In FY 2019, the target is to verify the successful modulation of gene expression of the identified metabolic switch in *Synechocystis*, showing biomass productivity enhancement of at least 10% relative to the wild-type grown under diurnal light conditions in benchtop photobioreactors (PBRs). By the 18-th month midpoint, this project will have demonstrated a 20% increase in biomass productivity using engineered *Synechocystis* strains and will deliver a strategy to transfer the technology to production-relevant species. The end of the project will be a successful platform for rapid testing of photosynthesis and biomass improvement hypotheses in cyanobacteria, including strains of industrial relevance, as well as the development of a route to implement these strategies in selected eukaryotic systems.

Weighted Project Score: 7.7

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



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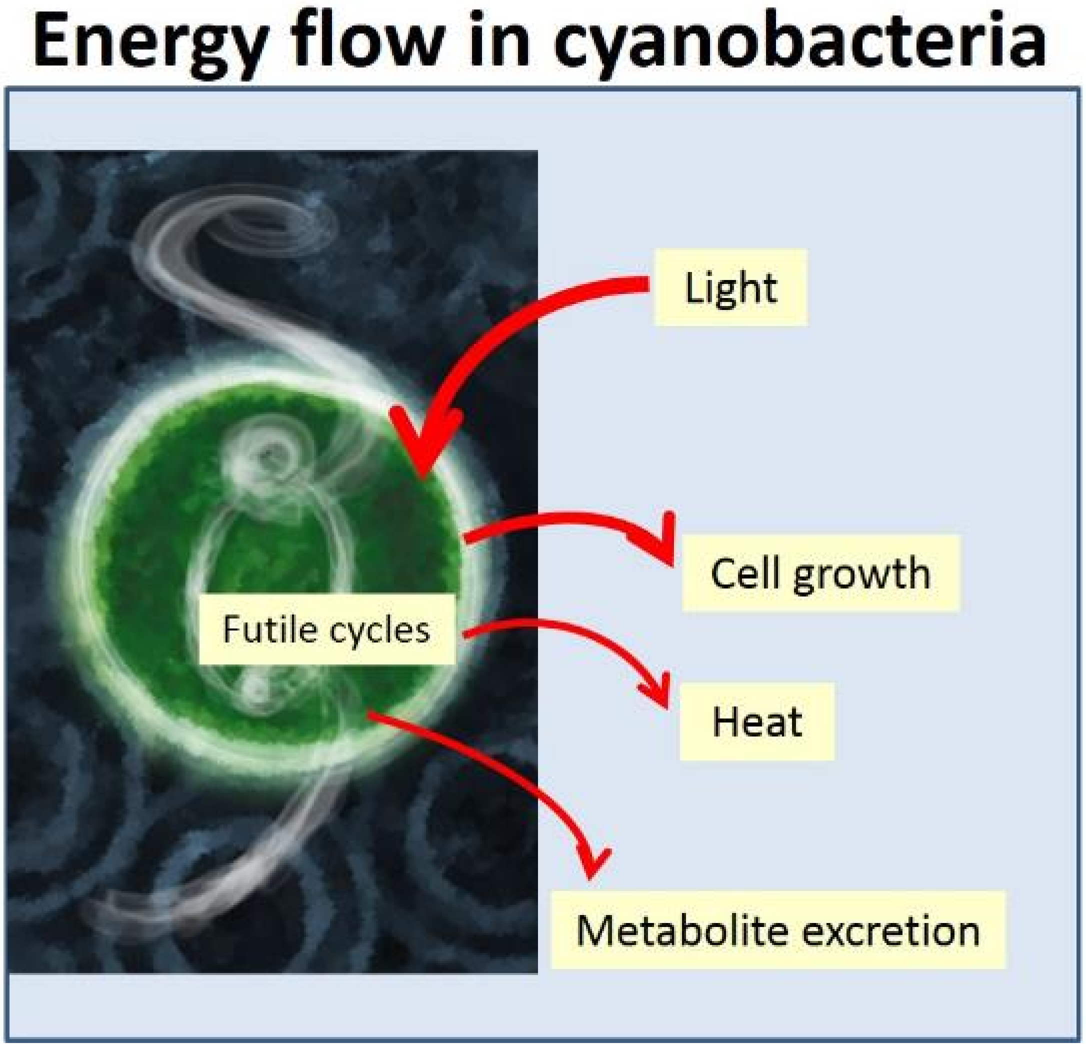


Photo courtesy of National Renewable Energy Laboratory

OVERALL IMPRESSIONS

- The end of the project will be a successful platform for the rapid testing of photosynthesis and biomass improvement hypotheses in cyanobacteria, including strains of industrial relevance, as well as the development of a route to implement these strategies in selected eukaryotic systems. Overall, I think this is an interesting project that is dealing with an important attribute in improving biomass productivity by improving photosynthetic productivity.

- This project is an AOP project at the beginning of its merit review cycle. The project appears to have appropriate technical and management approaches and good alignment with the MYP goals. Team members are encouraged to reach out to industry partners that have more direct experience with a focus on future planned research.

- This project leverages past research to further improve the photosynthetic efficiency of engineering algal strains. If successful, it will increase biomass productivity and contribute to BETO goals.

- The Cyanobacteria Photosynthetic Energy Platform project is focused on modifying the expression of photosynthetic pathways that have a high probability of impacting algal growth and productivity. The clear objectives and underlying data on gene targets are a strength of this approach. The upcoming completion of the proteomics and transcriptomics analysis will also be interesting to further the options for targets of modification. Additionally, the project team has outlined the potential partners for strain testing and potential commercial utility. Taking the next steps in evaluating these modifying strains in outdoor trials and assessing biomass quality will be critical to realizing the utility of the concept.

- The aim of the project is to develop genetic tools for cyanobacteria to improve photosynthetic efficiency through carbon pathway engineering, leading to improved biomass productivity and ultimately to reduced cost for fuels and chemicals. The target is to improve biomass productivity in cyanobacteria by 25%. The team will take a synthetic biology approach to manipulate the carbon sink in cyanobacteria by expanding the sink via ethylene production promoted by the insertion of the efe gene and overexpress/upregulate the photosynthetic apparatus. At the same time, the glycogen synthesis pathway will be blocked. The hypothesis is that this will increase biomass productivity for the organism. To do this, the team will develop new genetic manipulation and phenotyping tools. The project is very focused and has previous experience manipulating the organism with the efe gene. The project is initiating but has previous experience inserting the efe gene into cyanobacteria. Developing tools in model organisms allow for rapid hypothesis testing and then provide options for application to production cyanobacteria and eukaryotic species. The hope is to generate a small set of genetic manipulation tools applicable to cyanobacteria, which will enhance the carbon sink in biomass production. This could possibly generate a high-biomass-productivity organism. If successful, the project could advance BETO goals for high productivity and resilient algal crops. The project has recently been initiated and will be working on photosynthetic overdrive by PII overexpression and energy balancing by down-regulating the glycogen or sucrose flux using a weak promoter to drive glgC and sucrose-phosphate synthase expression. This is a simple strategy that can be demonstrated.

- No major weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

ALGAE DISCOVR – PNNL

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

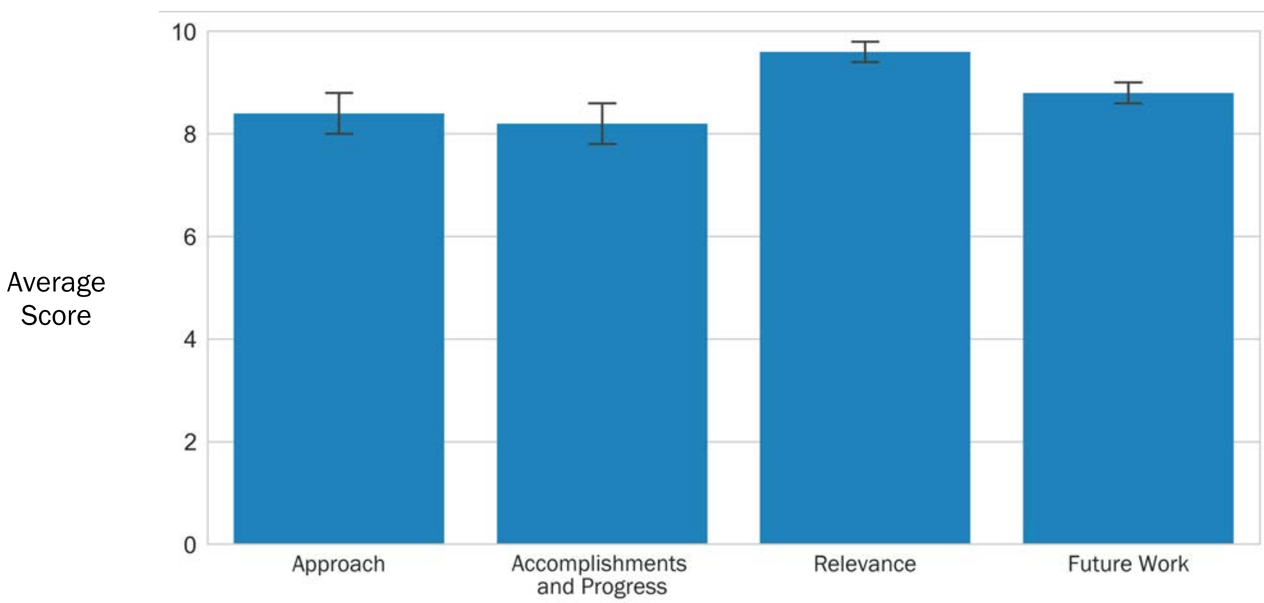
WBS:	1.3.1.501
CID:	NL0032208
Principal Investigator:	Dr. Michael Huesemann
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$6,780,000
DOE Funding FY16:	$0
DOE Funding FY17:	$1,500,000
DOE Funding FY18:	$2,390,000
DOE Funding FY19:	$2,890,000
Project Status:	Ongoing

New cultivars are needed for robust year-round algae cultivation. In addition, benchmarking and tracking the state of algal cultivation is a responsibility of the BETO. We are developing and applying an integrated platform and workflow for standardized, deep characterization of highly productive and resilient microalgal strains. Four national laboratories—PNNL, Los Alamos National Laboratory, NREL, and Sandia National Laboratories (SNL)—are collaborating to combine unique capabilities to significantly improve the TEA feasibility of algae-derived biofuels and products, as demonstrated in the context of SOT experimentation at the test bed of the Arizona Center for Algae Technology and Innovation (AzCATI).

New strains are screened using five consecutive tiers in a conceptual downselection funnel to arrive at the most promising strains. In Tier I, strains obtained from culture collections and industry partners are screened on gradient incubators to determine their temperature and salinity tolerance range. In Tier II, the strains' winter and summer season biomass productivities are quantified in the Laboratory Environmental Algae Pond Simulator (LEAPS) PBRs, biomass harvested from the LEAPS is analyzed for biochemical composition (lipids, protein, carbohydrates) and conversion susceptibility, and the resistance of strains to common grazers and pathogens is assessed in laboratory-scale stress tests. In Tier III, downselected strains are concurrently

Weighted Project Score: 8.8

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

improved by cell-sorting and adaptive evolution and evaluated in terms of culture stability in indoor test beds. In Tier IV, the best winter and summer season strains are evaluated in outdoor raceway ponds to confirm high-productivity biomass and culture stability and to provide biomass for detailed compositional and coproduct analyses.

The top strains are then evaluated in Tier V in seasonal SOT outdoor pond culture campaigns at AzCATI, the dedicated BETO SOT test bed. The core team supervises and coordinates the BETO algal SOT campaign at AzCATI. Management activities include the detailed experimental design of field trials, implementation of harmonized protocols and data management, and test bed oversight.

Major accomplishments so far include the successful execution of the DISCOVR strain downselection pipeline, starting with more than 40 initial strains; the identification of strains with biomass productivity up to 34% more than the benchmarks; the development of quantitative strain downselection decision rules based on maximum specific growth rate versus temperature data, LEAPS productivity, biomass composition, and grazer resistance; and the performance of 11 outdoor pond culture campaigns. Most importantly, a 13.6% increase in annual SOT biomass productivity (relative to 2017) was demonstrated at AzCATI, equivalent to a reduction in the biomass selling price of 10%, from $909 per ton to $824 per ton.

DISCOVR research direction and priorities are guided by a technical advisory board that convenes on a quarterly basis. A DISCOVR website has been designed and posted (https://discovr.labworks.org/), providing an overview of research activities and unique technical capabilities. To further solicit high-impact ideas to help reduce algal biofuel costs, we are requesting research proposals in a call for collaboration.

In summary, DISCOVR is a streamlined, coordinated, synergistic effort that leverages the member laboratories' complementary core capabilities in environmental simulation and productivity prediction, robustness evaluation, biomass valorization, and strain improvement. DISCOVR addresses several key research needs stated in BETO's MYP with the goal of delivering high-productivity biofuel-relevant algal strains through a standardized process for characterizing and comparing potential biofuels and bioproduct strains.

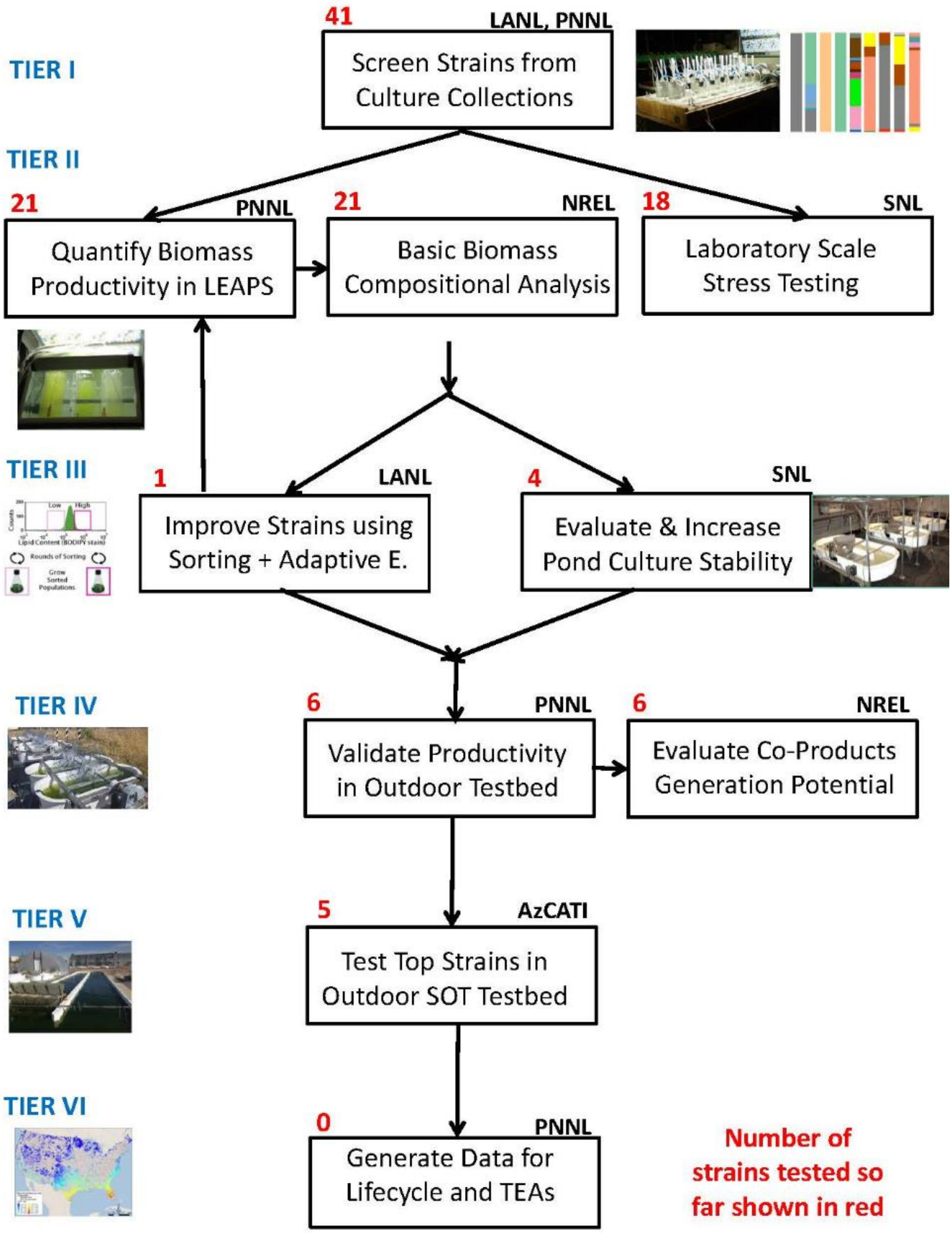


Photo courtesy of Pacific Northwest National Laboratory

OVERALL IMPRESSIONS

- The DISCOVR project team has established a highly integrated and collaborative process leveraging the expertise and capabilities of each member. The productivity targets, associated goals, and process for downselecting promising strain candidates is very clearly defined. The focus on field-relevant conditions in the laboratory testing and strains of highest interest for biomass production is also a strength of the approach. Significant learning has been achieved in the results shared to date. This project might benefit from additional outdoor testing capabilities in relevant, diverse environments.

- This project comprehensively aligns advanced technologies and analytic methods in a wholistic approach to evaluate, identify, and optimize high-potential algal strains. The goal for this pipeline approach is to contribute to the SOT annually because the summer productivity must improve 10% year over year to achieve the MYP 2025 target.

- This project is a large consortium focusing their efforts on a detailed and thorough strain characterization system, LEAPS, and validating their data by deploying their optimized strains to several large-scale sites. Future efforts need to focus on TEA/LCA modeling, but overall their efforts and results will become the next baseline for future projects.

- This was an incredibly ambitious project from its inception, and the team appears to be managed well, making great progress, and meeting its milestones. The broad collaboration has strong project management and is maintaining relevance to the algal industry and BETO's mission.

- The project aims to decrease the cost of biofuel production by screening and identifying high-productivity microalgae strains for biofuels and bioproducts that are resilient in year-round cultivation. To do this, the team has taken the approach of screening relevant strains from collections and submitted by industry collaborators through a complex six-tier selection, characterization, and identification pipeline, from which the best strains are downselected for outdoor trial cultivations and final TEA/LCA to determine their influence on the SOT. The project is very complex and includes selection, screening, productivity assessment, compositional analysis, stress testing, strain improvement, pond culture stability, outdoor testing, and coproduct evaluation; thus, it is a very thorough screening and identification process. The team management is well defined with participants from four national laboratories and the AzCati test bed, each bringing core expertise and capabilities to the project. The team has defined a set of key challenges and related success factors to ensure that strains are relevant and feed into BETO's other algal projects. The approach overall is seen to be very strong. The grazer testing, for cultivation resilience, is seen as an excellent component of the overall assessment of the resilience of strains. The project provides a direct interface to the development of BETO's MYP productivity goals, and it provides a method to show how new strains can be identified that will evolve and improve the SOT targets for BETO. Most importantly, the team is collaborating with several industry partners to test strains specific to their programs and has recently placed an open call for collaborations. The team is poised to continue strain characterization, LEAPS testing, biomass characterization, strain improvements, resilience testing, and further outdoor testing in test bed facilities. Thus, the project will increase the number of strains that go through the full screening, improvement, and valorization pipeline.

- The team is encouraged to consider adding tests to determine if the predefined screening protocols are biasing and predisposing the high-performing selected strains.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for the complimentary feedback on this project and the team's progress. We appreciate the opportunity to respond to some of the points raised here.

- We want to add that we are continuously using TEA as an underlying driver to prioritize the different research areas. The TEA integration is in collaboration with the algal TEA project (WBS: 1.3.5.200), specifically to the farm modeling, which calculates improvements in minimum biomass selling price (MBSP). For example, TEA has identified productivity as a critical driver of biomass production costs, and this area is the primary target pursued for strain comparison and improvement for the DISCOVR consortium. Our productivity measurements are integral aspects of the annual SOT framework. For example, in FY 2018, a 13% improvement in productivity yielded a 10% reduction in MBSP.

- It is a fair concern that the current screening protocol might be biased, and we are actively considering potential modifications to the initial wide-net screening approach to reduce the bias. Reducing the bias and making the selection procedures more relevant to selecting strong outdoor performing candidate strains will be a priority of our work moving forward. One bias that we recently realized is in favor of oxygen-sensitive strains because of the continuous stripping of photosynthetic oxygen out of the medium during the measurement of maximum specific growth rate, whereas in pond conditions, oxygen buildup to supersaturating levels is a common and nearly daily occurrence. High oxygen levels are problematic for photosynthetic biology, so mechanisms to tolerate high oxygen levels are critical to survival in outdoor ponds. Therefore, in the next 3-year cycle, we will add a screen for oxygen tolerance. Ultimately, all screening protocols need to deal with false positives and false negatives. The former adds to the burden of larger scale confirmation, and the latter results in missed leads. The true test of our screening protocol will come from outdoor trials as we continue to pursue our goal of annual quantified improvements in productivity.

ALGAL TRANSLATIONAL GENOMICS

Los Alamos National Laboratory

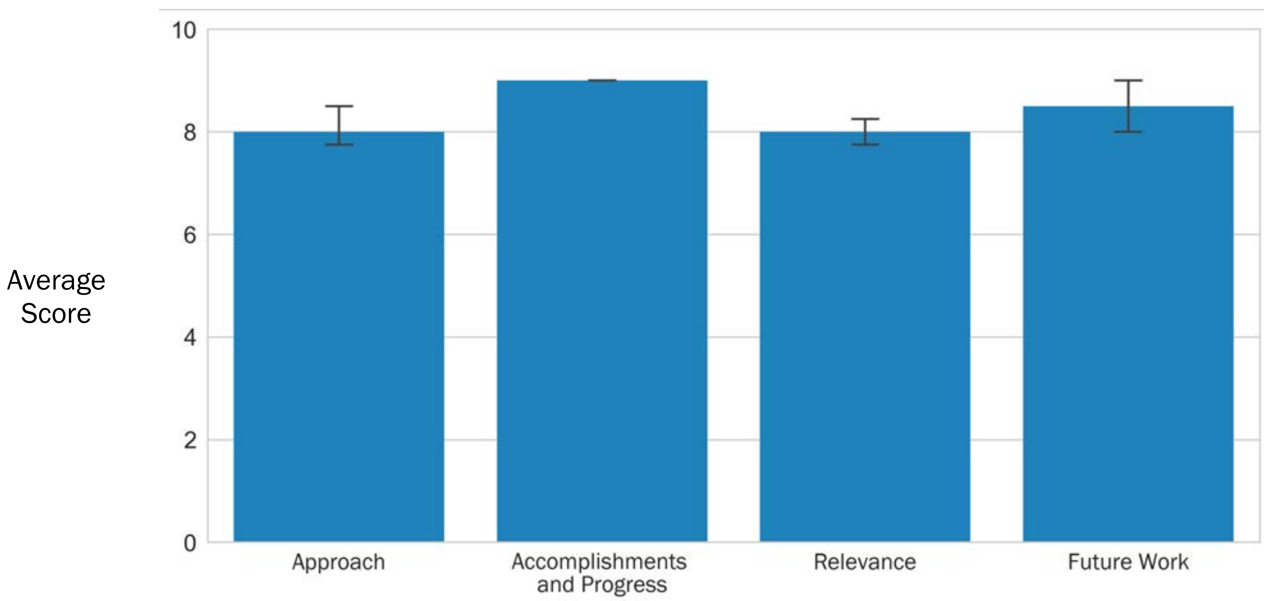
PROJECT DESCRIPTION

WBS:	1.3.1.600
CID:	NL0029949
Principal Investigator:	Dr. Shawn Starkenburg
Period of Performance:	10/1/2015–9/30/2021
Total DOE Funding:	$1,750,000
DOE Funding FY16:	$350,000
DOE Funding FY17:	$350,000
DOE Funding FY18:	$600,000
DOE Funding FY19:	$450,000
Project Status:	Ongoing

The primary goal of this project is to curate and expand the genomic information housed in the algal Greenhouse knowledge base to accurately characterize the metabolic potential of leading production strains to enable performance improvements. A significant barrier to advancing applied algal systems is that genome-wide metabolic models and regulatory networks are lacking, stemming from a dearth of knowledge of gene function for most production strains. Our research will focus on sequencing complete genomes and curating gene annotations through *in silico* and experimental approaches to expand fundamental knowledge of production strain physiology with a primary focus on inorganic and organic carbon assimilation and metabolism. Building on previous genomics efforts, we will accomplish the following major technical objectives: (1) maintain and expand the Greenhouse knowledge base; (2) develop and deploy new assembly methods to construct complete genomes; (3) curate carbon metabolism pathways for leading production strains, beginning with *Scenedesmus*; and (4) phenotype *Scenedesmus* inorganic and organic carbon use to functionally validate genome annotations. Understanding the molecular mechanisms of carbon assimilation and sequestration in eukaryotic microalgae as well as advancing our basic knowledge of biophysical and biochemical components of carbon uptake will generate useful hypotheses to enable strain improvement through downstream genetic and systems engineering. Further, exploiting the ability of

Weighted Project Score: 8.4

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

microalgae to grow mixotrophically on light and organic carbon has the potential to greatly improve productivity when grown in nonpotable, organic-rich wastewater sources, and improve carbon input cost sustainability through discovery and use of efficient carbon metabolism pathways.

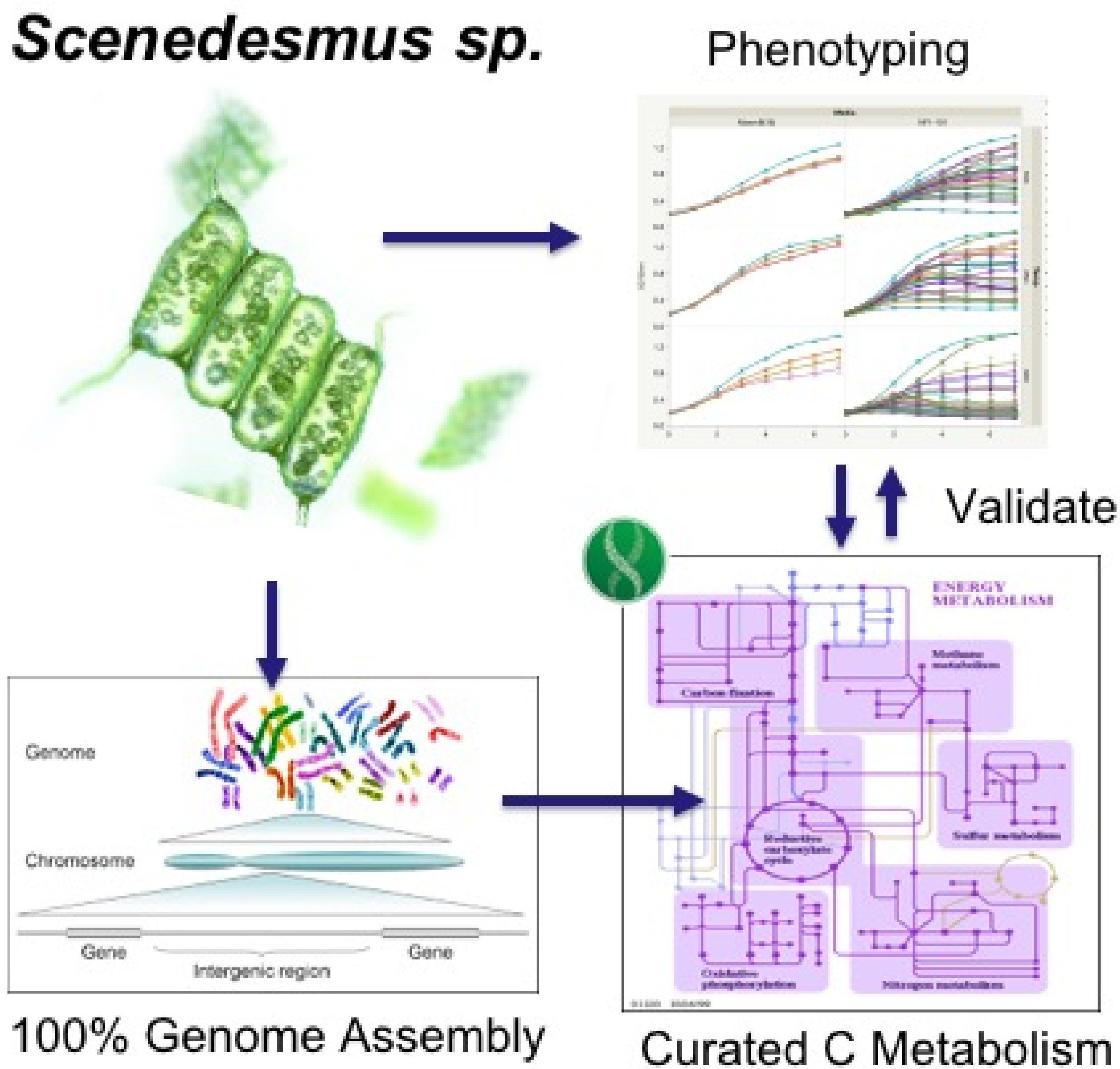


Photo courtesy of Los Alamos National Laboratory

OVERALL IMPRESSIONS

- This project has high potential to be a catalyst to the advancement of algal fuel production through deepening the omics toolbox. The focus on *Scenedesmus* for both relevance and pragmatic reasons is a strength of the approach. Given that the project has just begun, the team has created a strong foundation for their future work through the literature review process. If successful, the development of more efficient methods for complete genome assembly will be highly valuable. Focusing on carbon pathways for modification aligns with production cost targets to enable the use of wastewater for algal growth. The variability and complexity of the anticipated water sources in a production system should be considered when completing the phenotyping analysis.

- This project continues building on previous research by leveraging genomics to characterize the metabolic pathways of industrially relevant strains to farther enhance performance.
- This is a well thought out and ambitious project. The use of wastewater might be beneficial for this strain but not others, and that might limit the overall benefit of this project.
- This AOP project is early in its merit review cycle, and initial accomplishments are on track. The team provided clear justification for the work and strain of choice, and successful completion of this project should have strong relevance to the BETO portfolio and the broader algal industry. The objectives and approach appeared to be appropriate for the project goals around understanding carbon use and metabolism.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Thank you for the thoughtful review and critique. Industrial-scale production of algal biomass using wastewater that is rich with organic matter would provide a cost-effective means to maximize productivity and improve biofuel yield. Unfortunately, the genetic basis for the use of reduced carbon from these waste streams is larger unknown for any candidate production strain. We agree that the variability and complexity of waste sources (carbon types, organisms, and additional dissolved materials) must be considered when assessing productivity improvements; however, the curated carbon use pathways will provide knowledge of which waste carbon streams are best suited for *Scenedesmus*, will guide genetic modifications that are required to enable metabolism of additional carbon sources, and determine which carbon sources are likely consumed by other cocultivated organisms in open systems.
- We agree that the type and degree of organic carbon use is most likely strain specific. We chose to focus on *Scenedesmus* because of its outdoor growth performance, abundance of genomic material, and known ability to be cultivated in a variety of nonpotable wastewater sources. Further, curating and validating the functional annotations of *Scenedesmus* will improve the genome annotations of other chlorophytes because this new information will be available for homology assignments as new algal genomes are sequenced. As resources allow, curating the genome annotations in other lineages (i.e., *Nannochloropsis*) will maximize the value of this project for the user community.

ALGAL BIOMASS COMPOSITION

National Renewable Energy Laboratory

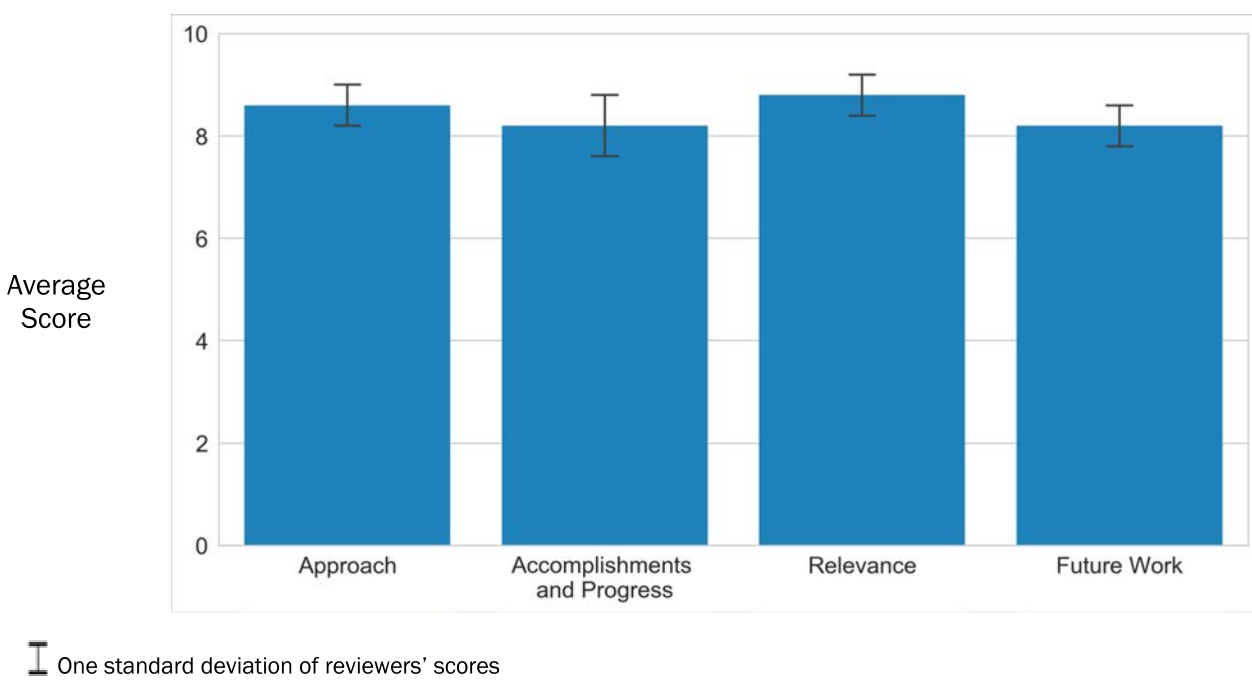
PROJECT DESCRIPTION

WBS:	1.3.2.001
CID:	NL0025629
Principal Investigator:	Dr. Lieve Laurens
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$3,100,000
DOE Funding FY16:	$850,000
DOE Funding FY17:	$750,000
DOE Funding FY18:	$750,000
DOE Funding FY19:	$750,000
Project Status:	Ongoing

Addressing critically needed improvements in biomass, bioproduct, and biofuel productivity is a priority for algae commercial development and is guided by supporting economic and sustainability principles. Exploiting pathways for the integration of engineering approaches with fundamental biochemistry of photosynthetic organisms might help to unravel the contentious nexus of growth rates, biomass productivity and composition, and nutrient load. This project focuses on identifying critical factors for economic development and deployment of algal biofuels, biomass productivity and conversion efficiency, and compositional characteristics and then providing a robust set of tools to allow for an unambiguous biomass quality and compositional description. This project is highly relevant to the DOE multiyear program targets of reducing costs and integrating dynamic biomass composition with downstream process characteristics by providing options for the development of fuel-relevant products derived from either the lipid, carbohydrate, or protein fraction of algal biomass. This project directly addresses two BETO barriers: (1) lack of understanding of the value of the feedstock (in context of seasonality and environmental variability) and (2) lack of information on the physical, chemical, and biological quality of the biomass in the context of the impact on biorefinery operations and performance. To address these barriers, this project (1) builds on the standardization effort for compositional characterization of algal biomass by supporting and validating the implementation of

Weighted Project Score: 8.4

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



methods and (2) gathers an understanding of the dynamic biochemical composition and carbon allocation in terms of biomass value and conversion yields and selectivity. The goal for this project's merit review cycle is to build and show a coproduct portfolio that, when integrated in a conversion pathway, can increase the intrinsic value of algal biomass by at least 30% of biomass cost value for a set of model species. This goal will be achieved by increasing the value of biomass and supporting a dynamic biochemical compositional profile in a set of model algae to ultimately quantify the value of the biomass for conversion or upgrading pathways. To establish this conversion interface, a highly granular compositional profile should be established during a dynamic growth profile to link with lipid upgrading, nutrient and water recycling, and with integrated fuel and bioproduct conversion. As an outcome of the experimental valorization subtask, we will continue to drive toward an inherent value of the components in the biomass composition, providing a better link with biomass production costs and eliminating the potential conflict between maximizing biofuel yields and maximizing potential revenue, which provides a better sense of the path to commercialization. To date, this project has developed and is maintaining an online repository of robust standard analytic procedures. We added a sterol quantification procedure to support the development of sterol-derived coproducts as well as help to close the overall descriptive mass balance of algal biomass. We built an amino acid analytic pipeline to aid with the more rigorous assessment of protein and protein-derived high-value products. Through the development of an experimental data collection framework for the quantitative assessment of biomass composition, bioproducts, energy, and productivity in different indoor PBR cultivation conditions, we compared continuous and batch cultivation on overall measured growth rates and project algal farm productivity. We used this framework to select species, growth scenario, and bioproducts based on the overall value of the biomass. This allowed for tracking quantitative increases in intrinsic value, with the aim of adapting cultivation conditions to outdoor pond operations toward increased overall intrinsic biomass value.

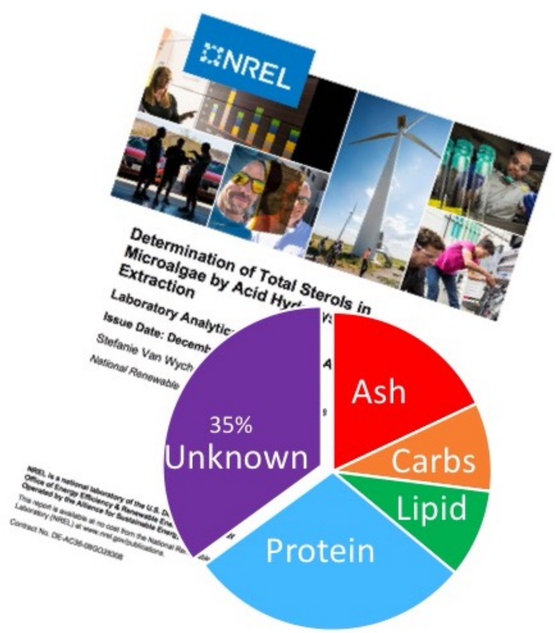


Photo courtesy of National Renewable Energy Laboratory

OVERALL IMPRESSIONS

- The group's best efforts are tied to developing innovative analytic techniques. The other aspects of this project are too big in scope and are loosely associated with each other.

- This project continues to have a clear benefit to the algal industry as a whole and is an important link to BETO priorities. Dr. Laurens continues to be a leader in biomass compositional analysis, and the team regularly interacts with industry and the modeling community to make sure critical compositional assumptions are clear. The project appears to be managed well, is meeting its milestones, and is maintaining its relevance to public and private algal research. The project is also commended for prioritizing information dissemination and community engagement.

- This project has the potential to, and likely will, dramatically reduce the cost of biofuels via understanding how to maximize the value of the coproducts. The current scope includes creating analytic techniques that accurately account for the entire biomass composition, cultivation variables impact on composition, as well as the downstream processing required to fully capture the value of the coproducts created.

- The development of methods to assess algal biomass composition in support of a developing bioproduct portfolio is critical to drive improvement in the economics of the production of fuel from algae. This project nicely combines novel methods to work toward closing the mass balance and enabling tools for novel compound discovery. In addition, the team has worked to define best methods for assessing algal product quality and made the physical materials available to the broader community. Continued work on biomass composition analysis under varied cultivation conditions will be critical to determining quality variance in real-world conditions.

- The projects goals are to reduce the cost of biofuels by increasing the algal biomass value and to reduce the uncertainty around process inputs and outputs. The first goal will be approached through a quantitative development of biomass composition, energy, and productivity for model algal species. The second goal will take an approach of standardized characterization and development of standards for the quantitation of properties. Therefore, the project helps address key barriers in sustainable production of algae, biomass characterization, and material properties. The project's main strength is in the development of robust standardized analytic procedures. This is seen through the large download of methods and the standardization of methods across the ATP3 partners. Further, the identification of key valuable products that can influence the overall valorization of the biorefinery process can provide incentives toward processes that help reach BETO's strategic goals in this area. The future work planned activities are deemed appropriate.

- A possible weakness is noted in the approach to use the tools generated to valorize biomass components because many factors affect the market value of raw components.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their complimentary feedback on this project. Several comments by the reviewers were reiterated, and we appreciate the opportunity to respond on the relevance of the compositional shift experiments. It is our primary goal to establish tools and techniques required to test whether there are cultivation conditions, light, temperature, or semicontinuous harvesting that impact composition without inhibiting overall biomass productivity. If we can unlock this potential in indoor PBR, we can implement this approach in relevant outdoor cultivation trials. Details on the cultivation strategies implemented to obtain shifts in biomass composition needed to be minimized, and we want to clarify that the growth experiments experienced high-incident light, which is likely one of the primary drivers of productivity. We have shown that maintaining high cell density cultures, by frequent harvesting, yielded higher storage products (carbohydrates and lipids), which translates to increased intrinsic value when following the novel TEA modeling approach. By comparing the rates of accumulation of storage products and shifting biomass composition in a consistent manner under respective strain optimized media and nutrient conditions, we aim to elucidate the differences in inherent capacity of the strains to adapt their underlying composition.

- In summary, we have developed an experimental setup that allows us to test hypotheses in PBRs, and we are making progress on using these experiments to provide biomass for biochemical mapping of metabolites and thus validating different valorization models. Since the peer review presentation, we have finalized our work with the TEA team on integrating compositional dynamics into a valorization framework. By setting up this conceptual process, we are now able to directly assess and leverage the impact of additional products identified in this project.

ATTACHED PERIPHYTIC ALGAE PRODUCTION AND ANALYSIS

Sandia National Laboratories

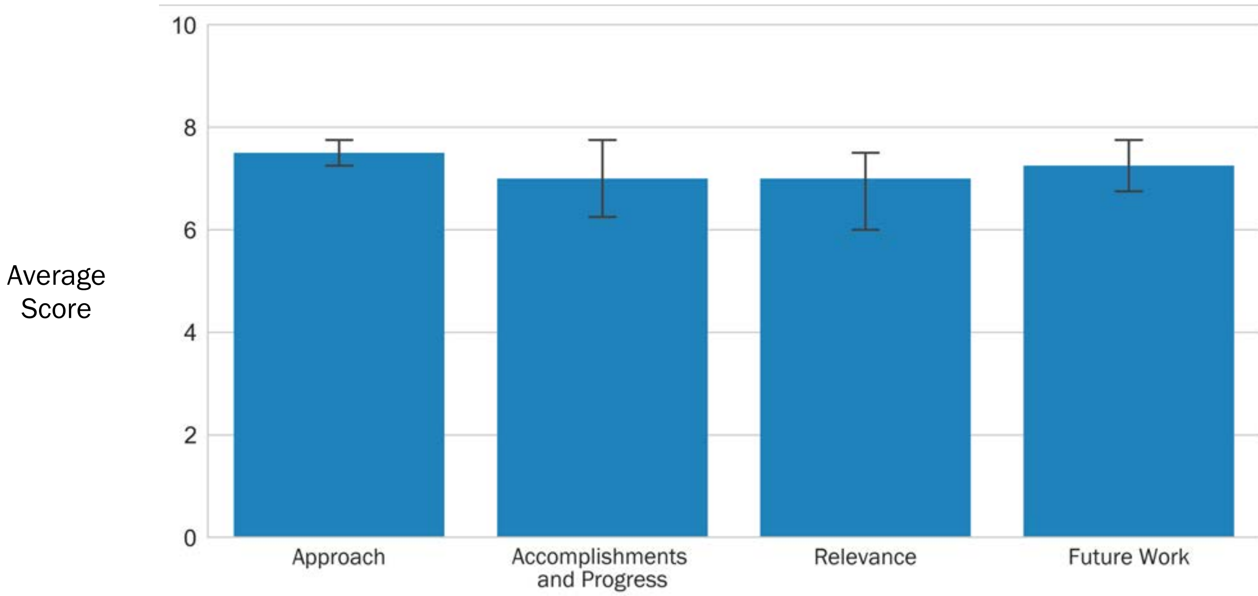
PROJECT DESCRIPTION

WBS:	1.3.2.130
CID:	NL0027375
Principal Investigator:	Dr. Ryan Davis
Period of Performance:	10/1/2015–9/30/2021
Total DOE Funding:	$3,200,000
DOE Funding FY16:	$950,000
DOE Funding FY17:	$750,000
DOE Funding FY18:	$750,000
DOE Funding FY19:	$750,000
Project Status:	Ongoing

This project aims to test, evaluate, and show the proposition that attached periphytic algal polyculture systems are capable of achieving high annual average biomass productivities that at least equal production from raceway ponds and can exceed to 24 g/m^2/day (ash-free dry weight), thereby providing a path to 5,000 gal acre-year algae-derived fuel. The approach uses compromised surface waters with nutrient contamination (N, P, trace metals) concentrations that are much lower than those required for recirculating raceway pond production of planktonic algae. The same surface waters will also generally have adequate levels of carbon dioxide (CO_2) and/or carbonate to avoid the need for supplying supplementary CO_2, which is also otherwise required for recirculating raceway pond production of planktonic microalgae. By periodically pulsing the inflow, the shallow turbulent medium provides several mechanisms that contribute to the capability of an attached periphytic culture to achieve relatively high productivities despite lower nutrient concentrations. These include a high exposed surface-to-volume ratio of cultivation, turbulence-induced rapid light dark cycles that improve photon use efficiency of the cellular photosynthetic apparatus, and improved nutrient and gaseous exchange and breakup of boundary layer gradient limitations that otherwise exist among the algal cellular matrix, water, and atmosphere. These mechanisms are partially

Weighted Project Score: 7.2

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

shared with observations of high algal productivity seen using thin-layer cascading cultivation systems, which have system configuration and operational similarities.

To optimize yield and minimize ash based on new substrate materials and culture seeding processes, we apply SNL's computational fluid dynamics expertise and algorithms with experimental validation to predict the residence time of seed culture cells in the floway and substrate matrix in the absence and presence of an established turf. A key component of this R&D effort will be to evaluate whether culture seeding and substrate optimization can provide synergistic yield enhancement, minimize biomass productivity losses through maturation latency and sloughing, and reduce ash in the presence of a native (i.e., unseeded) periphytic turf because the native periphytic cohort is expected to confer the culture stability observed to date. As part of this work, we are evaluating the impacts of sloughing and evaporation losses based on changes to the substrate material and as a function of biomass yield because scale-up of this technology will likely face significant hurdles in arid regions, such as the U.S. Desert Southwest, if increased evaporation losses are incurred by the system compared to already existing surface waters. The current year goal is to demonstrate the ability for attached algal cultivation to simultaneous achieve 24 $g/m^2/day$ and 25% ash using compromised surface waters. The 3-year goal of the project is to demonstrate attached algal cultivation coupled to water treatment with a net biomass cost of $450 ton ash-free dry weight.

OVERALL IMPRESSIONS

- This AOP project includes a novel approach to low-input and low-cost production of algal biomass. The project appeared to be managed well and had clear relevance to the BETO mission and MYP goals. The new merit review cycle is building on directly relevant previous work. The team is encouraged to incorporate TEA results from a fully integrated process into the project's experimental design.

- This project strives to increase algal biomass productivity in environments with excess nutrients (from agriculture runoff) with as little additional infrastructure and equipment as possible. The project has made significant progress in baseline understanding and implementing work to overcome learnings and challenges.

- The attached periphytic algal project has been a successful collaboration between research and public offices to solve a very specific water contamination challenge. The team has developed a valuable data set in a relatively large production setting that can drive TEA assumptions and future work in this area. The challenges around biomass quality are significant and will require considerable effort to overcome for this system to be useful in the production of feedstock for fuel.

- The aim of this project is to develop cost-effective means for cultivating easily harvestable algal biomass using nutrients from compromised surface waters and attached algae cultivation methods. This project is currently working to couple remediation of agricultural/storm runoff with algal biomass production >24 $g/m^2/day$ and ash content <25%. Scale-up of a domestic algal biomass industry requires identification of value propositions, including remediation of runoff, to achieve production costs that are commensurate with high-volume commodities and energy. The high ash content of biomass from turf systems is a challenge that the project will be addressing. The team is using three facilities for turf algal growth but mainly focusing on the Salton Sea floway. Biomass from this system has been thoroughly characterized for chemical composition and microbial ecology. The team is working on ash reduction and reduced nonbiogenic ash by 77% using a mesh to filter waters and will be working on seeding the turf algal system with non-diatomaceous strains of algae. The approach is reasonable and should work as long as the system is not naturally reseeded by diatoms. Although turf algal systems have challenges, as noted by the project team, the opportunity for high growth, biomass productivity, and water remediation are excellent. If the biomass productivities are achieved with reduced ash content, the project can help achieve productivity targets for the BETO program. The team will deploy flow-rate feedback, seed cultures with non-diatomaceous periphyton, evaluate species and biomass production, and quantitate evaporation. The approach is reasonable.

- The only concern noted is related to the possibility of natural reseeding by diatoms.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

CONTINUOUS BIOLOGICAL PROTECTION AND CONTROL OF ALGAL POND PRODUCTIVITY

Lawrence Livermore National Laboratory

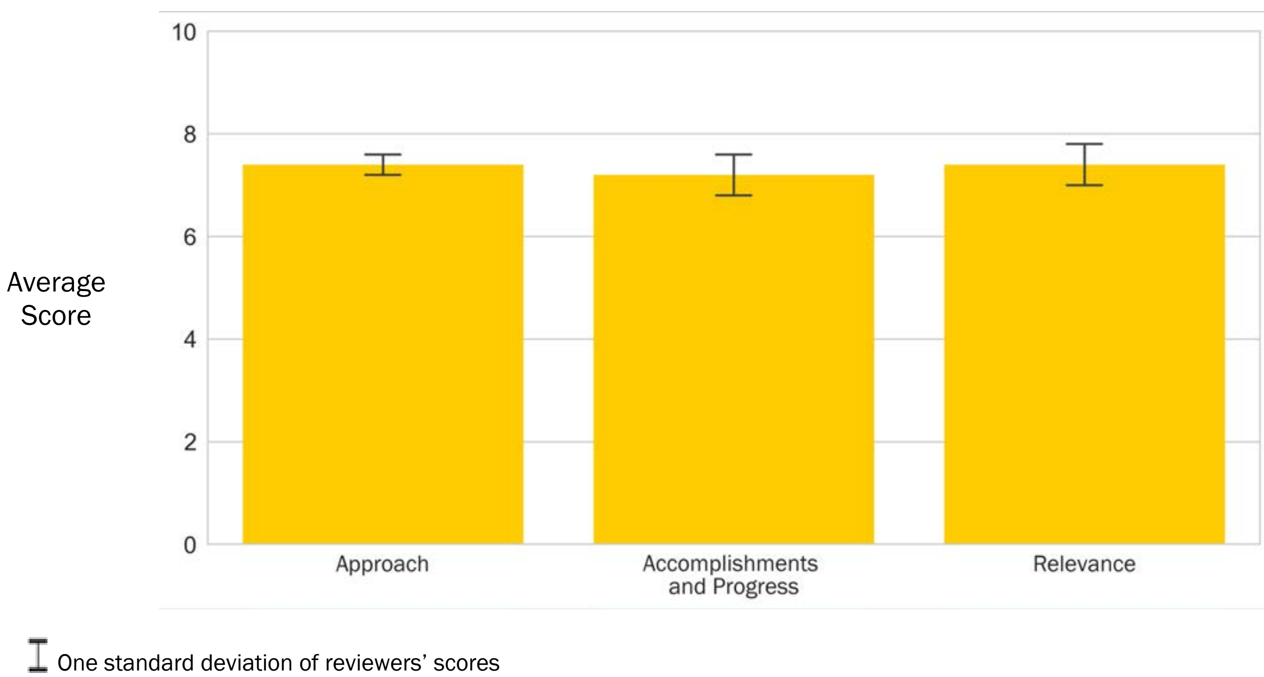
PROJECT DESCRIPTION

WBS:	1.3.2.300
CID:	NL0029886
Principal Investigator:	Dr. Michael Thelen
Period of Performance:	9/1/2015–9/15/2018
Total DOE Funding:	$1,080,000
Project Status:	Sunsetting

We present on two projects, one funded under the Targeted Algal Biofuels and Bioproducts (TABB) competitive FOA, concluding this year, and another follow-up project, funded as an AOP, beginning this year. The projects are aimed at improving algal cultivation through the application of microbes to protect algal crops from losses caused by predators and pathogens. Ultimately, both projects aim to increase annual algal biomass yields. Our projects address barriers from the TABB FOA of the high costs of producing biomass and low yields of target feedstocks and of translating laboratory success to outdoor cultivation environments. In addition, we focus on the MYP barriers Aft-B: Sustainable Algae Production and Aft-A: Biomass Availability and Cost. The TABB project team was led by LLNL and included SNL (Livermore), the University of California at Davis, and the microalgal development company Heliae, LLC (Arizona). Our goals were to identify probiotic bacteria that protect algae against predators and pathogens and rapidly test these bacterial applications at increasing scales. During our 3-year project, we identified both a bacterial isolate and consortium that protect against rotifer predation at the laboratory and mesocosm scales. This project highlights the importance of (1) leveraging the algal microbiome to increase algal resistance to predators and pathogens and (2) rapid translation from laboratory to outdoor testing. We performed a feasible industrial-scale (10,000 L) probiotic addition and incorporated laboratory and outdoor experimental data into a relevant TEA model to assess sensitivities and future opportunities. Our new project will apply what we have learned, addressing the sensitivities of the TEA

Weighted Project Score: 7.3

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



model to make our probiotic application more economic. This new project forms a new collaboration with Lawrence Berkeley National Laboratory and aims to determine the mechanisms of bacterial protection and to induce the protective effect, increasing the efficiency and duration of the application.

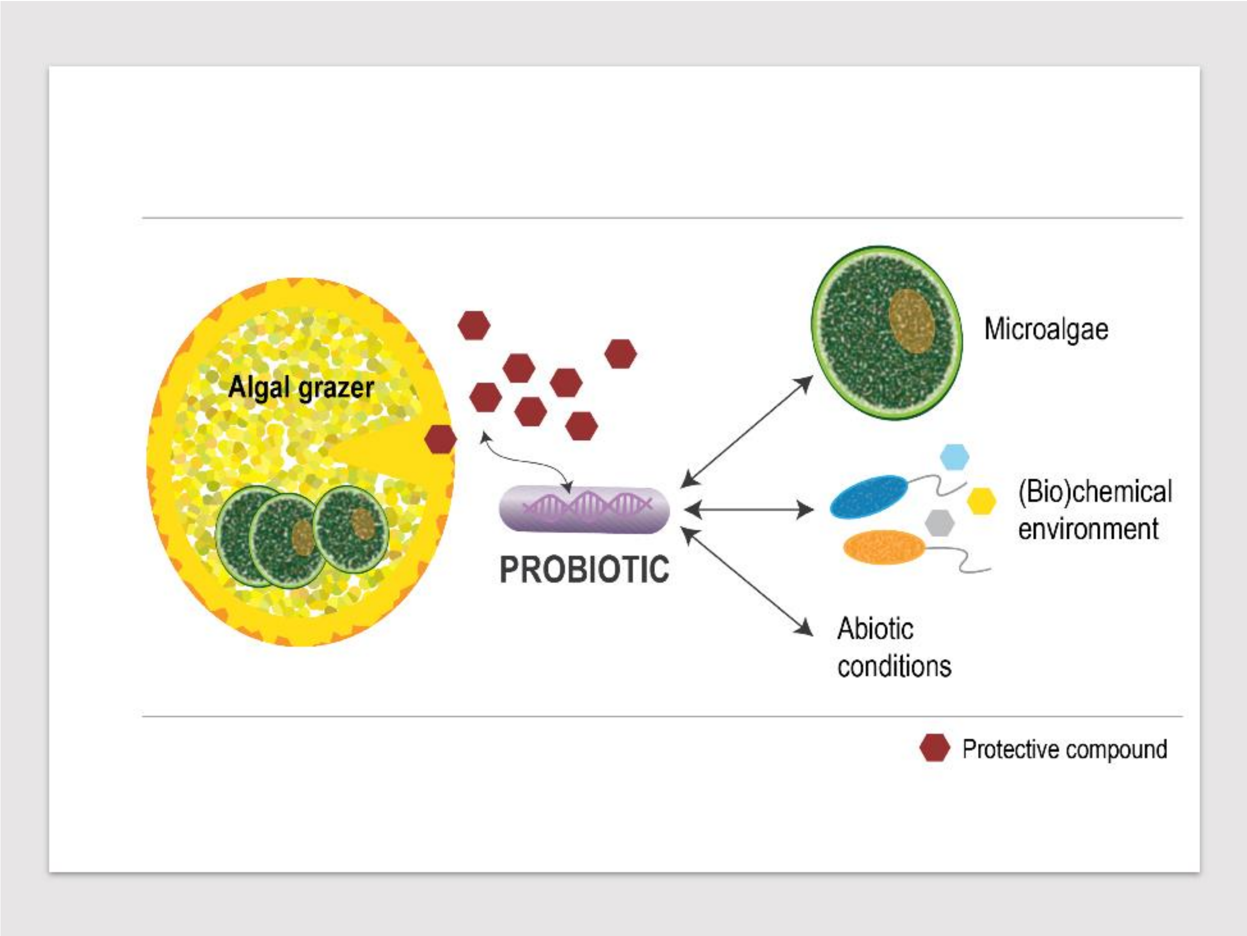


Photo courtesy of Lawrence Livermore National Laboratory

OVERALL IMPRESSIONS

- The next phase of this project continues to develop strategies for creating a "probiotic" that can be added to open pond algal raceways to improve the productivity and robustness of commercial farms.
- This project review is a combination assessment of the completed work and the new plan that is a continuation of this project. There were many key accomplishments exploring the use of probiotic bacteria in the cultivation of *Myrmica salina* as a protective approach to rotifer predation, including the development of a TEA around this strategy. The team is now building on this work to understand the potential mode of action and develop the process to scale. For the upcoming work, the project team will benefit from clarity on objectives in the outdoor trials to ensure project success.
- To reach the overall project goals of BETO, minimizing pond crashes is essential. This project has the potential to establish new and innovative solutions for pond pest management, but they seem to be focused only on mitigating saltwater rotifer. Rotifers are only one of many pests that hinder algal productivity. The project would make the most impact if the team focused on expanding their work of yearly sampling and characterizing the microbiome community to more sites while simultaneously using their techniques to find a solution for the different pests that arise during the different seasons.

- This project is focused on identifying, understanding, and scaling probiotic microorganisms for large-scale outdoor production, which has clear relevance to the BETO mission and MYP goals. The project included an appropriate proportion of laboratory, mesocosm, and field-testing, with experimental design appropriate for each scale. The group has completed most objectives and will be building on these accomplishments in a future AOP merit review cycle. The team is encouraged to incorporate outdoor testing early in the project to ensure bench-level successes will have relevance in an industrial environment in the presence of more challenging ecological pressures.
- The aim of the project is to improve the resilience of algal crops to predators and pathogens by using probiotic bacteria that will increase annual algal biomass yields above the 2015 SOT baseline. The team plans to improve the protective effect of probiotic bacteria by demonstrating probiotic application regimes that significantly increase the magnitude and duration of the probiotic protective effect by 25% each, above the current baseline, and significantly decrease *in situ* algal carbon loss compared to untreated, ultimately contributing to improved algal cultivation yields. The approach involves studying single cultivation relationships between algae and bacteria and identifying microbial communities that enhance algal cultures and scaling these up in a stepwise fashion. In the new AOP, the team will take an approach to identify protective genes because they hypothesize that violacein is the compound produced by bacteria that infers protection. The approach is deemed reasonable. Progress in the previous AOP is deemed reasonable. Success in developing probiotic mesocosmsmecocosms for algal cultures could potentially enhance robustness of cultures and overall productivity, helping to meet productivity goals for the BETO program.
- The team should provide a vision on the use of the identification of the gene(s) responsible for violacein production.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

INTEGRATED PEST MANAGEMENT FOR EARLY DETECTION ALGAL CROP PRODUCTION

University of California, San Diego

PROJECT DESCRIPTION

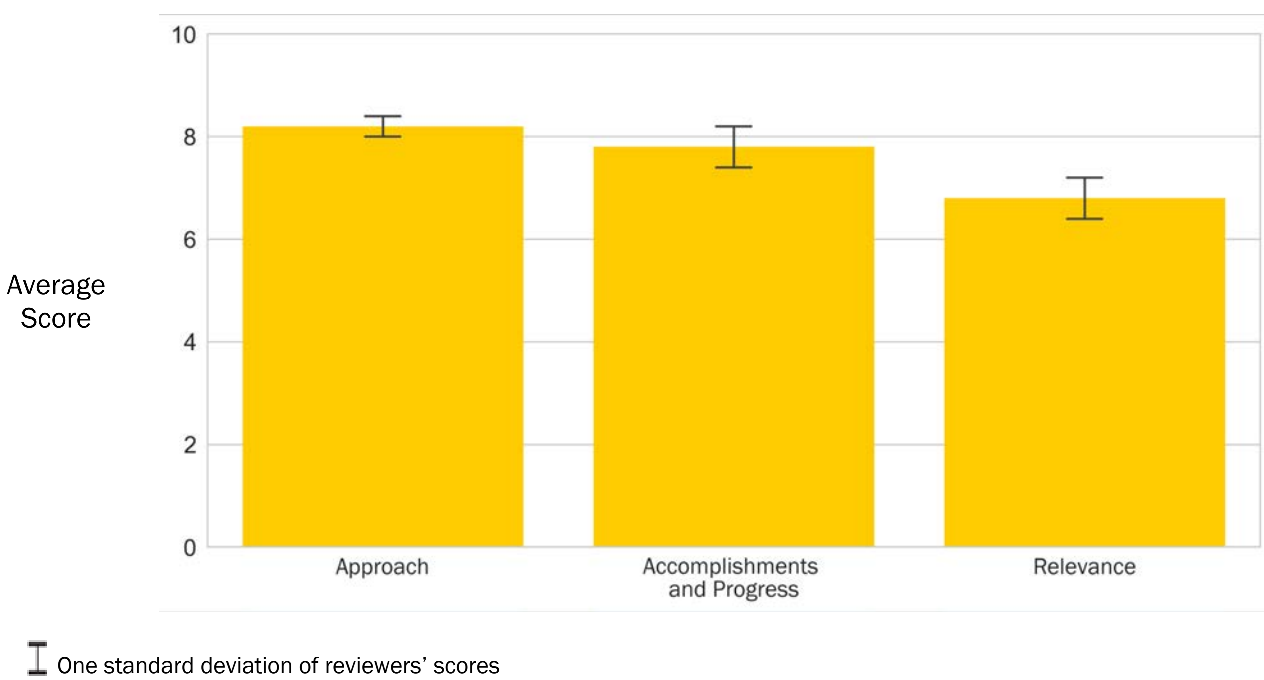
WBS:	1.3.2.310
CID:	EE0007094
Principal Investigator:	Dr. Robert Pomeroy
Period of Performance:	10/1/2015–3/31/2019
Total DOE Funding:	$820,327
Project Status:	Sunsetting

Contamination of industrial-scale growth systems by pathogens, predators, and nonproductive contaminating species continues to be a major obstacle to the robust and economically sustainable production of algal biomass and bioproducts. Appropriate management of these pests requires a sensitive and continuous monitoring system that can detect and identify contaminants and competitors as early as possible. Compared to current detection methodologies, such as quantitative polymerase chain reaction (PCR) or FlowCam monitoring systems, a mass spectrometry-based detection system is orders of magnitude more sensitive and can be readily automated for continuous monitoring of multiple production ponds.

We developed a chemical ionization mass spectrometry-based detection system capable of real-time monitoring of volatile compound abundances in the air over an algal culture. Using this system, we examined the headspace over healthy algal cultures throughout multiple growth phases, under abiotic stresses, and through culture crashes resulting from infecting the cultures with predators. The resulting data allowed us to confirm previously characterized molecular signatures derived from breakdown pathways occurring in the culture liquid. These experiments allowed us to expand our catalog of molecules that indicate the health of the algae or contamination as well as to determine thresholds for detection of contaminants and the kinetics of

Weighted Project Score: 7.7

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



culture crashes post-infection. Altogether, this research is rapidly advancing the development of a field-deployable instrument for monitoring the contamination in algal cultures.

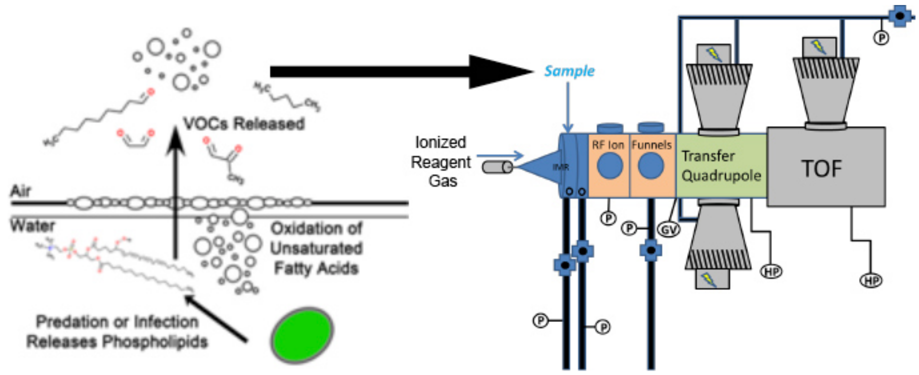


Photo courtesy of University of California, San Diego

OVERALL IMPRESSIONS

- Tools for integrated pest management are critical for large-scale stable outdoor production, and the relevance to the BETO mission, MYP goals, and the algae industry was clear. The team discussed a suite of promising results that aligned with originally planned objectives. The team is encouraged to think about how lower cost detectors might be able to build on these accomplishments and increase the potential for field deployment.

- This project demonstrates innovative use of already established instrumentation for early detection of contamination. This process, although interesting, is not viable at the production scale but could be used by other research groups that are working on contamination issues of outdoor systems.

- Significant expertise and progress were demonstrated evaluating samples and developing reliable analytic methodologies to predict pond crashes. Future work is required to further fine-tune a portable prototype.

- Crop protection at the commercial production scale will continue to be one of the most important areas for R&D to achieve reliable, high-quality algal biomass production. This project focused on very early identification of pest presence, which has very high potential, allowing algae farmers to act before the crop is lost. The team produced exciting results demonstrating the ability to detect pest pressure well before the health of the culture declines. The challenge in this system is the likelihood of success in scaling. There is a great opportunity to continue to use this method in laboratory work supporting the development of cultivation practices that mitigate biotic pressure in the algal field.

- The objective of this effort was to develop simple, automated, affordable, and robust technologies for the early detection and identification of pathogens, predators, and nonproductive competitors in algal cultures. The team used two major technologies in their approach: mass spectrometry and quantitative PCR with high-resolution melt analysis. This is a small project that is ending. To meet the goal of the project, the team developed mass spectrometric methods to evaluate possible signature compounds indicative of an imminent crash caused by a pathogen. They developed approaches to monitor cultures with molecular tools. The approach is deemed innovative and well implemented.

- Several weaknesses were noted associated with the implementation of the technology in the field.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their comments. In our detailed responses to scaling, costs, and alternative devices, we provide a detailed argument for continuing to develop this technology toward field deployment.
- We thank the reviewer for their acknowledgement of our project's successes.

ALGAE PRODUCTION CO_2 ABSORBER WITH IMMOBILIZED CARBONIC ANHYDRASE

Global Algae Innovations, Inc.

PROJECT DESCRIPTION

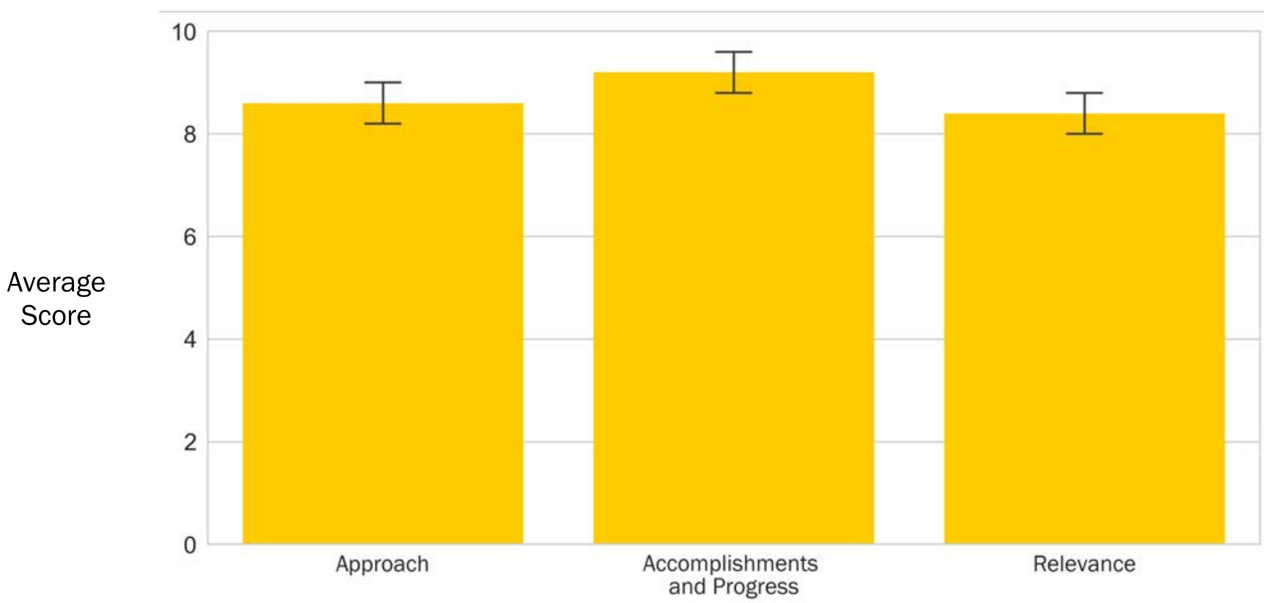
WBS:	1.3.2.320
CID:	EE0007092
Principal Investigator:	Dr. David Hazlebeck
Period of Performance:	10/1/2015–12/31/2019
Total DOE Funding:	$998,962
Project Status:	Sunsetting

GAI is a leader in low-cost algae production technologies. A suite of advances in open pond algal growth is being developed and demonstrated to achieve commercially viable production of oil and high-protein meal. An essential part of GAI's algae production method is the efficient and economic transfer buffering storage distribution of CO_2 from power plant flue gas to the actively growing algae, ensuring an ample supply of CO_2 for photosynthesis at all times.

GAI is partnering with TSD to apply immobilized carbonic anhydrase and alternative absorber designs to improve the capture efficiency and reduce the cost of CO_2 management in algae production. Additionally, the outdoor, open raceway cultivation methods will be developed to improve CO_2 use efficiency. The capture and use improvements will be demonstrated in an integrated outdoor raceway testing with flue gas.

Weighted Project Score: 8.8

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



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OVERALL IMPRESSIONS

- This project focused on improved CO_2 capture and delivery using an industrial flue gas source and demonstrated performance in outdoor ponds. The relevance to the BETO mission, MYP goals, and the algal industry were clearly described. Project objectives were completed and TEAs were discussed and used to prioritize efforts. The project was well managed, and it appears that the technology could have wide applicability for a variety of flue gas sources.
- This is an innovative and detailed system for sites that will colocate with a power plant and have access to their flue gas.
- This project showed success in decreasing the cost of CO_2 captured; most advantages are attributed to alternative design, not the absorber material.
- The project team delivered on goals on time or early across all facets of the project objectives. An integrated system has been developed to significantly improve carbon use efficiency in a large-scale algal production facility. The improvement of CO_2 delivery and use is critical to achieving economic feasibility in algal production and directly addresses MYP goals. The cultivation system and design are directly linked to the methods employed in this process and will need to be considered holistically.
- The goal of the project was to use carbonic anhydrase and/or other absorbers to increase CO_2 capture use efficiency from flue gases. The team, including all industrial partners, operated the GAI facility in Hawaii near a power plant that provided flue gas, some of which can be used for algal cultivation. The approach taken was to capture CO_2 from the flue gas using an absorber, converting the CO_2 to a $NaHCO_3$ media that could be fed directly to a pond. The team faced major challenges in the complexity of the system and the efficiency and lifetime of carbonic anhydrase. The approach the team took was successful in implementing this CO_2 concentrating technology. The project defined the baseline and used it to determine success. The project set target goals, which have been met, showing high carbon capture and use efficiencies. The result is that the system indicates an ability to reduce the CO_2 supply cost significantly. The team showed 83% capture efficiency with more than 95% use efficiency for the algae. The team moved to a modified packed column for CO_2 capture that showed better capture efficiency and stability than carbonic anhydrase. The team plans to move into direct carbon capture from air. The organization of the project is deemed to be part of the success because the team was able to move the technology forward by redesigning the CO_2 absorber and increasing the overall performance.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

ATMOSPHERIC CO_2 CAPTURE AND MEMBRANE DELIVERY

Arizona State University

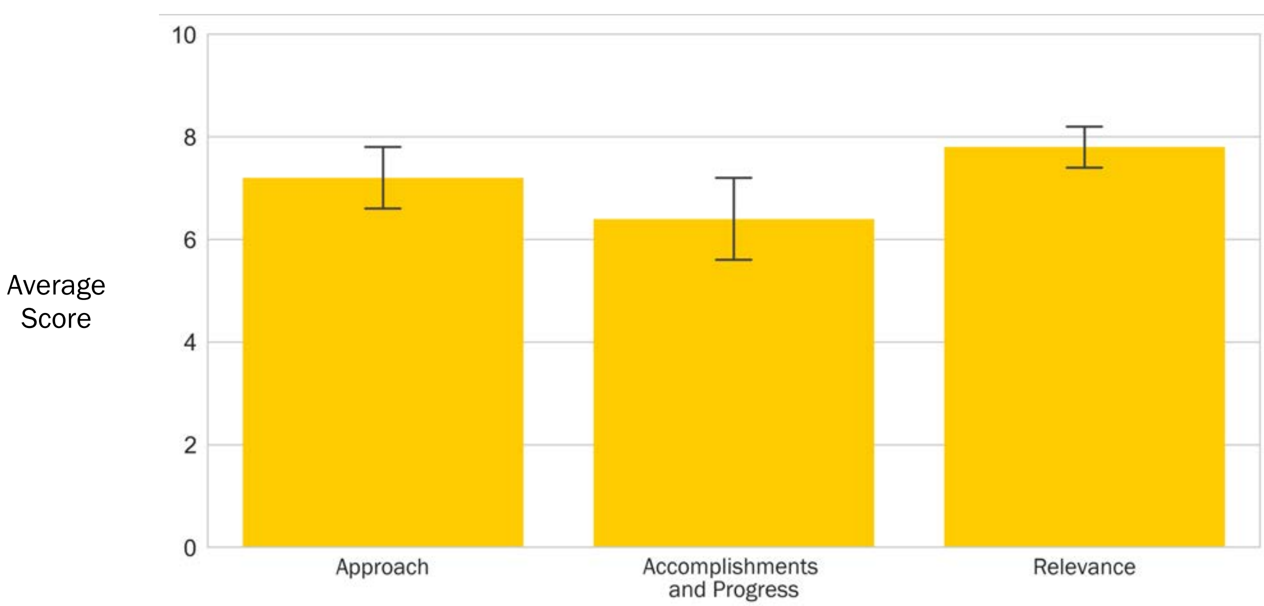
PROJECT DESCRIPTION

WBS:	1.3.2.330
CID:	EE0007093
Principal Investigator:	Dr. Bruce Rittmann
Period of Performance:	10/1/2015–9/30/2018
Total DOE Funding:	$1,000,000
Project Status:	Sunsetting

A major bottleneck for growing microalgae as a sustainable alternative to fossil carbon in economically producing fuels and chemical products is the cost of delivering CO_2 in enough concentrations for it not to limit growth. This project sought to develop and integrate two innovative technologies for capturing and concentrating CO_2 from air and delivering it to microalgae with high efficiency into an Atmospheric CO_2 Enrichment and Delivery (ACED) system. The CO_2 capture technology is based on moisture swing sorption, where specialized resin materials selectively capture CO_2 when dry and release it when wet into a confined space where the concentration can be increased up to 500-fold. The CO_2-delivery technology is based on membrane carbonation, which uses hollow fiber membranes that allow CO_2 to diffuse into the algae-containing liquid without forming bubbles, achieving nearly 100% delivery efficiency.

Weighted Project Score: 7.0

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



One standard deviation of reviewers' scores

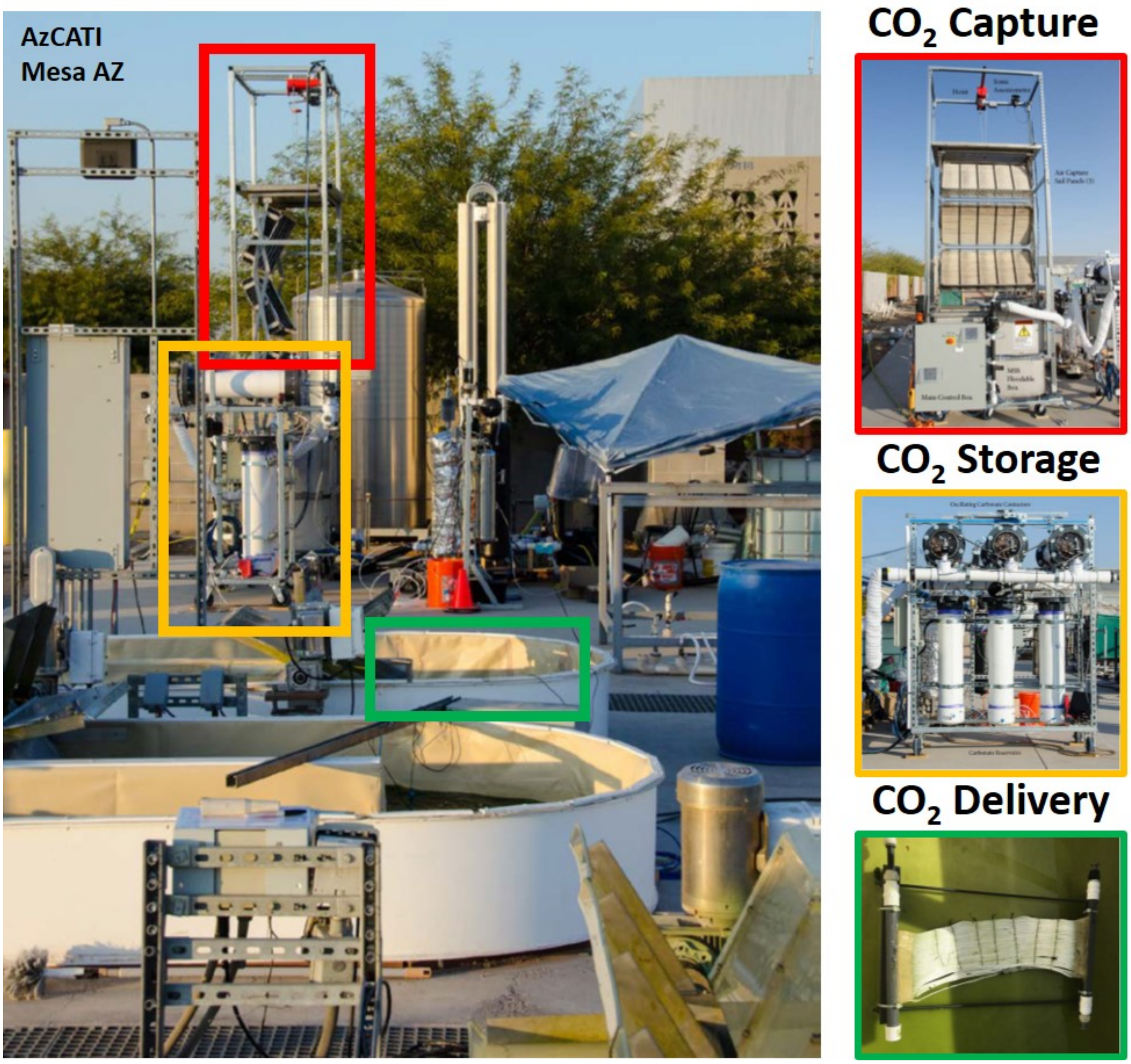


Photo courtesy of Arizona State University

OVERALL IMPRESSIONS

- The purpose of this project was to understand and develop innovative ways to capture and deliver atmospheric CO_2 to pond culture. If successful, the study would have clear benefits to the algal industry and would align with BETO priorities and MYP goals. Unfortunately, the CO_2 capture portion of the study encountered hardware, software, and integration hurdles that were not able to be mitigated, combined with economics that did not appear to advance the SOT. Membranes used for CO_2 delivery appeared more promising, and the team indicated that the membranes met several performance targets during the study period.

- This is a novel approach but not a robust deployment of technology. It is very capital intensive, and it seems that the maintenance cost is very high as well.

- This project uses novel membrane technology previously developed for wastewater treatment and adapts it to capture and concentrate CO_2 from ambient air. The team successfully demonstrated the concept at commercially relevant scales, but further fine-tuning required for seamless integrated operations.
- The ACED projects aims to combine two independent systems to significantly reduce the cost of CO_2 delivery for algal biomass production. The atmospheric capture component of the project experienced significant challenges and will require additional effort to be evaluated. The membrane delivery system has shown promise in significantly improving the carbon use efficiency compared to the currently used system of CO_2 sparging. Because this project has closed, the evaluation of the membrane delivery at scale through modeling and large-scale evaluation should be considered for future work.
- The goal of this project was to design, build, and demonstrate a system to capture and concentrate CO_2 from ambient air and deliver the CO_2 to microalgae. The ACED concept uses anionic exchange resin sheets to capture CO_2 when dry and release when wet. The CO_2 is transferred to sodium carbonate/bicarbonate brines to buffer capture and demand rates; and it is thermally extracted and pressurized. This allows for nearly 100% CO_2 to be delivered on demand to microalgae using membrane fibers. The technology is very innovative. The team was able to develop mitigating approaches for most of the challenges faced in the development and deployment of the technology.
- A few minor weaknesses were noted related to the complexity of the technology and need for some simplifications.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for the feedback.
- Although hardware and software issues limited the operation time of the CO_2 capture and storage system during the project, our project advanced the SOT for sustainably sourcing concentrated CO_2 for cultivating microalgae. Current sources include bottled and industrial waste CO_2 that are not sustainable for large-volume fuel applications. We developed and demonstrated a first-of-its-kind system for direct CO_2 capture from ambient air (410 ppm), a novel energy-efficient gas-liquid contactor for transferring low-concentration CO_2 into a liquid brine for storage, and a thermal extraction system for releasing the CO_2 on demand from storage with > 90% CO_2 on a laboratory scale and > 70% CO_2 in a much larger outdoor system. The system was operated for several extended periods within a harsh desert environment with +40°C temperatures, sustained +10 ultraviolet index, periodic intense +40-mph wind, dust, and rainstorms. The accompanying graphic shows the field implantation of the entire system.
- Detailed TEA, made possible through this award, identified the most critical parameters for optimizing the system, including a path to drastically improve the economics of CO_2 capture and storage using continuous active CO_2 transport (not batch cycles) through membranes with much lower capital and maintenance costs; this is being pursued through a follow-on award, DE-FOA-0001858 (Advanced Research Projects Agency-Energy). This much simpler design should alleviate some of the hardware and software challenges encountered in the BETO project.
- The TEA also highlighted the value of delivering captured CO_2 with near 100% efficiency, which was shown effectively using the membrane delivery technology during multiple month-long cultivation trials without performance degradation. This promising technology will be further evaluated for delivering industrial waste CO_2 as a part of award DE-FOA-0001908.

SOFAST: STREAMLINED OPTIMIZATION OF FILAMENTOUS ARTHROSPIRA/SPIRULINA TRAITS

Lumen Bioscience, Inc.

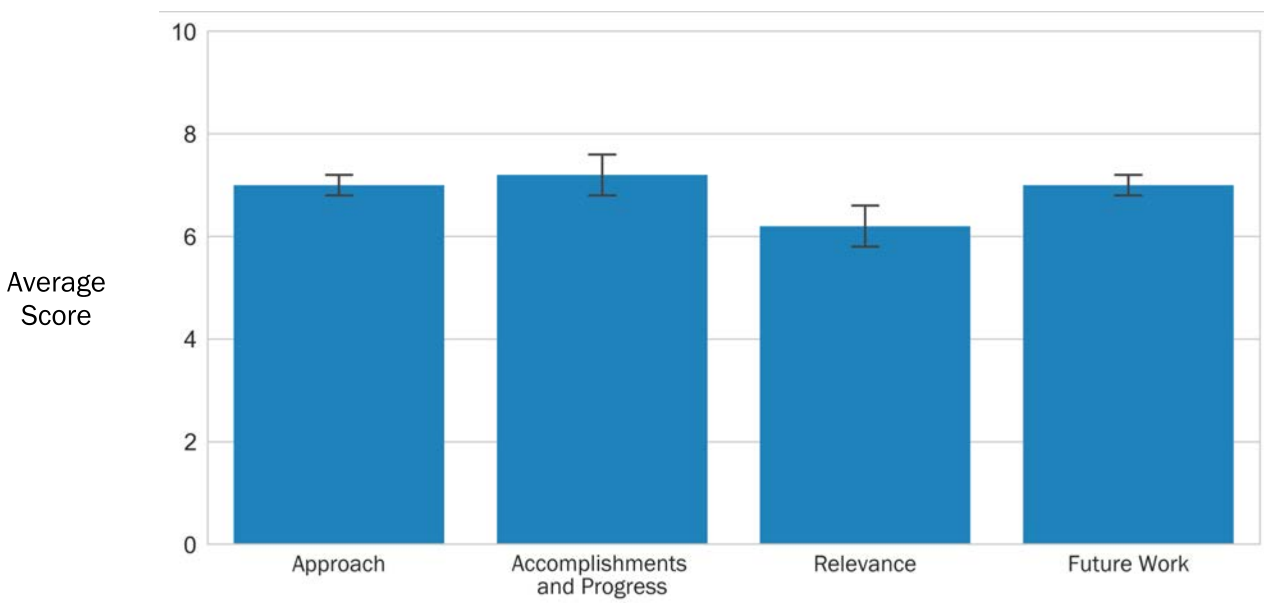
PROJECT DESCRIPTION

WBS:	1.3.2.601
CID:	EE0008120
Principal Investigator:	Dr. Damian Carrieri
Period of Performance:	10/1/2017–9/30/2020
Total DOE Funding:	$1,851,376
Project Status:	Ongoing

To enable the development of algae-based biofuels and bioproducts, this project is making fundamental improvements in the major areas of strain development, specifically tailored to an already highly productive and commercially deployed species *Arthrospira* (also commonly known as *Spirulina*), and aimed at achieving a doubling of the SOT of algal biomass productivity. *Arthrospira* species are attractive for commercial biofuel production because of their ability to grow in highly alkaline seawater as well as their relative ease of harvest; however, *Arthrospira* also suffer from notable deficiencies: the cells are sensitive to photodamage in bright sunlight, impeding growth, and they contain substantially less high-energy lipid than eukaryotic algae. This project leverages Lumen Bioscience's proprietary method of genetically engineering *Arthrospira* to build strains that are both more photodamage resistant and accumulate more lipids than unmodified *Arthrospira*. We have assembled functionally rich combinatorial overexpression libraries and used competitive selection coupled with pioneering methods in metabolic profiling at NREL and whole-genome sequencing to discover expression element combinations that have the best growth rates. We will continue these screens and characterizations and construct new strains using winning constructs. These newly made strains will be tested under indoor and outdoor growth conditions to demonstrate improved biomass productivity and lipid accumulation relative to their wild-type parent strains.

Weighted Project Score: 6.8

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

OVERALL IMPRESSIONS

- This project leverages previously developed high-throughput strain characterization and selection techniques to improve biomass productivity of *Spirulina*. The team demonstrated progress on most milestones. Unfortunately, however, the project pivoted away from outdoor raceway trials to indoor simulated trials.

- This project appears to have strong technical and management approaches that are appropriate for the project objectives. The team was forthcoming about project challenges, executed mitigation plans, and had clear plans to get the project back on schedule. It is unclear if the current plans for outdoor testing would contribute to advancements in MYP goals.

- This is a novel approach to increase biomass and biofuel productivity on a strain that is not commonly used for lipid production; however, there was no true explanation as to how the team is going to validate at scale their improvements.

- The Streamlined Optimization of Filamentous *Arthrospira/Spirulina* Traits (SOFAST) project addresses both the development of tools for strain improvement and characterization as well as the use of those tools to improve an industrially relevant strain. Focusing on *Spirulina* as a target is logical and relevant given the long history of outdoor cultivation at scale. Key steps have been made toward the project goals around tool development. The use of competition experiments to identify top-performing strains under selective pressure is clever and likely to result in a highly efficient methodology. It is unfortunate that the validation of the strain performance will not be conducted in outdoor trials.

- The goal of this project is to produce engineered *Arthrospira platensis* strains with improved photosynthetic and cold-tolerance traits and increased lipid content. The team is taking an approach to transform libraries with various traits and compete these. The approach is innovative and potentially very high-throughput. The team would then sequence to identify constructs that imparted best fitness or most wax esters, then rebuild these constructs into *A. platensis* to produce optimized strains. Stacking of growth, tolerance, and wax production traits would then take place. The approach is very focused and sound. The competition experiments are ongoing and showing successful and unsuccessful genes that might confer appropriate stress resistance. Metabolic profiling using mass spectrometry near-infrared spectroscopy show ability to understand lipid and protein composition. The high-resolution mass spectrometry imaging capability for phenotyping and selection is deemed to be very innovative within this concept. Both indoor and outdoor cultivation studies are underway. The project is working on developing a robust strain of *A. platensis* that is resistant to predation and other stresses and can be easily harvested. If the productivity goals are achieved, the work could approach the FY 2022 productivity targets. The work is also developing new genetic tools to help increase robustness and fitness of strains. Therefore, the project is deemed to be well aligned with the BETO goals.

- Some weaknesses were noted by this reviewer in the development of wax ester production and association of this effort with overall goals of the program.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- The project pivoted from outdoor raceway trials to outdoor flat-panel trials. Indoor simulations were always planned and remain in the project goals. We regret that this was unclear to some reviewers.

- Outdoor validation trials will indeed be completed.

PREVENTION OF LOW-PRODUCTIVITY PERIODS IN LARGE-SCALE MICROALGAE CULTIVATION

Global Algae Innovations, Inc.

PROJECT DESCRIPTION

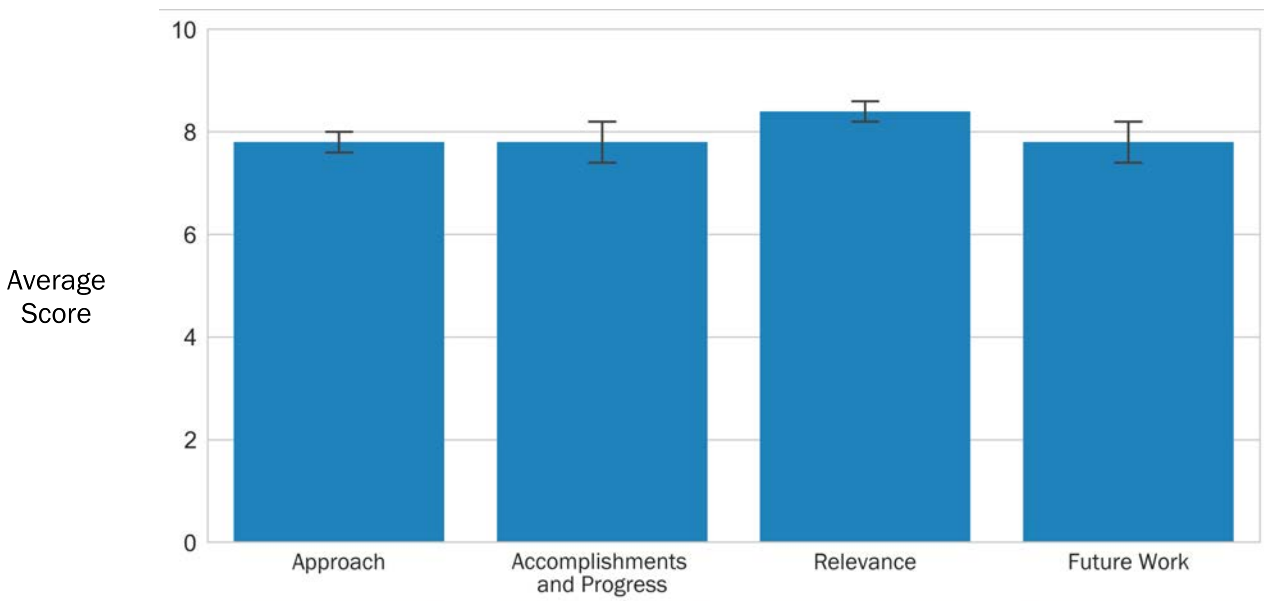
WBS:	1.3.2.630
CID:	EE0008121
Principal Investigator:	Dr. Aga Pinowska
Period of Performance:	10/1/2017–6/30/2020
Total DOE Funding:	$3,000,000
Project Status:	Ongoing

GAI is a leader in low-cost algae cultivation and has developed a high-productivity, crash-resistant cultivation system; however, even with this advanced cultivation system, periods of unexplained low productivity are observed. We will investigate the causes of low productivity and develop strategies to control microbial ecology and dissolved organic matter (DOM) released by microbes. These strategies will include tools to monitor the system ecology, methods to avert low-productivity conditions from microbes or DOM, and methods to promote microbes that improve productivity. We will collaborate with SNL to deploy a low-cost SpinDX detection system to monitor the cultivation pond ecology. The microbial and viral ecology of low- and high-productivity conditions will be analyzed and cataloged. Then target microorganisms will be cultivated to test their impacts as well as probiotic, antibiotic, and DOM remediation strategies.

The project team includes SNL, the University of California at San Diego Scripps Institution of Oceanography, and J. Craig Venter Institute.

Weighted Project Score: 8.0

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

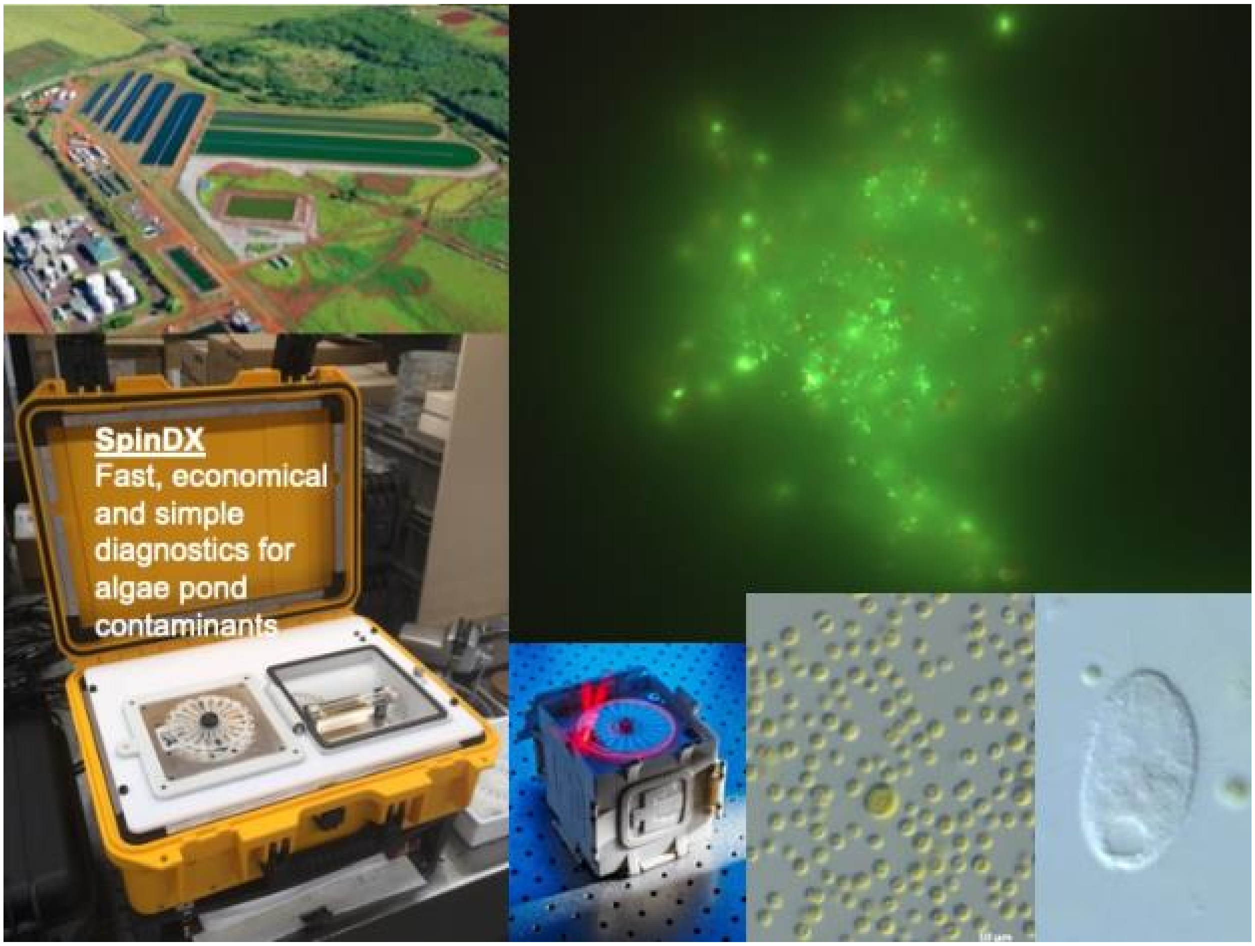


Photo courtesy of Global Algae Innovations, Inc.

OVERALL IMPRESSIONS

- This project is developing an easy-to-use, rapid analytic instrument to ultimately prevent pond crashes and unexplained periods of low productivity.
- This project addresses an important challenge in large-scale production and has designed a well-managed study to try to understand periods of abnormally low productivity. The project includes basic research and tool development. The team has a sound technical and management approach and appears to be on track in accomplishing its initial objectives. Future work was described in detail and appears consistent with original plans.
- This is one of several projects using microbiota to increase biomass production. This project is strongly coupled to this specific site but could help establish a baseline for real-time large-scale contaminant occurrence. These data could be used by all other sites and start building solutions to mitigate these contamination occurrences.
- This project is a great example of the potential value of collaborations between academic laboratories and industrial partners. The team used large-scale cultivation systems to generate large, relevant data sets aimed toward the reduction of crop loss in the field. The microbial population data collected to date and demonstration of the utility of the Spin DX tool are key accomplishments. The project will benefit from

continued effort on management and integration of the microscopy, sequencing, and productivity data to ensure a smooth handoff and broad usability.

- The project's goal is to reduce periods of unexplained low pond productivity by identifying and controlling microbiota cultivated with target algae. The partnership includes a strong team from industry, academia, and national laboratories. The team aims to build a broad genomic database of key organisms associated with the water ecology of algal cultures and will identify key organisms and determine their effects on productivity along with treatment methods when necessary. This is an area that is not well understood in the literature, and this work has great potential for impact. The team will also use a SpinDX instrument to identify and quantify organisms in the field. The instrument is innovative and carries some risks in implementation, but there is little reason it cannot be successful in the field as long as samples are properly pretreated prior to analysis. Then the team will implement a broad-spectrum treatment of culture to reduce organic contaminants in cultures. The approach is deemed reasonable, and major challenges have been identified with appropriate mitigating steps. The project's progress is deemed reasonable at this stage. The project addresses key areas in understanding pond ecology and effects on productivity, which can have major contributions to sustainable production and meeting the goals of the BETO program in the development of highly productive strains and cultures that are resilient and robust. Future work is deemed reasonable and should lead to high-impact strategies for cultivation controls.

- A minor weakness was noted in describing/envisioning strategies for the treatment of pathogenic organisms.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their thoughtful comments and suggestions. We agree with the reviewers that the biggest challenge in using SpinDX for the identification and quantification of organisms contaminating algal ponds is sample preparation and pretreatment. We are focusing on developing the right approach for extraction of DNA and RNA for specific microorganism identification. We know that approaches are different depending on the target organism that will be detected: eukaryotes, bacteria, or viruses. Another reviewer concern was on developing approaches to treat pathogenic organisms. We already have a few treatments developed to mitigate problems caused by bacteria and ciliates. We have very little understanding on what organisms they target and how specific they are. This will be addressed in the next phase of this project. We also expect to develop new specific treatments when SpinDX is available and as sequencing data continues to be processed. At this point, one new treatment approach to mitigate bacteria problems was already proposed based on the results of first round of sampling and sequencing. We have many potential treatments ready to test.

HIGH-THROUGHPUT DIRECTED EVOLUTION OF MARINE MICROALGAE AND PHOTOTROPHIC CONSORTIA FOR IMPROVED BIOMASS YIELDS

The Colorado School of Mines

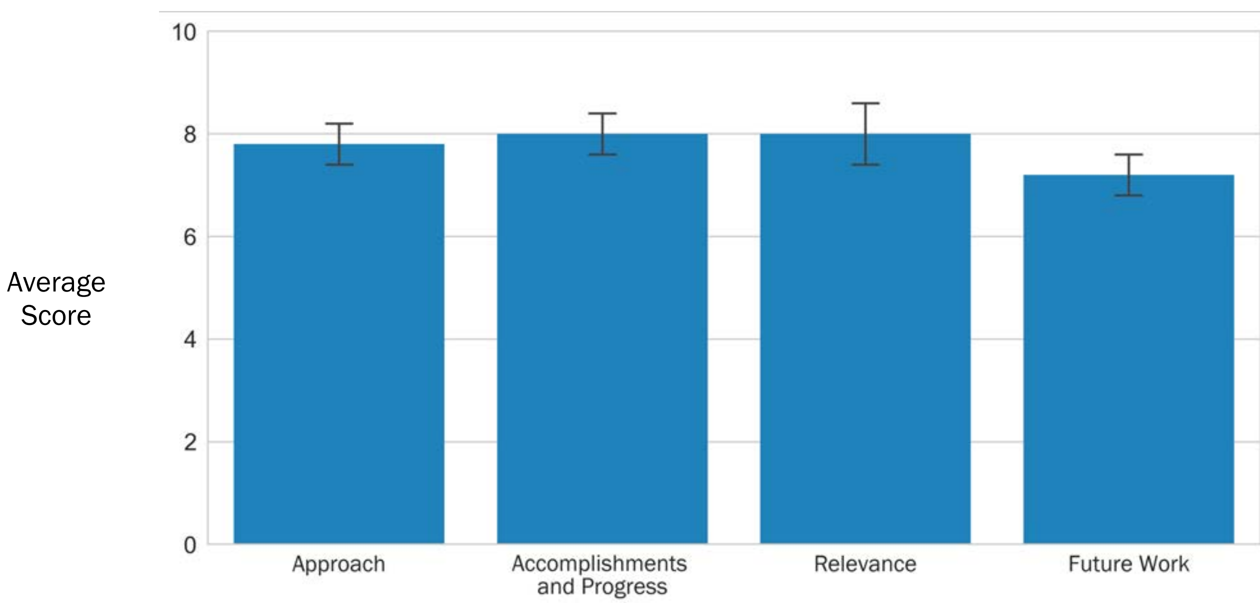
PROJECT DESCRIPTION

WBS:	1.3.2.640
CID:	EE0008245
Principal Investigator:	Dr. Matthew Posewitz
Period of Performance:	9/30/2017–9/30/2020
Total DOE Funding:	$2,459,178
Project Status:	Ongoing

Directed evolution and targeted trait selection remain among the most powerful and successful tools available for attaining the process improvements necessary to enable commercial success in many biotechnology sectors. To develop robust and efficient algal biotechnological chassis, we propose using “solar-simulating” bioreactors to select strains evolved for improved growth rates and improved tolerances to high levels of light, pH, and oxidative stress; and to identify photoautotrophic microbial consortia that are able to improve algal biomass yields. We will use deep sequencing (genome, transcriptome) in conjunction with comparative genomics approaches to probe organismal and consortia evolution and identify genomic alterations that enable increased biomass yields and adaptations to growth in outdoor open pond systems. Outdoor validation will be done in collaboration with GAI using their advanced open pond cultivation system that is shown to prevent contamination and attain a threefold productivity increase relative to standard raceways. Organism foci include a diatom routinely grown at GAI, rapidly growing cyanobacteria, as well as consortia of these organisms. We anticipate being able to exceed the targeted productivities for the spring and annual growth cycles. Despite the widespread use and proven track record of directed evolution in other bio-based applications, these tools have seen only limited use in the algal biofuel sector. This is primarily because of the cost, the engineering and human fiscal resource constraints imposed by these techniques, especially when attempting to simulate the extreme photon flux and sinusoidal nature of solar light and environmental temperature fluctuations. Additionally, currently available

Weighted Project Score: 7.8

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

commercial systems do not mimic outdoor growth well, have poor lighting systems that lack feedback measurements and adjustments, are not particularly robust, and/or are prohibitively expensive. We are using low-cost, modular, and scalable growth stations that mimic algal pond parameters of importance for outdoor growth. Importantly, these easily reach the highest photon intensities of sunlight and are capable of programmable temperature swings. We are using these "solar-simulating" bioreactors to select strains evolved for improved growth rates and improved tolerances to high levels of light, pH, and oxidative stress; and to identify photoautotrophic microbial consortia that can improve algal biomass yields. We will use deep-sequencing metagenomics to probe organismal and consortia evolution and probe alterations that enable increased biomass yields. Data from experimental work will be used to inform sustainability assessment work with results from modeling work used to understand the impacts of the research. Currently, we have finished determining pH, oxygen, and temperature optima, and we are now leveraging this information to improve tolerances.

Photo courtesy of The Colorado School of Mines

OVERALL IMPRESSIONS

- This project attempts to apply directed evolution (a well-known and used technique in adjacent fields) to increase productivity of current strains further. The team has made significant progress to date.
- This competitive project appears to be managed well and is directly relevant to MYP goals and the BETO mission. The project is on track to meet its objectives, and the team included quantitative goals that it still believes are achievable.

- The “directed evolution” path is not presented in a step-by-step process, which makes it hard to understand how the target strain was “adapted.” The presentation does not highlight how the phototrophic consortia will be maintained throughout the different laboratories and testing.

- The project team is strong because it includes academic and industry partners with a broad set of expertise. Several students were employed at the Colorado School of Mines to build the reactors, bringing creativity and student engagement to the project. Focusing on an existing industrial production strain, the team has developed a unique laboratory system to select for strain modifications under relevant environmental stresses. The method of strain improvement also enables rapid deployment of field-testing, avoiding any restrictions associated with strain importation or release of genetically modified strains, which could be advantageous. The field demonstration will be a critical step to evaluate the stability of the evolved strain in production and ensure that there are no unintended negative impacts on biomass quality.

- The team hopes to use directed evolution approaches as a tool to improve photoautotrophic microorganism biomass yields and select strains/consortia that attain 24 g/m^2/day in the spring growing season in Kauai. The approach is to evolve a specific strain to have good growth characteristics under the typical high-O_2, low-pH, and high-temperature conditions observed during culturing. The new evolved strains will be grown in environmental simulation bioreactors. They will also assemble and evaluate the productivities of consortia. Evolved strains will be cultivated outdoors, and TEA/LCA modeling will be performed. The approach is deemed to be appropriate and sound. The team represents a strong partnership between academia, industry, and national laboratories. The project is still starting and exercising major capabilities in growth characterization. The projects goals to reach productivities of 24 g/m^2/day for the spring growing season in Hawaii would help the BETO program reach and possibly surpass its targets. Further, because the algal species selected are being subjected to growth at extreme salt, temperature, and pH environments, these cultures would likely be very resistant to predation, thus addressing goals in fitness and robustness of cultures. The team’s efforts for future work are in-line with the project goals.

- No weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers and DOE BETO management for their insightful and constructive comments on our Productivity Enhanced Algae and Toolkits research project. We are encouraged that the review team acknowledges the power and potential usefulness of directed evolution strategies. We concur that these techniques have the potential to help realize MYP goals. We also agree with their assessment that maintenance of evolved strains might be challenging, which is why we established cryopreservation techniques and intend on maintaining selective pressures. We are currently evaluating the stability of our first-generation cell lines at multiple laboratories, and we are attaining the necessary regulatory approval to transfer strains to the GAI site. The evolutions are being conducted one parameter at a time and with all parameters applied simultaneously. Pressures between the two laboratories are similar and designed to mimic outdoor data from GAI. As noted, starting with an industrial strain in hand is encouraging, and careful mapping of pH, temperature, and O_2 optima is allowing quantitative comparisons between baseline and optimized strains. As suggested by the reviewers, if additional throughput is necessary, we could build and deploy smaller volume, higher throughput reactors. We also concur that important progress has been made since the initiation of the project and that the development of a more defined timeline of goals is appropriate.

SUCCESS THROUGH SYNERGY: INCREASING CULTIVATION YIELD AND STABILITY WITH RATIONALLY DESIGNED CONSORTIA

New Mexico Consortium, Inc.

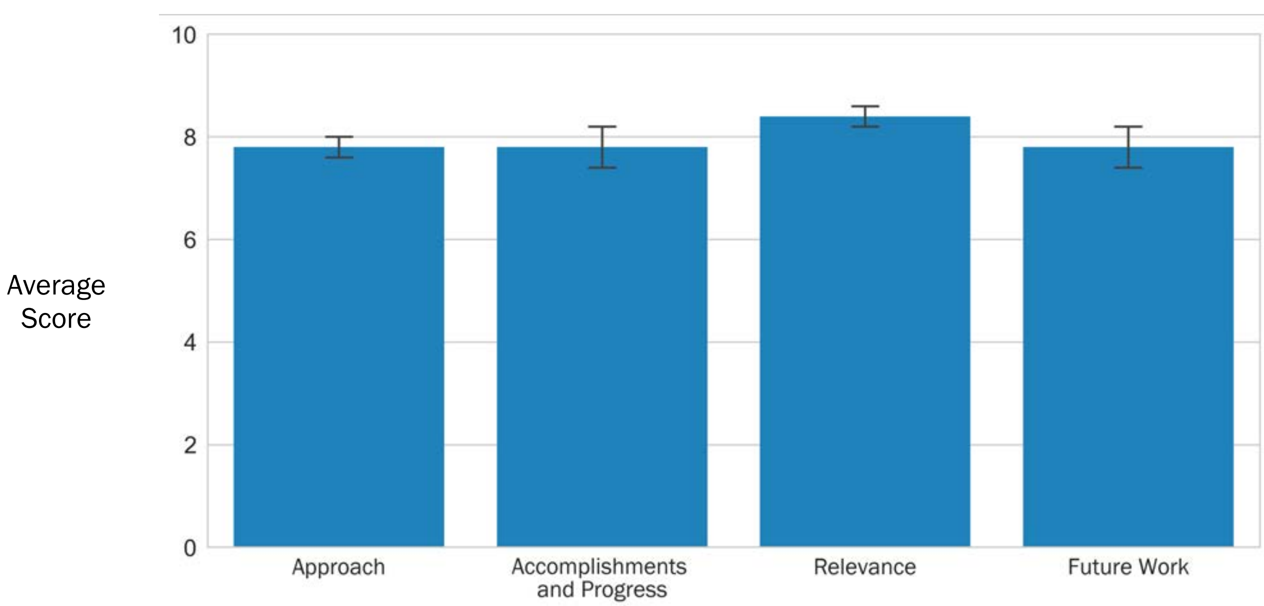
PROJECT DESCRIPTION

WBS:	1.3.2.641
CID:	EE0008122
Principal Investigator:	Dr. Alina Corcoran
Period of Performance:	10/1/2017–9/30/2020
Total DOE Funding:	$2,486,149
Project Status:	Ongoing

The commercialization of algal biofuels will not be realized until major technical and economic barriers are overcome. As outlined in BETO's update to the *National Algal Biofuels Technology Roadmap*, there is a need to improve culture productivity and stability as well as to better understand these metrics at commercial scales. In this project, we address these gaps. Specifically, we aim to increase productivity of open, outdoor *Nannochloropsis* cultures from 7 $g/m^2/day$ to 14 $g/m^2/day$ (doubling of fall SOT value) through a stepwise process to ecologically engineer consortia consisting of (1) multiple *Nannochloropsis* species and strains, (2) growth-promoting bacteria and individual *Nannochloropsis* strains, and (3) complex communities with multiple *Nannochloropsis* strains and bacterial taxa. Previous research focused on consortia has resulted in successes and failures. We argue that the failure to produce more productive and stable cultures has stemmed from two pitfalls: experimental approaches that employed haphazard inclusion of species into polycultures and technical limitations that prohibited screening of high numbers of consortium members. Our project addresses these limitations through rational design and high-throughput bacterial screening. The project, which started in February 2018 with verification, is currently 30% complete and on track with respect to budget. To date, we have met six milestones and have work in progress for an upcoming milestone. In our presentation, we highlight some of our major accomplishments. First, to rationally design *Nannochloropsis* consortia, we analyzed an existing data set and generated additional data sets of strain tolerances to field medium, salinity,

Weighted Project Score: 8.1

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

temperature, and pests. From this analysis, we constructed nine consortia and are currently testing their performance compared to a baseline field strain through high-throughput screening and bioreactor trials. Data collected thus far show that certain consortia outperform the baseline strain under certain conditions. Second, we are overcoming the limitations of traditional bacterial screening by using a high-throughput tool (High-throughput Screening of Cell-to-cell Interactions [HiSCI]) to isolate and select specific bacterial partners that enhance the productivity and stability of cultures. To date, we have successfully modified the HiSCI system for *Nannochloropsis*, collected environmental samples for input material, and conducted three HiSCI runs. Three bacterial strains have been scaled from these efforts and are being tested in field-relevant conditions. We have also sequenced multiple *Nannochloropsis* strains, not only to develop and implement molecular tracking tools for this project but also to contribute to the repository of algal genomes for the scientific community. Finally, we discuss future work, including lab and field trials, as well as mitigation efforts to address potential challenges.

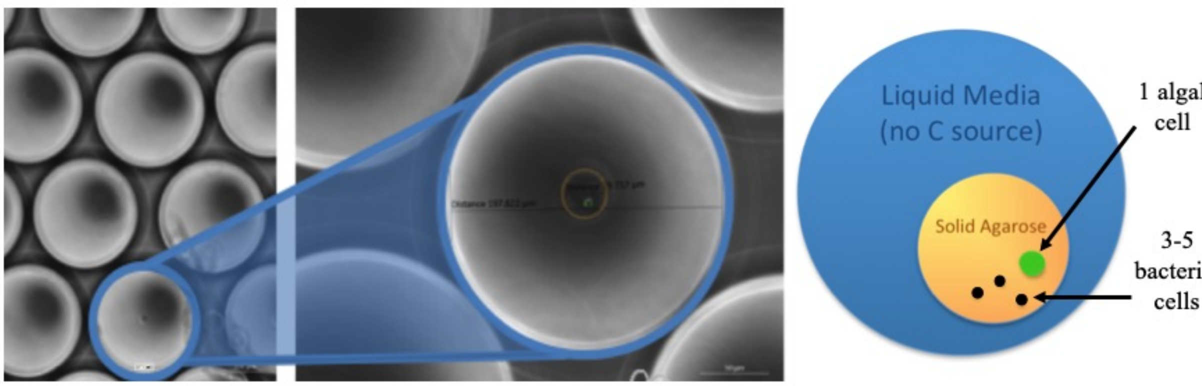


Photo courtesy of New Mexico Consortium, Inc.

OVERALL IMPRESSIONS

- The team is developing novel techniques to increase productivity and reduce crashes in open ponds. The work is significant and encouraging progress has been made to date.
- The team appears to have a strong technical approach for a project that is highly relevant to the BETO mission and MYP goals. Initial baseline accomplishments are on track, and strong project management will be required to complete an ambitious set of remaining objectives.
- The team is using a novel consortia-based polyculture approach to achieve higher production. This is a complex approach with high probability for false correlation.
- This approach to consortia development is based on targeted improvement to productivity while maintaining consistent biomass quality in-line with MYP targets. The use of strategic consortia designed to reduce productivity loss from abiotic and biotic stress has high potential. The inclusion of additional algal strains and sources of potentially beneficial bacteria should be considered for future work.
- The objective of this project is to rationally design intrageneric *Nannochloropsis* consortia and *Nannochloropsis*-bacteria consortia to increase productivity, stability, and yield of open, outdoor cultures, with the goal to reach a productivity target >14 g/m^2/day with consistent biomass composition. The team is developing tools to rationally design consortia of algae within the same genus but different phenotypes. To do this, a molecular tracking system to distinguish consortium members is being developed using the ccsA amplicon sequence. Consortia are integrated in a high-throughput screening system using agarose beads. The use of this tool is viewed as very innovative and a simple and fast approach to screening consortia of multiple compositions. The team has met all the milestones to date.

The ability to synthesize and control polycultures and microbial consortia could be an excellent approach to increase biomass productivity and crop resilience. This would help meet major BETO goals in these categories for the Productivity Enhanced Algae and Toolkits 2020 targets. The approach is seen as innovative with a direct impact potential on BETO program efforts. The team is on task on all milestones and performance.

- Only one minor weakness/concern is noted for this project, which is related to the high-throughput screening potential for biases in culturing diverse organisms.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We agree that our objectives are ambitious—and we believe they are attainable given our project management plan, which relies on traditional tools (e.g., statement of project objectives [SOPO], Gannt chart) as well as question-based and iterative/incremental approaches. The reviewers aptly asked that we consider (1) the inclusion of additional algal and bacterial strains, (2) potential biases in our high-throughput screening approach for bacterial identification and isolation, and (3) false correlations (in productivity improvements, we suspect) stemming from complexities in our approach.

- Although proposed in our SOPO as only a mitigation strategy, we are currently testing bacterial isolates that have been identified in the literature and/or by collaborators as having growth-promoting characteristics. We are also using non-*Nannochloropsis* algal taxa for the construction of intergeneric consortia; this activity was proposed during verification as a stretch goal of the project. With respect to biases, we agree with the reviewers that bacterial growth in the gel microdroplets (see image) might be different than growth in liquid cultures or raceway ponds. Yet, we also believe that our approach offers a chance of success that is worth this trade-off. Moreover, in the early stages of this project, we altered the scaling platform for captured bacteria from a chip-based platform to one in which microdroplets can grow with shaking in-field medium under fall cultivation conditions. In addition, built into our project plan is the isolation and scaling of captured bacteria for addition into liquid *Nannochloropsis* cultures. We are currently scaling one bacterium for algal-bacteria consortia testing within bioreactors to assess translatability to the field. With respect to the final criticism, we recognize that multiple ecological interactions can make interpretation of cause and effect difficult. Yet, we have a robust flask-to-field pipeline that should help minimize false correlations.

DEVELOPING ADVANCED GENETIC AND SYNTHETIC BIOLOGY TOOLS FOR IMPROVED ALGAE PRODUCTIVITY

University of California, San Diego

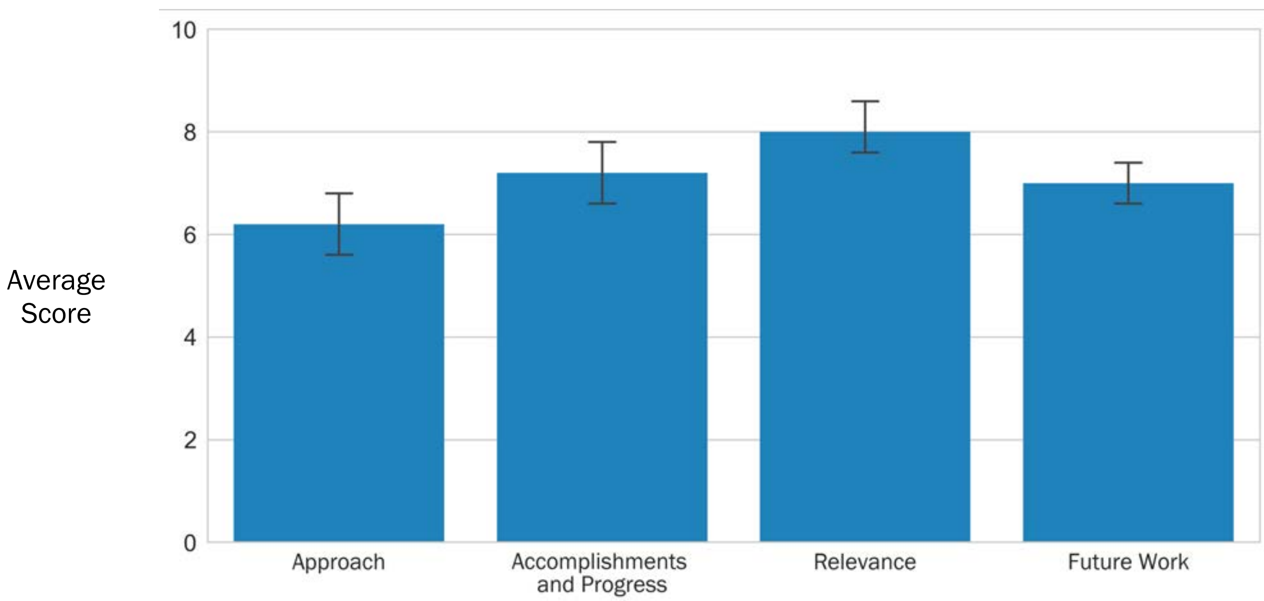
PROJECT DESCRIPTION

WBS:	1.3.2.650
CID:	EE0008246
Principal Investigator:	Dr. Stephen Mayfield
Period of Performance:	9/30/2017–9/30/2020
Total DOE Funding:	$3,000,000
Project Status:	Ongoing

Under this proposal, we will develop genetic tools, high-throughput screening methods, and breeding strategies for green algae and cyanobacteria commercial host strains. We will develop transformation strategies and vectors, synthetic promoters and regulatory elements, and improved methods for nuclear gene editing and transgene expression for engineering of any algae. We will demonstrate the metabolic engineering capabilities of these tools by engineering unique branched-chain wax esters into cyanobacteria and green algae for conversion into energy-dense jet fuels and renewable polymers. We will work with three key commercial partners—Triton Algae Innovations, Algenesis Materials, and GAI—to ensure that the tools and technologies developed are relevant to commercial algae production. For Triton, we will specifically develop robust nuclear genetic control systems that allow expression and secretion of recombinant proteins as high-value coproducts as well as develop breeding strategies to improve biomass productivity in their commercial strains. We will work with GAI to develop genetic tools and high-throughput technologies that will enable improved productivity in two of their commercial strains: a green alga and a cyanobacterium. We will develop these tools and technologies to be as universal as possible, allowing them to be adapted to any algae or cyanobacteria of commercial interest. Under previous support from DOE, we developed a suite of basic algae and cyanobacteria genetic tools that were made available to the entire algal community through the "Life Technologies" catalog, and we will continue to develop and distribute these new tools under a similar arrangement. As a proof of concept

Weighted Project Score: 7.1

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

under the Productivity Enhanced Algae and Toolkits challenge, we will demonstrate the utility of these developed tools by engineering and selecting commercial strains with improved biomass, protein, and lipid composition. We will take these strains into pilot-scale outdoor production at our University of California, San Diego algal pilot facility, and obtain a U.S. Environmental Protection Agency TERA approval for any testing of genetically modified algae in outdoor cultivation, as we have previously done. We will characterize biomass productivity and lipid and protein accumulation using TEA/LCA to evaluate product and coproduct production in our facility. In collaboration with Algenesis Materials, we will evaluate both protein and lipid products for their potential to make bio-based petroleum replacements products, especially renewable polymers, and again use TEA/LCA to assess economic viability and environmental sustainability of these potential coproducts.

OVERALL IMPRESSIONS

- This project continues key learnings of past DOE projects with hopes of creating a process tool kit for improving the algal yield of high-value coproducts.

- This is a great project working on important genetic tools that will be needed to improve algal strains to produce diverse products as well as increase overall productivity to reduce the cost of production.

- This project successfully identified valuable coproduct opportunities that would immediately improve the economics of algal biomass production while contributing to the longer term MYP targets. Improved processes in the development of genetic tools such as promoter suites and breeding are highly valuable to the BETO portfolio of projects. The team is clearly connected with relevant industry partners for both input to cultivation parameters driving testing conditions and opportunities for coproduct development. The project will benefit from additional structure around the prioritization of future activities and connection to TEA assumptions.

- This project appears to be managed well, has a strong technical approach, and is on schedule to achieve project objectives. The project contributes directly to the BETO mission, MYP goals, and to commercial advancements. The team is encouraged to add quantitative targets to its scale-up and demonstration efforts.

- The goal of this project is to develop a process for making advanced genetic tools using genomics, synthetic biology, high-throughput screening methods, and breeding technologies. The project is very ambitious, and any one of the objectives set forth can be (and in some cases is) a whole project within the BETO program. The team is working on synthetic promoters for recombinant gene expression in *Chlamydomonas reinhardtii*, reported gene expression in cyanobacteria, and mating in *C. reinhardtii*. The team shows a pipeline that can optimize a strain in 17 weeks. The development of improved molecular biology tools, along with breeding of strains, will help advance the goals of the BETO program by providing the tools necessary to create, breed, and adapt strains with the necessary traits to grow with high productivity and be resilient to environmental pressures. This project is just beginning, and future work will continue developing the genetic tool capabilities.

- The main weakness observed is related to the need for a comprehensive plan to move toward all the objectives delineated and providing focus for the project.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

A COMPREHENSIVE STRATEGY FOR STABLE, HIGH-PRODUCTIVITY CULTIVATION OF MICROALGAE WITH CONTROLLABLE BIOMASS COMPOSITION

The University of Toledo

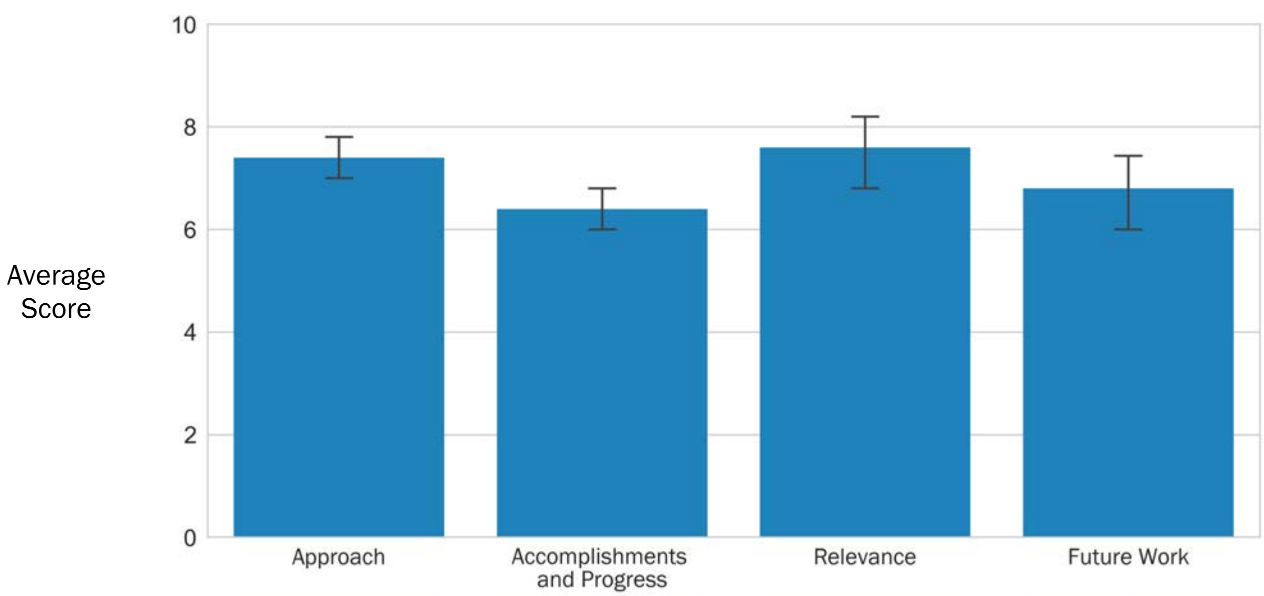
PROJECT DESCRIPTION

WBS:	1.3.2.651
CID:	EE0008247
Principal Investigator:	Dr. Sridhar Viamajala
Period of Performance:	9/30/2017–7/31/2020
Total DOE Funding:	$2,397,698
Project Status:	Ongoing

For algal biofuels to replace fossil fuels, it is imperative that cultivation systems are not constrained by (1) proximate availability of flue gas or other high-concentration CO_2 sources and (2) the energy and infrastructure burden to deliver CO_2 long distances. A recent study by Quinn and coworkers estimated that microalgae cultivation systems that are constrained by the availability of flue gases (in addition to low-slope barren lands and favorable climates) could achieve less than 10% of DOE's 2030 advanced fuel targets. We propose cultivation of microalgae in high-salinity and alkalinity media (pH greater than 10, alkalinity greater than 100 milliequivalents, salinity ~30 g/L) to achieve high biomass productivity and culture stability. Our cultivation media comprise high concentrations of dissolved inorganic carbon (DIC) (greater than 60 mM) at pH higher than 10. As alkaliphilic cultures grow, bicarbonate (HCO_3^-) is taken up by the algae, CO_2 is abstracted and fixed, and hydroxide (OH^-) is released. In parallel, because of the high pH of the medium, gaseous CO_2 is dissolving into the culture medium at a rapid rate, even from ambient air. The resulting high alkalinity in the growth medium ensures that enough HCO^-_3 remains available in the solution for continued carbon fixation. At night, when photosynthesis is absent, the bicarbonate depleted from the solution during the day is replenished via transfer of CO_2 from the atmosphere. Our strategy is to use this high-pH and high-alkalinity culturing

Weighted Project Score: 7.0

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

strategy to achieve high-productivity cultivation of strain SLA-04 without any inputs of concentrated CO_2. Additional productivity improvements are envisioned through the development of molecular tool kits, including metabolic modeling combined with targeted genome editing.

OVERALL IMPRESSIONS

- This is a novel application and development of molecular biology tool kit to prevent pond crashes and decouple algal farm locations from (expensive) CO_2 sources and delivery mechanisms. It leverages past DOE projects to demonstrate improved biomass productivities at a reduced cost in outdoor ponds by the conclusion of this project.
- Developing approaches to reduce dependence on CO_2 availability is highly valuable to reduce the cost of algal biomass production. The project team, led by The University of Toledo, has a long history in working together and appears to have the required resources to drive this initiative forward. Because this project is just beginning, there is an opportunity to ensure that the outdoor cultivation experiments are tightly connected to the tool development and economic analysis. It is critical that the biology impact is considered when refining models for CO_2 exchange with the pond media. Overall, the project would benefit from additional clarity and connection of biology to the experimental design and model assumptions.
- This presentation and project are well thought out. There is a possibility that the team might want to do too much, especially when they enter the later genomic editing deliverables. Their efforts do not seem to focus a on the biofuel production stages or open, large pond deployment. The multiseason experiments focusing on productivity and algal community dynamics should highlight the positives and negatives associated with this growth strategy.
- This project addresses an important challenge of large-scale algal production: the cost of CO_2 acquisition, distribution, and delivery. The goals, objectives, and management approach appear appropriate for the project. The project is on track, and early results are promising. The team is encouraged to make sure that the mixing and growth conditions tested will be representative of large-scale production.
- The goal of this project is to develop cultivation approaches that use high-pH and high-alkalinity media (1) for high rates of atmospheric CO_2 capture and (2) to provide nonlimiting DIC concentrations for growth. The main outcome is a high-biomass and biofuel-precursor productivities in outdoor open ponds using atmospheric CO_2 alone. The team plans to lay out and test the chemical and engineering principles associated with CO_2 transfer into alkaline media. They will use a *Chlorella* isolate for their evaluations. The team plans to develop a metabolic network model and genome editing approaches to improve the carbon uptake and tolerance of the selected strain. The project is just beginning and showing progress toward its main tasks. It is noted that the selected *Chlorella* strain shows productivities of 22 g/m^2/day to 32 g/m^2/day in high-alkalinity media. The development of high-productivity cultures that can grow on atmospheric CO_2 inputs would have major impacts on the implementation and success of BETO efforts. This project is deemed highly impactful for the overall program efforts. Because the project is just beginning, future work will incorporate all the main objectives and tasks for the project.
- No major weaknesses are noted for the project's approach and outcomes.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their overall positive comments on the project and for recognizing the "highly impactful" merit of our high-pH/high-alkalinity approach for decreasing/eliminating the cost of CO_2 acquisition, supply, and delivery. Per reviewer suggestions, we plan to integrate the biology tool kits more closely with the cultivation experiments as we progress through the project. Further, we will use first-principles mathematical modeling of the mass transfer process and correlate model predictions with experimental data to estimate mass transfer fluxes in commercial-scale systems and quantify the effects of mixing on the CO_2 mass transfer rates.

MICROBIOME ENGINEERING OF *DESMODESMUS* TO ALLEVIATE CARBON LIMITATION

Lawrence Livermore National Laboratory

PROJECT DESCRIPTION

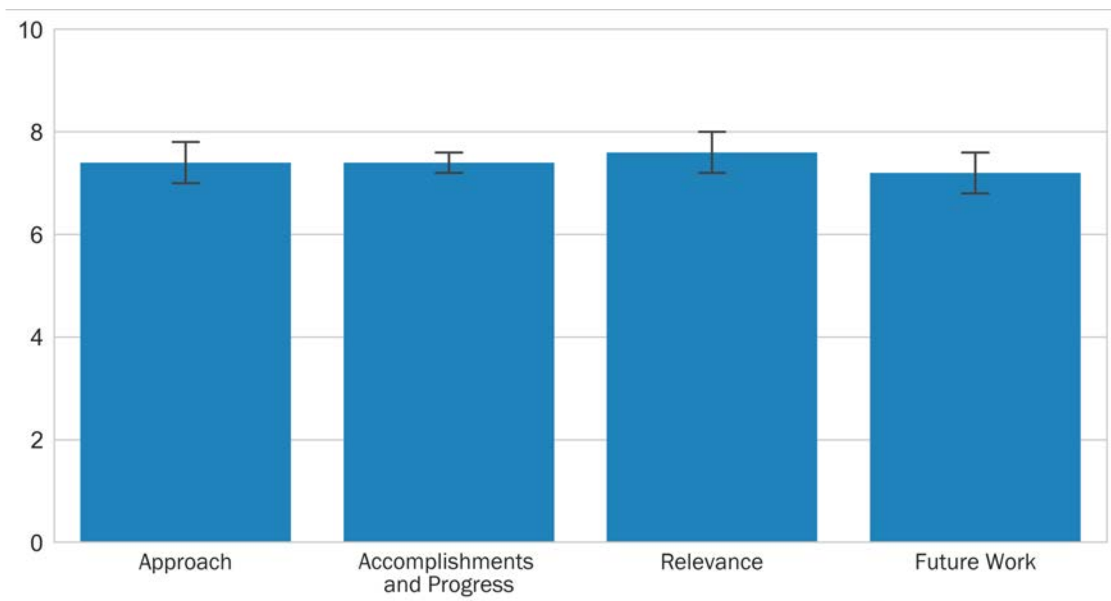
WBS:	1.3.2.652
CID:	NL0033320
Principal Investigator:	Dr. Xavier Mayali
Period of Performance:	10/1/2017–9/30/2020
Total DOE Funding:	$1,539,149
Project Status:	Ongoing

Roughly half of the carbon photosynthetically fixed by microalgae is lost through extracellular exudation of DOM. In high-biomass-density ponds, algal cells are also CO_2-limited and stressed from overproduction of O_2 from photosynthesis. In summer conditions, high light and temperature stress further decrease algal photosynthetic efficiency. To reach the goal of 26 g/m^2/day ash-free dry weight produced during summer months, and using *Desmodesmus* strain C046 as a model system, our research aims to ecologically engineer the algal microbiome to enrich for bacteria that efficiently remineralize DOM to CO_2 and simultaneously remove O_2. Our approach is to examine the microscale interaction between surface-attached bacteria and the *Desmodesmus* cells and use high-throughput sorting and screening with microfluidic incubation chambers developed by our industrial partner General Automation Lab Technologies. Using a combination of assays for DOM, microbial community analysis and algal growth quantification, we identify individual microbiome components with desired characteristics, such as high respiration rates and efficient DOM removal. In addition to alleviating CO_2 limitation and O_2 toxicity, the outcome of this synthetic ecological engineering approach will decrease algal pond DOM at harvest, which is a wasted resource, a supply of organic pollution if released into the environment, and which enables colonizing and possibly detrimental microorganisms to more easily invade the pond community. Our work includes scaling microbiome effects on algal growth from microwell to 1,000-L scale and the development of tool kits to be made available to the research community, including (1) a high-

Weighted Project Score: 7.4

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%

Average Score



One standard deviation of reviewers' scores

throughput assay approach for screening the effect of tens of thousands of distinct microbiomes on algal growth and (2) a medium-throughput assay for screening hundreds of microbiomes for high respiration and recycling of DOM.

OVERALL IMPRESSIONS

- This promising early-stage AOP project appears to be supported by a strong technical and management approach. Early results are consistent with project goals, and the team is well-equipped to complete its bench-scale objectives. Outdoor demonstration of the technology might be challenging given the additional ecological complexity, but the team is commended for including these larger scale tests in its objectives.
- This project strives to improve the productivity and robustness of algal strains by identifying microbiome consortia particularly well suited for different stressful environments—early results are encouraging.
- The positive synergetic relationship between algal cells and bacteria is not a new concept; many other groups have attempted to harness this relationship with somewhat minimal results. This project has the potential to achieve better results because they are using novel instrumentation for their initial high-throughput phases as well as isotope tracing for their later grow phases. This is a research area with great opportunity that could be a major driver for the improvement of productivity at scale. There might be some issues with running these experiments at scale, and the push to the field could be difficult.
- There is tremendous opportunity in engineering a beneficial microbiome to improve algal productivity. This project focuses on a naturally occurring consortia of *Desmodesmus* and associated bacteria. The demonstration of reduced DOM and increased productivity under temperature and light stress is a strong indication of the potential of this approach. There are several key steps for this concept to be applicable at the industrial scale. Of note, the biomass composition will need to be evaluated to ensure there is no negative impact of the presence of the microbial community. Overall, it is exciting to see this type of project within BETO's portfolio.
- The project's aim is to use a high-throughput microfluidic screening system to identify mutualistic bacteria with high respiration metabolism and protective pigmentation that lead to increased *Desmodesmus* growth under summer conditions and test at different lab and outdoor scales. The goal is to target 26 g/m^2/day biomass production under high-light and high-temperature stress (1,000-L scale). The approach will use microbial community analysis along with high-throughput cultivation in microwells and phenotyping of single cells using nanoscale secondary ion mass spectrometry for phenotyping. The approach is deemed to be reasonable. The team has characterized the microbiome associated and aggregated with different isolates of *Desmodesmus* showing differences in communities and growth characteristics. Xenic cultures in the microwell systems have higher cell yields. The project is just beginning and therefore has limited results to date. Controlling the bacterial microbiome in algal cultures provides an opportunity to enhance productivity. If successful, the project can directly impact the goal targets for the BETO program. Future work will focus on the main tasks delineated in the project.
- The main weakness noted was in providing a better explanation/justification on the use of isotope tracing for this project.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

DIRECT PHOTOSYNTHETIC PRODUCTION OF BIODIESEL BY GROWTH-DECOUPLED CYANOBACTERIA

Arizona State University

PROJECT DESCRIPTION

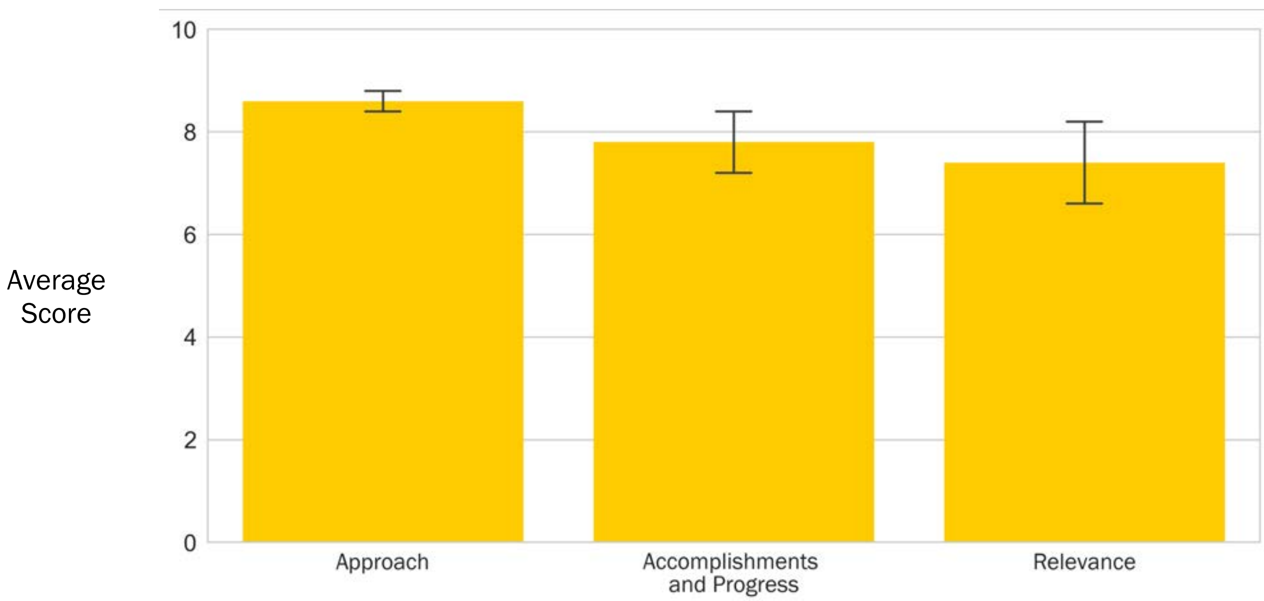
WBS:	1.3.2.910
CID:	EE0007561
Principal Investigator:	Dr. Wim Vermaas
Period of Performance:	9/1/2016–12/31/2018
Total DOE Funding:	$1,818,000
Project Status:	Sunsetting

The breakthrough catalyzed by this project is a direct production of excreted, drop-in, ready biofuel (methyl laurate or ethyl laurate) by cyanobacteria using CO_2, water, and light as the main inputs and not wasting carbon and energy by limiting the amount of biomass produced. The main advantages over current biofuel products are (m)ethyl laurate's immediate application as biodiesel and its limited solubility in water, thus reducing the availability to heterotrophs in the culture and increasing the ease of harvesting. The productivity of (m)ethyl laurate is increased by (1) boosting metabolic flux through the fatty acid biosynthesis pathway to (m)ethyl laurate and (2) reducing the production of extracellular polymeric substances such as exopolysaccharides. A further research aim is to uncouple growth of the culture (biomass production) from production of the biofuel, thus increasing (m)ethyl laurate yield and productivity while reducing the production of biomass. The resulting combination is disruptive to current biofuel production because of the type of biofuel produced and the reduction in exopolysaccharides, whereas decoupling cell growth and fuel production might enable the organism to put even more resources into biofuel.

This approach provides a "one-stop-shop" cyanobacterial platform that generates liquid transportation fuel from CO_2 and water with sunlight as the energy input. As established biodiesel molecules, methyl and ethyl laurate are suitable for direct use as a diesel replacement. Moreover, lauroyl esters have many additional

Weighted Project Score: 7.9

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



One standard deviation of reviewers' scores

applications. The ester moiety, meanwhile, provides a "protective cap" to the fatty acid molecule, reducing product degradation by heterotrophic scavengers, a key consideration for large-scale PBR systems. The product is readily harvested because of its poor solubility in aqueous media. This simplicity streamlines the biofuel production process and improves overall economic viability.

The concept builds on the team's prior success in efficient photosynthetic production and scale-up of laurate by a modified strain of the cyanobacterium *Synechocystis sp.* PCC 6803; this strain contains a thioesterase from the plant *Umbellularia californica*, which releases the fatty acid laurate when native fatty acid biosynthesis reaches the C12 stage. This platform strain is further engineered to improve laurate production and to convert laurate to (m)ethyl laurate. Conversion of laurate to methyl laurate is done by a S-adenosyl methionine-dependent enzyme, and conversion to ethyl laurate proceeds via lauryl-CoA and ethanol coproduction; the latter is done using constructs provided by Algenol. Inducible gene circuits are engineered to control the expression of decoupling mechanisms to arrest cell growth without harming viability and metabolic activity. Genetic changes to reduce the level of exopolysaccharides help to further push fixed carbon toward biofuel production and to reduce the level of nutrients available to heterotrophs.

Although ethyl laurate production was unstable, the majority of laurate was efficiently converted to methyl laurate by appropriately modified cyanobacteria, and the methyl laurate was excreted from the cell and captured in a biocompatible organic solvent, which greatly aids in harvesting. This provides an efficient method to produce and excrete biodiesel using a cyanobacterial culture, CO_2, and light.

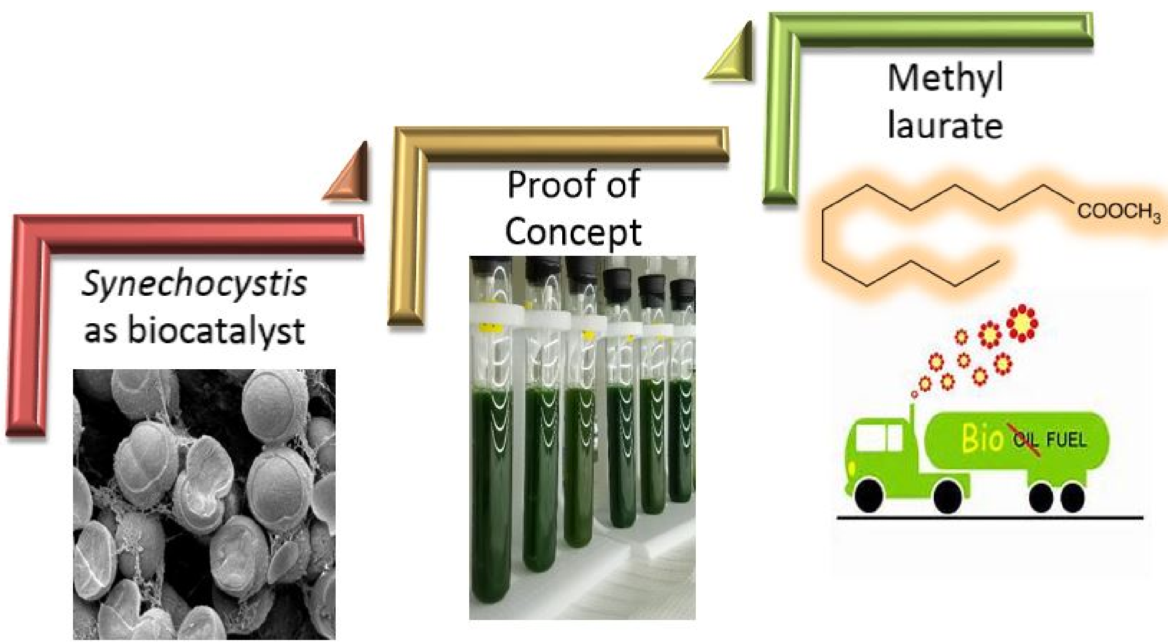


Photo courtesy of Arizona State University

OVERALL IMPRESSIONS

- This innovative project was able to accomplish its bench-level objectives after successfully navigating a pivot in target products. The team was managed well, and if the approach could be scaled up successfully, it could make a strong contribution to MYP goals. It was unclear if scale-up efforts were able to replicate the bench-level accomplishments.

- This project employs molecular biology tools to engineer the photosynthetic algae to directly excrete the biofuel, significantly limiting the downstream processing involved. This project successfully accomplished the goal, and the preliminary TEA is promising.

- The production of novel compounds from algae as a coproduct or precursor to biofuel is critical for economic viability. This extremely unique approach to the production of methyl laurate provides a demonstration of feasibility. The secreted coproduct can be relatively easily separated from the algal culture for a continuous production system. This is a great example of a stretch project providing potentially disruptive approaches to increase the value of algal production systems.

- The team appears to have been successful in engineering a strain that can produce methyl laurate. Questions remain about how this could be used for production, but this is a good first step toward a viable production system. Methyl laurate is an easily labile substrate for many marine alkane-degrading organisms. It is possible that if this were excreted extracellularly at scale in open air ponds, a large bacterial bloom would be observed, resulting in consumption of the product. This is a concern that should be addressed in operational designs if methyl laurate production is ever taken to scale.

- The main objective of the project was to develop cyanobacteria that excrete photosynthetically produced biodiesel with increased carbon flux into this pathway. The team took the approach of engineering *Synechocystis cyanobacterium* for fatty acid synthesis and overexpression. They developed a stable methyl laurate producing strain. They attempted to decouple growth and laurate production and reduce exopolysaccharide production. Then the team demonstrated production in a 55-L PBR system and perform a TEA. The technology is deemed very innovative. The team met several obstacles and developed strategies around these. Some weaknesses were noted in the association with the separation and collection of the methyl laurate and the modeling efforts.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their thorough evaluation of the project and their overall very positive take on the progress.

- We appreciate the comment that methyl laurate might be consumed by specific marine organisms. The compound appeared stable under our experimental conditions, including when bacterial contamination was introduced; however, we realize that there could be culture conditions where methyl laurate is less stable than desired in open pond conditions. If this proves to be an issue, then covered ponds might be considered.

- The value of methyl laurate is on the order of $1,000 per metric ton. A major consideration for the growing laurate and methyl laurate market is that cyanobacteria produced methyl laurate is significantly more sustainable than current production mechanisms, which involve palm oil plantations on recently converted tropical rain forest land. We agree that the production split between biomass and methyl laurate production is not yet firmly established and that TEA analyses done in this project are preliminary, without a thorough analysis of required downstream infrastructure; however, these preliminary results show that in principle the approach is promising.

- In the presentation, we did not sufficiently stress that an organic overlay of the culture (e.g., dodecane), functioning to harvest produced methyl laurate, did not negatively affect the culture in any way. Separation of the aqueous and organic layers typically is straightforward, but sometimes an emulsified interface will form that complicates clean separation of the phases. This is one issue we ran into at the 55-L scale, together with the loss of much of the organic overlay (along with the product) as a result of aerosolization and capture into the aerosol-quenching bleach solution that is required for larger scale cultures of transgenic cyanobacteria. This will need to be investigated further if commercialization is contemplated.

A NOVEL PLATFORM FOR ALGAL BIOMASS PRODUCTION USING CELLULOSIC MIXTROPHY

Arizona State University

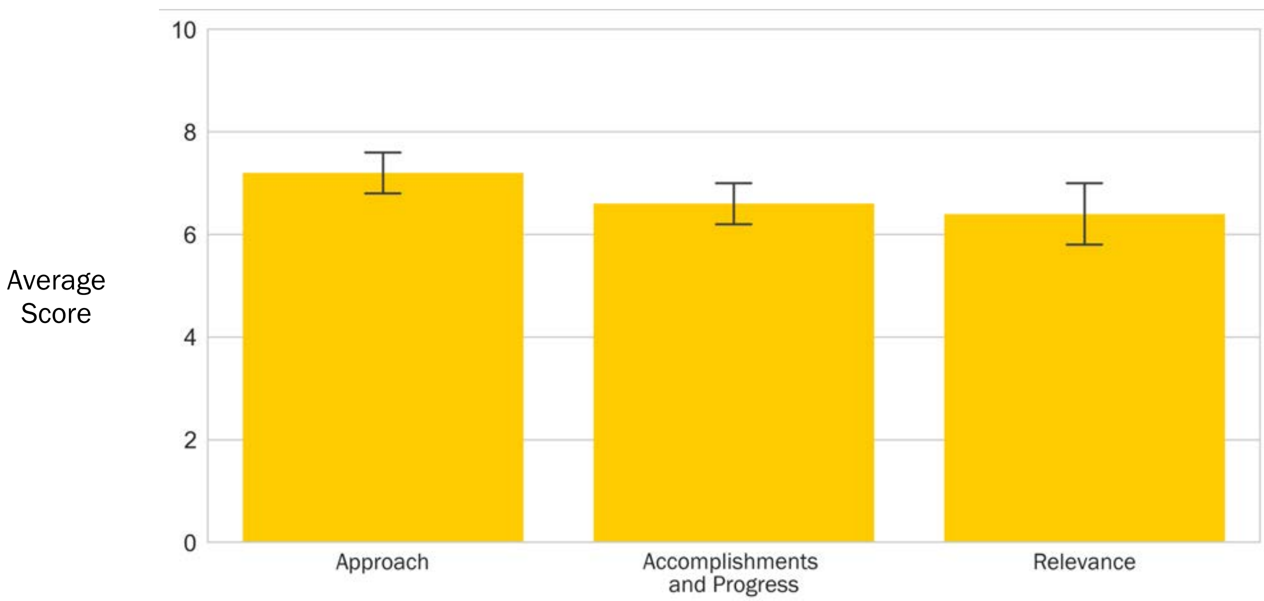
PROJECT DESCRIPTION

WBS:	1.3.2.920
CID:	EE0007562
Principal Investigator:	Dr. Peter Lammers
Period of Performance:	10/1/2016–9/30/2018
Total DOE Funding:	$1,689,791
Project Status:	Sunsetting

Algal feedstock production platforms specifically designed for scale-up on land with limited water resources remain an important gap in the DOE BETO algal R&D portfolio. As a result, abundant flat land in the southwestern United States plays little or no role in current DOE resource assessments, yet this region offers significant potential for algal biofuels that would not compete with food production if the water barrier could be addressed. The BETO portfolio has focused on open pond cultivation systems, but these systems have struggled with stability and productivity in outdoor scale-up trials. The goal of the proposed research is to close these gaps in the BETO portfolio. To this end, we will grow thermostable, acidophilic algae mixotrophically in PBRs to improve biomass productivity and reduce water consumption by reducing evaporation and eliminating cooling requirements. We will determine if the expected order-of-magnitude improvement in productivity outweighs the added costs of mixotrophic cultivation.

Weighted Project Score: 6.7

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



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OVERALL IMPRESSIONS

- This novel project on carbon metabolism appeared to be managed well and accomplished many of its objectives. The project included an extensive list of publications in preparation or in print. It is unclear if the approach has relevance to biofuel production scenarios because it seems to require a unique bioreactor and a high-value coproduct to support the TEA.

- The team's goal was to create a system suitable for arid regions of the country by using cellulosic sugars as a feedstock and closed PBR systems to reduce water consumption. Iterative modeling with the TEA team provided useful experimental direction.

- The project team has taken a unique approach to the challenge of water limitation in arid regions where algal production might otherwise be highly successful. In addition, the team has an industrial partner with a focus on a valuable coproduct that will drive the economics of the system. The resulting TEA from this project has created a focus for the project team toward improved cultivation systems. There is also an opportunity in projects of this nature to assess the strain choice and further explore the assays for assessing mixotrophy potential of the culture.

- This is an interesting model, but it is not clear if the approach address BETO's goal of reducing fuel costs or generating biomass for fuel purposes. This system does not appear to be a coproduct as much as a system designed solely to generate high-value products.

- The objective of this project was to demonstrate mixotrophic cultivation on cellulosic sugars from acid-pretreated cellulose hydrolysate by the alga *Galdiera sulphuraria* and produce a phycocyanin coproduct. The approach is deemed innovative and unique to the BETO portfolio. The project was successful in demonstrating biomass productivities over 1 g/L/day on corn stover hydrolysate, with yields of 07–1.1 g biomass/g sugars. These high productivities and yields can be beneficial to the overall economics of the process. The team showed that mixotrophic conditions provide an opportunity to produce high yields of biomass with high contents of polysaccharides. Further, the team showed the ability to optimize the system for high productivity of phycocianin. The TEA showed the need for reduced PBR costs and production of a high-value product. Thus, the project addresses major goals of the BETO program in crop resilience and productivity.

- The main weakness for the project is providing supporting information from the TEA/LCA showing that this pathway to fuels would be more beneficial than a direct pathway that converts the stover into a biofuel.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

ALGAL FEEDSTOCKS LOGISTICS AND HANDLING

Idaho National Laboratory

PROJECT DESCRIPTION

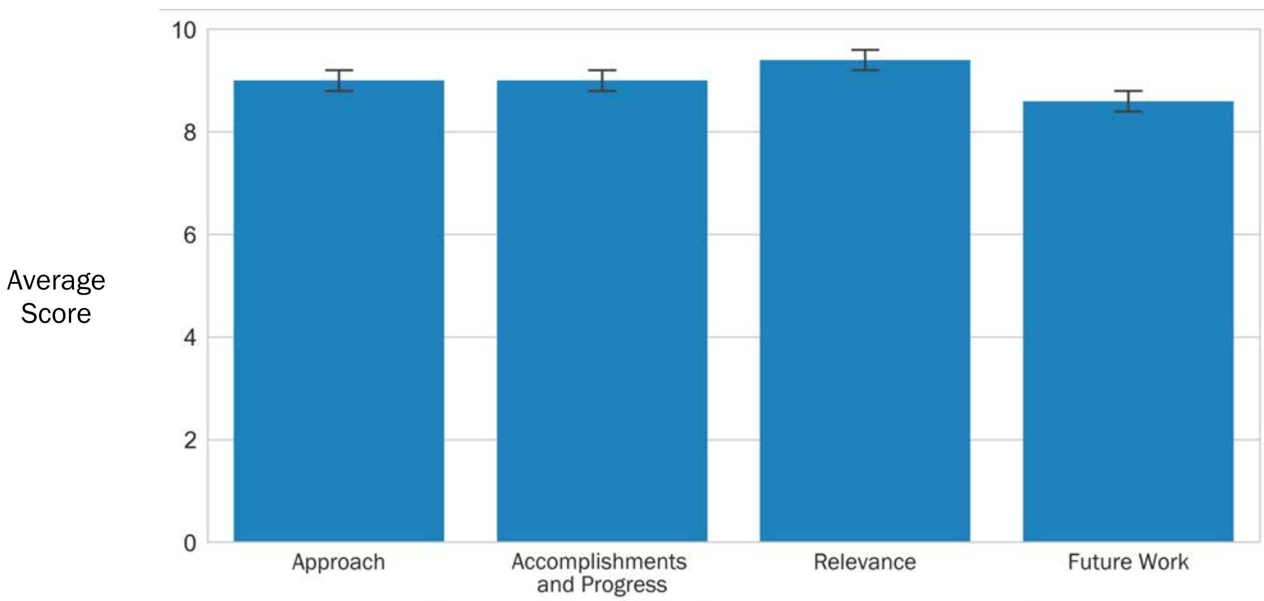
WBS:	1.3.3.100
CID:	NL0032357
Principal Investigator:	Ms. Lynn Wendt
Period of Performance:	4/1/2015–9/30/2019
Total DOE Funding:	$2,360,000
DOE Funding FY16:	$0
DOE Funding FY17:	$800,000
DOE Funding FY18:	$760,000
DOE Funding FY19:	$800,000
Project Status:	Ongoing

This project focuses on understanding the post-harvest physiology of algal biomass to enable the development of process technologies that preserve, and even improve, the quality of algal biomass prior to downstream conversion. Post-harvest physiology spans the time frame between harvest and conversion, which can range from minutes to months. This research project will provide solutions to feedstock supply chain and logistics challenges that occur between algal biomass production and conversion, meanwhile reducing the risk of feedstock loss. The two objectives of this project are to (1) solve the problem of seasonal variation in algal biomass production by developing a long-term storage approach that can reliably stabilize algal biomass while reducing energy input in a cost-competitive manner compared to the current state of the art and (2) increase conversion throughput by reducing ash content in periphytic biomass originating from an algal turf scrubber (ATS).

We have shown that seasonal variation can be mitigated through the development of a wet anaerobic storage process for algal biomass. This effort has used lab-scale experimentation, process optimization, characterization of stored materials, and TEA to achieve algal biomass stability in a cost-competitive manner. Algal biomass, 20% solids blended with lignocellulosic biomass and stored using existing practices for silage production, was accomplished at the 20-L scale for 3 months with 3% dry matter loss. Continuous, bench-scale

Weighted Project Score: 9.0

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

HTL tests of the stored material conducted at PNNL suggest no change in biocrude yield as a result of storage. We also demonstrated a stabilization approach for algal biomass at 20% solids that results in 10% total loss in dry matter over 6 months, and this approach reduces costs compared to drying followed by dry storage. This project also provides continued support to the BETO's annual SOT algal production effort by assessing the post-harvest stability of algae harvested from seasonal cultivation trials in outdoor test beds.

Physical and chemical ash-removal approaches conducted to date have revealed multiple pathways for ash reduction in biomass harvested from the ATS floways. Despite high biomass productivities and water remediation opportunities for ATS systems, their adoption as biofuel feedstock production platforms is limited because of high ash content, which is consistently 60 wt %–65 wt % for this biomass. Elemental ash analysis revealed that biomass from ATS systems sourced from seawater contain ~20% sodium and chloride, which are easily removed by water extraction. Water extractions reduced ash by 28%–40%, although overall ash content was 40 wt %–50 wt % and is considered too high for many conversion approaches, including HTL. Alkaline treatment was successful at removing silica, which can constitute 50% of the ash because of large contributions from diatoms, such that final ash contents ranged from 20–40%.

In summary, this research is providing solutions to feedstock supply chain and logistics challenges that occur between algal biomass production and conversion, meanwhile reducing the risk of feedstock loss. This effort supports multiple MYP barriers (e.g., storage systems, quality) and contributes to the goals of increasing biomass value through coproduct formation and ultimately for producing $2.50/gal biofuels.

Photo courtesy of Idaho National Laboratory

OVERALL IMPRESSIONS

- The incorporation of algae into existing bioenergy feedstock streams is a clever and actionable approach. The project update was exceptional with clear communications of the value drivers, challenges, accomplishments, and critical next steps. The opportunity to improve the economics of biofuel production through combining feedstock sources has been shown through this innovative and well-run project. I look forward to seeing the future development of this exciting work.

- The relevance of the project was clearly linked to MYP goals, such as biomass storage challenges and preservation of biomass quality. The team is guided by strong technical and management approaches. An extensive publication list is an indication that the team is committed to project results being used to advance the SOT for bioenergy applications. Project results have been incorporated into BETO's SOT and resulted in improved future fuel selling prices.

- This research focuses on the vital, but often overlooked, aspect of logistics (between biomass harvesting and downstream processing). Promising progress to date related to maintaining quality of biomass during storage. Task 2, ash reduction, will be incredibly valuable across the industry if successful.

- Wet storage of algal biomass is a novel concept that needs to be further explained. This project has made great progress in addressing many questions, but it is lacking additional results from large-scale harvests. The team's collaboration with AzCATI should be expanded to include multiple strains grown in different seasons to see if there is a variability. The data already show significant variability from different strains, and further evaluation of seasons and how growing conditions affect the storage will be also important.

- The team will use feedstock logistics operations to develop process technologies that preserve and even improve the quality of algal biomass prior to downstream conversion. The key outcomes are (1) a process that both preserves harvested microalgal biomass during a 6-month period to manage seasonal production variation and enables a biorefinery to run year-round with a consistent feedstock supply and (2) a process to improve the quality of biomass derived from an ATS through ash reduction that could be applicable to multiple high-ash algal species. In feedstock stabilization, the team is blending the algal feedstock with terrestrial feedstock under anaerobic conditions and stabilization with lactic acid to understand fundamentals in preservation process. They are also working on ash composition analysis and removal from algae coming from scrubbers through several methods. This is overall a well-conceived and unique project that can have great impact on the industry. The team has shown algal preservation for 6 months for all components, with only minor changes in carbohydrates, under the blending with corn stover. No change in performance is seen in the HTL of the blended material that was stored. The team also found that adding lactic acid decreased storage losses from 44% to 6%, but this is not economically feasible. The ash characterization studies are being performed in water from an estuary that contains up to 75% ash. The team is working on systematic removal of ash by alkaline washes and determining removals and effects on remaining algae. The team is working on ash accumulation studies in an ATS floway in California. Progress has been steady, and the project has great potential for impact. The TEA shows that successful wet storage contributes to a decrease in the MFSP of the SOT. The relevance and direct impact of these efforts are deemed to be very high for the BETO program. Future work is deemed reasonable and appropriate.

- No weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for the positive feedback. We agree that addressing biomass availability and quality in the feedstock logistic supply chain is important in advancing the commercialization of algal-based biofuels. We will continue to seek a fundamental understanding of algal post-harvest physiology in support of BETO MYP goals of reducing costs of algae-based biofuel production. This project has been collaborating with AzCATI since Spring 2018 on assessing the storage stability and ash content of multiple strains grown outdoors from each season, and we will continue this effort to understand strain-to-strain variability, seasonal variability, and the fundamental aspects of preservation through wet storage with a goal to make wet stabilization approaches universally applicable. Ash mitigation in outdoor systems is a growing concern for the algal industry. We will expand our work to include algal biomass cultivated in raceways and in wastewater effluent to develop solutions that result in reduced ash content in a variety of sources of algal biomass.

ALGAE TEST BED PUBLIC-PRIVATE PARTNERSHIP (ATP3) – A RAFT PARTNERSHIP

Arizona State University

PROJECT DESCRIPTION

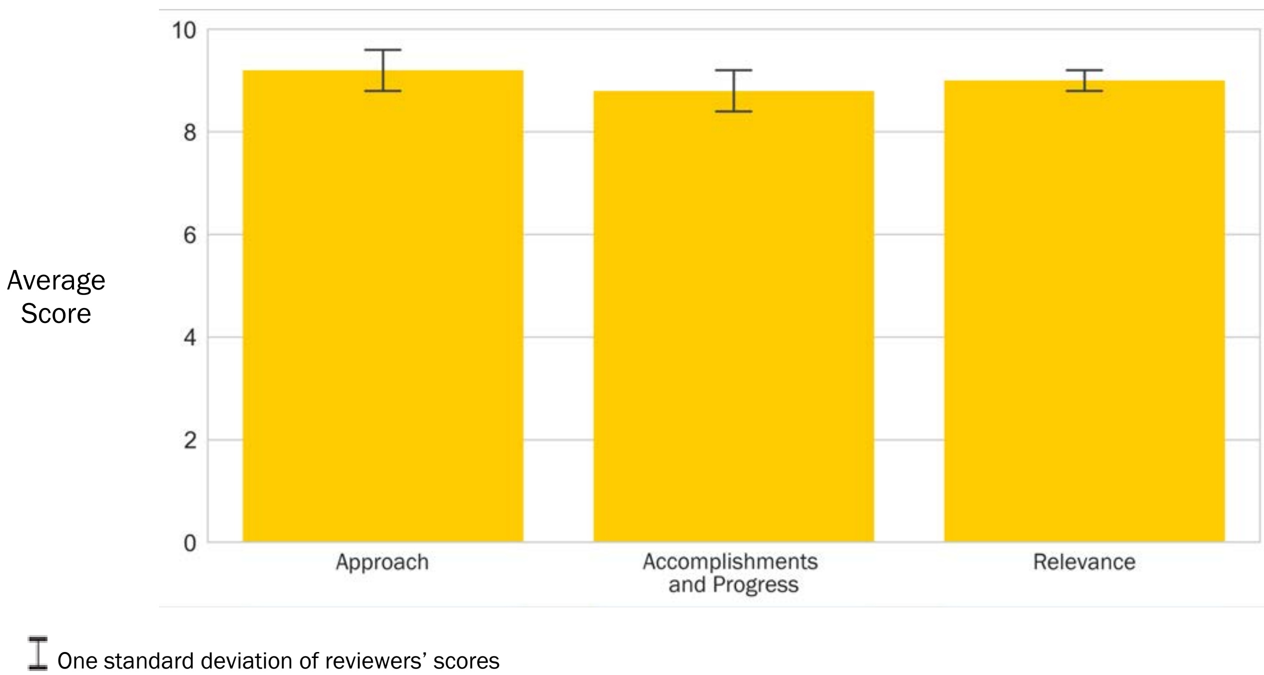
WBS:	1.3.5.100
CID:	EE0005996
Principal Investigator:	Dr. John McGowen
Period of Performance:	1/31/2013–9/30/2018
Total DOE Funding:	$14,999,658
Project Status:	Sunsetting

The ATP3 is a multi-institutional effort funded by DOE to establish a network of operating test beds that bring together world-class scientists, engineers, and business executives with the goal to increase stakeholder access to high-quality facilities by making available an unparalleled array of outdoor cultivation, downstream equipment, and laboratory facilities to the algal R&D community. ATP3 used the same powerful combination of facilities and technical expertise to support the TEA/LCA and resource modeling and analysis activities, helping to close critical knowledge gaps and inform robust analyses of the SOT for algal-based biofuels and bioproducts. ATP3 included test bed facilities at AzCATI and augmented by university and commercial facilities in Hawaii (Cellana), California (California Polytechnic State University, San Luis Obispo), Georgia (Georgia Institute of Technology), and Florida (Florida Algae), in addition to partners at the NREL, SNL, Valicor Renewables, University of Texas at Austin, and Commercial Algae Management, Harmon Consulting, Evodos and Litree.

ATP3 aimed to make significant advancements in the algal biofuel arena by promoting opportunities through an open collaborative test bed network. Our regional test beds were equipped to develop and evaluate value propositions in support of multiple algal market value chains. We successfully served clients across a range of applications and needs from water testing and species identification, productivity, and analytic measurements from biomass grown in novel PBRs and ponds, biomass supply from gram to multiple kilogram quantities,

Weighted Project Score: 8.9

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



identification of bioactive molecules that promote animal health and associated scale-up processes, and equipment testing, in particular novel PBRs and dewatering equipment. Our network offered access to such desirable features as open and closed, small to large pilot cultivation systems; access to natural saltwater, wastewater, and CO_2 streams; and integrated harvesting unit operations. During the program, ATP3 engaged more than 50 clients from academia, national laboratories, and industry; included international deliveries of hundreds of kilograms of algal biomass to partners; conducted 15 educational and training workshops; and completed more than 80 client projects generating additional revenue in support of the overall test bed network.

ATP3 used our facilities to perform coordinated long-term cultivation trials producing robust, meaningful data sets from this regional network determining the effects of seasonal and environmental conditions. These data are critically important to support the modeling community and guide R&D toward the transformative goal of cost-competitive algal biofuels and bioproducts by 2030. ATP3 implemented an experimental framework, termed "unified field studies" which were conducted across six distinct geographic regions using standardized mini-raceway ponds, standardized protocols, and data acquisition and tracking methodology. In addition, we leveraged specific site capabilities, including PBRs, access to wastewater and CO_2, larger pilot-scale raceway ponds, and dewatering equipment to conduct targeted cultivation trials termed "advanced field studies." From September 2013 through December 2018, more than 95 individual experiments were conducted across the network, with an average duration of at least 50 days. These experiments included standardized, validated methods with an emphasis on continuous improvement, more than 15 strains used in outdoor cultivation experiments with the majority of multiseason data coming from 5 strains with an average run time of more than 40 days. ATP3-generated productivity data continue to be the primary data sets supplied to the DOE-sponsored SOT metrics for 2015, 2016, 2017, and 2018 and under the DISCOVR for 2019 and beyond.

OVERALL IMPRESSIONS

- The ATP3 project successfully implemented a multilocation algal field trial network using standardized protocols and cultivation practices. This type of field testing is instrumental to the advancement of the industry by providing sites to host outdoor experiments and continue to refine the TEA accuracy. The team has done an extraordinary job of collaborating across the industry by providing an important service and quality data as well as education opportunities. The continued support of programs like this and expansion of field-testing locations would be highly valuable to the continued development of algal biofuel production.

- Large consortia focusing their efforts on year-round large-scale deployment of top three algal strains at three outdoor test bed locations. All data accumulated during the 3 years is available to the public. Their efforts and results will become the next baseline for future projects just as other large consortia projects have done before the RAFT and ATP3.

- ATP3 is a large project addressing a key goal for the BETO program by establishing and operating a test bed capability to facilitate innovation and growth of the algal biofuel and bioproduct R&D and industrial community. To meet this goal, the ATP3 team formed a national partnership/network with entities that could provide a diverse set of capabilities across the United States. As part of the process, the team made the facilities available to a broad range of investigators from academia, national laboratories, and industry. Further, the team provided access to much of the cultivation data collected during various large-scale experiments to the community through appropriate web portals. The list of accomplishments by the ATP3 team is impressive, covering supply of biomass (>1,000 kg), equipment testing, analytic methods testing and validation, culture maintenance, scale-up of cultures (including setting up initial stages of GMO trials), along with education and training workshops. The test bed capability was developed by the BETO program in response to a stakeholder outcry for capabilities to supply biomass, standardize cultivation practices, provide facilities for testing and evaluation, and concerted development of training and outreach. The ATP3 partnership clearly addressed these needs and provided the algal community and the BETO program with an excellent capability. The capability has evolved to become an integral component of the BETO program and engages stakeholders broadly. Further, the ATP3 project addressed key needs in workforce development and technology dissemination. Very few weaknesses were noted. The ATP3 partnership and the BETO program are commended for this excellent success.

- This ambitious project has served a critical role in generating publicly available data in outdoor conditions at a variety of scales. Outputs include an impressive list of peer-reviewed publications and numerous workshops, training sessions, and other forms of stakeholder engagement. The project has played a central role in supplying SOT data and continues to be closely aligned with BETO priorities and MYP goals. The team and assets also serve as an important collaborator on many other BETO-funded projects.
- ATP3 has contributed—and continues to contribute—a significant wealth of knowledge and baseline data to the algal industry. It is proven to be a great resource for researchers to trial new concepts.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- ATP3 thanks the reviewers for the comment. Our commitment to facilitating stakeholder access to facilities and expertise was core to our formation and through this program.
- ATP3 conducted harmonized, highly coordinated cultivation trails across five sites with three commercially relevant strains for more than 2 years, and we leveraged additional experimentation for another 3 years and another five commercially relevant strains. We agree there is a continued and important place for large, well-run consortia projects, and we were honored to participate and contribute to the BETO AAS portfolio.

REGIONAL ALGAL FEEDSTOCK TESTBED PARTNERSHIP

University of Arizona

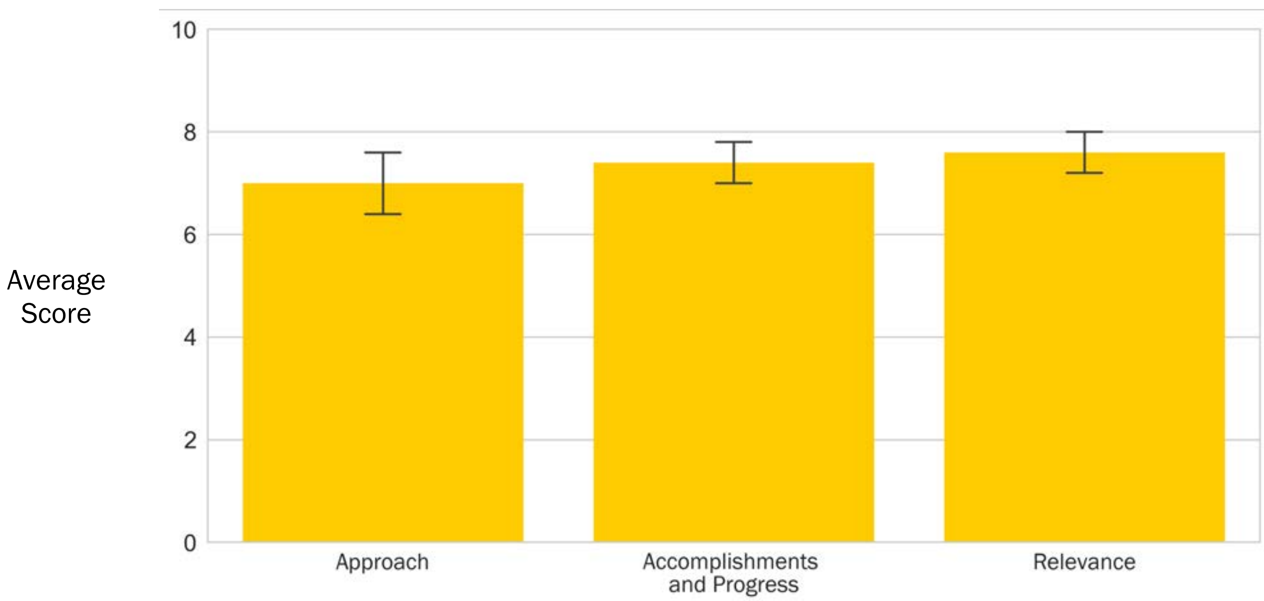
PROJECT DESCRIPTION

WBS:	1.3.5.111
CID:	EE0006269
Principal Investigator:	Dr. Kim Ogden
Period of Performance:	9/1/2013–5/31/2018
Total DOE Funding:	$8,000,001
Project Status:	Sunsetting

The RAFT project completed detailed characterization of 12 potential production strains and extensively tested the three best strains in long-term seasonal cultivation trials. During these trials, the team completed 272 cultivation experiments at three outdoor test bed locations during 3 years, testing seasonal effects as well as various harvest, crop protection, and strain rotation strategies. The team developed a sensor to continuously monitor cell density and submitted a patent application. The team also developed and validated models for predicting strain performance, determining biomass productivity, and evaluating cultivation system design and operational strategies in large-scale production scenarios. In addition, the project established a detailed database for long-term cultivation trials with discrete data from the test bed cultivation experiments. The database will be maintained by the University of Arizona (https://raft.arizona.edu/cultivation-data/) for public access and continued academic evaluations. The RAFT project also produced numerous presentations, peer-reviewed publications, patents, theses, and dissertations.

Weighted Project Score: 7.3

Weighting for Sunsetting Projects: Approach-25%; Accomplishments and Progress-50%; Relevance-25%



One standard deviation of reviewers' scores

OVERALL IMPRESSIONS

- This project included a balanced set of tasks spanning strain optimization, scale-up, field-testing, and modeling. The team appeared to be managed well and was able to accomplish its project objectives. Results had clear relevance to the BETO mission, MYP goals, and the algal industry. Data generated in outdoor growth trials are publicly available, and the project resulted in an extensive publication list.

- The RAFT project has supported the development of an outdoor open pond algal productivity data set for use by the public. Facilities to test cultivation practices and new strains are critical to the development of the algal biofuel program. The team has taken the first step in building a database of production data collected across the U.S. Southwest. The continued funding of work for this purpose is critical to ensure resources of this nature are available to researchers working toward the achievement of MYP targets.

- This is a large consortia focusing their efforts on year-round, large-scale deployment of the top three algal strains at three outdoor test bed locations. All data accumulated during the 3 years are available to the public. The team’s efforts and results will become the next baseline for future projects, just as other large consortia projects have done before RAFT and the ATP3.

- RAFT has captured and disseminated a large amount of knowledge regarding long-term algal cultivation to the broader research community. Data and key learnings are available on the website and in the final report.

- The RAFT project goals included setting up long-term cultivation processes that would provide cultivation data to understand and promote algal biomass production. This would include algal biomass production and support the BETO goals toward cost-competitive algal biofuels by 2022. To achieve these goals, the four partners put together a data management plan and information management that would support and provide data on the long-term cultivation of two strains during two seasons, the growth and productivity of three strains as a function of abiotic parameters, two strains cultivated in impaired waters and nutrient recycle, along with cultivation of two strains in three different regions. The team showed the ability to perform multiple productivity runs across the partnership sites, some showing excellent productivities. The RAFT team selected three primary strains to work with, which came from a previous program. These were cultivated during various seasons using a number of cultivation strategies, including polycultures, municipal wastewaters, saline well waters, etc. To do this, the team evolved a number of best practices in characterization and laboratory analysis, strain maintenance, scale-up, outdoor cultivation, control strategies, and molecular diagnostic tools. The RAFT final report will be a great asset for the program and a resource to the community. The team’s efforts resulted in 34 publications, 37 presentations, and three patents. The RAFT program created a successful strategy to obtain cultivation data on algal biomass production. This is a major effort, which, if performed on a continuous and well-characterized basis, could feed very relevant information to growth and assessment tools critical to the program. Further, the project was able to incorporate the use of a sizable number of doctoral, Bachelor of Science, and high-school students, along with postdoctoral researchers. This inclusion will help evolve the algal workforce of the future.

- Several weaknesses were noted, primarily in the development of external efforts and the diversity of strains and length of cultivation processes.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers’ overall impressions of their project.

ALGAL BIOFUELS TECHNO-ECONOMIC ANALYSIS

National Renewable Energy Laboratory

PROJECT DESCRIPTION

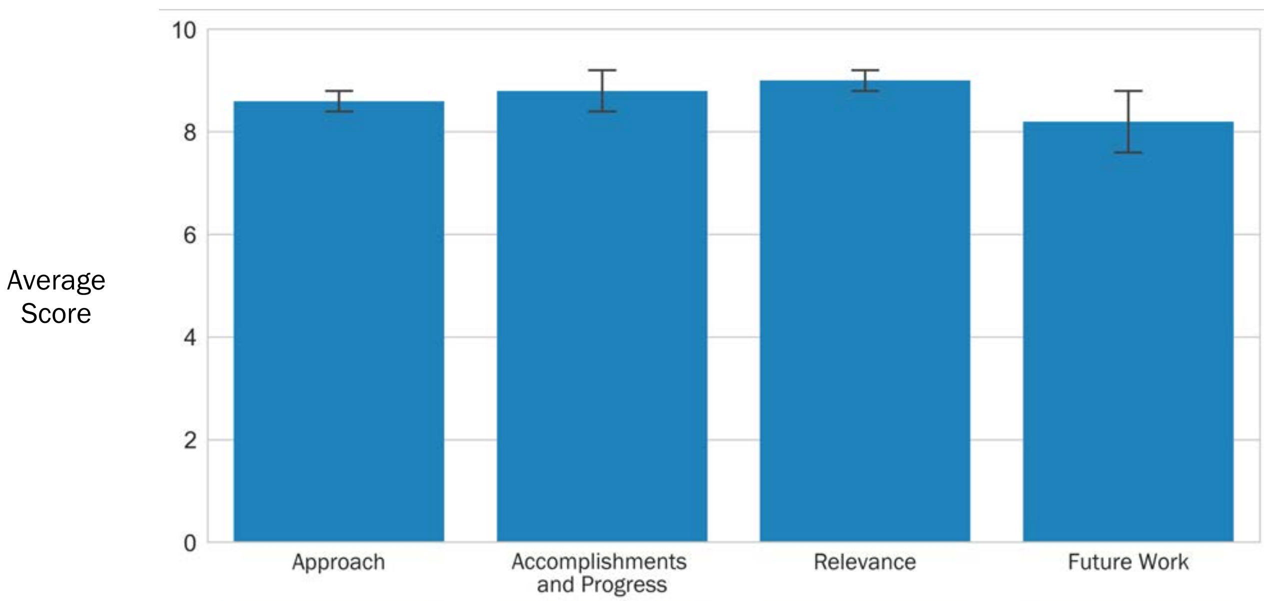
WBS:	1.3.5.200
CID:	NL0021975
Principal Investigator:	Mr. Ryan Davis
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$1,300,000
DOE Funding FY16:	$300,000
DOE Funding FY17:	$300,000
DOE Funding FY18:	$350,000
DOE Funding FY19:	$350,000
Project Status:	Ongoing

The objective of NREL's algal biofuel TEA project is to provide process modeling and analysis to support algal program activities using TEA models to relate key process parameters with overall economics for cultivation, processing, and conversion of algal biomass to fuels and coproducts. By quantifying economic implications of key process metrics, TEA models highlight the technical requirements to achieve future program cost goals as well as enable a means to track progress toward these goals.

This project is highly relevant to BETO in that the project produces critical cost data tied to funded research at NREL and other collaborators, with the analyses subsequently exercised by BETO to guide program plans, FOA priorities, and other strategies to direct bottom-up research toward achieving cost targets that are set from the top down. This includes costs for both algal biomass production (dollars per ton) and downstream conversion to fuels (dollars per gallons gasoline equivalent), most notably to support BETO's fuel cost targets to less than $2.5/GGE by 2030. Moreover, our work strives to address the large disparity in public claims regarding cost potential for algal biofuels by establishing rigorous, peer-reviewed cost models based on multiple input sources. This project also interfaces closely with other BETO-funded efforts, such as the DISCOVR consortium and algal test-bed partners to align TEA models with current data and future planning priorities.

Weighted Project Score: 8.7

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

The algal TEA project has made significant achievements since the 2017 peer review, including (1) coordination of a recently published *Harmonization Report* update (joint with PNNL and ANL) quantifying opportunities for national-scale algal biomass biofuel production enabled by carbon capture and the inclusion of high-value coproducts, (2) highlighting potential paths to achieving future $2.5/GGE fuel cost goals based on multifuel/product biorefinery concepts under NREL's combined algal processing (CAP) pathway, and (3) benchmarking progress toward those goals through SOT updates based on the latest experimental data for both algal cultivation and CAP conversion.

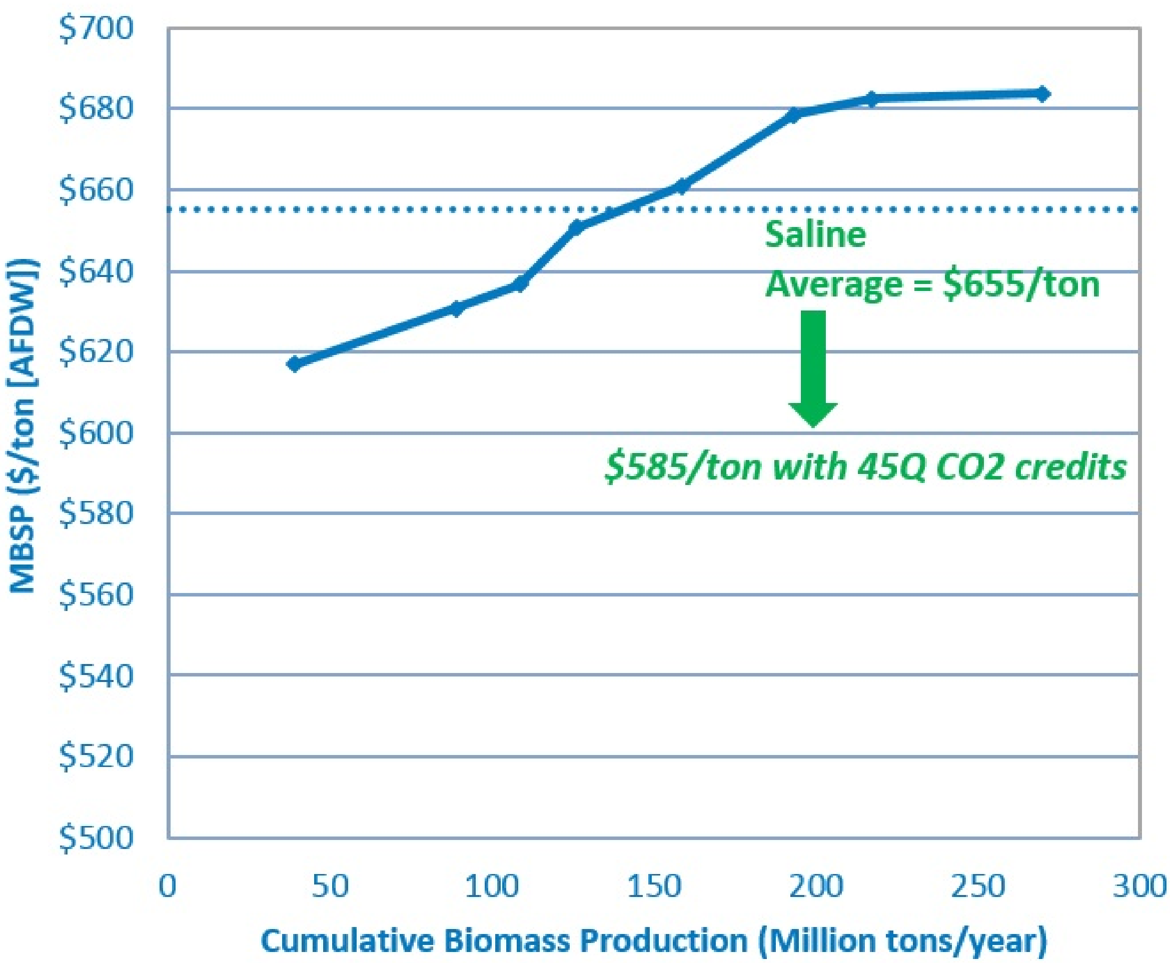


Photo courtesy of National Renewable Energy Laboratory

OVERALL IMPRESSIONS

- Since 2010, this project has contributed much insight to algal productivity and commercialization. CO_2 sourcing from carbon capture in the new model is interesting because it decouples sites from certain areas. A negative is that TEA modeling is highly relevant only if the approaches are relative to the field. Siloing all growth information with one grower in one location is problematic.
- This project appears to be managed well, is meeting milestones, and continues to provide a valuable service to the community. The SOT modeling is a core tool for BETO and tracking progress toward MYP goals. The project is commended for making the underlying Excel model available to the public.

- This project directly relates to and collaborates with many other AAS projects. These TEAs provide valuable insight into identifying crucial factors across the entire algal platform that result in high-value coproducts that have the potential to reduce the gallons gasoline equivalent to achieve MYP targets.
- The current version of the TEA provides a comprehensive model for the drivers of algal fuel production. Especially of note is the inclusion of extraction and a broad diversity of coproducts. The project team continues to build on their experience strengthening the value of the TEA data to drive the prioritization of R&D activities. There is a now a great opportunity to explore the interactions between cost and value drivers as well as refine areas such as the impact of crop protection in cultivation. It will be great to see further development of the tool as stakeholders begin to use the model and provide feedback.
- The aims of this project are to provide TEA to support algal program activities. This includes the creation of process/TEA models for cultivation, processing, and conversion of algal biomass to fuels and coproducts (CAP conversion), relating key process parameters with overall economics. The approach is rigorous and builds checks to ensure credibility of models. The team has delivered several products since the last review. This project provides direction, focus, and support for industry and BETO by providing bottom-up TEA to show R&D needs for achieving top-down BETO cost goals. This project will guide R&D toward economic viability and the eventual adoption of algal biofuels/products into the U.S. market. In the future, the project will put together a number of assessments in algal cultivation on wastewater (Q1 FY 2019, complete); biomass growth versus quality modeling; FY 2019 CAP design report update; SOT benchmarking for biomass production plus CAP conversion; and TEA support for the DISCOVR Consortium.
- No weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their positive feedback in recognizing the utility of this project for BETO and the algal community. We hope to further develop and refine the newly published algal farm TEA tool to maximize its utility based on feedback from stakeholders. Moving forward, we also plan to continue expanding on recent efforts to further explore cost-versus-value trade-offs around algal cultivation practices, biomass growth rates, and compositional quality as applicable for conversion to fuels and value-added products.
- Regarding the comment about focusing on growth in only one location, for the SOT data sourcing this was done out of necessity given that only one location (AzCATI) has been available to furnish year-round outdoor cultivation data for informing inputs to annual SOT updates. Beyond SOT efforts, however, the algal farm TEA models have considered a large range of possible locations, e.g., as presented in the recent 2017 *Harmonization Update*, which evaluated TEA and resource potential for more than 500 plausible algal farm locations spread across the contiguous United States.

ALGAE TECHNOLOGY EDUCATIONAL CONSORTIUM

National Renewable Energy Laboratory

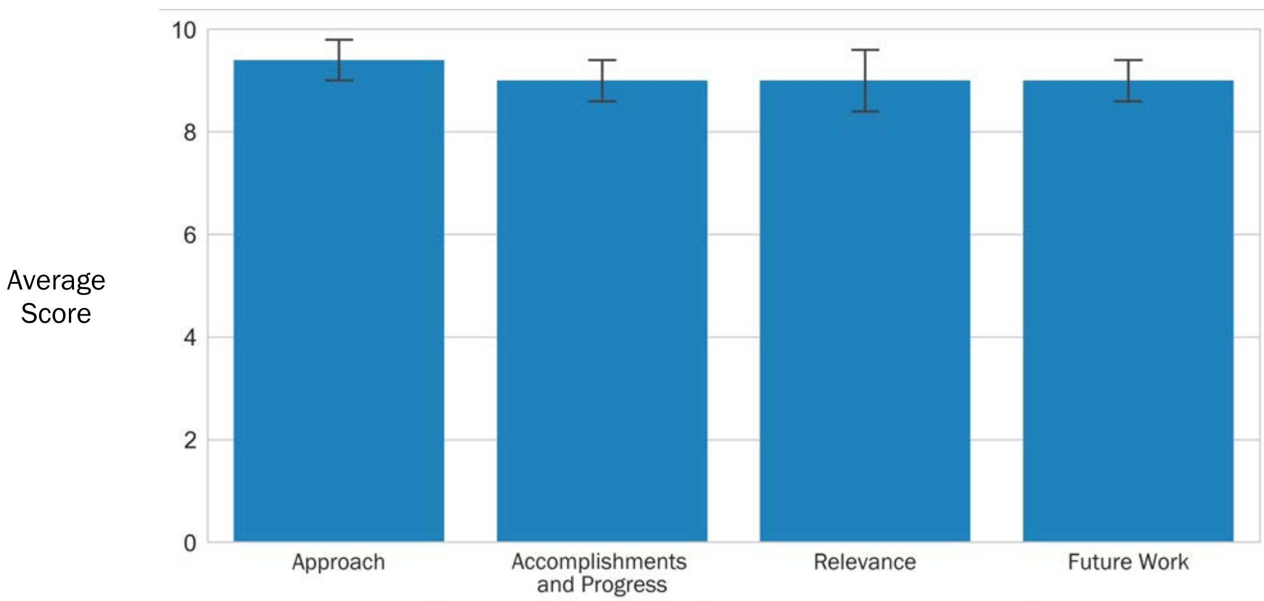
PROJECT DESCRIPTION

WBS:	1.3.5.201
CID:	NL0029628
Principal Investigator:	Ms. Cindy Gerk
Period of Performance:	10/1/2015–9/30/2021
Total DOE Funding:	$1,875,000
DOE Funding FY16:	$55,000
DOE Funding FY17:	$600,000
DOE Funding FY18:	$600,000
DOE Funding FY19:	$620,000
Project Status:	Ongoing

The ATEC project is a collaboration of academic and commercial algal experts that created two separate community college degrees in algal farming and biotechnology, providing an educational platform resulting in the next generation of algal professionals. Additionally, ATEC has assembled an IAB comprising senior management from leading U.S. algal companies to ensure that the ATEC skill set meets industry needs. Future efforts include formalizing relationships with more community colleges; online courses; institutionalization of the intensive, in-person laboratory courses; distribution of the Algae Cultivation Extension short-course learning modules; distribution and analysis of the second-generation algal-based jobs survey, targeting the biotechnology and wastewater treatment industries; and curriculum and learning outcome assessment by an external educational assessment team and the ATEC IAB. ATEC continues to engage all stakeholders and pursues collaborative relationships with algal companies, academics, and community colleges as this program grows.

Weighted Project Score: 9.1

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

Photo courtesy of National Renewable Energy Laboratory

OVERALL IMPRESSIONS

- ATEC is garnering interest for and building algal industry-related community college curricula for a variety of skills and interest to support the industry by creating a skilled workforce. The team successfully met the original intention, goals, and objective of this project.
- This project is both workforce development and a forward-facing approach to support the integration of algal biomass in the bioeconomy. The program has been very successful in terms of the reach, expansion, and feedback of program participants. This type of outreach is also valuable to spur interest in the sciences at large to ensure a strong future for algal biofuel development.
- This is a great project with a well thought-out plan to get students and teachers more interested in algae.
- The focus of this project was to improve workforce development for the nascent algal industry through K–12 and community college education. The team presented an impressive list of accomplishments, spanning Massive Open Online Courses (MOOCs); K–12 science, technology, engineering, and math materials and coursework; and community college engagement. The team presented impressive enrollment in all programs and discussed successful outcomes from early participants, such as job offers, increased salaries, and college acceptances.
- The objective of this project is to develop and implement collaborative educational programs ranging from K–12 to community college degrees and extension short courses. The approach includes online education modules, extension training, and programs in community colleges. Each area includes courses and training programs. The community college effort, in particular, sets a program that is reviewed by an IAB and independent educational assessment team. In addition, the team is setting up micro and macro algal extension short courses targeting personnel already involved in the aquaculture industry. The approach is thorough and well thought out. The team has set up collaborations with nine community colleges and universities, including Santa Fe Community College and Austin Community College. It is notable that the online algal MOOC has received more than 3,500 students, with 17% reporting increases in pay. Further, each approach has gained acceptance in the community and is being promoted actively.

The program clearly supports BETO's efforts in helping create the workforce for the future of algae. The industry stakeholders are implementing and using the programs to educate and recruit employees. The team plans to continue the expansion of the ATEC courses to additional community colleges and have targets to reach K–12 students. The team anticipates completion of the algal biotechnology MOOC and an increased number of students taking the online modules. They will also market the extension training short courses. The team is setting up an external certification and endorsement program for college curricula. They plan to expand the curriculum into more specialized areas and expand their collaborative network. Future activities are deemed very appropriate.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers' overall impressions of their project.

HYDROTHERMAL LIQUEFACTION MODEL DEVELOPMENT

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

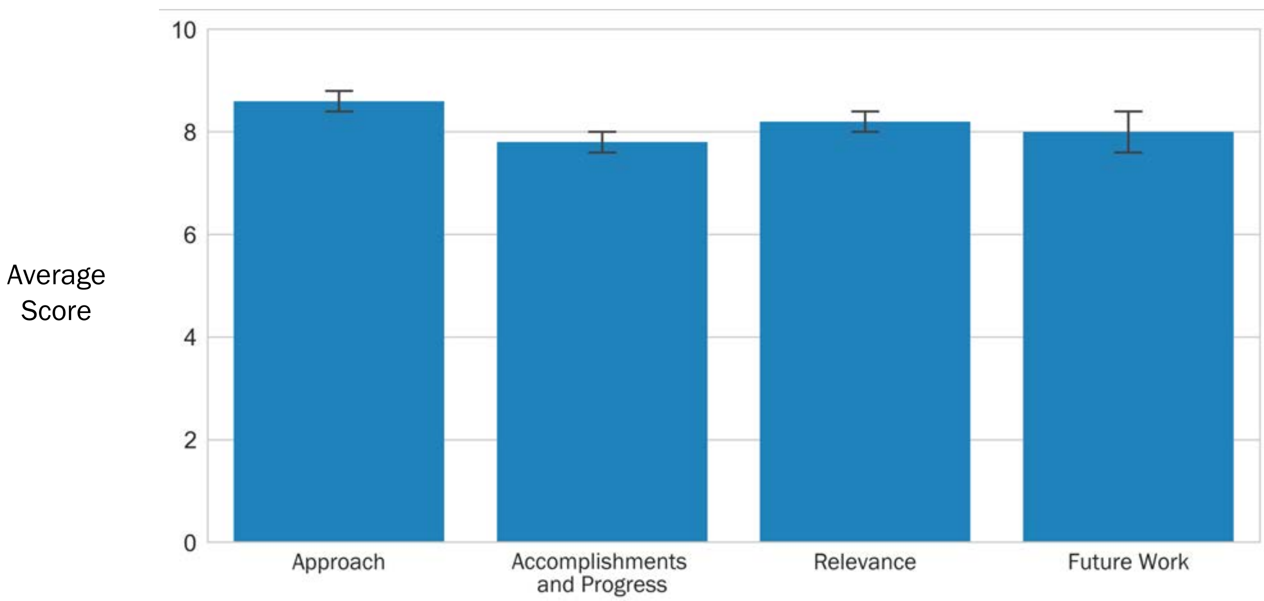
This project provides technical, economic, and sustainability analysis for algal conversion via HTL and upgrading to hydrocarbon fuels and chemicals to direct research toward high-impact results. HTL is a biomass conversion process that uses time, temperature, and pressure to produce a biocrude product that can be hydrotreated and distilled into hydrocarbon transportation fuels. A target conceptual biorefinery model was developed with researcher input and compared against benchmark models that incorporate currently achieved research results. This (1) identifies barriers, cost-reduction strategies, sustainability impacts; (2) helps to set technical and costs targets; and (3) tracks research progress.

WBS:	1.3.5.202
CID:	NL0025839
Principal Investigator:	Ms. Sue Jones
Period of Performance:	10/1/2017–9/30/2020
Total DOE Funding:	$800,000
DOE Funding FY16:	$0
DOE Funding FY17:	$0
DOE Funding FY18:	$600,000
DOE Funding FY19:	$200,000
Project Status:	Ongoing

A key outcome is the support for meeting the out-year conversion-only cost goals. The annual SOT is prepared reporting the modeled costs for that year and the associated research used in the modeling. Another outcome is the coordinated harmonized analysis between the PNNL work to estimate farm siting and scale; NREL analysis providing costs for cultivation, harvest, and dewatering this project; and ANL for sustainability considerations. Current and future work will focus on developing new conversion cost-reduction strategies to inform a revision to the original target case published in 2014. This will involve considering alternative HTL reactor and heat exchange configurations, valorization of aqueous phase carbon, and enhanced biocrude upgrading.

Weighted Project Score: 8.2

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

Data availability is a common challenge for all analysis projects. This is mitigated by frequent, close interactions with researchers to exchange information and review sustainable cost-reduction strategies. Collaboration with analysts at ANL, INL, and NREL enhance project effectiveness. Frequent communications with BETO technology leads ensure impactful outcomes. Disseminating results for use by stakeholders is achieved through publications and presentations.

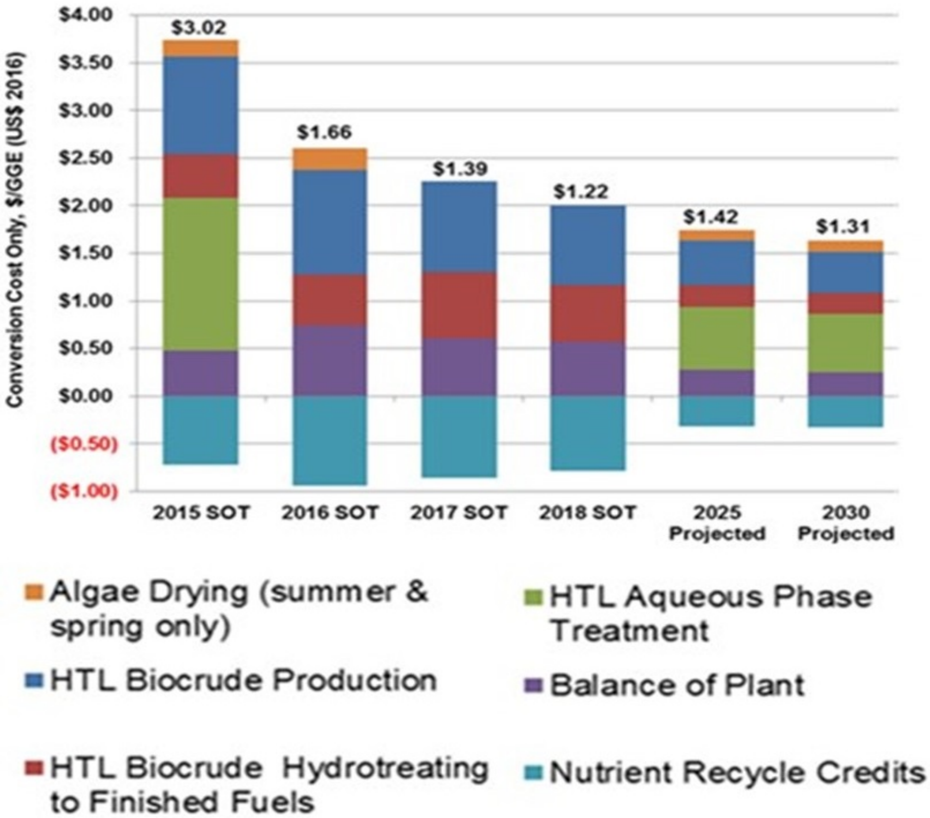


Photo courtesy of Pacific Northwest National Laboratory

OVERALL IMPRESSIONS

- This project appears to be managed well and is on track to meet stated objectives. The project has clear relevance to the algal industry and BETO's mission. The team is encouraged to remain focused on HTL when collaborating on related modeling efforts.
- This project applies broadly across the AAS portfolio and provides valuable guidance to directing research dollars to the biggest opportunities for improvement. As experimental data continues to be generated, it will be vital that this type of analysis continues to be performed in an iterative way.
- The group would like to incorporate a lot of data from other programs, which are essential for a robust model. Working closely with the researchers helps to strongly enforce or assist in identifying future research opportunities.
- The HTL model development team has successfully partnered with key stakeholders, making great progress toward the project goals. The inclusion of statistical tests of confidence is a strength of the accomplishments to date. The project will benefit from further inclusion of value drivers, such as the interaction of nutrient recycle and the impact on biomass quality. Overall, the support toward establishing the SOT with the inclusion of multiple scenarios will be highly valuable to achieving BETO's program goals.

- The project develops process and cost models for the HTL pathway that inform the annual SOT assessments. Thus, the project has a direct impact on the economic analysis published in the BETO MYP. The team takes a rigorous analysis approach using process models in AspenPlus and cost models in Excel. The team follows technology development for the HTL R&D (R&D) portfolios and uses data-driven approaches for their models and analysis. Therefore, the project relies on the quality of the data provided by the R&D portfolio in this area. This is seen as an excellent approach to understanding the progress in R&D based on quantitative assessments of the technology, which can provide the BETO program and R&D investigators with an analysis of the impacts of technology development and gaps in research. Since the last review, the team has published an Algae Farm Cost Model and worked on the SOT model for HTL conversion using three pathways. The team has also developed a predictive algal HTL model based on biomass composition and Monte Carlo uncertainty analysis. This project directly supports the algal processing pathway toward meeting the 2030 BETO performance goal. The work develops experimentally based modeled production costs indicating high-impact research areas for conversion. The models help advance the current knowledge on the SOT for HTL processes and provide a view to gaps and areas that need further analysis and development. The team plans to work on the HTL FY 2019 SOT assessment and HTL biocrude upgrading.
- No major weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We sincerely thank the reviewers for their time and efforts in providing critical evaluation and valuable feedback.
- We plan to incorporate experimental learnings to enhance predictive modeling and uncertainty analysis, identify cost drivers, and consider alternative processing options (such as multistage HTL) that lead to improved economic viability.
- We agree that leveraging the learnings and methods by researchers outside of PNNL is important. To that end, although we based our predictive model on a consistent set of continuous experiments conducted at PNNL, we used calculation methodology found in the literature and compared our results to published HTL models (which were based on batch HTL processing at a variety of conditions).
- The PBR cost model was a preliminary estimate to consider whether there might be an advantage over open ponds. Because of the limitation of real field data viability and the large variability in data from different sources, we concluded that more data are needed. A sensitivity study for key parameters based on expert suggestions and the literature search was included.
- We agree that ash content is important for the cost analysis and that different feedstock can have different ash contents. Our algal HTL model is on a dry ash-free mass basis and thus avoids the different ash content impacts on plant scale. This is consistent with the algal feedstock flow rate information provided by NREL, which is also at a dry ash-free basis. For HTL, much of the feed ash is soluble and ends up in the aqueous phase.
- Nutrient recycling testing work is ongoing, and in the future we will have more data available for improving our cost analysis regarding the nutrient recycling credits. For ongoing work, algal cultivation, harvest, and dewatering inputs are provided by NREL from their open pond model. We do not use the PBR cost model for annual SOT assessments and reporting to BETO.
- In our ongoing and future work, we are considering coproducts from HTL aqueous in single-stage and multistage HTL to offset the high cost for algal production.

MICROALGAE ANALYSIS

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

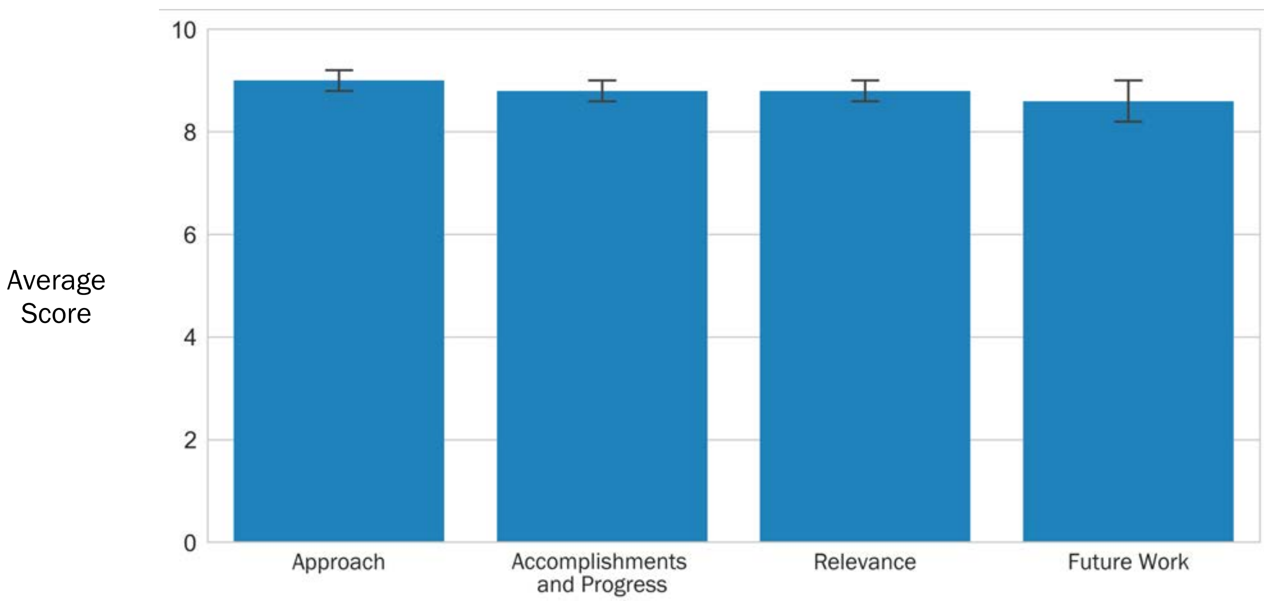
WBS:	1.3.5.203
CID:	NL0022302
Principal Investigator:	Dr. Mark Wigmosta
Period of Performance:	10/1/2016–9/30/2019
Total DOE Funding:	$2,550,389
DOE Funding FY16:	$800,389
DOE Funding FY17:	$600,000
DOE Funding FY18:	$600,000
DOE Funding FY19:	$550,000
Project Status:	Ongoing

Fundamental questions must be considered for the realization of algal biofuels, including where production can occur; how much nutrient, land, and water resource are required; how much energy is produced; where ideal production sites are located; as well as trade-offs inherent to algal enterprises. The Biomass Assessment Tool (BAT) is a biophysics-based analysis platform for BETO and industry research activities to achieve high-impact objectives. The BAT provided the first high-spatiotemporal, national assessment of potential open pond algal biomass production and water demand. Results from this study were referenced in a 2012 Presidential Energy Policy speech.

Accounting for “climatic conditions, fresh water, inland and coastal saline water, and wastewater resources, sources of CO_2, and land prices” in a “national assessment of land requirements for algal cultivation” is recognized by the National Research Council as a need for informing how algal biofuels could be produced economically in the United States (NRC 2012). The BAT, developed and advanced in this project, using high-resolution spatiotemporal data and models (i.e., 30-m x 30-m topography, soils, and land cover—43.3 billion locations on the landscape; hourly meteorological data; and biomass growth simulations), is used to address these questions. Multiple criteria must be considered, and the highest biomass production site might not be the “best” site because of infrastructure, resource, or economic constraints. The BAT is uniquely suited to this task

Weighted Project Score: 8.8

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers’ scores by considering suitable lands; hourly meteorology; fresh saline alternative water demand and supply; strain-parameterized biomass growth modeling in an open pond or PBR; waste CO_2 colocation and transport; electrical transmission lines; gas pipelines; road, rail, and barge transportation networks; known and quantified nutrient demand and available supply; and refinery infrastructure.

Results from this study are used to inform BETO's annual SOT and have resulted in 19 peer-reviewed publications of direct benefit to industry to evaluate optimal site locations, strains, and operations.

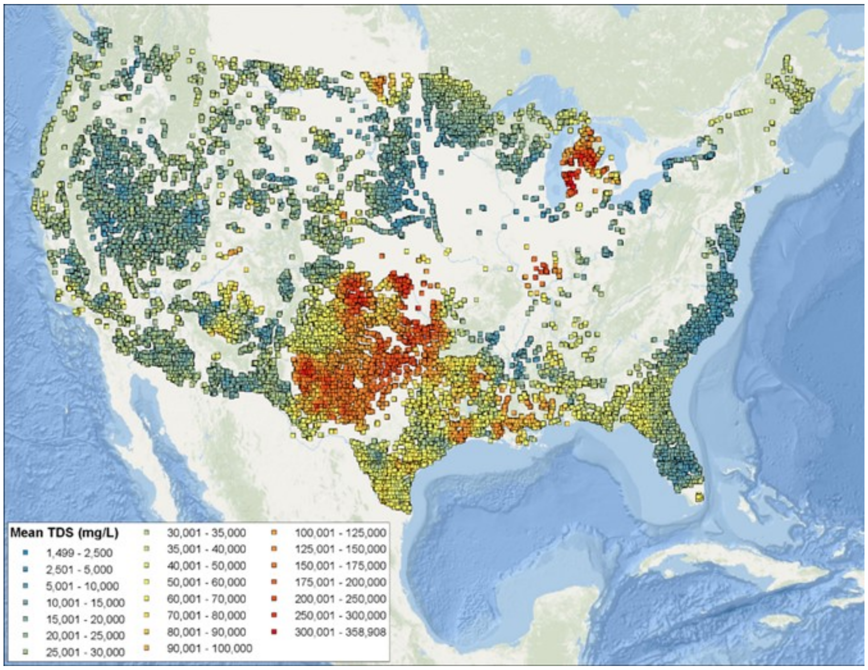


Photo courtesy of Pacific Northwest National Laboratory

OVERALL IMPRESSIONS

- The project is considering multiple important criteria that fall outside some other models currently being developed but that have great impact on deployment and environmental sensitivities, such as water demand, biomass growth, water supply, and CO_2 colocation. Detailed input of groundwater availability in addition to the reduction of water stress is a big step in the right direction. Incorporating actual groundwater salinity data and depth to salinity groundwater is a great tool for any future algal farm.

- The project appears to be managed well and is on track to meet its stated objectives. The team is commended for incorporating uncertainty in its modeling efforts. Challenges and limitations were acknowledged, and the team is committed to remaining relevant to the algal industry as well as BETO's mission.

- The BAT is an incredibly useful analysis tool relevant across the AAS portfolio. The tool strives to simplify the complexity associated with considering more than 15 factors that directly impact the cost of converting algae to biofuels. The locations that result in the highest biomass productions might not actually be feasible for achieving the BETO gallons gasoline equivalent goals given the other infrastructure and resource demands to grow, cultivate, and process algae.

- The use of modeling to optimize algal productivity by exploring both geographic placement and cultivation practice is critical to achieving the BETO MYP targets. As the available data set to feed the model continues to grow and becomes more relevant to commercial production, this project team is well positioned to incorporate these data and further refine the accuracy and utility of the tool. The team has set ambitious publication targets for the remainder of the project. Upon completion, the papers should be valuable to guide prioritization of future work in algal biomass production. There is an opportunity to explore advances in satellite technology in other fields to further enhance these modeling tools.

- The project aims to provide a national assessment of where algae production can occur, along with nutrient, land, and resource requirements. They have taken an approach that reviews the full U.S. landscape with superimposed geo-specific climate as well as resource supply and demand to map appropriate production strains through cultivation, conversion, and downstream logistics. The project addresses key barriers in biomass availability, cost, sustainable production, and resource management. The approach is to be sound and robust. Progress continues to be steady, with significant publications in the field. The team provides assessment tools for BETO that help link national resources to potential industry efforts in the cultivation and processing of algae. The project underpins national resources with key national and industry goals. This is seen as a vital part of the BETO algal program and is of great interest to stakeholders. Future work will assess sustainable collaborative BETO targets for FY 2020.

- There are no major weaknesses noted for this effort.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- For all aspects of the BAT, we develop components and a level of detail as BETO, MYP goals, and the algal research community requires. A systematic process is used to provide additional detail as the needs and data support increases. We agree that satellite imaging is a vital resource; our team has more than 20 years of experience using multimodal imaging and analytics in various domains, including land use/land cover, ground-truthing, and water resource assessments. Additional imaging applications will be investigated.

- We agree that highest productivity biomass locations might not be economically feasible because of required infrastructure. We addressed this in a collaborative study with Sapphire Energy and continue to address various aspects of this (2017 Algae Harmonization Study). We continue to evaluate PBRs and examine operational strategies to improve year-round biomass yield, along with alternative sources for water and nutrients, including colocation with wastewater treatment facilities and CO_2 emitters.

- Brackish/saline groundwater resources (>2.5 practical salinity unit) have the potential to reduce freshwater demand while providing a largely noncompetitive year-round supply, but they come with variable geochemistry and increased cost to acquire and manage. We used more than 500,000 brackish/saline groundwater well records to characterize depth, salinity, pH, and 18 nonorganic chemical constituents. Currently, depth and salinity data are used in strain-specific open pond productivity and blowdown calculations to quantify groundwater pumping demand/cost and volume of brine on a site-specific basis; costs for various brine disposal pathways are being investigated.

- We feel it is important to acknowledge challenges and limitations in our approach and capture of uncertainty in our results. Our evolving approach allows an end user to examine, with direct consideration of uncertainty, the likelihood that a particular action will achieve the desired result (e.g., MYP target) or which alternative shows the most promise.

DEVELOPMENT OF ALGAL BIOMASS YIELD IMPROVEMENTS IN AN INTEGRATED PROCESS

Global Algae Innovations, Inc.

PROJECT DESCRIPTION

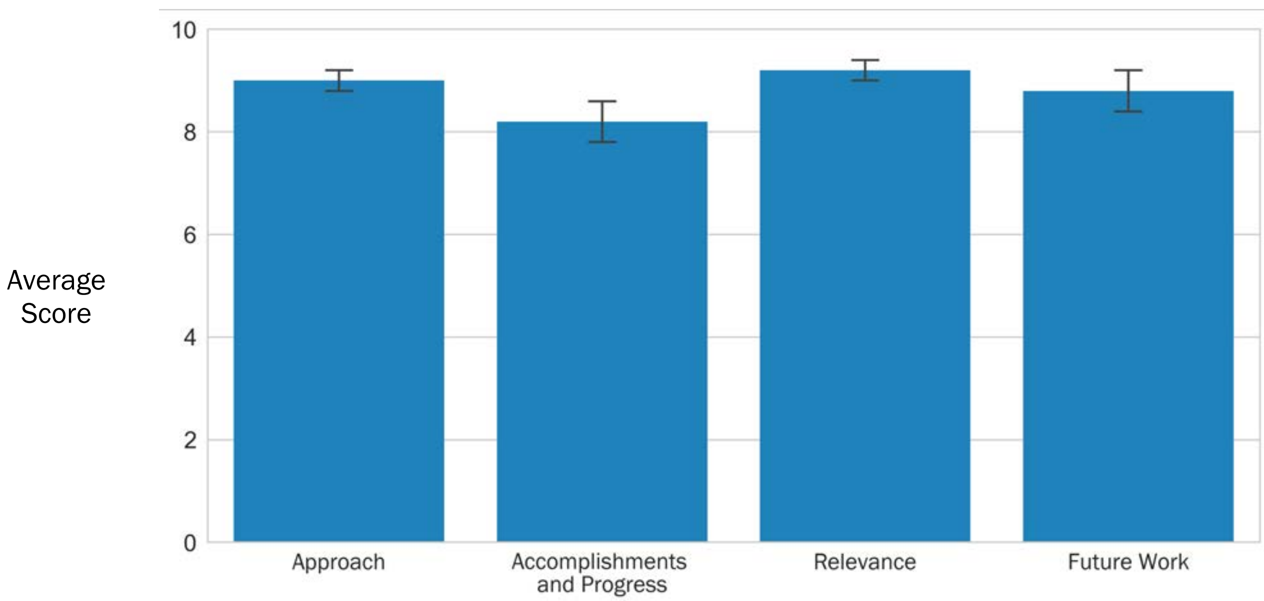
WBS:	1.3.5.211
CID:	EE0007689
Principal Investigator:	Dr. David Hazlebeck
Period of Performance:	10/1/2016–6/30/2019
Total DOE Funding:	$6,250,000
Project Status:	Ongoing

This project will build on the success of phase one of the GAI Algae Biomass Yield project to accelerate the commercialization of algal biofuels through the development of an integrated, economic, photosynthetic, open raceway system to produce algal oil. Two parallel pathways to a biofuel will be investigated. In the algal crude oil pathway, the dewatered algal biomass slurry is used as a feed to HTL to produce an algal crude oil and recycle aqueous stream. In the algal lipid oil pathway, the algal biomass slurry is dried and the oil is extracted to produce an algal lipid oil biofuel intermediate and a high-protein algal meal coproduct. Upgrading to drop-in fuels has been demonstrated for the biofuel intermediate in both pathways. Because the algal lipid oil pathway requires lipid accumulation, the productivity is generally less than the algal crude oil pathway, but the required productivity for economic algal biofuel production is also less because the product value is greater. Thus, we have separate biofuel intermediate productivity targets for each pathway. Our phase two targets for these two pathways are:

- 5,000 gallons per acre per year at a projected minimum lipid oil selling price of $1.60/gal
- 8,000 gallons per acre per year at a projected minimum lipid oil selling price of $4.10/gal.

Weighted Project Score: 8.8

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

The project team provides expertise across the full breadth of strain development, advanced algal cultivation, open raceway contamination control, CO_2 supply, harvesting, dewatering, extraction, HTL, TEA, and LCA. The approach is to combine best-in-class cultivation and preprocessing technologies with some of the world's leading strain development laboratories. The strain development task is bolstered by expertise from the Hildebrand laboratory at Scripps Institution of Oceanography and the Mayfield laboratory at the University of California, San Diego. TSD Management Associates provides expertise in CO_2 supply, algal harvesting and dewatering, and algal drying and extraction. Texas A&M University provides algal harvesting and extraction expertise. PNNL brings HTL technology, and GE Water and Power brings membranes technology. NREL rounds out the team with extensive expertise in algal TEA/LCA.

Our phase one project resulted in tremendous productivity and preprocessing improvements in an integrated, large-scale, low-cost cultivation and preprocessing process that has moved algal technology closer to economic viability for biofuels than ever before. Phase two combines these advances with better strains through teaming with world-class algal strain developers, additional open pond cultivation innovations, and a new drying and extraction unit operation to facilitate the development of a commercial algal biofuel industry.

OVERALL IMPRESSIONS

- The commercial ambitions of the lead participant in this team bring focus and relevance to the project objectives. TEAs are used to inform project priorities, and the project scope is aligned with the BETO mission and MYP goals. The team presented a strong technical and management approach, and most tasks are either complete or in the final stages of completion. The project was able to make several advances across the value chain and demonstrated these accomplishments in an integrated industrial environment.

- This holistic, integrated project is nearly complete and achieved all the original goals (in some cases, exceeded significantly) because of strong project management implementation. The Zobi harvester®, patent pending, is commercially available.

- The development of the harvester is a significant contribution of this work and directly focuses on reducing the cost of algal biofuel production. The team has employed several methods to improve strains and cultivation practices while developing laboratory and research pond systems designed to reflect commercial outdoor conditions. There is an opportunity to further explore the lack of translation of traits during field deployment that is critical to stability. Overall, this project is a huge success and exemplifies the opportunity in industry and academic collaborations.

- This is an interesting project with great results on non-GMO strain improvement tools. The two main tasks are completely disjointed, with the preprocessing energy task being essentially an introduction to the trademarked instrument.

- The goal of the project is to develop improved strains and cultivation methods to increase the algal biofuel intermediate yield by at least 70% and to develop a new drying and extraction technology to reduce the energy for downstream processing by at least 50% to work in an integrated outdoor system that reduces the projected minimum selling price of algal biomass by 20%. The approach benefitted from a rigorous technology assessment using cost models. The team used strain improvement by directed evolution, developed advanced cultivation methods, and evolved biomass harvesting and processing technologies to reduce costs and increase efficiencies. This is a very strong team that can appropriately take laboratory improvements into field-scaled cultivation quickly. In the strain improvement category, the team showed the ability to develop new strains with two to three times higher lipid and protein contents than wild type. Two strains were shown to have excellent temperature tolerance and high productivity of up to 29 $g/m^2/day$ with good lipid content of approximately 36%, which brings the overall projected productivity very close to the targets at 3,500 gallons per acre per year. Further, improvements in the Zobi harvester and processing systems showed an ability to reduce energy requirements by 97%. These reductions are substantial improvements in the SOT. The project directly supports BETO goals in increasing yields of biofuel intermediate and reducing the energy requirements for processing algal biomass. The project advances the SOT significantly in all categories it is working on. The Zobi harvester technology is being made available as a commercial product.

- Few weaknesses were noted for this project because it is still developing successfully. The main goal should be to take the strains into scaled cultivation to verify modeled productivities.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- The two main tasks have separate objectives, although they were integrated in that the strains and cultivation technology from the first task were used to generate the feedstock for the second task. The preprocessing energy task included both harvesting and extraction/drying. The extraction/drying included the development of multiple new unit operations that were combined in a novel way to obtain an additional 85% energy reduction in energy use relative to the reduction already attained in phase one of the Algae Biomass Yield project. The harvesting energy savings were based on the development and demonstration of the Zobi harvester technology, which is patent pending and being produced as a commercial product.

INTEGRATED LOW-COST AND HIGH-YIELD MICROALGAL BIOFUEL INTERMEDIATES PRODUCTION

MicroBio Engineering, Inc.

PROJECT DESCRIPTION

WBS:	1.3.5.243
CID:	EE0007691
Principal Investigator:	Dr. John Benemann
Period of Performance:	10/1/2016–9/30/2020
Total DOE Funding:	$5,000,000
Project Status:	Ongoing

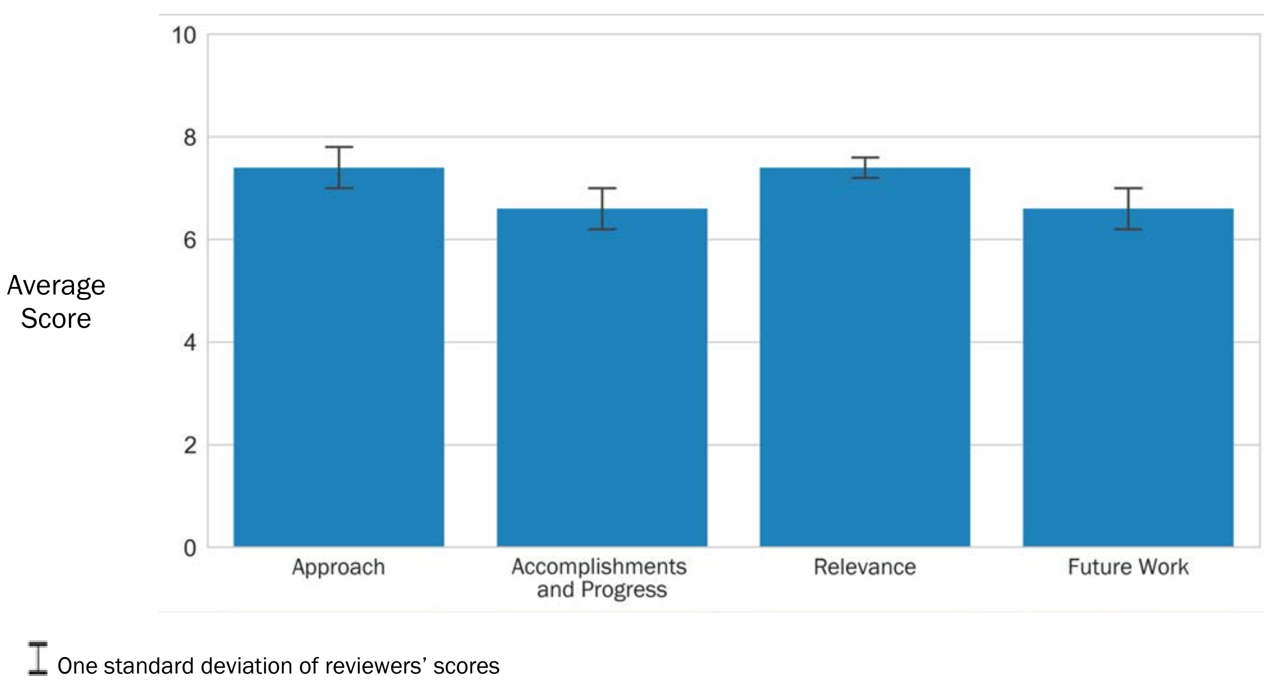
Improving algal biomass productivity and composition is required to meet BETO's MYP goals of 3,700 gal of biofuel intermediate per acre-year by 2020. This project will increase the baseline yields of wild-type algal cultures by integrating several innovative processes, including:

- Improving algal strains for increased biomass productivity and/or storage of oils or carbohydrates
- Increasing biomass productivity in open ponds by incorporating mixotrophic processes
- Producing biofuel intermediate by biomass extraction, fermentations, and anaerobic digestion.

Project collaborators with MicroBio Engineering Inc. (MBE) include California Polytechnic State University (Cal Poly) on strain improvements, outdoor cultivation, fermentations to ethanol and anaerobic digestion; SNL (Livermore) on genomic characterization of improved strains and protein fermentations; and Heliae Development LLC on mixotrophic cultivation of microalgae. Initial experiments by the MBE-Cal Poly team included screening 15 pure culture strains from culture collections and three native isolates in the lab for productivity and ease of handling. The eight most promising strains were further tested in outdoor wastewater ponds for productivity and culture robustness, defined as relative resistance to diseases, predation, and

Weighted Project Score: 7.0

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



competition from weed species. Three strains from the species *Scenedesmus* obliquus (DOE 0152z), *Desmodesmus armatus* (Utex B 2533), and *Tribonema sp.* (a new isolate) were identified as promising for further study and improvement. Biomass from *Scenedesmus* obliquus (DOE 0152z) and *Tribonema sp.* were used in protein and carbohydrate fermentations, respectively; and residuals from the carbohydrate fermentations were digested anaerobically to produce methane. In phase three of this project, baseline productivity, robustness, and composition (proteins, oils, and carbohydrates) of the wild-type will be compared to the improved strains outdoors.

Photo courtesy of MicroBio Engineering, Inc.

OVERALL IMPRESSIONS

- This ambitious project addresses a wide range of MYP goals, including as productivity, long-term cultivation, nutrient recycle, and whole biomass conversion. The team appears to be managed well, with a strong technical approach guiding project activities. The team is making strong progress on many of its original objectives. The team is encouraged to consider challenges encountered and TEA results to help prioritize future work.

- This project's integrated approach combines strain improvement, mixotrophic cultivation, and lipid extraction/fermentation downstream processing to achieve BETO's 2020 targets. If successful, it will provide more diversity to the AAS portfolio.

- The path to achieving improved algal productivity with reduced cost will require highly integrated approaches such as the one being followed in this project. The use of commercially relevant strains and focus on wastewater for cultivation is a strength for this work. It will be a significant challenge to pull together the many components of the project to achieve the goal of understanding integrated low-cost and high-yield production. This will be especially true as the improved strains enter outdoor testing. Overall, there is tremendous opportunity in this project that will be realized with strong project management support.

- This is a great project that is working to optimize several areas at once to reduce the cost of biomass production. Although all the areas the group is working on are important, there is ambiguity in how the tasks are approached and how the tasks interact with each other. The scope might be too broad for many tasks to be used to their full potential, such as the goal that states that optimization of strains will be done through non-GMO approaches, but there is a specific task that is using GMO approaches. Outdoor cultivation is getting great data on polyculture, which a novel approach to this project only, but the fermentation and HTL tasks are not using the polycultures that are being used in the field. Focusing on the tasks that are unique to this project would make the most impact on the industry.

- The goal of this project is to develop technologies that enable mature modeled annual average algal yields of 3,700 gallons per acre per year of biofuel intermediate by developing a non-GMO approach to produce strains with increased productivity for total biomass, or individually for proteins, carbohydrates, or lipids; and to demonstrate the long-term strain productivity and robustness in outdoor ponds on wastewater. The project will also leverage mixotrophy in algal cultivation to improve productivity and convert algal biomass to biofuel intermediate through fermentations of carbohydrates to ethanol and proteins to fusel alcohols and anaerobic digestion of the biomass residuals to biogas. This will be verified through TEA and LCA. This is a strong team led by industry with support from academia and national laboratories. The approach is sound and appropriate. Further, the team is progressing the goals of the project and providing appropriate quantitation through TEA/LCA modeling.

- Some weaknesses are noted in the approach to intermediates because multiple pathways are being taken for various fractions of the biomass that could interfere with each other.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We appreciate the positive feedback and recognition of the multifaceted nature of the project. Revisions have been made to streamline the overall work plan following the go-no-go project review. The TEA/LCA is being updated with a sensitivity analysis performed to provide a focus for future research efforts.

- The project has an integrated approach and includes multiple processes. Our project management is focused on keeping the integrative aspects on track for a successful project.

- Project management is a significant effort in this multifaceted project; monthly conference calls and routine in-person meetings and site visits have been employed to coordinate efforts between project partners. Further, task-specific (including strain improvement, pond cultivation, carbohydrate fermentation, and anaerobic digestion) meetings are held weekly to integrate workflow. Material for the peer review was based on results acquired up to the go-no-go project review, based on which the workflow was streamlined to focus on increased productivity for total biomass, which would also improve the productivity of specific biomolecules.

- Strain improvement through genetic engineering is not part of the current project; however, results from this project could be used to inform GMO approaches in the future. In this project, improved strains developed through a non-GMO directed evolution approach are tested in the laboratory and then moved outdoors for trials in open ponds. The focus of this project is on the improvement and validation of defined strains; therefore, the native polyculture serves as a control for productivity comparisons. Further, we used the polyculture as a resource for novel strains. For example, the *Tribonema* strain currently being used in the fermentation and anaerobic digestion effort was isolated from the native polyculture.

- The initial phases of this project focused on identifying suitable strains for improvement and testing approaches for improving the productivity of specific biomolecules. At the conclusion of these trials and following the go-no-go project review, the workflow was streamlined to focus on increased productivity for total biomass, which is necessary for the economic viability of large-scale biofuel production and would also improve the overall productivity of specific biomolecules. For example, rapid growth also leads to high protein content; therefore, the incorporation of selective pressure for enhanced faster growth and productivity is synergistic with improvements in protein content, a biofuel intermediate that can be fermented to produce fusel alcohols (as part of the SNL work).

PRODUCTION OF BIOCRUDE IN AN ADVANCED PHOTOBIOREACTOR-BASED BIOREFINERY

Algenol Biotech, LLC

PROJECT DESCRIPTION

WBS:	1.3.5.260
CID:	EE0007690
Principal Investigator:	Dr. Paul Roessler
Period of Performance:	10/1/2016–12/31/2019
Total DOE Funding:	$4,239,755
Project Status:	Ongoing

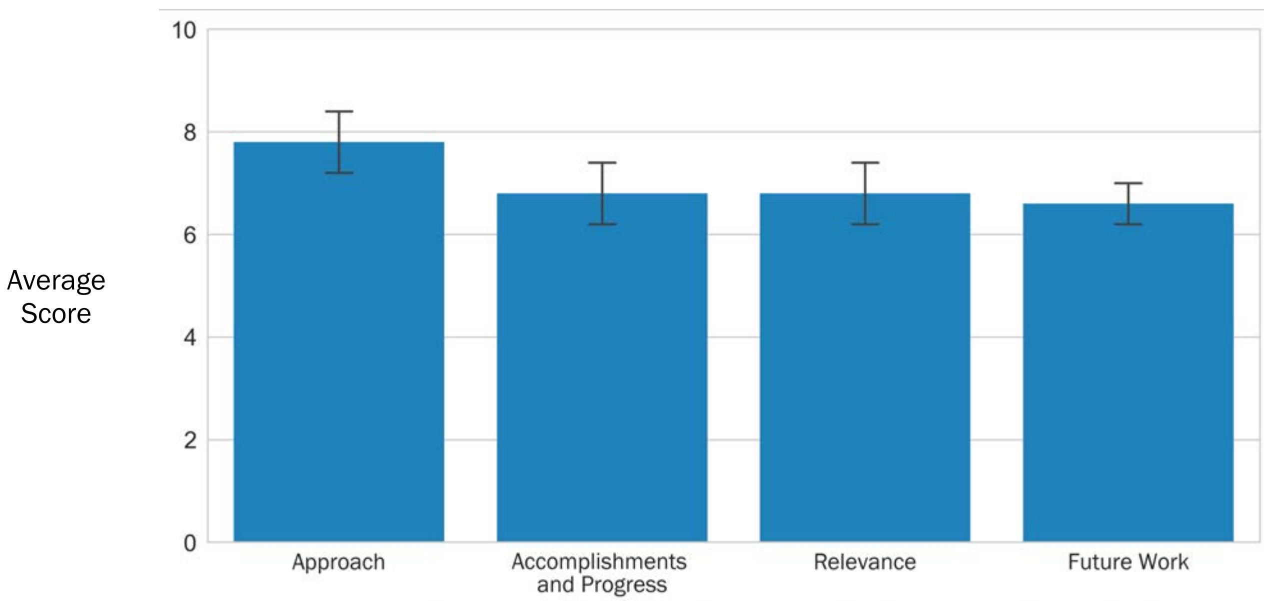
Algenol Biotech, NREL, Georgia Institute of Technology, and Reliance Industries have formed a team to advance the state of the art in algal production and downstream processing with the end goal a sustainable, economically viable biofuel intermediate (biocrude) product. The project includes examination of high-value coproduct production as a market entry strategy and to enhance the economics of a biorefinery for biofuel intermediate production. The project targets innovations in biology, operations, and engineering. It builds on the experience gained at Algenol in its DOE-funded project for an integrated biorefinery for ethanol production.

The goals of the project are: biofuel intermediate productivity greater than 4,000 gallons per acre per year of biofuel intermediate on an annualized basis; energy-efficient innovations in biomass harvesting, dewatering, and HTL, resulting in an energy expenditure less than 10% of the biofuel intermediate energy content and a carbon footprint reduction of more than 60% compared to fossil alternatives; and a comprehensive TEA that identifies limiting factors for commercial viability of a PBR-based biofuel product.

The project is on track to achieve the overall goals. Strain development efforts have resulted in the identification of a strain (AB1166) that, relative to existing strain AB1, exhibits a 10%–15% increase in productivity and also results in cultures with greater than 50% reduction in viscosity at low shear rates that

Weighted Project Score: 7.0

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

improve harvesting efficiency; these results represent the achievement of key phase two milestones. Progress has also been made in altering the biochemical composition of algal biomass to improve the yield and quality of biofuel intermediate produced via HTL. These strain enhancements plus improved outdoor cultivation practices, including semicontinuous operation, have increased areal productivity by nearly 80% over the established baseline productivity; this result greatly exceeded the 30% improvement metric required to achieve a “go” decision to enter phase three of the project. The annualized biomass productivity achieved (26.8 g/m^2/day ash-free dry mas), coupled with HTL conversion yields realized thus far (36% biofuel intermediate), results in 3,900 gallons per acre per year of biofuel intermediate, exceeding the FY 2020 BETO goal of 3,700 gallons per acre per year of biofuel intermediate.

Significant progress has also been demonstrated in PBR and large-scale production system design, operability, and cost reduction. A 24,000-L production module of interlinked PBRs was constructed and successfully operated. The productivities obtained convincingly demonstrated the scalability of laboratory measurements to large-scale outdoor operations covering a culture volume range of 2 mL to more than 20,000 L. The system was used to cultivate *Arthrospira platensis* (*Spirulina*), an industrially relevant cyanobacterium and source for phycocyanin, an approved blue food colorant that Algenol is developing as a risk-reduction strategy for future biofuel projects and as a potential business opportunity. A key project milestone to develop phycocyanin extraction and purification technologies was achieved ahead of schedule, and product samples have received positive feedback from potential customers.

The production and downstream operations data generated in this project are being used to conduct and refine TEA and LCA to provide research guidance to reduce the costs and environmental footprint of algal biofuel and coproduct manufacturing plants. These assessments will enable detailed comparisons of PBR versus open pond production systems.

The progress in this Algae Biomass Yield Phase Two project addresses many barriers identified for the AAS R&D program and are directly relevant to achieving the established BETO goals associated with large-scale biofuel production and cost reduction.

Photo courtesy of Algenol Biotech, LLC

OVERALL IMPRESSIONS

- The project appears to be managed well with an appropriate technical approach for project objectives. The project contributes to the BETO mission and MYP goals. Tasks include demonstration at meaningful scales in an industrial environment, and the team indicated that future coproduct development work will de-emphasize very niche products in favor of those with larger markets and moderate cost targets.

- Algenol is leading a fully integrated project to optimize algal strains, PBR configuration, downstream processing, and coproduct extraction to improve the economics of PBRs. The team shows strong project management coordination for such an ambitious project.

- The production of biocrude in an advanced PBR-based biorefinery project leverages significant commercial experience and enables access to experimentation at the industrial scale. The team has identified a very interesting strain with high tolerance to temperature that might have broad utility in algal biomass production. The project has established clear target goals with coproduct targets that will improve the economics of the process. The market opportunity for the coproduct and impact on potential for fuel production should be continually assessed to maintain commercial relevance.
- Focusing on the validation of results will add tremendous value to the project because currently there is much ambiguity on how the data are being generated and how their improvements are being measured.
- The goal of the project is to develop highly productive algal strains, a cost-effective PBR-based production system, enhanced cultivation practices, energy-efficient downstream processes, and a coproduct strategy that will advance the technology needed for economic, large-scale algal biofuel production. The approach taken includes strain development, cultivation engineering, downstream processing, and plant integration. A phycocianin coproduct intermediate supports the economic viability. This is a strong industry-led team with national laboratory and academic participation.
- A few weaknesses were noted that are associated with support of the modeling efforts with year-round cultivation at scale of one or two of the most productive strains.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Regarding the selection of phycocyanin as a coproduct, we fully recognize that the limited, albeit growing market would be saturated with a large-scale biofuel facility. As stated in the original proposal, phycocyanin production represents a market entry strategy. We expect the favorable economics of phycocyanin production to facilitate project financing for an initial, small-scale biofuel facility, which could be enlarged as the technology for biofuel production advances. Mid-volume, mid-value coproducts are also being evaluated at Algenol and will be incorporated as a scenario in our TEA modeling.
- With respect to the desire for further validation of results, productivity modeling, and general aspects of system performance, we would like to point out that the Algenol productivity model takes into account actual weather station data (light and temperature) and photosynthetic parameters derived from laboratory and small-scale outdoor experiments (quantum yield, photosaturation, light acclimation, respiration).
- Our outdoor growth data for *Arthrospira* is essentially year-round (at scales up to 24,000 L) and confirms the modeling results. By the end of the project, we will have data for *Cyanobacterium* from several seasons of the year and expect additional validation of our modeling approach. Further validation comes from year-round productivity data from past work with ethanol-producing *Cyanobacterium sp.* AB1. In addition, we have excellent agreement with PNNL with respect to laboratory performance parameters for AB1, the strain we provided to PNNL for inclusion in their DISCOVR project. The final project report will include extensive information on the development and validation of the productivity model, including indoor and outdoor testing at various scales and at several sites around the world (e.g., India, Arizona, Florida).

REWIRING ALGAL CARBON ENERGETICS FOR RENEWABLES (RACER)

National Renewable Energy Laboratory

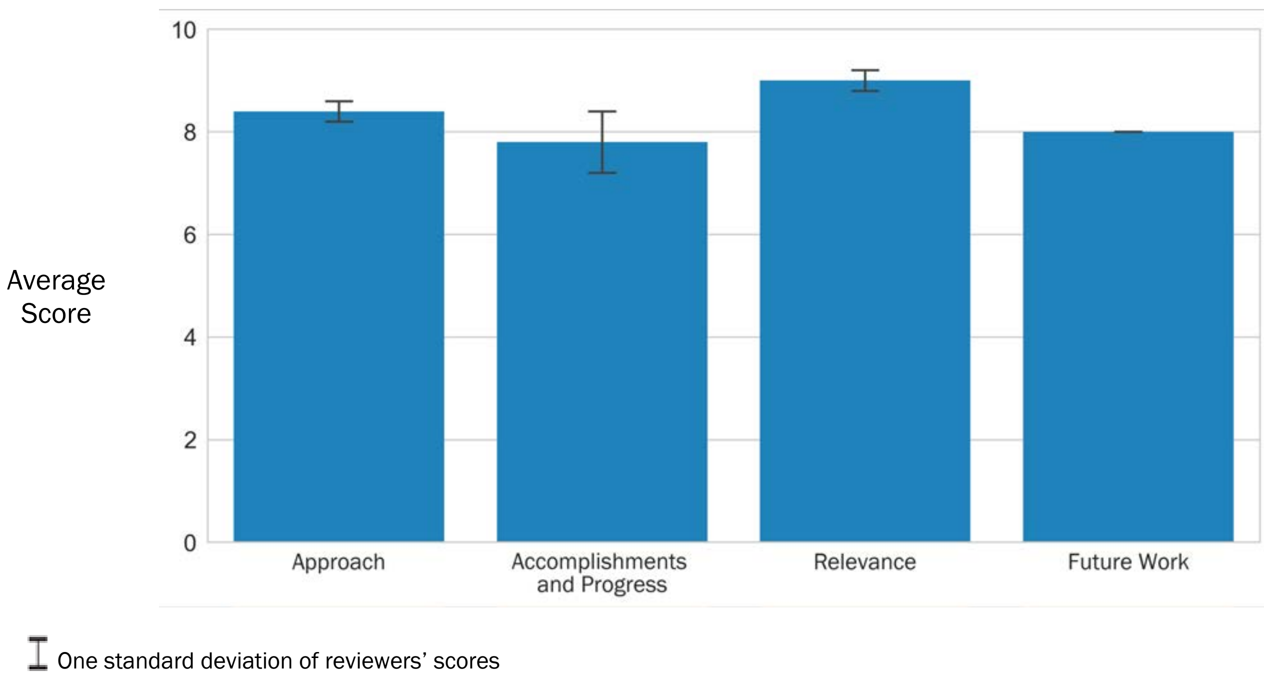
PROJECT DESCRIPTION

WBS:	1.3.5.270
CID:	NL0032697
Principal Investigator:	Dr. Lieve Laurens
Period of Performance:	8/1/2017–12/31/2020
Total DOE Funding:	$5,000,000
Project Status:	Ongoing

Critically needed improvements in biomass and biofuel intermediate productivity can be made by addressing fundamental inefficiencies in algal carbon conversion efficiency to biofuel feedstocks, cultivation performance, as well as the conversion to fuel intermediates. Algal photosynthesis is, at best, able to convert incident light energy to biomass at a carbon conversion efficiency of approximately 5%–7%, whereas downstream conversion to fuel intermediates in the current design process falls 15%–-25% short of its maximum potential because of inefficiencies along the pathway. There is room for improvement to achieve much higher biomass and biofuel intermediate yields through manipulating the basic biological processes of photosynthesis and carbon sink metabolism in the cells and integrating the upstream cell biology with downstream conversion process improvements. This presentation covers recent progress in the RACER project consortium and focuses on a means to address these carbon conversion inefficiencies in a pathway from algal biomass to a trifecta of fuel intermediates, ethanol, lipids, and green biocrude in a coordinated and integrated manner. The goal of this work is to engineer a single, commercially relevant algal species *Desmodesmus armatus* (SE 00107) to demonstrate both areal biomass productivity improvements of 64% relative to a conservative baseline of biomass productivity with an overall doubling of the fuel intermediate yields. The new algal biorefinery paradigm embodied in the RACER approach opens opportunities for algal engineering beyond efforts typically targeted solely at lipid content or improved light harvesting efficiency. We present progress on parallel

Weighted Project Score: 8.3

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



approaches toward improved (1) photosynthetic carbon conversion efficiency through the elimination of wasteful diversion of energy during photosynthesis and increasing carbon flux through carbon assimilation by increasing the transitory carbon storage in the cells; (2) outdoor operation and nutrient management strategies; and (3) fundamental operational efficiency of downstream conversion and extraction in a CAP approach to fuel and high-value product intermediates.

Photo courtesy of National Renewable Energy Laboratory

OVERALL IMPRESSIONS

- The RACER project aims to improve the productivity and biomass composition of a commercially relevant algal strain directly aligning with the MYP target goals. The team has overcome several challenges around the production of the transformants as well as production of biomass. The learnings from this project are likely as valuable as achieving the targeted goals. A clear strategy for 2020 including risk mitigation steps has been established by the team.
- This ambitious project clearly addresses BETO priorities and MYP goals. The team has been able to stay largely on track despite reductions in project budget. Accomplishments were explained in detail and matched original objectives. The team is encouraged to incorporate TEA in the prioritization of future tasks.
- This ambitious project attempts to integrate and coordinate strain engineering, cultivation operations, and conversion engineering of a robust, industrially relevant algal strain. Significant progress has been made to date, including contributing to the FY 2018 SOT less than halfway through the project.
- This is a good strategy to boost production. The multifaceted approach using multiple targets for improvement increases the chances for hitting strain enhancements. The timeline and goals to accomplish are well thought out and achievable within the period set forth. The project is yielding good results, but long-term yields are potentially being stunted because assumptions are being made for the amount of time that the culture might be induced for lipid accumulation at production scale.
- The team will improve the overall carbon-to-fuel intermediate productivity for a biorefinery using *Dromogomphus armatus* as a production species to reach at least 3,900 gallons per acre per year. This will be done through improvements in photosynthetic carbon conversion efficiency through random

mutagenesis and targeted engineering; cultivation management advances through the implementation of informed permutations of operations and nutrient management; and tailoring and optimizing conversion processes to extractable lipids, carbohydrate-derived fuel intermediates, and HTL biocrude from protein residue. This strain shows good productivity in year-round cultivations. The Sapphire project has made available the strain along with genome and transformation and mutagenesis protocols. The project tasks are specific and well defined. The transformation of *D. armatus* showing cNAT resistance and mCherry reported has been demonstrated by the team. The team has shown successes with overexpression transformants and has identified additional genes to effect photosynthetic apparatus. Mutagenesis has yielded one mutant with a 50% increase in lipid and no reduction in growth rate. The project's summer cultivation productivity has been included in the BETO FY 2018 cultivation SOT. The team has also demonstrated good efficiencies in fermentation and has engineered *Zymomonas mobilis* to metabolize mannose to 2,3-Butanediol. The team has demonstrated an extraction of lipids with a cosolvent and more than 30% yield on HTL of protein-rich stillage. They are working on the TEA/LCA of the integrated process. Progress to date is deemed reasonable. This is an end-to-end project aiming for carbon conversion efficiency improvements by coordinating photosynthesis and carbon sink engineering as the basis of biomass accumulation and biofuel production. The project is well aligned with BETO's AAS Program and likely to contribute significantly to additional improvements in the SOT. The project will continue to work on the major tasks and address milestones and has go-no-go decision points.

- No weaknesses are noted.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their complimentary feedback on this project. Several comments by the reviewers were reiterated, and we appreciate the opportunity to respond to some of the main questions, in particular on the integration of TEA in our decision-making process. When we planned this project, we included the TEA group to help prioritize different tasks. By targeting biomass productivity in the main tasks of this project, we directly address the primary factor driving the biomass selling price and, by extension, fuel selling price, one of the metrics called for in the Algae Biomass Yield Phase 2 FOA announcement. Further, the respective fraction yield improvements in the CAP (fermentation of soluble sugars, extractable lipids, and protein to HTL crude) that we are targeting in the conversion task were calculated to have a significant impact on the projected biofuel yield and price. We are continuously communicating progress with the TEA team, and a comprehensive analysis of the quantitative improvements achieved in the first phase of this project will be presented to the interim verification team.

- We want to clarify our experimental and cultivation approaches in that this work does not include a dedicated lipid induction phase during the outdoor cultivation experiments. The targeted metabolic engineering tools that are in the process of being developed will focus on genetic targets impacting central carbon assimilation and carbohydrate storage. It is thus much more likely that carbohydrates will accumulate prior to lipids, which will function more as overflow storage of metabolic energy. The outdoor cultivation approach on nutrient and pond operational management will include different permutations on the harvesting strategy, e.g., keeping the cultures at high cell density but low depth will increase light stress with the potential to rapidly shift the composition and maximize biomass productivity, both of which would be highly beneficial in the overall integrated process operations.

PRODUCING ALGAE FOR COPRODUCTS AND ENERGY (PACE)

Colorado School of Mines

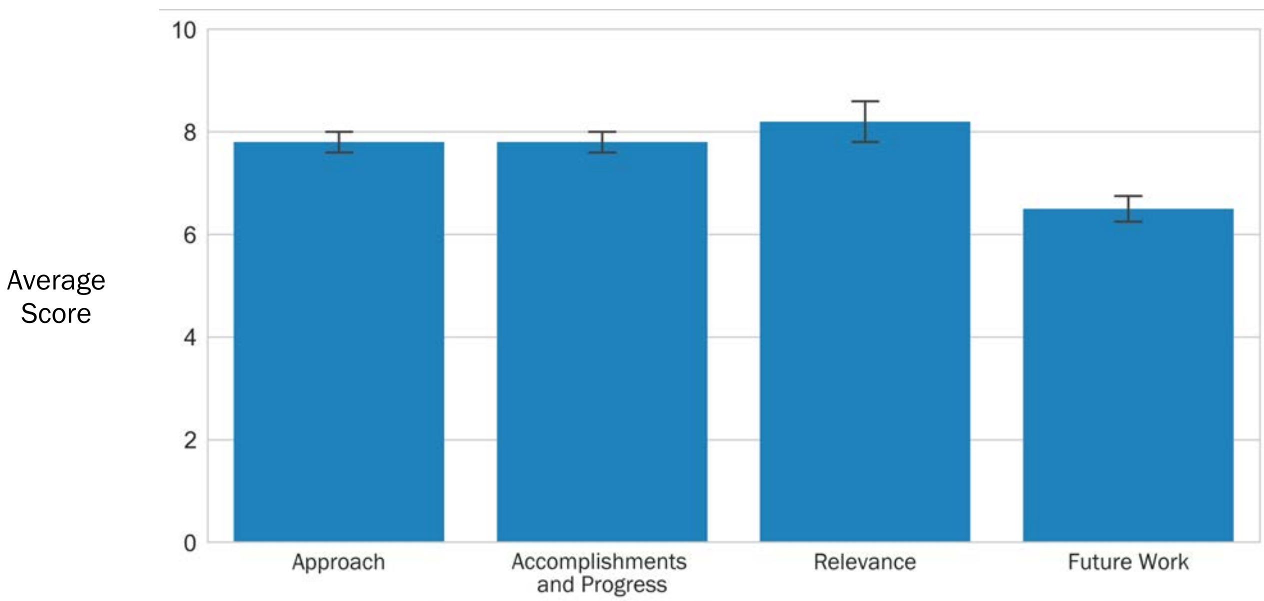
PROJECT DESCRIPTION

WBS:	1.3.5.300
CID:	EE0007089
Principal Investigator:	Dr. Matthew Posewitz
Period of Performance:	10/1/2015–3/31/2019
Total DOE Funding:	$9,000,000
Project Status:	Ongoing

Producing Algae for Coproducts and Energy (PACE) is a consortium of academic, industrial, and national laboratory partners that is addressing major technological challenges that remain for the sustainable and economic production of algal biofuels at scale. Beginning with the best technologies developed through the National Alliance for Advanced Biofuels and Bioproducts (NAABB) consortium, our overall objectives are (1) to reduce the cost of fuel produced from algae; (2) to enhance the stability and performance of integrated algal biofuel production systems at scale; and (3) to enhance the energy return on investment for algal biofuel production while reducing CO_2 emissions, water consumption, and demands for fertilizer supplements. Specifically, we are targeting improved biomass yields, the production of value-added coproducts, and the optimization of process integration. Reliance Industries is our primary cost-share partner, enabling access to their large and integrated algal biofuel facility in Gagva, India. Targeted goals include 25 g/m^2/day algal biomass yields, the production of coproducts (guar gum and phenylethanol), an energy return on investment of three, and a carbon index of 55 grams CO_2 per megajoule. At the Gagva facility, yields of approximately 20 g/m^2 can be achieved and integrated with harvesting and processing to algal biocrude. Liter quantities of photoautotrophic algal biocrude are readily being produced, and a suite of processing approaches are being investigated for conversion to fuels and engine testing. The NAABB identified the green alga *Chlorella sorokiniana* as a promising algal biofuel candidate. The PACE consortium has developed a suite of genetic manipulation tools to enable *C. sorokiniana* strain engineering. Overexpression of sucrose nonfermenting (SNF)-related kinase

Weighted Project Score: 7.6

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

(SNRK) in *C. sorokiniana* resulted in lines with elevated rates of photosynthetic activity and higher levels of starch accumulation relative to wild-type control cells in solar-simulating environmental PBRs. A TERA was submitted and approved by the Environmental Protection Agency. Testing of this promising engineered strain for improved biomass phenotypes at the AzCATI is anticipated in 2019. Putative genes for the generation of the coproducts guar gum and phenylethanol were transformed into *C. sorokiniana*, and phenotypes are being analyzed. Multiple lines produced phenylethanol, and process conditions are being optimized to maximize yield. Strains expressing phosphite oxidoredutase and Pyrroline-5-carboxylate synthase (P5CS, a key enzyme controlling proline levels) were successfully attained, and phenotypes show potential applications in crop protection. The PACE consortium is also investigating marine species of *Nannochloropsis* that have established genetic tools. Constructs overexpressing a suite of carbonic anhydrases, bicarbonate transporters, and Calvin-Benson cycle enzymes were successfully generated, and biomass phenotyping is underway. In sum, multiple hypotheses are being tested to engineer biomass improvements. Kilogram quantities of algal biomass are being generated and used to optimize the processing of biomass to fuels, including two-stage hydrothermal processing to recover carbohydrates. Additionally, a suite of promising coprocessing and blending approaches are being investigated to generate transportation fuels. Last, an end-to-end PACE Aspen model for fully integrated TEA and LCA was developed.

Photo courtesy of Colorado School of Mines

OVERALL IMPRESSIONS

- This broad partnership includes academic, federal laboratories, and industry working together to address a set of barriers that is clearly aligned with BETO priorities and MYP goals. The team has been able to accomplish a wide range of its planned tasks, including strain improvement and demonstration in an outdoor industrial environment. An ambitious set of activities remains prior to project completion, and the group believes a no-cost extension can be used to accomplish all tasks.

- Despite encountering many roadblocks, the PACE project has now delivered on multiple fronts toward the MYP targets. The access to extracted oil from a large-scale facility is a great asset to this program. Several high-potential modified strains have been developed to improve productivity, enhance crop protection, and produce potentially valuable coproducts toward economic targets of algal biofuel production. The team now aims to stack these traits and run field trials to validate the observed laboratory performance. Given the progress made to date, I expect that the team can deliver on the significant challenges ahead.

- The project has made good progress in the genetic modification field, yielding much higher growth rates and carbohydrate production. Focus on validation of results will add tremendous value to the project.
- This ambitious project set out to investigate multiple aspects along the process flow (genetic engineering, cultivation, harvesting, and downstream processing) to increase algal biomass production to more than 25 $g/m^2/day$ while reducing costs by more than two times. Significant results have been demonstrated (and the remainder are planned to be completed by the end of the extension), whereas other objectives have been de-emphasized because of time and resource constraints (which is not entirely surprising given the enormous scope of this project).
- The main goal of the PACE project is to improve areal biomass productivities and enable the production of high-value coproducts using strain engineering. Further, PACE will integrate the cultivation and processing steps and demonstrate a reduction in the overall cost of algal-derived biofuel. The project set several objectives and metrics to determine progress and success factors. This is a large effort, under the TABB FOA, with a very strong partnership including academia, national laboratories, and industry. The management team demonstrated good practices for regular meetings and reporting. The team efforts addressed technical challenged in genetic transformation, gene stacking, producing biofuels and valuable products simultaneously, crop protection, and efficient harvesting. The success indicators for the project are clearly delineated and measurable. The effort is very well defined, and the team is deemed as very strong, involving key players in all areas of performance.
- Several areas deemed as possible weaknesses in the approach and future efforts are noted. The team is encouraged to develop focused strategies that can determine potential failures and develop mitigations, if necessary.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the program reviewers and DOE for their time and constructive advice regarding the PACE project. As noted by the reviewers, the national laboratory, academic and industrial partners on the research team have made significant progress in several challenging areas and MYP goals. Specifically, several successful strain engineering targets were achieved, and these strains are enabling the consortium to test strategies aimed at biomass yield improvements, coproduct production, and enhancing culture stability. Additionally, promising and novel culturing, processing, and fuel conversion strategies are being developed. Comprehensive TEA/LCA demonstrate that we are now approaching several of the project's programmatic economic targets. As noted by the reviewers, trait stacking and outdoor testing/validation of engineered strains remain a significant challenge. The SNRK expression line has been approved for outdoor growth at AzCATI, and these trials are currently being planned and initiated. Trait-stacking efforts are ongoing with a focus on P5CS/SNRK. A primary objective in the summer trials is to attain outdoor growth data for SNRK grown at AzCATI and determine whether enhanced biomass yields are attained from this transgenic line. These areas are among the primary foci of the no-cost-extension period, and we are confident that we will be able to insert multiple genes into *C. sorokiniana* (trait stacking) and test an engineered strain in the AzCATI outdoor test bed. In sum, many programmatic goals have been attained, and the no-cost-extension period should enable the completion of most of our remaining objectives.

MARINE ALGAE INDUSTRIALIZATION CONSORTIUM (MAGIC): COMBINING BIOFUELS AND HIGH-VALUE BIOPRODUCTS TO MEET RFS

Duke University

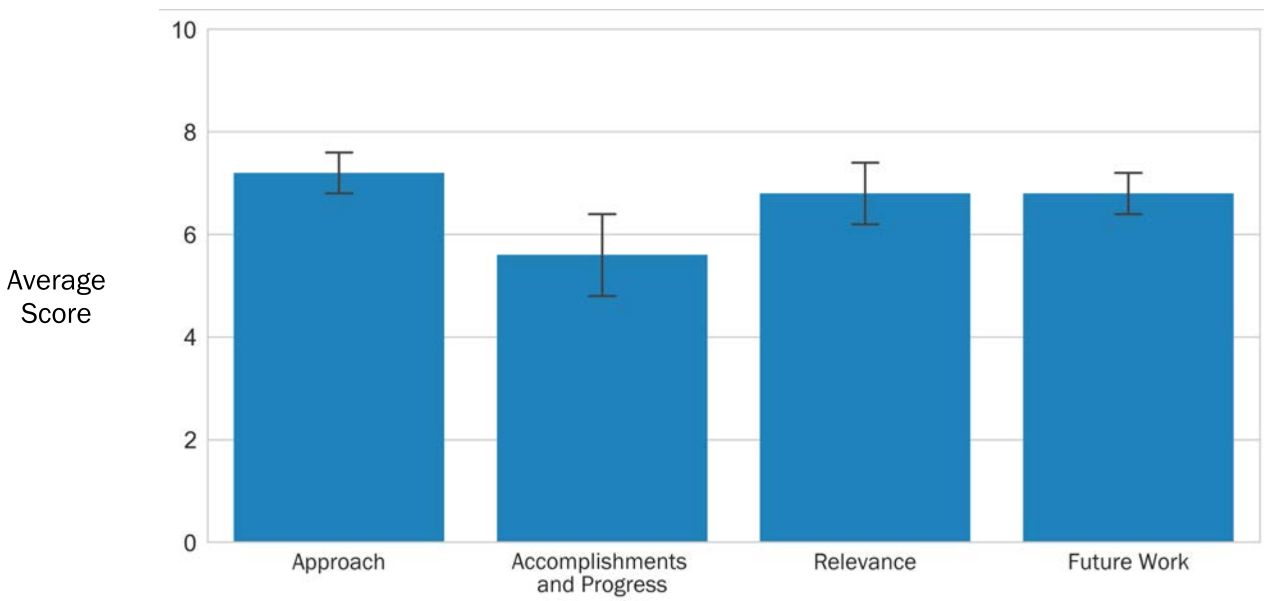
PROJECT DESCRIPTION

WBS:	1.3.5.310
CID:	EE0007091
Principal Investigator:	Dr. Zackary Johnson
Period of Performance:	10/1/2015–8/31/2019
Total DOE Funding:	$5,240,313
Project Status:	Ongoing

The objective of this project is to demonstrate, using a multiproduct commercialization path, an algal biofuel at commercial scale with a positive energy return that achieves the Renewable Fuel Standard, (RFS), and sells at a price of less than $5/GGE. The approach is founded on our achievements to date in algal biofuel and coproduct development, and it is aimed at increasing the overall algal product value (and thereby decreasing the net cost of algal biofuel). This project is driven by product specifications in four markets, including (1) drop-in fuels, the foundational product; (2) a salmon feed ingredient equivalent to fishmeal in protein content and biochemically superior to soy and other protein meals; (3) a poultry feed ingredient that is superior to commonly used soymeal in protein content and contains other important micronutrients; and (4) a dairy food replacement for human food, equivalent in protein content to soy, rice, and other plant-based replacement products but superior in the content of essential fatty acids and micronutrients. All three coproducts approximately scale with fuel and thus are viable solutions on the commercial scale. To demonstrate product value for each product, we will (1) define product specifications and commercialization opportunities in collaboration with commercial partners; (2) match product specifications for these products as closely as possible to marine algal strains from our highly characterized collection of hundreds of strains; (3) cultivate selected strains at the bench scale to

Weighted Project Score: 6.6

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%



One standard deviation of reviewers' scores

provide a comprehensive assessment of their performance against product specifications; (4) cultivate, harvest, and process approximately 10 strains in quantities of approximately 50 kg per strain, providing ample material for product development trials; and (5) continuously evaluate commercialization potential by using economic and LCA iteratively as a design tool at each stage to guide product development. Results from this project will have a substantial impact on the environmental and economic benefits of algal biofuels. The proposed bioproducts, to the extent they replace current feed and energy crops, would also confer a significant increase in benefits with regard to recognized greenhouse gas emissions by virtue of the positive effect of indirect land use change brought about by the 50-fold land-use intensification achieved by algal production. Core members of this consortium—who have during the past 10 years taken the initial leading steps in product development and demonstration with substantial industry and government support—are joined by members with specific commercial interests in the development of biofuels, aqua feeds, poultry feeds, and human foods. The combined abilities and experience of this proposed consortium position it for success and will broadly benefit the U.S. algal industry, from growers of algae, to developers of harvesting and extraction tools, to end users of bioproducts.

OVERALL IMPRESSIONS

- This project identified a broad set of goals that were directly related to the BETO mission and MYP goals. Many tasks remain prior to project completion, and the group plans to use a no-cost extension and several mitigation steps to complete the work. The team is encouraged to report quantitative productivity and coproduct targets (value and specifications) to enhance the relevance and future value of their work to the broader algal community.
- The project seems to have a thorough process for validation. An assessment of the barrier to enter certain markets and the cost required to meet certain product specification is lacking detail.
- This project is identifying, demonstrating, and validating high-value coproduct production by investigating optimizations along the entire process chain (from strain selection, to cultivation, to end-use applications). Preliminary results are encouraging, but significant gaps will require more trials and market research of the intended end use to build a meaningful TEA/LCA model.
- The MAGIC team has worked through many challenges associated with a complex project spanning geographies and objectives. The construction and operation of the research ponds at Duke University is a highlight of the work to date. The ability to produce both animal feed and fuels from algal biomass significantly improves the economics and near-term commercialization opportunity. The team has identified promising strains with the potential of improving animal feed quality and has now initiated the feeding trials. The results of this work will be critical to understanding the value and economic potential of this approach.
- The main goals of the MAGIC consortium are to increase the valuation of lipid-extracted algae by testing across algal strains and coproduct types. Specifically, the team will develop 10 strains to meet product requirements, produce 30 kg–50 kg of 10 strains for algal feedstock for biofuels and feed ingredients, assess product efficacy and value, and analyze the potential of commercial-scale facilities. The team comprises academic and commercial partners. The team developed a method for ranking strains according to key variables, e.g., growth rate, sinking index, along with ash, lipid, and protein content. Ten top strains were selected from a large culture collection for scaled cultivation. The team delivered more than 120 kg of biomass of four strains. Product characterization and assessment were performed for three strains; two of these strains had poor recovery. An Xcel-based formulator was used to develop optimal feed compositions for further testing and analysis. TEA/LCA were performed on the strains. The results showed large variabilities in the final values.
- Several weaknesses were noted, mostly associated with the lack of optimization of processes, which causes large variations in TEA/LCA analyses.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

The recipient choose not to respond to the reviewers’ overall impressions of their project.