ADVANCED ALGAL SYSTEMS

TECHNOLOGY AREA

CONTENTS

INTRODUCTION

Six external experts from industry, academia, and other government agencies reviewed a total of 39 projects during the Advanced Algal Systems session. This review addressed a total U.S. Department of Energy (DOE) investment of approximately $115,631,328, which represents approximately 16% of the Bioenergy Technologies Office (BETO) portfolio reviewed during the 2017 Project Peer Review. During the Project Peer Review meeting, the principal investigator (PI) for each project had 30 minutes to deliver a presentation and respond to questions from the Review Panel.

Reviewers evaluated and scored projects for their approach, technical progress and accomplishments, relevance to BETO goals, and future plans. This section of the report contains the results of the Project Review, including full scoring information for each project, summary comments from each reviewer, and any public response provided by the PI. This section also includes overview information on the Advanced Algal Systems Program, full scoring results and analysis, the Review Panel Summary Report, and the BETO Programmatic Response.

BETO designated Daniel Fishman, Technology Manager, as the Advanced Algal Systems Review Lead. In this capacity, Mr. Fishman was responsible for all aspects of review planning and implementation. Lead Reviewer, Dr. Eric Jarvis, prepared the Advanced Algal Systems Review Panel Summary Report with support and contribution from the entire Review Panel.

ADVANCED ALGAL SYSTEMS OVERVIEW

The role of the Advanced Algal Systems Program is to fund the research and development (R&D) of sustainable algae production, logistics, and conversion to biofuels. Projects within the portfolio 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 co-products, techno-economics, sustainability, and resource availability.

Advanced Algal Systems R&D focuses on demonstrating 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 macro-algae, as well as cyanobacteria. Algal feedstocks include concentrated whole algae 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 Strategic Goals

The strategic goal of Advanced Algal Systems R&D is to develop algae production and logistics technologies that, if scaled up and deployed, could support the production of 5 billion gallons per year of sustainable, reliable, and affordable algae-based advanced biofuels by 2030. The strategic goal directly addresses and supports production of algal feedstocks for use by all potential conversion pathways to both biofuels and bioproducts.

Advanced Algal Systems Support of Office Performance Goals

The Advanced Algal Systems performance goal is to increase the projected productivity of large-scale algae

cultivation and preprocessing while maximizing efficiency of water, land, nutrient, and power use to supply a stable biofuel intermediate for conversion to advanced biofuels. Specifically, the program will validate the potential for algae supply and logistics systems to produce 5,000 gallons of oil (or an equivalent biofuel intermediate) per acre of cultivation per year at the pre-pilot scale by 2022; this will achieve a modeled nth plant minimum selling price of $3.00/gasoline gallon equivalent (gge) of algal biofuel. For details on the technology area goals, please review BETO's 2016 *Multi-Year Program Plan* (MYPP).

Advanced Algal Systems Approach for Overcoming Challenges

The Advanced Algal Systems approach for overcoming challenges and barriers is outlined in its work breakdown structure (WBS), organized around five key activities. Current activities are focused on (1) assessing current and potential sustainable biomass feedstock resources and corresponding costs; (2) developing improved algal strains and sustainable feedstock cultivation systems; (3) improving the capacity and efficiency of harvesting, preprocessing, storage, and handling; (4) characterizing algae to interface appropriately with conversion methods; and (5) scaling integrated algae R&D systems. These activities are performed by national laboratories, universities, industry, consortia, and a variety of state and regional partners.

ADVANCED ALGAL SYSTEMS REVIEW PANEL

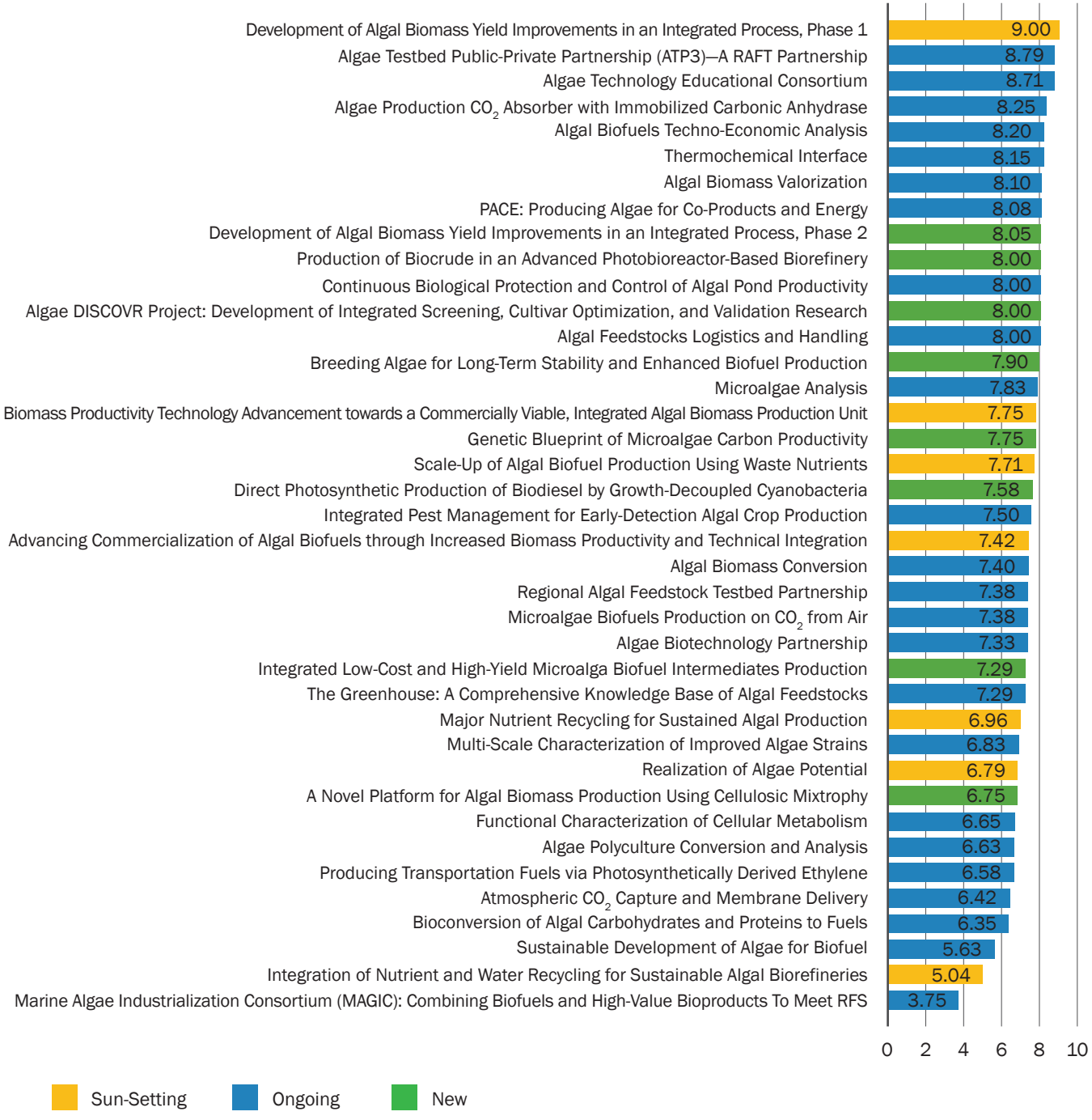
The following external experts served as reviewers for the Advanced Algal Systems Program during the 2017 Project Peer Review.

Name	Affiliation
Eric Jarvis*	Independent Consultant
Toby Ahrens	U.S. Department of Agriculture, National Institute of Food and Agriculture
Louis Brown	Synthetic Genomics
Bill Crump	Leidos
Sarah Smith	A.E. Allen Laboratory, Scripps Institution of Oceanography
Rebecca White	Qualitas Health

* Lead Reviewer

TECHNOLOGY AREA SCORE RESULTS

Average Weighted Scores by Project



ADVANCED ALGAL SYSTEMS REVIEW PANEL SUMMARY REPORT

Prepared by the Advanced Algal Systems Review Panel

Overall, the projects within Advanced Algal Systems have demonstrated excellent productivity over the past 2 years. The projects are well aligned with the goals of BETO's MYPP and show a high degree of diversity, touching on all of the critical technology barriers. High levels of innovation are evident in many of the projects, and there is an excellent balance between lower-risk, incremental reward and higher-risk, potentially transformational approaches.

BETO has effectively molded the research directions of the ongoing projects through the guidance of multiple funding opportunity announcements (FOAs). This has led to significant changes in emphasis since the last review, including a focus on marine strains, increased attention to co-products, and novel approaches for atmospheric carbon dioxide (CO_2) utilization. An across-the-board emphasis on reaching productivity targets is evident.

The three top-scoring projects reflect the overall diversity of the portfolio:

- The first of three presentations by Global Algae Innovations Inc. (GAI) outlined its Advancements in Algal Biomass Yield (ABY1) project, which targeted improved strains, cultivation, and novel harvest/dewatering technology to demonstrate an integrated process at large scale. The work was tightly linked to GAI's cost models and demonstrated significant progress toward BETO goals in terms of biofuel intermediate yield and reductions in preprocessing energy. Specific outcomes include commercialization of the Zobi Harvester® dewatering system and claims of a modeled $3.33/gge minimum fuel selling price (MFSP) for GAI's integrated process.
- The Algae Testbed Public-Private Partnership (ATP3) stood out as an outstanding resource for the entire community. The team has served many stakeholders and provided high-quality, multi-regional, long-term growth data to establish the BETO state of technology (SOT). Methods development, standardization, and harmonization have been critical. ATP3 is one of several foundational projects that support the program, including the National Renewable Energy Laboratory's (NREL's) Algal Biofuels Techno-Economic Analysis and Algal Biomass Valorization and Pacific Northwest National Laboratory's (PNNL's) Microalgae Analysis projects.
- The Algae Technology Educational Consortium run jointly by NREL and University of Southern Maine scored highly as filling an important role for the algal biotechnology industry. By working to establish degree programs and coursework through academic partners, the project has already made progress toward satisfying industry's need for trained workers.

The Advanced Algal Systems Program is on track. Over the coming years, it will be critical to maintain momentum, particularly in productivity improvements and identification of viable co-products. Improvements in agronomic practices at scale and strengthening of connections between researchers and industry will also be critical for establishing a viable algal biofuels industry.

Impact

The projects BETO is funding are clearly advancing the SOT, and future work planned should continue to move the industry toward BETO's targets. Extensive work has been completed over the past 2 years to solidify the current SOT, both through ATP3's long-term productivity data and through NREL's in-depth techno-economic analyses (TEAs). The unavoidable conclusion from this work is that achieving $/gge MFSP targets will require significant advances across the entire value chain and must include valorization of other components (i.e., co-products). Private-sector investment cannot yet substitute for government funding because of the degree of risk that must still be overcome to achieve cost-ef-

fective algal fuel production. For the foreseeable future, it is appropriate for BETO to continue funding projects at a variety of technology readiness levels all the way through large-scale, pre-commercial deployment.

Projects in three main areas stood out as having the highest impact in the past or are expected to have high impact over the next 2 years:

- A few annual operating plan (AOP) projects led by national laboratories serve as a foundation for all of the other projects in the portfolio. These projects have enabled an in-depth understanding of the current SOT and have brought much needed standardization of methods. Stand outs include Arizona State University's (ASU's) ATP3 testbed and NREL's Algal Biofuels Techno-Economic Analysis and Compositional Analysis projects.
- Three industry-led deployment projects are seeking to demonstrate an integrated process at scale. The top-scoring project was GAI's ABY1, which appears to have demonstrated particularly impactful advances in productivity and harvesting/dewatering and claims to be well on the way to meeting BETO's targets.
- Given the understanding that co-products will be critical to meeting cost targets, several pursuits may be poised for high impact. This includes projects such as NREL's Algal Biomass Valorization and Algal Biomass Conversion, Algenol's Photobioreactor-Based Biorefinery, and the Colorado School of Mines-led Producing Algae for Co-Products and Energy (PACE) consortium.

Innovation

A high level of innovation was evident within the 39 projects presented. The flexibility of BETO's target-driven FOA process has been very successful in attracting innovative approaches. Some of the research areas that stood out to the Review Panel as being particularly innovative include the following:

- **Strain improvement:** Many investigators reported incremental but significant improvements in productivity. However, innovative tools and datasets coming online have the potential to greatly accelerate this progress. Several labs are exploring promising Cas9 methodologies, which may even allow the generation of non-genetically modified organisms engineered strains. Other advances—such as rapid strain screening methods, cell-sorting capabilities, classic breeding techniques, functional genomics databases, etc.—will further accelerate progress.
- **Carbon capture:** Several approaches to improving carbon capture are being explored, including a large-scale GAI absorber unit, ASU's membrane capture system, University of Toledo's high alkalinity cultivation, and PNNL's carbonic anhydrase studies. The potential for these innovative approaches to enable atmospheric CO_2 capture, if successful, could be transformational in relieving siting constraints.
- **Crop monitoring and protection:** Some investigators are addressing issues of culture instability due to predators and pathogens. Lawrence Livermore National Laboratory (LLNL) is seeking to understand pond microbiomes and select beneficial probiotic bacteria. A University of California, San Diego project is searching for chemical signatures of impending crashes. Such work is critical to increasing yields through crash prevention.
- **Algal polyculture using impaired water:** Cultivation of mixed algal cultures—such as Sandia National Laboratories' (SNL's) Algae Turf Scrubber and California Polytechnic State University's and MicroBio Engineering's wastewater projects—show promise for good productivity while providing water remediation benefits and simultaneously addressing nutrient recycle.
- **Direct fuel/product production systems:** Two of the most innovative projects seek to produce fuel/chemical intermediates directly in photobioreactors

(PBRs) using cyanobacteria (ethyl laurate at ASU and ethylene at NREL).

Synergies

Overall, the synergies between projects are excellent. Reviewers noted an unprecedented level of collaboration between groups. Several of the consortium projects—such as the Development of Integrated Screening, Cultivar Optimization, and Validation Research (DISCOVR); ATP3; and NREL's Algae Biotechnology Partnership—stood out as models of inter-laboratory cooperation. There is little duplication of effort; approaches that appear similar are yielding different outcomes in different settings (i.e., some degree of redundancy is valuable).

Some additional opportunities for synergy were noted:

- Sharing knowledge between corporate and national laboratory projects has been limited and needs to be encouraged at all levels for cross-fertilization. This is particularly true in order to benefit calibration of TEA and life-cycle analysis (LCA) models.
- Interactions with industry are also lacking when it comes to product and co-product quality and integration. For example, several of the projects have attempted to quantify the impact of HTL for conversion of algal biomass to intermediates, and using the value of the intermediate in their TEA. However, there is no clear consensus on the value of these intermediates to a refiner, or the cost of upgrading the intermediates to an acceptable specification.
- Standardized methods (developed by NREL/ATP3) and biomass productivity units ($g/m^2/day$) have been embraced by most, but not all, teams in the portfolio. Productivity reporting for PBRs is particularly problematical.
- There is still little consensus on the best algal species, cultivation methods, and downstream processing approaches (e.g., combined algal processing versus HTL) to be pursuing, and projects are underway to further broaden the scope of possibilities (e.g., DISCOVR and the Algae Biotechnology Partnership). A tighter focus would enhance cross-fertilization between projects. Given the number of unknowns and the fact that different situations may require different organisms and strategies, BETO should not impose down-selection at this point. However, the Office should monitor whether lack of focus is due to entrenched interests or true value in continuing to pursue alternatives.
- Projects proposing to integrate herbaceous feedstocks—such as the Idaho National Laboratory (INL) feedstocks logistics and ASU mixotrophy projects—should explore synergies with the cellulosic biomass industry.

Focus

The 39 projects reviewed represent very broad coverage of the entire value chain, including strain improvement and tool development, cultivation practices, crop protection, storage, downstream processing, fuel intermediates, co-products, sustainability, techno-economics, etc. The emphasis placed on each of these areas seems appropriate.

No notable gaps were identified; however, one consistent theme is lack of interaction with end users, particularly refiners that would purchase fuel intermediates or industries that would purchase or market co-products. It would be useful for DOE to sponsor projects with recognized industrial experts to determine the cost of upgrading fuel intermediates and co-products and the corresponding value of the upgraded materials.

There appears to be relatively little ongoing work on algal harvesting and dewatering. Some players might argue that this has been solved; if so, that needs to be definitively demonstrated since this is a critical barrier.

Finally, there is always room for more disruptive technologies and out-of-the-box thinking that could lead to greater than incremental improvements. This can

continue to be encouraged through FOAs that allow for flexibility in the investigators' approach.

Commercialization

BETO is appropriately funding projects across the technology readiness level spectrum, from early stage research to later-stage deployment at relatively large scale. There has been an excellent tie-in between TEA and LCA results and BETO's decision making. Some of the national laboratory efforts could benefit from closer interactions with industrial growers to ensure their developments will satisfy commercial needs. Promoting more direct interaction between PIs and end users would help to obviate surprises or showstoppers, such as was found with devalued HTL-derived fuel intermediates due to quality issues such as high metals content.

It is clear that identifying appropriate co-products with relatively large market size and sufficiently high value will be a critical challenge over the next 2 years. It is also important to identify co-products where algae pose a unique advantage, rather than simply competing with other commodities such as corn starch. There is little, if any, consensus on what the best target co-products will be. Perhaps BETO could work to help refine the co-product options through engagement of industrial interests. Currently, the value of many algae-derived co-products is assumed rather than demonstrated; funding research on actual co-product utilization (e.g., algal meal feeding trials or studies of algal co-polymers) could be of value.

Regulatory issues need to be dealt with more systematically, including genetically modified organism (GMO) deployment issues and genetically modified product requirements for many co-products.

The Review Panel's recommendations fall into three categories:

1) **Productivity improvements:** Improving the yield of algal biomass and useful compounds therein should continue to be of highest priority.
   - Ongoing work on upstream components is critical. This includes tool development, strain engineering, photosynthetic efficiency enhancement, carbon uptake, product/co-product yields, cultivation practices, crash resistance, etc.
   - Further improvements are needed in realistic lab-scale testing and iteration between lab and field (demonstrations under real-world conditions, long-time scales, and large pond sizes).
   - Current FOAs are limited to pond sizes of 60,000 liters (L) and smaller, which is too small to really demonstrate commercial applicability.
   - Funding of both further incremental improvements and high-risk, potentially transformational concepts should continue.
2) **Connections with industry:** Better ties between laboratory researchers and industrial interests are needed.
   - Concepts aimed at growth enhancement or crop protection need buy-in from real-world algal producers.
   - Modelers need more input from producers to realistically capture effects of nutrient loss, harvesting schedules, downtime, etc.
   - Thorough market assessments and sensitivity analyses are needed for the evaluation of potential co-products.
   - Researchers and industry should work together toward consensus on what types of products are particularly well suited to production in algal systems.
   - Buy-in from end users of fuel intermediates and co-products is needed, including an understanding of cost penalties associated with quality issues.
   - The value of algae-derived co-products should be demonstrated (e.g., feeding trials for algal meal).
   - Mechanisms are needed to leverage valuable data confined within companies that are receiving funding from BETO. This should include lessons learned, not just the successes.

3) **Agronomic approaches:** The Review Panel would like to see algal cultivation treated more as agriculture than biotechnology. Agronomic approaches to crop improvement and integrated pest management are needed, possibly in collaboration with the U.S. Department of Agriculture and trained agronomists. Approaches should be evaluated under realistic conditions at large scales with best management practices; long-term field testing (greater than 1 year, across seasons) is needed for meaningful results. Associated regulatory issues need to be addressed, including both GMO deployment and genetically modified product protocols for co-products.

ADVANCED ALGAL SYSTEMS PROGRAMMATIC RESPONSE

Introduction/Overview

The Advanced Algal Systems Program would like to thank the Review Panel for recognizing the success of the BETO-funded portfolio in advancing the SOT and the effectiveness of the program strategy, as informed by our rigorous analysis portfolio. The program is working to continually advance in areas identified by national laboratory-led analysis as the most impactful: productivity improvements and increasing the value of biomass through investigation of co-products. BETO also thanks the panel for acknowledging how critical government funding is in supporting these innovative technologies in order to reduce risks for private investors. The program will continue to focus on improvements at a variety of pre-pilot R&D technology readiness levels at the national laboratories and through competitive FOAs.

The Review Panel affirmed that BETO funding has enabled innovative approaches, significant advances along the SOT, and unprecedented synergies among projects. While the panel noted that gaps were not significant, we acknowledge that the program's dedicated work in harvesting, dewatering, and logistics was absent from this review because it is focused within the Small Business Innovative Research portfolio, though the Algal Biomass Yield portfolio also includes "downstream" logistics within project plans, notably the GAI Zobi Harvester®. With regard to regulatory issues related to GMO deployment, BETO is coordinating closely with the Environmental Protection Agency through the Biomass Research and Development Board to provide guidance on permitting requirements managed by that agency. We also concur that there is always room for more disruptive technologies, and the program will continue to encourage non-incremental improvements through FOAs that allow for flexibility in the investigators' approach.

Recommendation 1: Focus on improving the productivity of biomass production

The Review Panel's key recommendation to the program is to focus on improving the productivity of biomass production. The program fully agrees with this recommendation and has focused R&D on achieving productivity targets for the last several years. Biomass productivity and biofuel yield have been key targets in the program's competitive funding opportunities since 2013. Future work will continue to keep productivity as our highest priority, focused on biological improvements tested in outdoor-relevant conditions. In addition to the existing portfolio's efforts in strain improvement, the program is initiating a new program under the Productivity Enhanced Algae and ToolKits funding oppor-

tunity—announced in Fiscal Year (FY) 2017—to not only set new targets for productivity and yield, but also to create tools and methods to advance the SOT across the field.

Recommendation 2: Establish better ties between laboratory researchers and industrial interests

The program agrees that there needs to be better ties between national laboratory researchers and industrial interests, and we hope to continue fostering interactions with industry boards. The program discusses industrial interests regularly with the Algae Biomass Organization through stakeholder meetings, listening days, workshops, and conferences. The program will continue to leverage the industrial boards of ATP3 and other competitive projects and encourage relationships between national laboratories and private partners. The national laboratory DISCOVR consortium, for example, is soliciting strains from industrial partners to test through its "pipeline" process. Going forward, the program will be sure to critically evaluate and improve upon "tech-to-market" plans of the national laboratories, as well as support partnerships between industry and fundamental R&D.

Recommendation 3: Develop agronomic approaches to crop improvement and integrated pest management

The Advanced Algal Systems Program solicited agronomic approaches to crop protection and pest management in topic two of the FY 2015 Targeted Algae Biofuels and Bioproducts FOA, as well as the FY 2017 Productivity Enhanced Algae and ToolKits FOA. The program agrees that agronomic approaches are a necessary element in this field of study and is pursuing R&D strategies to improve the state of cultivation technology of the NREL Algae Farm design case.[18] We concur that algal cultivation should be envisioned as a large-scale agricultural practice and not only as a biotechnology. Long-term outdoor field testing at meaningful scales is planned per our MYPP, within a 5-year time frame. BETO will continue to coordinate with the U.S. Department of Agriculture and Environmental Protection Agency through the Biomass Research and Development Board on issues such as GMO deployment.

[18] Ryan Davis, Jennifer Markham, Christopher Kinchin, Nicholas Grindl, Eric C. D. Tan, and David Humbird, *Process Design and Economics for the Production of Algal Biomass: Algal Biomass Production in Open Pond Systems and Processing Through Dewatering for Downstream Conversion* (Golden, CO: National Renewable Energy Laboratory, February 2016), NREL/TP-5100-64772, http://www.nrel.gov/docs/fy16osti/64772.pdf.

MICROALGAE ANALYSIS

(WBS #: 1.3.1.102)

Project Description

An important step toward realizing the biofuel potential of algae is quantifying the demands commercial-scale algal biofuel production will place on water, land, and nutrient resources. This project developed and advances a high-resolution spatiotemporal Biomass Assessment Tool (BAT) focused on fundamental questions of where production can occur; what are the associated demands for nutrient, land, and water resources; how much energy is produced; and, by evaluating numerous trade-offs, where the ideal production sites are located. To help answer these questions, the BAT considers site-specific, high-fidelity climate information; existing land use/land cover; transportation networks; known and quantified nutrient sources; and refinery infrastructure. The BAT provides a biophysics-based analysis tool for linking key BETO and industry research activities to achieve high-impact objectives. Results from this study have resulted in 15 peer reviewed publications of direct benefit to industry for evaluating optimal site locations, strains, and operations.

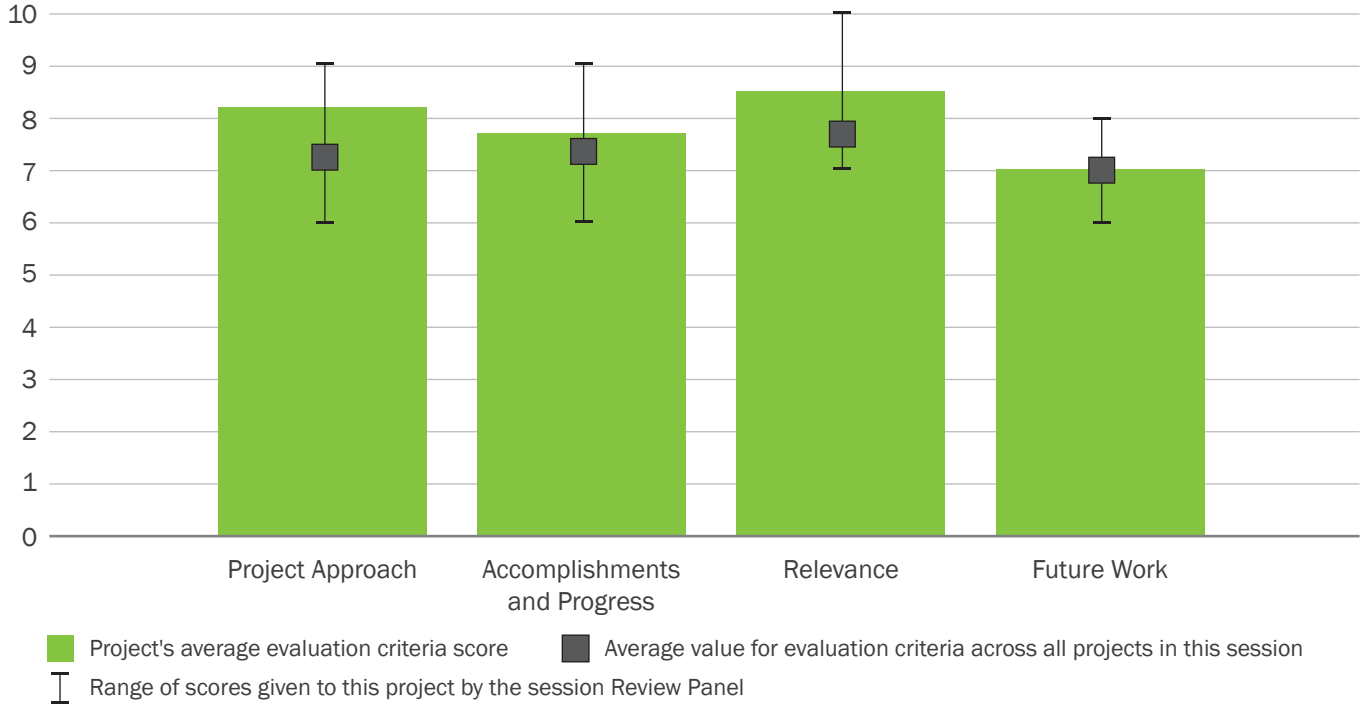
Recipient:	Pacific Northwest National Laboratory
Principal Investigator:	Mark Wigmosta
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$600,000
DOE Funding FY 2015:	$900,000
DOE Funding FY 2016:	$800,389
DOE Funding FY 2017:	$600,000

Overall Impressions

- The model they are developing is very detailed in terms of selecting the parameters required to make an informed site selection. For cases where they model strain rotation, it would be a good addition

Weighted Project Score: 7.8

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



to include the scheduling inefficiencies encountered using this approach. This model will be very helpful for assisting industry with their decision making and helping policymakers in evaluating the potential of the algae industry.

- This is one of the bedrock projects in the portfolio, and key findings from this work have continued to help guide the program. Integrating soil-type data to glean information on where pond liners would be required could be a valuable addition.
- The continued development of sound site-specific TEA analysis is an important component of the successful deployment of large-scale algal biomass productivities. Overall, this project addresses the majority of questions and issues related with certain large-scale production sites. The model could incorporate more detailed information on water availability on a state-by-state basis to provide more clarity as to how much water resource is available given a specific state's regulatory issues.
- The BAT should be a key tool for the industry as it expands. Anyone considering building or buying algae production facilities should be using it as part of their due diligence efforts. Proposed improvements to the tool via the future work will enhance it greatly, particularly the inclusion of alternative paths to biomass production.
- This project fits well within the BETO portfolio by providing a tool focused on assessing resources required for the large-scale production of fuel from microalgae, an essential component of determining the viability and scale-up of algal-derived fuels. The project has been productive with important key findings, such as the significant potential for production using saline water and co-location with CO_2 sources.
- The team has made strong contributions to biomass assessment efforts in the past with clear links to BETO MYPP goals. Overall, the goals and milestones for the new 3-year effort were unclear, including how those goals differed from previous efforts.

PI Response to Reviewer Comments

- We thank the reviewers for their valuable and encouraging input. For all aspects of the BAT, we have strived to develop components and a level of detail required by BETO, MYPP goals, and the algae research community. By design, we use a systematic process of building a best representation and then subsequently add additional detail as the needs and data support. This approach helps to identify research needs and data gaps, for BETO and others in the algae community. When gaps are filled, we are able to utilize these results and continue to build more detail and rigor into the BAT.

  We agree with the reviewers that BAT/TEA integration is critical. BAT calculates some spatially derived TEA components (cost of pipeline/well drilling/water transport, land valuation, land preparation, transport costs of moving biocrude to refineries, etc.). Through BETO model harmonization efforts, we continue to integrate resource assessments with TEA models (i.e., NREL). This combination allows site-specific analyses to be conducted throughout the United States and allows results and research to be shared, which has been done through direct working relationships with industry, trade groups, conference talks, and publications.

  Major cost barriers associated with pond liners drives the need to evaluate soils and respective infiltration of pond water into those soils. We built a preliminary soil-plugging model based on a national soils database that uses soil texture, organic matter content, and cation-exchange capacity to develop estimates of saturated conductivity under both natural and compacted soil conditions to aid with this assessment. This analysis would benefit from additional research.

We are developing more detail into our water availability models, including sustainable water use that considers environmental flow requirements and seasonal use, along with other existing competitive uses. This will significantly improve our previous approach. In addition, we are evaluating site operation strategies (e.g., pond operating depth, harvest strategies, water recycle) to gain a more realistic representation of water use. In the past, we have proposed evaluating state regulatory water issues and factoring these into the BAT and agree that this topic should be considered—at least for the states that show the most promise for production.

We agree with the reviewers in that our strain rotation work needs additional 'operational' detail, particularly in how we represent the logistics of switching strains. What has been presented shows a first-order evaluation of the possible production potential by implementing rotations to reflect seasonal environmental conditions at a monthly time-step. We need to obtain operational data of strain rotations so we can better represent the processes and effects. To this end, we are also using the BAT to help inform experimental design under the Regional Algae Feedstock Testbeds (RAFT) program. We expect data and feedback from the RAFT and ATP3 programs to provide data on relevant operational details.

Key efforts over the next 3 years include the following:

- Developing an improved estimate of sustainable algal biomass productivity for harmonized assessment (with Argonne National Laboratory, NREL, and Oak Ridge National Laboratory), including CO_2 co-location, saline versus fresh water, requirement for liners, and maximum farm size.
- Using improved site-specific operations to increase seasonal and annual feedstock production.
- Establishing improved metrics for sustainable algal feedstock production to reduce impact to water and land resources (with Oak Ridge National Laboratory) considering environmental flows, water quality, and additional saline water constraints.
- Gaining an improved understanding of the trade-offs associated with alternative pathways to meet MYPP 2018 and 2020 production targets considering the use of PBRs and CO_2 co-location.
- Quantifying the feedstock production potential associated with alternative sources of nutrients (wastewater; concentrated animal feeding operation) that contribute to a continental United States-wide trade-off analysis.
- Providing BAT model support to SOT reports.

ALGAE POLYCULTURE CONVERSION AND ANALYSIS

(WBS #: 1.3.1.103)

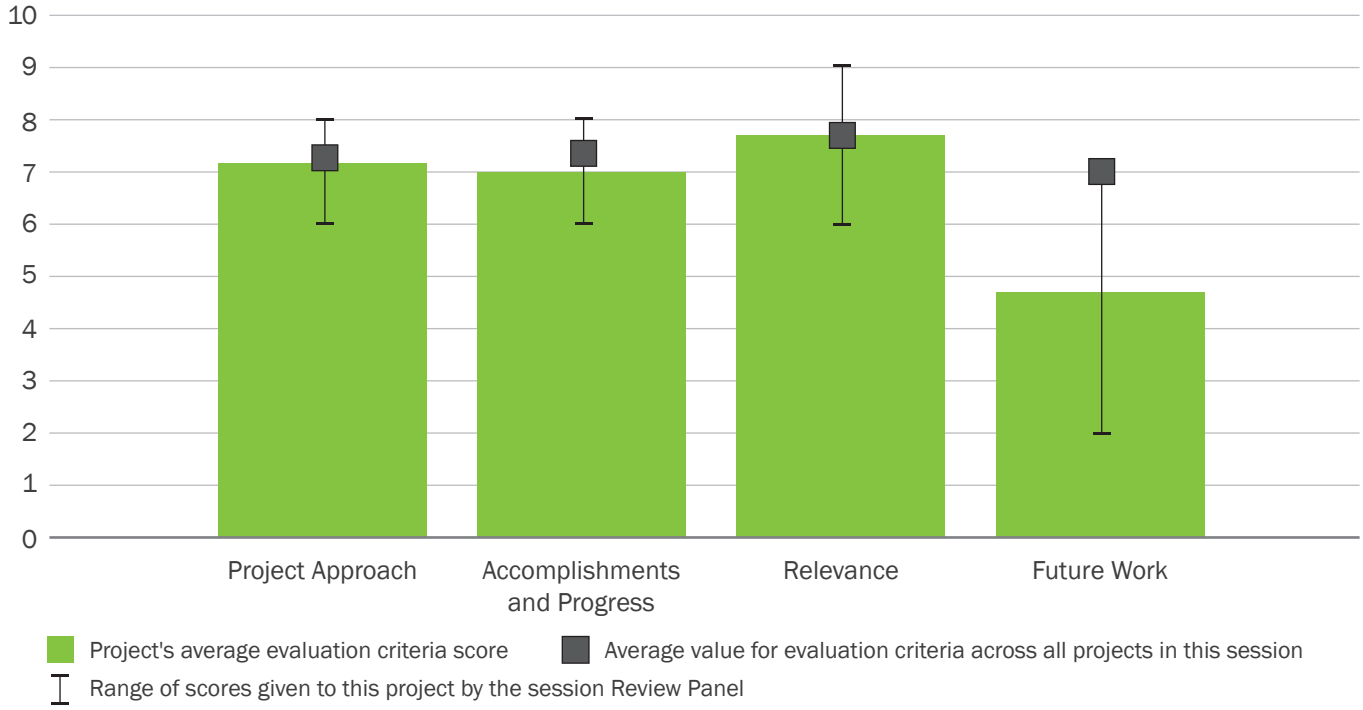
Project Description

The overall objective of this project is to establish the technical and economic feasibility of achieving and scaling high and reliable production of easy-to-harvest algal turf polyculture for biofuels using fresh and estuarine/marine surface waterways without supplemental CO_2 and nutrients addition. Commercial algal turf scrubber systems deployed for cleaning water have not been optimized for biomass production, but rather for the treatment and reduction of nitrogen and phosphorous contamination in surface waters in the environment to extremely low levels at discharge. Extrapolation of the productivities observed over a year give an annual yield of more than 34 tons per acre, per year. Through this project, we seek to increase productivity to 18–25 g/m^2/day ash free dry weight by optimizing cultivation and harvesting system operations and reducing biomass losses. Finally, combining techno-economic, resource, and geographic information assessments are being applied to determine U.S. scale-up potential.

Recipient:	Sandia National Laboratories
Principal Investigator:	Ryan W. Davis
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$440,000
DOE Funding FY 2016:	$950,000
DOE Funding FY 2017:	$750,000

Weighted Project Score: 6.6

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



Overall Impressions

- This is a promising project for economical water cleanup. Future work should include improving the productivity, working on algae cleanup, and developing a strong TEA for an identified site. While this approach may be a difficult method to meet the productivity goals of the MYPP, it may be better suited for meeting sustainability goals.
- This approach lends important diversity to the portfolio. The project has both revealed promise (such as good productivity and the scale of available resources) and challenges (such as the very high ash content). Critical deliverables for the remainder of the project should include detailed water analysis before and after treatment, analysis of the suitability of the biomass for downstream processing, and completion of TEA/LCA.
- Overall, this is a well-planned and deployed project that could provide a feasible methodology for the production of algal biomass using wastewater technology and phycoremediation. Issues of scaling the system could be problematic. Setting this system up in a manner that is "off the grid" provides an interesting approach for stand-alone automated systems that could reduce the overall operating cost of this type of system.
- This is an excellent contribution for algae biomass production, and making the best of waste from one industry into products for another, if this can scale as predicted in the project and is economically feasible.
- Using the algal turf scrubber system to cultivate algae for fuel is very interesting, though there may be some critical issues to overcome, such as lower-than-expected productivity and high ash content. This project has made some demonstrable progress toward producing biomass with this system, but should focus on a clear plan for future work to ensure continued progress and relevance.
- The project has installed, operated, and generated data from an innovative technology package. Early data suggests productivities approach current microalgal SOT. It is unclear whether the biomass generated from this technology package will be compatible with conversion technologies for fuel applications, but the technology may provide valuable water treatment services in certain situations.

PI Response to Reviewer Comments

- The AOP team would like to thank the reviewers for their generous contributions toward the success of this and the associated BETO Advanced Algal Systems projects. Their feedback was greatly appreciated.

ALGAL BIOFUELS TECHNO-ECONOMIC ANALYSIS

(WBS #: 1.3.1.200)

Project Description

The objective of this project is to provide process modeling and analysis to support Advanced Algal Systems Program activities, utilizing process and economic models to relate key process parameters with overall economics for cultivation, processing, and conversion of algal biomass to fuels and co-products. By quantifying economic implications of key process metrics, TEA models highlight the requirements to achieve future program cost goals, as well as provide a means for tracking progress toward these goals. This project is highly relevant to BETO objectives because it produces critical cost data tied to funded research at NREL and elsewhere, with the analyses subsequently exercised by BETO to guide program plans, FOA priorities, and other strategies to guide research toward achievement of cost targets that are set from the "top-down." 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. The Algal Biofuels TEA project has made significant achievements since the 2015 Peer Review, including publication of a new "algae farm"

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Ryan Davis
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$350,000
DOE Funding FY 2016:	$300,000
DOE Funding FY 2017:	$300,000

Weighted Project Score: 8.2

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.

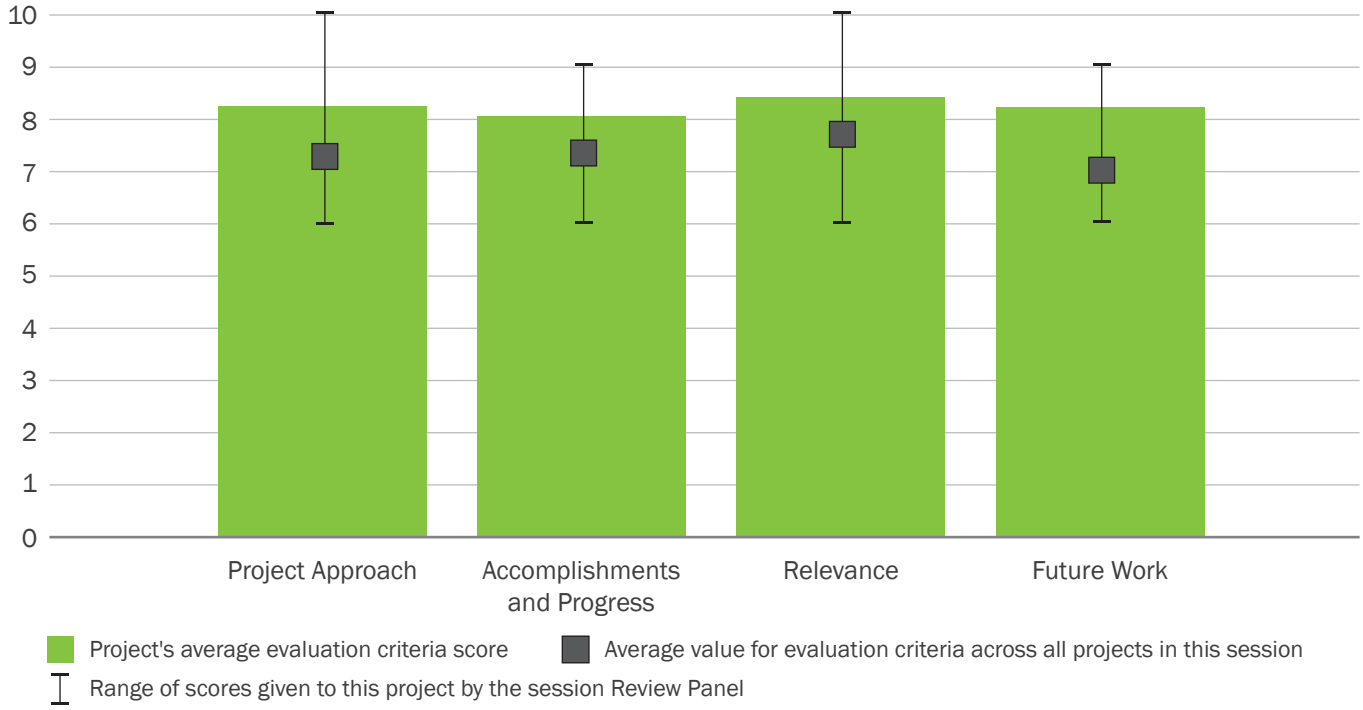
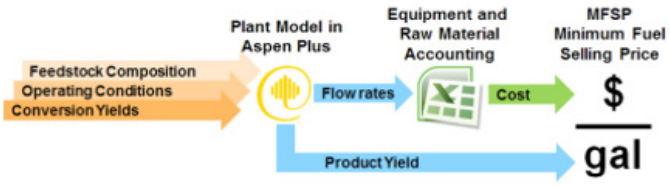


Photo Courtesy of National Renewable Energy Laboratory



design report that documents cost projections for algal biomass cultivation and harvesting, as well as demonstrates viable paths to achieving $3/gge fuel cost targets by 2022 as set by BETO, based on co-production of fuels and value-added co-products.

Overall Impressions

- This project is one of the essential core elements of the portfolio. Its past and future work provides unbiased data for BETO and industry to direct their resources. Continued emphasis on co-products is critical.
- The continued development of sound and relevant TEA for the use of defining and identifying gaps within the current knowledge base of large-scale algal biofuel production is important. These studies have a great research impact, as they provide a blueprint for current or needed optimization within the field. More interaction with large-scale production cultivators may help to focus the TEA. Possibly, interaction with commercially viable nutraceutical systems that are currently in operation may shed some light on areas or processes that have already been or are being explored.
- This TEA project is large and complex, but it provides clear direction for areas that must be targeted for additional work in order to meet cost per gge (or cost per ton of biomass).
- This project has been an essential part of setting BETO benchmarks and thereby has driven progress across several other projects in the BETO portfolio.
- This project has made strong contributions to benchmarking the state-of-the-art in algal biofuel technology pathways. The proposed future work is expected to continue being highly valuable to BETO in helping to monitor the progress of the Office's algal portfolio and helping to prioritize future funding efforts. The team has a strong track record of collaboration, and the proposed future work should continue that trend.

PI Response to Reviewer Comments

- We thank the reviewers for their positive feedback in recognizing the utility of this project for BETO and the algae community. We plan to continue leveraging the expertise established for both algal biomass production and conversion TEA models to update SOT benchmarks and track progress against future cost goals, and to provide insights that industry may build upon regarding algal biomass valorization opportunities for fuels and co-products. We do have a number of working relationships with stakeholders in industry, and we hope to continue those discussions and reach out to others to leverage existing knowledge they've established in validating or improving our models. We always welcome such inputs and also would gladly seek similar guidance from other related industries, such as nutraceutical producers (although recognizing that there may be some differences in processing practices, constraints, and costs between nutraceutical production and commodity-scale fuels and other products).

ALGAE TECHNOLOGY EDUCATIONAL CONSORTIUM

(WBS #: 1.3.1.201)

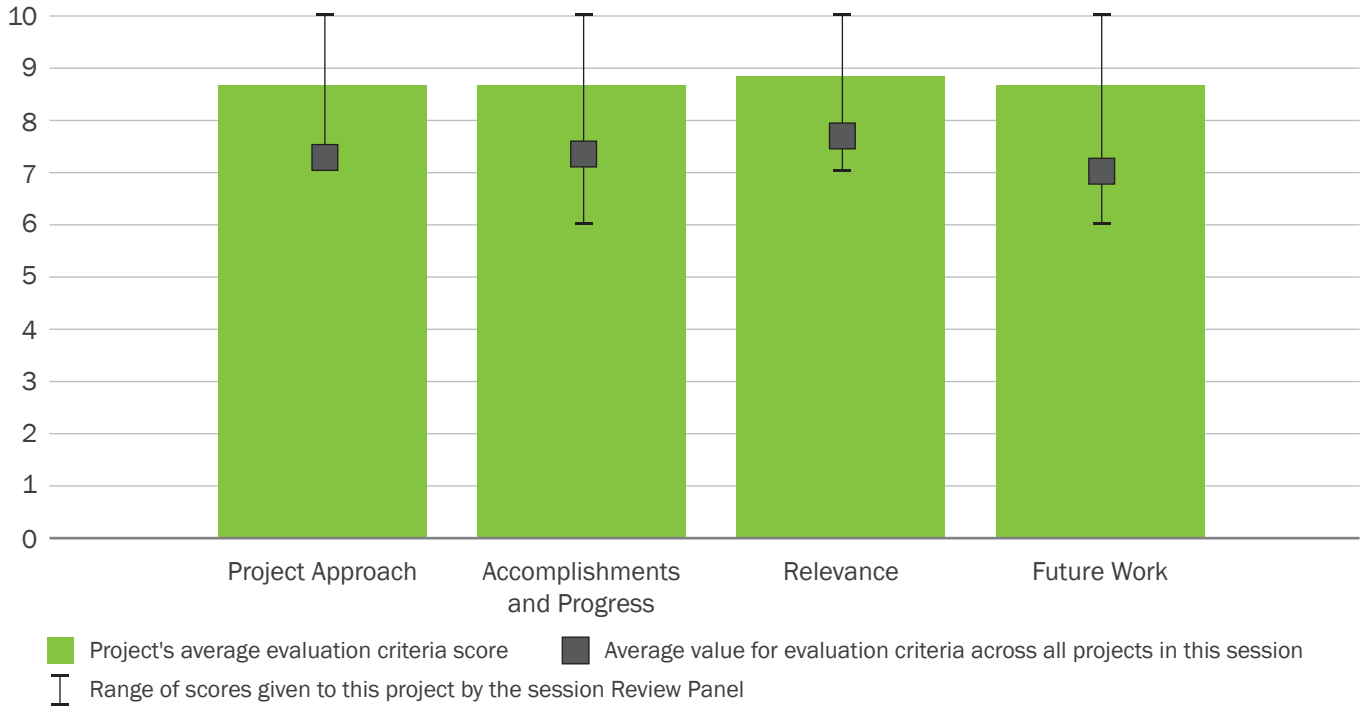
Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Cindy Gerk
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$250,000
DOE Funding FY 2016:	$55,000
DOE Funding FY 2017:	$600,000

Project Description

The Algae Technology Educational Consortium (ATEC) project was created to support BETO's vision of the algal industry and bioeconomy growth by training technicians to meet the 12,000 positions anticipated by 2021. The Algae Foundation and NREL have collaborated to lead a consortium of academic and commercial algal experts to develop 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 industrial advisory board comprised of senior management from America's leading algal companies to ensure that the ATEC skill set meets industry needs. An ATEC jobs survey identified present and future job opportunities. 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 industrial advisory board. ATEC continues to

Weighted Project Score: 8.7

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



engage all stakeholders and pursues collaborative relationships with algal companies, academics, and community colleges.

Overall Impressions

- This is a very exciting project. The project effort is important and relevant for meeting several DOE objectives, and it addresses industrial challenges for finding and retaining trained professionals (in particular, in some of the rural regions). This project would also be very relevant for the U.S. Department of Agriculture's goals. The project is very excellent at identifying schools that already have biotechnology or related degrees and adding in the algae component, other colleges, and an online course. The project has good insights on methods and goals for how to teach college students and for coordinating with algae companies to make the course relevant and acceptable to the industry.
- This project is already yielding great 'bang for the buck' and is gaining traction on the introduction of algal technology degree programs and coursework. Assuming growth of the industry proceeds as anticipated by the survey, such a program will be essential to meet the industry's workforce needs in the coming years. The diversity of approaches and functions of ATEC, such as the proposed free Massive Open Online Courses (MOOC) and internship coordination, will go far toward serving the community.
- Overall, this project is a forward-thinking approach to combat the growing need for skilled workers in the future.
- Training programs are dearly needed; in addition to providing required skills sets, they also help promote awareness and interest in the industry. It would be a wonderful addition if this project could modify its curriculum or promote its MOOC to extend to continuing education or job training for existing employees.
- This is an excellent, thoughtful, and creatively approached project that will undoubtedly support a growing industry by providing a skilled workforce to companies that don't necessarily have the resources to train their own. Likewise, this effort will serve students by preparing them to be effective contributors to a developing field.
- The team appears to have met all original goals and continues to make a strong contribution to workforce development for the algae biofuels industry. The group is building early learnings into future plans and is poised to reach a wide audience with education and training activities that should be applicable to adjacent industries, as well as the nascent algal biofuels industry.

PI Response to Reviewer Comments

- ATEC is grateful to the reviewers for their insightful comments and suggestions. We thank the reviewers for their support and encouragement as ATEC moves forward in achieving more successes, generating additional momentum from academia, and producing the first class of graduates to enter the workforce in the growing algae industry.

  The ATEC flowchart clearly indicates that the MOOC is the 'interest generator' for the entire ATECs program. We have always envisioned the MOOC would come first for all participants, including our two community college degrees; Algae Cultivation Extension Short-course learning modules; or alternative existing programs, including ATP3, the University of California, San Diego Edge Program, or Maine Kelp Farming educational efforts. The Algae Foundation fully intends to disseminate and advertise the MOOC in our publications, professional presentations, social media, and all of our degrees and Algae Cultivation Extension Short-course efforts.

The concept of pre-training employees is the very essence of the ATEC philosophy. As the owner of an algal farm, two of three new hires didn't last more than 1 week. Either they were ill prepared or we were poor teachers. Either way, the existence of the ATEC farming degree would have been invaluable to our operational efficiencies and budget. ATEC degree programs are built around understanding the skills and mindset expected in new employees, and we instill these experiences and values into our training and educational program.

SUSTAINABLE DEVELOPMENT OF ALGAE FOR BIOFUEL

(WBS #: 1.3.1.500)

Project Description

This project supports the development of a sustainable and cost-effective domestic supply of algal biomass, biofuels, and bioproducts. Environmental and socioeconomic indicators for measuring and modeling aspects of sustainability were developed, published, and are being used to identify sustainability synergies and trade-offs. Co-location of algae with waste CO_2 sources can improve profitability while also improving key environmental indicators and energy return on investment. We estimated, for the first time, both the potential algal biomass and qualitative environmental effects across the United States, and results were presented in the *2016 Billion-Ton Report*. We have completed proof-of-principle experiments showing that the addition of carbon sources and/or algae can reduce soil conductivity—a prerequisite to determining locations and conditions for which unlined or minimally lined ponds could be feasible while maintaining water quality and quantity. We identified algal strains that are synergistic, out producing monocultures when grown together in model wastewater conditions. These polycultures have the potential to decrease susceptibility to pond crashes, moving toward energy security, and improve water quality and quantity. Food security from joint production of protein

Recipient:	Oak Ridge National Laboratory
Principal Investigator:	Rebecca Efroymson
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$200,000
DOE Funding FY 2015:	$800,000
DOE Funding FY 2016:	$465,000
DOE Funding FY 2017:	$83,000

Weighted Project Score: 5.6

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.

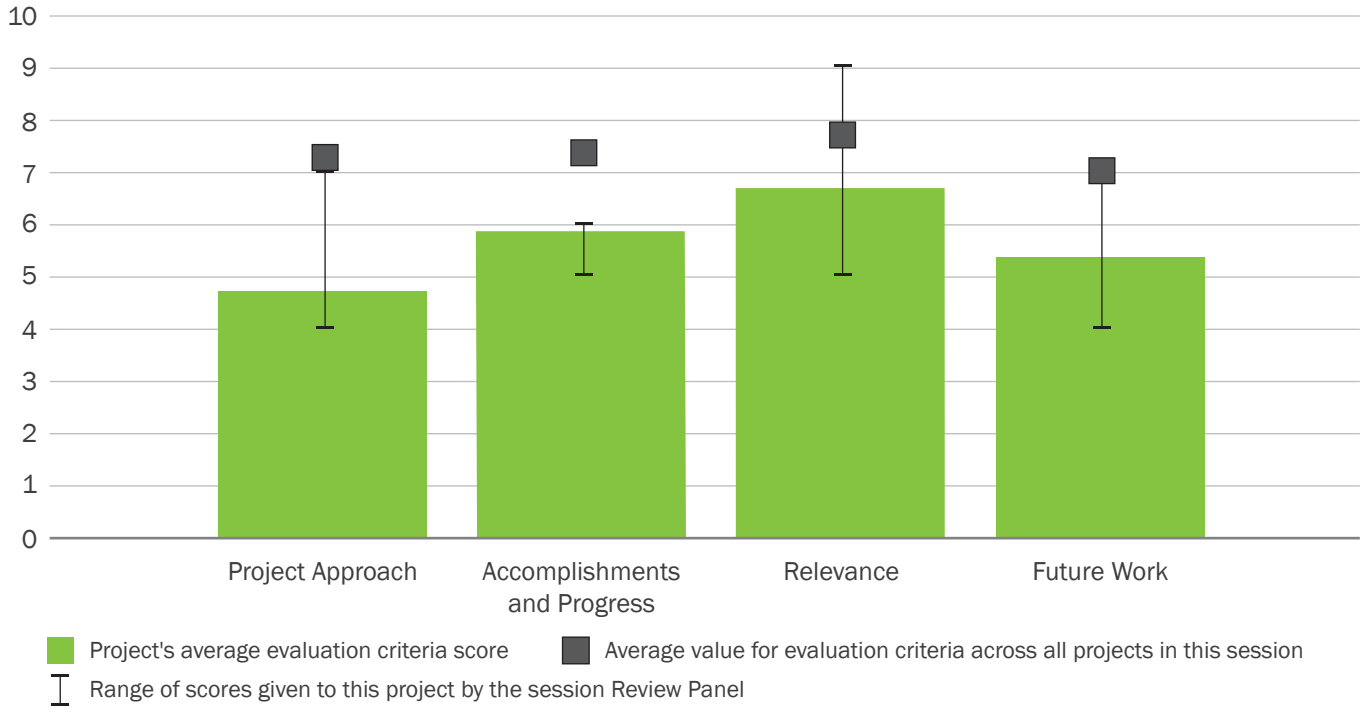


Photo Courtesy of Oak Ridge National Laboratory

and energy from algae will be considered in the future. Efforts are underway to identify criteria, data, models, and regions to meet DOE's goal to model 1 million tons of sustainable algal biomass.

Overall Impressions

- This effort seems very disjointed. It is difficult to understand the goals, how they are related, and what was performed as part of this project or performed under other projects. It appears that this project changed over time, and that the accomplishment and emphasis end up not 100% aligned with original goals. The stated conclusions appear to be more directional in nature, and they are not very definitive. I think this presentation would have benefited from a "cold-eyes" review to help the presenter communicate to the Review Panel.
- Important areas of sustainability (economic, environmental, and socioeconomic) are being investigated. The project made important contributions to the *2016 Billion-Ton Report*. Overall, though, the project appears disjointed and relatively lacking in quantitative deliverables.
- Overall, the analysis and incorporation of sustainable practices to large-scale production systems has importance.
- Fully evaluating all potential inputs for sustainability and issuing a best management practices tool will be extremely useful for the industry; however, there needs to be strong industry involvement to make it relevant.
- Vaguely, this project aims to make algae fuels sustainable through defining indicators of sustainability, establishing best practices, and identifying challenges for commercialization. However, the overall approach to accomplish this is not particularly cohesive or well-defined.
- Overall, the project has made strong contributions to BETO's goals for modeling the supply of sustainable algal biomass. The project seems to include several bench science efforts that are not integrated with the modeling work in terms of management, goals, or relevance. Future work also seems beyond the scope of original project goals and seems unlikely to succeed given the current team members.

PI Response to Reviewer Comments

- Reviewers found that the project is important and relevant to the platform goals and objectives of the BETO MYPP ("…the project has made strong contributions to BETO's goals for modeling the supply of sustainable algal biomass.") The CO_2 co-location contributions to the *2016 Billion-Ton Report* were highlighted as a significant accomplishment.

  Some reviewers felt that the objectives were well framed, but a few reviewers felt that presenting the

breadth without a more comprehensive discussion of the links among tasks, the project history, and the integrated management approach made the project seem disjointed. The presentation involves a few different tasks related to sustainability and resource analysis of algal biomass production. In the time allotted for the presentation, we were not able to clearly convey how all tasks were linked, but we have made a concerted effort to coordinate objectives among tasks and between labs. Also, the resource analysis work was moved to a feedstock platform project to focus this sustainability project more on its sustainability roots, yet the resource analysis task was presented in the algae platform to get expert feedback.

The overall focus of this project is now on sustainable biomass, indicators, targets, and best practices, with a broad definition of sustainability. The project's two proof-of-concept tasks (unlined ponds and polycultures in wastewater) were responses to the following key needs for sustainable algal production: (1) reducing costs, (2) reducing freshwater consumption and nutrient consumption, (3) maintaining water quality, and (4) increasing productivity. The specific lab studies were not being done elsewhere, and the expertise to do them was at Oak Ridge National Laboratory. These lab studies end this year. The project will continue to identify sustainability needs and conduct needed research on different indicators, as well as best management practices.

There were some concerns about our future work in food security; we have a diverse team of economists, agricultural engineers, landscape ecologists, and other experts who have focused on food security issues for many years. We believe that food security is a real advantage of algae (potential co-products, use of non-agricultural lands, etc.), and that it is relevant to sustainability goals.

FUNCTIONAL CHARACTERIZATION OF CELLULAR METABOLISM

(WBS #: 1.3.2.100)

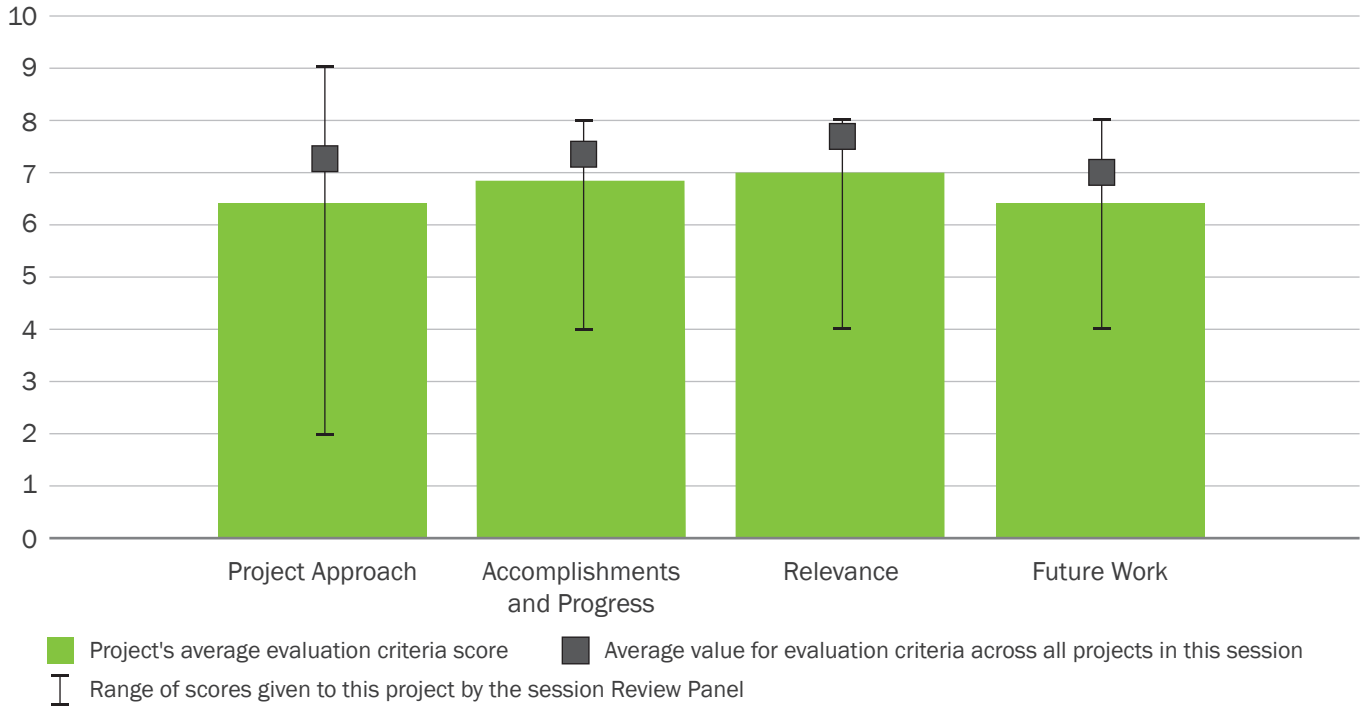
Project Description

The goals of this project are to advance technical capabilities for rapid strain improvement in productivity and robustness. We will expand the molecular toolbox through Cas9 editing of key regulatory genes involved in nitrate sensing and signaling in Nannochloropsis salina CCMP1776. Overexpression of assimilatory proteins, including chloroplastic and cytosolic glutamine synthetase, and asparagine synthase will be explored for altering carbon/nitrogen balance in coordination with overexpression of phosphoenolpyruvate carboxylase for anapleurotic carbon backbone biosynthesis. This project will also focus on developing novel applications of flow cytometry probes for rapid characterization of algal cell physiological status. Phenotyping cells for lipid accumulation, intracellular pH, REDOX status, autophagy responses, and cell cycle/ DNA ploidy will be developed and applied to multiple production species. We have demonstrated a linear pH response with pHrodo Green AM in Picochlorum and DNA ploidy responses with DyeCycle Orange. Application of these rapid assays will allow for developing a basis for understanding population responses to environmental stimuli and optimizing algae systems.

Recipient:	Los Alamos National Laboratory
Principal Investigator:	Scott Twary
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$1,000,000
DOE Funding FY 2015:	$1,000,000
DOE Funding FY 2016:	$1,000,000
DOE Funding FY 2017:	$650,000

Weighted Project Score: 6.7

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



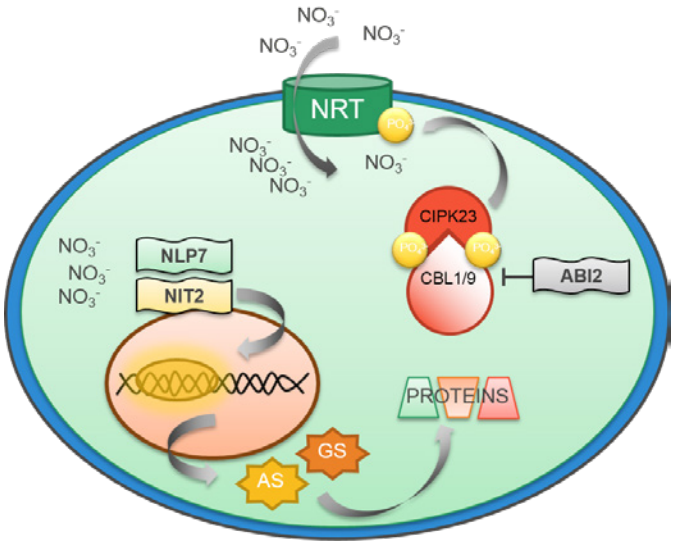


Photo Courtesy of Los Alamos National Laboratory

Overall Impressions

- This long-term project has resulted in some significant advances in pathway engineering. The improved strains require further characterization, trait stacking, and real-world testing. Future work on nitrogen regulatory responses and non-GMO Cas9 strategies could result in important advances. Future cytometry work is perhaps of less value, particularly if it is envisioned as a real-time cultivation diagnostic tool.
- Overall, continued improvement in the creation of genetic information on potential cultivation species is important. The project seems to have an understanding that taking things to scale will provide substantial information. Looking into the optimization of nutrient management and the increase of lipid accumulation through optimizing the nitrogen pathway shows forethought and the ability to create an impact at scale. The project seems to have developed a robust pipeline to generate cohesive results while taking information from the lab to scaled systems.
- Development and application of Cas9 techniques for genetic modification will significantly improve the chances of improved strains making it to commercial readiness.
- The overall objectives of this project are commendable and relevant to BETO, as understanding and engineering algal metabolism will likely be a key part of developing algae as an agricultural crop. This project suffers from a lack of focus and is not realistic regarding the magnitude of and potential challenges with the endeavor. Though the overarching ideas and goals presented are interesting and valuable, the lack of specific and clear targets and dearth of data included give the sense that this project is on track to overpromise and under deliver.
- The team is on track to make valuable contributions to expanding the molecular toolbox to include more methods (e.g., CRISPR Cas9) that need methodological improvements to work in algal systems. Work on understanding the genetic and metabolic basis for carbon partitioning is important for understanding the limitations on lipid production during the growth phase, which has implications for large-scale algal biomass production goals.

PI Response to Reviewer Comments

- We greatly appreciate the independent reviewers' insights into our research approach. These perspectives help us to focus on the identified important issues and direct resources to make the most significant improvements. Specifically, in the past year, we have refocused and consolidated our AOP portfolio into two main endeavors: flow cytometry physiological characterizations and genetic knock-out toolbox advancements. These will be applied to relevant BETO challenges for developing algae strains for enhanced productivity and improved robustness in the wake of environmental stress. These recent redirections have allowed us to complete the initial foundational work for both objectives and now quickly advance meeting the future milestones for strain improvements and performance testing. One strong, consistent message is the need to continue to push developed strains forward for extensive environmental testing at production-scale cultures.

One of our goals is to create a diagnostic toolbox, not only for the transgenic lines we are engineering, but also for use with other algal strains for rapid, high-throughput, single-cell analysis that other researchers will find useful. We had significant success in the first months developing these probes requiring experimentation for different strains, growth conditions, and physiological states. We now have functional protocols for three probes for two different strains achieved in the first quarter. Our goal is to have developed at least six total probes for different strains by the end of this AOP. These probes provide a significantly more comprehensive evaluation of cellular responses, thereby yielding more information to deepen our understanding of physiological responses. We agree that developing the real-time diagnostics will require methodology that is easily transportable to the field. However, to determine in-depth phenotypic characteristics, a robust, reliable, efficient, and user-friendly methodology is required. Thus, flow cytometry is the ideal method for these types of assessments. Protocols for phenotypic characterization in algae using appropriate flow probes that will not interfere with algal auto-fluorescence signals and will not undermine the fitness of the organism have not been developed to date.

We will continue to develop the molecular toolbox for Nannochloropsis, but are now directing efforts to design effective engineering platforms and protocols for applications to many algae strains. We have achieved significant foundational advancements, identifying many challenges but also recognizing overlap for optimization. The metabolic targets and expected response outcomes for our engineered pathways are conserved among species based on our genomic and bioinformatics analysis. Results from our studies can then be applied for broader impact across many algae species.

MULTI-SCALE CHARACTERIZATION OF IMPROVED ALGAE STRAINS

(WBS #: 1.3.2.102)

Project Description

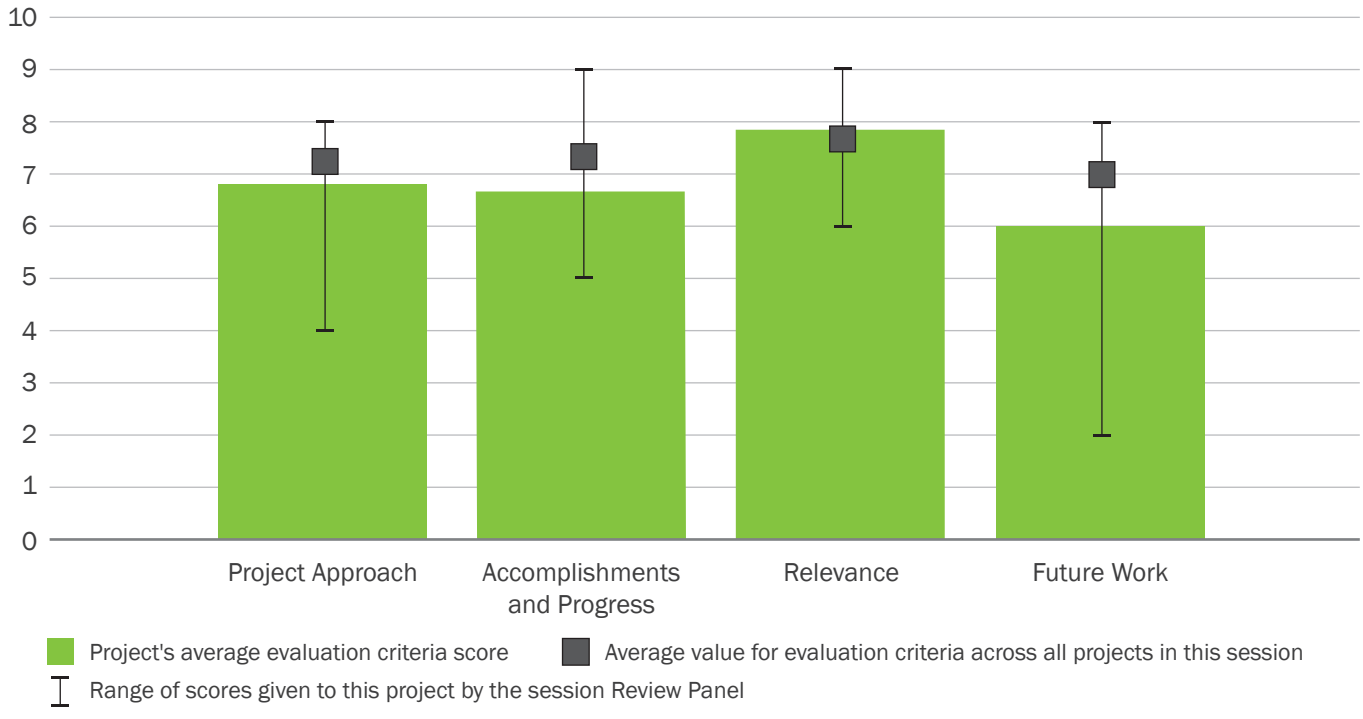
The primary goal of this project is to generate improved algae strains and characterize their performance at multiple scales, from the bench to outdoors. By achieving this goal, this project aims to tackle two challenges. First, algal biofuel costs are highly sensitive to algae biomass and lipid productivity, but identifying or generating strains that have a productivity that is sufficiently high for profitable fuel production has been a challenge. Second, strategies for accurately down-selecting strains indoors, in an outdoor-relevant manner, require development. We specifically address these challenges by generating improved algae strains, testing them at multiple scales, and examining the metabolic changes responsible for the new phenotypes.

Recipient:	Los Alamos National Laboratory
Principal Investigator:	Taraka Dale
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$250,000
DOE Funding FY 2015:	$350,000
DOE Funding FY 2016:	$500,000
DOE Funding FY 2017:	$600,000

We have three objectives: (1) Strain Improvement: use non-genetic modification strategies to improve strain productivity and robustness, including flow cytometry and adaptive evolution methodologies; (2) Strain Transition: develop and utilize strategies for transitioning our improved strains from laboratory flask experiments to outdoor ponds; and (3) Molecular Mechanisms: uncover the mechanisms by which sorted and adapted strains show improved phenotypes. Taken together, these ef-

Weighted Project Score: 6.8

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



forts are generating improved strain phenotypes, as well as a better understanding of algae performance across scales, both of which will enable BETO to reach $3/gge advanced biofuels.

Overall Impressions

- This project has shown significant ability in being able to sort for lipid concentration and separately for productivity. The work they are doing to down-select strains from in-door experiments and have the results translate to outdoors performance addresses issues that the industry has experienced. However, I think that having a goal of biomass accumulation instead of growth rate can be very misleading due to potential growth lag times.
- The project is focused on important questions of strain improvement and "flask-to-farm" issues. Some of the stated accomplishments require considerably more validation. Future plans are quite ambitious and may require a narrower focus.
- Overall, this is sound project with clear and relevant objectives. Integration of this project with others helps to standardize results relevant to the field. Clear and successful transition of lab-based assessment to field-scale pilot studies show a sound approach and well-thought-out pipeline for bio-feedback and relevant results.
- Very interesting work on productivity improvement, but I would like to see work at a scale larger than 1,000 L ponds to demonstrate that productivity improvements would hold as you move up in scale, as the project has demonstrated in moving from the lab to the field.
- Work conducted in this project is highly relevant for BETO and should be particularly commended for working with production strains and considering applications of project outputs for algal production at scale. It is also effective at interactions with other national facilities and leveraging resources such as national testbeds. The overall technical approach would benefit from improving experimental design to increase confidence in the validity of strain improvements.
- This project addresses an important issue of utilizing advanced cell sorting and genetic tools to develop improved cell lines. If the project is able to overcome the transience of advanced phenotypes, the project has a strong platform to benchmark performance in a pipeline that spans the lab to the outdoor field conditions.

PI Response to Reviewer Comments

- Thanks to the reviewers for their thoughtful comments and general support of this work. We agree that ongoing validation is important for project success and aim to continue to do so. We agree that larger-scale data (greater than 1,000 L) would be relevant to collect. Should resources become available for such a task, we may pursue it; meanwhile, we will leverage similar work conducted at the testbed facilities and by our industry collaborators. Regarding validation of the improved strains and transience of phenotype, we have noted multi-year stability of improved phenotypes, in the lab for multiple strains and up to the 1,000 L scale for the one strain tested with that particular question in mind. We could have communicated this more clearly, as well as our onboarding of mutagenesis strategies to isolate even more stable phenotypes, which we did not have time to discuss but is ongoing. We take the reviewer's point regarding productivity calculations and will be extensively characterizing the new improved-growth phenotypes to better understand the impact on relevant productivity measurements.

ALGAE BIOTECHNOLOGY PARTNERSHIP

(WBS #: 1.3.2.103)

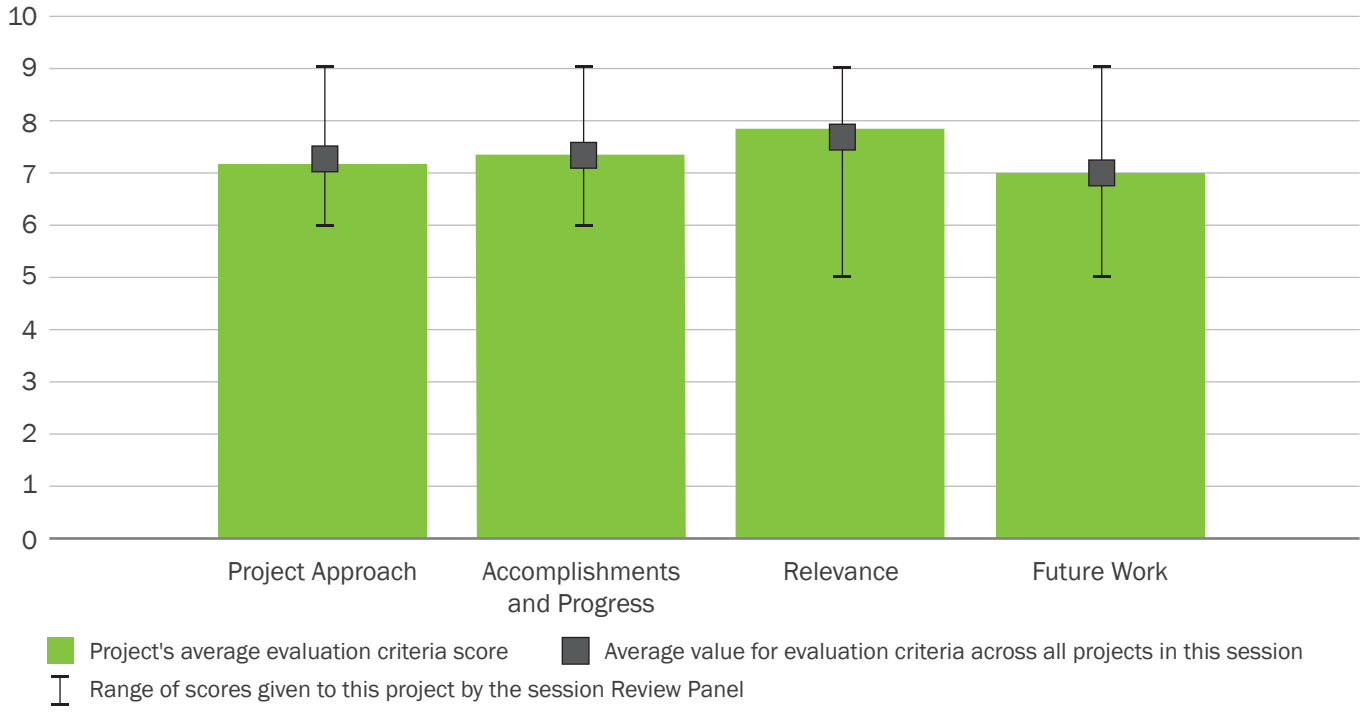
Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Michael Guarnieri
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$300,000
DOE Funding FY 2016:	$200,000
DOE Funding FY 2017:	$500,000

Project Description

The commercial viability of algal biofuel pursuits requires improved biological productivity across the entire value chain, which comprises a function of growth rate, biomass accumulation capacity, and a robust biosynthetic capacity for target molecules. Additional characteristics related to sustainability and deployment, such as tolerance to a wide range of salt concentrations and temperatures, must also be considered. To this end, the Algae Biotechnology Partnership aims to identify novel, halotolerant algal strains with productivity superior to current SOT strains, suitable for outdoor deployment in saline water as summer and winter crops. We are targeting the development of broad-host-range genetic tools in these halotolerant strains in an effort to achieve the level of development seen in the top-performing freshwater strains. To date, we have successfully screened, isolated, and characterized a series of halotolerant algal strains with exemplary productivity metrics, validated strain productivity in outdoor ponds, and developed baseline genomic and genetic tools for targeted strain engineering pursuits. Importantly, these strains have demonstrated productivity superior to BETO 2016 SOT metrics. This work directly targets identified BETO MYPP barriers, including feedstock availability and cost, sustainable production, and feedstock genetics and development.

Weighted Project Score: 7.3

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



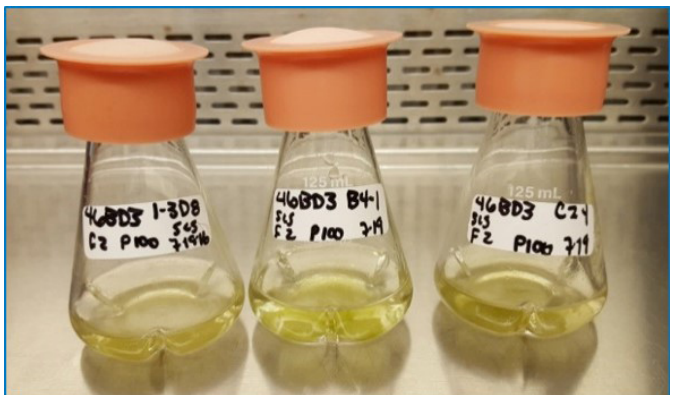


Photo Courtesy of National Renewable Energy Laboratory

Overall Impressions

- This project is working on finding strains that can grow in adverse, non-fresh-water environments and are suitable for typical downstream processing. The work to develop a high-productivity strain is relevant to the industry and meeting MYPP goals. To this point, the work has been done partially indoors and/or batch systems. The future work to enable targeted strain engineering to improve the productivity and demonstrate in outdoor ponds is appropriate for their presented plan.
- Strain screening and characterization has identified some promising-looking salt tolerant strains with high productivity and suitable compositions. Genetic toolbox development is making good progress. More complete characterization should be forthcoming. The future plans for the project as presented were quite vague, especially for FY 2018.
- Overall, this project has substantial merit and potential for the discovery of higher-productivity microalgae species. Integration of simulated outdoor screening blends well with the ability to transition for lab to field pilot-scale data collection.
- Advanced genetic tools (especially newly developed CRISPR technology) for algae will be important for advancing toward productivity targets and improving strain robustness.
- Screening several halotolerant algal strains for modest improvements in productivity relative to SOT is a key accomplishment of this project to date. Regarding genetic tool development, this project is ambitious at the risk of being somewhat unfocused. Beneficially, this project leverages core capabilities of several partner institutes and has effectively utilized testbed facilities (ATP3).
- This project couples strain improvement efforts with scale-up and testing in outdoor ponds. Results to date include identification of several fast-growing strains and demonstrated performance above a benchmark strain under several conditions and scales. Identification of fast-growing, halotolerant strains will have clear relevance to industry and BETO goals if successful.

PI Response to Reviewer Comments

- We thank the Review Panel for their encouraging and constructive critique. We are optimistic that our progress to date to identify high-productivity halotolerant algal strains and develop associated genetic and genomic toolkits represents a critical advancement for the BETO algae portfolio and the larger algal research community as a whole. We look forward to continued efforts to enhance productivity in top-candidate strains via the further development of robust genetic and functional genomic tools.

BREEDING ALGAE FOR LONG-TERM STABILITY AND ENHANCED BIOFUEL PRODUCTION

(WBS #: 1.3.2.104)

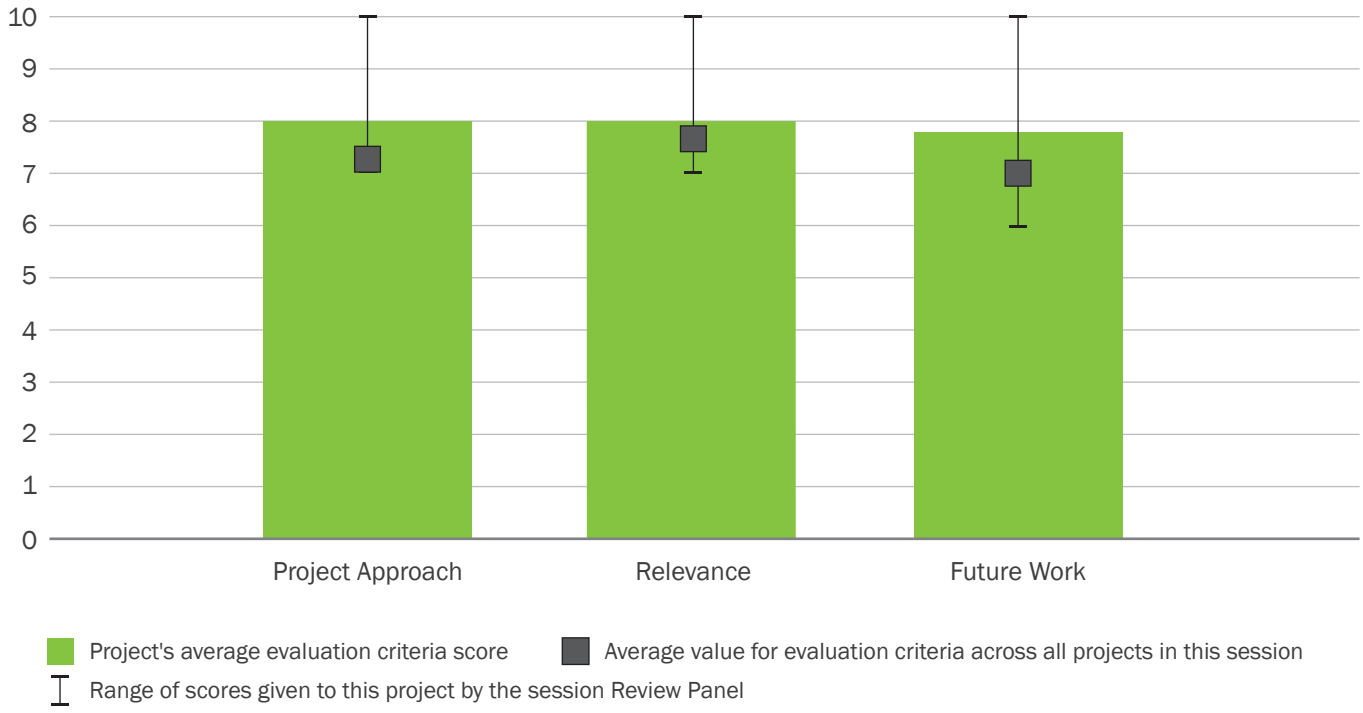
Recipient:	Los Alamos National Laboratory
Principal Investigator:	Richard Sayre
Project Dates:	10/1/2016–9/30/2017
Project Category:	New
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$0
DOE Funding FY 2017:	$150,000

Project Description

With molecular-assisted breeding, great strides have been made in developing higher-yielding and more robust crops. To date, however, these techniques have not been applied to commercial strains of algae for crop improvement. This has been due to a lack of understanding of the genomics and reproductive life cycles of algae. Recently, we and collaborators completed the genome annotation of three independent isolates of the commercial algal production strain Chlorella sorokiniana. We discovered tremendous genetic diversity in these strains and the presence of genes involved in meiosis and encoding the flagellar proteins required for mating. Thus, the potential to use molecular-assisted breeding strategies for algal improvement exists. Over the first quarter of the project, we have developed conditions to induce breeding in Chlorella sorokiniana, as well as demonstrated induction of the flagella following meiosis and cytoplasmic exchange. We have developed a novel genetic transformation system to introduce genetic markers to track mating events and saturated the three genomes with genetic markers spaced on average 37 kilobase apart. Next, we will demonstrate generation of new recombinant lines and backcross these lines to

Weighted Project Score: 7.9

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.



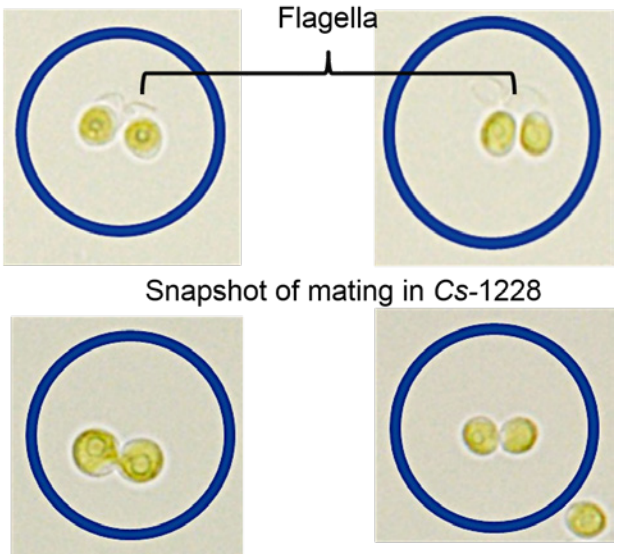


Photo Courtesy of Los Alamos National Laboratory

develop stable inbred lines. Longer-term goals are to select for traits of interest and to map the genes conferring those traits. The toolkits developed through this project will be made available to the community.

Overall Impressions

- Molecular-assisted breeding systems are being developed for a promising green algal strain. The success of such systems in higher plant improvement, and the fact that resulting strains would be non-GMO, makes this a worthwhile option to pursue. The species specificity of the approach is one downside, but methods developed could benefit others.
- This project employs a somewhat different approach than that of the other genetic manipulation projects, which I find to be very interesting. The stated goals of this approach try to lend the practices of what was done in conventional agriculture to that of the current algae farming industry. Though the project does have some challenges with deploying these strains and maintaining these improvements through the breeding process, it could be a step in the right direction.
- Great advancement in breeding systems—marker assisted for trait fixation and work on sterility systems is critical for progress in improved strains for production.
- Understanding algal reproduction is essential to harness potential (introduction of stable traits) and mitigate potential problems (trait loss in production settings). Most studies aiming to improve strains for large-scale biomass production don't consider the potential benefits and problems associated with algal breeding in a production setting, and researchers should be commended for developing a strategy to investigate the potential with this project.
- With a single year of funding, this project is on track to make significant accomplishments that could lead to methodologies that are widely applicable in the algae community. The rigorous approach to developing stable lines could help modernize algae breeding efforts, which appears to be a gap in the algal industry today.

PI Response to Reviewer Comments

- We thank the reviewers for their efforts and helpful comments.

MAJOR NUTRIENT RECYCLING FOR SUSTAINED ALGAL PRODUCTION

(WBS #: 1.3.2.200)

Recipient:	Sandia National Laboratories
Principal Investigator:	Todd Lane
Project Dates:	10/1/2013–9/30/2016
Project Category:	Sun-setting
Project Type:	FY 2012—Advancements in Sustainable Algal Production (ASAP): DE-FOA-0000615
Total DOE Funding:	$2,145,126

Project Description

A consortium of researchers from SNL, Texas A&M AgriLife Research, and Open Algae have developed a novel, cost-effective, and efficient remineralization process to convert organic forms of nitrogen and phosphate present in algae to chemical forms that can be liberated from the harvested algal biomass, then readily captured and returned to algal mass culture systems, and that are capable of supporting algal growth. We have developed methods for the rapid remineralization of up to 70% of the cellular phosphate from osmotically shocked, non-denatured algal biomass using endogenous enzymes under a range of relatively mild incubation conditions. Our phosphate remineralization process, which we have demonstrated at both laboratory and pilot-pond scale, supports equivalent algal growth and does not contain any growth inhibitory compounds, as evidenced by multiple sequential cycles of growth and nutrient remineralization. We have also demonstrated the remineralization of approximately 60% of cellular nitrogen through the fermentative conversion of amino acids to ammonium. We have precipitated re-mineralized phosphate and ammonia through the formation of struvite and demonstrated growth of algae on these recaptured nutrients. Finally, we have demonstrated the potential to integrate

Weighted Project Score: 7.0

Weighting: Approach–25%, Relevance–25%, and Accomplishments and Progress–50%.

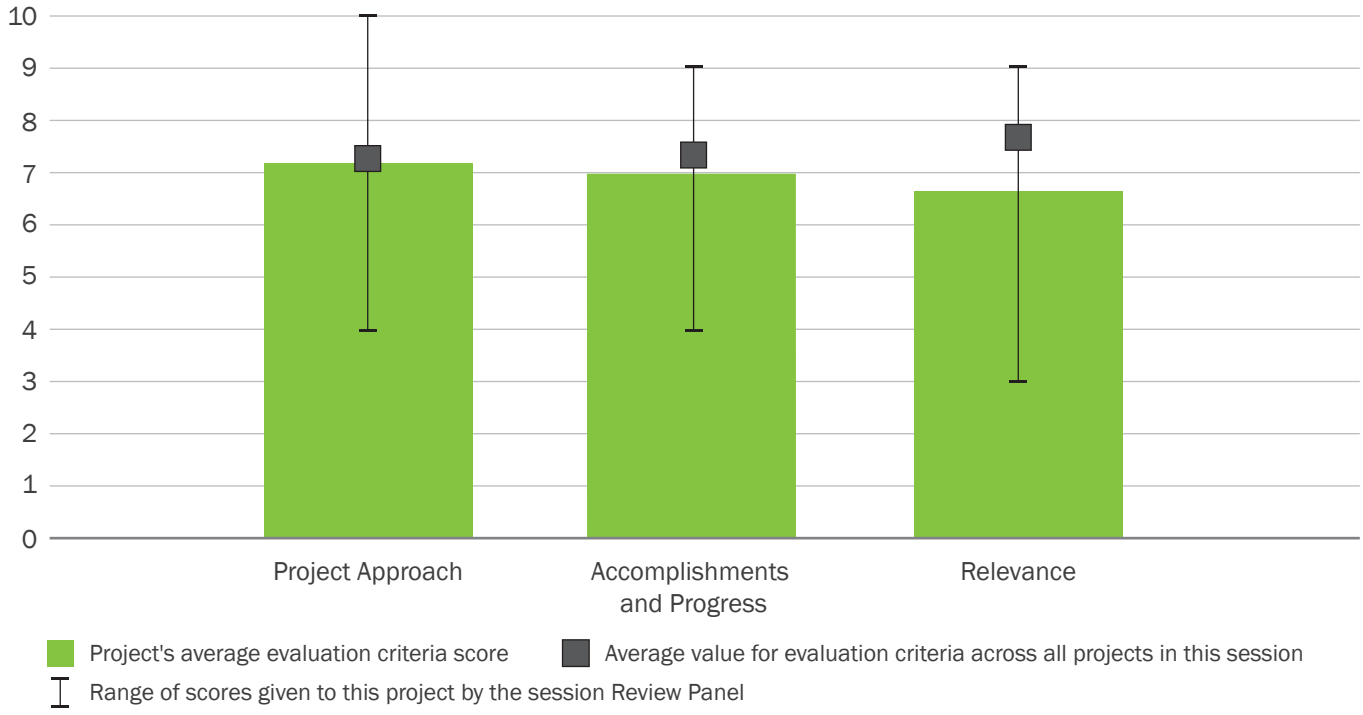


Photo Courtesy of Sandia National Laboratory

our nutrient recycle protocols with biomass processing methods, such as those for the extraction of neutral lipids.

Overall Impressions

- Excellent presentation. The goals, methods, and conclusions were clearly communicated and to the point. This is a well-focused project with clearly stated results and conclusions. I appreciate the work done to demonstrate the results from bench scale to out-of-doors. The project demonstrated a feasible method for recycling phosphorus and the majority of nitrogen. Additional work to determine the economics of this approach may be warranted.
- This project was an important demonstration of a mechanism for recycling nitrogen and phosphorus to enhance the sustainability and economic viability of algal biofuels. This is particularly critical work given potential global phosphate supply limitations. Though perhaps somewhat "dated" now, the goals of the project were achieved, including a demonstration of biological accessibility. Integration with TEA should have been discussed.
- Overall, the recycle of nutrient stream back to the main production systems has significant value. The main issues may relate to how this process scale and integrates itself into the larger-scale production system in a cost-effective manner. Value may be found for the resale of recovered nutrients as a potential co-product.
- This project demonstrated promising results for nitrogen and phosphorus recycling and should be continued—particularly the work around struvite.
- Nutrient recycling efforts have a clear link to BETO priorities for resource use efficiency, and the presenter articulated a clear rationale for a need for phosphorus use efficiency in general terms. It is unclear if this project would have relevance to large-scale algae production.

PI Response to Reviewer Comments

- We would be happy to continue work should additional resources become available to address the TEA, engineering, and marketing issues that were not in the scope of the original project.

INTEGRATION OF NUTRIENT AND WATER RECYCLING FOR SUSTAINABLE ALGAL BIOREFINERIES

(WBS #: 1.3.2.202)

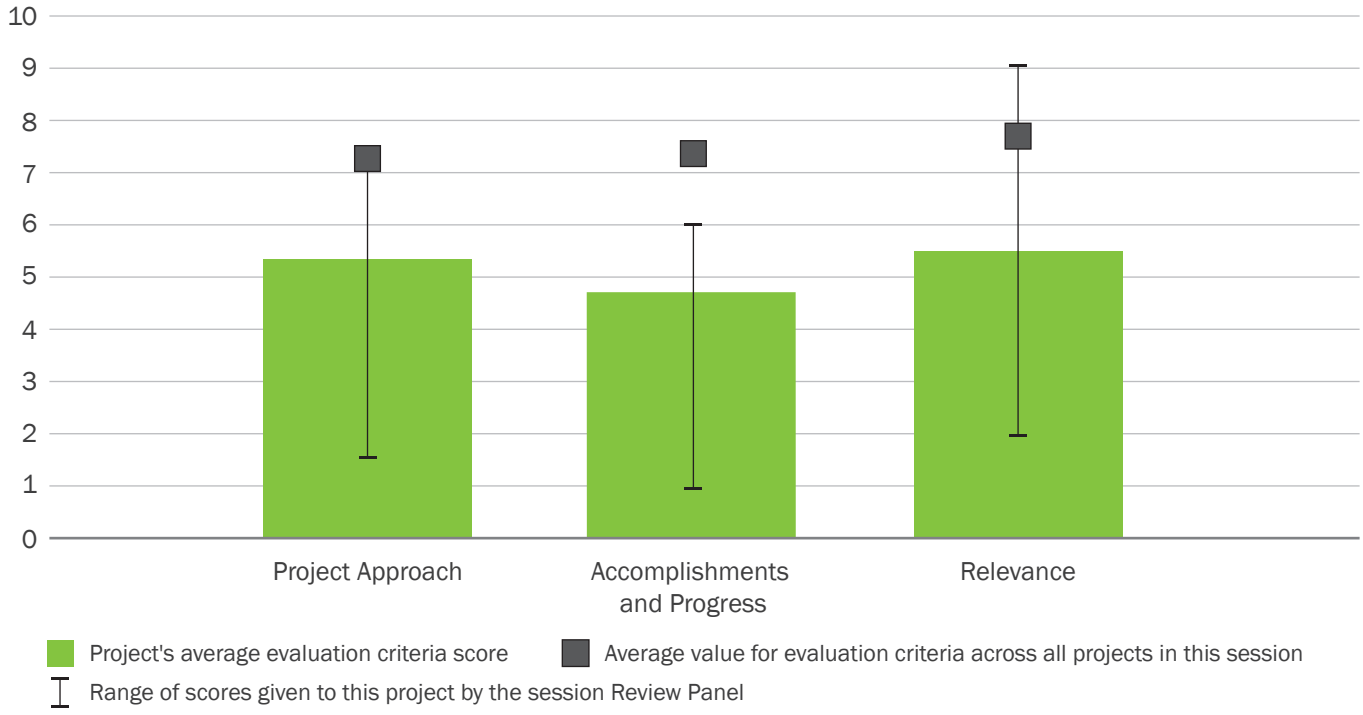
Recipient:	University of Toledo
Principal Investigator:	Sridhar Viamajala
Project Dates:	2/1/2013–8/31/2016
Project Category:	Sun-setting
Project Type:	FY 2012—Advancements in Sustainable Algal Production (ASAP): DE-FOA-0000615
Total DOE Funding:	$2,999,934

Project Description

Our project has focused on isolating and characterizing high-productivity microalgae strains that thrive in alkaline conditions. The microalgae are cultivated under conditions that simultaneously provide (1) a high pH (approximately 10.2) to effectively scavenge atmospheric CO_2, and (2) a high alkalinity (greater than 100 milliequivalents) to maintain high, non-limiting bicarbonate concentrations (greater than 30 millimolar) for photosynthetic carbon fixation. Under these growth conditions, we have demonstrated sustained high productivity (approximately 20 g/m^2/day) of strain SLA-04 under outdoor conditions, even in the absence of supplemental CO_2 input. Furthermore, in 2 years of outdoor experiments, the cultures resisted detrimental contamination and culture "crashes," likely due to the high pH values. Finally, we demonstrated that the cultures grow well in fresh water, high salinity waters, and with a low input of synthetic fertilizers. The resulting low-N-content biomass (approximately 3%) is favorable for biofuel production due to the higher relative proportion of carbohydrates and lipids. Our project has thus addressed cultivation challenges related to (1) sourcing limitations and high cost of CO2 delivery, (2) high culture productivity while maintaining culture stability, and (3) mini-

Weighted Project Score: 5.0

Weighting: Approach–25%, Relevance–25%, and Accomplishments and Progress–50%.



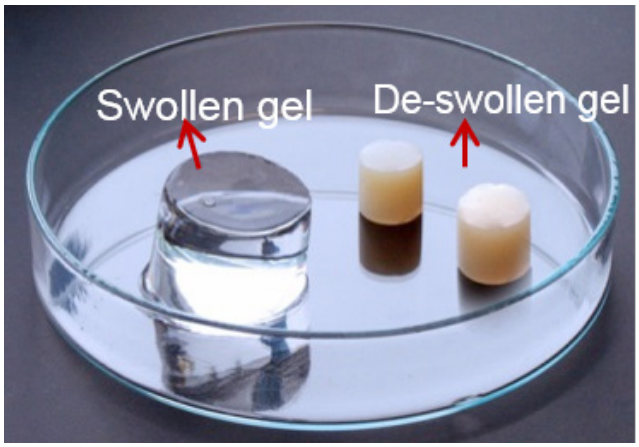


Photo Courtesy of University of Toledo

mization of nutrient inputs. We have also developed and tested novel "smart hydrogel"-based solid-liquid separations that allow for low-energy harvesting and effective water recycle without chemical contamination.

Overall Impressions

- The cultivation studies performed as part of this project have produced some unexpectedly high productivities while relying only on CO_2 transport from the atmosphere. It is my understanding that these tests occurred over the time frame of 4 to 8 days. To validate the results, it would be beneficial if the investigators would increase the testing period to several months. The use of hydrogels is an innovative approach for dewatering. The hydrogels should be tested to see what effect media contaminants have on the gel performance and a TEA prepared to explore potential commercial uses or limitations.
- The project explored two approaches that touch on critical limitations of algal biofuels production (CO_2 cost and pond siting, culture stability, and dewatering efficiency). Results are promising when taken at face value, but were not convincingly presented.
- The reduction of needed CO_2 for the operation of a large-scale system does have merit. Decoupling the sites from co-location lends to the possibility of more attractive siting opportunities. There are potential issues with the overall scaling of this system with site-specific water sources causing precipitation issues due to the heavy loads of bicarbonate, which in turn could increase the overall operating costs.
- This is an interesting project, but there are many issues with how experiments were conducted (i.e., length of growth trials) relative to the conclusions drawn.
- Conceptually, cultivating algae at high pH/alkalinity is beneficial for both CO_2 delivery and crop protection. This project provides data demonstrating that high productivities can be obtained in a high pH/alkalinity system, which is one of the most important findings from this work. Few publications have resulted directly (yet), and the effort could be more productive.
- This project addresses high-priority issues of improving productivity, culture stability, and low-cost dewatering through a targeted set of technologies (high pH growth and hydrogel dewatering). Results appear promising, but the short-term nature of the experimental approach and the lack of economics for the dewatering approach make it unclear how results will be used by the broader community.

PI Response to Reviewer Comments

- We thank the reviewers for their careful review of our project and acknowledgement of its many strengths. The reviewers also expressed some concerns, which are addressed below.
  - Duration and scale of growth experiments: The scope of our project is to develop fundamental science and engineering-based solutions to some of the critical challenges associated with microalgae cultivation—high cost of CO_2 supply and frequent culture crashes. As such, using first-principles mass transfer models coupled with aquatic inorganic carbon chemistry, we assessed that a

high pH and high alkalinity media would allow high-productivity microalgae cultivation without concentrated CO2 inputs. The high pH media would also mitigate frequent predator infestation. Conceptual validation of this approach was achieved through outdoor pond cultivations at 30 L and 1,000 L scales. As correctly pointed out by the reviewers, the next steps would be pilot-scale studies to address (1) scale-related issues, if any; and (2) long-term culture performance. We have ongoing work to complete longer-term studies (of 3-month duration), and we anticipate that these will be completed by the end of July 2017 (before the end date of the project).

- Potential for precipitation: We agree with the reviewers that precipitation of carbonates can become a concern in high carbonate alkalinity media. Until now, the team has not observed significant issues with precipitate formation since pH values, carbonate concentrations, and multi-valent cation concentrations will have to be high for carbonate (e.g., calcium carbonate) precipitation to occur. Geochemical modeling using MINTEQ and PHREEQC have been accompanying the work, and we continue to be vigilant regarding the possibility of mineral precipitation having an impact on the potential of the high-alkalinity culturing approach.
- Publications: Two patent applications (one provisional and one utility) and one publication have been submitted, describing aspects of the high-pH and high-alkalinity cultivation and hydrogel harvesting. The bulk of the work on this aspect of our project has only recently been completed (and some work is still ongoing), and additional manuscripts are under preparation for submission.
- TEA of hydrogel harvesting: This has been addressed in (1) a recent publication from our group,[19] and (2) a recent master's thesis.[20]

[19] Adam M. Hise, Gregory W. Characklis, Jordan Kern, Robin Gerlach, Sridhar Viamajala, Robert D. Gardner, and Agasteswar Vadlamani, "Evaluating the Relative Impacts of Operational and Financial Factors on the Competitiveness of an Algal Biofuel Production Facility," *Bioresource Technology* 220 (2016): 271–281.

[20] Adam Hise, "Interdependence of Financing Parameters and Processing Improvements in the Design of Economically Competitive Algal Biofuel Production Pathways" (master's thesis, University of North Carolina at Chapel Hill Graduate School, 2015), https://cdr.lib.unc.edu/record/uuid:981f952c-f518-4f0f-ad25-599178aaaf8a.

CONTINUOUS BIOLOGICAL PROTECTION AND CONTROL OF ALGAL POND PRODUCTIVITY

(WBS #: 1.3.2.300)

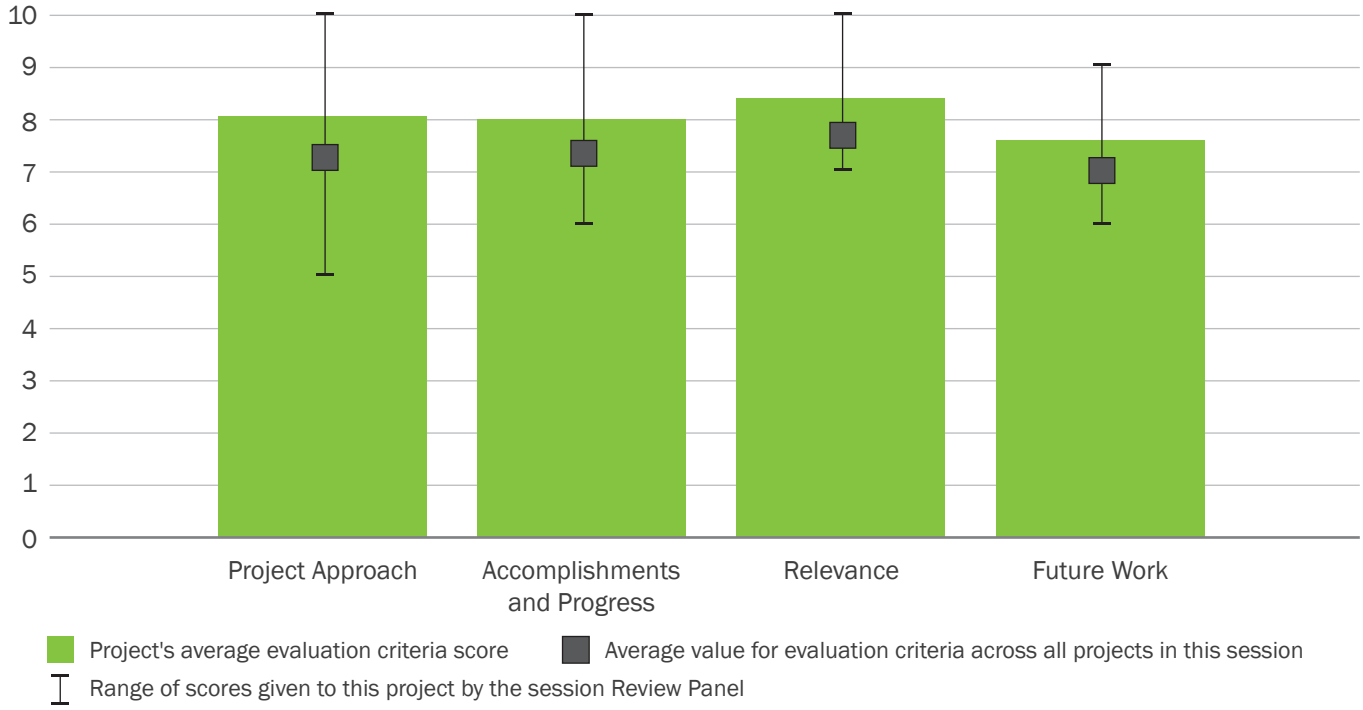
Recipient:	Lawrence Livermore National Laboratory
Principal Investigator:	Michael Thelen
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	FY 2015—Targeted Algae Biofuels and Bioproducts (TABB): DE-FOA-0001162
Total DOE Funding:	$695,000

Project Description

A team of researchers that brings together complex microbial community analyses (Lawrence Livermore National Laboratory), cutting-edge algal monitoring (Sandia National Laboratories), a leading commercial algal company (Heliae Inc.), and techno-economic modeling (University of California, Davis) are developing protective bacteria for crop protection that will increase algal pond stability and improve predictability and annual yield. Pipeline development to identify probiotic species and conditions will also provide a path forward for translating microbiome work from bench scale to process development. While causative pests in pond crashes often are unknown, grazing in general is estimated to result in a 20% loss in biomass productivity annually. By targeting common culprits of ponds crashes, we estimate that, at minimum, a 5%–10% increase in annual yield is possible if our goal is achieved. To date, we have developed assays to determine grazing and parasitism rates of model pests (rotifers and chytrids) and set up a screening pipeline to identify novel probiotic consortia and isolates that decrease these rates. We have demonstrated isolates and consortia that decrease grazing rates under variable bench-scale conditions, and we profiled an industrial system microbiome over an entire year to identify conditions for optimal probiotic application.

Weighted Project Score: 8.0

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



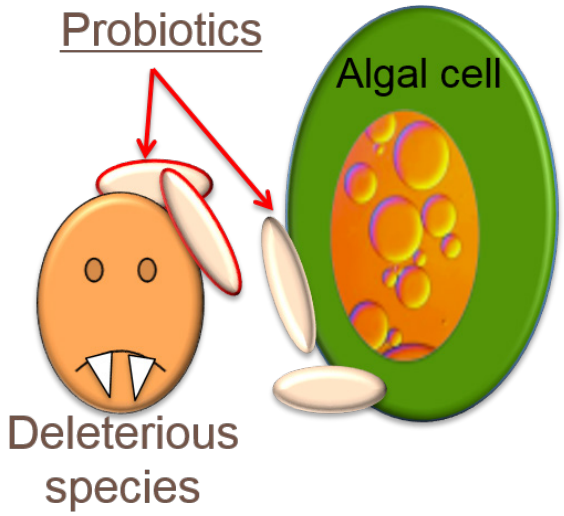


Photo Courtesy of Lawrence Livermore National Laboratory

Overall Impressions

- This project is good initial work for determining the potential protecting properties of probiotic bacteria. Future work should include the ability to sustain the effects long term. The long-term effects may be very complicated to control and predict as a pond's susceptibility to pests can be due to accumulative stress factors, which makes determining a solution complex. The future work will include a TEA, which should be revealing. I appreciate the scale-up to commercial size this project will use to test their conclusions.
- The investigators have developed powerful tools to screen for probiotic bacteria for crop protection. This is a very valuable approach and has already yielded promising bacterial candidate strains. The microbial community interactions that will be revealed through this work will be of fundamental scientific interest, but also will have practical implications for increasing real-world algal productivities. It is important to include studies to assess bacterial effects on productivity with no predation challenge. Elucidating the protective mechanisms would be an important contribution if the project scope allows.
- Overall, this project is an innovative approach that could show significant value moving forward. Though there may be some issues with the ability to apply this at scale due to regulatory and co-product issues, this project could provide a new and innovative way to combat pond predation and improve overall crop productivity.
- This project demonstrates the potential of non-chemical crop protection of outdoor raceway ponds and the importance and potential leveraging of the microbiome of an algal culture. Alternative crop protection methods are required to move the industry to the scale needed for the MYPP. However, the methods must be deployable at scale and cost-effective.
- The team presented a clear rationale for the project, and the technical approach appropriately addresses interaction between scales. A TEA was integrated through the team's efforts. The team appears to be on track, and future work is clearly aligned with the original work plan. This project is highly relevant, as pond stability is absolutely critical to all long-term BETO MYPP productivity and cost targets.

PI Response to Reviewer Comments

No official response was provided at the time of report publication.

INTEGRATED PEST MANAGEMENT FOR EARLY DETECTION ALGAL CROP PRODUCTION

(WBS #: 1.3.2.310)

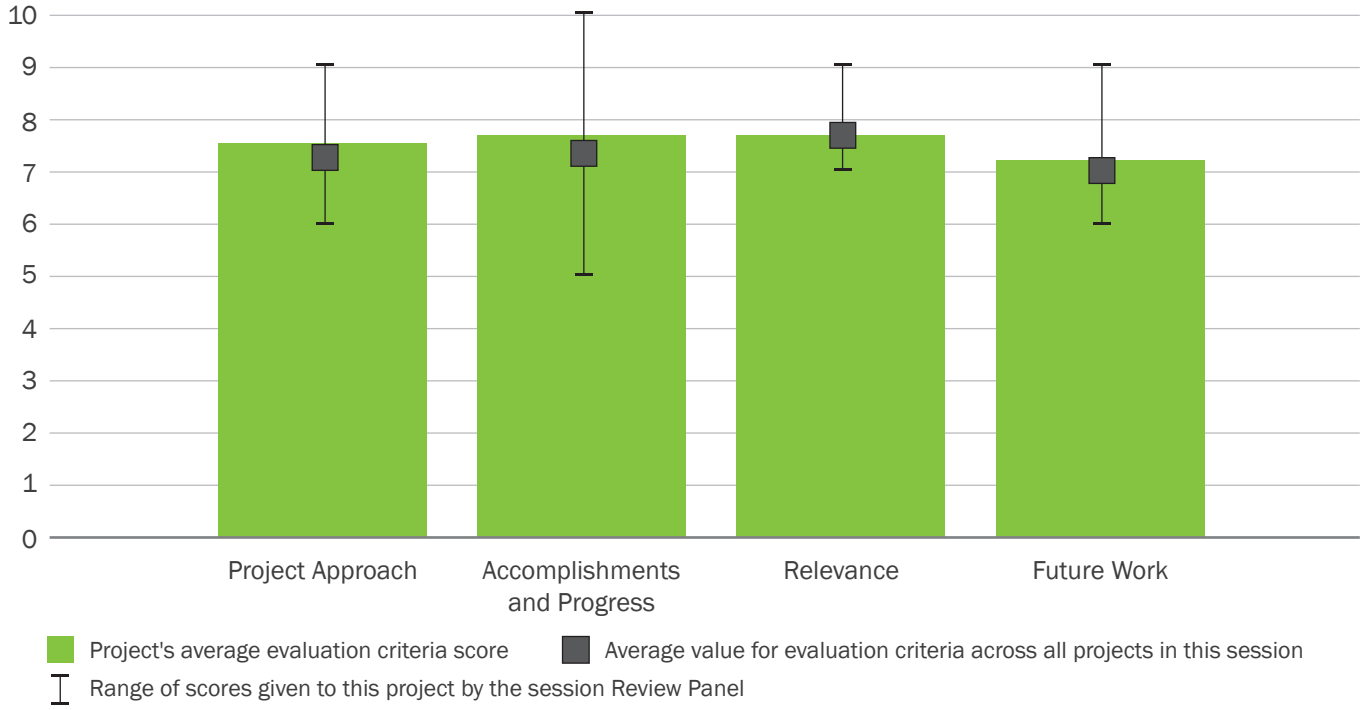
Recipient:	University of California, San Diego
Principal Investigator:	Robert Pomeroy
Project Dates:	10/1/2015–3/31/2019
Project Category:	Ongoing
Project Type:	FY 2015—Targeted Algae Biofuels and Bioproducts (TABB): DE-FOA-0001162
Total DOE Funding:	$760,725

Project Description

Large-scale, outdoor algal biomass growth, production strain(s) are subject to attack by pathogens, predators, and non-productive competitors. This project focuses on the early detection and identification of pests with the intent of guiding the best practices for prevention and control. An automated mass spectrometry-based crop protection monitoring system coupled with quantitative polymerase chain reaction/high-resolution melt analysis that will be integrated into existing environmental control systems is under development. These systems will enable robust biomass production through automated, early, and potentially pest-specific crop protection and will greatly reduce the costs and labor associated with current monitoring systems and resultant losses of valuable biomass from contaminations. The project has encountered problems related to sample collection and strain purification. To date, we have a mass spectrometry-based platform that has shown promising results as an early detection system; a cataloging of molecules specific to prey-predator interactions, both in the head

Weighted Project Score: 7.5

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



space and bulk liquid has been collected and will be expanded; a Chemical ionization mass spectrometry capable of continuous monitoring is in development; a set of primers has been optimized for quantitative polymerase chain reaction/high-resolution melt as a simple closed-tube method of class/species identification; the techno-economic impact of this platform is underway; and intellectual property in the form of chemical marker identification, primer sequences, and new application-specific instrumentation is in development.

Overall Impressions

- Interesting project and detection method for determining if a pond is being stressed by predation. I would like to see a more detailed plan on how this system would be deployed in a commercially sized field, along with costs and operations plans. I think it will be difficult to quantify the benefit of this approach in a TEA. However, the early detection seems intuitively to be a good idea.
- The sensitivity of the approaches being used should allow for significantly earlier detection of upcoming culture crashes. If appropriate intervention strategies are available, this should lead to reductions in the number and length of downtimes. This is early-stage research and could elucidate specific markers for various biotic challenges. However, it is not clear how this will transition into a low-cost, field-deployable detection system (i.e., "simple, automated, affordable, and robust technologies").
- Overall, this is a very interesting and forward-thinking project. Though the described system at scale may not be feasible for deployment due to various complications with maintaining and the costs of installing specific high-cost infrastructure, the premise of the project could have a dramatic impact on the ability to maintain crop integrity and stability moving forward. The concept of this project may find a better deployment option as the system is tested and costs are considered at scale.
- Novel approach to pest detection that seems reasonable for field deployment; it could be used by field operators in real time (depending on prototype), or, depending on the cost of infrastructure, put online for real-time monitoring. This would enable early detection of pests, which is critical for crop stability and productivity improvements.
- Significant progress has been made during this project toward understanding the biology of predator-mediated pond crashes, which is critical knowledge necessary to develop systems aimed at early detection. Additionally, investigators have shown the economic value of early intervention through the use of TEA, making this work directly applicable for industry and increasing relevance. Knowledge gained from this project has the potential to contribute significantly toward production at scale, and future work should focus on developing industrially relevant prototypes.
- Improving detection sensitivity for pond crashes has clear relevance to mitigation strategies for maximizing uptime. The team presented a nice TEA to quantify the advantage of early detection, and the technology also may have applications in adjacent industries. It is unclear how the technology in its current form would be deployed in a large-scale algae farm, but the approach could be used for low-cost sensor development in the future.

PI Response to Reviewer Comments

No official response was provided at the time of report publication.

ALGAE PRODUCTION CO_2 ABSORBER WITH IMMOBILIZED CARBONIC ANHYDRASE

(WBS #: 1.3.2.320)

Project Description

GAI is a leader in low-cost algae production technologies. A suite of advances in open pond algae growth is being developed to achieve commercially viable production of oil and high-protein meal. An essential part of GAI's production method is the efficient and economical capture, storage, and distribution of CO_2 from power plant flue gas to the actively growing algae, ensuring an ample supply of CO_2 for photosynthesis. GAI has operated a large-scale open raceway algae cultivation system with all of the CO_2 supplied from power plant flue gas for the past 3 years. An advanced flue gas CO_2 supply method, which incorporates an absorber and carbonate shuttle, overcomes the permitting and engineering/cost limitations of other state-of-the-art approaches.

Recipient:	Global Algae Innovations Inc. (GAI)
Principal Investigator:	David Hazlebeck
Project Dates:	7/1/2016–7/1/2019
Project Category:	Ongoing
Project Type:	FY 2015—Targeted Algae Biofuels and Bioproducts (TABB): DE-FOA-0001162
Total DOE Funding:	$900,000

The objective of this project is to further improve the efficiency of this CO_2 supply method. The goals are to achieve 80% CO_2 capture efficiency and 90% carbon utilization efficiency in integrated operation of a high-efficiency absorber with open raceway algae cultivation. This project will improve the efficiency of GAI's proven system for utilizing power plant flue gas to supply CO_2 to large-scale open raceway cultivation, which is important to BETO objectives because low-cost CO_2 is necessary to achieve algal biofuel cost metrics, and high CO_2 capture and utilization efficiency are necessary to achieve biofuel life-cycle and production potential metrics.

Weighted Project Score: 8.3

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.

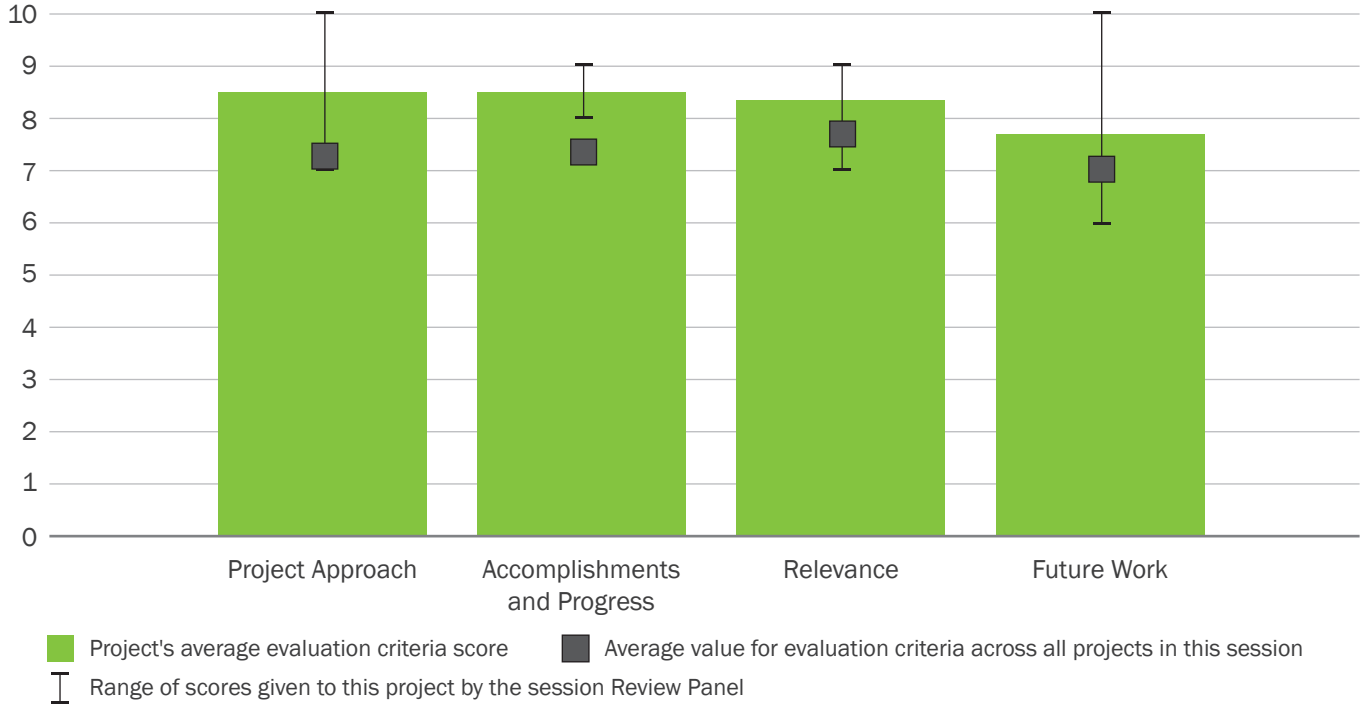


Photo Courtesy of Global Algae Innovations Inc. (GAI)

Overall Impressions

- Very innovative approach to use enzyme to speed the absorption of CO_2 into the media. While this approach is being investigated by others, this project will test, and hopefully improve, its robustness in an actual industrial application. Great job.
- GAI is taking a novel approach to issues around carbon delivery that many had not perceived as issues, but compelling arguments are presented to support the value of such an approach. The concept has the potential to reduce capital and operation expenditures around flue gas delivery to ponds. Good progress has already been made. Hopefully the economic case for (or against) this approach will be made available at some point to the greater community.
- Overall, this project has made significant progress in the completion of its deliverables. Utilizing a system of this nature provides the opportunity to reduce loss of conventional CO_2 injection systems currently used by industry. These advancements can and will have a significant impact on the industry.
- This project is highly relevant—growing algae exclusively on flue gas at over 5 acres for 3 years and developing commercially deployable technology to advance the industry.
- This straightforward and worthwhile project is aimed at developing technologies to improve sustainability and reduce the cost of producing fuel from algae at scale. It is particularly constructive to work to address engineering challenges (such as the capture and delivery of CO_2) in a facility involved in all aspects of production.
- Large-scale deployment of algal biofuels depends on high CO_2 utilization efficiency, and the proposed approach appears to result in large efficiency improvements compared to gaseous approaches in neutral-pH, unbuffered media. Strong progress toward technical targets was reported for carbon use efficiency and cost of CO_2 capture. The project appears ahead of schedule and on target for milestones.

PI Response to Reviewer Comments:

- CO_2 delivery is a crucial component of making algae biofuel a reality. Flue gas delivery for open-pond systems is particularly challenging with difficult permitting and engineering/cost issues. Our 8-acre open-pond operations have been integrated with CO_2 supplied from flue gas for 3 years, and our approach resolves the permitting and engineering/cost issues. We are excited to lead this project, which will provide a good option to the algae industry for low-cost CO_2 delivery by making further improvements to the efficiency of the CO_2 supply system.

ATMOSPHERIC CO_2 CAPTURE AND MEMBRANE DELIVERY

(WBS #: 1.3.2.330)

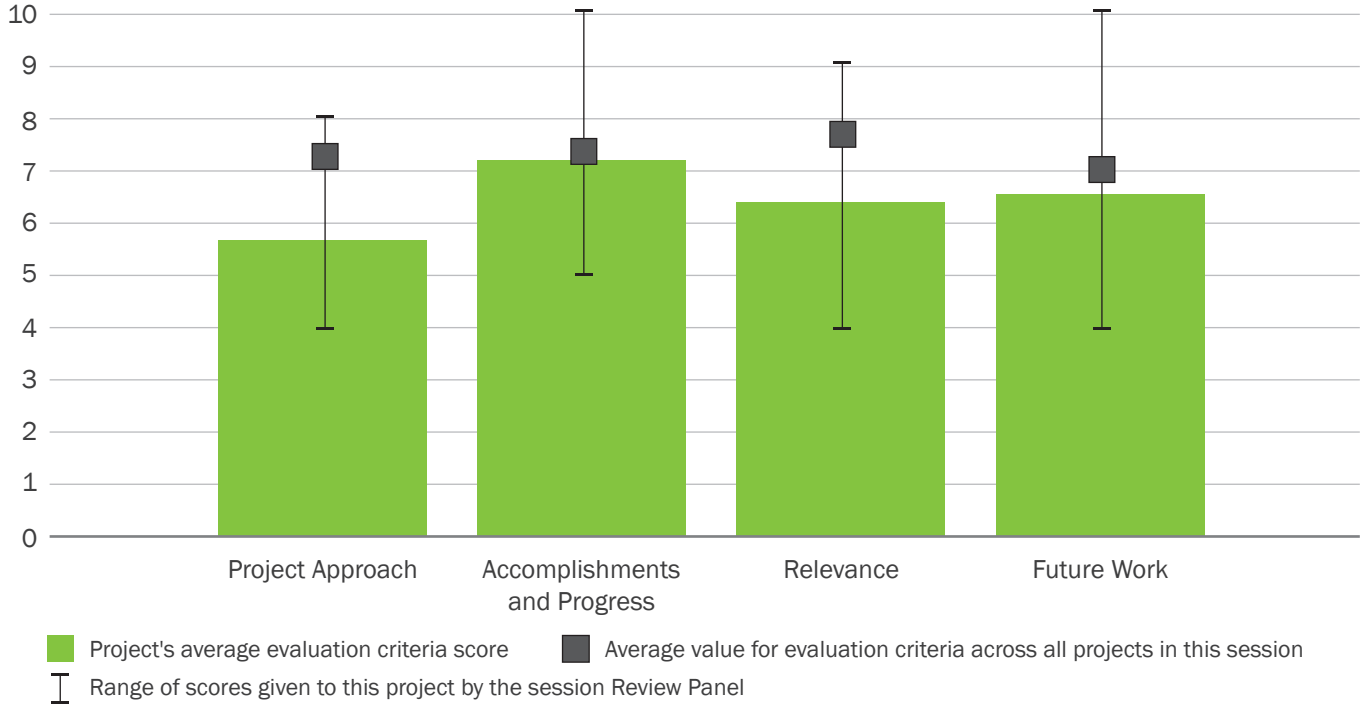
Project Description

Increasing the CO_2 concentration in gas supplied to a microalgae growth system can improve its productivity many fold over using atmospheric air. While flue gas seems like a good source of CO_2-enriched gas, it is not sustainable, can require significant transportation costs, and can include contaminants that can be toxic to the microalgae and contaminate fuel or other high-value products. We will overcome this obstacle by concentrating atmospheric CO_2 at the site of microalgae growth using moisture swing sorption (MSS) to provide a sustainable source of CO_2 without contaminants. MSS uses a dry/wet cycle to passively collect and concentrate ambient CO_2 about 100 fold. We propose to deliver the concentrated CO_2 using special bubble-less gas-transfer membranes, called membrane carbonation (MC). MC delivers CO_2 via diffusion on demand with efficiencies near 100%, which is a significant improvement over sparging. We will combine MSS and MC to deliver CO_2 to microalgae growth systems with high efficiency and at a rate that is high enough to promote high biomass production rates in closed or open systems. The project's objective is to scale up both approaches and integrate them into a single system that we will test at prototype scale outdoors using various algal strains (Scenedesmus, Chlorella, and Syn-

Recipient:	Arizona State University
Principal Investigator:	Bruce Rittmann
Project Dates:	10/1/2015–2/28/2018
Project Category:	Ongoing
Project Type:	FY 2015—Targeted Algae Biofuels and Bioproducts (TABB): DE-FOA-0001162
Total DOE Funding:	$1,000,000

Weighted Project Score: 6.4

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



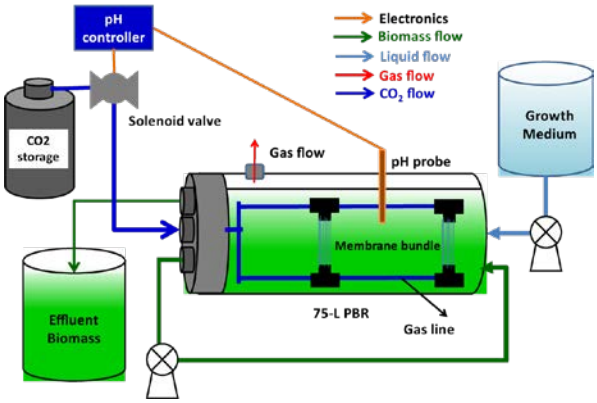


Photo Courtesy of Arizona State University

echocystis) that grow optimally with different concentrations of inorganic carbon.

Overall Impressions

- This project is attempting two innovative developments. One is for capturing atmospheric CO_2 using a "filter unit," and the other is a new method for delivering CO_2 to PBRs using membranes. I would rather these were separate projects so the focus would be strengthened. The approach for releasing CO_2 from a carbonate mixture and delivering gaseous CO_2 to the media using membranes has several processing steps, equipment, and requires energy input. It is not clear to me the cost-benefit of this approach, as we could much more directly feed the carbonate solution to the media. The cost of the filter unit is prohibitive. I understand that the project is exploring cost-reduction ideas for the filter unit. If the team is not able to significantly reduce the cost of the filter unit, maybe they will be able to recommend where additional research is needed to potentially make this approach feasible in the future.
- This project addresses an important goal of atmospheric CO_2 capture for algal cultivation, which could be game-changing. Good progress has been made in demonstrating the underlying technologies and evaluating the economics. However, the complexity and energy requirements of the system would seem to make economic viability of this approach extremely unlikely (as supported by the TEA). The value proposition for the CO_2 delivery system is particularly doubtful, and resources for that part of the project should be redirected to reducing costs in the CO_2 capture technology.
- Overall, this project illustrates an innovative approach that could provide significant impact. Some particle concerns with the potential scalability of a system of this nature may be difficult to overcome. But, this level of out-of-the box thinking drives innovation.
- Novel carbon capture and delivery system that is definitely applicable to PBRs, but needs significant work on cost reduction, open-pond suitability, and scalability.
- Improving delivery of CO_2 to algal ponds is important, and this project utilizes a novel technology—anionic resin sheets. The concept is interesting; however, there should be careful attention paid to system engineering and TEA to ensure this system/process is viable for industry.
- The proposed technology concentrates atmospheric CO_2 for delivery into algae production systems, which, if successful, would add considerable flexibility to land available for deployment of algae farms. The project appears to be on schedule and meeting proposed milestones. It is unclear if the proposed approach will be able to de-risk a technology that appears to have cost-prohibitive capital requirements.

PI Response to Reviewer Comments

- In principal, the carbonate/bicarbonate storage solution could be fed directly to the PBR in lieu of extracting and compressing the CO_2 gas for delivery via MC. However, this approach presents several problems: (1) photosynthesis normally drives up the

pH by consuming inorganic carbon and reducing nitrate, so delivering the acidic form avoids the need for adding acid to regulate the pH; (2) delivering bicarbonate requires a balancing cation, usually Na+, which increases the salt concentration; and (3) the storage tank contains a mixture of carbonate and bicarbonate at high pH such that it can more efficiently take up CO_2 delivered from the capture system, whereas PBRs are typically operated at a pH near 8.5. Thus, adding bicarbonate from storage will tend to increase the pH of the bioreactor, requiring compensating forms of acidity. Put another way, extracting accumulating alkalinity from the algae pond would be expensive. The storage system, as designed, retains the alkalinity in the storage tank and only transfers CO_2 to the microalgae.

Another more direct approach might be to deliver captured CO_2 directly to the PBR using a fabric contactor and bypassing the storage subsystem. As part of the final report, the team will suggest future lines of research to address commercial feasibility.

ALGAE DISCOVR PROJECT: DEVELOPMENT OF INTEGRATED SCREENING, CULTIVAR OPTIMIZATION, AND VALIDATION RESEARCH

(WBS #: 1.3.2.501, 502, 503, 505)

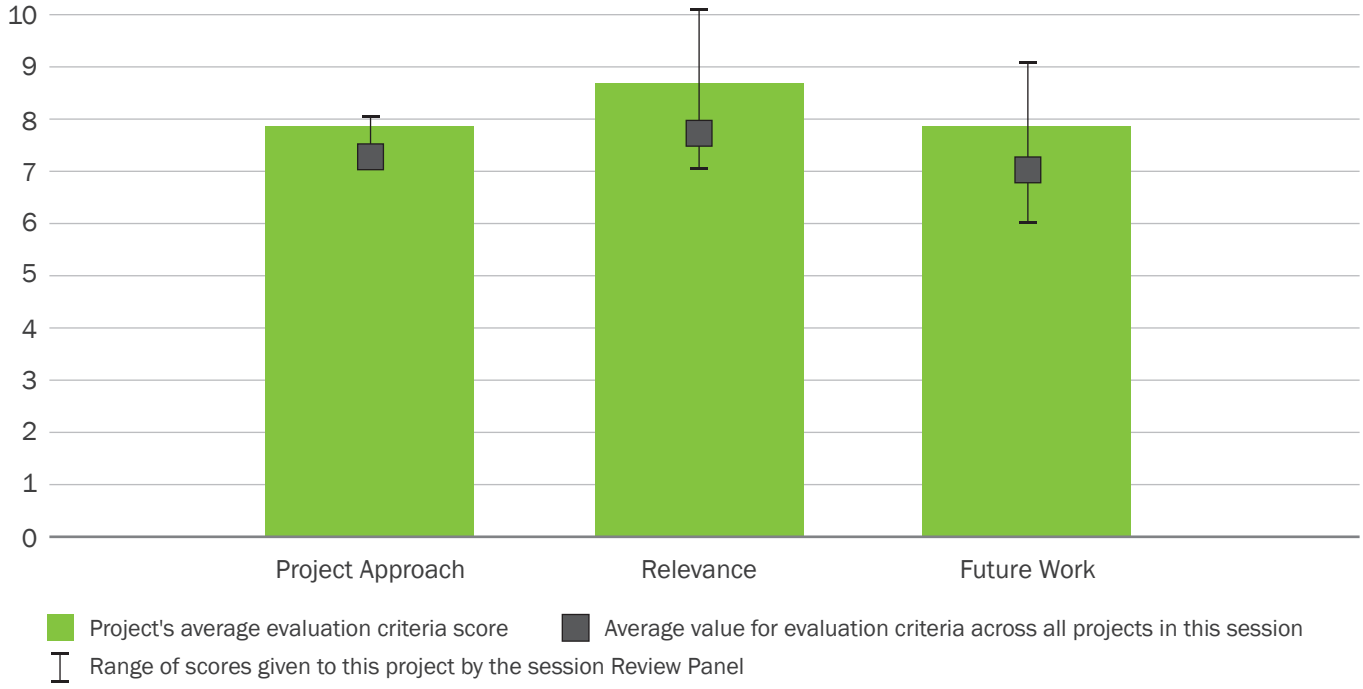
Recipient:	Multi-lab
Principal Investigator:	Michael Huesemann
Project Dates:	10/1/2016–9/30/2019
Project Category:	New
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$0
DOE Funding FY 2017:	$1,500,000

Project Description

The Algae DISCOVR Project is a national laboratory consortium consisting of PNNL, LANL, NREL, and SNL. The overall objective of the Algae DISCOVR project is to develop an integrated platform for standardized, deep characterization of high-productivity microalgae strains for robust year-round outdoor cultivation. In this 3-year project cycle, we will characterize at least 30 selected strains in terms of their detailed growth characteristics (TIER I); evaluate 10 strains in terms of their seasonal areal biomass productivity, basic biomass composition, and resilience to biological stressors (TIER II); improve and further characterize 6 strains in terms of more detailed biomass composition and biological stress resistance in indoor ponds (TIER III); and test 4 strains in outdoor ponds (TIER IV) to provide inputs to life-cycle and techno-economic analyses (TIER V). This streamlined, coordinated effort capitalizes on the consortium labs' complementary core capabilities in environmental simulation and productivity prediction, robustness evaluation, biomass valorization, and strain improvements. In summary, the DISCOVR project will develop a standardized, industrially relevant process for characterizing

Weighted Project Score: 8.0

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.



potential biofuels/bioproduct strains and aims to deliver the best strains to industry to assist in meeting BETO's goal of producing biofuels at less than $3/gge.

Overall Impressions

- This project appears to be very competently planned with good use of industry/academic expertise. The project goal of identifying specific strains and then going through a process of strain down-selection until finally testing in outdoor ponds and then developing a TEA should provide good characterization data and may also achieve a new SOT level. However, I suggest the project test how well their outdoor results in a small outdoor pond on a controlled site translates to larger ponds that are operated as would be expected for a large commercial facility.
- This relatively massive project seeks to identify and thoroughly characterize new strains with high potential for fuels and co-products. The overall approach is excellent and inclusive of many critical screens. It is also a model for inter-laboratory cooperation. There were some questions raised by industrial growers in the review session as to how well maximum specific growth rate of dilute cultures in microtiter plates will ultimately relate to productivity.
- The project's goal of setting the "gold standard" of strain evaluation is certainly within reach; however, the size limitation on final outdoor trials will eventually be a significant limitation.
- The development of a gold standard algal prospecting and strain development pipeline is highly meritorious, and the DISCOVR project is an organized, coordinated effort aimed at doing this. Strengths include leveraging national lab core capabilities and integrating efforts across multiple institutions. There are some concerns with workflow organization. Waiting until strains are completely characterized in the lab prior to conducting pond trials may be misguided because some strains that perform well outdoors may appear inferior to strains that perform well at the bench.
- This project represents an ambitious integration of national lab capabilities from strain screening, to optimization, to field validation. The project is clearly organized and managed. Early iterative indoor/outdoor testing is encouraged.

PI Response to Reviewer Comments

- Regarding screening at low cell densities: The DISCOVR screening process is based on our long-term experience in biomass growth modeling (validated for more than five strains) where we found that cells with a high maximum specific growth rate (measured in dilute cultures) and low light extinction coefficient have high biomass productivity in dense cultures.[21] Thus, strain screening in dilute cultures is predictive of productivity in high-density cultures.

  Regarding conducting outdoor trials at different scales: The testing of strains in ponds of different sizes is beyond the proposed scope of the DISCOVR project. However, depending on budget availability, it may be possible to test selected strains in ponds of different sizes at the ATP3 testbed site. ATP3 testbeds were developed to address algae production scaling challenges, and productivity comparisons have been performed across different scales in the past (for example, we are aware that the Cellana Kona Demonstration Facility, an ATP3 testbed site, ran comparative outdoor pond trials at 1,000 and 60,000 L). Furthermore, depending on industry interest in se-

[21] M. Huesemann, B. Crowe, P. Waller, A. Chavis, S. Hobbs, S. Edmundson, M. Wigmosta, "A Validated Model To Predict Microalgae Growth in Outdoor Pond Cultures Subjected to Fluctuating Light Intensities and Water Temperatures," *Algal Research* 13 (2016): 195–206, https://doi.org/10.1016/j.algal.2015.11.008.

lected strains, these could be grown at larger outdoor facilities. We will consult with our Technical Advisory Board regarding their knowledge of the effects of scale and will take advantage of any interest in potential outdoor testing at industrial facilities.

Regarding testing strains outdoors at TIER IV and not earlier in the 'pipeline:' There are many reasons why we developed a tiered approach for strain testing, such as time- and cost-effectiveness (i.e., similar to drug screening), knowledge of a strain's temperature and salinity tolerance, and pre-confirmation of high biomass productivity potential in PNNL's laboratory environmental algae pond simulator PBRs. In addition, in evaluating robustness in terms of predator or pathogen resistance, indoor testing under simulated environments has numerous advantages over a program of outdoor testing. Pond crashes are somewhat stochastic and unpredictable, so a dependence on 'naturally occurring crashes' severely limits the amount and quality of data that can be recovered. With indoor climate simulation ponds, we can control the conditions under which studies are conducted and are not hostage to the vagaries of weather, nor limited to specific growing seasons, single growth conditions, or naturally occurring environmental inputs to cause crashes. Furthermore, uncontrolled environmental inputs from outdoor culture can confound the measurement of resistance to specific predators or pathogens. In short, we can collect much more useful information on resistance through controlled challenge experiments. Finally, through the use of environmental simulation ponds, we do not run the risk of contaminating other portions of the outdoor test facility or compromising other experiments.

Lastly, there may be a misunderstanding regarding the outdoor testing schedule. Outdoor testing will start early in the project (not at 2.5 years): there will be two rounds of outdoor strain testing with the first round starting as early as the second quarter in Year 2 and the second round starting in Year 3.

GENETIC BLUEPRINT OF MICROALGAE CARBON PRODUCTIVITY

(WBS #: 1.3.2.504)

Project Description

Recipient:	Lawrence Berkeley National Laboratory
Principal Investigator:	Igor Grigoriev
Project Dates:	10/1/2016–9/30/2019
Project Category:	New
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$0
DOE Funding FY 2017:	$125,000

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. Furthermore, 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. The project objective is to gain a better understanding of algal biology, in particular (1) mechanisms regulating carbon production and switches from rapid growth to stress-induced carbon storage, and (2) growth responses at varied temperatures critical to overcome these limitations. Improving the productivity and robustness of algal strains against perturbations will require extensive advanced genetic, genomic, and molecular biology tools, which currently are lacking for most algal species. This project directly addresses barriers to genetic modification and development, as described in the BETO MYPP. 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 bio-

Weighted Project Score: 7.8

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.

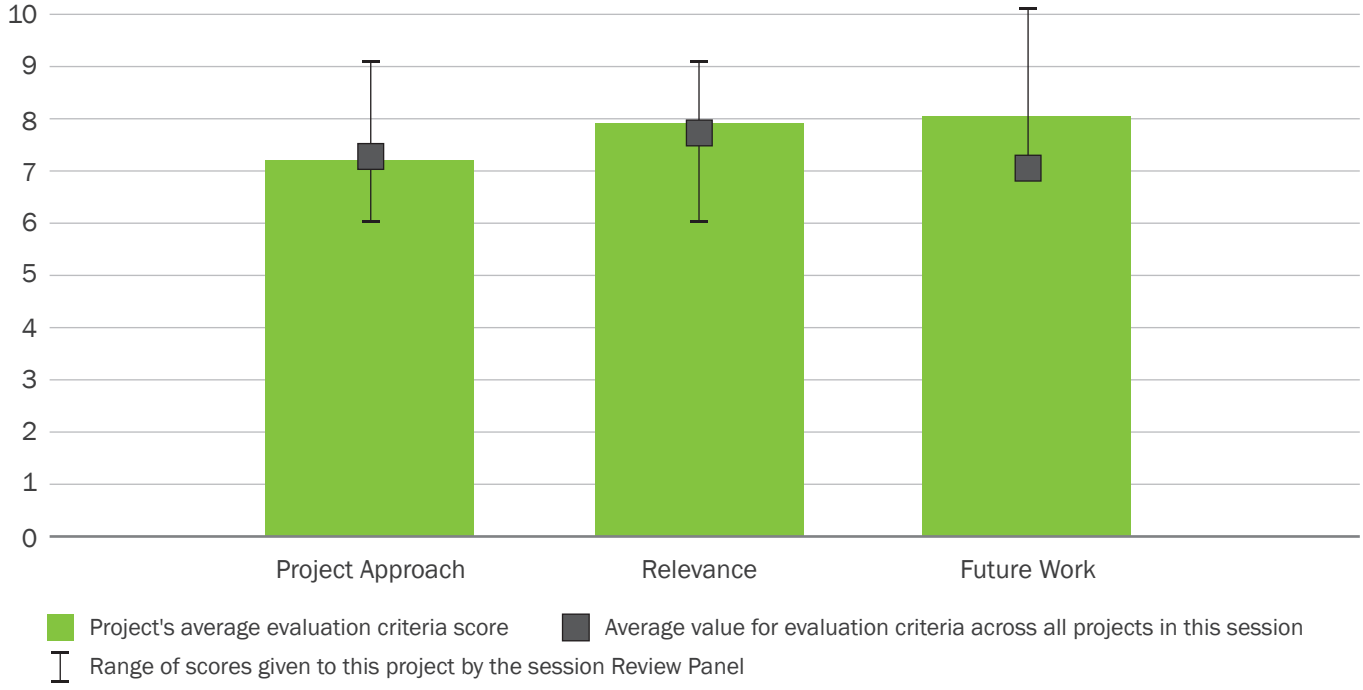


Photo Courtesy of Lawrence Berkeley National Laboratory

mass production rates by 25% and significantly decrease the lag time for genetic modification.

Overall Impressions

- Hopefully this project is successful in speeding up the time it takes to understand a strain. It would be good if the presenter provided more information on the extent of the issue they are attempting to address and the real benefits to industry if their intended target for reducing the schedule is realized.
- Development of functional genomics tools for new strains and new cultivation conditions is of potentially high value. A massive amount of data will be collected under this project, but at this point the nature of the expected deliverables is quite vague. Choice of temperature variations as the first conditions to study is questionable.
- Overall, this project is another genetic development plan to enhance overall large-scale productivity.

  Issues with using small-scale optimization information and a slow throughput may be an issue. But the overall improvement of genetic information in the algal community, which is lacking in many respects, would lend greatly to the overall understanding of certain variables that can lend to the overall improvement of algal biomass production.
- Great tool for strain improvement work; integrating the omics and deciphering the regulatory network will help elucidate not only seasonal transition information, but can also help to maximize product/co-product production.
- This straightforward project aims to sequence the genome and do initial/exploratory functional genomics studies on an industrially relevant strain (Chlorella luteoviridis). Eventually, this project will enable strain engineering for enhanced production traits in this species. While it is difficult to say whether results from this project will directly contribute transformational knowledge for large-scale cultivation, it will certainly enable additional investigations on an organism with industrial potential, making this project a relevant and important part of the portfolio.
- The proposed work seeks to demonstrate a method for using a 'pipeline approach' for coupling genetic and genomic technologies to improve strain performance. It is unclear whether the project will result in an improved strain that has industry relevance, but demonstration of the pipeline approach should have wide applicability.

PI Response to Reviewer Comments

- Expected deliverables for this project include a (1) functional genomics pipeline applicable to various strains produced by other BETO projects, (2) accelerated cycle to identify gene targets for algal strain improvement, and (3) list of targets for the Chlorella luteoviridis improvement.

  Regarding choice of temperatures, we selected the Chlorella strain as the first pilot because it outperformed other strains in the Mesa, Arizona, outdoor pond experiments, and we aim to simulate these conditions with perturbation experiments in an environmental PBR to identify a ranked list of gene targets for strain improvement.

Regarding comments on low throughput, small scale, and industrial relevance, we intend to accelerate the target selection process, completing the first phase within 2 years (50% improvement over current cycle time). In the first small-scale experiments, we will develop a robust and customizable pipeline that can be applied to other industrially relevant strains at different scales. In addition, we will collaborate with NREL and its partners to further develop and improve Chlorella luteoviridis as an industrially robust strain.

THE GREENHOUSE: A COMPREHENSIVE KNOWLEDGE BASE OF ALGAL FEEDSTOCKS

(WBS #: 1.3.2.600)

Recipient:	Los Alamos National Laboratory
Principal Investigator:	Shawn Starkenburg
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$0
DOE Funding FY 2017:	$350,000

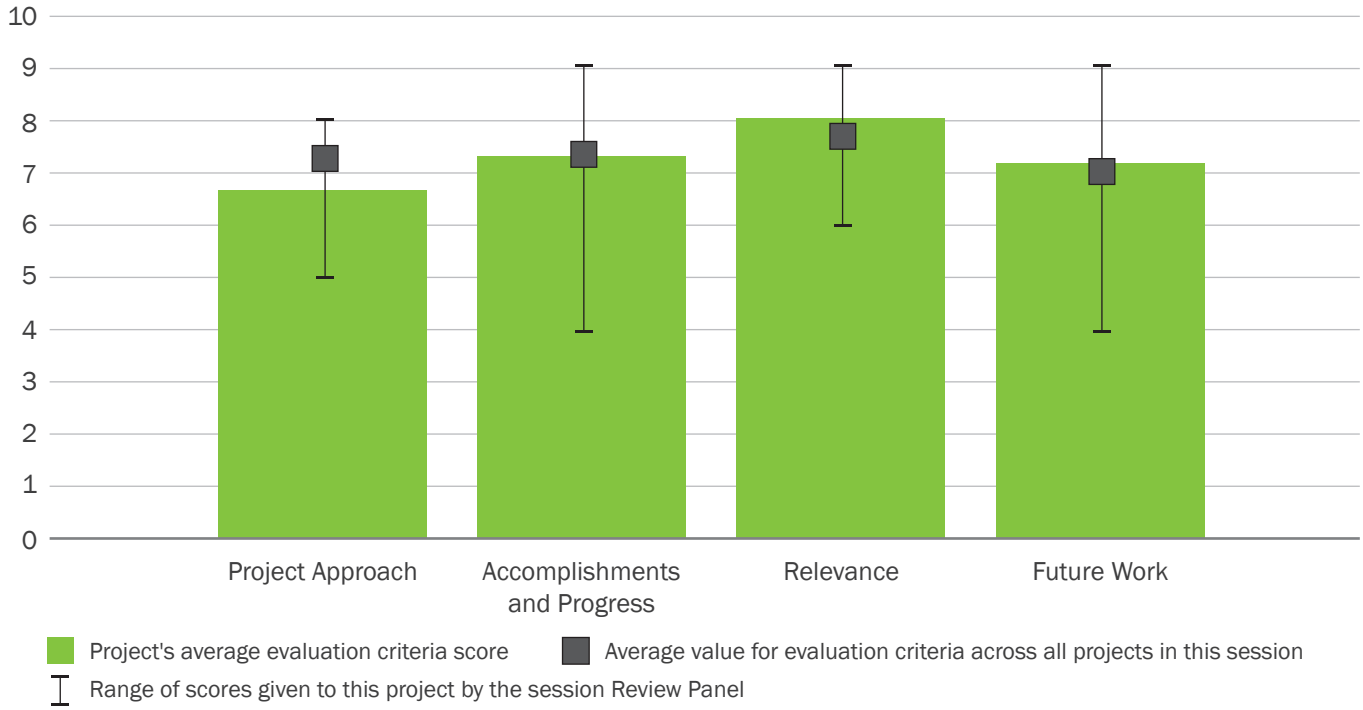
Project Description

The main goal of this project is to create a multi-functional, web-based repository and novel software to organize and integrate metadata with algal'-omics' profiles to standardize and accelerate algal strain improvement. Improving productivity in the presence of abiotic and biotic stressors requires extensive ecological, genetic, and biochemical information, which currently is lacking for most algae. Successful completion of this project will help mitigate these barriers to achieve cost parity with petroleum-based fuels by 2022.

Specifically, this project will maintain and expand a web-based platform (www.greenhouse.lanl.gov) by developing and deploying (1) "user management" functionality to enable both public and private data sharing for individual users or large consortia; (2) genomes of production strains; (3) strain-level diagnostics tools for crop monitoring and pathogen/predator detection; and (4) a pathway viewer and annotation tools that integrate and display genomics, metabolomics, transcriptomics, and proteomics data on biochemical pathway maps. These tools and resources enable academic and industrial entities to both contribute to and reap the benefits of BETO-funded national laboratory programs and serve as the foundation of

Weighted Project Score: 7.3

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



future BETO-funded, targeted strain-engineering efforts; transgene selection; molecular assisted breeding; genome editing; and identification of critical enzymes used to produce fuel precursor molecules and co-products.

Overall Impressions

- This website seems to be very useful and is currently being used by private industry. This website has the potential to include a wide array of metrics.
- The Greenhouse project is making excellent progress and is providing vital tools to the algal research community that are centralized and standardized. This project is on track to become one of the core foundational projects in the portfolio. Close ties to complementary projects and end users should be maintained.
- Overall, this is strong project with great collaboration and potential. The open-source system for accessing public data on specific species could lend greatly to the advancement of improving overall algal productivity. Issues with sourcing relevant material—not just lab-based material—could slow progress. It would be sound to source material that has been cultivated at the larger scale to strengthen the project.
- The Greenhouse tool has a lot of potential and very exciting plans for upcoming work; however, it needs to get into the hands of more people.
- In theory, a web portal that hosts genome data and -omics data and incorporates other knowledge of algal feedstocks is an incredibly valuable tool/resource. However, in reality, Greenhouse suffers from a bit of an identity crisis in that it isn't clear if it's meant to be a data repository or an interactive analysis interface (or both). Current project progress has been made primarily on the data repository end, but its current iteration isn't functionally differentiated enough from other data repositories to offer a unique utility. This is a massive and commendable undertaking. Ultimately, the project may not have sufficient resources (personnel, expertise) to be successful in every aspect, but it has the potential to be very successful in certain elements, provided the focus is more finely honed.
- The project is in the process of developing a robust web-based portal to facilitate storage and dissemination of omics and metadata to accelerate strain improvement efforts. The team has made clear progress, and the web portal is currently functional. A clear set of improvements will be added to the portal, and the project appears to be on schedule.

PI Response to Reviewer Comments

- In its infancy, Greenhouse was strictly a data repository. Going forward, the focus will shift to building and deploying 'interactive' analysis tools customized for algal production strain characterization. Differentiation from other data repositories will improve as the aforementioned omics analytical tools are built in the later stages of the project. Furthermore, industrial relevance is a top priority. We will continue to seek consultation from industry to ensure the tools and omics resources disseminated through the Greenhouse website bolster strain improvement efforts.

MICROALGAE BIOFUELS PRODUCTION ON CO_2 FROM AIR

(WBS #: 1.3.2.900)

Recipient:	Pacific Northwest National Laboratory
Principal Investigator:	Michael Huesemann
Project Dates:	10/1/2015–9/30/2017
Project Category:	Ongoing
Project Type:	FY 2013—Incubator: DE-FOA-0000974
Total DOE Funding:	$900,000

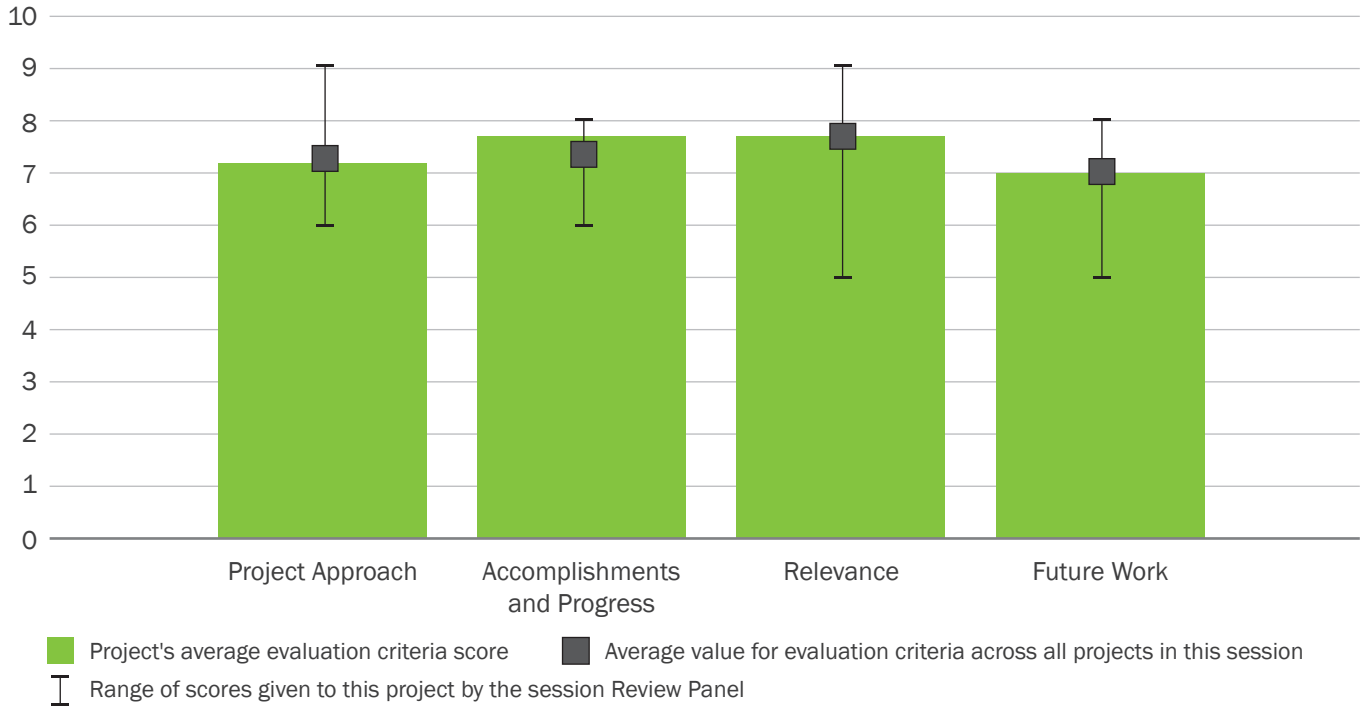
Project Description

The project objective is to demonstrate the potential feasibility of the AlgaeAirFix™ process. AlgaeAirFix™ uses the enzyme carbonic anhydrase (CA) to accelerate the mass transfer rate of CO_2 from the atmosphere into the algal culture, allowing microalgae biomass production without use of enriched CO_2 sources. The goal is to determine the enhancement of CO_2 mass transfer from air and resulting increases in algal productivity by adding commercially available CA. Experiments have been carried out with laboratory and climate simulation bench-scale reactors at PNNL, as well as outdoor ponds operated by the industrial partner, MicroBio Engineering.

The CO_2 mass transfer rate coefficient has been determined as a function of CA concentration, mixing speed, temperature, salinity, and alkalinity. The baseline biomass productivity in outdoor ponds has been determined as a function of paddlewheel speed and alkalinity. A TEA and resource assessment has been carried out based on the data obtained to detail the cost-benefit potential of this technology and further R&D required to move the process to industrial reality. Favorable results would enable the production of algal biofuels without use of enriched CO_2 sources—such as power plant flue gases—thereby greatly increasing the potential of algae to contribute to reducing fossil energy use in the transportation sector.

Weighted Project Score: 7.4

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



Overall Impressions

- This project has a good start on developing baseline values, identifying mechanisms, and exploring some approaches for increasing the CO_2 transfer rate to a pond media using a CA. Their future work includes some innovative ideas on increasing the efficacy of the CA and for altering the media properties to increase the CO_2 transfer rate.
- This work directly addresses the need to decouple production from CO_2 point sources, thus increasing the number of suitable sites and overall sustainability. Excellent demonstrations of the potential for enhancing atmospheric CO_2 mass transfer to the culture have been made. The current limitations of the project around the number of strains and water types are currently being addressed, and future work includes several promising avenues to reach the end goal.
- Overall, this project is an innovative approach that cost-effectively decouples large-scale algae farms from co-location issues.
- The most significant outcome of this project is the potential to uncouple algae production from CO_2 co-location. Much work is needed on how this would deploy at a relevant scale.
- Developing a cost-effective CO_2 delivery system to algae in order to boost productivity is a worthwhile endeavor. However, there are some major barriers to the success of AlgaeAirFix™, the most prominent one being the requirement for external addition of an expensive enzyme. The solution presented (endogenous CA production) is problematic, as there will necessarily be issues like unanticipated energetic costs reducing productivity. There are several other potential feasibility issues that should be investigated, such as enzyme stability in ponds due to both abiotic and biotic factors.
- Co-location with CO_2 sources could severely limit algal biofuel siting options, and viable technologies that enable use of atmospheric CO_2 could broaden deployment options dramatically. If the proposed technology is able to be deployed without reducing current or productivity targets, results from an initial TEA suggest that it could be economically viable. The project appears to be on schedule and has met a go/no-go decision on time.

PI Response to Reviewer Comments

- Regarding barriers to the success of AlgaeAirFix™: There are, certainly, major barriers to successfully growing microalgae on just air levels of CO_2. Extracellular CA will be a key factor in this process; however, at present, it is undefined exactly how extracellular, if, as in essentially all cases studied, it is only periplasmic or possibly excreted into the culture medium. Without going into a long discussion, these issues will remain for future study. The main objective of the present project is to demonstrate that there is a path to enhancing atmospheric CO_2 transfer into algal ponds sufficiently to allow for a relatively high productivity. Follow-up research will need to address translating this work into an actual working that is economic process. However, we emphasize that, at this point, the need is for a proof of concept from which the economic issues can be addressed via conducting a TEA. Also, there are several approaches to this process, from shallow cultures and attached biofilms to use of alkaliphilic algal strains operating at high pH (where chemical enhancement will also play a role). These will be addressed in our follow-on proposal.

DIRECT PHOTOSYNTHETIC PRODUCTION OF BIODIESEL BY GROWTH-DECOUPLED CYANOBACTERIA

(WBS #: 1.3.2.910)

Recipient:	Arizona State University
Principal Investigator:	Wim Vermaas
Project Dates:	9/30/2016–3/31/2018
Project Category:	New
Project Type:	FY 2014—Incubator II: DE-FOA-0001320
Total DOE Funding:	$1,818,000

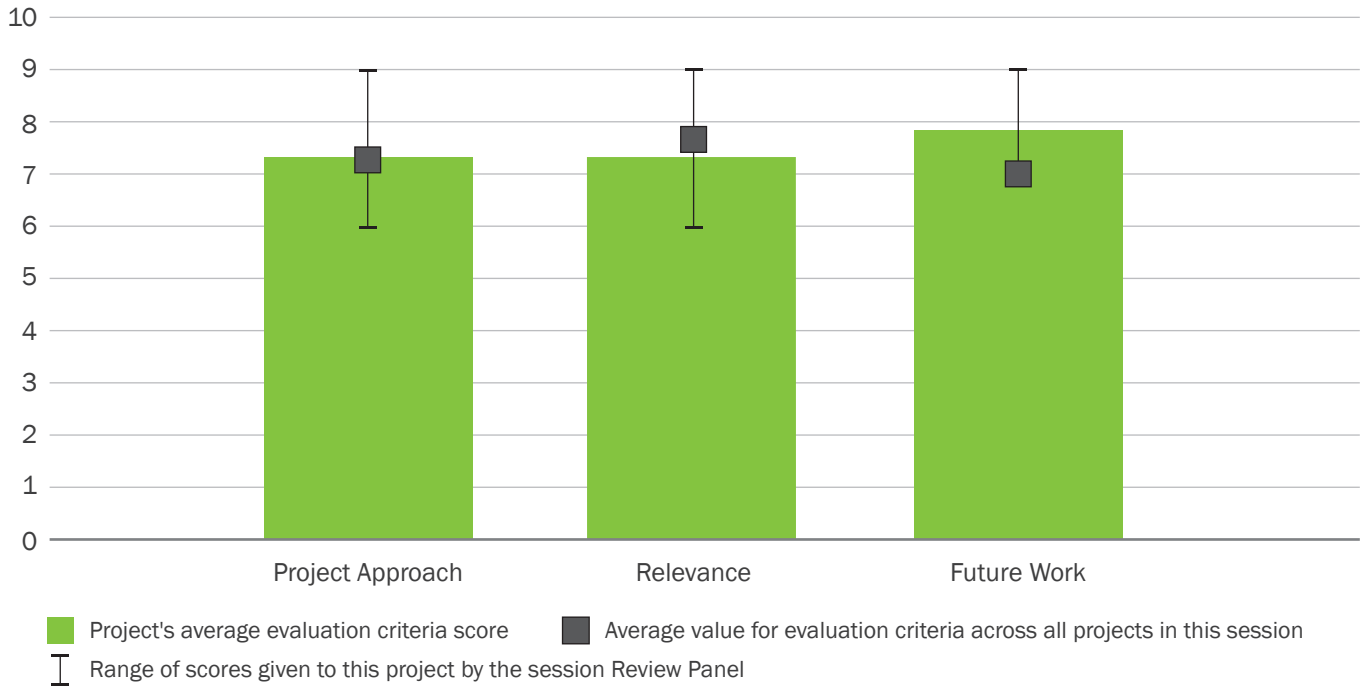
Project Description

This project aims to engineer the photosynthetic production of ethyl laurate—an excreted, "drop-in" biodiesel alternative resistant to microbial scavengers—using cyanobacteria and inputs of just CO_2, water, and light. Also, we will induce growth arrest of the culture, decoupling ethyl laurate production from culture growth, to enhance ethyl laurate productivity. Ethyl laurate production will be further increased by (1) boosting metabolic flux through the fatty acid biosynthesis pathway to intermediates used for ethyl laurate production, and (2) reducing the production of exopolysaccharides.

The project builds on the team's prior success in efficient photosynthetic production of laurate by an engineered strain of the cyanobacterium Synechocystis sp. PCC 6803; this strain contains a thioesterase that cleaves off laurate from the native fatty acid biosynthesis machinery. This platform strain will be engineered further to improve laurate production, convert laurate to lauryl-CoA, co-produce ethanol (using constructs provided by Algenol), and synthesize ethyl laurate from lauryl-CoA and ethanol. Inducible gene circuits are being engineered to dynamically arrest cell growth without harming viability and metabolic activity. Additional genetic changes to reduce the level

Weighted Project Score: 7.6

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.



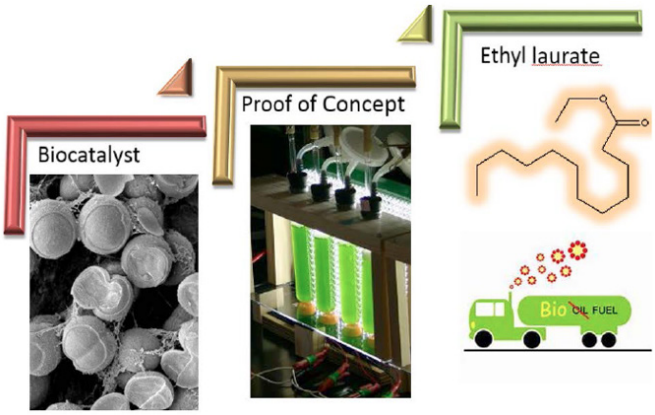


Photo Courtesy of Arizona State University

of exopolysaccharides will help to further direct fixed carbon toward biofuel production while also reducing the level of potential substrate available to opportunistic heterotrophic contaminants.

Overall Impressions

- Great start on getting the bacteria to produce both laurate and ethanol. This project has identified the need to modify the cyanobacteria to form the ethyl laurate and for increasing the carbon flux to product. I believe the TEA they generate will be fairly uncertain until they can develop a solid plan for harvesting methods and cleaning methods; I believe both of these hurdles will be difficult to overcome.
- This is an exciting, potentially transformational approach to direct fuel production by cyanobacteria. This is high-risk research with many challenges, including some that are not considered, but the potential payoffs of obviating harvest, extraction, and downstream processing are huge. This adds valuable diversity to BETO's portfolio. Near-term production of the product as an industrial chemical could help with deployment. Future work is quite ambitious and could benefit from early TEA to help narrow focus.
- This project has an innovative concept that could produce significant results moving forward.
- Novel concept for meeting fuel production goals. Because the project is so new, commercial scalability is unclear; TEA work will need to address this.
- Production of ethyl laurate in cyanobacteria has the potential to be transformative, provided there is a clear path to scaling up the technology. Early results are exciting and promising, and the investigators clearly possess the technical expertise needed to explore the production of this compound in this organism and move it in the right direction. This project could be improved by assessing the potential of this product by contextualizing the work with engineering and economic analyses.
- The proposed plan of work for this high-risk, high-reward research seems appropriate for the FOA. The project identified a clear plan of work guided by appropriate management tools. Potential challenges were identified and incorporated into the project plan. If successful, the project will contribute to yield and cost goals of BETO's MYPP.

PI Response to Reviewer Comments

- One reviewer viewed the project to be potentially transformational, but also high risk with many challenges. We concur with this assessment and note that this project is a first step to prove feasibility of the approach. Details of harvesting, extraction, and downstream processing are not yet worked out extensively, as production rates and product accumulation on top of cultures first will need to be known and analyzed better. At that time, TEA for the whole process will be more useful. TEA will inform commercial scalability and potential.

  We share reviewers' concerns regarding scalability, but we are still too early in the project to have a reasonable idea about the challenges that we will actually face. We anticipate that we will have further information on this at the next review. We have

engineering and TEA expertise on the team, and we can further expand the team in a next phase of the project after the current project comes to an end.

One reviewer shared the excitement and high-risk nature of the project with the first reviewer. Indeed, it is a high-risk project, but excellent progress has been made thus far. Another reviewer lauded the strong start of the project, but views definition of harvesting and cleaning methods to be critical in developing an optimal and detailed TEA. We fully concur, and while development of harvesting and cleaning methods is beyond the scope of the current project, these are very important research directions once we have gathered information on the production rate and accumulation (on top of the culture) of ethyl laurate.

A NOVEL PLATFORM FOR ALGAL BIOMASS PRODUCTION USING CELLULOSIC MIXTROPHY

(WBS #: 1.3.2.920)

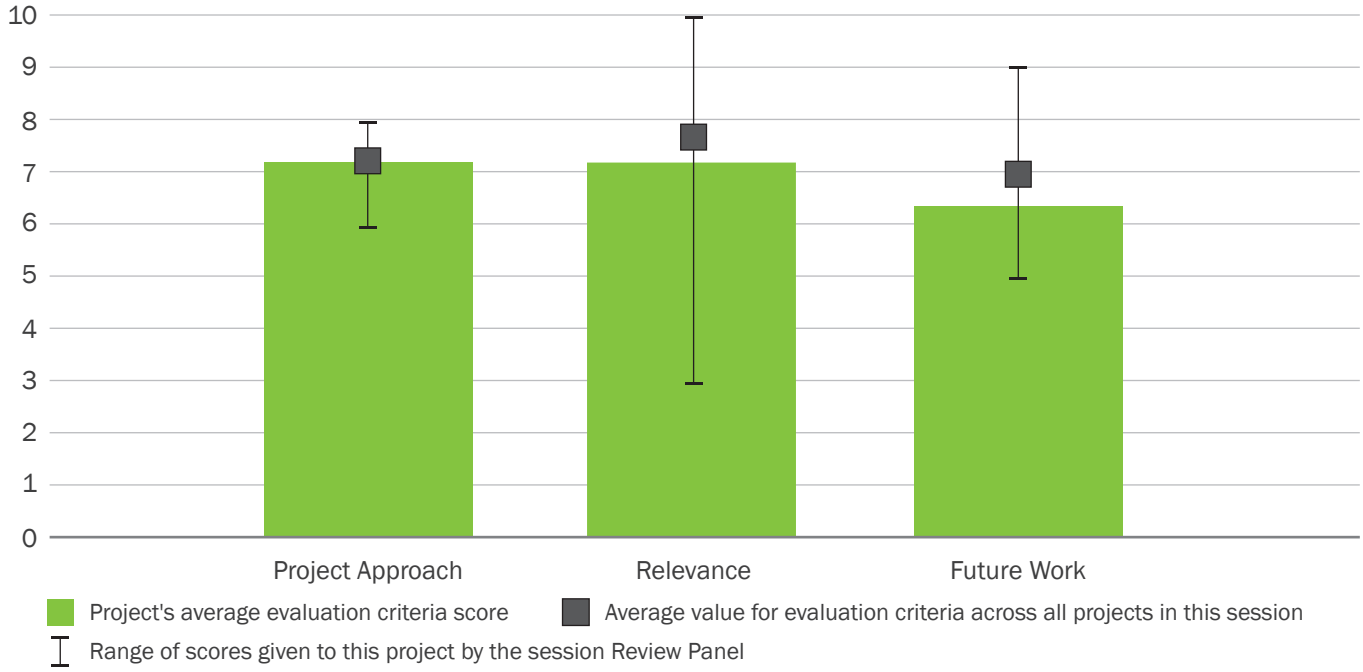
Recipient:	Arizona State University
Principal Investigator:	Peter Lammers
Project Dates:	9/1/2016–8/31/2018
Project Category:	New
Project Type:	FY 2014—Incubator II: DE-FOA-0001320
Total DOE Funding:	$1,956,515

Project Description

Abundant, flat land in the southwestern United States plays little role in the current BETO algal portfolio, yet the region offers significant potential for algal biomass production if evaporative water loss issues can be addressed. The goal of this project is to utilize mixotrophic metabolism of cellulosic glucose and xylose by red algal extremophiles in the genus Galdieria to maximize biomass productivity. Mixotrophy couples stoichiometric oxygen-dependent sugar oxidation to photosynthetic CO_2 capture, thus reducing CO_2 supply costs and bioreactor mixing energy—two major contributors to greenhouse gas emissions in current algal biofuel models. Preliminary results show the low pH and high temperatures preferred by Galdieria allow it to outcompete other microorganisms for sugar utilization, achieving volumetric productivities between 0.5 and 1.0 g/L/day outdoors. Our biomass productivity target is to exceed 50 g/m^2/day, which is a minimum fivefold increase over baseline photoautotrophic cultivation. The project includes techno-economic and greenhouse gas emission modeling for both vertical and tubular PBRs for overall decision support and down-selection of the best reactor for deployment in the southwestern United States. Resource assessments will identify cellulose sources available in the deserts of this region. The project also will identify direct and indirect practices that limit catabolic repression of photosynthesis by cellulosic sugars.

Weighted Project Score: 6.8

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.



Overall Impressions

- This project appears to be mostly about advancing the state of making phycocyanin. Addressing the MYPP goals of high productivity with this method seems of minimal value, as the industry has already shown high productivities with certain cellulosic sugars. I expect this project to encounter issues with running a system with multiple strains that need to be kept in performance balance, keeping the system clean of competitors, and developing systems that can be effectively cleaned in place.
- This is an intriguing mixotrophy concept with potential to reduce gas delivery and mixing costs. Significant challenges include temperature dependence, cellulose resource availability, and PBR costs. Overall, this is a high-risk but potentially breakthrough approach that adds diversity to the portfolio. Given high production costs in PBR systems, addressing the purification and marketing of phycocyanin will be critical to successful commercialization, and this was poorly addressed in the presentation. The path of future work was not clearly articulated.
- Overall, this project seems like an innovative way to produce a high-value product and possibly consider the biofuel as a co-product in a sense. The production of this material in a PBR could become cost prohibitive, but the potential of high-value products may be beneficial in reducing the capital impact of the PBR system.
- Complex biology (Galdieria and Cyanidioschyzon merolae synergistic relationship) was put to use to "unlock algal productivity potential." Additionally, stability of the system should be increased because crop protection issues are due to an acidophile environment and high temperatures.
- The advantages and disadvantages of mixotrophic cultivation of algal biomass have not been fully tested or explored, making this project an interesting addition to the BETO portfolio. Though it is just getting started, and has demonstrated progress toward meeting the overall objective of maximizing algal productivity (to more than 50 g/m^2/day). The short time frame during which this project will receive funding means that it will need to be highly focused in order to be productive. The path moving forward should be described more clearly.
- The team presented a clear history and rationale for the project. The technical and management approaches were identified and appropriate for the project. The team is encouraged to make an effort to measure the relative contribution of fixed carbon that comes from heterotrophic versus autotrophic growth. Without these measurements, it is unclear how mixotrophic efforts (and results reported for areal productivity) are aligned with BETO MYPP goals.

PI Response to Reviewer Comments

- This project is based on a novel cultivation route to fuel feedstock plus co-product designed to achieve a 5–10-fold increase in biomass productivity. Previous BETO-funded work by members of this team has established HTL-based conversion to biocrude at yields up to 40% from Galdieria feedstock. The TEA, LCA, and resource assessment tasks will evaluate fuel-scale manifestations of the cellulosic mixotrophy approach in the arid southwestern United States with phycocyanin co-product analysis built into the TEA. Phycocyanin yield maximization is directly addressed in the proposal, including glucose/xylose limitation prior to harvest and other strategies. Heliae will focus on nutraceutical-grade phycocyanin in the $100–$200/kilogram price range. A 10% market share could be achieved with a 25-acre mixotrophic facility.

  A detailed TEA is included (Task 8) to guide the proposed work. We specifically target the arid

southwestern United States region for deployment with a major design objective to minimize evaporative water loss. Thus, PBR use is a strategic necessity. Various PBR design concepts (Task 5) will be evaluated to minimize the cost of the system. Plans to extend the mixotrophic growing season through winter will evaluate cultivation at pH values of 0.2–1.0 to suppress contaminants.

The 24-month period of performance is divided into two 1-year phases. The first year focuses on establishing the degree of mixotrophy when growing on cellulosic hydrolysate, completing an initial round of PBR design and cost reduction and building the TEA and LCA tools. The second year combines cultivation and analysis to co-optimize the cultivation conditions and PBR design. In this phase, seasonal productivity data will be collected and leveraged by TEA and LCA to document achievement of final targets at the end of the project based on cost, emissions, and scale-up potential.

The team is well aware of the importance of maximizing photosynthesis in the context of a mixotrophic production platform. The relative contributions of photoautotrophy and heterotrophy will be addressed in Task 1 by quantifying metabolic gases in an off-gas, mass spectrometry analyzer. These values will be used as criteria to screen and down-select from six different Galdieria strain candidates. Metabolite analysis of Calvin-Benson cycle intermediates (Task 4) will help reveal underlying mechanisms. A potential route to maximizing photosynthesis is to restrict the rate of aeration in PBRs. The consequences of variable aeration rates with respect to cultivation and the balance of photo-/hetero-trophic metabolism will be evaluated in the context of minimizing mixing energy for the LCA modeling (Task 9). The project presentation included elements of a dual culture approach to mitigate the risk of partial catabolic repression of photosynthesis by sugars. While this is a common sense approach, it is outside the approved scope of work and will not be pursued with the current funding. The PI apologies for this confusion.

Our team is not aware of any stable, high-productivity mixotrophic system using cellulosic sugars other than our own. The team fully understands that mixotrophic systems must exceed the productivity and/or economics of any fermenter-based fuel-feedstock production system in order to be adopted. Project funding is based on the assumption of phycocyanin co-production along with fuel feedstock generation. The approved scope of work is for cultivation of a single strain that does not require any clean-in-place procedures (other than those used to clean the PBRs) to maintain culture stability based on the extreme pH and temperature conditions used. The full pH range of 0.2–4.0 will be used to extend the mixotrophy growth season along with rotation of Galdieria strains with high- and low-temperature optima for full-year production potential.

ALGAL FEEDSTOCKS LOGISTICS AND HANDLING

(WBS #: 1.3.3.100)

Recipient:	Idaho National Laboratory
Principal Investigator:	Lynn Wendt
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$350,000
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$0
DOE Funding FY 2017:	$800,000

Project Description

This project addresses feedstock logistics challenges occurring between algal biomass production and conversion in order to provide solutions that assist in meeting conversion cost targets. The goal of this project is twofold: to manage seasonal variation in algal biomass production through stabilization, and to reduce ash to increase conversion yield. Algal biomass is susceptible to rapid degradation once harvested; losses have been measured at 2%–3% per day. This project will demonstrate a wet storage approach that preserves harvested algal biomass for 6 months and enables a biorefinery to run year-round with a consistent feedstock supply.

Wet, anaerobic storage, or ensiling, is a low-cost storage approach that is commonly used to preserve wet herbaceous feedstock. Initial storage studies with algae/herbaceous blends have demonstrated losses of less than 10% over 1 month and 10%–17% over 6 months, meeting a go/no-go milestone target of 30% loss. Wet storage of algae alone is also promising; preliminary results show 1-month storage losses as low as 6.5%. This project will also identify a process that stabilizes algal turf scrubber biomass and reduces its ash by more than 70%, which could be applied to other high-ash algae species. In these tasks, optimal conditions will be determined in the laboratory, followed by estimation of conversion performance

Weighted Project Score: 8.0

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.

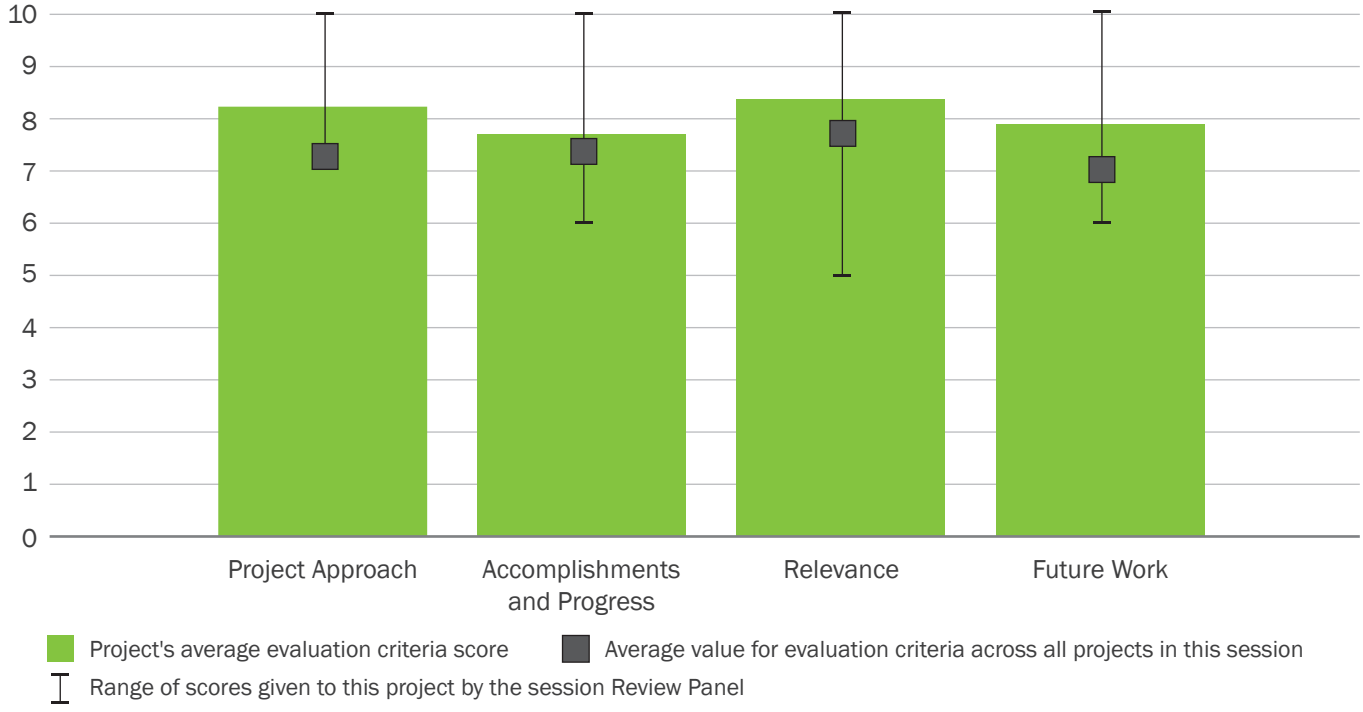


Photo Courtesy of Idaho National Laboratory

made in collaboration with national laboratory partners, and TEA will be used to compare to baseline targets.

Overall Impressions

- This project is very relevant for long-term storage, quantifying feedstock changes, and being able to feed at constant rate during low-productivity months. The project objectives are going to be relevant to the algae industry and potentially to other industries with high moisture content feedstocks and intermediates. A TEA that includes the cost of delivering blending material to the plant, the loss of dry matter, and conversion versus blending would be very helpful.
- This project is addressing key algal feedstock logistics barriers in novel ways. Adaptation of existing agricultural methods is a plus. Use of turf scrubber biomass might seem to be an unnecessary diversion from the industry mainstream.
- This project has interesting and innovative ideas. The blending of algal with other types of feedstocks presents an interesting case for the supplementation of material for the larger-scale refining process. Ideas for the stabilization of harvested material to reduce the loss of target products could lend to production systems maintaining greater recoverable yields and in turn make the system more profitable. High ash quantities are a serious problem facing the industry, so steps to reduce the ash quantities provide a serious benefit to the industry as a whole.
- Storage and shipping is too often overlooked; this project provides a novel solution that appears to be more sustainable than traditional drying or refrigerated storage.
- Developing a baseline understanding of the effectiveness of algal biomass stabilization techniques and optimizing these techniques are essential elements of developing algae as an energy crop. With that in mind, this project represents a valuable step toward industrialization of microalgal biomass. Commendably, this project considers both technical and economic barriers of algal biomass stabilization, but could benefit from a more organized experimental approach to generate more concrete data that would improve the impact of the contribution.
- This project has a strong management plan with clearly defined goals and a rigorous technical approach. The scope addresses a clear bottleneck for large-scale production and may have near-term relevance for industry as well. The project does a good job building off of approaches in other agricultural disciplines, and early results have achieved project goals to minimize biomass losses during storage. Progress appears to be on track to meet project goals and milestones.

PI Response to Reviewer Comments

- We thank the reviewers for their thoughtful comments and suggestions. We agree that addressing feedstock logistics barriers and proposing solutions

for stabilization and maximizing conversion yield will advance the commercialization of algal-based biofuels. We agree that TEA and LCA should be conducted alongside experimental work such that the full impact of our stabilization approaches can be assessed. We have defined the unit operations and capital and operating costs of a system for stabilization of microalgae through blending and long-term storage and have found it as a cost-competitive approach to drying algae in order to manage seasonal production variation. We will continue to incorporate TEAs, in collaboration with NREL, for the storage of algae alone, and in conjunction with SNL on the impact of ash removal from biomass derived from a turf scrubber.

We have, and will continue to, incorporate multiple microalgae strains in our experimental plans to ensure that our approaches are applicable to a wide range of industrially relevant strains. We are also working with our partnering national laboratories to understand the impact that wet storage designs have on conversion potential.

While biomass derived for an algal turf scrubber was described by one reviewer as less relevant to mainstream processes, it does represent a feedstock that could benefit from advanced logistics (e.g., stabilization, ash mitigation). The ash-reduction approaches determined through this research effort may also be applicable to microalgae grown specifically for fuel production, where open-pond configurations could introduce significant ash contamination.

THERMOCHEMICAL INTERFACE

(WBS #: 1.3.4.101)

Project Description

This project is focused primarily on developing advanced HTL-processing methods to improve process efficiency and reduce capital and operating costs for the production of drop-in biofuels from microalgae. The algal HTL pathway is also developing processes to enable nutrient recycle/bioavailability from HTL waste streams and upgrading technology for the production of finished fuels. The project is validating process scale-up at engineering scale using a newly acquired HTL-processing system at PNNL with three skids for (1) feedstocks preparation, (2) HTL processing, and (3) product separations. All data from these efforts will directly support the pathway model TEA/LCA and SOT. Scale-up and technology transfer are important components of the project.

Recipient:	Pacific Northwest National Laboratory
Principal Investigator:	Daniel Anderson
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$1,000,000
DOE Funding FY 2015:	$1,100,000
DOE Funding FY 2016:	$1,520,000
DOE Funding FY 2017:	$1,200,000

Overall Impressions

- This project is making progress on characterizing algal biomass, making some optimization improvements on the HTL process, and exploring methods for increasing nutrient recycle. I think the TEA of this project would benefit greatly by including a reputable member of industry practiced in upgrading oils to make them suitable as a refinery feedstock.

Weighted Project Score: 8.2

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.

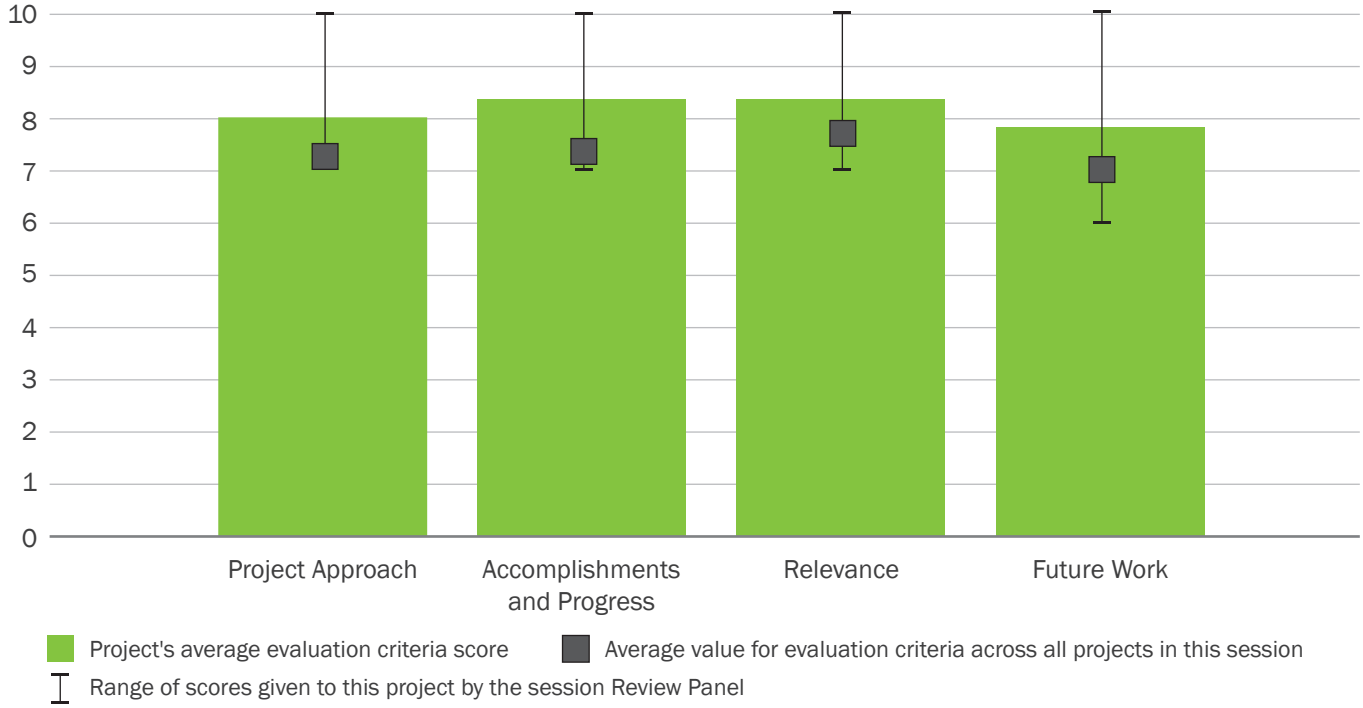


Photo Courtesy of Pacific Northwest National Laboratory

The industry partner should advise on the cost of upgrading (including the removal of metals) the different oils generated by different HTL operating conditions to make them acceptable to a refiner and at what discount the refiner would want in order to take the oils.

- This project is clearly on BETO's critical path. Impressive technology demonstrations and improvements have been made to date, with significant cost-reduction implications. The vision for the blending work is unclear. Further opportunities for aqueous stream valorization and interactions with end users of fuel products should be sought.
- Overall, the HTL platform has shown great progress since its inception. The development of the system has provided a substantial amount of data, as well as opportunities for cultivators and industry partners.
- This project demonstrates significant advancement of HTL development, particularly with the pilot unit to enable further field testing at multiple sites (unit is relocatable). Phosphate recovery from the HTL solids is an important step forward.
- The team continues to lead the field in quantifying the SOT for a promising conversion technology (HTL). The work is clearly aligned with BETO MYPP goals for low-cost conversion of algal biomass into biofuels. The team continually engages with leading algae producers (whether in industry, academia, or national laboratories) and has validated a number of process improvements that help increase efficiencies and drive down capital costs.

PI Response to Reviewer Comments

- Thank you for your review and insights.

  The vision for future feedstock blending with algae will be based on (1) an ongoing resource assessment in a related project of the available feedstocks for potential blending with algae, and (2) the experimental results from testing selected blendstocks in the HTL process.

  We believe you have an excellent suggestion in getting industrial participation, and this is the basis for future planned work through a new NREL-PNNL experimental project aimed at understanding the requirements needed for petroleum refinery acceptance. Key to this effort will be assistance from refining experts.

BIOCONVERSION OF ALGAL CARBOHYDRATES AND PROTEINS TO FUELS

(WBS #: 1.3.4.200)

Recipient:	Sandia National Laboratories
Principal Investigator:	Ryan W. Davis
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$150,000
DOE Funding FY 2015:	$300,000
DOE Funding FY 2016:	$350,000
DOE Funding FY 2017:	$350,000

Project Description

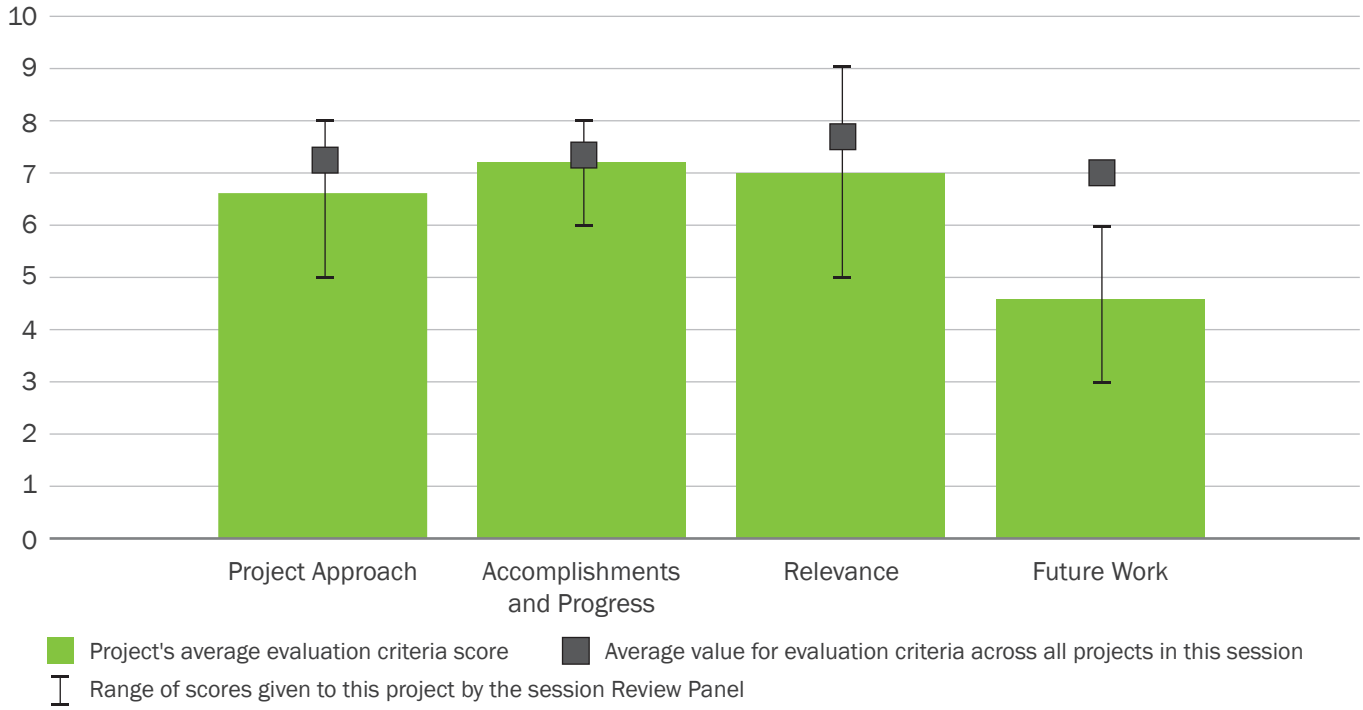
Algae biomass is an intrinsically heterogeneous feedstock consisting predominantly of proteins and carbohydrates under high growth conditions. Therefore, the objective of this project is to develop algae conversion technologies that enable utilization of multiple substrates for generation of algal-derived fuel products. Our approach was to engineer fermentation microbes and apply process optimization and intensification in order to biologically produce more than 10 g/L of fusel alcohols at productivity and yield of more than 0.5 g/L/hour and 0.2 g/g (equivalent to greater than 70% net theoretical yield), respectively, from whole benthic and microalgae hydrolysates. This objective supports BETO's 2022 MYPP goals to demonstrate algae biofuel yield of 5,000 gallons of biofuel intermediate per acre per year at $3/gge.

Overall Impressions

- This project has potential for a combination of water cleanup and as a potential pathway for fuels/chemicals production. I think this is a useful first step for the next scale-up stage and the generation of a third-party-verified TEA.

Weighted Project Score: 6.4

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



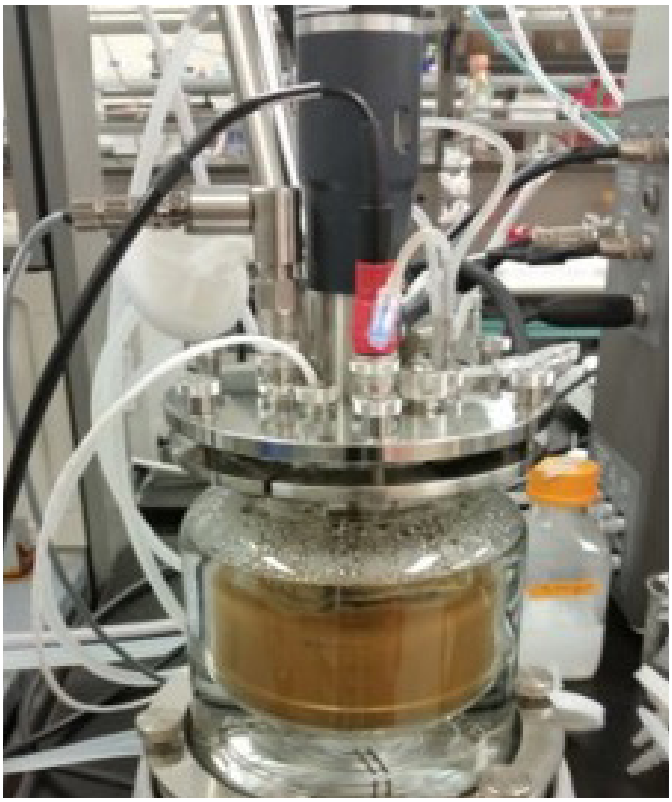


Photo Courtesy of Sandia National Laboratories

- This project explores an interesting alternative pathway for the utilization of algal proteins and carbohydrates. Excellent progress was made in the optimization of fermentation for fusel alcohol production. Relevance should be considered in the context of fusel alcohol as a higher-value co-product rather than a fuel. In order to decide whether future development of this approach is warranted, TEA needs to be addressed.
- Overall, this project has merit and the potential to meet the program's goals and deliverables moving forward. Innovative approach using algal turf systems for production of new biomass. Productivity of benthic algal material may be an issue moving forward, as well as high ash content.
- This project demonstrates an excellent use of turf scrubber biomass from water cleanup to also generate fuels.
- This team's efforts to increase biofuel yields and co-products through algal hydrolysate fermentation are clearly aligned with BETO MYPP goals to increase per-acre algal biofuel yields. There appears to be nice market flexibility in the target fusel alcohol products, from industrial solvents to biodiesel. It is unclear if the approach will have market relevance given several inherent disadvantages in the fermentation feedstock relative to competing technologies in the marketplace. High-density fermentation could be difficult with hydrolysates without expensive pre-concentration steps.

PI Response to Reviewer Comments

- The PI would like to thank the reviewers for their valuable insights.

ALGAL BIOMASS CONVERSION

(WBS #: 1.3.4.201)

Project Description

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Philip Pienkos
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$500,000
DOE Funding FY 2016:	$600,000
DOE Funding FY 2017:	$500,000

The Algal Biomass Conversion (ABC) project identifies and develops bolt-on unit operations for the Combined Algal Processing (CAP) scheme to valorize all algal components to fuels and chemicals to accelerate biofuel commercialization. ABC leverages work begun by the Sustainable Algal Biofuels Consortium, becoming an AOP project in FY 2015. CAP is the SOT for the algal lipid upgrading pathway. TEA models indicate that the cost of algal biomass production will never become low enough to support a process designed to produce only biofuels, and thus the addition of a scalable portfolio of co-products is the only way to achieve the target of less than $3/gge. ABC's challenges include identifying production algal strains (especially halotolerant) with high carb/lipid composition, identifying co-products with sufficient price and volume to justify consideration, and developing co-product processes that can plug and play with CAP. We have identified a number of halotolerant strains with high lipid/carb content; demonstrated the fully integrated CAP process at bench scale, including the upgrading of the lipids to hydrocarbons; substituted succinic acid for ethanol with significant reduction in MFSP; demonstrated the conversion of algal sterols to surfactants; and demonstrated the conversion of algal proteins to four

Weighted Project Score: 7.4

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.

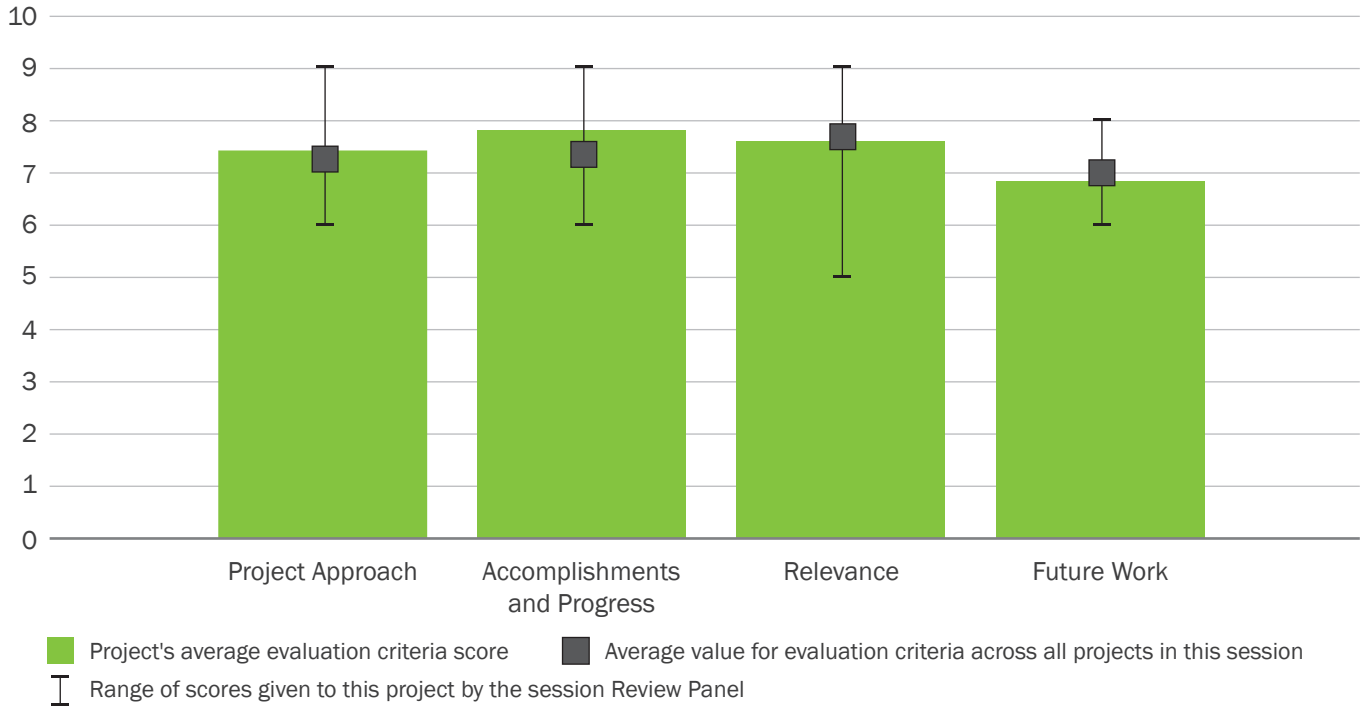
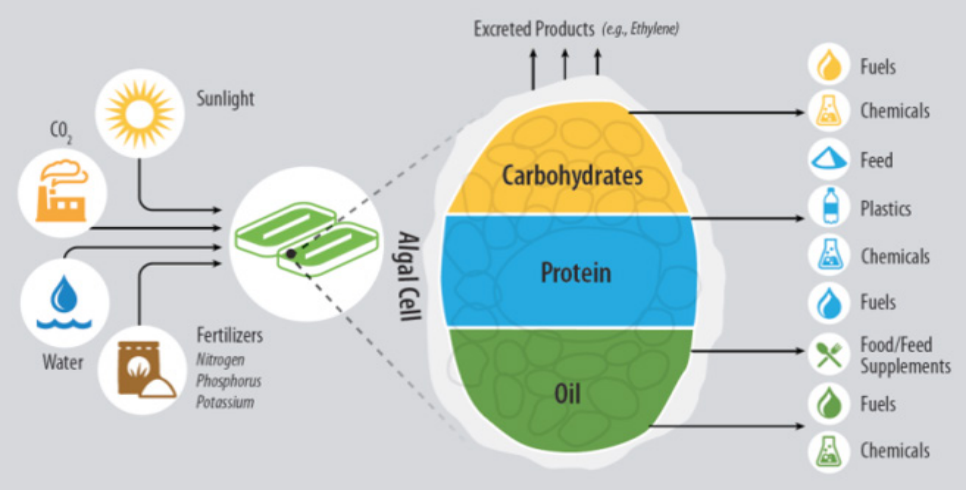


Photo Courtesy of National Renewable Energy Laboratory



co-products. A biorefinery concept producing hydrocarbon fuels, succinic acid, surfactants, and bioplastics can achieve an MFSP of less than $3/gge.

Overall Impressions

- This project is a good start on characterizing algal biomass with a goal of providing products into certain higher-value markets. They have made good definitive progress in their stated goals. I would have liked to hear more information on how the project envisioned controlling the culture to optimize their intended composition. They provided some encouraging results toward meeting certain unit cost goals, but I believe considerable more work needs to be done to allow for the creation of a credible TEA.
- This clearly presented project is fleshing out one of the two front-running pathways for bioprocessing of algal biomass to glean maximum value. It is extremely well integrated with other projects, in particular, NREL's TEA and Algal Biomass Valorization efforts, but also serves as the process design basis for many other projects in the portfolio. A myriad of options exists for co-products, and how future directions will be decided is not completely clear.
- Overall, this is an innovative approach that could provide significant impact in regard to large-scale algal production. By setting up a multi-product pipeline, it allows the farmer to attack various markets as they shift; this will in turn help maintain overall product value while achieving biofuel production goals.
- This project demonstrated the feasible production of several co-products and the necessity of having a portfolio of co-products to ensure target $/gge is met.
- The project has demonstrated a conversion technology that could significantly improve areal yields of fuels and co-products from algal biomass, which is aligned with BETO MYPP near-term yield goals. The project appears to be on schedule and has achieved many of the stated conversion yield goals. Development of co-product strategies appears promising. It is unclear whether the fermentation portions of the CAP process will be competitive in the marketplace with competing technologies that utilize concentrated glucose feedstocks.

PI Response to Reviewer Comments

- We thank the reviewers for their thoughtful and encouraging comments. Our TEA models suggest that a co-product approach is the only way that MFSP targets can be achieved regardless of the conversion platform. Working with our colleagues in TEA and Algal Biomass Valorization, we have developed a

strategy to identify a broad portfolio of co-products rather than focusing on a single option, spending our resources trying to justify that decision. As noted by reviewers, the portfolio allows for flexibility in decision making for a conceptual algal biorefinery and also allows for better opportunities to develop partnerships with industrial stakeholders.

- It is only in the past calendar year that we have begun to demonstrate feasibility for some of our more promising co-products, such as succinic acid, sterol-based surfactants, and protein-based bioplastics. The shift to biomass from halotolerant strains as feedstock could present unique co-product opportunities that we have not yet identified with Scenedesmus. We believe that a strategy of early feasibility studies, coupled with preliminary TEA analyses, will help to identify an integrated process with highest economic potential with least complexity. Detailed TEA modeling is a time- and labor-intensive activity and cannot be easily incorporated into early-scenario casting. However, our initial prioritization was greatly helped by a high-level overview of the potential value of eight different co-product scenarios, allowing us to justify development of detailed TEA models. This provides an optimal list of co-products for a conceptual biorefinery, as well as guidance on how to deploy research resources. Thus, co-products that are promising on a theoretical basis might be rejected in favor of less promising ones that require less developmental work. At the same time, we will continue to leverage process improvements coming from the work of others that would otherwise be out of this project's reach. A case in point is the work done by colleagues on projects within BETO's Conversion R&D Program to isolate an improved strain of Actinobacillus succinogenes, which has shown promise for higher yields on algal sugars (a critical metric). The ABC project does not have the resources to make this sort of improvement, but we will take advantage of such crosscutting opportunities whenever possible.
- But we realize that the time to make an impact with this project is fleeting and that decisions on ideal co-product scenario must be made soon. It is our plan to continue to evaluate new opportunities into FY 2018 and then wrap up (or greatly reduce) the exploratory phase of this project in favor of process development in the areas that can provide the greatest benefit to the overall economics. This will be incorporated into the FY 2018 goal to "Demonstrate all unit operations for conversion of algal biomass to optimal fuels and co-product portfolio for 20% reduction in modeled MSFP relative to FY 2016 SOT."
- Finally, it is important to respond to the criticism that it will be challenging to develop processes with algal sugars that can compete with concentrated industrial sugar feedstocks. It is clear that algal biomass is always likely to be more expensive than terrestrial biomass and thus all components must be valorized to the maximum extent. There are certainly process options that currently require highly concentrated sugar streams, and we have deliberately stayed away from these (e.g., the use of algal sugars to grow oleaginous yeast) for precisely that reason. This is the reason we continue to be interested in ethanol (where the penalty for low titers have been quantified in our TEA model) and succinic acid, which is toxic to the organism at titers much higher than 30 g/L so that concentrated sugars do not provide much of an advantage. We will continue to be guided by TEA to note the economic impacts of titers constrained by low sugar concentrations and develop mitigation strategies where appropriate.

ALGAL BIOMASS VALORIZATION

(WBS #: 1.3.4.300)

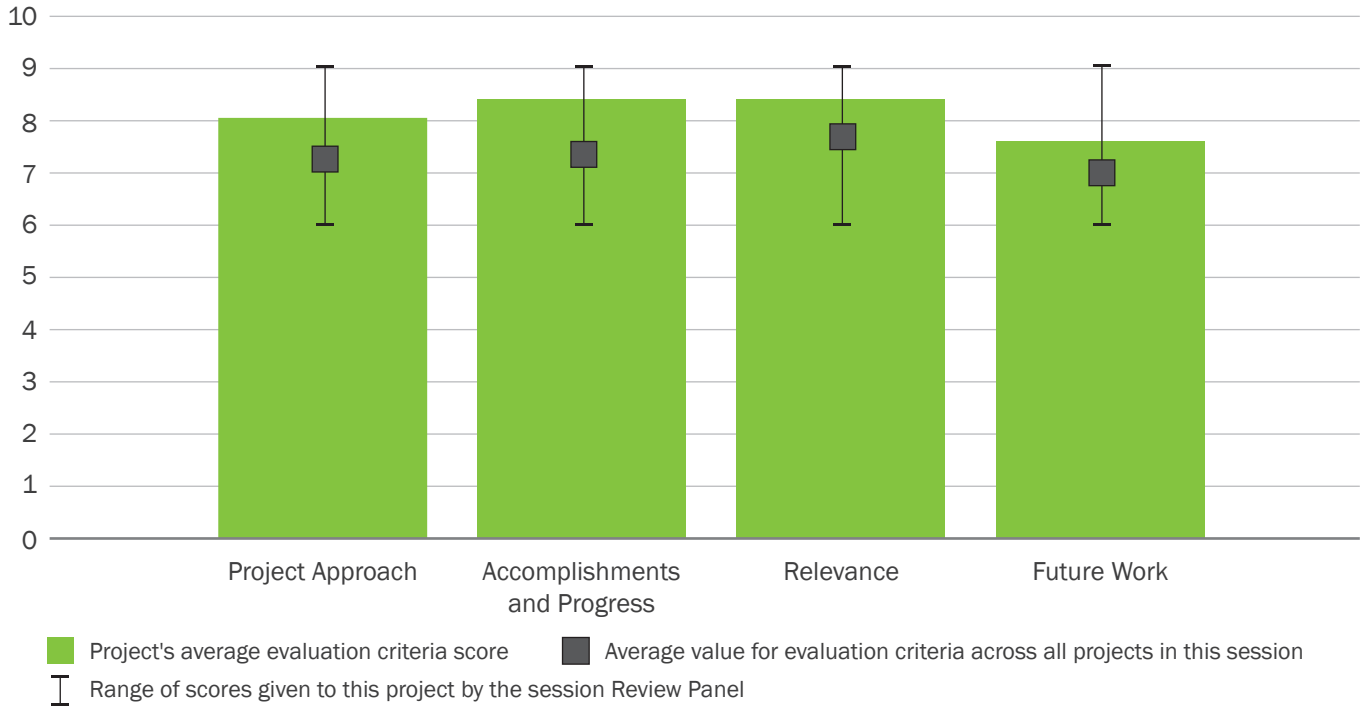
Project Description

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Lieve Laurens
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY14:	$0
DOE Funding FY15:	$800,000
DOE Funding FY16:	$850,000
DOE Funding FY17:	$750,000

To drive the critically needed transition from research to commercialization scale, demands increase for rigorous experimentation and characterization to provide the requisite data on biomass products. The isolation of co-products from algae processing routes is the only way to reduce the cost of fuels to $3/gge by 2022. By implementing an integrated cost-value framework, this task focuses on increasing the intrinsic value of algal biomass. Experimental work is focused on the identification and isolation of key co-products beyond lipid-based fuels in a multi-product algae biorefinery model, commensurate with the Combined Algal Processing conversion process. A second major objective focuses on uncertainties surrounding current harmonized models and productivity assessments; analytical experimental procedures support the generation of verified data to underpin the economic base case and set realistic process and cost targets for future strain improvements. Similarly, robust data are needed to assess progress toward the targets using standardized measurements. This task publishes experimentally validated procedures that can advance the field of algal biofuels with reference analytical methods, data for techno-economic modeling, and analysis and help set quantitative metrics for process and strain improvement strategies.

Weighted Project Score: 8.1

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



Overall Impressions

- The identification and extraction of valuable co-products is critically important for advancing the economic viability of this industry. The approaches and methodology for identifying valuable co-products and doing initial work on the economics is a good start. Additional work on the capture and purification of the co-products and harvest strategies would be good ongoing work.
- This continues to be one of the essential core projects in the portfolio. Expansion into algal biomass valorization via co-products is taking advantage of the core expertise of this group and providing assistance to many other projects, both within BETO's portfolio and within the algal community in general. Close interactions with TEA modelers and industry are essential as this project moves forward.
- Overall, this project has the potential for significant impact and importance within the field of scaled algae production. The potential impact of the standardization of metrics and process is very great and could help to harmonize information across the industry.
- Full biomass valorization and the measurement methods required to achieve it give important direction and tools to both research and industry for maximizing the potential of algal biomass.
- This project has made strong contributions to method standardization across the algal industry. The team should be commended for their methods work, as well as stakeholder engagement and outreach efforts (both for input and information dissemination). Efforts related to co-product development and market analysis are highly relevant to BETO MYPP goals to achieve $3/gge selling price for algal biofuels. The team is encouraged to engage with industry in future co-product development efforts to ensure co-product TEAs capture appropriate regulatory compliance and downstream-processing costs.

PI Response to Reviewer Comments

- We appreciate the complimentary remarks by the review team and welcome the opportunity to respond. Overall, the comments reflect a deep understanding by the reviewers of the critical value this work brings to the algae bioeconomy and in particular of how this project is helping to drive down the cost of algal biofuels and bioproducts. As we move forward in the experimental work for this project, we will address the points brought forward by the reviewers in that we will continue to engage industrial partners to help prioritize products and pathways and, ultimately, to help shape the regulatory landscape where it is applicable.

  Quantitative pursuit of the feasibility and cost assumptions for isolation and purification of products is a standard part of our detailed TEA modeling. As we move forward with the development of promising new co-product options, we will work those, with their respective purification pathways, into our models. Simultaneously, we will integrate a detailed cost-value framework around cultivation productivity and the respective trade-offs in biomass composition. This will allow us to more fully understand and optimize opportunities for algal biofuels and bioproducts over a range of cultivation conditions. Ultimately, we are driving this work toward a fully transparent and open framework for the integrated algae biorefinery concept and multi-product pathways, and we look forward to a continued collaboration with BETO and commercial partners.

PRODUCING TRANSPORTATION FUELS VIA PHOTOSYNTHETICALLY DERIVED ETHYLENE

(WBS #: 1.3.4.301)

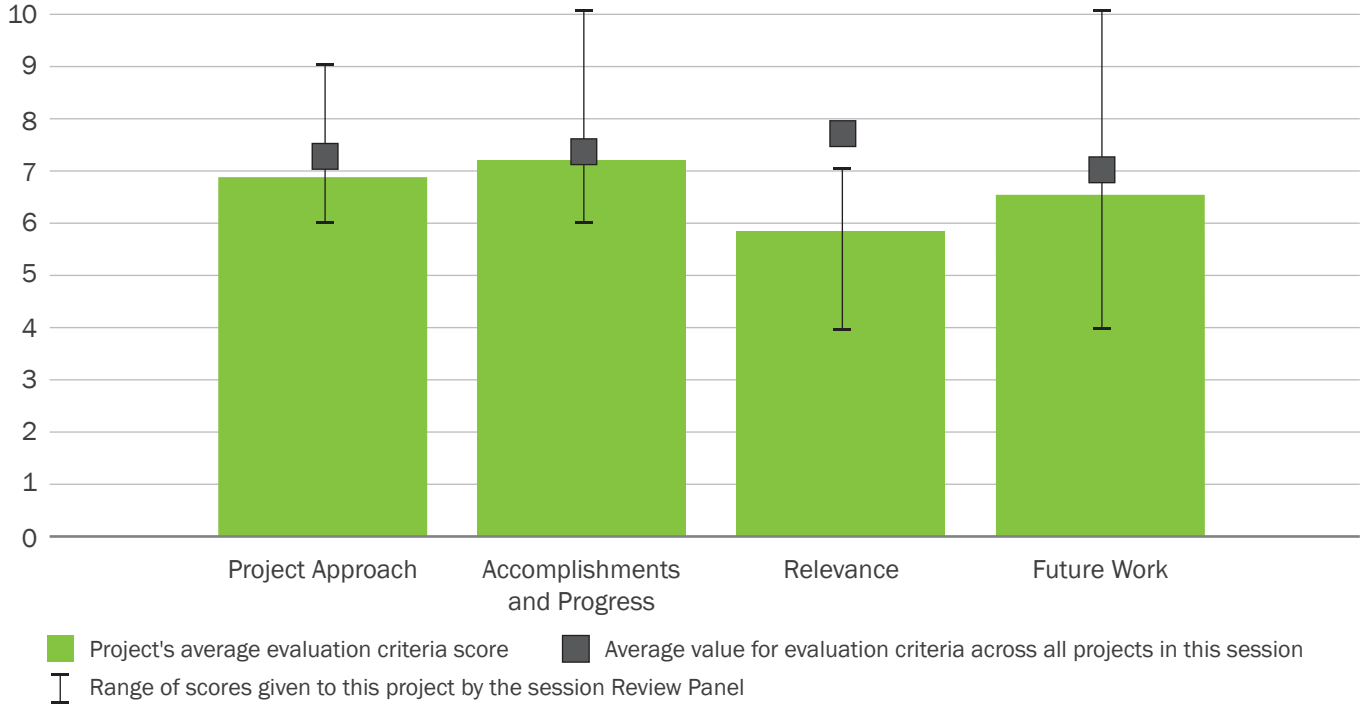
Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Jianping Yu
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$150,000
DOE Funding FY 2016:	$400,000
DOE Funding FY 2017:	$400,000

Project Description

Ethylene is the most widely produced petrochemical feedstock globally and a potential fuel intermediate. It is currently produced from fossil resources, and its production via steam cracking is the largest CO_2-emitting process in the chemical industry. A potentially more sustainable alternative is a biological process that converts CO_2 to ethylene by photosynthesis. The efe gene encoding an ethylene-forming enzyme was expressed in the cyanobacterium Synechocystis 6803, leading to continuous ethylene production. Ethylene production can be supported by seawater. The productivity has been increased by enhancing efe expression levels, such that up to 20% of photosynthetically fixed carbons are redirected from biomass growth to ethylene formation in simulated diurnal light conditions. Despite losing carbons as ethylene, biomass growth is not affected, indicating stimulation of photosynthesis. Detailed characterization using metabolic flux analysis identified global adjustments in carbon and energy metabolism. Both light reactions of photosynthesis and carbon uptake are stimulated by ethylene production. We are trying to identify and overcome current rate-limiting step, presumed to be the supply of arginine, which is a substrate of an ethylene-forming enzyme. A

Weighted Project Score: 6.6

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



process design and cost model is under development to drive future studies. Co-product guanidine, a versatile intermediate and fertilizer, is found to accumulate in the culture medium.

Overall Impressions

- The production of ethylene does not appear to directly advance the goals of the MYPP in terms of biofuel or biomass production (although ethylene can be converted to liquid fuels that are not the highest-value product). However, if this technology can be advanced to the commercial stage the benefits will be meaningful in terms of reducing petroleum usage for the production of products and providing an economically sustainable avenue for the development of methods and facilities for cultivating and processing cyanobacteria. After being over half done with the project, their carbon flux toward ethylene is very low. Their future work includes the use of PBRs and separation membranes. I believe they should put the PBR and membrane work on hold until they can significantly improve the production toward ethylene.
- This is an exciting concept that certainly has potential as a direct photobiological chemical production system. However, the vision of this concept at scale is still lacking many important details, and the economic feasibility has only been examined to a very rudimentary level. The guanidine co-product scenario being explored may or may not be feasible, and product purification (of ethylene and guanidine) needs to be worked out. Further dialogue with industry is essential.
- Overall, this project is very conceptual in nature and may provide a very interesting aside as to what alternative pathways there are to achieve the overall programs goals and deliverables. Continued investigation as to the merit of how this system will be deployed commercially will help to illustrate its viability to meet BETO's goals and deliverables.
- This is very exciting work, but more consideration needs to be given to commercialization.
- Significant knowledge has been generated regarding engineered metabolism toward ethylene production as a result of this project and should be commended. There may be issues with the economics for large-scale production, particularly if guanidine is to be used as a co-product as methods to harvest soluble guanidine haven't been developed.
- If successful, the project could result in a bio-based alternative to one of the most widely produced petrochemicals, as well as co-products and biofuels. The project approach appears to involve a number of high-risk, high-cost components even if the team is successful in achieving target production rates of ethylene, co-products, and biomass. It is unclear if the TEA accurately accounts for these costs.

PI Response to Reviewer Comments

- We sincerely appreciate all of the valuable comments and constructive suggestions. We strongly agree that strain development (to further improve productivity and carbon partition) and process development (to test ethylene production from bench to large-scale PBRs) remains the focus of our R&D efforts, while TEA remains an important tool to guide future R&D with integrated assessment of the overall process concept. As mentioned in slide 21,[22] the strain development effort is centered on identification of rate-limiting step and subsequent genetic modification. The process development effort stud-

[22] Jianping Yu, "Producing Transportation Fuels via Photosynthetically Derived Ethlyne" (presentation given at the Bioenergy Technologies Office 2017 Project Peer Review, Washington, DC, March 8, 2017), https://energy.gov/sites/prod/files/2017/05/f34/algae_yu_134301.pdf.

ies PBR operation targeting for a deployable production system particularly suitable for ethylene and other co-product production. Both efforts are needed to move the technology forward, including meeting our FY 2017 year-end milestone—a 50% increase in ethylene productivity in PBR. We also plan to have a TEA milestone in FY 2018 to incorporate ethylene, guanidine, and biomass valorization.

As algae platform research shifts from lipid-based biodiesel production to more integrated biorefinery with fuels and chemicals, high-value co-product is key to achieving $3/gge. This is also true for the cyanobacterial ethylene project. The project team will expand to study alternative, more integrated process concepts beyond just making ethylene for upgrading to transportation fuels. For example, an integrated biorefinery can photosynthesize CO_2 to ethylene, while also converting ethylene to value-added co-product; converting cyanobacterial cell biomass to renewable diesel blendstock, proteins, pigments, and other co-products; and harvesting guanidine and upgrading it to value added co-product. Guanidine accumulation in media was discovered only a few months ago. Guanidine salts can be major components of fertilizer or rocket fuel, among many other uses. Our engineering efforts will include purification of guanidine and converting it to high-value co-product. Guanidine can be converted to desired guanidine salts by reaction with a strong base, such as an alkali metal hydroxide or methoxide. The guanidine salts can then be separated from the solution by crystallization for high purity. Iron exchange column may be an alternative method to harvest guanidine based on its positive charges.

The success of this project (high-impact publications, patent, and major awards) has attracted collaborators from both academia and industry, maximizing the impact of BETO funding. Collaboration with industry has influenced this project in many ways. Our TEA manuscript received valuable feedback from major chemical and algal companies. We recently started to collaborate with a company on larger-scale cultivation located at ASU using tubular reactors. We have also collaborated with an algae cultivation company on attached growth, and with Colorado State University on thin film reactors. Ultimately, we will test various PBRs in order to (1) understand feasibility and robustness of continuous cultivation and harvesting of the main product and co-products for targeted productivity, yields, or carbon flux; (2) enhance both our and BETO's knowledge base for commercialization strategies; and (3) ensure technology development following the path toward a viable process. On product harvesting, we have been working with a company on membrane separation of ethylene. Moving forward, the process development effort will include co-product production/credits, PBR cost, gas-phase separation cost, and separations of dissolved products, and will be studied in collaboration with industrial partners.

As a component of the NREL algae platform, we are planning to coordinate with other projects and take advantage of our compositional analysis capabilities, as well as newly updated PBR designs and costs by the algae platform analysis team. We will look for opportunities and potential application of the reported PBRs to ethylene and co-products production.

ALGAE TESTBED PUBLIC-PRIVATE PARTNERSHIP (ATP3)

(WBS #: 1.3.5.100)

Recipient:	Arizona State University
Principal Investigator:	John McGowen
Project Dates:	1/31/2013–12/31/2017
Project Category:	Ongoing
Project Type:	FY 2012—Advancements in Sustainable Algal Production (ASAP): DE-FOA-0000615
Total DOE Funding:	$14,999,658

Project Description

The goal of ATP3 is to establish a sustainable network of regional testbeds that empowers knowledge creation and dissemination within the algal research community, accelerate innovation, and support growth of the nascent algal fuels industry. ATP3 increases stakeholder access to high-quality facilities (Function 1) by making an unparalleled array of outdoor cultivation, downstream-process equipment, and laboratory facilities available, along with world-renowned expertise from a tightly managed multi-institutional and transdisciplinary team. ATP3 utilizes a powerful combination of facilities, technical expertise, and proactive management structure to support DOE's techno-economic, sustainability, and resource modeling and analysis activities, helping to close critical knowledge gaps and inform robust analyses of the SOT by conducting coordinated, long-term cultivation feedstock trials at ASU's geographically diverse sites to provide a unique data set regarding reproducibility, scalability, seasonal, and environmental variability (Function 2). These data are critically important to support TEA and LCA activities that will guide R&D toward the transformative goal of cost-competitive algal biofuels by 2022.

Weighted Project Score: 8.8

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.

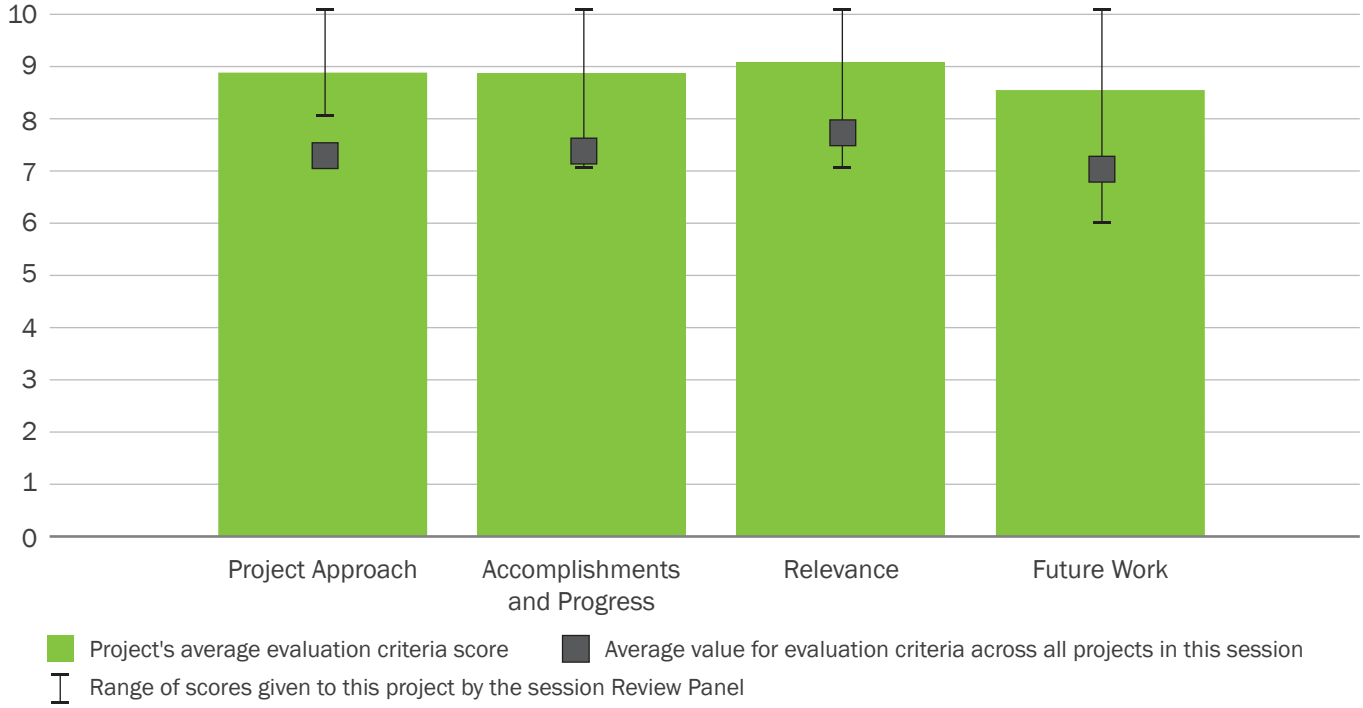


Photo Courtesy of Arizona State University

Overall Impressions

- This is a flagship effort for the algae industry, for both academia and industry. Excellent progress has been made in advancing protocols, methodologies, and training, and in being a reliable testbed. It appears to me that private funding for this facility may be necessary in the future. If they have not already done so, the project should consider providing third-party-verification services where vendors can have their equipment or processes verified by ATP3 (ATP3 personnel run the equipment, not the vender).
- ATP3 is serving a vital role in supporting the stakeholder community. Its record of high productivity and the feedback from stakeholders speak to the fundamental value of this project. It would be a serious loss to the community if this function were no longer available, and it seems too early in the evolution of the industry to expect this work to be self-supporting.
- Overall, the ATP3 testbed has provided a valuable resource to the algal production industry. The ATP3 team has helped move the industry forward and provide a valuable service to companies needed to test and prove technologies.
- A sustainable network of regional testbeds is required to continue the work on improvements to meet MYPP goals. Multi-regional, long-term field trials with publicly available and widely shared data are critical to achieving the goals set in the MYPP. Demonstration in outdoor conditions is critical not just for productivity, but for demonstration of viability of co-product production, providing biomass for downstream development, method development, method harmonization and variation reduction, etc.
- ATP3 is a keystone effort within the BETO portfolio and integral to the success of several other projects funded by BETO. The kind of standardization achieved and aimed for by ATP3 is essential for generating data and setting baselines that will drive the industry forward. The project should continue to develop standardization metrics to ensure a high quality of reproducibility moving forward.
- The project has had broad stakeholder engagement, with over 40 clients and 60 projects in 4 years. The testbed has engaged a mix of national labs, academics, and industry, and has served as a primary source of data for BETO's SOT for empirical data feeding into TEA efforts. The project has achieved major milestones and critical success factors. The project accomplished education and training goals and effectively served the broad stakeholder community, including a long list of collaborators.

PI Response to Reviewer Comments

- We appreciate the comments and look forward to continued dialogue with BETO's Advanced Algal Systems team about how we can continue to be a resource for the algae stakeholder community. Through critical focus areas such as Standards Development and Deployment; High-Impact Data from Lab-to-Field-to-Lab studies at pilot scales that support SOT assessments; and Open-Access Testbed(s) Education and Training for Workforce Development and Outreach activities, we are a key resource for R&D and technology and business risk reduction.

  We feel there is significant value in the partnership established under ATP3 and that there are several

critical capabilities lost if future support for the testbeds is not secured, including the following:

- SOT support with year-over-year integration/objective validation of
  - Cultivation systems improvements/testing
  - Geographic variability/seasonal variability
  - Quantitation of annual operating days
  - Pond contamination and reliability monitoring.
- National lab access to outdoor testing for validation of lab-based R&D.
- Access to facilities for validation/due diligence/standards for industry and early-stage technology company support.
- Standardized, genetically modified algae outdoor testing facilities.
- Workforce development through an established program centered on access to facilities; ATP3 is the only hands-on workshop providing participants access to outdoor cultivation facilities.

The ATP3 network is the first-ever demonstration of the value of implementation of standardized analytical and process-monitoring methodology across a multi-institutional group of researchers. We built on years of experience in developing and validating procedures,[23] and throughout the ATP3 project the implementation, training, and quality control of data was refined and is the subject of two recently accepted manuscripts to be published in *Algal Research* (Lieve M.L. Laurens et al. and John McGowen et al., *Algal Research* 2017; in press). This approach has been well received by the community, and at least two newly funded consortium projects are implementing a similar approach. Standardization is critical to a nascent industry establishing itself and achieving long-term viability. We are very proud of the efforts we have put in to our own standardization activities and look forward to continued stakeholder engagement on this topic.

The ATP3 testbed network provides easy access for researchers to an outdoor testbed site to validate and capitalize on improvements made in the laboratory, such as with genetically engineered strains, and provide established methodology and an easy cost structure that is invaluable to the support for the burgeoning algae industry in which different technologies can be road-tested and compared and technology and business risk reduced. We are eager to continue in that support role. And while we were not able to go into detail in our presentation, third-party validation is a key offering for ATP3 and in fact is something that we have already done and is part of the revenue we reported.

The investment BETO has made over the past 4 years has built a unique research group with demonstrated ability to carry out complex algae cultivation experiments, broadly increase access to outdoor cultivation facilities, and implement robust education and workforce development programs. We have provided robust, reliable, publicly available and outdoor algae cultivation, harvest, composition, and pond ecology and pathology data for SOT assessments and decision support for BETO's Advanced Algal Systems Program and are uniquely suited to continue to serve that platform. Reconstituting this capability in the future will be costly and time consuming, and we continue to pursue opportunities to remain active in this role.

[23] See: (1) Lieve M. L. Laurens, Thomas A. Dempster, Howard D. T. Jones, Edward J. Wolfrum, Stefanie Van Wychen, Jordan S. P. McAllister, Michelle Rencenberger, et al., "Algal Biomass Constituent Analysis: Method Uncertainties and Investigation of the Underlying Measuring Chemistries," *Analytical Chemistry* 84, no. 4 (2012): 1879–87, doi:10.1021/ac202668c. (2) Lieve M. L. Laurens, Stefanie Van Wychen, Jordan P. McAllister, Sarah Arrowsmith, Thomas A. Dempster, John McGowen, and Philip T. Pienkos, "Strain, Biochemistry, and Cultivation-Dependent Measurement Variability of Algal Biomass Composition," *Analytical Biochemistry* 451, no. 1 (2014): 86–95, doi:10.1016/j.ab.2014.02.009. (3) Stefanie Van Wychen, William Long, Stuart K. Black, and Lieve M. L. Laurens, "MBTH: A Novel Approach to Rapid, Spectrophotometric Quantitation of Total Algal Carbohydrates," *Analytical Biochemistry* 518, no. 1 (2017): 90–93, doi:10.1016/j.ab.2016.11.014.

REGIONAL ALGAL FEEDSTOCK TESTBED (RAFT) PARTNERSHIP

(WBS #: 1.3.5.111)

Recipient:	University of Arizona
Principal Investigator:	Kimberly Ogden
Project Dates:	9/1/2013–8/31/2017
Project Category:	Ongoing
Project Type:	FY 2012—Advancements in Sustainable Algal Production (ASAP): DE-FOA-0000615
Total DOE Funding:	$8,000,001

Project Description

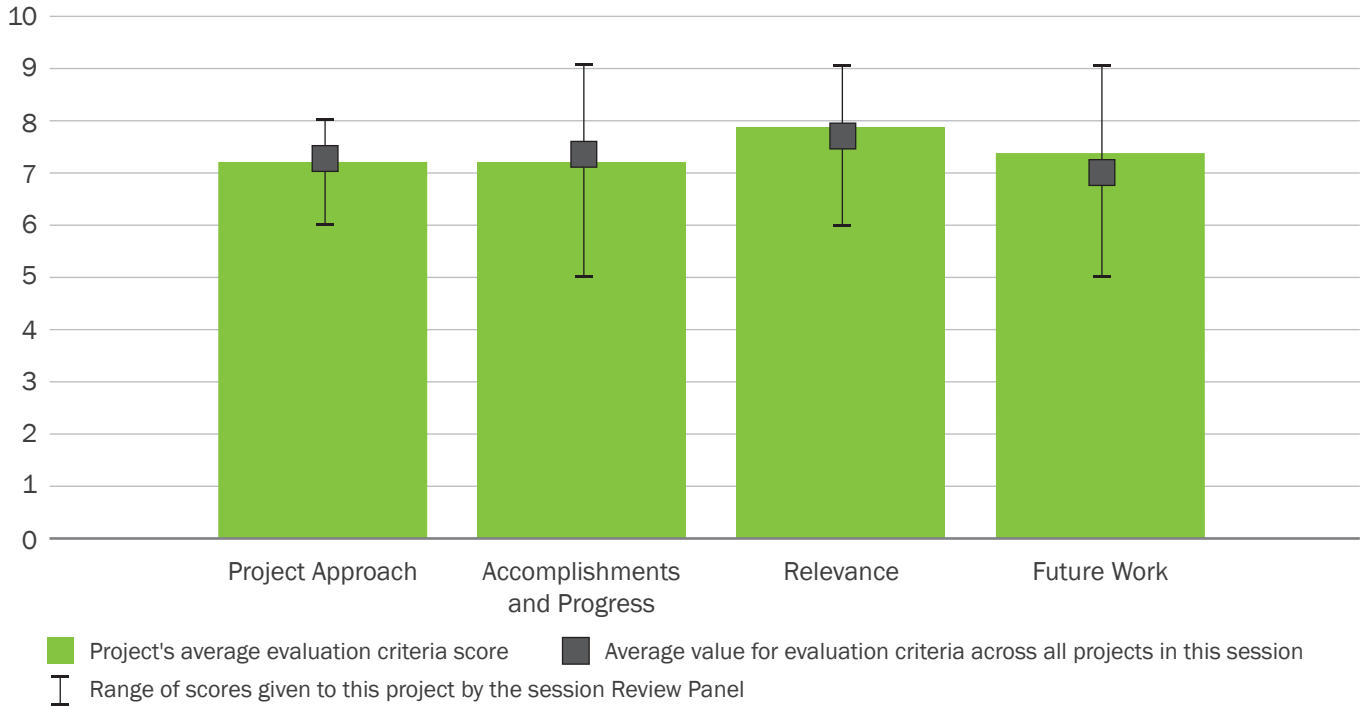
The overall goal of the RAFT Partnership is to obtain long-term cultivation data that is model driven. The project is a collaboration between the University of Arizona, PNNL, New Mexico State University, and Texas A&M AgriLife Research. Our approach is to characterize the growth rates of production strains as a function of light, temperature, and salinity. This data is then given to modelers to predict and design a crop-rotation strategy for the testbeds. Each testbed implements the rotation strategy to obtain cultivation data. RAFT researchers have grown three strains over three seasons in three sites. The team has also cultivated algae in recycled media and wastewater. All data will be publicly available at http://www.algaeknowledge.org. Additional work involves quantitative polymerase chain reaction data to monitor contaminants and development of an online optical density sensor to continuously monitor algal growth.

Overall Impressions

- This project is providing good baseline-type data that other projects can start with. In addition to their stated goal of cultivating algae year-round outdoors, this project has also developed operating procedures to help improve productivity, reduce downtime,

Weighted Project Score: 7.4

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



and detect contaminants. This project has developed equipment and procedures that I assume will be available for industrial use. It would be good if this program coordinated with ATP3 on developing and agreeing to standard operating and analytical procedures.

- This project casts a wide net. Useful data on the SOT, pond crashes, seasonal strain performance, etc., have been collected. Defined, unifying goals are lacking, and reviewers' concerns from the 2015 Project Peer Review regarding lack of interaction with the algae community have not been fully addressed.
- Overall, the RAFT project and team have combined to provide relevant algal production data over the course of the project. The information generated can and will provide important information as to the processes of scaling algal biomass production systems and integrating various species and pond designs in different locations.
- Multi-location, multi-season field trails for validation of Biomass Assessment Tool and other tools combined with the pond ecology work demonstrates that these collaborative field trial efforts are critical to continue productivity improvements. This is required to meet the productivity goals in the MYPP.
- RAFT is generating real productivity measurements and dealing with problems that arise during cultivation at scale in different environments, making it a valuable effort toward developing systems for sustainable production of algae fuels. The data management system currently being developed will be useful for transparency in sharing data and moving the industry forward.
- The project is making strong contributions to publicly available, large-scale outdoor production tests at multiple locations. The project has resulted in several pond-monitoring and crop-protection improvements, and biomass produced has been used by a number of groups for downstream-process testing and product development. The team has made a strong commitment to making publicly available data sets user-friendly and will continue to improve data accessibility moving forward.

PI Response to Reviewer Comments

- A near-term goal is to send biomass to NREL to determine how well our techniques for determining ash-free dry weight, protein, carbohydrate, and lipid compare with theirs, and then we can have a discussion regarding standard operating and analytical procedures.

  Thank you for your feedback, both written and in person at the Project Peer Review.

DEVELOPMENT OF ALGAL BIOMASS YIELD IMPROVEMENTS IN AN INTEGRATED PROCESS – PHASE II

(WBS #: 1.3.5.211)

Recipient:	Global Algae Innovations Inc. (GAI)
Principal Investigator:	David Hazlebeck
Project Dates:	10/1/2016–9/30/2019
Project Category:	New
Project Type:	FY 2016—Advancements in Algal Biomass Yield Phase 2 (ABY2): DE-FOA-0001471
Total DOE Funding:	$6,250,000

Project Description

GAI's Phase 1 project resulted in tremendous productivity and processing improvements that have moved algal technology closer to economic viability for biofuels than ever before. This project builds on these successes to accelerate the commercialization of algal biofuels. Best-in-class cultivation and processing technologies are being combined in some of the world's leading strain development laboratories to develop yield and energy use improvements. Yield improvements will come from better strains generated by world-class algal strain developers and from open-pond cultivation innovations. Energy use improvements will come from new drying and extraction unit operations.

Two parallel pathways to a biofuel are being investigated. In the crude oil pathway, an algal slurry is converted to oil and an aqueous recycle stream via hydrothermal liquefaction. In the lipid oil pathway, the algae biomass slurry is dried, and oil is extracted to produce lipid oil and a high-protein algae meal co-product. Generally, the algal lipid oil pathway has lower productivity because of the need to accumulate lipids, but it has better

Weighted Project Score: 8.1

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.

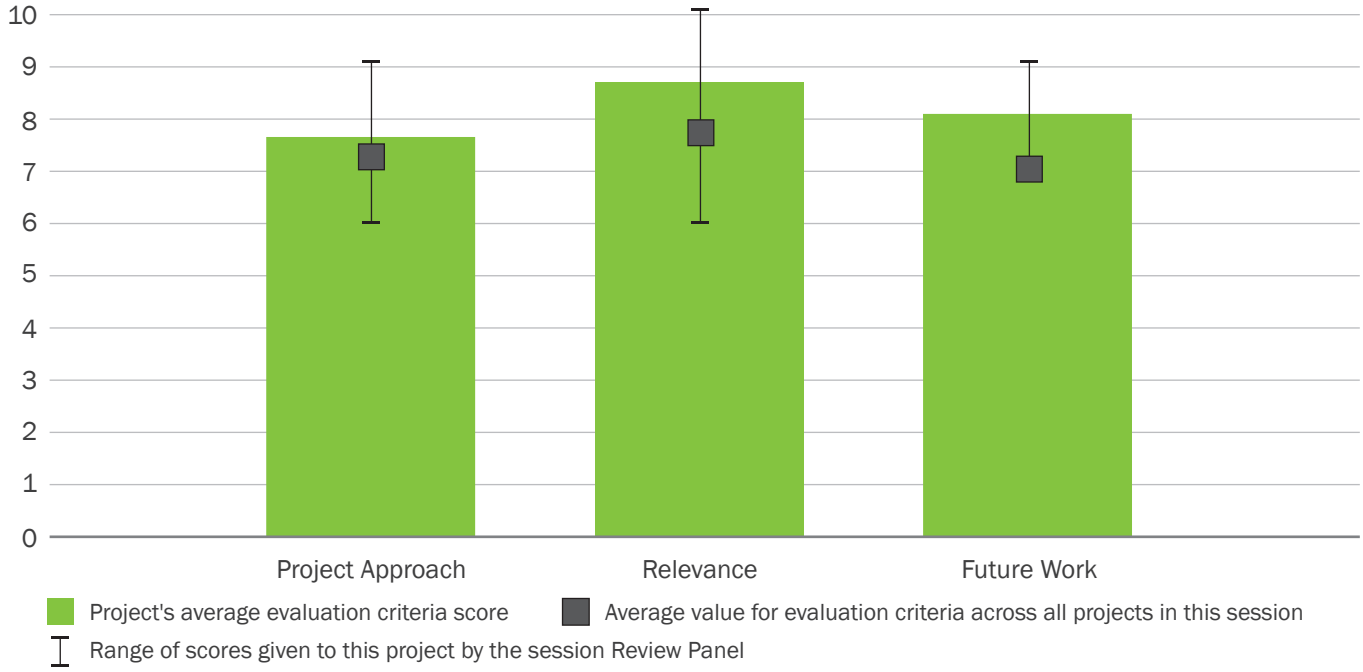


Photo Courtesy of Global Algae Innovations Inc. (GAI)

economics because the lipid oil is higher quality than crude oil and a protein meal co-product is produced. The productivity goals for the project are 5,000 and 8,000 gallons per acre per year for the lipid and crude oil pathways, respectively. The energy use reduction goals for this project are 58% and 19% for the lipid and crude oil pathways, respectively.

Overall Impressions

- The advances are very encouraging in terms of pond design, operating procedures, and equipment development. The development of a full TEA with independently validated technical inputs will be important for determining the future of these advancements as a whole.
- The lack of detail presented in project approach and future work is understandable for a for-profit entity, but it makes evaluation difficult. Ambitious metrics have been set, and the comprehensive approach that addresses every facet of the process will likely result in significant advances.
- Overall, this is a solid project with a solid team of investigators. By doing most of the work at a commercial scale, data and information can be directly translated to provide clarity into further TEA assessments. Optimization of CO_2 stream into the production systems show significant promise for the improvement of productivity while reducing over facility capital cost.
- The integrated approach is useful; viewing process from end to end allows for synergies between unit operations. Continued iterative optimization of ABY1 technologies and improvements, with a focus on energy reduction, will hopefully lead to additional similar breakthroughs as in ABY1.
- The proposed project has a clearly defined plan for strain improvements, cultivation improvements, and efforts to decrease downstream-processing energy. The team has a track record of meeting project goals within proposed timelines and budgets. The work will be executed in an integrated, industrial environment and has clear relevance to industry, as well as BETO MYPP goals.

PI Response to Reviewer Comments

- This project builds on the success of the ABY1 effort with ambitious goals that will require development of new transformational technologies. The approach is to combine best-in-class cultivation and processing technologies with some of the world's leading strain development laboratories to develop yield and energy use improvements. All of the technologies are filtered through a comprehensive techno-economic model so that only work is restricted to approaches that will contribute to formation of an economically viable, sustainable algae commodities industry that can provide oil for drop-in biofuels.

BIOMASS PRODUCTIVITY TECHNOLOGY ADVANCEMENT TOWARDS A COMMERCIALLY VIABLE, INTEGRATED ALGAL BIOMASS PRODUCTION UNIT

(WBS #: 1.3.5.220)

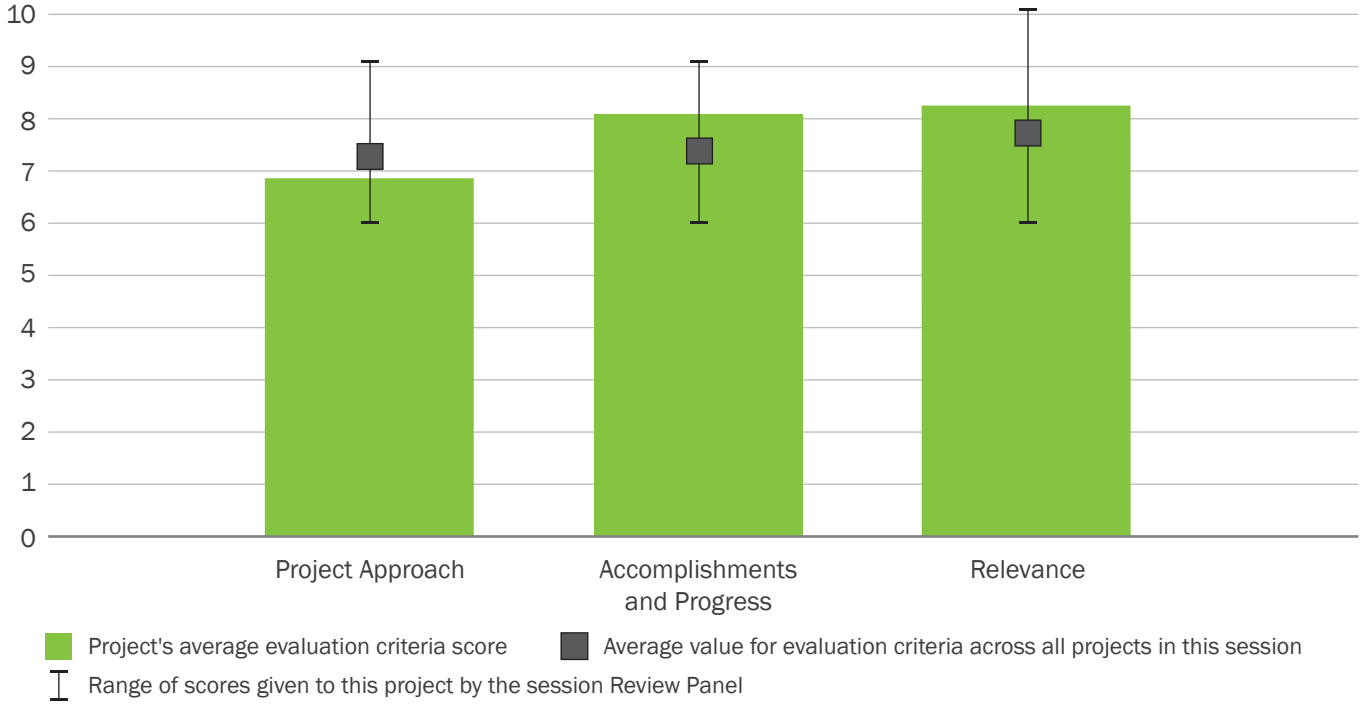
Recipient:	Sapphire Energy Inc.
Principal Investigator:	Craig Behnke
Project Dates:	10/1/2013–8/31/2016
Project Category:	Sun-setting
Project Type:	FY 2013—Advancements in Algal Biomass Yield (ABY1): DE-FOA-0000811
Total DOE Funding:	$5,000,000

Project Description

Sapphire Energy Inc. (Sapphire) has developed an end-to-end process to produce renewable, algae-based fuel that is fungible with existing refinery streams. The project addresses three priority areas: (1) improve algal biomass productivity in outdoor cultivation environments relevant to commercial scales, (2) improve preprocessing technologies that can be integrated at scale with biomass production, and (3) successfully integrate priority areas 1 and 2 to ensure that target yields are met at a scale that enables production of cost-competitive fuels and products. In meeting each of these objectives, Sapphire aims to demonstrate sufficient improvements in algal biomass yield at lab and outdoor pilot scales to provide a positive indication toward success of a 1-acre demonstration of 2,500 gallons per acre per year oil productivity by 2018. In a highly integrated process, Sapphire aims to increase intrinsic algal biomass productivity by employing evolution-based strain engineering and developing a systems biology approach to identify the regulatory networks associated with controlling both biomass productivity and oil content. To improve its cultivation process, Sapphire is

Weighted Project Score: 7.8

Weighting: Approach–25%, Relevance–25%, and Accomplishments and Progress–50%.



constructing ecologies to minimize yield loss in the ponds by increasing robustness across biotic and abiotic stresses. Improvements are also developed in nutrient recycle, harvest and extraction, and hydrothermal treatment and extraction methodologies by increasing efficiency and decreasing cost.

Overall Impressions

- This is a well-executed project. The project considered many of the factors other projects are trying to address in more detail. Great approach and demonstration at larger scale out of doors and for an extended period of time. This project demonstrated exceeding the MYPP goal of 2,500 gallons per acre in an outdoors raceway and reduced the estimated cost to $5.20/gge from their baseline of $7.60. I believe the TEA was done with the cost of de-metaling the HTL oil included. In the presentation, the HTL was done at severe conditions of 400oC and 60-minute residence time. If the severity was lessened with corresponding oil yield loss, I assume the cost for treating the HTL oil would decrease as the oil quality improves. How would this "better oil" at a lesser yield impact the TEA? I believe other projects assuming upgrading of HTL oils would benefit from this presentation and an understanding of what a refinery might be willing to accept as a feedstock.
- The lack of specifics presented (due presumably to both time/space considerations and proprietary data) make it difficult to assess the specific approaches and accomplishments. However, it appears that the overall productivity goal was reached. Valuable lessons were learned, including the importance of HTL oil quality for acceptance by refiners. It is hoped that these lessons will become available to the greater community through publications.
- Overall, this project shows good lab-to-field testing and implementation. Lessons learned in the field with larger-scale growth trails can lend to significant improvements in large-scale culture optimization.
- This project demonstrated impressive advancement in crop protection, leading to increased crop stability.
- The project appeared to meet many stated technical targets, including productivity improvements leading to a demonstration that exceeded BETO's MYPP goals for areal production (2,500 gallons per acre per year by 2018). The project has clear relevance to the algal biofuels industry and virtually all work has been completed.

PI Response to Reviewer Comments

No official response was provided at the time of report publication.

REALIZATION OF ALGAE POTENTIAL (REAP)

(WBS #: 1.3.5.230)

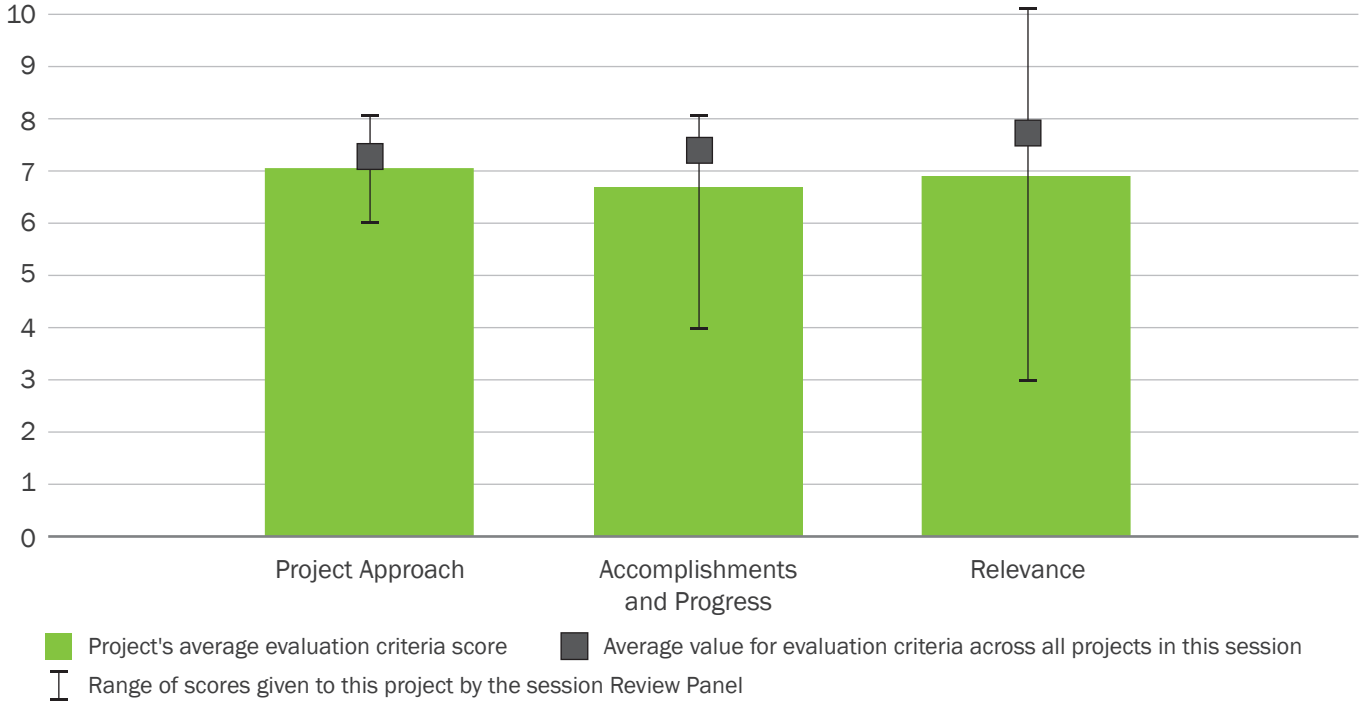
Project Description

Recipient:	Arizona State University (ASU)
Principal Investigator:	Pete Lammers
Project Dates:	9/30/2013–3/31/2016
Project Category:	Sun-setting
Project Type:	FY 2013—Advancements in Algal Biomass Yield (ABY1): DE-FOA-0000811
Total DOE Funding:	$5,000,000

The goal of the REAP R&D program is to develop an integrated process for producing at least 2,500 gallons of biofuel intermediate per acre per year within 30-months (Performance Period 1). This outcome will advance the DOE goal of demonstrating 5,000 gallons per acre per year by 2022. We take a translational approach, building on only the most promising technology options for each unit operation. The focus is on implementation and integration driven by cost, scale-up potential, and energy-balance design criteria. Process integration trials will occur at ASU's algal cultivation testbed. This work will produce engineering data for system modeling so that those data will be coherent and integrable. Simultaneously, REAP members at Washington State University will explore improvements to oil production by sequential HTL; work at Los Alamos National Laboratory will improve strains; work at PNNL will validate quantitative growth models required for resource assessment modeling; work at Pan Pacific Technology and Argonne National Laboratory will produce energy- and material-balanced system models in Aspen based on the REAP process data; and work at Algenol Biofuels will support cultivation studies and provide assistance for techno-economic readiness.

Weighted Project Score: 6.8

Weighting: Approach–25%, Relevance–25%, and Accomplishments and Progress–50%.



Overall Impressions

- I think the TEA of this project would benefit greatly from including a reputable member of industry practiced in upgrading oils to make them suitable as a refinery feedstock. The industry partner should advise on the cost of upgrading (including the removal of metals) the different oils generated by different HTL operating conditions to make them acceptable to a refiner and at what discount the refiner would want in order to take the oils.
- This project explored important issues across the entire process. Perhaps it was a failing of the presentation rather than the actual work, but it is not clear what, if any, important takeaways resulted from this work. The demonstration of a novel mixotrophy strategy did result in funding of a new project.
- Overall, this project has shown some interesting and relevant results that should provide some significant impact on further development.
- This project was well executed and demonstrated that more than 5,000 gallons per acre per year is feasible in a PBR system using mixotrophy, as well as over a year of stable cultivation with multiple crop rotations (called strain switches in the presentation).
- Somewhat marginal progress was made in strain improvement during this project; however, the hallmark accomplishment of this project was mixotrophic cultivation in outdoor PBR systems that improved productivity above targets. This is a new accomplishment since the last review, making this an overall successful endeavor/partnership.
- The team presented a clear management and technical plan and achieved several of the project's original goals. Long-term stability of heat-tolerant cultures was demonstrated, and high-density harvests were possible with mixotrophy. Productivities exceeded BETO MYPP goals, although it is unclear if areal productivities using mixotrophic approaches are meaningful for these targets. The TEA suggested that BETO MYPP biofuel cost targets could not be met with this approach.

PI Response to Reviewer Comments

- The team acknowledges the broad scope of the REAP project. There are several important project conclusions that may have been underemphasized in the presentation. The ABY1 program goal for areal fuel feedstock yield was achieved (or missed by a small margin). This was accomplished with wild-type organisms in PBRs operated continuously without culture failures for more than 1 year. Mixotrophy was then shown to provide a route to 6,700 gallons of biocrude per acre per year, more than double the ABY1 yield goal and more than the 2022 goal of 5,000 gallons per acre per year.
- Obstacles to transformation were overcome and genetically improved strains are now in the BETO pipeline. While the team could not evaluate the yield impact and TEA implications of the genetically improved strains, the BETO portfolio was significantly advanced by the entire REAP team's efforts. TEA modeling included analyses that meet or go beyond other TEAs in BETO's portfolio. The LCA analysis identified critical barriers to PBR design with respect to mixing energy and CO_2 mass transfer. Threshold values for air/CO_2 supply were established, providing critical design standards for scalable PBR systems.
- Statements that PBR systems are too expensive may be premature. Quantifying the economic benefit of the low-crash rates in REAP PBRs should be a goal for future TEA studies. The effect of additional co-products toward achieving BETO cost targets in nth generation PBR systems cannot be assessed at present. The strategic design decision made during proposal preparation to focus on water conservation for arid-region deployment rests on sound reasoning

that has not changed. The REAP vision was ambitious and made within the context of a limited-competition, two-phase project plan. With no phase two funding, it is inevitable that some key REAP assessment parameters will be lacking at the end of stage one.

- The value proposition for extremophile growth in PBR systems for deployment in arid regions to reduce evaporative water loss was firmly established. Cyanidiales strains demonstrated over 35 weight percent biocrude oil yields from high-protein/low-lipid biomass, eliminating the need for lipid induction via nutrient limitation and thus the associated productivity losses. Significant progress toward nutrient recycling was obtained and published.[24] The algae crop rotation system was effective in minimizing winter/summer productivity differences, and the PBR systems operated without any rain or wind associated culture crashes seen so often in open-raceway ponds. This was true for the green and red algal strains in the rotation.
- Direct and sequential HTL systems were compared and benefits for both approaches identified. Sequential HTL offers a potential route to co-product recovery, but direct HTL afforded better results in terms of yield and oil quality in batch processing. With a no-cost extension in place, the PI and HTL team are conferring with UOP engineers regarding the upgrading of biocrude feedstock and solids separation to include metals and additional phosphorus removal, plus associated cost estimates.
- Finally, BETO-REAP funding has resulted in 12 papers published to date in the primary scientific literature with 4 more submitted and under review.

[24] T. Selvaratnam, H. Reddy, T. Muppaneni, F. O. Holguin, N. Nirmalakhandan, P. J. Lammers, and S. Deng, "Optimizing Energy Yields from Nutrient Recycling Using Sequential Hydrothermal Liquefaction with Galdieria Sulphuraria," *Algal Research* 12 (2015): 74–79, doi:10.1016/j.algal.2015.07.007.

SCALE-UP OF ALGAL BIOFUEL PRODUCTION USING WASTE NUTRIENTS

(WBS #: 1.3.5.240)

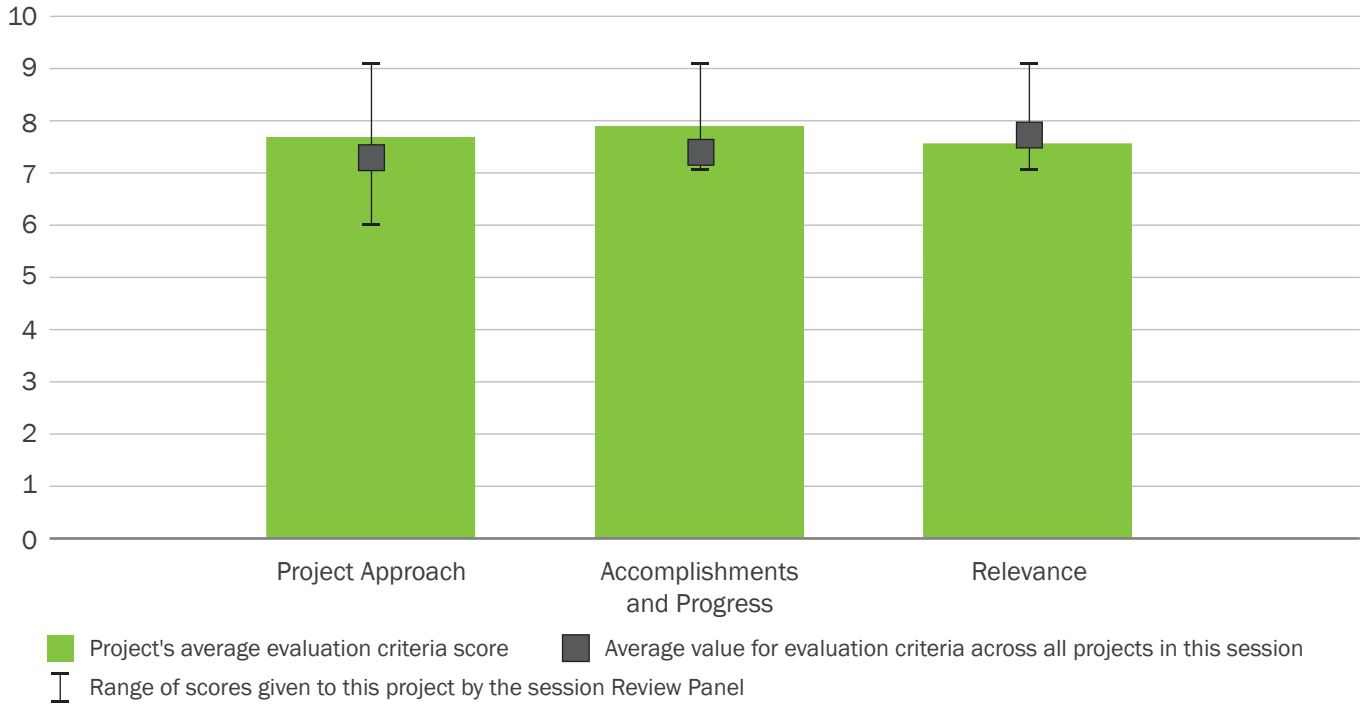
Recipient:	California Polytechnic State University
Principal Investigator:	Tryg Lundquist
Project Dates:	10/1/2013–3/31/2016
Project Category:	Sun-setting
Project Type:	FY 2013—Advancements in Algal Biomass Yield (ABY1): DE-FOA-0000811
Total DOE Funding:	$1,480,883

Project Description

Growing algae in wastewater media utilizes waste nutrients and carbon, supporting mixotrophic and heterotrophic growth in addition to autotrophic, leading to high areal productivities and biofuel potential with relatively low greenhouse gas intensity. Experimental studies were conducted at a full-scale wastewater treatment plant (0.6 million gallons per day, 7 acres of raceways) in California. Pilot raceway ponds were also installed at the site to investigate optimization of biomass production, wastewater treatment, and harvesting through the manipulation of pond dilution rates, diel influent timing, and CO_2 addition. Biomass harvested from these systems (33 g/m^2/day annual average productivity) was transformed to biocrude through HTL, and yields of biocrude and fuel distillate were measured. Wastewater from HTL was found to be a suitable medium for additional algal growth. Productivity and bioflocculation of cultures was found to correlate to certain community genetic compositions. Finally, laboratory PBR technology was developed that allows rapid estimation of areal raceway productivity of test strains under stimulated climates. TEA and LCA using the CA-GREET model and BETO harmonized inputs resulted

Weighted Project Score: 7.7

Weighting: Approach–25%, Relevance–25%, and Accomplishments and Progress–50%.



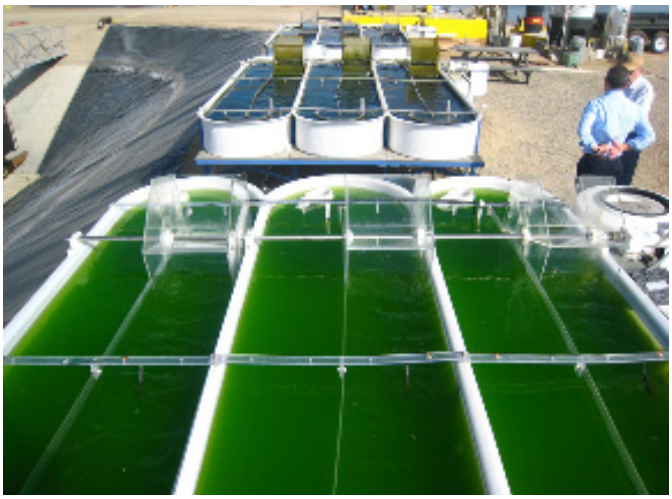


Photo Courtesy of California Polytechnic State University

in a MFSP of $4–$9/gge (2011$) depending on revenue streams. Greenhouse gas emissions were estimated to be 13 g of CO_2 equivalent per megajoule biofuel intermediate.

Overall Impressions

- This project did an excellent job of focusing on using wastewater for algal growth and water cleanup. I think the TEA of this project would benefit greatly from including a reputable member of industry practiced in upgrading oils to make them suitable as a refinery feedstock. The industry partner should advise on the cost of upgrading (including the removal of metals) the different oils generated by different HTL operating conditions to make them acceptable to a refiner and at what discount the refiner would want in order to take the oils.
- Algae wastewater systems represent low-hanging fruit that will help to jumpstart the algal biofuels industry. This work has demonstrated excellent productivities and $/gge that can be achieved with such an approach. The concept of a closed loop with complete recycle of the HTL aqueous stream is encouraging, but will require longer-term studies. It is not clear how identification of high-performance strains and community genetics studies will be used to influence polycultures at scale when using wastewater in outdoor systems.
- Overall sound project that shows the impact of using nutrient from wastewater streams, which could have severe impact on the cost of large-scale cultivation.
- Using wastewater treatment service as main nutrient inputs, demonstrating such high productivities, and meeting or exceeding several MYPP goals and the accompanying TEA/LCA demonstrates the clear feasibility of this method for biomass production.
- Overall, this project should be commended for a multi-faceted approach toward addressing several relevant barriers for BETO, as well as generating data indicating progress toward improved productivity and processing toward the objective of 2,500 gallons per acre per year. Uniquely, this work incorporates wastewater as a source of water and nutrients, differentiating it from many other projects in the BETO portfolio and making it a valuable endeavor.
- The project has accomplished many of its original goals. Work appeared to be fully integrated—from strain selection through outdoor production, harvesting, conversion, and regrowth on recycled nutrient streams. Models of the integrated approach suggested that BETO MYPP goals for productivity and carbon intensity of algal biofuels could be met. Wastewater treatment credits could be used to drive down the cost of fuels to values close to BETO MYPP goals. The project did not evaluate the relative contribution of autotrophic and heterotrophic growth to the system's overall productivity, so it is unclear how much of the growth comes from photosynthetic growth.

PI Response to Reviewer Comments

- Regarding the value of community genetics characterization for scaled-up wastewater polyculture: If organisms are identified that correlate to culture properties (e.g., bioflocculation, productivity), then

perhaps the community composition of the cultures could be influenced to promote desirable characteristics. Such influences might be through inoculation, enrichment, or otherwise changing the operation of the culture reactors. At a minimum, genetic techniques might give algae farmers forewarning of culture changes, allowing early response. This community genetics work is among the first ever conducted on algae polycultures. The modest subcontract to SNL to improve our understanding of algal-bacterial community dynamics was seen by the PI as a relevant investment. SNL has to develop new methods to analyze and understand the large amounts of data generated from our polyculture sampling. This development process is continuing at SNL beyond the end of the ABY1 project. Additional results and recommendations are expected in the coming months.

An important outcome of Dr. Huesemann's work at PNNL-Sequim was development of the benchtop Laboratory Environmental Algae Pond Simulator (LEAPS) reactors as part of this project. LEAPS is meant to more accurately simulate outdoor pond culture performance than other available technologies. During ABY1, LEAPS performance was tested against 3.5-square meter raceways operating at the Delhi, California, site. Chlorella sorokinana (DOE 1412) cultures in LEAPS had similar growth curves as the same strain in the Delhi raceways. During our ABY2 project, this pure culture validation will be extended to wastewater polycultures using new continuous-flow benchtop reactors, similar to LEAPS. If successful, such benchtop reactors should greatly reduce the cost and increase the rate of R&D related to both mono- and poly-culture raceway cultures. The ultimate promise of this type of research equipment is that culturing methods and strains could be optimized in the lab for any future outdoor scale-up location, saving a larger onsite R&D effort and more quickly reducing uncertainties for scale-up investments.

Data to calculate the contribution of heterotrophic and autotrophic growth to the systems overall productivity has been collected for this project, and this is currently being calculated for inclusion in the final report. In past projects, the amount of heterotrophic biomass (both algal and bacterial) has ranged from one-half of the biomass in the winter to approximately one-third of the biomass in the summer. Therefore, historically depending on season, the photosynthetic biomass is approximately one-half to two-thirds of the total biomass. Preliminary analysis of this project's data seems in line with these conclusions.

PNNL advised and continues to advise on HTL oil upgrading and oil conditions needed for refining for the TEA.

The PI appreciates the reviewers' helpful comments and critiques.

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

(WBS #: 1.3.5.243)

Recipient:	MicroBio Engineering
Principal Investigator:	John Benemann
Project Dates:	10/1/2016–9/30/2019
Project Category:	New
Project Type:	FY 2016—Advancements in Algal Biomass Yield Phase 2 (ABY2): DE-FOA-0001471
Total DOE Funding:	$5,000,000

Project Description

This project will develop improved microalgae strains for higher productivity of biofuels and biofuel intermediates. Microalgae will be cultivated on treated wastewater, providing nutrient removal along with biofuel production. This will allow achievement of DOE's goal of 3,700 gallons of algae biofuel intermediate per acre per year in an outdoor test environment by 2020, as well as $3/gge by 2022. The project includes the integration of several innovative processes, including the development of algae strains with improved carbohydrate and lipid productivity; increased biomass productivity through mixotrophic processes; and conversion of the entire biomass to biofuel intermediates utilizing extractions, fermentations, and HTL of residual biomass. Strain selection will be conducted on mutagenized cells through the manipulation of environmental parameters in PBRs operated to enrich for strains with desired traits. Selected strains will be cultivated outdoors in wastewater ponds and biomass harvested by bioflocculation. Challenges to be overcome include the successful selection of enhanced strains and the maintenance of desirable traits in the absence of continued selective pressure, and their applications in mixotrophic cultivation. The initial stage of this recently funded

Weighted Project Score: 7.3

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.

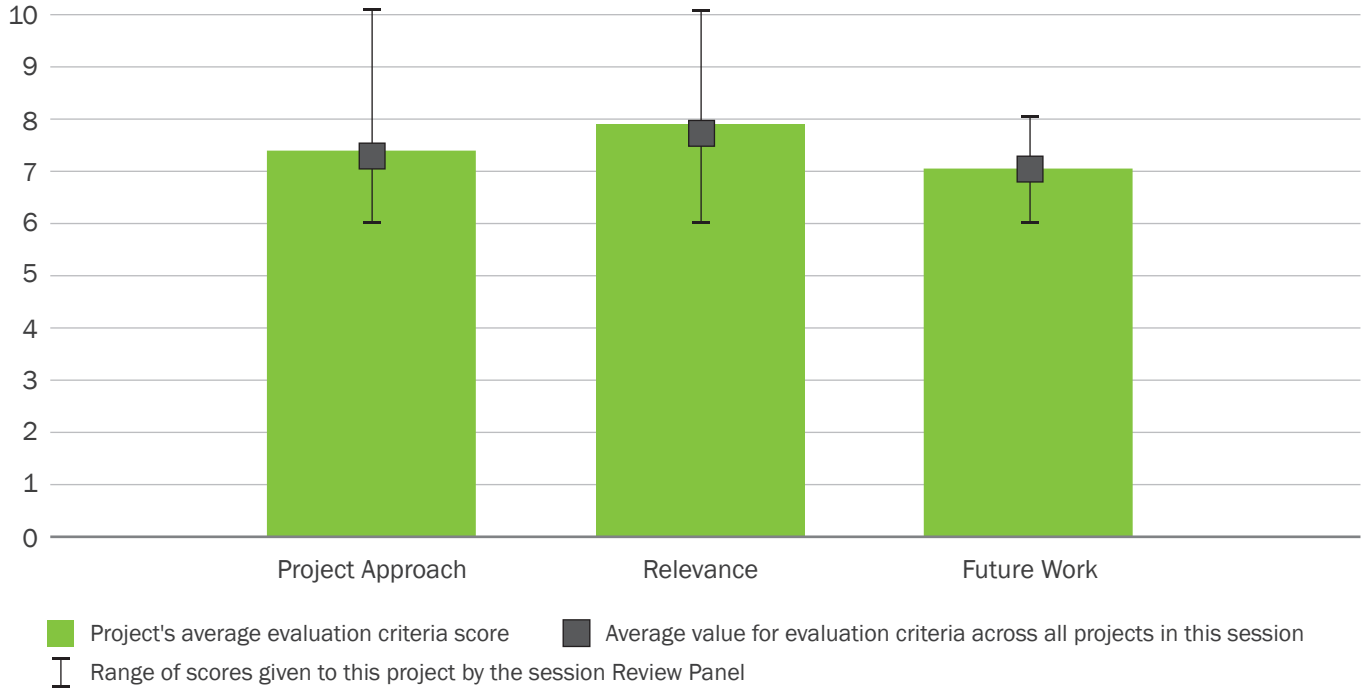


Photo Courtesy of MicroBio Engineering

project, currently underway, focuses on the acquisition of baseline data from wild-type strains and the development of PBRs for enrichment cultures.

Overall Impressions

- I think the TEA of this project would benefit greatly from including a reputable member of industry practiced in upgrading oils to make them suitable as a refinery feedstock. The industry partner should advise on the cost of upgrading (including the removal of metals) the different oils generated by different HTL operating conditions to make them acceptable to a refiner and at what discount the refiner would want in order to take the oils.
- This will be a large effort with an excellent team. A variety of approaches will be used to increase overall fuel productivity, including strain improvements, mixotrophy, and downstream-process optimization. However, it was not clear from the presentation what sort of innovation will be implemented that is expected to enable productivity targets to be met. Trying to simultaneously generate strains with high lipid, carbohydrate, or protein would suggest the lack of a clear path to improved economic outcomes, and strains that are enriched for one component may exhibit lower productivity overall.
- Overall, this project shows some innovative ideas in order to best take advantage of wastewater streams. The use of wastewater could potentially reduce the overall operational costs by the reduction of nutrient inputs, though care may need to be taken as high biomass yield can sometimes not be achieved without supplemental nutrients.
- This is a straightforward project addressing strain selection and improvement with clear targets.
- This project aims to improve several aspects of algal biomass production for biofuels, ranging from strain selection through conversion technologies, making it highly relevant for BETO. A strength of the project is the focus on using reclaimed wastewater to cultivate strains, making it stand out from other projects in the portfolio. Overall, this project takes on a lot of varied efforts and will likely lead to significant advances in at least a few technology areas that will demonstrate concrete advances in improving areal productivity of fuels derived from algal biomass.
- This new project seeks to address BETO MYPP goals for productivity (3,700 gallons per acre per year by 2020) and price ($3/gge by 2022) through optimized growth on wastewater and low-energy downstream processing, though it is unclear how much of the yield target is accomplished from heterotrophic growth versus photosynthetic growth. The team presented a clear plan aligned with original project objectives. The team is encouraged to build off of previous accomplishments during resource allocation for this project.

PI Response to Reviewer Comments

- The central objective of this project is to select algal strains that preferentially produce either carbohydrates or oils, not both, without decrease in productivity. (Wild-type strains can produce high levels of

oils or carbohydrates, but only when nutrient-limited and with reduced productivities). High protein content is typical for wild-type strains; therefore, protein fermentations are included to cover all options, including fermentation of residual biomass (after extraction of oil or fermentation of carbohydrates).

The reclaimed (secondary treated) wastewaters used in the pond cultivation studies have all the required nutrients for maximum growth, as already demonstrated by prior work by the Cal Poly-MicroBio Engineering team.

In response to the question on how much of the yield target is accomplished from heterotrophic growth versus photosynthetic growth, the objective of the mixotrophic growth is to minimize actual heterotrophic growth, at least by the non-algal actors.

The PNNL team is highly qualified and suffices to cover upgrading oils to make them suitable as a refinery feedstock.

ADVANCING COMMERCIALIZATION OF ALGAL BIOFUELS THROUGH INCREASED BIOMASS PRODUCTIVITY AND TECHNICAL INTEGRATION

(WBS #: 1.3.5.249)

Recipient:	Cellana LLC
Principal Investigator:	David Anton
Project Dates:	7/18/2014–1/30/2015
Project Category:	Sun-setting
Project Type:	FY 2013—Advancements in Algal Biomass Yield (ABY1): DE-FOA-0000811
Total DOE Funding:	$3,500,000

Project Description

Using top-performing algal Nannochloropsis sp. strains, sustained improvements in biomass productivity and lipid yield were explored throughout the different unit operations at Cellana. The goal was to integrate approaches representing the "best of the best" results achieved in three primary areas: strain improvement, cultivation, and downstream dewatering. Improvements leveraged from work across all sites in this team yielded algal biomass with a lipid content of more than 40% and culture productivity of 14 g/m^2/day, sufficient to enable yields consistent with BETO's advanced biofuel intermediate goal of 2,500 gallons per acre per year by 2018. In addition, this project demonstrated the use of recycled flue gas as a CO_2 source without compromise in productivity. Improved energy efficiency was achieved through process changes in the dewatering step, and supercritical CO_2 extraction proved to capture more than 80% of the high-value Omega 3's from the neutral lipid fraction. Challenges included replication of indoor cultivation results at one site to outdoor production at another, as well as translation of productivity and compositional results from the 1,000 L outdoor scale

Weighted Project Score: 7.4

Weighting: Approach–25%, Relevance–25%, and Accomplishments and Progress–50%.

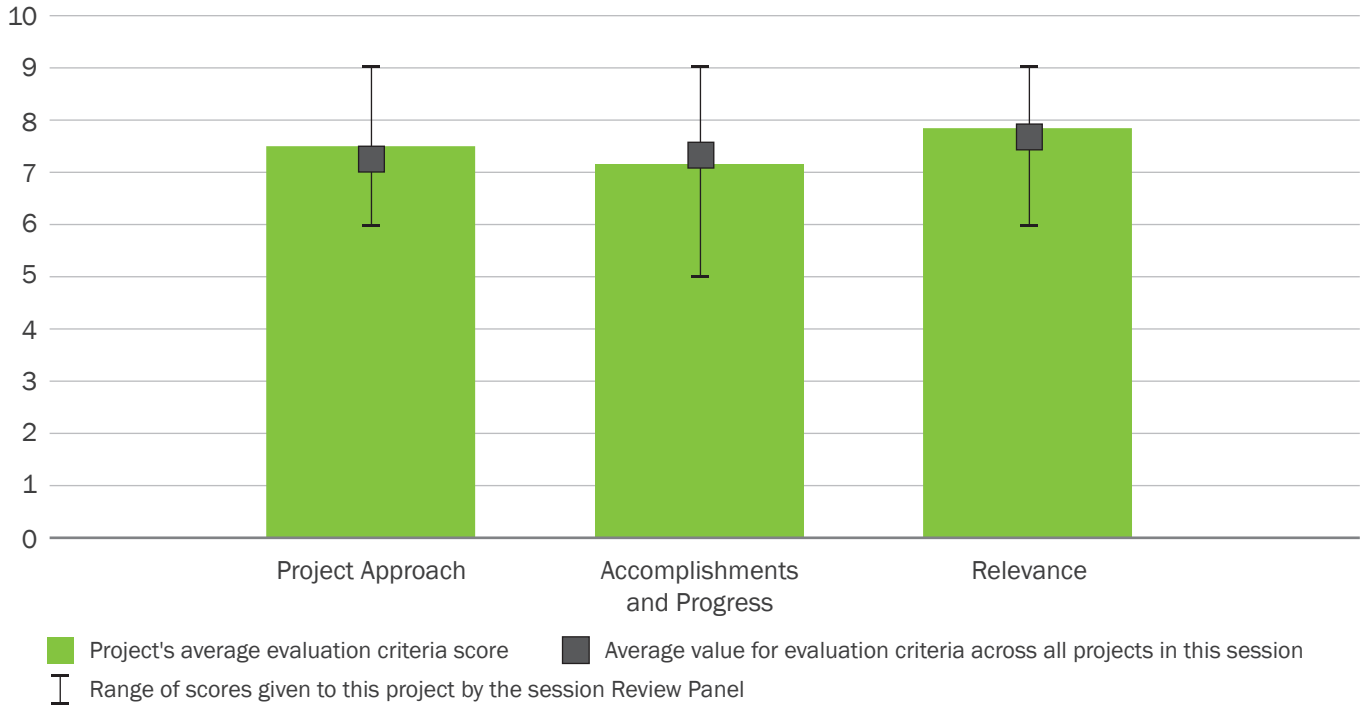


Photo Courtesy of Cellana LLC

to the 60,000 L scale. Project results confirm the techno-economics to support commercial-scale production of algae-based energy products and nutritional co-products, representing a highly sustainable source for oils, proteins, and Omega-3s that can be produced in the United States.

Overall Impressions

- Great project. The trajectory is promising to increase productivity further. I appreciate the full integration and extended testing. Additional work on scaling up further would be necessary to understand if the results can be replicated at larger facilities.
- It appears that productivity improvements have been made that enable the MYPP targets; however, enough detail is lacking that it is difficult to make a thorough evaluation. Details and lessons learned from this project should continue to be disseminated through peer reviewed publications.
- This project shows significant accomplishments that show the viability of using strains that have commercial value in the high-value product space that may also have an impact on the need for algal biomass for fuel production.
- Achieving sustainable high productivity is still a challenge, but addressing it by batch production may be part of the answer.
- The project reported incremental progress toward specific focus areas, including meeting project goals in all three priority areas. None of the technical accomplishments stand out as particularly important. This project considered the applications of the expected outputs, and worked to scale up, making it more relevant.
- The project appeared to meet many stated technical targets, including strain improvements, productivity improvements, dewatering energy use, and conversion yields. The project goals and accomplishments are clearly in line with BETO MYPP goals for areal production (2,500 gallons per acre per year by 2018). The project has clear relevance to the algal biofuels industry and involved entities with commercial ambitions tied directly to project results.

PI Response to Reviewer Comments

- The team thanks the Review Panel for its comments and looks forward to future continued and expanded collaboration opportunities to sustain and advance progress toward the MYPP goals, which, in turn, will move the algae industry forward. Sustained DOE support and its recognition of the need for multi-product business models to drive initial economic viability in the commercial development of algal biofuels has been critical to success in this field to date. Further proof of concept at industrially and commercially relevant demonstration scales is required to ensure that additional improvements necessary to this emerging and expanding area are realized. Given the expense and associated timing with work at larger scale, the substantial involvement of DOE, the near real-time feedback from DOE program officers, and their close coordination and oversight of BETO projects truly enable differential flexibility and response to results generated in these projects. Our team is truly grateful for BETO's ongoing support of work on algal biofuel development.

DEVELOPMENT OF ALGAL BIOMASS YIELD IMPROVEMENTS IN AN INTEGRATED PROCESS – PHASE I

(WBS #: 1.3.5.250)

Recipient:	Global Algae Innovations Inc. (GAI)
Principal Investigator:	David Hazlebeck
Project Dates:	10/1/2013–9/30/2016
Project Category:	Sun-setting
Project Type:	FY 2013—Advancements in Algal Biomass Yield (ABY1): DE-FOA-0000811
Total DOE Funding:	$4,996,784

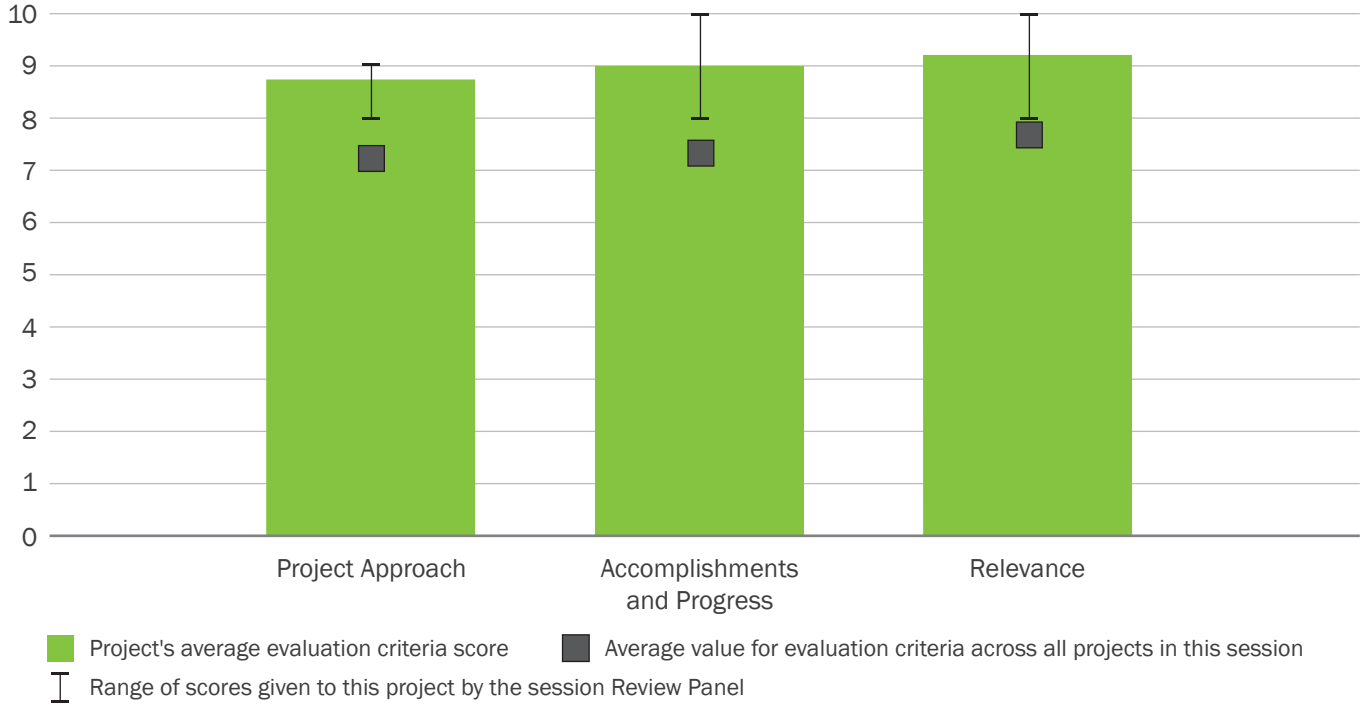
Project Description

GAI collaborated with the Scripps Institution of Oceanography at the University of California, San Diego; TSD Management; GE Power & Water; Kuehnle AgroSystems; and other organizations to increase algae oil yield and optimize unit operations to create a clear path for successful commercial development of algal-derived, drop-in fuels. The project goals include (1) developing improved strains and cultivation methods for a 40% increase in algal biofuel intermediate yield, (2) developing new harvest and dewatering technology for an 88% reduction in downstream-processing energy, and (3) achieving these improvements in an integrated outdoor system that reduces the projected algae biomass production cost by 58%.

All project objectives were exceeded, two major breakthroughs were achieved, and the project was completed on schedule and within budget. The first breakthrough is an improved cultivation system approach that increases algal productivity by 80% in growth phase while simultaneously reducing the energy use for cultivation by 67%. The second is a harvesting and dewatering technology that reduces energy use by 95%–99%, achieves 100%

Weighted Project Score: 9.0

Weighting: Approach–25%, Relevance–25%, and Accomplishments and Progress–50%.



harvest efficiency with crystal clear permeate, and produces a 15%–20% algae slurry. This technology is now available to the algae industry commercially as the Zobi Harvester®. These revolutionary technologies enabled the project to exceed the BETO MYPP 2020 target of 3,700 gallons per acre per year and exceed the 2022 targets for energy use and production cost.

Overall Impressions

- Excellent project. Well thought out and demonstrated at a meaningful scale. The approaches are innovative and transformative and have achieved future goals of the MYPP. The dewatering system development is relevant to the industry as units have already been sold. It will be useful to find out the performance of the dewatering system once it has been in operation at other, more extreme environments (higher loading of contaminates and agricultural residue) and how well the productivity gains translate to other environments.
- GAI has presented a very strong package of improvements throughout an integrated and proven process. Lack of detail requires us to take some aspects on faith.
- Overall, this project exhibited a solid technical approach in achieving stated goals and deliverables. Innovative ideas for productivity improvement through improved process improvement.
- This project accomplished its goals in a fully integrated industrial environment. Accomplishments in productivity improvement and low-cost dewatering are particularly notable. Productivity enhancements were made primarily through improvements in cultivation strategies and operations rather than strain improvements. Project results are highly relevant to industry, as well as BETO's MYPP goals.

PI Response to Reviewer Comments

- The revolutionary improvements in cultivation and harvesting achieved in this project have brought economical algae biofuel production within reach. Support for longer-term operations at scales of 1 acre or more to solidify process consistency and product quality could lead to a commercially viable algae commodities industry. An algae industry operating with the high agricultural productivity demonstrated in this project, which is 50-fold over terrestrial crops, would be transformational in terms of creating high-quality, rural jobs and increasing our standard of living.

PRODUCTION OF BIOCRUDE IN AN ADVANCED PHOTOBIOREACTOR-BASED BIOREFINERY

(WBS #: 1.3.5.260)

Recipient:	Algenol
Principal Investigator:	Ronald Chance
Project Dates:	10/1/2016–9/30/2019
Project Category:	New
Project Type:	FY 2016—Advancements in Algal Biomass Yield Phase 2 (ABY2): DE-FOA-0001471
Total DOE Funding:	$5,000,000

Project Description

Algenol Biotech, NREL, Georgia Institute of Technology, and Reliance Industries Limited have formed a team to advance the state of the art in algal production and downstream processing with the end goal of a sustainable, economically viable biofuel intermediate product. The project includes examining the production of high-value co-products as a market entry strategy and to enhance the economics of a biorefinery for biocrude 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 to achieve a biofuel intermediate productivity of greater than 4,000 gallons per acre per year on an annualized basis; pilot energy efficient innovations in biomass harvesting, dewatering, and HTL to deliver an energy expenditure less than 10% of the energy content in biofuel intermediate and an overall greater than 60% carbon footprint reduction compared to fossil sources; and deliver a comprehensive TEA that identifies limiting factors for commercial viability for a PBR-based biofuel product.

Weighted Project Score: 8.0

Weighting: Approach–25%, Relevance–25%, and Future Work–50%.

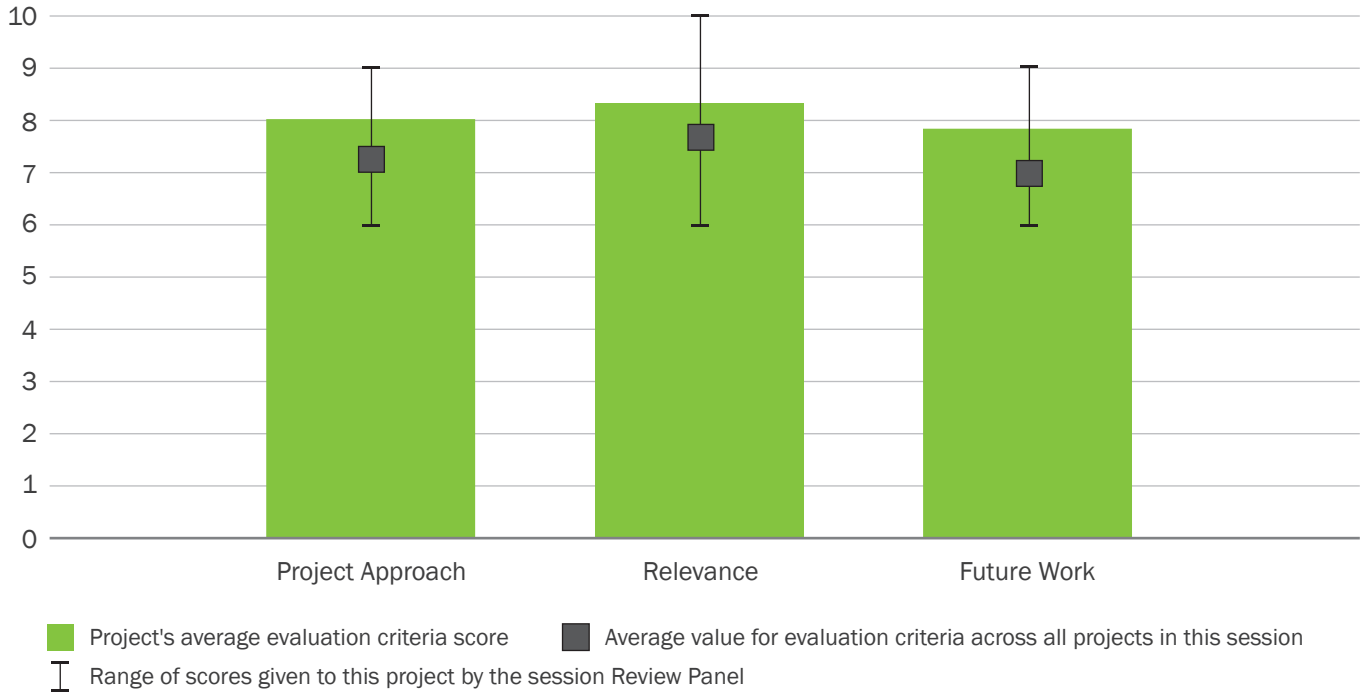


Photo Courtesy of Algenol

Overall Impressions

- This is a very strong project that should provide reliable information on costs and productivity for some unique algal strains. The presentation contained good and relevant details for their intended technical approach. This project has the potential to set the SOT for PBR in terms of both economics and lower-cost biomass production.
- This team is well poised to explore the potential for PBR-based biofuel production through a combination of strain and process improvements. Few details are provided, but opportunities throughout the pathway are being considered. A systematic TEA comparison with open ponds will be of particular value. The chosen co-product strategy is excellent and may enable fairly rapid commercialization.
- Overall, this project is well laid out and clearly has objectives that meet the current program's goals and deliverables. Generally, large-scale PBRs for fuel production are not able to scale up due to large capital and operational expenses. Due diligence should be performed in analyzing capital and operational cost when compared to large-scale open-pond systems. Phycocyanin market analysis should be done in order to better understand the market pricing, as well as the potential impact of new input streams.
- It is important to include PBR production of biomass in the overall evaluation of potential biomass production from algae, and having a detailed comparison between PBRs and open-pond cultivation will give direction to development and improvement of PBR systems.
- The plan forward is polished and organized; however, it is somewhat short on detail regarding specific strategies, making this project somewhat opaque. An advantage of this project is the aim and ability to improve multiple aspects of fuel production from algae in a highly integrated way. It's anticipated that this effort will lead to advances in technology that will directly benefit industry and lead toward more cost-effective production of algal biomass for biofuels.
- This new project presented a clear work plan aligned with original project objectives and, if successful, could meet a number of BETO MYPP goals. The project has clear relevance to the bioenergy industry, uses co-products to drive down the cost of fuels, and involves team members that could pull the technology through to commercialization if the project meets its stated goals.

PI Response to Reviewer Comments

- As discussed above, Algenol believes that PBR-based technology can compete favorably with open-pond production systems when viewed from a broad and comprehensive perspective. As part of the planned program, a comparison of growth of Algenol's proprietary cyanobacterial strain in ponds versus PBRs will be made, and the results will help to further refine our existing, highly detailed techno-economic model. We have already shared our TEA analysis with NREL in a PBR-pond comparison exercise separate from the project under discussion. Their independent work showed PBRs and open ponds to be much closer in costs than previously thought, mainly due to the advancements we have made in PBR manufacturing combined with the productivity enhancement that PBR operation enables.

In support of the selection of phycocyanin as the leading co-product candidate, we have performed a detailed assessment of the existing phycocyanin market along with anticipated future growth trends. This analysis has supported our conclusion that phycocyanin represents an excellent co-product opportunity, which was further validated through discussions with several potential customers and partners, who have provided detailed guidance on phycocyanin pricing and quality requirements.

PRODUCING ALGAE FOR CO-PRODUCTS AND ENERGY (PACE)

(WBS #: 1.3.5.300)

Project Description

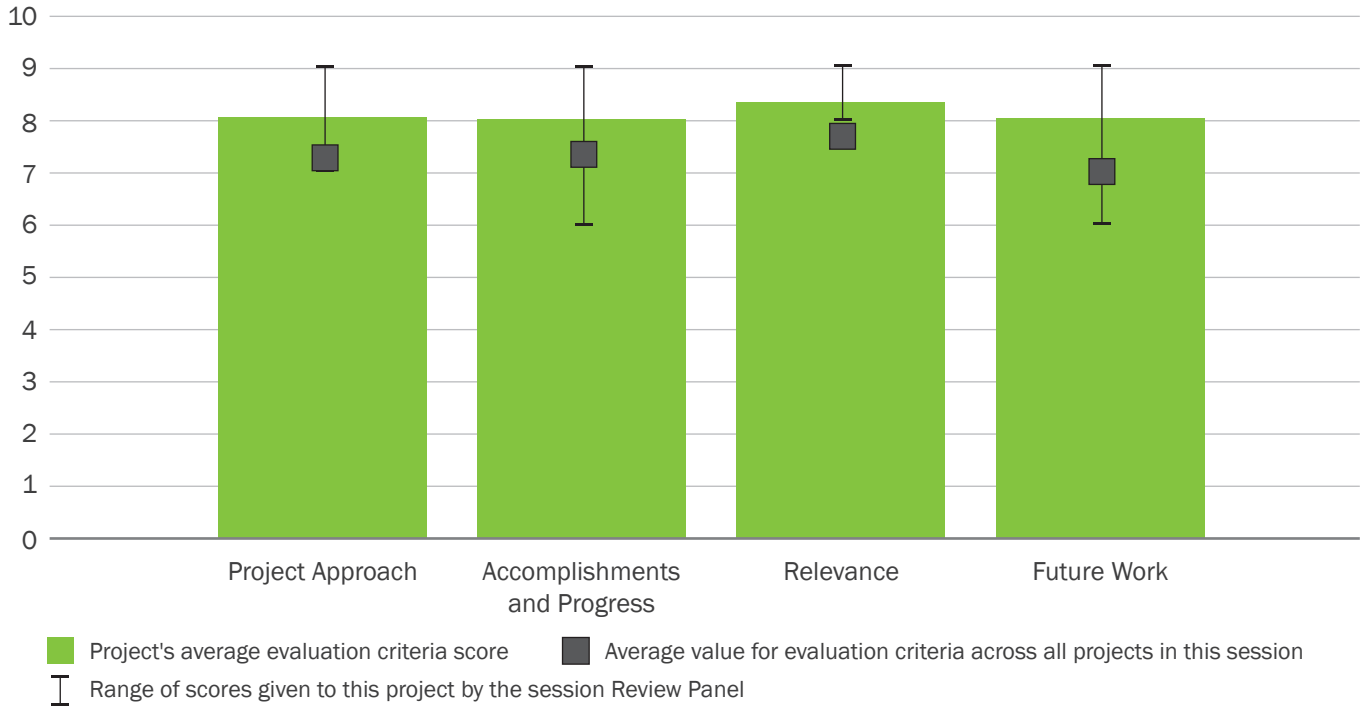
Building off the most advanced algal biofuels systems designed to date, PACE is developing and integrating emerging and leading-edge technologies to (1) produce algal biofuel at less than $5/gge; (2) achieve an energy return on investment greater than 8; (3) achieve a carbon index less than 40 g CO_2 per millijoule; and (4) recover and recycle more than 80% nutrients and CO_2 while reducing water use by 50%. These objectives will be achieved by (1) improving sustainable biomass yield greater than two-fold; (2) producing co-products to offset fuel costs by 30%–50%; and (3) optimizing integrated processes through LCA/TEA studies. PACE uniquely addresses each of BETO's MYPP objectives for algal biofuels.

Recipient:	Colorado School of Mines
Principal Investigator:	Richard Sayre
Project Dates:	10/1/2015–3/31/2019
Project Category:	Ongoing
Project Type:	FY 2015—Targeted Algae Biofuels and Bioproducts (TABB): DE-FOA-0001162
Total DOE Funding:	$9,000,000

Major challenges include (1) developing robust genetic transformation and genome editing tools for C. sorokiniana, (2) producing and recovering valuable co-products from biocrude, and (3) developing energy efficient algal harvesting systems that are scalable. To date, PACE has developed robust genetic engineering and genome editing protocols for C. sorokiniana; demonstrated cultivation of algae in 80% seawater at normal growth rates; achieved substantial progress on improving acoustic harvester efficiency; chemically converted algal carbohydrates to fuel

Weighted Project Score: 8.1

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



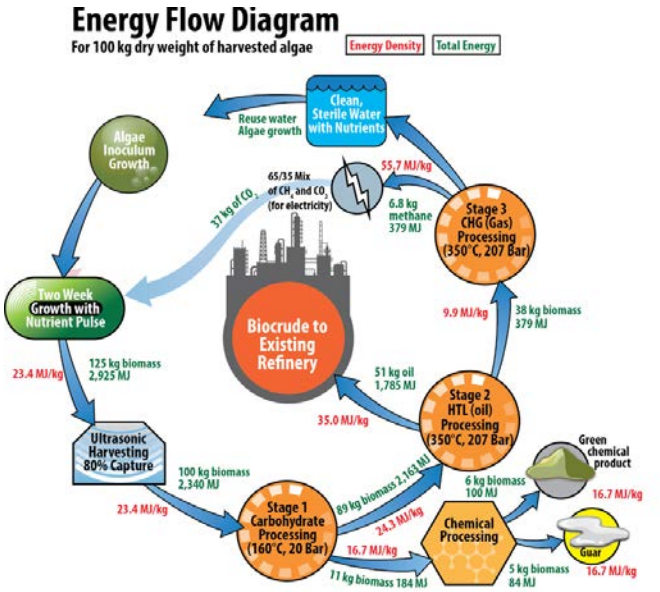


Photo Courtesy of Colorado School of Mines

and co-products; developed a continuous flow, two-stage HTL process to separate carbohydrates from the oil fraction—partner Reliance Industries has established the first fully integrated algal biofuels system at Gagwa, India; and carried out extensive LCA/TEA modeling to identify process bottle necks and constraints.

Overall Impressions

- This project team is a collection of leaders in their fields brought together to address the project's technical tasks. I suggest the project consider how a commercial facility producing these products (or any types of products) impacts the market price and how this would impact their cost evaluation. I think the TEA of this project would benefit greatly from including a reputable member of industry practiced in upgrading oils to make them suitable as a refinery feedstock. The industry partner should advise on the cost of upgrading (including the removal of metals) the different oils generated by different HTL operating conditions to make them acceptable to a refiner and at what discount the refiner would want in order to take the oils.
- This is a massive project exploring many promising strain and process enhancements. The specific approaches appear, in general, to be well considered. The choice of co-products is questionable, particularly the PEA product, which has a tiny market. Integration of all project improvements will be challenging, particularly in the stacking of multiple traits of interest. It will be critical for the investigators to be flexible as the project evolves and not be locked into a particular technology path.
- Overall, this project shows significant promise in the ability to provide data and improvements essential to meeting the program's goals and deliverables.
- The project demonstrates several key improvements in crop stability and crop protection via genetic engineering, and the suicide gene system development will be critical as well. There are a lot of tasks in this project (full process from cultivation to extraction/co-product production), and it seems well executed so far.
- This large consortium is clearly organized into three main project areas that are aimed at reducing the overall cost of producing biofuel from algae. Within these three main areas, there is a diversity of more specifically focused projects leveraging expertise and infrastructure developed from previously funded efforts, making this a valuable continued investment.
- The project is clearly aligned with BETO MYPP goals for genetic advances, increasing productivity, and using co-products to enable competitively priced fuels. If successful, the project should have broad impact on the algal biofuels industry by demonstrating utilization of the genetics toolbox to improve system economics for fuels and co-products. Timelines related to production of the improved organism at a reasonable scale for downstream processing work may present a challenge, but the project team is aware of these challenges and will work to mitigate them to the extent that they are able.

PI Response to Reviewer Comments

- We thank the reviewers again for their comments. We have a very reputable full-scale algal production, harvesting, fuel conversion, and refinery operations partner in Reliance Industries Limited. An additional relevant partner is Genefuel Corporation, with experience in commercial-scale HTL. Additional academic input comes from the energy conversion group at Colorado State University. These and other partners' inputs are integral to the TEA development planned for Budget Period 3. By continuously integrating LCA/TEA analyses throughout the project to meet milestones and deliverables, we are confident that we can address challenges to fully integrate operations.

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

(WBS #: 1.3.5.310)

Recipient:	Duke University
Principal Investigator:	Zackary Johnson
Project Dates:	10/1/2015–8/31/2019
Project Category:	Ongoing
Project Type:	FY 2015—Targeted Algae Biofuels and Bioproducts (TABB): DE-FOA-0001162
Total DOE Funding:	$5,240,313

Project Description

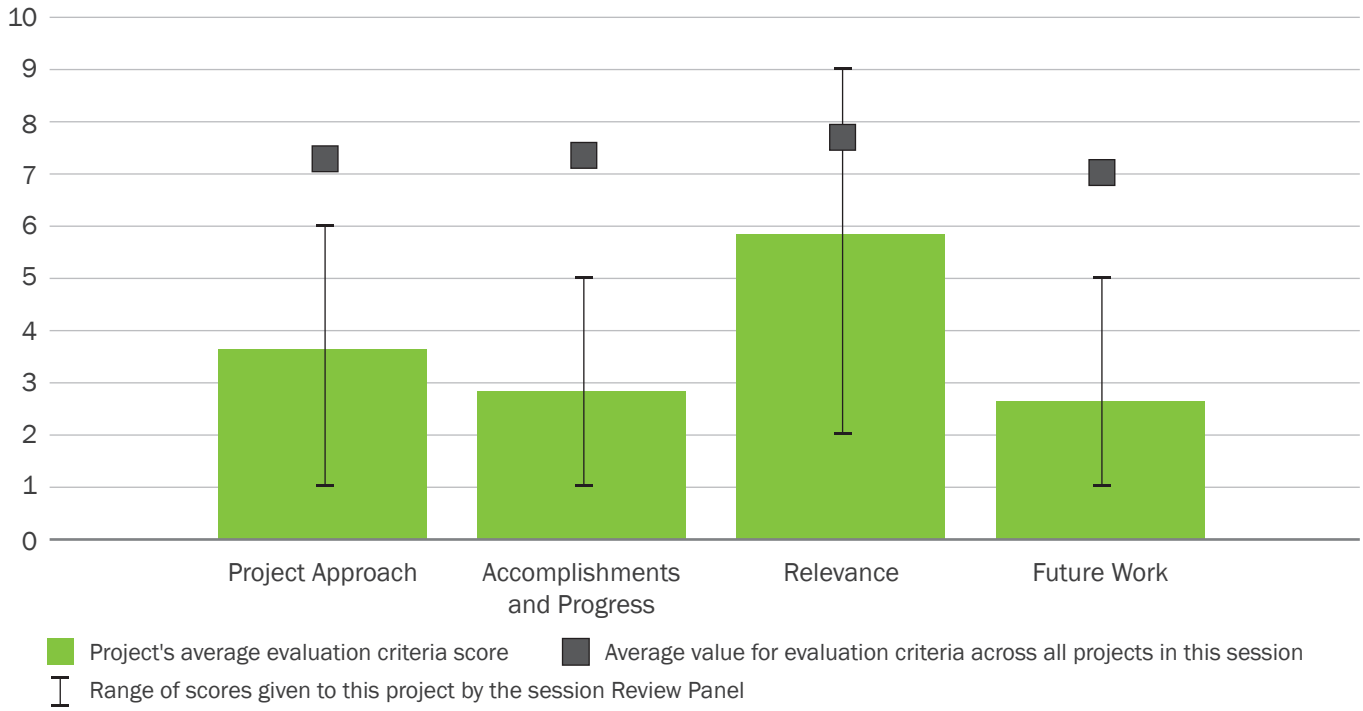
Not provided by project team.

Overall Impressions

- This project team is composed of many experienced individuals and companies. However, it seems this project is disjointed and in many separate areas that may make this project difficult to manage and execute. It also appears that the technical plans are not clear and potentially in flux. This project has been struggling with funding. Minimal information was given on the future work details, and I am concerned about the trajectory of this project.
- The presentation for this project was devoid of any substantive information, making it impossible to make a fair evaluation. The project's underlying approach and work plan may be excellent, but the reviewers were unable to score it well based on what was presented. The relevance to BETO's goals appears to be on target based on the focus on marine species and adding value through co-products.

Weighted Project Score: 3.8

Weighting: For ongoing projects, there is equal weighting across all four evaluation criteria: Approach, Relevance, Accomplishments and Progress, and Future Work.



- Overall, this project seems to have potential, but current issues with starting certain aspects of the project, as well as stated goals seeming to be off of the proposed goals, may create irrelevant data or information.
- Not enough details were given to properly evaluate this project. No work was presented, and no details of the work plan were presented.
- Unfortunately, neither the approach nor future work of this project was presented during the Project Peer Review, making it impossible to evaluate these aspects of the work. The only technical accomplishments reported were a list of publications, and it could not be determined which (if any) of these publications directly resulted from work conducted as a part of this project. The overall impression that was given was that the actual project was not well represented by the presentation given.
- The presentation for this project was not responsive to most review requirements. For this reason, it is difficult to determine the relevance of the project to BETO goals or whether the work plan (not discussed) is appropriate for project goals (also not covered).

PI Response to Reviewer Comments

- The MAGIC team thanks the reviewers and DOE for their time and comments on the presentation. Within the minimum space allotted, we have provided additional details that we hope will help with the evaluation process.

  It is important to note that this peer review of our project was effectively conducted prior to the full group receiving funding, and so the progress made to date only represents a small fraction of what we expect in the future. Nevertheless, the progress documented in this response, as well as the Project Peer Review presentation, represents work toward this project's goal and does not come from past projects. As documented with previous evaluation criteria, with the successful completion of the validation process and the recent (days before the Project Peer Review meeting) release of funds, the work on this project has just started. The original proposal; validation process, including modified statement of project objectives with revised milestones and decision points (go/no-go); and this peer review all provide us with the solid framework for completing this future work.

  We welcome the opportunity to answer any other questions or provide additional clarification.