ADVANCED ALGAL SYSTEMS PROGRAM

TECHNOLOGY AREA

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INTRODUCTION

The Advanced Algal Systems Technology Area is one of 12 technology areas that were reviewed during the 2021 Bioenergy Technologies Office (BETO) Project Peer Review, which took place virtually March 8–12, 15–16, and 22–26, 2021. A total of 46 presentations were reviewed in the Advanced Algal Systems session by eight external experts from industry, academia, and other government agencies. For information about the structure, strategy, and implementation of the Technology Area and its relation to BETO's overall mission, please refer the corresponding Program and Technology Area Overview presentation slide decks, which can be accessed here: https://www.energy.gov/eere/bioenergy/2021-project-peer-review-advanced-algal-systems-program.

This review addressed a total U.S. Department of Energy (DOE) investment value of approximately $107.4 million, which represents approximately 16% of the BETO portfolio reviewed during the 2021 Peer Review. During the Project Peer Review meeting, the presenter for each project was given 25 minutes to deliver a presentation and respond to questions from the Review Panel.

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

BETO designated Christy Sterner as the Advanced Algal Systems Technology Area Review Lead, with support from Jamie Meadows (American Association for the Advancement of Science [AAAS] Science & Technology Policy Fellow). In this capacity, Christy Sterner was responsible for all aspects of review planning and implementation.

ADVANCED ALGAL SYSTEMS REVIEW PANEL

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ADVANCED ALGAL SYSTEMS REVIEW PANEL SUMMARY REPORT

Prepared by the Advanced Algal Systems Review Panel

INTRODUCTION

The entirety of the 2021 BETO Peer Review of the Advanced Algal Systems (AAS) served to reconfirm the U.S. Department of Energy's standing as a global leader in its commitment to use algae as a basic platform to address critical societal challenges. The projects reviewed reflect the workstreams and technology development efforts needed to develop sustainable algal feedstocks and conversion processes in service of producing renewable fuels, bioproducts, and chemical intermediates. While the overall goals remain ambitious and challenging, the status of the projects showcase a steady and compounding progress that results in our continued, confident, and optimistic view for the technology area and its future successes.

The AAS Peer Review Team reviewed a portfolio of 46 projects as part of the 2021 BETO Project Peer Review. The reviews were conducted by two panels. Panel A reviews were undertaken on March 9 for Strain Development Projects (WBS 1.3.1), March 10 for Strain Development and Systems Integration Projects (WBS 1.3.5), and March 11 for Systems Integration Projects. Panel B reviews were undertaken on March 22 and 23 for Cultivation System Improvement Projects (WBS 1.3.2), and on March 24 for Cultivation System Improvements, Logistics (WBS 1.3.3), and Conversion Interface (1.3.4).

The Peer Reviewers were able to access project materials online via the Peer Review Tool beginning on February 22, 2021, for their review and preparation prior to the formal Peer Review. The reviewers were subsequently active participants in the individual project presentations and the follow-up question-and-answer periods. After each day of presentations, the Peer Review Team and the AAS Team met to debrief and engage in discussion regarding the daily session.

The Peer Reviewers provided comments and scores for each individual project using the Peer Review Tool. All reviews and scoring were completed by April 1, and the scoring was in accord with the assigned DOE Project Scoring Rubric, with scores ranging from 1 (Unsatisfactory) to 5 (Outstanding).

A follow-up video meeting was held on May 14 by the Peer Review Team to discuss general and specific topics and to gather input for this summary report. An initial draft of the summary report was circulated among the team members to provide an opportunity for comments and review ahead of its issuance in final form.

As otherwise expected, this summary does not necessarily reflect a uniformity of opinion by all of the reviewers. Where appropriate, the important differences are reflected herein. The inherent value provided by differing professional backgrounds, training, experiences, perspectives, and expertise of each individual reviewer serves to inform the review as a whole.

It is important to emphasize that the materials reviewed and the corresponding project presentations were all limited in presentation time, the question-and-answer period, and especially in the scope of the entire endeavor to solely reflect publicly appropriate data and technology disclosures. These combined conditions limit the public dialogue and the natural curiosity regarding confidential project data, commercial sensitivities, intellectual property, and other similar related matters. Deference is needed in these regard to BETO and AAS's overall responsibilities that are part of the standing mechanisms of active management for the portfolio and all individual projects. These formal efforts directly address procedural and contractual reviews, verification processes, milestone reviews, decision points, independent reviews, stakeholder engagement, and other similarly important elements.

Regardless of these limitations, the materials, accomplishments, and achievements presented during the Peer Review collectively report a very healthy rate of success by any measurable scientific, technical, or business metric. The portfolio represents a very successful return on investment for BETO and AAS as they continue to serve as the thought and investment leaders for this important and promising component for the future economy.

STRATEGY

1. The Advanced Algal Systems technology area has a very well-defined strategy regarding mission, goals, and sets of technical targets.

   AAS has, by virtually every measure, become the de facto thought and investment leader in this space. It is the primary sponsor for much of the basic research; drives the applications of learnings; has developed and defined all the important metrics and measures; has established all the important short-, medium-, and long-term goals in service of commercialization; provides the line(s) of sight needed to achieve success; and supports the development of virtually all tools and techniques of relevance.

   AAS continues to refine, expand, and adjust its efforts and portfolio to reflect learnings, new discoveries, and uncovered opportunities. AAS remains practically focused and responsive to feedback, commercial interests, and new opportunities. The efforts both in detail and in the aggregate are intended to attract and develop commercial interests and investment in service of developing an entire industry to deliver renewable bioenergy and profitable coproducts from the algae platform.

2. AAS has been able to qualitatively evaluate, set parameters, identify process integration needs, and highlight specific areas and priorities needed for improvement to achieve the defined targets and metrics necessary to advance concepts and opportunities into viable forms of renewable bioenergy and related coproducts.

   Industry and stakeholders often repackage their own needs and interests in terms of AAS goals and parameters, but often with vague, tangent, or oblique application to bioenergy end products. Much of the laboratory, industry, and stakeholder community place their emphasis on scientific inquiry, products, or coproducts that in some instances are not of tangible bioenergy commercial interest. Additionally, some other individual stakeholder's view of the governing dynamics or larger circumstances often results in default outcomes where despite good project progress, future opportunities for next steps in deployment or scale do not seem viable.

   While remaining respectful of the underlying integrity and obligatory self-interest of all the awardees, we can illustrate these dynamics using three of the projects reviewed. The Lumen Bioscience project (1.3.2.601) as a part of the Productivity Enhanced Algae and Tool-Kits (PEAK) portfolio openly represents that their work is in service of the company's commercial ambitions and are not intended to produce any energy products, but rather their technology advances may at some point be useful to other interests in pursuit of energy products or coproducts. Separately, the MicroBio Engineering projects (1.3.5.243 and 1.3.2.600) present important information and report good progress that can directly support bioenergy products and coproducts, but they also readily acknowledge the difficulties in pursuing larger scales and attracting industry investment.

   It is noteworthy to highlight a project that does appear to be tracking toward meaningful commercial success. The UC San Diego project (1.3.2.650) presented the advancement of its efforts to a stage where coproducts are nearing a point where outside commercial partner interests and associated investment potentials seem quite viable. This project may serve as a success example and possible template for other coproduct or first product applications.

3. The Peer Review Team generally believes that the AAS approach and portfolio are very well designed, balanced, and managed. The staggered nature, informed decision-making, and aggregating benefits are reflected in the program structure, the national laboratory efforts, and the ongoing funding opportunity announcement (FOA) award. Adjustments in details, emphasis, priorities, and topics are made in real

time and reflect prior learning, and the lessons from earlier efforts are incorporated into AAS's next endeavors.

We believe that AAS is at a point in the program where they are in a good position to make thoughtful and appropriate upselections, downselections, augments, and other modifications to the technical areas that reflect their direct and comprehensive insights at project, topic, and program levels.

Based upon the public reviews, we highlight the need to concentrate additional efforts targeted toward some standardization, repeatability, and scale, along with the ability to share learnings and finally create a broader opportunity for the application of outdoor cultivation using genetically modified strains of algae.

The portfolio has been advanced to a point where we believe it is both possible and appropriate to consider the standardization of some baseline techniques, measurements, equipment, and experiment durations. Examples include measuring algal productivity to be more comprehensive than just a single parameter that has inherent limits in translatability; adoption of standards for laboratory photobioreactors to allow meaningful comparisons of results across the range of projects in the portfolio; adoption of duration and seasonal standards for experiments used to establish any measured or performance value; and further alignment and definition of input parameters and methods of analysis used for techno-economic analysis (TEA) and life cycle assessment (LCA).

We believe there may be an opportunity to review the portfolio for possible redundancies and overlaps, as well as to concentrate efforts to monitor or otherwise gauge the repeatability or expected repeatability of experimental results across the projects.

BETO and the AAS Team are well aware of the challenges faced by the portfolio and the industry as a whole regarding the ability to scale any and all of the individual components, as well as the larger integration of unit operations. This remains a serious obstacle for larger adoption and commercial opportunities. We believe that it is important to implement a disciplined and systematic program to establish accurate scaling methodologies, techniques, validations, calibrations, and sensitivities that can be of great service to the industry, and that BETO is uniquely poised and qualified to undertake and support such an effort.

While appreciating all the issues associated with coordinating and sharing data across projects, as well as the challenges and practical legal limits whenever technology, commercial interests, and intellectual property are involved, we feel it is important to always attempt to improve, however possible, the ability to share information and learning between projects and interested parties. Formalized group workshops and encouraging the use of mutual and reciprocal nondisclosure agreements between projects may be approaches that can improve the current condition.

It was very encouraging that a few of the projects had efforts incorporated to run some outdoor experiments using genetically modified algae. We understand the difficulties associated with these types of endeavors and applaud those projects, their principal investigators (PIs), and the AAS Team for the leadership in this area. We believe this area of work represents a tremendous (although not necessarily immediate) AAS opportunity that as of this date has been a gap in the portfolio. A systematic and structured plan to develop interest, methodologies, facilities, infrastructure, regulatory support, and access to other federal agencies that are well versed in the subject matter could serve as a tremendous resource to advance the AAS technology area.

4. Based on our reviews, we believe that the AAS Team is making very good use of the available funding mechanisms. The program structure, the wide variety of awardees, and the AAS Team's active management of the portfolio have served to achieve strong results.

The eight rounds of FOA procurements have been logically sequenced and structured to address specific known challenges and topics. They reflect previous learning and experience and are designed to make adjustments that reflect the states of knowledge and technology.

The National Laboratories Harmonization, Collaboration, and Conversion and the Analysis Strain Characterization and Improvement efforts have created an overall program framework that will continue to benefit and improve all future endeavors. Most importantly, perhaps, these efforts can help create overall alignment and common standards for the entire program.

Some annual operating plan (AOP) efforts seemed less clear than the FOA programs. The national laboratories remain well poised to undertake direct efforts that can close some gaps and address important AAS challenges. Perhaps the AAS Team can provide an assessment regarding how prior efforts were scoped and identified and then measure the results against the AAS goals. Using this effort as a baseline and using the most current or updated set of AAS goals or gaps, the AAS Team and the national labs can better coordinate any future AOP research efforts to create better alignment and measure of results.

STRATEGY IMPLEMENTATION AND PROGRESS

1. The AAS project portfolio relates very accurately to the area's strategic direction, with a healthy distribution of approaches and investigators that allows for a risk-balanced approach to many critical areas that are recognized as needing improvement to achieve Multi-Year Program Plan (MYPP) goals. Utilization of atmospheric carbon dioxide (CO_2), compounding improvements in growth rates, and projects focused on understanding and improving algae health and crop protection are all reporting significant success and are in direct service of supporting AAS's strategic direction.

   We would like to acknowledge the productive efforts and flexibility of the AAS Team and the individual project teams in navigating the many challenges that resulted from the COVID-19 pandemic. Most projects were able to maintain progress or construct repair schedules going forward to recover end-of-project goals.

   The National Renewable Energy Laboratory's (NREL's) Algae Technology Educational Consortium (1.3.5.201) represents a standout example of a very successful and highly impactful project. The project is developing a nationwide education and workforce training network that will be of service to the nation's future bioeconomy. This project is an outstanding example of the completeness of the portfolio, reflects the promise inherent in AAS, and is confidently premised on future success so as to prepare, engage, and be ready to mobilize a workforce for the future.

2. Similar to success in achieving strategic results, the AAS project portfolio is also supporting and advancing leading-edge work within the field. Notable and meaningful progress is being achieved by the Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVR) project and the national laboratories harmonization efforts integrating resources assessment; productivity modeling; site-specific productivities, yields, and water balances; and TEA and LCA. Similarly, the national laboratory conversion analysis for hydrothermal liquefaction (HTL) and combined algae processing (CAP), including Aspen modeling, provide a stand-alone framework for consistent and accurate independent evaluation for developing baselines, comparing alternatives, strain types, and locations. These tools will also go a long way into providing meaningful insights and conclusions regarding the mass and energy balances of integrated systems and the technical and economic viability of fuels and companion coproducts.

3. Based on the materials that were publicly presented, growth rates, CO_2 utilization, harvesting, and crop protection criteria are approaching targeted goals. Larger-scale integration and commercial applications for bioenergy and coproducts do not yet seem entirely viable and continue to remain elusive. There does

not seem to be any meaningful commercial interest for investment at hand or being lined up or explored. A coproduct or first coproduct (footwear) does seem viable in the near term and is attracting commercial interest and support. The Peer Review Team notes a wide variety of methodologies and assumptions being used for the LCA/TEA among many of the projects, with corresponding representations that most project goals are being achieved. We can only comment that despite these and the otherwise overwhelming declarations of individual project success, the apparent inability to attract outside commercial interest or partnership is unfortunate and disappointing.

4. The public presentations advertise strong success and achievement of goals. We might note some small degree of redundancy, as well as the completion of some long-term projects, where the original basis of the work has been superseded by more recent undertakings and developments. These are both reasonable and expected outcomes of a multiyear, multiportfolio assembled program. Regardless, the uniform view is that the AAS Team is actively managing the projects and the entire portfolio in an effective and productive fashion that ensures good stewardship of the committed resources and drives for the best possible outcomes for the project performers and the government.

   Lastly, we note that in a few instances, the reviewers had difficulty determining how much actual progress some projects had made since the previous Peer Review.

RECOMMENDATIONS

1. Institute a project (or subprogram) to address the translational gaps between lab and field results that currently inhibit the development of commercial opportunities.

   A. Ensure that laboratory-scale measurements for growth rates or any other performance characteristic can be accurately translated as scales increase and locations vary.

   B. The project or subprogram should also consider the scaling of all unit operations that are relevant to converting algae to a product, establish reliable methodologies to accurately translate lab to field results, and ensure that the results are appropriate for the scaling needed to achieve commercial-scale and viable production volumes and economics.

2. Augment and expand the current national lab analysis and modeling efforts (such as the LCA, microalgae, and TEA analysis projects; thermochemical interface project; algal feedstocks logistics and handling project; HTL model development; and DISCOVR projects) to establish nationwide platforms to model and evaluate the overall performance, required inputs and resources, environmental impacts, and economic viabilities of proposed strains, individual geographic locations, technologies, and products.

   A. Predict strain growth rates geographically.

   B. Establish mass balances for processes being proposed, developed, or considered.

   C. Ensure standardization of metrics and assumptions so all suitable projects in the portfolio can be evaluated using these as reliable diagnostic tools.

   D. Consider requiring all suitable projects to submit key data that can be used by these tools to independently assess key performance criteria and metrics separate of the project participants own efforts—especially with regard to strain, site selection, TEA, LCA, and mass and energy balances.

3. Ensure that entities that receive funds from BETO are advancing the algae biofuels industry and are addressing AAS programmatic goals. For research scientists (academics and national lab researchers), ensure that all research projects are working toward addressing AAS program goals and not simply pursuing science for its own sake. For industry partners, include a stipulation in FOAs requiring they pursue support, interest, and possible funding from outside sources other than BETO. The final project

goals should require documentation of efforts to attract outside interest that may result in additional investment or some form of commercial support. The purpose of both of these refinements is to incorporate additional incentives for the performers and to deliver additional tangible project outcomes that can be used by AAS in subsequent upselection and downselection decisions.

ADVANCED ALGAL SYSTEMS PROGRAMMATIC RESPONSE

INTRODUCTION

The AAS Program Team thanks the reviewers for their detailed review and assessment of the AAS portfolio of projects, as well as of the program overall. We appreciate the acknowledgement that while the goals are ambitious and challenging, steady, continuous progress is being made toward a viable and sustainable algae industry.

As noted, the Peer Review is the culmination of independent reviews of a minimum of 80% of the program's project portfolio, as well as the program's strategy, implementation of that strategy, and the progress made toward the goals defined within that strategy. The Peer Review meetings are open to the public, limited in time (in order to include a detailed program overview and as many project presentations as possible), all materials are developed for public disclosure, and all discussions are limited to publicly available information. BETO and the AAS Team recognize reviewers' and participating parties' natural desire for more details and data related to the specific technologies and processes presented throughout Peer Review. Consequently, the AAS Team greatly appreciates the reviewers' acknowledgement of this and their efforts to limit their reviews and comments to reflect the information as it was provided in the presentations, question-and-answer sessions, and any additional discussions. Furthermore, we cannot stress enough our appreciation for the importance and significance of the reviewers' following statements, "Regardless of these limitations, the materials, accomplishments, and achievements presented during the Peer Review collectively report a very healthy rate of success by any measurable scientific, technical, or business metric. The portfolio represents a very successful return on investment for BETO and AAS as they continue to serve as the thought and investment leaders for this important and promising component for the future economy." We work very closely with our stakeholder community—academia, national laboratories, and industry—to identify and support the areas of research and development necessary to make the greatest impact on advancing the algal biofuels and bioproducts industry. Input from our stakeholder community through multiple avenues such as Peer Review, workshops, requests for information, conferences, individual project reviews, and meetings between our staff and stakeholders is vital to our collective and continued success as an industry.

The AAS Program's multiyear strategy incorporates the outcomes and lessons learned from the project portfolio, input from stakeholders, literature from industry-relevant scientific journals, and modeling and analyses. As the reviewers note, the strategy is fluid in that we refine the strategy to reflect "learnings, new discoveries, and uncovered opportunities." We appreciate the reviewers' positive comments relative to our strategy, approach, and portfolio. We plan to continue in this fashion, striving to constantly increase stakeholder interaction and input and adjust our strategy to reflect feedback, industry needs, and new opportunities.

The reviewers note that for a handful of projects, the tie to AAS metrics and goals is not clear, and it appears that the research is geared toward their own corporate interests. Our funding opportunity announcements, as well as our annual operating plans for the research at the national labs, are developed to address the research and development (R&D) needs of the industry in support of the overall AAS goals. The AAS Program will continue to align projects with AAS and BETO goals.

While the reviewers indicate strengths of the program's approach and portfolio, they offer some suggestions for efforts going forward such as standardization, repeatability, scaling, sharing learnings, and creating opportunities for outdoor cultivation using genetically modified strains of algae. We agree with the reviewers that standardization relative to some baseline techniques, measurements, equipment, and experiment durations have the potential to further advance the portfolio and the industry. We have efforts underway regarding all of these aspects and will continue to work with our stakeholders and industry partners to proactively address these suggestions. For example, NREL has established standard operating procedures (SOPs) for various experimental techniques utilized in algal research and development. The SOPs are posted on NREL's website. We regularly encourage our projects to utilize these SOPs in their research. The AAS Team welcomes engagement with the industry on measurements and parameters that are or can be applicable and comparable across the many different algae technologies and processes. These are ongoing conversations and have extended beyond the industry stakeholders to include other government offices and agencies such as the Office of Fossil Energy and Carbon Management and U.S. Department of Agriculture.

Scaling and commercialization are the ultimate goals of all of the R&D programs within BETO. As the reviewers note, BETO and the AAS Team are well aware of the challenges faced by the portfolio and industry relative to the ability to scale and integrate unit operations. We appreciate the reviewers' comments about BETO being uniquely poised and qualified to implement a program to establish scaling methodologies, techniques, validations, calibrations, and sensitivities to further scaling and commercial opportunities. BETO has a specific program in place to address scale-up and commercialization, and the AAS Program includes scale-up and commercialization considerations in our strategy and both short- and long-term goals.

The AAS Program recognizes the importance of sharing information and learnings between projects and interested parties. We also agree with the reviewers that workshops and encouraging partnerships and mutual and reciprocal nondisclosure agreements between projects can further these activities. The AAS Program has instituted FOA-specific workshops designed for awardees from those FOAs to share plans, lessons learned, ideas, and results over the course of their projects. For example, the PEAK FOA included a specific workshop for the awardees at the planning stages for the required PEAK challenges in order for the awardees to share ideas, plans, and lessons learned as each prepared their own specific plans for their respective PEAK challenge experiment. The first workshop was very successful. This same approach has been implemented in the latest FOA. The AAS Team includes partnering in all of their funding opportunities and will continue to do so going forward.

Another component of the AAS strategy is to require outdoor testing (or, depending on the particular FOA or AOP request, testing in outdoor-simulated conditions with a plan for future outdoor testing). We recognize the value of iterative testing between indoors and outdoors and working to close the translational gap between the two. Of course, outdoor, real-world environment testing is critical for advancing the organisms and technologies toward scale-up and commercialization. This includes testing of genetically modified organisms. The AAS Program's strain development activities allow for genetically modifying organisms. We do not have any control over permitting for testing outdoors, but we do work closely with our sister agency counterparts that do. Therefore, we understand the timelines and the data and information needed for the permitting. Our FOAs allow the use of genetically modified organisms (GMOs) with the requirement that applicants include the time in their project schedules needed to pursue and receive the proper permitting.

The AAS Team appreciates the reviewers' comments regarding the FOAs and their relevance to the states of knowledge and technology. We base our FOA strategy on identified gaps in the most impactful research and development as noted through lessons learned in our research portfolio, stakeholder input and feedback, modeling and analyses, and assessment of how to reach out-year goals. The reviewers suggested using a comparative analysis of previous AOP scoping efforts and how the results from those efforts feed into and/or meet AAS goals, and then use that analysis as a baseline with the current AAS goals to better align AOP research efforts with those goals. Similar to our FOAs, AOPs are developed with the AAS barriers, metrics, and goals in mind. This includes research from lab-scale bench testing to the outdoor testing within the DISCOVR AOP project. The AAS Team recognizes this may not have been conveyed well during the Peer Review and agree that presenting our approach and rationale for AOP work through the results of an assessment such as the one suggested may make the alignment clearer to stakeholders and future reviewers.

As noted by the reviewers, the AAS Program is not only achieving strategic results, but supporting and advancing leading-edge work within the field. The harmonization efforts bring together resource assessments, productivity modeling, and TEAs/LCAs to provide information and valuable tools to the industry. We appreciate the reviewers' acknowledgement and comments regarding the valuable and long-term impacts of these efforts. They similarly note the progress being made by the DISCOVR project as well as the HTL and combined algae processing efforts. We agree and continue to support and advance these efforts to provide baseline and comparative information for the industry. We are currently planning additional harmonization efforts between all of these activities to strengthen each with even more consistent and validated data, information, and informed detailed assumptions where real data are not currently available. As the data related to those assumptions become available, they are updated accordingly. We also agree that while it is disappointing that outside commercial interest is not at a level this industry needs and that would make a substantial impact, we do believe that continuing to work together with our vast array of stakeholders on these activities, as well as the rest of our portfolio and our plans for future work, commercial interest and commitment can and will ramp up in the short term and be maintained long term as we continue to make progress.

Recommendation 1: Institute a project (or subprogram) to address the translational gaps between lab and field results that currently inhibit the development of commercial opportunities.

A. Ensure that laboratory-scale measurements for growth rates or any other performance characteristic can be accurately translated as scales increase and locations vary.

B. The project or subprogram should also consider the scaling of all unit operations that are relevant to converting algae to a product, establish reliable methodologies to accurately translate lab to field results, and ensure that the results are appropriate for the scaling needed to achieve commercial-scale and viable production volumes and economics.

As noted previously, the AAS Program Team recognizes the need to close the translational gap between lab and field results. This includes advancing and optimizing prediction tools and models, and understanding and supporting scaling needed to achieve commercial-scale and viable production volumes and economics. We have multiple efforts currently underway to address all of these aspects and will continue to do so going forward, adjusting real time for successes, lessons learned, and new opportunities within each. For example, within the DISCOVR project, the project partners utilize the data and parameters from real outdoor experimentation at the Arizona Center for Algae Technology and Innovation (AzCATI), Arizona State University, to run indoor experiments to identify and close the gaps on the indoor/outdoor translation of predictions and results. This is an iterative process and includes indoor/outdoor/indoor efforts. Many projects within the portfolio take this approach, such as Los Alamos National Laboratory's Multi-Scale Characterization effort, which is also very synergistic with and informative to the DISCOVR project. The resource assessments, as well as the productivity models and predictive data sets and tools, continue to advance, allowing the projects and industry in general to better utilize these tools to make informative plans and decisions for their own work.

The ultimate goal of any research and development program is to advance from lab R&D through the necessary scaling stages to commercial-scale and production volumes. We work closely with our stakeholder community to understand what those scales are (e.g., R&D, pre-pilot, pilot, demo, and any identified necessary in between) and how and when to best pursue them. As the reviewers noted previously, BETO is knowledgeable and experienced in scale-up and well positioned to make it happen. We also understand the risks involved in scaling, particularly new technologies, and the importance of unit operation integration and ultimately market assessment and commercialization. We agree that there are technologies within our portfolio and the algae industry that are ready to scale up and others that are very close. We will continue to work with our stakeholders to pursue these efforts through the mechanisms and funding available to us.

Recommendation 2: Augment and expand the current national lab analysis and modeling efforts to establish nationwide platforms to model and evaluate the overall performance, required inputs and resources, environmental impacts, and economic viabilities of proposed strains, individual geographic locations, technologies, and products.

A. Predict strain growth rates geographically.

B. Establish mass balances for processes being proposed, developed, or considered.

C. Ensure standardization of metrics and assumptions so all suitable projects in the portfolio can be evaluated using these as reliable diagnostic tools.

D. Consider requiring all suitable projects to submit key data that can be used by these tools to independently assess key performance criteria and metrics separate of the project participants own efforts—especially with regard to strain, site selection, TEA, LCA, and mass and energy balances.

The AAS Team appreciates this recommendation and agrees there may be ways to expand and augment the analysis and modeling efforts to address the many suggestions noted above. Many of the analysis and modeling efforts such as the LCA and TEA and the resource assessment have been "harmonized" to standardize many items like inputs and outputs, resources, metrics, targets, assumptions, unit operations, scales, and operating parameters. Projects are always encouraged to engage participants in other projects, share information, publicize their results and lessons learned, and share as much information about their efforts as possible. The DISCOVR consortium is another good example of harmonizing efforts across the analysis and modeling platforms, as well as the lab portfolio *and* the competitive FOA portfolio. Many industry stakeholders, as well as project participants, work directly with the DISCOVR consortium to share and test strains, data, operating logistics and parameters, biomass characteristics, crop protection information and strategies, and gap analysis. As noted, many of the projects have growth, productivity, TEA, and LCA models. Some use the TEA and LCA models utilized by the program, whereas others have their own or augment the program models with their specific technologies' information. The program's state-of-technology reports, as well as the TEA and LCA models and analyses and resource assessments, are shared to provide a framework for standardization across the industry.

We have additional mechanisms in place to standardize metrics, key data, assumptions, and other project data across the many projects in the portfolio as well. For example, all of our FOAs include a requirement to complete a technical data sheet. The technical data sheet is designed per FOA, representing the metrics and goals not only required within the specific FOA, but individual project metrics and goals (as a project management tool) as well. The idea behind utilizing this data sheet is that it can be used to standardize measurements, units, metrics, volume requirements, economics, and goals across all projects awarded from that FOA. Beyond this capability, it can be used as a project management tool to monitor and track progress. Even though the data sheet is specific to each FOA, there are common measurements and metrics relatively applicable to many, if not all, such as productivity and yield. This also allows the program a way to assess, harmonize, and compare the various fields across the projects. Similarly, we also require TEAs and LCAs from all of our projects. This is a way to get project personnel thinking more long-term about the economic viability and feasibility of their technology, as well as the environmental impacts of their technology. Many projects also include requirements for mass and energy balances. Other projects are required to provide specifications for their proposed product regime, including the characterization of their algal biomass, to see if they can meet those specifications.

The activities noted above are already underway; however, they are not standardized across the portfolio. They are not generally included in our AOP projects, and they are not generally harmonized with our assessment, analysis, and modeling efforts with the national labs in order to provide an apples-to-apples comparative basis and/or validation tool. There is definitely value in doing so. As the reviewers note in their summary statements, we also work within the intellectual property realm and consider business-sensitive/confidential/proprietary information related to data sets, technologies, and the applied research and development itself. Therefore, we also have to be sensitive of how this information can be harmonized and utilized. Many of our current competitive projects (from FOAs) provide data and input into the TEA, LCA, HTL, and CAP projects. In fiscal year (FY) 2021, we are initiating an effort to advance the harmonization and collaboration between the resource assessments, TEA, LCA, HTL, and CAP efforts even further. We believe this will address much of the recommendation above, as a deliverable from this effort can be a set of measurements, metrics, units, and information we can potentially request from all of our projects to support and validate project and portfolio/program success and advancement.

Recommendation 3: Ensure that entities that receive funds from BETO are advancing the algae biofuels industry and addressing AAS programmatic goals. For research scientists (academics and national lab researchers), ensure that all research projects are working toward addressing AAS program goals and not simply pursuing science for its own sake. For industry partners, include a stipulation in FOAs requiring they pursue support, interest, and possible funding from outside sources other than BETO. The final project goals should require documentation of efforts to attract outside interest that may result in additional investment or some form of commercial support. The purpose of both of these refinements is to incorporate additional incentives for the performers and to deliver additional tangible project outcomes that can be used by AAS in subsequent upselection and downselection decisions.

The AAS Program Team appreciates this recommendation as well. Like the other two recommendations, we have current activities that address this recommendation. For example, in an effort to continually improve on aligning our research activities with AAS program goals, in FY 2021, the AAS Team participated in issuing a formal “lab call” for new AOPs (along with the other BETO Programs). Each year, we have AOP projects ending, some continuing, and some new projects beginning. This particular year, the AAS Program had a large number of AOP projects ending and chose the lab call mechanism as a more formal way (as noted in the recommendation) to pursue new AOP efforts closely aligned with AAS goals and the R&D needs and priorities of the industry. This effort is currently open and underway.

Relative to near-term, field-oriented and technology development projects, although we have not required what is proposed in the recommendation in the past, we have always strongly encouraged partnering, particularly with industry partners to drive the research in a commercialization direction. We have had a few FOAs where including industry partners and actually producing and characterizing proposed products is required. As the technologies and industry continue to advance toward commercialization, the AAS Team agrees that it is absolutely necessary to attract and involve outside interests for a variety of reasons. We will continue to explore this recommendation as to how to best incorporate this idea into future funding opportunities and mechanisms within our regulations.

IGET: INFORMATICS-BASED GENETIC TOOLS FOR RAPID ENHANCEMENT OF PRODUCTION STRAINS

Los Alamos National Laboratory

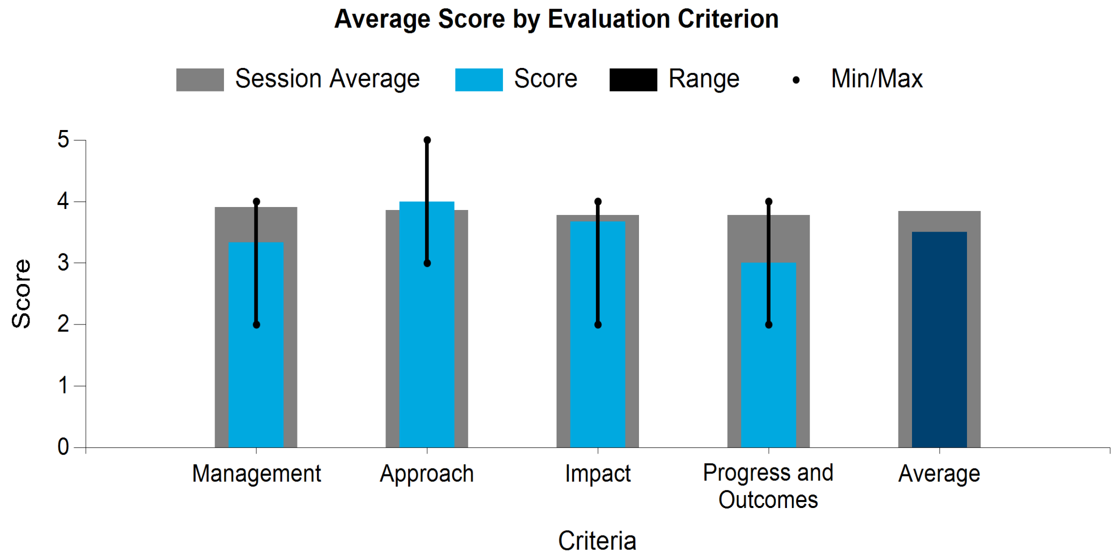
PROJECT DESCRIPTION

WBS:	1.3.1.001
Presenter(s):	Blake Hovde
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2022
Total DOE Funding:	$200,000

The algal research community needs genetic engineering tools and protocols to reach BETO's Advance Algal Systems' production targets of algae biomass yields. Targets of 30 tons ash-free dry weight algae biomass per acre per year with conversion yields of 80 gallons gasoline equivalent (GGE) per ton of biomass by 2023 support the 2029 goal of $2.5/GGE mature modeled minimum fuel selling price for advanced biofuels. To reach these goals, we need to increase the number of algal characterizations and experiments being performed and increase the number of genetically engineered algal strains in active development. Focusing on developing basic genetic engineering toolboxes is recommended to support strain improvement efforts. This proposal aims to address two significant problems hampering genome and metabolic engineering efforts across the algal research space. The first problem is the lack of available resources regarding promoter choice in genetic engineering expression tools for algal production strains. Currently, only a handful of extremely strong promoters are being used to drive gene overexpression for products of interest. While this approach will work to improve productivity in some cases, the algal community requires a more varied library of promoter sequences to fine-tune expression of certain genes and multi-gene pathways. As we continue to improve informatics and biochemical pathway annotations, new target genes and pathways for genetic engineering are emerging. For this reason, we are working to develop curated promoter libraries of varying expression strength and inducible conditions, so these libraries are ready for immediate use as synthetic biology and genome engineering efforts continue to expand and improve.

A second problem is a lack of precise and rapid generation of new genetic mutants. Currently, most effort spent during algal genome engineering projects is on simply generating the mutants. This leaves minimal time for characterizing mutant properties, let alone generating lots of mutants for characterization. During this project, we will also develop CRISPR (clustered regularly interspaced short palindromic repeats) Cas9 stable expression cell lines for the BETO-relevant algal production strain *Scenedesmus obliquus* UTEX393 that can be utilized to generate newly engineered algal strains in a streamlined manner.

Key deliverables:
IGET: Informatics-based genetic tools for rapid enhancement of production strains will provide the algal engineering community with crucial, verified tools and methods that will increase both the pace and approachability of algal genetic engineering research. We will generate practical genetic toolboxes using *Nannochloropsis salina*, *Scenedesmus obliquus* UTEX393, and *Picochlorum celeri*. The genetic toolboxes delivered will include a native promoter library for the selected algal strains with constitutive promoters of varying gene expression strength ranging over one thousand fold from strongest to the weakest promoter in the library, a stably expressing *Scenedesmus obliquus* UTEX393 Cas9 cell line to be used for rapid genome engineering applications, and application of our new promoter libraries to transcription factor gene enhancement to improve lipid and/or biomass accumulation in *Nannochloropsis salina* and *Scenedesmus obliquus*.



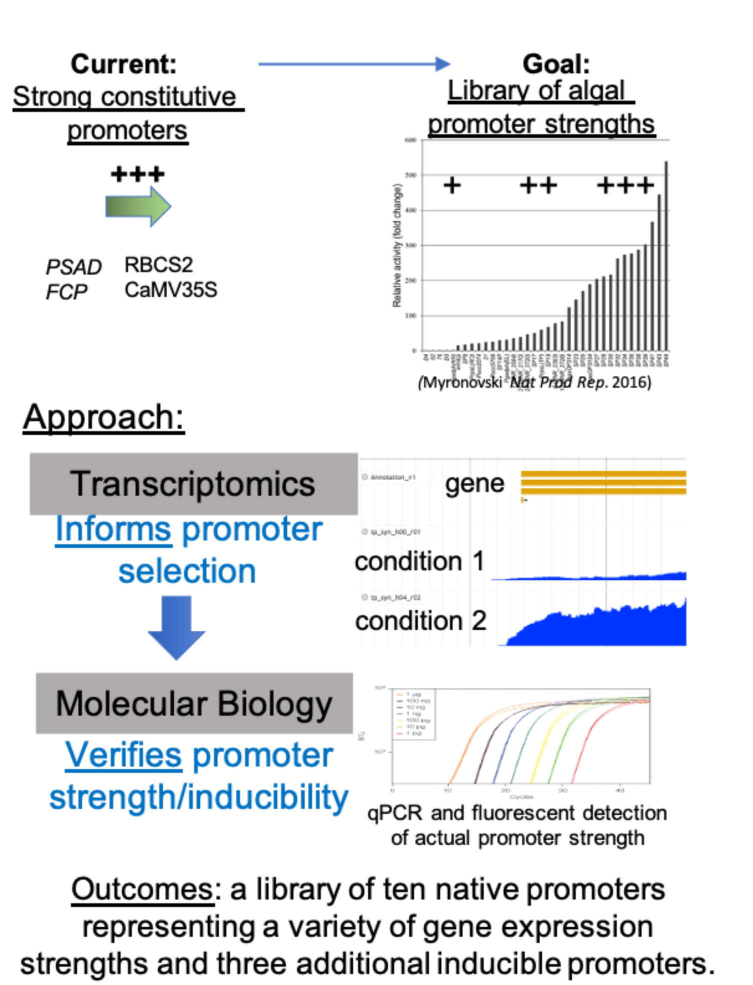


Photo courtesy of Los Alamos National Laboratory

COMMENTS

- It may well be the case that this reviewer does not have a deep enough appreciation of the technical challenges involved and practical challenges that this project may have encountered. From the materials presented, it is difficult to ascertain the degree to which the project was organized to interact with and in support of other BETO programs and goals. Some of the possible risks did materialize without any apparent effective follow-up mitigation or adjustments. The absence of an abstract and the lack of outcomes for measuring progress or addressing the end-of-project milestone make it hard to gauge the project's final end state.

- Overall, this project seems slightly behind schedule due to the initial experimental approach involving randomly integrating promotors transgene. The proposed strategy to achieve more consistent gene expression is promising. The project team meets weekly and project structure is clearly defined. Primary risk of validating gene expression is identified. Overall approach seems sound. Initial approach involving random integration to study gene expression was an unfortunate choice; positional effects of transgene expression in algae are well documented in the literature. New strategy to target specific integration sites using CRISPR is good. Release of the promoter libraries for the three target strains would be useful for the academic and industrial communities. Progress toward validating gene expression of the promoters was hampered by selecting random integration strategy; new strategy involving CRISPR should yield more fruitful results.

- Technical risks appear unclearly described and unmitigated in this project. The team's constrained staff size lends well to clear and constant communication. The team's late pivot away from random integration of promotors and toward more precise engineering strategies reflects a capacity to identify real risks and underscores the need to identify and ameliorate them before they arrive. The choice to use native promotors eliminates many regulatory hurdles and increases the likelihood these data will contribute to real-world successes. The approach of utilizing transcriptome to inform a bioinformatics pipeline and validate assumptions using quantitative polymerase chain reaction (qPCR) is straightforward logic. Creation of a publicly available promotor library for industrially relevant algae strains has significant potential impact. The express train tool described could reduce research and development timelines for future strain development. The poor correlation between predicted and measured expression levels and variability of expression between clones shows more work is needed to complete several unanticipated tasks in the short time left on the period of performance. This work is valuable even if not completed within schedule constraints. Successful completion of this project appears on track now that a plan for consistent promoter insertion is planned.

- The team has developed an approach for the rational selection of stably expressing promoters of various strengths by using transcriptomics to build a native promoter library for a given algal strain. Enabling the public to access this method through the development of the tool, ExpressTrain, would greatly enhance the capabilities of the algal research community and industry when developing molecular tools for diverse algae strains. Promoter libraries have been developed for *Nannochloropsis* and *Scenedesmus*; however, the team is facing significant challenges validating promoter strength due to random integration of the CRISPR/Cas9 expression construct. As a mitigation strategy, the team is developing CRISPR/Cas9 safe harbor cloning methods to improve the accuracy of promoter strength measurements. If molecular validation problems persist, the PI might also consider validating the bioinformatics pipeline by applying it to a model alga with well-established molecular tools so that molecular validation of predicted promotors could be achieved as a proof of concept. If predicted promoter libraries can be validated, the results of this project would be a significant contribution to the field.

- The team will use gene expression-level analysis and informatics tools to inform promoter identification and selection. Then they will clone promoter candidates into algal strains. Finally, they will validate the promoter induction strength using qPCR. Due to uniqueness of algal strains, promoters will need to be tested across a variety of strains to determine effectiveness. Three initial strains have been selected for implementation and work—*Scenedesmus*, *Nannochloropsis*, and *Microactinium* species. The team will try to establish native promoters for the selected strains. The approach is rational and could establish a quick pipeline of promoters for molecular engineering on target algae strains. The impact of these approaches to the BETO program is based on investigating and developing tools to enhance productivity of algae. The overall development of genetically engineered organisms is constrained by GMO-based regulations for field deployment. Therefore, developing non-GMO-based technologies, either by trait sorting and/or CRISPR/CRISPR-associated (Cas) regulation could possibly avert some GMO regulations when placing these strains into the field. This is a very high-impact project if successful in selecting implementable promoters for CRISPR/Cas9 engineering into production strains. The approach will then be made publicly available through an "Express Train" tool that can be implemented on new target strains. The team has developed promoter libraries for *Nannochloropsis* and *Scenedesmus*. However, there are variable expression levels of these libraries, likely due to random integration effects causing variability in integration events. To remedy this, the team is developing CRISPR/Cas9 safe harbor cloning methods to consistently insert the test expression constructs into the same genomic location to improve replication and accurate promoter strength measurement. Without the latter mitigation, the project will be at risk in identifying promoters that are proven to be effective in the selected strains. The team and management plan described are appropriate for this project. Two risks were identified with no discussion on mitigation—contradiction of predicted promoter constructs and validation of gene expression. A third and most critical risk was identified after cloning processes were not successful due to random and variable integration events. Overcoming this through CRISPR/Cas technology will be critical to the success and usefulness of the technology. The presentation would have benefited from direct discussion of tasks, milestones, and metrics achieved to date and left to perform through the life of the project.

- This project is augmenting the variety of promoter sequences available to researchers by developing a publicly available promoter library, starting with three commercially relevant algal strains. This work supports future advancements in genetic engineering of algae, including enabling fine-tuning of target product and coproduct pathways. The project progress experienced setbacks as the promotor libraries developed for two algal cell lines had high variability in results and are not effective. The project is due to complete later this year with a pace of one strain per year. The team was able to pivot to an alternate methodology and is working to address this challenge.

PI RESPONSE TO REVIEWER COMMENTS

- Our team would like to thank the reviewers for the engaged feedback regarding the IGET project. As recognized by the reviewers, there has been an active pivot over the course this project regarding the challenges we presented on random transgene integration effects of promoter testing cassettes, and we appreciate the ideas and suggestions regarding the CRISPR-driven safe harbor site approach moving forward. We are also optimistic that this updated approach will yield critical results that we will have published by project end this year. The team also appreciates the validation of this project providing useful output for the greater algae engineering community regarding rapid and accessible methods for developing novel promoter libraries, including the potential future development of Express Train software package if our current approach proves fruitful for promoter library development. The suggestion of bringing this approach to first validate in a model alga, such as *Chlamydomonas*, is very valuable if issues with promoter cloning continue. Another piece of valuable feedback was the suggestion that utilization of native promoter elements may also support regulatory issues of GMO generation. Overall, the reviewer comments are extremely insightful and will help us continue moving this project forward in a productive direction.

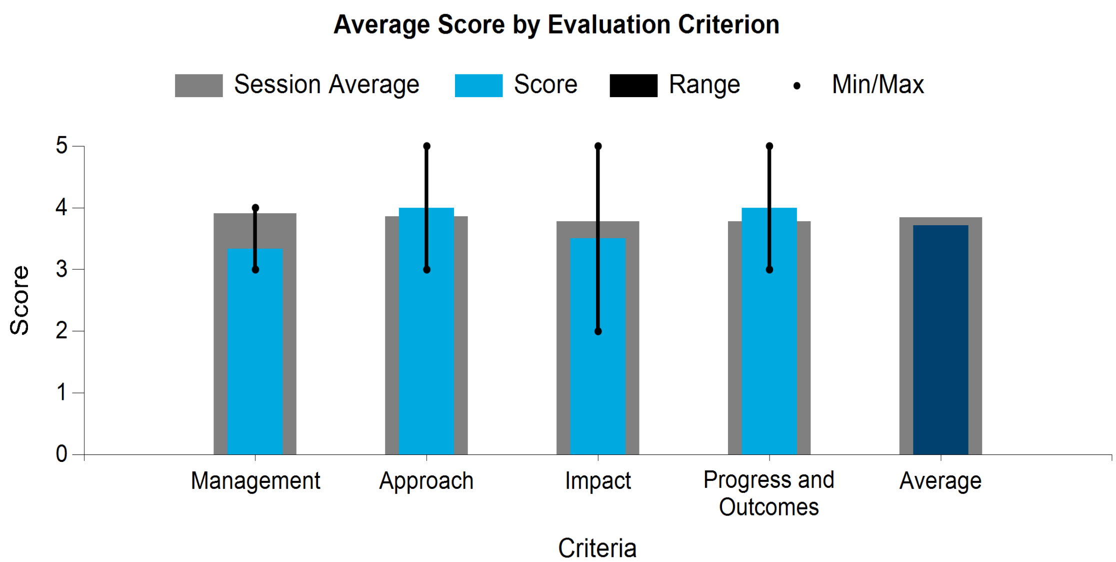
FUNCTIONAL CHARACTERIZATION OF CELLULAR METABOLISM

Los Alamos National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.1.100
Presenter(s):	Scott Twary
Project Start Date:	10/01/2016
Planned Project End Date:	12/31/2020
Total DOE Funding:	$1,950,000

This project integrates flow cytometry physiological characterization assays, epigenomic profiling, and molecular genetic engineering to advance line development in algae. The focus is on understanding nitrogen stress responses and regulation of lipid biosynthesis. Six flow cytometry assays were optimized for three algae species: *Nannochloropsis salina* 1776, *Picochlorom soloecismus*, and *Tetraselmis striata* LANL1001. These assays characterized individual cellular responses within populations for reactive oxygen species, metabolic activity, DNA content, intercellular pH, actin structural changes, and neutral lipid content. We applied these assays to facilitate discoveries for the other two project components. Molecular engineering was optimized with significant increased efficiency after identification of key cell cycle timing of DNA replication after staining by flow. We generated overexpression lines for multiple genes involved in nitrogen uptake, assimilation, and regulation. These lines resulted in greater nitrogen assimilation rates or greater lipid accumulation. In addition, Cas9 lines were generated for targeted gene knockouts. Stability and efficiency of Cas-directed engineering was challenging. Epigenetic investigations identified unique regulatory mechanisms between the three algae species. *Nannochloropsis* does not utilize DNA methylation in its genome. *Picochlorum* has a low frequency of DNA methylation but is responsive to nitrogen stress. *Tetraselmis* has a high frequency of methylated genome and hypermethylates its genes in response to nitrogen stress. Epieffector molecules for both methylation inhibition and histone deacetylation inhibition significantly alter algae phenotypes. The DNA methylation inhibitor 5-AZA significantly alters lipid productivity in both *Picochlorum* and *Tetraselmis*, suggesting a regulatory model of these genes. Integrating these components for strain improvement has identified novel pathways to further advance algae productivity.



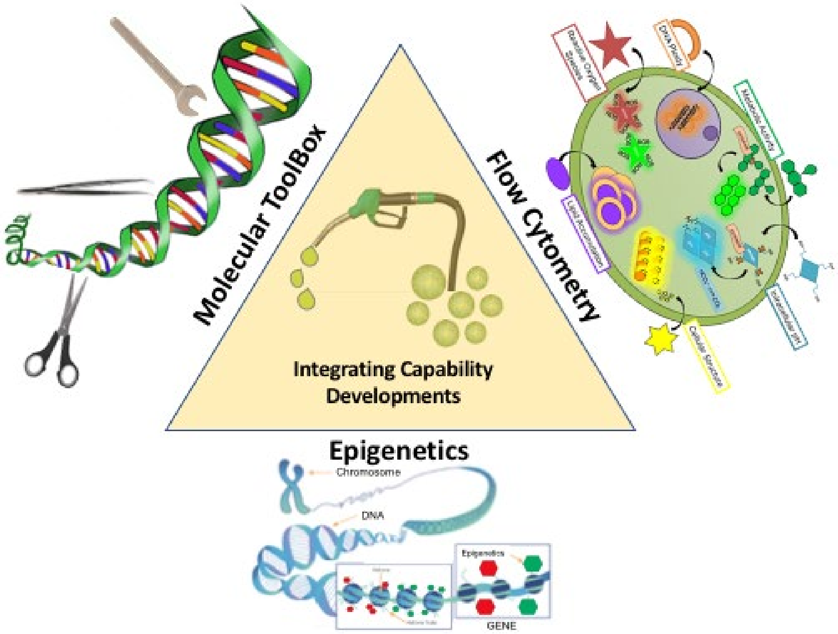


Photo courtesy of Los Alamos National Laboratory

COMMENTS

- Overall, this project seems to have gone well, and several useful molecular tools and insights were achieved. Project team met weekly, and the project seems to have been managed effectively. A summary of risks and how they were addressed during the project would have been helpful. The integrated approach of combining epigenetic characterization, molecular genetic engineering, and flow cytometry physiological characterization is solid and has the potential to advance the state of the art. The impact of the work seems good. The project team published six papers and has two in preparation. Molecular engineering tools and strategies developed here will likely assist other AOP projects. Most project goals seem to have been accomplished. Project team is still working on RNA-seq, whole genome bisulfite sequencing, and achieving more stable lines for *Nannochloropsis* and *Tetraselmis*.

- The overall project approach is clear and logical, with an appropriate management plan. The project leverages phenotyping via multiple flow cytometric assays to inform rational genetic engineering approaches. The project team has made significant progress toward project milestones. To date, the team has expanded the molecular toolkit available for multiple algal species, including improving transformation efficiency and creating stable overexpression lines for *Nannochloropsis* to investigate nitrogen metabolism. Quantification of epigenome modification and the effect on metabolism and productivity are novel and potentially impactful. The group has experienced difficulties with CRISPR/Cas9 genome engineering, however, and should consider focusing instead on the successful cell lines and epigenetic studies for the remainder of the project period. Developing an assay to determine the epigenetic regulation status of diverse algal species is a logical extension of this project and should be supported. It would benefit the project for the PI to more clearly demonstrate the significance of the project's impact on the industry and the potential commercial applications of the technologies being developed.

- The project is staffed and managed by a very qualified and accomplished team. It is difficult to gauge the degree that the work is coordinated with other adjacent BETO programs and goals, with an appreciation that a toolkit is an inherently overbroad definition. The strains used represent the best current candidate strains for commercial use. It remains difficult to gauge the impact of the completed work given the inability or restrictions otherwise to take next steps in an outdoor environment to evaluate any benefits that may be possible from this basic work. The goal of trait stacking was only achieved for one of the three strains. The work's efforts are being well disseminated via publications.

- The team described an approach to non-GMO strain improvements for *Picochlorum soloecismus*, *Nannochloropsis salina* 1776, and *Tetraselmis striata* LANL1001. The approach includes population sorting based on a set of assay traits. In parallel, the team is investigating epigenetics of these organisms. Finally, they are developing a CRISPR/Cas genome engineering toolbox for the organisms, and overexpression of nitrogen (N) assimilation and utilization pathways toward greater lipid productivity. The use of three strains for the development provides a wide array of target organisms of interest to the BETO program. The epigenetics work is unique in the program and can result in basic knowledge for regulation and enhancement of productivity in these organisms. Implementation of epi-effectors would not be directly possible in a nonlaboratory environment, but the knowledge gained can result in new approaches to regulation that can be engineered into the organisms in future programs. The CRISPR/Cas toolbox can serve to establish components that can be used to regulate the organisms' productivity and enhance specific traits. Tackling nitrogen regulatory pathways provides an excellent model to exercise the tools developed under the project. The impact of these approaches to the BETO program is based on investigating and developing tools to enhance productivity of algae. The overall development of genetically engineered organisms is constrained by GMO-based regulations for field deployment. Therefore, developing non-GMO-based technologies, either by trait sorting and/or CRISPR/Cas regulation, could possibly avert some GMO regulations when placing these strains into the field. The team has made excellent progress and completed milestones in each of the targeted approaches listed above. In addition, there have been six publications and two more are in preparation. Their work in epigenetics to understand regulation is unique and can result in new pathways for future molecular engineering projects. The team and management plan described are appropriate for this project. The presentation would have benefited from direct discussion of tasks, milestones, and metrics achieved to date and left to perform through the life of the project, along with risks associated with achieving project goals.

- This project is nearing completion on an integrated approach to applying, developing, and expanding genetic engineering tools to advance algal strain improvements, with a particular focus on nitrogen stress response.

- This team seems well managed and poised for collaboration with national lab teams and other potential partners. The team's constrained staff size lends well to clear and constant communication. This project does not seem to have a true mitigation strategy, but instead uses a somewhat shotgun-style approach to work on many parallel approaches in hopes that one or more will yield meaningful results. Each of the approaches used for strain development seems well-thought-out and capable of yielding success in the context of meeting project milestones. The overall significance of the project seems limited though, as the epigenetic work does not translate to any imagined use in field application at this time, and the molecular engineering does not appear to be yielding promised improvements. The transporter regulation proteins and lipid accumulation both seem like good targets for rational engineering of improved strains. The flow cytometry assay development seems to be a sound method for translating strain improvement to field application by avoiding regulatory scrutiny. This technique is not novel, though, and the impact of this project seems limited to fundamental scientific inquiry for a small set of strains with limited translation to general bioengineering pipeline development. The choice of *Nannochloropsis* and *Tetraselmis* as model organisms makes sense from an ease-of-research perspective. Both are well characterized with well-annotated genome sequences and large volumes of research data available. This choice may make less sense from an industry impact perspective as these strains have not been described as among the most productive or promising for energy or product cultivation by other close collaborators. The discovery that *Nannochloropsis* utilizes histone winding proteins and not DNA methylation for epigenetic regulation appears to be a surprise and an unmitigated risk for the project that hindered intended progress. This insight is potentially significant if a control mechanism can be employed. This research does not seem to suggest how to utilize new epigenetic understanding but does provide important fundamental insights and molecular tools to the public that may be integrated into application at some point. These findings are translatable to rational strain engineering and will shorten research and development timelines for groups working on this strain in the future.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the insightful comments and critiques from the reviewers for our BETO project. The focus of this work was to establish foundational tools for broader future applications. Focusing on multiple integrated approaches has allowed for developing a greater understanding of algae physiological responses. These methods have currently been applied across multiple BETO projects through data and protocol sharing, potentially advancing the success of other projects and identifying paths forward for industry impact. One of the major challenges in algal bioengineering is developing an efficient targeted genome editing system. We recognized this limitation and scoped our work to only explore engineering conserved metabolic targets across species to characterize interspecies differences in responses to the perturbation of these targets. Our results provide a unique perspective to molecular engineering of future production species. The epigenetics research we performed in this project provides baseline information for using epigenic manipulation as a non-GMO strategy for outdoor or production cultivation. Our research is the first to characterize epigenomic information (gene-by-environment interactions across the genome) in several production species. First, we have one particularly strong publication showing a non-GMO epigenetic manipulation strategy (EpiEffector) for enhancing lipid production in *Picochlorum*. EpiEffectors are drugs that are used to treat cancer, neurological disorders, and other diseases. We are using these drugs as tools to understand epigenetic regulation in algae. These drugs alter the epigenome of the algae, and in *Picochlorum*, increase lipid production by 66%. Although these drugs are expensive, there are several analogs that could be used in open or closed cultivation systems. We would like to pursue future work based on this initial EpiEffector research to develop capabilities for epigenetic manipulation in a production setting or in field applications. Second, we have completed RNA-seq and whole genome bisulfite sequencing for *Tetraselmis* and are currently finalizing that manuscript. The preliminary data analysis demonstrates a high degree of epigenetic regulation of gene expression in *Tetraselmis*. Similar to *Picochlorum*, we have data demonstrating that this species is amenable to epigenetic manipulation for enhanced lipid production. Third, it was very intriguing to discover *Nannochloropsis* does not have DNA methylation. This extremely interesting and novel "negative" result for the project has further advanced our understanding of the genomic structure of *Nannochloropsis*, which will support more targeted approaches for genetic engineering. We currently have another publication in progress on this "missing link" of DNA methylation in *Nannochloropsis*. These key discoveries have been recognized by other algal researchers, impacting the importance of considering gene-by-environment interactions. Importantly, this finding demonstrates that not all algae utilize the same epigenetic mechanisms. As the reviewers noted, it would be prudent to support further development of an assay to determine epigenetic regulation in diverse algae species, as this is a logical extension of the project. The reviewers also noted that the epigenic assessment and its impact on metabolism and productivity are unique, novel, potentially impactful, and can result in new pathways for future engineering approaches. We appreciate that the reviewers see the uniqueness and great potential for understanding epigenetics in algae.

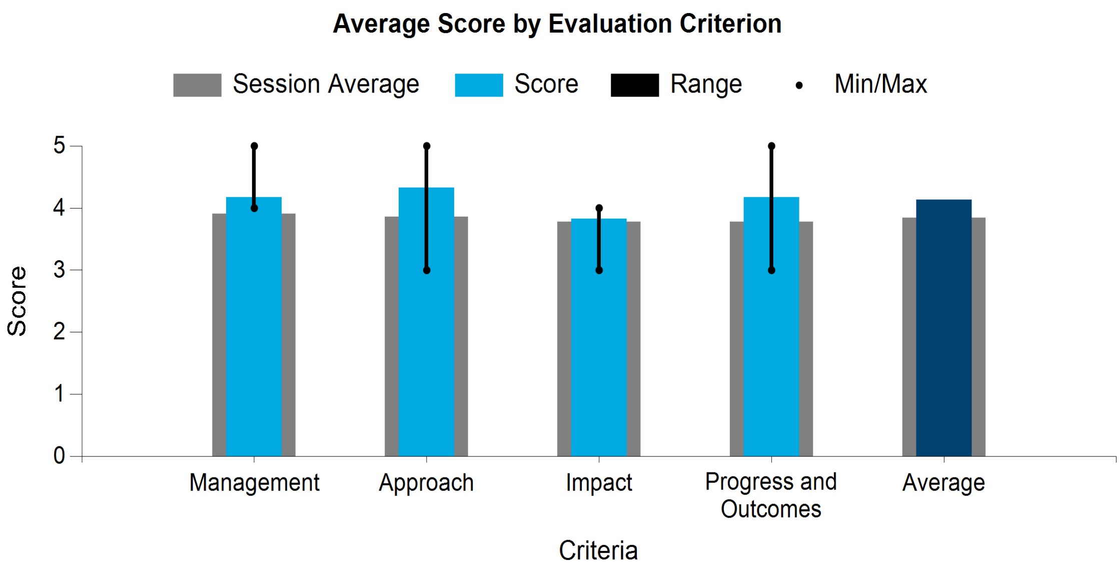
REAL HYPE: RESPIRATION ENGINEERING OF ALGAL LOSSES FOR HIGH YIELDS AND PRODUCTIVITY ENHANCEMENT

Sandia National Laboratories

PROJECT DESCRIPTION

WBS:	1.3.1.105
Presenter(s):	Anne Ruffing
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2021
Total DOE Funding:	$200,000

At night, most algae metabolize carbon that was fixed during the day as carbohydrates and lipids to provide energy, reducing equivalents, and carbon building blocks for cell division. Through this process, carbon dioxide is released, leading to the loss of up to 60% of total algal biomass that was accumulated during the day. As this "dark loss" occurs each night, the cumulative effect of this carbon loss has a significant impact on biomass productivity. Through the Respiration Engineering of Algal Losses for High Yields and Productivity Enhancement (REAL HYPE) project, we seek to reduce dark loss to improve biomass yields by at least 20% in *Microchloropsis gaditana* CCMP 526. Using CRISPR-Cas9 genome editing tools, we have knocked out five genes associated with dark respiration in *M. gaditana*. Knockout of one gene target resulted in a 14.5% improvement in ash-free dry weight (AFDW) accumulated during 21 days of growth under simulated outdoor light and temperature conditions. Additionally, knockout of a lipase gene resulted in 1.7-fold greater lipid productivity in *M. gaditana* under nitrogen-replete conditions. In order to rapidly test multiple combinations of gene targets, we are developing CRISPR interference tools for multiplexed gene knockdown in *M. gaditana*. Through multiplexed CRISPRi, we aim to demonstrate a 20% improvement in AFDW for engineered *M. gaditana* strains under simulated outdoor light and temperature conditions.



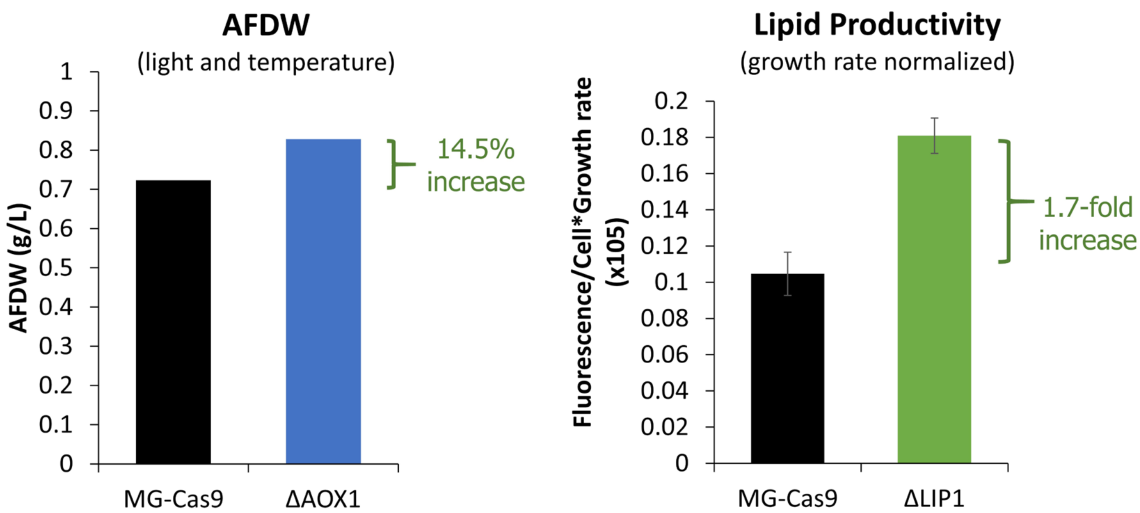


Photo courtesy of Sandia National Laboratories

COMMENTS

- During this seed project, the team successfully employed previously developed CRISPR/Cas9 tools for *Microchloropsis* to produce two gene knockout mutants with improved biomass productivity (AOX1) and lipid productivity (LIP1) at lab scale. It will be important to establish if these mutants' strain phenotypes are stable, and if results can be replicated in outdoor 100-L raceways to meet the project end goal of a 20% increase in biomass productivity. In addition to monitoring growth and lipid productivity, the PI should also consider measuring dark respiration directly for mutant strains compared to the parental strain to validate the mechanism for the improved phenotype and link results back to the project rationale. The team is experiencing issues with CRISPRi and are continuing to troubleshoot. Even if CRISPRi can be demonstrated by targeting nitrate reductase as a proof of concept, it should be noted that only two of the five target single-gene knockouts yielded improved phenotypes, suggesting that it is unlikely that multiple gene knockdown combinations for these would yield significant improvements over the single-gene-knockout mutants.

- Overall, the project seems to be going well and the project team is making progress in achieving its project objectives. Project management seems good. The project team meets biweekly and risks are being addressed. It appears that the hiring of a postdoctoral associate was delayed due to COVID-19, which could have impacted progress and outcomes. The approach has the potential to advance the state of the art. Both the gene knockdowns and CRISPR interference approaches would be useful for improving industrial strains. Project impacts are clear and significant. A 20% improvement in biomass productivity would be a significant improvement toward the 2030 target productivity of 25 g/m^2/day. The genes targeted in this project would likely have homologs in industrial strains, and thus the insights gleaned from this work would likely help industrial efforts. The project team achieved the FY 2020 Q4 annual milestone of 10% improvement in biomass productivity by demonstrating improvements of 14.5% of AOX1 mutant under light and temperature conditions. Work on the CRISPR interference approach seems to be slower and the results are inconclusive.

- Risks seem well identified with practical and specific mitigation strategies. The choice of multiple gene targets is in itself a sound risk mitigation strategy that does not require a programmatic shift to accommodate. The team's constrained staff size lends well to clear and constant communication. The potential impact of knocking out genes associated with dark losses seems potentially impactful, and the stated final goal of 20% biomass improvement does not seem overly ambitious given the seemingly novel nature of the approach. Making these strains available for other researchers increases the impact of this work tremendously. This plan defies conventional intellectual property protection-based paradigms and is an excellent model for collaboration in this sector. Making molecular toolkits developed publicly available is equally impactful and well aligned with other BETO consortium efforts. Capacity to decouple lipid accumulation from nitrogen starvation could result in step-change advances in what has been a critical barrier for algae compositional optimization. Strain characterization methods seem well aligned with other BETO consortium measures of productivity and lend to reproducibility of work. Work seems focused on a small set of rational and achievable targets. The project plan seems free of clutter and distraction from key objectives. Translating this work to greenhouse and outdoor testing is critical to validate lab results, but work done in the lab seems to have met objectives. More detail about what sort of troubleshooting is being done to verify inconclusive CRISPRi results or determine the root cause of the problem is needed to understand the ultimate success of this project.

- The project addresses the issues associated with losses due to algae respiration during the dark periods, causing loss of carbon through CO_2. For the species studied in this project, the carbon loss can account for >60% of the biomass loss. The approach is quite fundamental, in that it incorporates the engineering of gene knockouts for five gene targets that would block access to lipid and laminarin stores along with oxidation pathways from the respiration process. CRISPR/Cas engineering of gene knockouts/knockdowns would be implemented in each of these paths. Strains can then be competed in laboratory growth experiments for selection. The approach clearly delineates gene knockouts to be implemented using CRISPR/Cas9 technology on *Microchloropsis gaditana* targets (five). The goal is to improve productivity by 20% by end of project. The impact of these approaches to the BETO program is based on investigating and developing tools to enhance productivity of algae. The overall development of genetically engineered organisms is constrained by GMO-based regulations for field deployment. Therefore, developing non-GMO-based technologies, either by trait sorting and/or CRISPR/Cas regulation, could possibly avert some GMO regulations when placing these strains into the field. Furthermore, a 20% improvement in productivity would provide a path toward the BETO 2030 target of 25 g/m^2/day in biomass productivity. The team hopes to publish their results and has already made several presentations. Improved strains and plasmids will be made available upon request. The team has made excellent progress in developing strains that have significant biomass and lipid productivities over wild-type—all five gene targets have been successful in knockouts—successfully completing the go/no-go (AOX1 knockout demonstrate 15%–25% AFDW improvement). LIP1 knockout improves lipid content 3.5-fold with reduced growth, but productivity is still 1.7-fold higher. There are some issues with CRISPRi systems identified for which mitigation plans are being put in place and needs further explanation and assessment. Scale-up into 100-L mini-raceways is planned for successful strains. In future work, the team will be developing omics platforms to understand mechanisms of action in the engineered strains. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated—two of the risks not realized after experimentation; CRISPRi risk is the main remaining risk. Some hiring issues were identified due to the COVID-19 situation, the impacts of which were minimal and being managed with time from existing personnel. The presentation would have benefited from direct discussion of tasks, milestones, and metrics achieved to date and left to perform through the life of the project.

- The project team has done an admirable job of maintaining progress and schedules despite the challenges posed by COVID-19 delays. They have done a nice job identifying risks and developing and implementing effective mitigations and recoveries. The increases in AFDW and lipid productivity without nitrogen deprivation are nice milestone achievements as the work proceeds into next steps. It is noteworthy that their efforts attempt to use and calibrate against outdoor data from the Algae Test Bed Public-Private Partnership (ATP3), and most critically expect to be able to attempt scale-up in outdoor and open-pond conditions. This is an absolutely necessary step in evaluating the opportunities that may be possible by synthetic biology methods and techniques. This is noteworthy and very much in support of BETO's programs and goals.

- This seed project used CRISPR gene modification to reduce carbon losses at night with two of the genes, resulting in significant improvements in productivity and lipid content, respectively. The project and team are relatively small such that project management and coordination are more streamlined. The CRISPRi tool development work was identified as more broadly useful over a wider range of future work and differing algal species. The team has shown good progress despite staffing challenges primarily related to COVID-19. Outdoor testing is planned as an important next step, and it will be interesting to see if the modified organism is robust and resilient in replicating the same results and sustained health in outdoor conditions.

PI RESPONSE TO REVIEWER COMMENTS

- We agree that outdoor testing of the engineered strains developed in this project is critical for determining industrial relevance. However, greenhouse or outdoor testing of the strains developed in this project is beyond the scope of the current seed project due to budget and schedule limitations. We are currently looking for funding opportunities to support outdoor testing in our 100-L greenhouse raceway ponds. To demonstrate CRISPRi in *Microchloropsis gaditana*, we first attempted to introduce a dead Cas12a (dCas12a) protein with associated guide RNA (gRNA) targeting a yellow fluorescent protein that was previously introduced into *M. gaditana* CCMP 526 using random integration. While preliminary testing of the dCas12a CRISPRi transformants showed reduced yellow fluorescence, we determined that this reduction in fluorescence was due to knockout of the yellow fluorescent protein construct with transformation of the CRISPRi construct. Therefore, we shifted our CRISPRi demonstration target to knockdown of nitrate reductase, a native gene target with a single copy in the genome. Additionally, we have designed a CRISPRi system based on dead Cas9 (dCas9) as a parallel risk mitigation strategy. CRISPRi with dCas9 has been demonstrated in many other organisms and may therefore be functional in *M. gaditana* if the dCas12a system fails. We agree that phenotype stability of the modified strains should be confirmed to demonstrate the potential for long-term, large-scale cultivation. To this end, we have tested the knockout strains for stable maintenance of the gene knockout after more than 6 weeks of laboratory growth without selection. So far, four out of the five knockout strains have been tested using PCR to screen 50 colonies, and 96%–100% of the colonies tested retained the gene knockout, demonstrating high stability. This provides preliminary evidence that these genotypes and associated phenotypes should be stable over time without selection. We recognize that GMO regulations are a potential limitation for transitioning the strains developed in this project to industrial-scale production. One promising technology for introducing targeted gene knockouts or knockdowns without producing a strain containing recombinant DNA (and therefore avoiding GMO status) is CRISPR-Cas9 ribonucleoprotein (RNP) for genome editing. While our preliminary testing of CRISPR-Cas9 RNP for editing of *M. gaditana* was unsuccessful, we will continue to pursue research opportunities to establish this technology, as it will have a significant industrial impact.

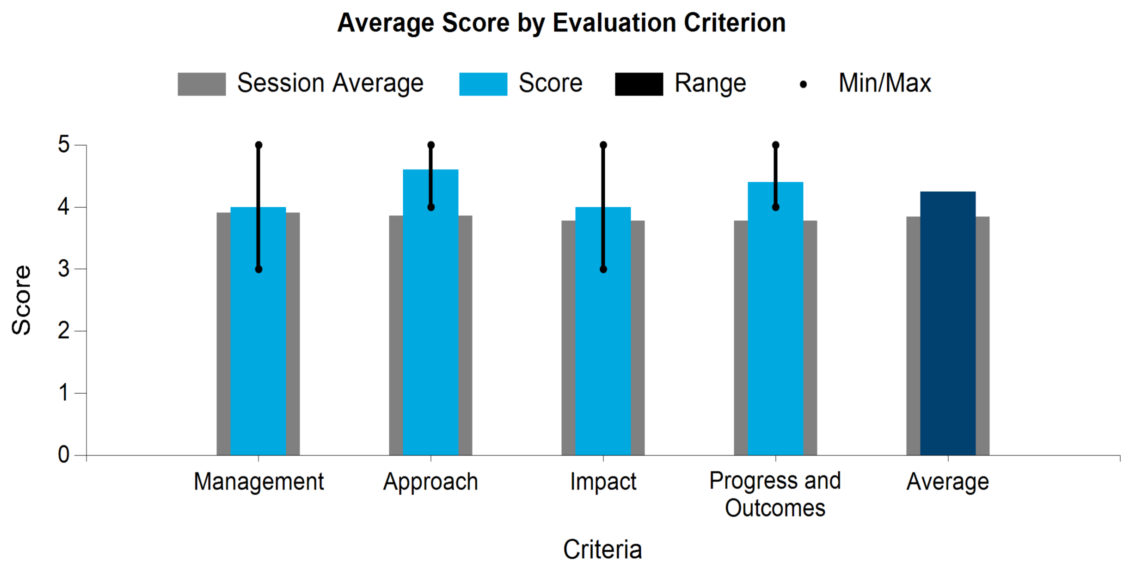
GENETIC BLUEPRINT OF MICROALGAE CARBON PRODUCTIVITY

Lawrence Berkeley National Laboratory and Los Alamos National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.1.110 & 1.3.1.111
Presenter(s):	Igor Grigoriev
Project Start Date:	10/01/2016
Planned Project End Date:	09/30/2022
Total DOE Funding:	$1,500,000

The potential of microalgae to emerge as major biofuel producers is limited by the fact that extensive ecological, genetic, and biochemical information are lacking for most candidate production strains. A better understanding of algal biology—particularly mechanisms that regulate (1) carbon metabolism and storage and (2) response(s) to various environmental perturbations (temperature, light, salinity stress, and pest pressure)—is critical to overcome these limitations. Thus, the 3-year project goal is to conduct a comprehensive systems-level analysis of promising strains grown under industrially relevant cultivation conditions and perturbation challenges to identify genetic targets and biochemical pathways that when modified, enhance stability and productivity of algal cultivation systems. By combining our expertise in algal genomics, transcriptomics, metabolomics, and proteomics, we propose to apply this suite of tools to novel algae with the highest potential to facilitate significant progress in strain improvement. In this continuation project, we apply the "Blueprint" functional genomics pipeline with additional multi-omics capabilities for three highly producing algal strains characterized by the DISCOVR pipeline.



COMMENTS

- Project seems to have been slightly delayed due to COVID-19 but seems to be making good progress. Project communication seems good and involves biweekly calls. Risk mitigation strategies that were specific to the project need to be identified, and it would be helpful to update their status. Functional genomics pipeline approach leverages national lab capabilities and thus seems good. Project methodology has been published and work seems to be being shared with industrial stakeholders. Progress seems to have been made on improving *S. obliquus* UTEX 393, understanding the metabolic flux analysis of UTEX 393, identifying the genes responsible for high salt tolerance for UTEX 393, and sequencing *Picochlorum celeri*.

- The project has assembled a highly qualified team that has done a nice job of managing through COVID-19 while being able to maintain progress. There is very good alignment between the abstract, progress, and outcomes to date when measured against the project goal and end-of-project milestone. The approach is especially focused on addressing and targeting specific deliverables for BETO's desired toolkit. The strains selected reflect the best thinking of the most viable strains that are being advanced by DISCOVR and industrial firms. The targeted genes can also be validated in partner labs. Both of these leverage the larger BETO portfolio efforts to best advantage in support of BETO goals. It was difficult to determine the starting baselines for the work.

- The team's goal is to develop a functional genomics pipeline. They will use multi-omics measurements of multi-state perturbations experiments to identify gene targets for strain improvement and commercialization. In order for this pipeline to be effective, the team will produce high-quality reference genomes of targeted strains of interest to the BETO program. The omics platform is holistic in that it incorporates sequencing and annotation, transcriptomics, and proteomics and metabolomics. The pipeline incorporates collaborations with other AOP projects for validation of targeted genes and engineering of mutants. The team is first focusing on *Scenedesmus* sp. through the pipeline. The impact of these approaches to the BETO program is based on investigating and developing tools to enhance productivity of algae. The overall development of genetically engineered organisms is constrained by the availability of genome sequences and other omic information. Therefore, exercising these base technologies on key strains of interest to the BETO program will be key in the ability to engineer strains that will help reach key goals for the program. The hope is that these omics-characterized strains and resultant mutants will effectively contribute to a 30% improvement in productivity over the baselines, which would provide a path toward the BETO 2030 target of 25 $g/m^2/day$ in biomass productivity. The team is already publishing their results and has already made several presentations. The project may have very high impact if selected strains and omics information can be transferred to the industrial community. The team has improved the genome sequence for *Scenedesmus obliquus*, along with curation of transcription factors. The incorporation of transcriptomics and metabolic flux analysis allowed them to reconstruct the metabolic maps for different cell compartments and compare these with other organisms. They are next focusing their efforts on *Picochlorum celeri*, which is currently in progress. They have selected four other candidates for their pipeline from the DISCOVR project, two winter strains and two summer strains, which will be studied in the third year of the project. The progress is excellent and continues to contribute across platforms. The project can establish clearer goals as to how far genomes need to be finished and how extensive and/or diverse omics analysis needs to be performed for organisms in order to have a good balance between completeness of data for one organism vs. coverage of a variety of organisms. The team and management plan described are appropriate for this project. The presentation would have benefited from direct discussion of tasks, milestones, and metrics achieved to date and left to perform through the life of the project, along with risks associated with achieving project goals.

- This broad collaboration between multiple labs is focused on producing high-quality reference genomes for target algal strains, generating multi-omics data for system-level analysis and modeling, and identifying gene targets for genetic modification to improve biomass production rates. Four primary targets were selected for the third-year work based on performance in the DISCOVR program and industrial relevance. The project was impacted by the COVID-19 pandemic, which has forced some parallel work paths rather than attacking the strains sequentially.

- This is a well-managed project that leverages extensive functional genomics capabilities at Lawrence Berkeley National Laboratory and Los Alamos National Laboratory. The Blueprint pipeline has been developed for multi-omics characterization of algal strains, with the aim to support rational strain improvement. The project has made significant progress and has most recently incorporated strains identified by the DISCOVR consortium into the pipeline deliverables. The project has produced annotated genomes of highly productive, salt-tolerant strains (*S. obliquus* and *P. celeri*) to inform metabolic modeling and regulatory network analysis. The project team has done a good job at showing how extremely large data sets can be distilled and applied to enable future strain improvements.

PI RESPONSE TO REVIEWER COMMENTS

- We thank reviewers for the comments. The strains and baseline data for this project were generated by the DISCOVR project. In year 3, we will choose one additional high-performing DISCOVR strain, while the remaining candidates will be targeted in the next phase (III) of the project. The current phase (II, FY 2020–FY 2022) expands both the number of algal strains (third strain added) and additional omics capabilities (proteomics from Los Alamos National Laboratory and DAP-Seq from Lawrence Berkeley National Laboratory) in comparison to phase I (FY 2017–FY 2019). Each systems-level profile will be assessed in out-years to find a balance of throughput vs. depth of multi-omics exploration. While the presentation was focused on achievements and completed tasks, the remaining tasks and milestones for each of the strains have been summarized in supplemental material. Despite complications/delays caused by COVID-19-related lab closures, parallel sample processing and analysis have helped mitigate risk.

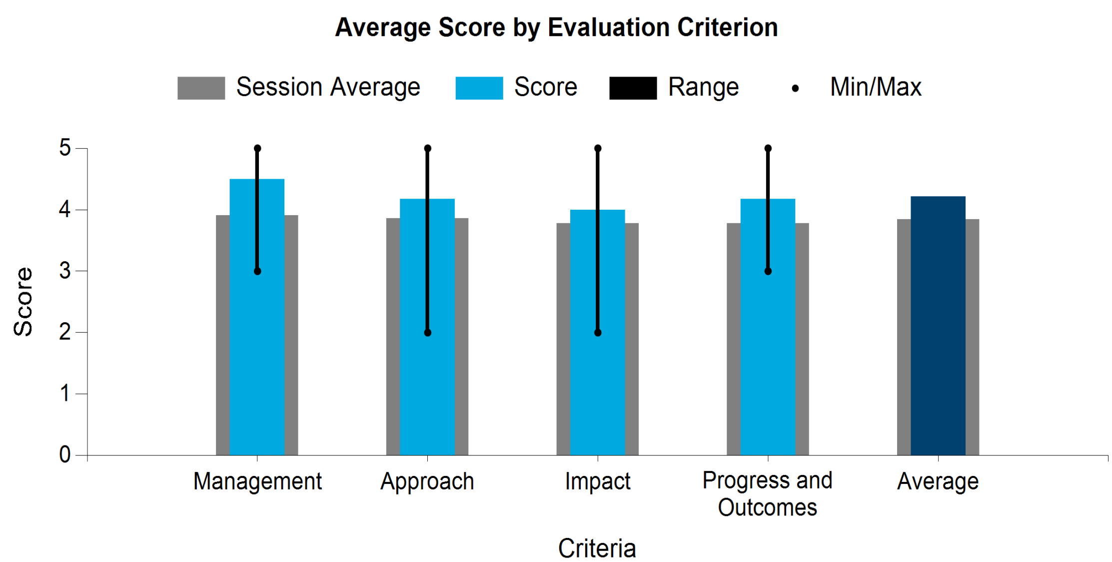
MULTI-SCALE CHARACTERIZATION OF IMPROVED ALGAE STRAINS

Los Alamos National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.1.120
Presenter(s):	Taraka Dale
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2022
Total DOE Funding:	$1,800,000

Several challenges must be overcome in order to realize the full potential of algae-based biomanufacturing. First, TEAs continue to point to algae productivity as a major contributor to biomass costs. Second, continued understanding of the relationship between indoor and outdoor phenotypes of algae strains is needed. Our working hypothesis is that algae strains with improved outdoor productivities can be generated indoors, by using a suite of algae strain improvement strategies and by giving close attention to harmonizing indoor experiments with outdoor conditions. Thus, our goal is to develop this suite of improvement approaches using cell sorting, adaptation, and genetic modification techniques, with a focus on tools for generating algae strains with increased biomass and carbon storage, as well as environmental robustness (e.g., salinity tolerance). We partner with BETO's outdoor test bed at AzCATI and employ an iterative approach to bring learnings from outdoor experiments back inside for the next round of improvement. Here, we share our progress on developing these tools and testing these strains across a range of scales, from indoor flasks to outdoor ponds.



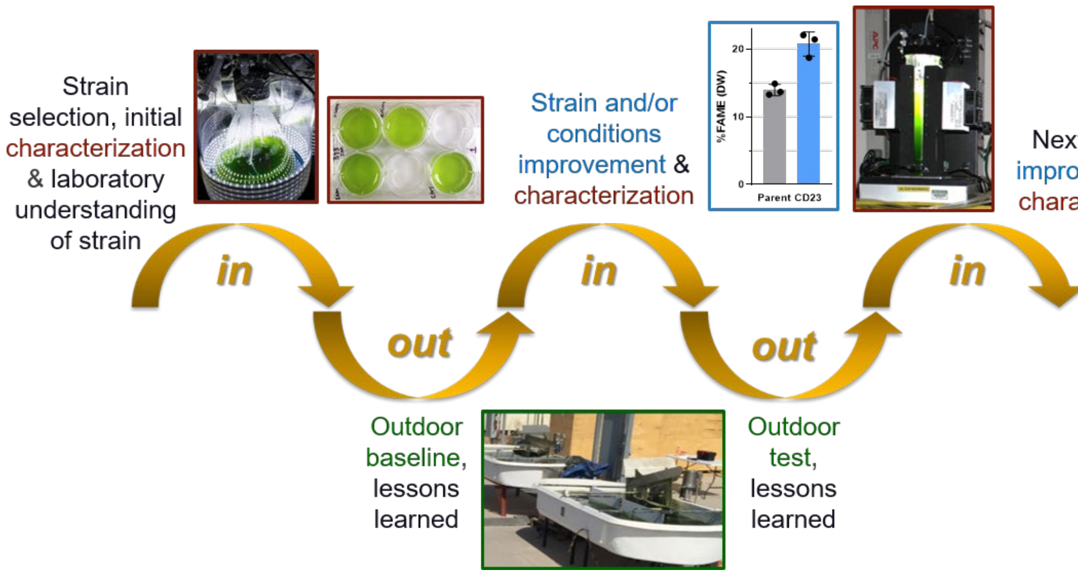


Photo courtesy of Los Alamos National Laboratory

COMMENTS

- An iterative approach has been established toward strain selection methods that will be tested indoors and outdoors through cycles. The goal is to identify best strains and conditions for improvements. The approach incorporates strain characterization procedures, improvements using non-GMO and molecular engineering tools, and testing in indoor and outdoor test beds. The goal is to deliver two microalgae strains with 30% increase over baseline performance within the 3-year project. The approach is described very well along with timelines, milestones, and go/no-go decision points. The impact of these approaches to the BETO program is based on investigating and developing tools to enhance productivity of algae. The overall development of genetically engineered organisms is constrained by GMO-based regulations for field deployment. Therefore, developing non-GMO-based technologies, either by trait sorting and/or CRISPR/Cas regulation could possibly avert some GMO regulations when placing these strains into the field. Furthermore, a 30% improvement in productivity over the baselines would provide a path toward the BETO 2030 target of 25 $g/m^2/day$ in biomass productivity. The team is already publishing their results and has already made several presentations. The project may have very high impact if selected strains can be transferred to the industrial community. Excellent progress with *P. solo* showing great productivity improvements and no crashes in outdoor testing. Improvements were established through the use of ammonia (rather than nitrate) and pH adjustment. A genetically modified strain showing 50% increase in carbohydrates is planned to be grown in outdoor hanging bags at AzCATI. The project's FY 2021 Q1 go/no-go for *P. solo* failed for number of strains but was successful in identifying a mutant with 40% and 50% increase in carbohydrates and lipids, respectively. The *C. sorokiniana* milestones have been met, providing strains with high salt tolerance and high fatty acid methyl ester (FAME) content. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project.

- Overall, the project appears to be on track and is going well. Project management plan is excellent, and risks and mitigation strategies are addressed. There seems to be significant collaboration involving other ongoing DOE-funded projects. Iterative approach using indoor-outdoor-indoor algae growth along with supporting characterization/selection methods is good. Project aligns well with BETO strategic goals and seems likely to deliver useful insights for improving algae productivity. Increases in *P. soloecismus* outdoor productivity were achieved as well as a 50% increase in *C. sorokiniana* growth and 35% in FAMEs outdoors via salinity adaptation.

- The project has a highly qualified team and coordinates/collaborates very well with other BETO projects and programs. The project has identified and been properly managed for a variety of risks and properly reflects prior experience and learning. The in/out/in/out/in strategy is the essential workflow to advance the biology and operational learning needed to support BETO's productivity and ultimately end-state success goals. This approach should be reflected in every single funded effort targeting strain improvement. Additionally, these types of efforts will help establish the important diagnostic relationships that still need further development/refinement for measurements between laboratory and outdoor field applications. The results of this work are being widely distributed via presentations and publications. The efforts could be further enhanced if instead of testing possible genetically modified improvements in outdoor bags at AzCATI, they could be experimented using the open indoor AzCATI ponds that have approval for genetically modified use.

- This project is well connected with collaborators and partners in such a way that all learnings generated will be rapidly integrated. This project is described as so intertwined with other AOP projects that it is difficult to distinguish unique goals and deliverables in this project from others in the portfolio. Risk mitigation strategies described seem to mostly overlook known challenges or plan to ameliorate limitations of indoor cultivation through use of more frequent bench-scale simulation. The choice to adapt freshwater strains to high salinity instead of using natural saline strains as a platform for development does not seem clear. This project seems fragmented and may have achieved more impact if it had been focused on a single significant deliverable. Moving strains indoors and out iteratively appears to be a reasonable way to increase odds of translating new strain development into robust outdoor cultivation. Longer outdoor cultivation cycles will be needed to verify improved sustained performance. Challenges associated with weather and extreme conditions should be evaluated. These periods of time are critical for all modeled plans, and productivity is assumed to be seasonal but not interrupted. Selecting a low-productivity baseline in artificial media makes the end-of-project target less impactful than many projects of this type. Adapting laboratory experiments to better reflect outdoor cultivation conditions does not seem novel, and if unique work has been done to address that challenge in this research, it does not seem clear. All milestones are reported on track or successfully completed. While this is true and progress has occurred, benchmark and target growth rates are both so much lower than the current state of technology (SOT) that this incremental progress appears to make limited impact on the development of the industry as a whole. Outdoor cultivation timelines do not appear to be long enough to validate long-term genetic stability or environmental viability of the strains developed in this project, and the tangible or lasting impact from this work is not clear.

- This project successfully applies the iterative approach of indoor-outdoor-indoor cultivation to focus efforts on improved productivity targeted to relevant outdoor conditions for future scale-up, addressing the ongoing challenge of how to transfer indoor studies to outdoor performance. This project is on track against milestones, and this was clearly communicated in the project Gantt chart. Outdoor conditions were closely mimicked by the indoor environmental photobioreactor (ePBR) as shown in the June/July data set supporting indoor-outdoor transitional work. Increased productivity has been demonstrated, and improved nutrient management of nitrogen and phosphorus were identified as the critical factors in maintaining increased productivity, particularly in outdoor operations.

- This project team demonstrates strong interactions with many other BETO-funded projects across strain characterization, strain improvement, and outdoor cultivation efforts. This exchange of information between project teams to share learnings and expertise is a significant strength of this project and demonstrates outstanding project management and leadership by the PI. The iterative approach for indoor-outdoor-indoor strain improvement, with multiple year/season cultivation campaigns included in the project schedule for each strain, is essential for demonstrating that strain improvements observed in the lab will be stable and scalable. The effort to harmonize measurements and cultivation conditions with the algae SOT is laudable. Improvements made to cultivation conditions (N-source and pH) have led to a >2x increase in *Picochlorum soloecismus* outdoor productivity over baseline, reaching a max of 25 g/m^2/day in peak summer months and showing resistance to pond crashes. Other strain improvement techniques have also yielded promising results for growth and lipid storage potential, and the project is on schedule to downselect modified strains for outdoor summer cultivation campaigns. Overall, the project has made significant progress toward meeting milestones and is on track to meet the project end goal of 30% improvement in biomass productivity or carbon storage molecules compared to preestablished baselines.

PI RESPONSE TO REVIEWER COMMENTS

- Thanks to the reviewers for their many supportive and insightful comments. We agree that this project is tightly integrated with other efforts in the BETO portfolio and that it aligns well with BETO's strategic goals. We perceive it to be an advantage to be closely intertwined with other AOP projects. Close communication across projects allows us to accelerate advances through shared information, strains, and approaches, thereby reducing redundancy and maximizing BETO investment. Many projects in the BETO portfolio may have a similar overarching goal of increasing productivity because that is a primary barrier to reducing algae biomass costs and a major goal of BETO's Algae Program. In addition to this, we described specific metrics associated with our end-of-project deliverable. We are excited that the panel values our approach and the significant progress we've made. To our knowledge, we conduct some of the longest outdoor trials in the AOP portfolio next to DISCOVR, using outdoor-relevant media, in fully open systems exposed to the most challenging pest period of the year (summer). Baseline values were appropriately set at the start of this merit cycle, 3 years ago. Since then, this project has realized large (>2x) increases in productivity, commensurate and on par with advances in other projects (such as DISCOVR). With respect to outdoor cultivation of our genetically modified *Picochlorum soloecismus* strain, we agree that it would be ideal to test these in outdoor open ponds. Given more time and resources, we might have been able to gain the needed approvals to test this strain in an open system. However, cultivation in outdoor hanging bags will still provide useful information, allowing us to decide if we want to pursue further outdoor work with this strain. Thanks again for the thoughtful review of our project and the recognition of the need and impact for this type of work. We especially appreciate the comment that this "in/out/in/out/in strategy is the essential workflow to advance the biology and operational learning needed to support BETO's productivity and ultimately end-state success goals."

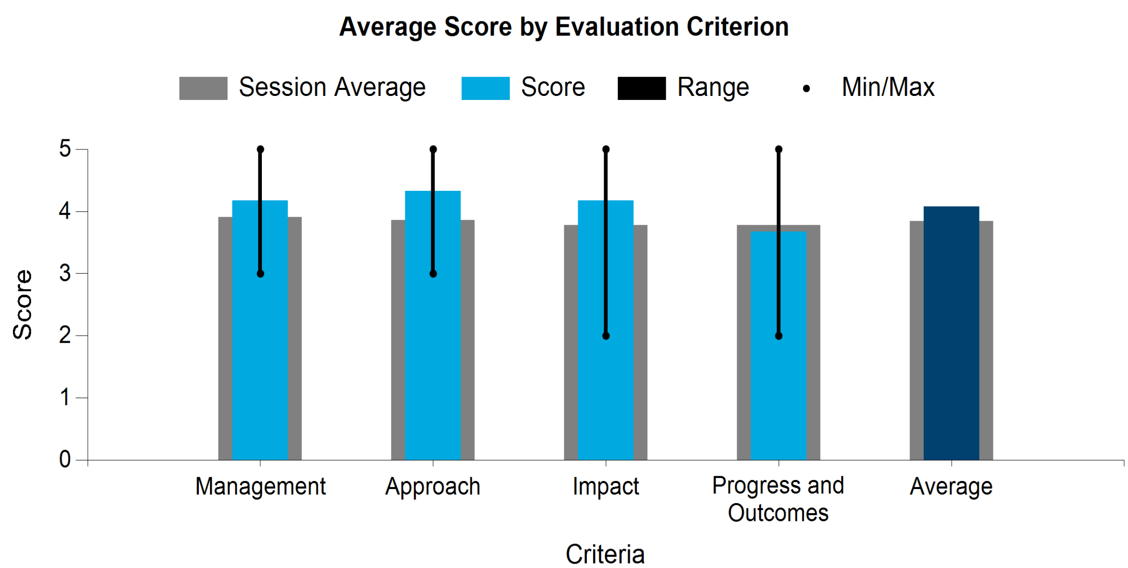
ALGAE BIOTECHNOLOGY PARTNERSHIP

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.1.130
Presenter(s):	Michael Guarnieri
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2021
Total DOE Funding:	$860,000

Development of advanced genetic and genomic tools for targeted algal metabolic engineering pursuits will be integral to achieving target biomass productivity and, ultimately, the BETO goal of cost-competitive biofuels derived from algal biomass by 2022. However, at present, broad host-range tool development is currently hindered by inhibitory host regulatory mechanisms. Indeed, the biological processes controlling algal transcription and translation are subject to complex host regulation, which often presents hurdles for targeted genetic engineering strategies. Advanced genetic approaches offer a means to rewire these regulatory systems and/or introduce novel functionality into algal biocatalysts. Synthetic systems biology approaches also present a means to construct novel genetic regulatory networks and rewire natural biological systems to establish an "orthogonal central dogma," wherein non-native control elements are introduced into or evolved in host microbes for bypass of host control. The Algae Biotechnology Partnership aims to develop advanced genetic editing tools, synthetic and orthogonal genetic regulatory systems, and functional genomic pipelines to enable universal metabolic engineering strategies in top-candidate deployment algal strains. Successful development will ultimately open the door for targeted strain-engineering strategies, aimed at maximizing algal outdoor biomass production, composition, and strain robustness.



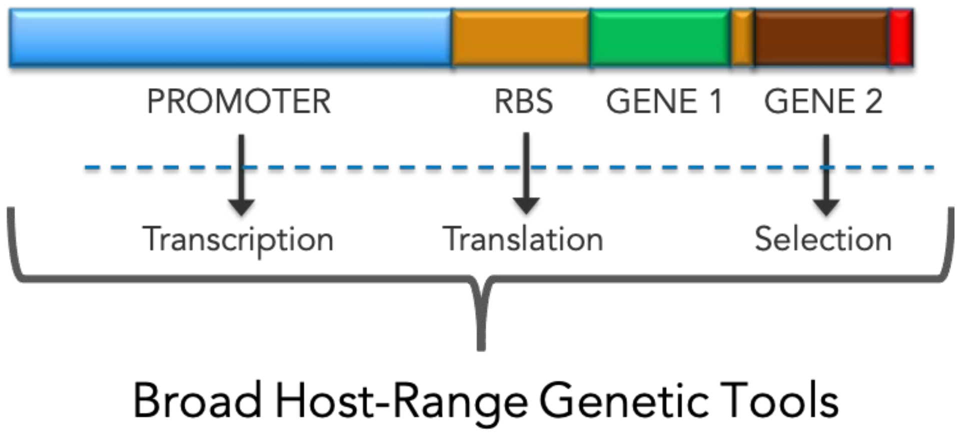


Photo courtesy of National Renewable Energy Laboratory

COMMENTS

- A good technical and management team has been assembled and is working toward progressing on BETO's goal of creating elements of a toolkit. There is a general approach to risk management without follow-up illustrations or examples. Similarly, there is mention of some synergy with adjacent programs and possible partners without meaningful details. The presentation generalizes early adopters, Toxic Substance Control Act (TSCA) Environmental Release Application (TERA) permitting, and genetic modification adoption but does not identify or address any specific issues that could help advance BETO's goals relative to these crucial elements. The summary of accomplishments does not appear to align or measure against the project goal or end-of-project milestone.

- Overall, this project seems to be on track and significant progress has been made developing tools for the top-candidate BETO strain. Project team meets weekly, and the project seems well coordinated. Risk mitigation strategy is well thought out and clearly addressed. Highlighting a couple of examples of the risk mitigation strategy would have been helpful. Approach involving *P. renovo* and one additional top-candidate strain selected from the DISCOVR AOP in order to demonstrate multi-organism applicability seems good. Project team is actively working on strain dissemination and several agreements have been developed, so the project seems likely to have a positive impact on academia and industry. Project team has made good progress toward developing tools for the top-candidate deployment strain and plans to demonstrate tool efficacy in four top-candidate BETO deployment candidates in FY 2021.

- The aim of this AOP is to develop modular genetic editing tools, synthetic and orthogonal genetic regulatory systems, and functional genomic pipelines to enable broad host-range metabolic engineering strategies in top-candidate deployment algal strains. The approach incorporates screening over 300 strains for rapid growth, biomass productivity, and halotolerance. The team will develop modular orthogonal genetic parts for bypass of host regulation, along with synthetic promoter and ribosomal binding site libraries for tunable gene expression, and heterologous RNA polymerase for orthogonal transcription. They aim to establish a broad host range of auxotrophic selection marker(s) in order to bypass the necessity for antibiotic resistance markers. Finally, they will demonstrate broad host-range efficacy of these tools. The impact of these approaches to the BETO program is based on investigating and developing tools to enhance productivity of algae. By aiming the genetic engineering tools to a broad set of organisms, the team hopes to provide a high-impact platform that is easily adopted. If the approach is successful in one strain and can be transferred to other strains, GMO deployment will be de-risked across implementation platforms. The team is already publishing their results and has already made several presentations. Tool dissemination is envisioned through the deposition of plasmids and enabling molecular engineering tools to the Addgene data repository. The project may have very high impact if tools are effectively used by the industrial community in outdoor platforms. The team has focused their development work using *Picochlorum renovo* as a model strain due to its established high productivity. Promoter/RBS libraries have been developed. A unique selection marker requiring phosphite as the sole phosphorous input allows for easy selection of effectively engineered strains. The team has been able to show effective secretion of peptides and proteins using the engineering tools and rewiring of fatty acid synthesis toward high-value products. They will next demonstrate the tools in the four top strains identified through the DISCOVR project. The team has shown excellent publication and presentation record and a number of provisional patents. Random integration of the engineered components poses a challenge to successful implementation across diverse organisms. The work would be enhanced by showing that biomass and/or fatty acid productivity can be effectively altered using these molecular engineering approaches. Management and communication team and strategies were adequately described. Further, interactions with other projects were clearly delineated. Risk identification and mitigation processes were discussed. Some challenges were discussed throughout the presentation providing approaches to mitigation of risks. Go/no-go decision points and performance metrics were adequately described, and the performance was discussed through a review of critical success factors.

- The purpose for incorporation of many partners for this project seems unclear, as all of these institutions have capable microbiologists on staff. The same level of cooperation and collaboration seems possible through the relationship with DISCOVR's management system and team, which includes most of the same researchers already engaged in what appears to possibly be overlapping work. The goal of transferring tunable gene expression across a strain development pipeline is a good one that takes focus away from a single strain or cultivation paradigm. Creating broadly applicable tools like these seems like an excellent use of the national labs' resources that enables a burgeoning industry without playing favorites in terms of strain or other preconceived constraints. The choice of using high-productivity strains instead of simply well-characterized strains increases project risk, but with good reason that increases the chances of this work enabling industry. The capacity to integrate a protein secretion mechanism to a broad range of target algal hosts would dramatically expand the uses of algae as a model organism for a variety of practical functions. A transferable phosphite auxotroph would provide a good alternative to common antibiotic selection markers used in lab-scale selection media that cannot be transferred to outdoor or large-scale operations. The public dissemination of these tools is critical, and it seems apparent that the team is not only dedicated to accurately and openly sharing data, but also engaging the public by answering questions and updating information instead of only providing documents and publications. With the period of performance coming to an end for this project, it remains highly uncertain that the team will successfully demonstrate translation of engineering orthogonal regulatory mechanisms in model strains to four new hosts. The work done so far is good but does not seem to make this last challenge any easier. Mitigation steps taken to reduce this highly risky final deliverable seem unclear.

- The team has a solid management plan that shows a commitment to risk identification and mitigation and a plan for aggressive data dissemination. The project is developing broad-use, host-agnostic molecular tools, which would enable advances across the BETO algae technology platform. The project has successfully developed molecular tools for tunable gene expression and bypass of host regulation for highly productive *Picochlorum* and *Scenedesmus* strains. To date, the project has demonstrated that these tools can be transferred rapidly to other strains and has successfully implemented tools in three top-performing strains, with plans to include an additional three strains that would cover both winter and summer strain candidates. Using these tools, the team demonstrated the ability to alter fatty acid profiles. Engineering of phosphite metabolism is a useful selection tool, bypassing the need for antibiotic selection and reducing false positives. However, it is not entirely clear if its commercial application as a potential crop protection strategy at scale would be feasible. The project would benefit from investigating this further and soliciting some feedback from industry on the potential use of phosphite in agriculture, to determine if this strategy would be viable from a TEA/LCA standpoint. Overall, the project is clearly on track to meet or exceed end-of-project performance goals.

- This large, multidisciplinary project team develops a range of genetic tools with a goal of applying broadly to multiple algal strains. They appear to be on track to meet their end-of-period performance target of demonstrating tool efficacy in four candidates. The management processes and strategy for coordinating across a large team were clearly detailed. A project goal includes efforts toward de-risking commercial GMO deployment by engaging with the U.S. Environmental Protection Agency to better assess and understand their biggest concerns such as the development and release of antibiotic-resistant microbes. It seems like even more concrete steps might be taken in this direction to bridge the gap toward de-risking GMO algae and timely approvals for safe deployment of genetically modified algal strains in open systems.

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 develop genetic and genomic toolkits in high-productivity, halotolerant algal strains represent a critical advance for the BETO algae portfolio and the algal research community at large. We agree with the reviewers' assessments that the project may have very high impact if tools are effectively used by the industrial community. Indeed, we believe the integration of high-productivity strains with genetic and genomic toolbox development offers a strong path forward to target algal deployment hurdles identified via techno-economic analyses (namely, biomass and fuel intermediate productivity). Further, as noted by the review team, the public dissemination of metadata and genetic tools to the algal community is a critical component of this project, and our team is dedicated to public engagement and accurately and openly sharing data therewith.

  With regard to the multi-institutional nature of this project, we believe this is a key component of successful orthogonal genetic toolbox development and deployment. Although all project partners have molecular microbiologists on staff, Los Alamos National Laboratory and Lawrence Berkeley National Laboratory contribute unique expertise in algal genomic and functional genomic analyses, including ready access to Joint Genome Institute and Los Alamos National Lab Greenhouse informatic pipelines. These analyses have enabled rapid identification of key sequence regions (e.g., plastid integration sites), genetic regulatory elements (e.g., constitutive strong promoters and signal peptides), and gene targets (e.g., transcription factors regulating fatty acid biosynthetic machinery), which have in turn informed and enabled rapid deployment of associated genetic tools. Similarly, partners at the Colorado School of Mines bring recognized expertise in the development of CRISPR tools in non-model systems (including *Nannochloropsis* and *Picochlorum* species), which allows for direct knowledge transfer to other BETO deployment organisms. Additionally, we note that this scope of work—integrating genomic and broad host-range orthogonal genetic tools—is not currently being pursued on other projects, thus filling a distinct gap in the BETO portfolio. We apologize that due to time constraints, specific examples of risk management were not discussed in detail. To date, we have accomplished all project milestones in a timely manner, with relatively few hurdles, which has largely mitigated the necessity to deploy the formal risk mitigation strategies presented in our presentation. However, to avoid redundancies in research efforts with regard to CRISPR tool development by numerous labs, we have notably shifted NREL milestone efforts to focus more explicitly on expansion of novel tool suites (to include auxotrophic marker selection, protein secretion tools, and synthetic transcriptional regulation) in diverse hosts and shifted CRISPR pursuits exclusively to the Colorado School of Mines to maximize our project output and team strengths.

  With regard to the feasibility of deploying phosphite as a crop-protection strategy at scale, we note that the utilization of phosphite as a crop-protection strategy is a secondary outcome for our project; our primary goal is to deploy phosphite utilization as a selectable marker within a larger suite of genetic tools. We have shown stable transgene integration in both the nucleus and chloroplast using phosphite in this manner, notably including *off* selection (when cultivated on phosphate). Thus, large-scale deployment of phosphite would not be requisite to employ phosphite as a broad host-range selectable marker. However, we agree with the reviewers' assessment that phosphite deployment for both selection and crop protection at scale merits further evaluation from a techno-economic perspective, and we are actively collaborating with the DISCOVR AOP to determine efficacy and economics at relevant scales.

- Lastly, with regard to likelihood of successful demonstration of project end goals (engineering orthogonal regulatory mechanisms in four deployment-relevant strains), we note that we have already successfully demonstrated our toolbox efficacy and transferability to three top-candidate strains in the BETO deployment portfolio. Efforts are underway to transfer these strains to three additional hosts, and we expect to achieve and/or exceed our end-project goal by the end of our current period of performance. Future efforts will target expansion and high-throughput, automated construction and deployment of our genetic toolbox, which we hope will enable rapid expansion into additional hosts in the BETO portfolio. Finally, we again thank the Review Panel for their time and input and look forward to continued efforts to develop facile genetic and functional genomic tools in top-candidate deployment strains.

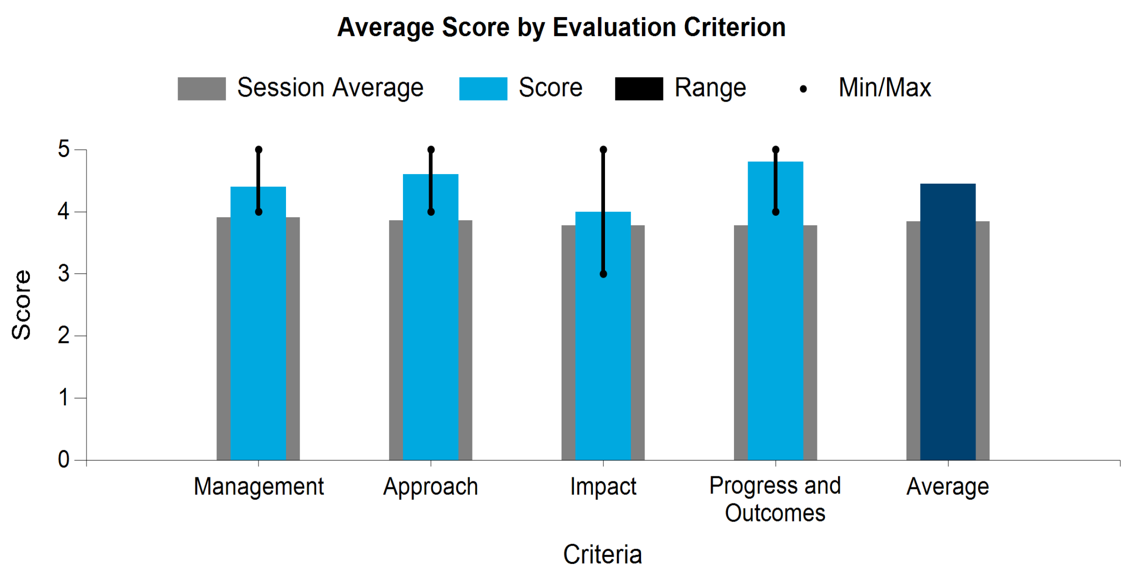
ALGAL TRANSLATIONAL GENOMICS

Los Alamos National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.1.600
Presenter(s):	Shawn Starkenburg
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2021
Total DOE Funding:	$900,000

The primary goal of this project is to use genomics to accurately characterize the metabolic potential of leading production strains to enable performance improvements. A significant barrier to advancing applied algal systems is that genome-wide metabolic models and regulatory networks are lacking, stemming from a dearth of knowledge of gene function for most production strains. Our research focuses on sequencing complete genomes and curating gene annotations through in silico and experimental approaches to expand fundamental knowledge of production strain physiology with a primary focus on inorganic and organic carbon assimilation and metabolism. The 3-year project objective is to improve and curate genomic annotations of central carbon metabolism, carbon fixation, and organic carbon uptake in *Scenedesmus*, a high-performing, industrially relevant feedstock. The curated carbon metabolic models will be validated by evaluating the direct contribution of various organic compounds to growth and biomass composition. Collectively, this research lays the groundwork for improving biomass production rates under carbon- and light-limited conditions in large-scale ponds, enables genetic manipulation and breeding of production strains to improve biomass yields/traits, and will help achieve carbon input cost sustainability through discovery and utilization of efficient carbon metabolism pathways.



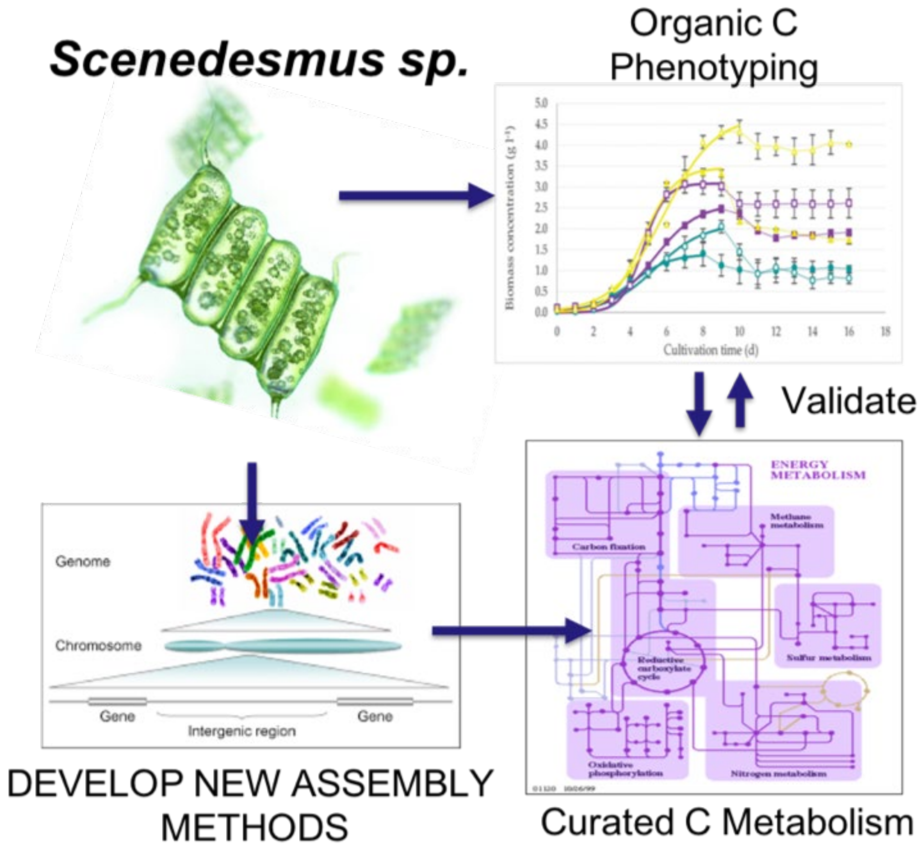


Photo courtesy of Los Alamos National Laboratory

COMMENTS

- The goal of assembling a 100% complete genome for *Scenedesmus* is aspirational, but the project team appears to have a clear strategy and timeline for meeting that goal. The improved genome assembly methods developed here are of value to the general field of genomics, and the completed algal genomes will be a foundation for genetic manipulation and breeding of production strains to improve biomass yields and traits. Initial characterization of carbon metabolism in *Scenedesmus* and availability of a high-quality curated metabolic map will also support future strain improvement strategies.

- The main goal of this project is to use genomics to accurately characterize the metabolic potential of leading production strains to enable performance improvements. The team will achieve this though annotation, curation and metabolic pathway construction, phenotyping and validation studies, and advancement of genome assembly methods. The team is first focusing on *Scenedesmus* strains due to their productivity performance established in other projects. Further, there are existing genomic resources available for multiple strains that will help assess metabolic properties. The team also feels that the genome of this organism is large enough to challenge scaffolding methods, which will help develop robust approaches to genome characterization. Further, the work can result in knowledge of breeding properties and approaches to trait improvement for this organism. The impact of these approaches to the BETO program is based on investigating and developing tools to enhance productivity of algae by providing the basic knowledge needed to improve biomass production through discovery and use of efficient carbon metabolic pathways. Curation of genome annotations will help establish comprehensive models of metabolic pathways, which can be informed through experimental validation. The impact discussion could be enriched by showing how metabolic pathways can be used to understand specific points for regulation that would generate higher biomass productivity or high-value product production, but due to poor state of current models for organisms such as *Scenedesmus*, such inferences cannot be properly made. The team has completed a literature review on the metabolism of *Scenedesmus* and published it. They further have constructed a carbon metabolism model and curated metabolic maps with phenotyping data. This achieved a key milestone in model construction. They have also completed carbon utilization studies. Further, they have completed 100% genome assembly and are working on publishing these data. The team has further work to complete in phenotyping and validation of metabolic responses. The discussion on how this knowledge base can be used to understand breeding and target trait enhancement would enrich the impact of the project. The work has generated six publications and a number of presentations. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project.

- The project team developed and implemented a detailed and rigorous plan and was able to mostly achieve the project goal and the end-of-project milestone. There were no significant impacts from possible risks. It is possible that the results can be used as an example for BETO's toolkit in support of strain improvement via biologic methods. The project does not represent, nor is it apparent, that work was coordinated with other important BETO projects and programs (most notably DISCOVR) that are focusing on algal strains with significantly higher potentials of achieving BETO stretch and commercialization goals.

- This project appears to be on track and has accomplished most of the proposed milestones. Project team meets weekly, and risks and some mitigation strategies are identified. Approach seems good, *Scenedesmus* is a promising strain, and having its genome sequence should aid strain improvement efforts. Impact of developed genome assembly methods seems likely to help with academic and industrial efforts to improve carbon input cost sustainability. Significant progress has been made on all three project tasks and project team is on track to complete all tasks by the end of the project.

- This project is working toward the complete assembly of the *Scenedesmus* genome, which requires a lot of painstaking work. This provides foundational work for algal genomics, and the hope is that developing the tools and experiences around this strain will facilitate an easier effort to replicate the same results for other strains in the future.

PI RESPONSE TO REVIEWER COMMENTS

- Many thanks to the Review Panel for their time and thoughtful responses. Through this project, we strive to develop state-of-the-art genomics tools to accelerate performance improvements in algal feedstocks. Building curated and validated gene annotations from high-quality genome assemblies helps the algal industry by providing reliable knowledge of strain physiology to enable optimization of cultivation practices, genetic manipulation/breeding, and quantitative trait locus analysis of production strains to improve biomass yields/traits. *Scenedesmus* was chosen based on its consistent performance in outdoor SOT trials and its ability to efficiently utilize nutrients from waste streams. The tools and methods developed herein can and will be applied to other promising feedstocks to maximize utility/value of this investment.

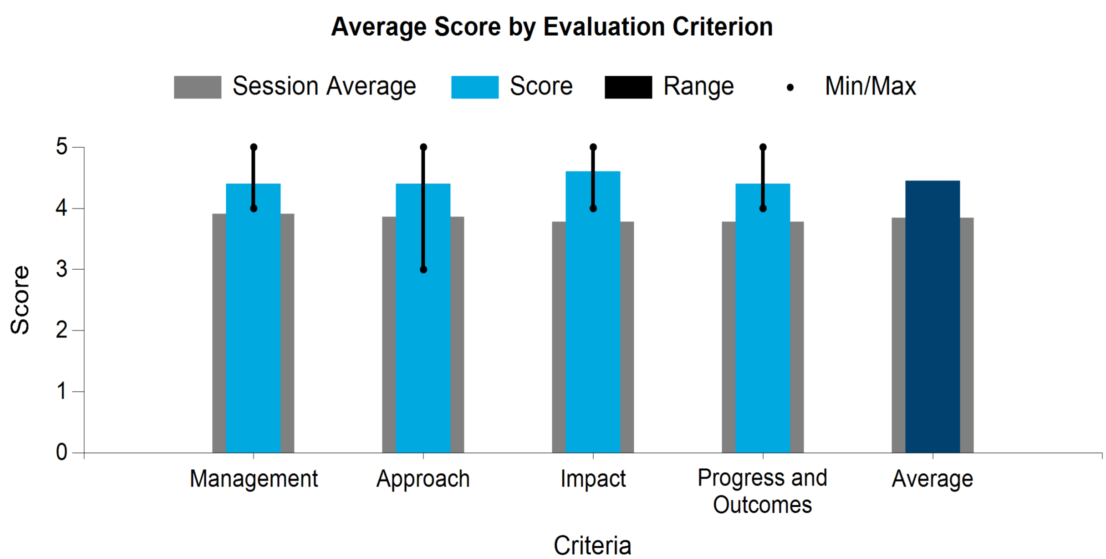
ALGAL BIOMASS COMPOSITION

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.2.001
Presenter(s):	Lieve Laurens
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2022
Total DOE Funding:	$2,200,000

Addressing critical improvements in biomass productivity and associated biochemical composition is a priority for the economic and sustainable commercial development of biofuels and bioproducts from algae. Capitalizing on pathways that integrate engineering approaches with fundamental biochemistry of photosynthetic organisms will lead to a better understanding of the complex nexus of algae growth rates, productivity, and composition. This project focuses on identifying the critical factors for economic development of fuel and bioproduct technologies. Algal compositional characteristics form the foundation of robust economic and business models. This project supports that foundation by developing and validating accurate compositional methods and disseminating them to the greater community. Simultaneously, we build a deep understanding of the dynamic biochemical composition and carbon allocation for biomass value and conversion yields. A coproduct portfolio developed under this project demonstrated a 30% increase in intrinsic value. Additionally, an integrated pipeline of molecular diversity mapping for product discovery with quantitative demonstration across species was deployed over the BETO algae program. The advances made here are highly relevant to BETO's multiyear program targets of reducing costs and integrating dynamic biomass composition with conversion processes to provide options for bioproducts, all leveraging the molecular diversity of algae.



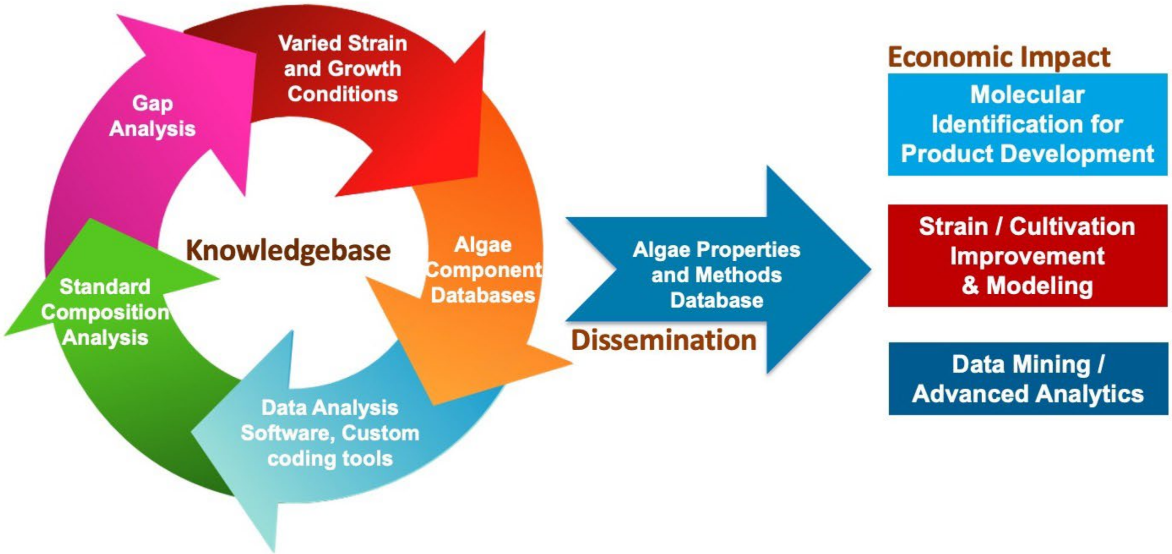


Photo courtesy of National Renewable Energy Laboratory

COMMENTS

- More information on project management and within-group data sharing and communications would have been helpful for the review. The open-access aspect is critical to the success of the project and harmonizing externally. This will definitely be a huge asset to the community overall and certainly further the capabilities of the industry as well as academic research. The only concern here is that people might get less creative with what they can make, because obviously it is lower risk to make a product with known value than to come up with a more novel biosynthesis. But in general, this is a great thing to have. The translation directly to surfactant chemistry is excellent—congratulations! This is really critical work, especially when it comes to the unknowns, which seem likely to turn out to be useful as many plant metabolites are. The analytical work was meticulous and had a direct commercial result, demonstrating why it is so critical to get these things right. Well done! Hopefully, this will get researchers and industry thinking more about non-petroleum synthetic routes.

- The project addresses important areas needed for commercialization of biproducts from algae biomass. The wide variability in growing conditions and among species types leads to a wide range of outcomes that makes product planning and corresponding economics difficult to predict or otherwise establish. The project coordinates and is informed by adjacent BETO projects and takes input from industry and outside interests. The presentation highlighted significant interest via website visits and downloads. The project's results and insights regarding the inherent issues present in current commercial laboratory compositional analyses may serve to represent a new and improved starting point for evaluating the best uses for commercial algae strains. The apparent and significant overstatement in carbs and protein content using commercial methods should trigger deep reconsideration of coproduct scale and viability for almost all applications. Similarly, the remaining and significant volumes of unknowns must be seriously considered for all purposes going forward. The project may or may not fully achieve its goal and end-of-project milestone but may have finally established a starting point and line of sight for the work needed to really understand the fundamental approach needed to establish the composition of algae. This in turn will greatly enable more accurate mass and energy balances, then product profiles and meaningful data for use in TEA and LCA. This greatly benefits industry and all BETO programs.

- The project aims to identify critical success factors, associated with biomass-derived products, for economic opportunity. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The team's approach is to develop and apply advanced compositional analysis of biomass and associated bioproducts, model value of components, integrate information in databases, and convene a technical standards working group to establish methodology and metrics. The approach is deemed holistic, covering essential components to assess biomass value. The effort has broad impacts to the algae research and industrial community through the development of methodology, reference materials, and standards. The team's efforts in understanding variations in analytical data for biomass composition are unique in the community. The problem is confounded by variations in methodology between laboratories. Developing methodology that accurately provides a mass balance of algae components is a critical factor for the community. Hopefully these efforts will be enhanced with future development and adaptation of standard methods and materials that will be utilized by the community. The efforts in developing an understanding of the intrinsic value of biomass components is progressing. The team should look into heuristic approaches in assigning value, along with ensuring that product values (or value ranges) are associated with market size, purity and characteristics of the products, and availability of the component.

- The project's interaction with collaborative partners strengthens this project, as well as all associated projects, in a highly impactful way. Conflict with existing nutritional panel analytical methods seems well understood, and the team seems well equipped to adapt methods and motivate revisions to antiquated standards. The translation of this work to macroalgae demonstrates the robustness of analytical methods and transferability of biomass components. Understanding of sensitivities and diversity of biology is well addressed while still creating uniformity and continuity in this project's general approach. Development of standards and common characterization metrics is critical for the development of this industry. Developing this work in the context of valorizing biomass is a critical step in proving the investment viability in this industry. Creating clear on-ramps to convert biomass into commodities has been tremendously challenging for many teams working in the space. This work begins to address that challenge but still fails to link algae cultivators with potential offtake agreements. Direct access to a market of potential consumers would take the existing database of valuable biomass fractions and link it to tangible sales. For this project to be completely successful, industry adoption will be required. Adoption by the Algae Biomass Organization is a significant step in that direction. Rigorous use of these standards by the NREL Systems Integration team could also be used to create a standardized comparison of technology and technological progress to the benefit of transparency and understanding the true state of the art. It's concerning that 200 kg of high-quality biomass appears to be the bottleneck for validation required for large-scale industry adoption, as this should be addressed by industry partners working in collaboration with BETO and national lab efforts.

- This project seeks to address critical improvements in biomass productivity and associated biochemical composition by identifying critical factors for economic development and deployment of algal biofuels, biomass productivity, conversion efficiency, bioproduct technologies, and compositional analysis. This is relevant to the BETO mission and addresses BETO barriers of lack of understanding of the value of feedstock and lack of information on the physical, chemical, and biological quality of biomass. The team has a clear management plan with well-defined task structure and leads by leveraging team expertise and previous experiences. They identified risk and outlined mitigation strategies, as well as established channels of communication and collaboration amongst team members. Their approach is to standardize compositional characterization of algal biomass by quantitative development and validation of analytical methods for biomass composition, productivity, and energy, as well as development of standards for quantitation. That approach seems reasonable, and successful execution will progress understanding of algal biomass value and impact on process changes on biomass composition and productivity, thereby optimizing the value of algae and reducing the cost of production. The team has made significant progress. They have developed standard analytical methods that are currently utilized in the algal industry, developed novel concepts for algal valorization, implemented a fractionation approach, and built a novel and validated algae-lipidomics database. They have done tremendous work in narrowing the gap of unknown fractions of algal biomass.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their complimentary feedback on this project. Several comments by the reviewers were reiterated and we appreciate the opportunity to respond. While we realize that there is still a significant quantity of the unknown fraction of biomass that is as of yet uncharacterized, we do believe that with our future approach of combining an in-depth dynamic metabolic understanding of the biomass, we will be able to elucidate at least respective pathways and metabolite intermediates that in our opinion will help partially close the gap of missing components. This more granular definition of algal biomass will also pave the way for carbon metabolism rerouting to make room for novel biosynthesis, as was mentioned by one of the reviewers, and novel petrochemical replacement routes based on identified new products.

- We realize that the project management aspect could have been explained in more detail, and it was not the intention to suggest that different areas are working in respective isolated silos. We have a close-knit team and meet weekly on experimental details and biweekly as a full group to align expectations and share advances made toward the quarterly milestones. With respect to the application of heuristic approaches to assigning value of products, we want to highlight that our close collaborations with the TEA group (WBS 1.3.5.200) and the conversion team (CPR project, WBS 1.3.4.201) provide a direct line of sight toward value and production/purification feasibility and integration of novel discovered products. We have over the past couple of years developed a flexible approach that allows for the integration and rapid testing of novel composition and products on the bottom line of algae-based bioenergy. We believe that our role is in communicating the "how" of reporting biomass quality and providing open-access tools to support widespread adoption by algae cultivators to consumers. Ultimately, the growth of the industry is outside of the reach of this project. We continue to prioritize publishing our work to present new ideas to the wider community, and within the limits of our control, industry adoption. The availability of a standard reference material will aid with the communication highlighted here and provides a tangible product that may be used by the algae industry to demonstrate improvements and method validation. Such reference material is not, in our opinion, the bottleneck for industry adoption, but rather a tool to help align and set expectations around reporting requirements.

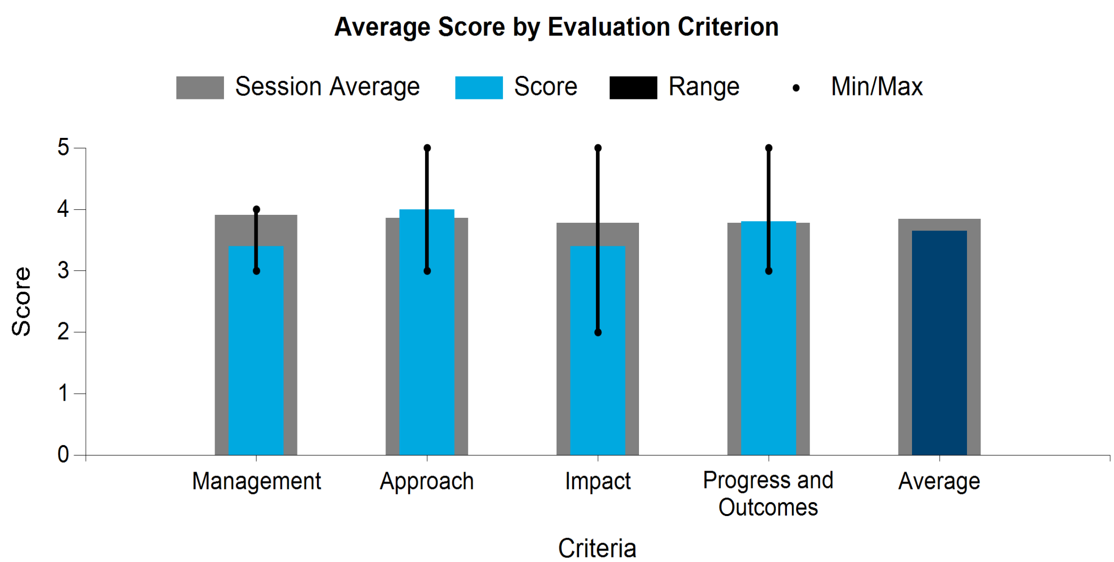
PROTECTIVE BACTERIA IN ALGAL PONDS: INDUCIBLE PROTECTION TO MAXIMIZE RESPONSE

Lawrence Livermore National Laboratory and Lawrence Berkeley National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.2.002 & 1.3.2.003
Presenter(s):	Rhona Stuart
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	$140,000

The end goal of the project is to demonstrate bacterial application regimes that measurably improve algal crop loss due to grazing: increasing the magnitude of protective effect by 25% and doubling the duration over current baselines. From a previous BETO competitive award, we had identified a particularly promising probiotic and demonstrated significant protective effects against rotifer predation. In this project, we focused on identifying probiotic ecophysiological regulation of protective compound production, considering the influence of surrounding chemistry, algae, rotifers, and the microbial community on the generation of these compounds. We then conducted experiments to apply this knowledge to induce and maximize the protective effect. Our approach included (1) genome analysis for elucidation of genes/pathways involved in protective mechanisms, (2) determining and inducing the mechanism of protection using our knowledge of these pathways, (3) comparing the protective effects using grazing assays with induced and uninduced (baseline/control) applications with increasing complexity, (4) modification of our TEA model to calculate economic feasibility of induced probiotic application regimes, and (5) scale-up of successful probiotic application regimes to 100-L pond trials. The project is currently in its third and final year, so aims 4 and 5 are ongoing.



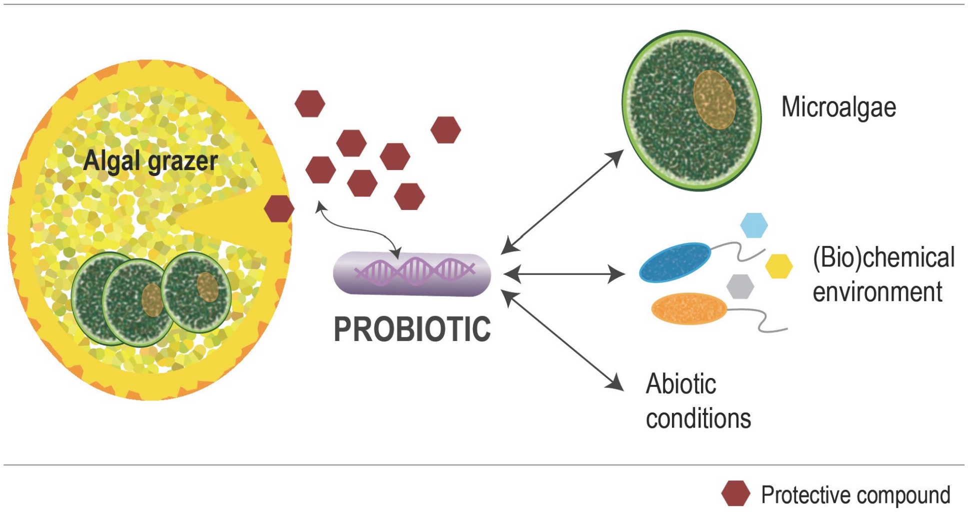


Photo courtesy of Lawrence Livermore National Laboratory and Lawrence Berkeley National Laboratory

COMMENTS

- Having a smaller team makes communication simpler. Roles and responsibilities are well defined. Risk management strategy was not clear. Identified a gap in crop protection; previous work indicated that this had some potential for optimization. Connection to outdoor performance and scalability is good. There is a clear connection between approach and outcomes, and the potential usefulness of the *Janthinobacter* strain development. However, the work doesn't seem significantly guided by TEA. The recycling of spent media to prime the inoculum of the next batch is particularly interesting. The assay development for empirical effects on predators is very good work. Oddly enough, violacein is useful for much more than antibiotics/antifungals—it's a great starting material for semisynthesis of a lot of chemicals. There is likely to be a Red Queen predator/prey resistance effect with bacterial toxins. The concern is that if this is implemented in a raceway, environmental safety will require additional containment and wastewater recapture to avoid large quantities of antibiotics being dumped into wastewater treatment plants and killing off their whole biological oxygen demand system. That could blow up the TEA completely. However, if the water is recycled, enough violacein/*Janthinobacterium* could potentially be captured to get that head start on the rotifers that resulted in no grazing. It is unclear how the algae will be harvested.

- Partnerships seem well grounded in project goals and research needs. Multiple TEA experts may not serve the team well at this bench scale. Grazing assays are difficult, and these seem well designed and executed. Insertional mutagenesis strategy seems poorly developed and difficult to translate into a field-ready crop protection product. *Janthinobacter* consortium member has potential and was a soundly made choice. Identification of violacein role in crop protection is significant. Introduction of an effective probiotic is impactful. The project would be more impactful if it demonstrated the capabilities of a probiotic development pipeline capable of screening and selecting more than one organism in a reproducible manner. The team seems to have met all goals and milestones. It does not appear that any work to regulate the induction of violacein production was successful or that violacein alone is an effective crop protection tool. The mechanism of efficacy for violacein should be explored more completely if this method for contamination control is going to be used in the field.

- The goal of the project is to understand the probiotic mechanisms of *Janthinobacter lividum* in algal cultures to help improve the effects and overall productivity of the algae. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The team's approach to identify protective genes, induce these at scale, and assess improvements is a unique approach within the BETO program. The use of a targeted probiotic minimizes complexity and allows for focused approaches to increase productivities. The impacts at the BETO level and project specifics were discussed with sufficient detail. The choice limits application of the probiotic to a specific pest control: rotifers. Rotifers are ubiquitous pests in many algae cultures, and therefore impact may be broad. The team has found that the main mechanism of action of *J. lividum* comes from the production of violacein and demonstrated improvements in protection of outdoor algae cultures. The team is working on violacein induction, but results have not been fruitful. They have been able to amend microcosms with *Janthinobacter* and induce protection, and have found that different strains also induce protection, indicating other potential toxins may be at play. Progress is deemed adequate, and the results are quite relevant to the goals of the project. The presentation can be enhanced by providing a quantitative assessment of milestones achieved and future work.

- The goal of the project of reducing algae losses from grazing using probiotics may provide meaningful insight and possible applications in pursuit of BETO's MYPP and other program goals. The admitted limitations may be the cost-prohibited nature of this type of approach, and that remains to be evaluated during the TEA portion of the work that is yet to be undertaken. The project team has done an admirable job managing through changes in staffing as well as schedule and logistical challenges created by COVID-19 and the California wildfires. The presentation documents good progress against the project goal and some progress against the end-of-project milestone.

- This project focuses on improving affected algal crops due to grazing by increasing the magnitude of protective effects by 25%. This is building upon the team's previous BETO award, where the team identified a probiotic, *Janthinobacter lividum*, and demonstrated significant protective effects against rotifer predation. The previous project did not address inconsistences with protection in complex communities and the cost impact of increasing the dose. In the current project, the team seeks to understand the biological mechanism and regulation of protection and utilize knowledge to induce and increase the protective response. If successful, the project will advance BETO goals of minimizing pond crashes. The team has a clear management plan with a well-defined task structure leveraging expertise of team members. The team did not identify risk and mitigation strategies. Established channels of communication and collaboration amongst team members are outlined. The team should consider clarifying strategies for outdoor trials to validate performance with probiotics and how this strategy will be deployed at commercial scale. The team's approach in designing grazing assays to assess effects of treatments to reduce grazing, identify protective genes, test induction conditions at scale, assess improvements with increased complexity, and develop methods to understand effects of protection on algal carbon loss is reasonable. Outdoor validation of proposed algal protection strategies should be implemented earlier in the program. Overall, the team has made progress toward goals. The identified violacein and the mechanism of protection and showed induction can be used to improve protection at laboratory scale. However, it's uncertain if this improved protection will translate to improved performance outdoors.

PI RESPONSE TO REVIEWER COMMENTS

- Our team greatly appreciates the feedback of the reviewers, which is very useful in guiding both how we present and how we may implement our results. We have taken the opportunity here to respond to some of the points raised that were not clear in our presentation. Regarding how much we were guided by TEA results, we were initially guided by our earlier TEA, which indicated that the dose cost was the area with the most sensitivity, so that is what we chose to focus on optimizing for this project. Recycling of spent media is important to explore, but the level of violacein added is quite low (1–10-nanomolar range, and undetectable by standard assays once added). *Janthinobacter* itself only persists for 4–5 days in the mesocosms, and thus we do not envision either the violacein or the bacterium as an environmental safety issue downstream. Additionally, at this level, the application does not selectively alter the native microbiome, so we do not have evidence that we are increasing the antibiotic-like activity in the cultures or spent media.

To clarify what we have found with regard to violacein, we have, in fact, been successful at inducing violacein in *Janthinobacter* quite consistently, through several distinct compounds and media formulations. These were mentioned in the presentation and are the subject of a paper currently in review, comparing the genomes of different isolates and their violacein production capabilities. Since some of these compounds were already known from the literature (e.g., mannitol), we did not highlight them. We should have mentioned that purified violacein does not protect, so alone it is not an effective tool. In fact, engineered violacein-producing *E. coli* and their extracts also do not appear to protect, whereas *Janthinobacter* and *Janthinobacter* extracts do, indicating that although violacein is involved, the mechanism of protection is more complex than just violacein. We agree this mechanism should be further studied, but it was beyond the scope of the current project to look at the physiological effects on the pest.

Regarding the Red Queen dynamics, while this is definitely possible, we see evolved resistance of the pests as much less likely than pesticide applications, for example. Adding purified violacein does not confer protection, but *Janthinobacter* extract does, so there are clearly additional compounds or delivery mechanisms involved that will require more complex evolution to evade. Additionally, a live bacterium will evolve more quickly than the predator due to faster doubling time. We agree that outdoor validation is very important, and its early testing was critical in the previous iteration of this project. Given that we had already validated outdoors in the previous project, we planned to wait until early in year 2 of this project (which would have been February or March 2020), so pandemic shutdowns delayed. We have now validated outdoors at the mesocosm scale and will test at 100-L scale at the end of the project. Regarding quantitative description of progress, this project has been very successful at meeting our goals. Our go/no-go was met 2 months ahead of schedule and all our milestones were met on time, except one that was discussed and for which mitigation is ongoing (also mentioned below). We did include a milestone table in our additional slides but agree we could have been more quantitative in the presentation.

To clarify our risk mitigation strategy, when the project was proposed, we outlined several risks and mitigation strategies for each year of the project. Most of these we have not needed to implement due to success of our proposed strategy, so were not discussed. However, we have had to manage risk on a few aspects of the project, one of which was highlighted—namely the difficulties with insertional mutagenesis to verify the mechanism. In this case, we turned to transposon mutagenesis, which has led to more insight into regulation of induction as well, so we see this as successful mitigation. Additionally, to clarify, insertional mutagenesis was not intended for application, but merely to confirm that the mechanism of protection involved violacein genes, the mutants themselves would not be applied. The other risk mitigation actions were regarding wildfire and pandemic shutdowns and were mentioned in the presentation. Regarding the impact of generating a probiotic development pipeline for screening and selection, our earlier project did just this, successfully. We developed a pipeline using selective pressure to find protective consortia, which is published and available for use (https://doi.org/10.1016/j.algal.2019.101500). This follow-on work focused on one of the promising isolates we found. We think this is also quite impactful by developing and presenting a strategy for optimization and application of an isolate, which can be replicated going forward for other strain and pest combinations.

CHYTRID CONTROL ADVANCING ALGAL TARGETS

Lawrence Livermore National Laboratory

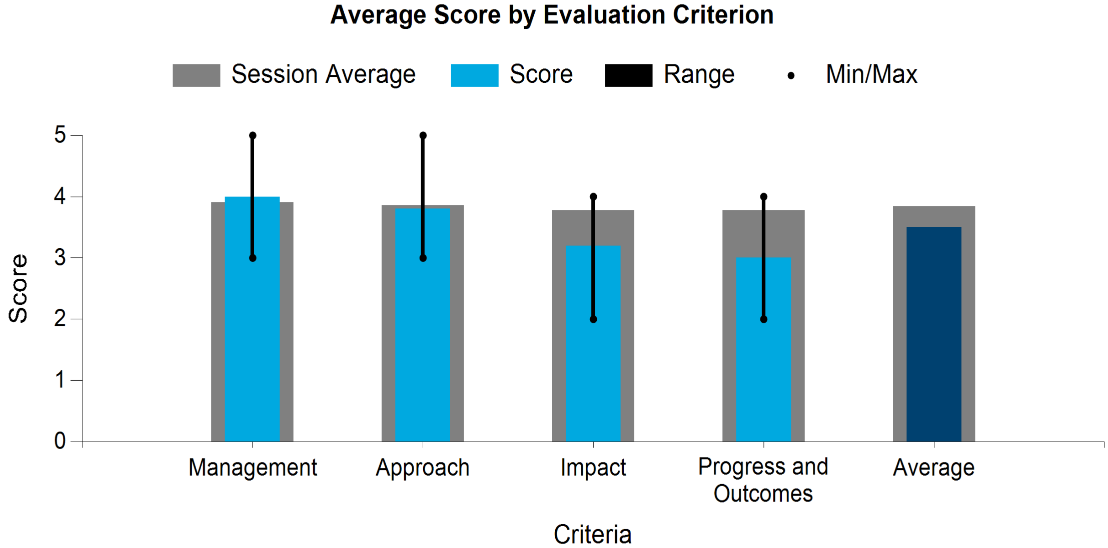
PROJECT DESCRIPTION

WBS:	1.3.2.040
Presenter(s):	Ty Samo
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2022
Total DOE Funding:	$200,000

This seed AOP project is developing new techniques, experimental platforms, and laboratory resources that will facilitate continued research into the design and application of treatments against chytrid fungi that infect algae. Chytrids are the primary culprits of frequent, unpredictable pond crashes that hamper algal biomass production, infecting numerous promising biofuel algal strains, including *Scenedesmus*, *Desmodesmus*, *Monoraphidium*, and *Haematococcus*. While data from production ponds are limited or inaccessible, it is well known that chytrid infections drastically reduce crop productivity for entire growth seasons and can be roughly estimated to reduce the annual yield by at least 30%. This seriously threatens the economic viability of algal cultivation and production of biofuels and bioproducts. Currently, most algal cultivation facilities rely on one of two approaches toward prevention, control, and mitigation of chytrid infections: (1) routine application of caustic agents (e.g., bleach, ammonia, hydrogen peroxide) and broad-spectrum fungicides, or (2) full harvest of algal biomass immediately upon detection of infection. Both of these approaches have severe drawbacks. Chemical treatments can be costly and harmful to the algal crop and may still be ineffective at killing chytrid spores. Due to these considerations, some growers opt to harvest algal biomass prematurely—despite considerable loss of potential productivity—since this ensures that at least the biomass that has already been produced at the time of infection is not lost. Neither approach works as a long-term solution for improved production.

Currently needed is a transformative approach to algal disease management that is informed by chytrid biology. This work aims to generate foundational understanding of chytrid biology and ecology that is necessary to design treatments against those that infect *Scenedesmus* spp., a workhorse oleaginous alga grown globally as renewable and highly productive feedstock for biodiesel fuel. To this end, we have developed a high-throughput metabolic phenotyping assay to screen for heterotrophic growth capacity of chytrids on various carbon substrates. This approach will help determine potential substrates to administer to infected cultures and induce chytrids into a non-infective state by providing an energy source that does not require parasitism of an algal host. *Rhizophydium* sp. and *Paraphysoderma* sp. were capable of growing on some but not all of the substrates, demonstrating the utility of the method and the potential to use these substrates as infection deterrents. Meanwhile, we developed a rapid and sensitive infection assay that builds upon the knowledge of fluorescent probes capable of staining chytrids and applies flow cytometry to quantify chytrid infection prevalence within *Scenedesmus* obliquus cultures. Results were highly comparable to those obtained from the traditional and accurate microscopy-based visual assessment of chytrid infection prevalence. Such rapid assessments are critical to determine onset of and treatment efficacy against chytrid proliferation.

Our work thus far highlights how considerations of chytrid ecophysiology can be leveraged to establish and validate approaches that enable reliable algal biomass production in the face of ever-present and voracious pest species.



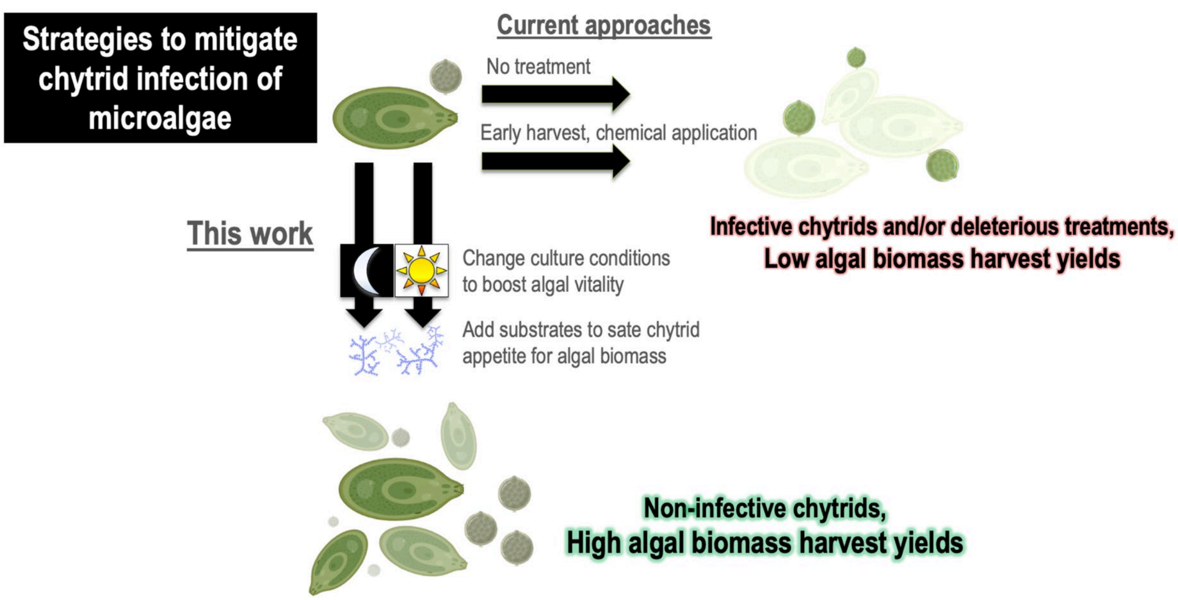


Photo courtesy of Lawrence Livermore National Laboratory

COMMENTS

- Roles and responsibilities are defined, and communication lines are straightforward, as it's not a large team. Risks and mitigation plan are clear. Novel crop protection strategies, especially from chytrid predation, are certainly needed. The strategy is certainly creative and interesting, though it seems like it may have the effect of keeping chytrids in log growth instead of maintaining satiety. Connection of approach to outcomes doesn't seem guided by TEA. It's unclear how this would be implemented. It seems like some percentage of the algal production would be sacrificed to feeding the predator. It's interesting in terms of understanding the basic biology, but the commercialization potential is not straightforward. The assay development is good. Answers are certainly needed to this problem, but really it is one of many problems. TEA of spent media recycling needs to be done but should be a straightforward addition to capital expenditures (CapEx) and operating expenditures (OpEx). The thing is, this is really a Red Queen problem, as all predation/prey relationships are. No matter what is found, eventually there will be some resistance mechanism developed for everything short of mechanical separation.

- The goal of this project is to develop treatments of chytrid infections by understanding the biology of these organisms. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The approach in developing tools to quantify chytrid infections and strain identification and identifying pest control mechanisms is deemed appropriate. Impacts were discussed based on the understanding of the biology of these organisms and their infectivity. The impacts presentation can benefit from discussion of overarching impacts to the algae cultivation community and to the BETO program. The team has made progress in identifying microbial constituents that enhance chytrid growth. They are developing spectroscopy methods for detection of chytrid-algae infected cells. The presentation can be enhanced by providing a quantitative assessment of milestones achieved and future work to be developed.
- The project presents a novel approach for managing chytrid infections in algae ponds by creating tools or management approaches that sate the chytrid and/or otherwise protect the algae. These approaches would be required to be cost-effective when compared with more traditional methods. Materials presented show progress toward development of a chytrid toolkit and the identification of two substrates that may be good candidates for chytrid treatment; however, the development and implementation of these treatments remains to be undertaken. The general ambition for treatment is apparent, but without details or supporting data remains an open question. This being a primary goal for the project that is now at its halfway point may become difficult to achieve.
- The team is small and well suited for this effort. Risks seem poorly defined and may represent cost challenges but fall short of preparation for unexpected outcomes in biological experiments. Genus-agnostic substrate plans seem to have sound inherent risk mitigation built into them, even though not specifically described in the management plan. Goal of eliminating harm done by chytrids without removing them from the environment seems practical and feasible. Use of traditional light microscopy lacks the glamor of many of the methods described by other projects but shows a commitment to understanding details of system dynamics that are often lost when using only omics and sequencing-based methods. The strategy to revert chytrids to non-infective status through direct feeding is very different. This novel approach seems to have obvious risks as it is similar to cocultivation instead of more typical destruction, removal, and starvation strategies. The approach still seems sound and meritorious and has the potential to be impactful if successful. The details of the technical approach do not seem well communicated. Chytrids are a narrowly defined but important target. The productivity lost to chytrid contamination is not well described, though. This leaves the overall impact of this work uncertain. Comparing flow cytometric assays to light microscopy makes these data very robust and more transferable. Work does not point to a useful field product at this time and work does not seem well aligned with any plans to control contamination in an affordable or practical way.
- This project focuses on developing tools to study chytrid metabolism and ecology and then using the information to design improved pest management strategies that are targeted, rational, and sustainable. The plan is to build upon a previous project that characterized chytrid infections of *Haematococcus pluvialis*. The project's goal is in line with the BETO mission and MYPP and addresses barriers in sustainable algal production (Aft-B) and biomass genetics and development (Aft-C). The team provided a clear management plan and outlined some risks and mitigation strategies. The team needs to provide more clarity on the implementation strategy and include outdoor testing. Baseline biomass productivity and the target improvement baseline was not discussed. The approach is to develop tools to understand chytrid ecophysiology as well as evaluate manipulations that will mitigate their negative effects on algal

growth. The team intends to evaluate approaches that convert chytrids from parasite to commensal by manipulating culture conditions to maintain adequate organic levels, adding substrate that sate chytrid appetites, and assessing efficacy of treatments by measuring onset and intensity of infections. The approach is innovative but unclear how substrates that support chytrid growth are good candidates to induce a non-infective state, and if the extracellular polymeric substances in algae play a role as substrate. The team should consider providing a detailed mechanism of the proposed approach. The team has made progress in developing a flow cytometric assay to quantify chytrid infection quickly and sensitively and demonstrated comparable data using microscopy-based assessment. Progress still needs to be made in identifying treatment/cultivation strategies at lab scale that diminish infectivity of chytrids and show improved biomass productivity.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the thoughtfulness and thoroughness of the reviewers' comments, and we are excited that the reviewers all see the merit of the project and novelty of the approach. We are further encouraged that reviewers ask for scaling up of treatments to outdoor demonstration and developing a TEA—though these both fall outside the reasonable scope of our 2-year seed AOP, we take this as a vote of confidence for our approach and hope that continuing funding will allow us to test the treatments resulting from the seed project. Several reviewers call for a more detailed technical approach and explicit mechanisms of potential treatments. Though we have this in the project plan, we did not fully go into them during the short Peer Review presentation. We appreciate this criticism and look forward to improving on this front in future presentations and Peer Reviews. A very important point made by two reviewers is the need for data on chytrid-related biomass loss. We fully agree with this and considered including this in the initial proposal. Very limited data are available to the research community, likely owing to the less-than-flattering optics of a production system hampered by crop loss. Test bed facilities and commercial growers should be encouraged to share valuable data and samples to better constrain loss estimates. AzCATI, in particular, should have information on *Scenedesmus*, *Desmodesmus*, and *Monoraphidium* crashes—directly relevant to our study.

LEAF: LEVERAGING ALGAE TRAITS FOR FUELS

Sandia National Laboratories

PROJECT DESCRIPTION

WBS:	1.3.2.043/1.3.2.042
Presenter(s):	Amanda Barry
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2022
Total DOE Funding:	$1,110,000

Building on our preliminary work on the utilization of plant substrates by microalgae, we will accomplish the following objectives by the end of the 3-year BETO/U.S. Department of Agriculture collaborative project Leveraging Algae Traits for Fuels (LEAF): scale up and optimization of mixotrophic growth on plant substrates for several strains of microalgae, demonstrating biomass and/or fuel yield improvement of 15% versus baseline (algae and plant alone), biochemical pathway mapping of plant substrate utilization in *Nannochloropsis* sp., characterization of plant biomass changes after algae growth, and microbiome characterization in algae/plant substrate cultivation. The LEAF project examines algae growth and performance with plant substrates at multiple scales, collaborating with two industry partners to maintain production-scale relevance. To date, the team has published on the utilization of plant substrates by *Nannochloropsis* sp. at multiple scales, describing improved growth with a 30% improvement in growth rate, shift in lipid production to omega-3s, changes in plant composition due to algae growth, and preliminary insight into the role of the bacterial microbiome. We will continue our examination of carbon utilization pathways by transcriptomics and plant substrate labeling, *Scenedesmus* sp. and *Desmodesmus* sp. growth at multiple scales with plant substrates, characterization of plant biomass in these cultures, and microbiome dynamics in mixotrophic raceway cultivation.

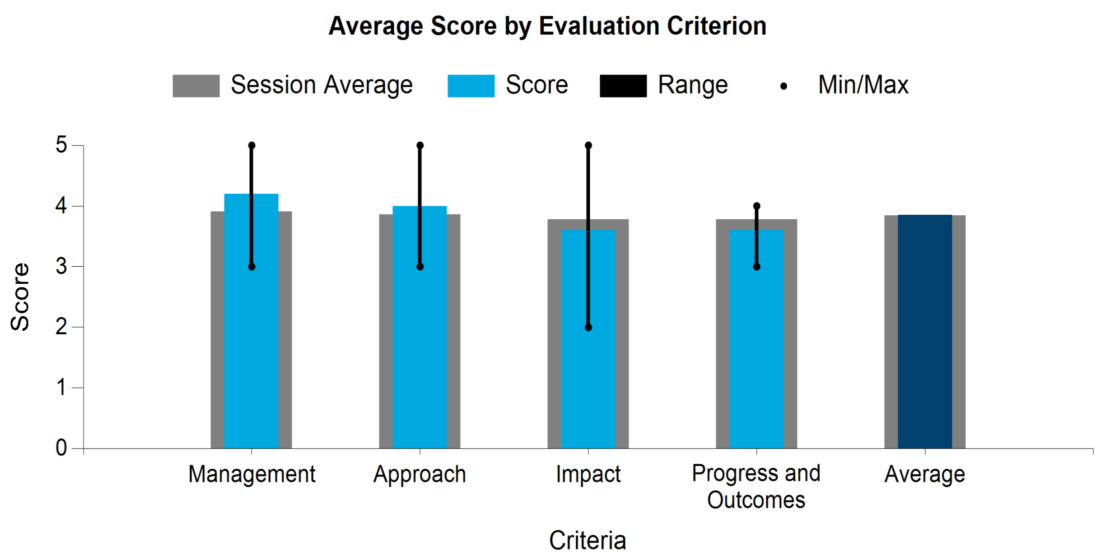


Photo courtesy of Sandia National Laboratories

COMMENTS

- Roles and responsibilities are defined. Risk management is clear and mitigation strategies described. Communication methods are mentioned. The experimental design is reasonable. It would have been helpful to see/hear more hypotheses about why algae might be eating plant material. Many questions surface from this related to whether or not the algae are scavenging nutrients or possibly cycling between benthic and pelagic phases like invertebrates; maybe the nutrients are broken down by biofilms and the algae just happen to enjoy some relationship with the biofilms. These questions are a bit like asking "Why do humans eat steak?" but it's just an odd thing since it's known that algae "eat" CO_2. Seeing/hearing more hypotheses here would have been interesting. It would be nice to see productivity increases, but this work is still in the very early development stage. It does not seem appropriate to evaluate on commercial ability at this point. There is some improvement, but it is still extremely early-stage work. The TEA should probably include biomass logistics costs at least a little or consider an instance where logistics costs are included.

- The goal of this AOP project is to design, develop, and understand mixotrophic growth cultivation with plant substrates for industrially relevant algae strains to improve biomass and lipid productivity. The team has previously demonstrated 30% improvement in growth rate of *Nannochloropsis* sp. by utilizing plants substrates, and a shift in lipid production to omega-3s. Building upon previous knowledge and success, the proposed work will improve biomass and lipid productivity while addressing BETO barriers Aft-B and Aft-C. The team had a clear management plan with a well-defined task structure and leads leveraging team expertise and previous experiences. They identified risks and outlined mitigation strategies, as well as established channels of communication and collaboration amongst team members.

Their approach in exploring industrially relevant algae strains with multiple plant substrates, determining impact of the substrate on biomass and lipid productivity, and understanding biochemical pathways involved and the role of the microbiome, as well as scale up validation, is reasonable. The team has made major progress toward the goals. The team has demonstrated biomass and lipid improvements with three strains at the shake flask scale and one strain at the 50-L raceway scale. Further studies are needed to understand why plant substrates show improvement in some strains and not others, and if there are differences in biochemical pathways for these strains. A major finding was that biomass improvements were significant without CO_2. This may be promising for commercial-scale algae ponds where CO_2 limitation impacts biomass productivity and can be coupled with direct air capture (DAC) technologies for improved CO_2 utilization and biomass productivity. The team needs to provide some clarity on how the plan to address AFDW determination of algae given the challenge of algae colonization to plant material, as well as how the proposed strategy can be commercially deployed.

- The management team seems well qualified for this work. The role of the U.S. Forest Service in this project is not clear. Risk mitigation strategy seems overly general and may not address some of the greatest technical risks to the project's ultimate success. The work described in this project seems well focused on key deliverables without distraction from tangential research activities. Collaboration with the U.S. Department of Agriculture for scanning electron microscopy analysis is a smart way to get critical information about the mechanism of cellulase activity that might not be found through transcriptomics alone. Plans to use microbiome analysis seem unclear and may not have been completely developed before collecting these data sets. Use of real—not modeled or neat—feedstocks make the impact of this research immediately translatable. It is unclear how biological discoveries made in this work redefine strain biology as described. It is unclear what work is being done with microbiome dynamics except community analysis, or how this work will translate into impact. The team's inability to determine AFDW or isolate algal biomass makes it difficult to assess the progress of this work in direct comparison to other projects in the portfolio. Structural and sugar content changes of plant matter with algae are not described in detail, so it is difficult to determine if this finding represents progress. It is unclear how growth rate improvements were demonstrated if algae biomass was not separated from general biomass in the mixed system. The conclusions that productivity improvements can vary across scales and that the microbiome may play a role in substrate accessibility seem like they should have already been known, and it is unclear how those conclusions help address any challenges without more detail or actionable recommendations.
- The project is novel and explores an intriguing topic for some in the algae community. The project involves and coordinates with a good set of industry players and other interested parties and projects. They have done a nice job managing COVID delays and appear able to complete the planned work. The project has achieved some interesting results that will be helpful. The mixed results in growth and composition at small scales are probably not good success predictors for larger-scale and in-field conditions. This would limit the value of these approaches in support of BETO's MYPP and other goals.
- The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The team will look for mixotrophic growth in four different strains of algae, using corn stover, switchgrass, and sugarcane bagasse as substrates providing the main source of carbon. They will determine the productivity changes, determine biochemical pathways involved, examine the plant substrate, and determine the role of the microbiome. The approach will determine the ability of these algal strains to grow mixotrophically and provide a new approach to algal biofuels and bioproducts through plant substrates. The impacts discussion was limited to justifying the work. This can be enhanced through a broader discussion of plant substrate utilization on emissions, along with impacts to the BETO and DOE goals such as productivity enhancements. The team has made progress toward project goals. *Nannochloropsis*,

Scenedesmus, and *Desmodesmus* show biomass and lipid productivity improvements on one or more plant substrates. Microbes may play a role in the process but are not necessary to the algae in-plant utilization. The team has demonstrated achievement of 15% improvement in productivity at the 50-L mini pond scale. The TEA shows that this lowers the biomass price from baseline. Determining the mechanism for utilization of plant substrate will be key in determining what improvements can be made in future production systems.

PI RESPONSE TO REVIEWER COMMENTS

- The LEAF project team thanks the Review Panel for their time and thoughtful comments. The team understands the importance of quantifiable metrics for biomass productivity. In all our experiments, algae are separated from plant substrate to determine algae biomass productivity improvements. While AFDW is difficult to calculate directly with algae colonization of plant substrate in cultures for some strains, we have had success separating algae from rinsed plant material by filtration. In this way, we are able to calculate AFDW in our mini-pond raceway experiments. We can also correlate AFDW with cell counts for estimated AFDW in our smaller flask experiments. In a fully integrated process at industrial scale, we do not foresee the separation of plant substrate from algae will be required for processing to fuels or products. We agree with the reviewers that results at small scales need to be validated at larger scale in outdoor conditions; LEAF is examining growth of algae with plant substrates at multiple scales, including 50-L mini-pond raceways in a greenhouse and in 700-L outdoor raceways with our industry partners. Our preliminary TEA, which included biomass (crop) logistics, is also our validation that this technology could indeed impact biomass selling price at large scales. Integral to this research effort is the exploration of carbon utilization of alternative carbon substrates, such as plants and plant waste, by several algae strains. We share the reviewers' enthusiasm for understanding algal biology. Our future work will tease out the mechanisms of plant substrate utilization in these strains and should also shed light on the role of the algal microbiome in these processes. Although our work with some strains, such as *Nannochloropsis* sp., indicates the microbiome is not required for plant substrate utilization, this population does appear to enhance accessibility to these substrates (https://doi.org/10.1016/j.algal.2020.102041). This will be explored with future microbiome and ^{13}C studies within the LEAF project. We see the potential to identify key bacterial members of the microbiome, which could be employed as a probiotic in the culturing of other algae strains on alternative carbon substrates in which this utilization is currently not observed. We will continue to apply the tools that this project is developing, such as scanning electron microscopy of plant substrate after algae growth and cultivation of algae with plant substrates at multiple scales. The U.S. Forest Service is an agency of the U.S. Department of Agriculture and has been an active team member in the LEAF project, performing scanning electron microscopy and analyzing plant structure and sugar content after algae growth. Our published manuscript in 2020 reports in detail the structural and sugar content changes of plant substrate after growth with *Nannochloropsis* sp. at multiple scales (https://doi.org/10.1016/j.algal.2020.102041). We will continue this methodology with other strains.

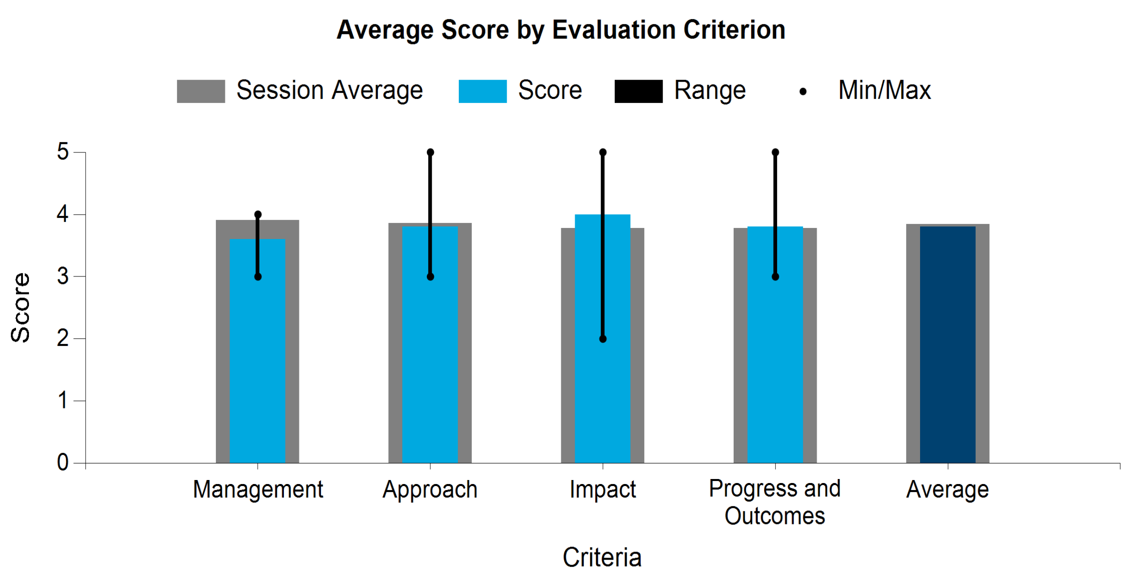
ATTACHED PERIPHYTIC ALGAE PRODUCTION AND ANALYSIS

Sandia National Laboratories

PROJECT DESCRIPTION

WBS:	1.3.2.130
Presenter(s):	Ryan W. Davis
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2021
Total DOE Funding:	$1,500,000

The objective of the Attached Periphytic Algae Polyculture project is to establish the technical and economic feasibility of achieving and scaling high and reliable production of attached algae biomass using fresh and estuarine/marine surface waterways without supplemental CO_2 and nutrients. We are working to improve the biomass yield in polyculture "algal turf" production by culture seeding and systems optimization in environmental simulation flow-ways at Sandia's Algae Testbed Facility. We are performing algae polyculture sample characterization and processing, and GIS resource scoping assessment to identify potential sites for deployment of single-pass or recirculating operations coupled to compromised surface waters or treated water outfalls to allow aggregated scale-up to meet BETO MYPP targets. We have successfully achieved monthlong production performance of >24 $g/m^2/day$ with ash content <25% dry weight by seeding filamentous periphyton in a controlled environment in 30-ft-long flow-ways, for comparison with experimental results and machine-learning models obtained from coastal estuarine/marine testing (Corpus Christi, Texas, and Savannah River, Georgia) and inland fresh riverine (Alamo River, Brawley, California) outdoor deployments. We are testing systems engineering improvements to the general "Algae Turf Scrubber" technology format, including a channeled flow-way prototype for extending flow rate and nutrient concentration regimes, and automated harvesting for minimizing biomass loss and costs associated with harvesting, which is a dominant operating expense for the system. Furthermore, we are characterizing and supplying periphytic algae biomass to support conversion testing and evaluation at increasing scales, in shared scope with Sandia National Laboratories and Pacific Northwest National Laboratory (PNNL) AOPs to assess the potential for efficient and cost-effective production of fuels and bioproducts from attached algae biomass. Finally, we are conducting and updating end-to-end TEA, LCA, GIS resource scoping assessment, and concept of operations and logistics (CONOPS/Logistics) analyses in collaboration from Colorado State University to evaluate the ecosystem service benefits of impaired surface water treatment using algae, including harmful algae bloom prevention by nutrient interception.



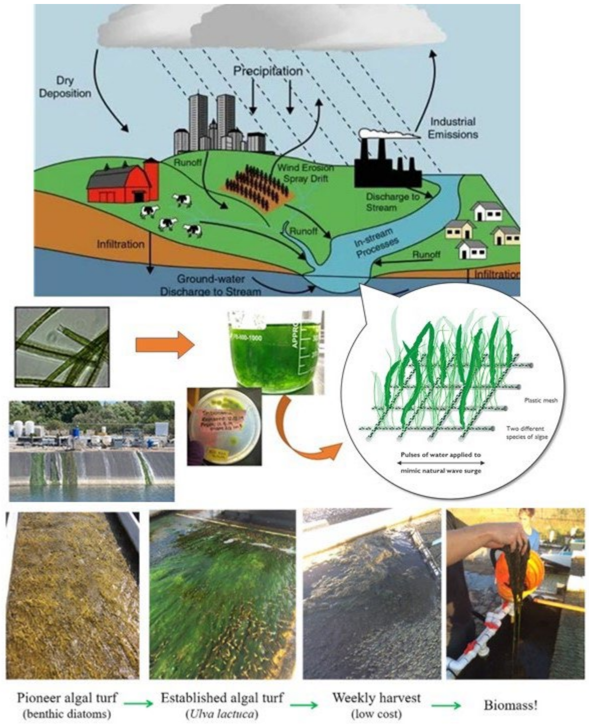


Photo courtesy of Sandia National Laboratories

COMMENTS

- A lot of people were involved in this project, with many collaborators, but it isn't clear who is doing what and how data are shared amongst the group. It would have been helpful to see roles and responsibilities more defined. No risk mitigation is described. This represents an interesting method for bloom mitigation, which is anticipated to become more of an economic problem than it already is due to climate change. Bioprospecting in the 16S results could have useful information. Applications for low-grade heat from cooling water returns, mitigation of algal bloom and zebra mussel/other invasive species overgrowth, pollution control, and remediation have direct and obvious uses. Productivity was relatively high given that the strains are not manipulated strains. It's interesting that tourism was a major driver of the TEA in traditional waste control systems, but in the decentral–conservative instance, technology is a higher driver.

- The goal of the project is to demonstrate the ability of attached periphytic algae polyculture to simultaneously achieve 24 $g/m^2/day$ and <25% ash using compromised surface waters. Success in the work will result in algae biomass costs of less than $50/ton AFDW. This is a novel approach to low-cost production of algal biomass. This is of clear relevance to the BETO mission and MYPP milestones. The team has a clear management plan with well-defined task structure and leads leveraging team expertise and previous experiences. The team has a good mix of academic and industrial partners. The project did not address risks and mitigation strategies. The team management demonstrates a well-established channel of communication and collaboration amongst team members. Progress has been made previously on understanding the impact of the environment on periphytic algal biomass composition and has generated data to drive the TEA. The approach is to deploy fields and pilot test beds to determine biomass productivity and composition and identify periphytic cohorts that sustain increased biomass productivity and low ash content. This approach is reasonable. The team has successfully established biomass productivities in marine/estuarine and riverine source water. Values of 4–10 $g/m^2/day$ and >50% ash were achieved in pilot-scale outdoor systems. Values of >24 $g/m^2/day$ and 20%–25% ash were achieved in environmentally controlled pilot-scale cultivation using inoculation strategies that include polycultures. Outdoor validation of this strategy is critical for success with the risk that the polyculture may not thrive or may be overtaken. The team is also deploying a new harvest strategy with a potential to increase biomass yield. Significant progress has been made and successful deployment of this technology will decrease the cost of algae biomass production and CO_2.

- The goal of this project is to demonstrate the ability of attached algae cultivation to achieve 24 g/m^2/day biomass productivity with <25% ash. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The approach is to develop periphytic algae mats, characterizing their composition and productivity; quantify water source impacts and produce biomass; and conduct sustainability and economic modeling. The use of attached algal mats is unique in the BETO program and viewed as a very practical approach to biomass production. Impacts of the technology development were adequately explained, along with impacts to BETO's targets and goals. The team has demonstrated algal turf systems in various locations. Although the systems are capable of producing at target levels under simulated conditions, in multiyear pilot-scale outdoor conditions, the productivities are 4–10 g/m^2/d with >50% ash content. TEAs/LCAs are very positive in the use of turf biomass through an HTL process even at very high ash content. It is not clear how the project will improve productivity and ash content under outdoor conditions in future work. The presentation can be enhanced by providing a quantitative assessment of milestones achieved and future work to be developed.

- The project and its approach are novel and will be informative. The original application of turf scrubbers and algae troughs is almost entirely in the areas of environmental cleanup and remediation. Even there, they remain rather limited in application and efficiency. A possible fundamental limit in the data presented and the subsequent analysis involves the achieved growth rate of 24 g/m^2/day. It is unclear what surface area was used for calculation. Most turf scrubbers are modest in scale and are not equivalent surface area representations when compared to pond surface areas. The commercial viability of a 5,000-acre system of turf scrubbers seems unreasonable, as does the footprint of the system needed to prevent the algae fouling of Lake Erie. The possible application of algae to treat wastewater has long been appreciated. In general, practical applications have been limited due to better performing alternative methods of treatment, the scale of algae footprints needed to match treatment plant flow rates, and the disruption sensitivity of the algae when compared to other more stable treatment systems. All of these remain inherent to the application of algae as considered in this project, but without identification as risks and without mitigation strategies. Using algae with or as a part of a wastewater treatment system also requires co-location with a wastewater treatment plant, limiting the number of possible sites.

- The team consists of many industry partners with a vested interest in sustainable water management. Collaboration with Algix shows clear ability to link environmental services with sustainable product development. The large number of participants seems difficult to manage but roles and responsibilities seem clearly defined by deliverables. The risks associated with deployment in rugged environments with minimal support infrastructure seems well understood, but mitigation of this risk or technical solutions do not seem to be described. The quality of the biomass generated by this method would always present challenges for fuel use. Displacing fuel use by generating plastic alternatives is a practical and bright solution that is well aligned with BETO goals and real industry pull. Plan to establish contamination interception potential of polyculture based on mass balances seems to answer critical questions at an appropriate level of depth. Future investigations of the microbial community dynamics driving metal accumulation would be an interesting focus for future work in optimizing environmental service potential of the technology. Pairing environmental services with algae biomass production seems to be the most appropriate use of algae technology at this time. Approaching the challenge of harmful algal blooms means that this technology could be potentially impactful in rehabilitating and protecting this country's most precious freshwater resources. This outcome is more impactful than any fuel product development that has occurred as part of any AAS efforts. It appears that ash content is still a quality problem for many uses of this biomass. Regional inconsistencies of this material will also continue to challenge its potential as a fuel feedstock. Focus on improved bioremediation aspects of this technology seems appropriate for the current deployment landscape.

PI RESPONSE TO REVIEWER COMMENTS

- The presenter and full project team would like to thank the reviewers for their thoughtful evaluation of this effort. The primary concern about the presentation of the material was that the risks and mitigation points were not thoroughly conveyed. The risks were specifically discussed in Section 2 – Approach (slide 6); however, we understand that these should have been better demarcated among the presentation materials. To summarize, since this project included significant efforts on field deployments in remote/austere environments, the major risks include those arising from lack of physical security at the deployment sites (potentially resulting in equipment theft, vandalism), difficulties for consistent biomass logistics (potentially resulting in variable biomass quality assessment), and potential for environmental safety and health risks from source waters laden with runoff. These risks were mitigated by including multiple levels of deployment-local operations and management support, including algae subject matter experts for each site, to efficiently support systems operation and data collection/verification, respectively. The other technical concern is that the results from the seeded cultures in environmentally simulated (test bed) cultivation, which were found to provide improved productivity and ash reduction, may not be stable in outdoor settings. It is our intent to validate our test bed findings in future work at the field sites and determine any potential added costs and related trade-offs for culture seeding and maintenance.

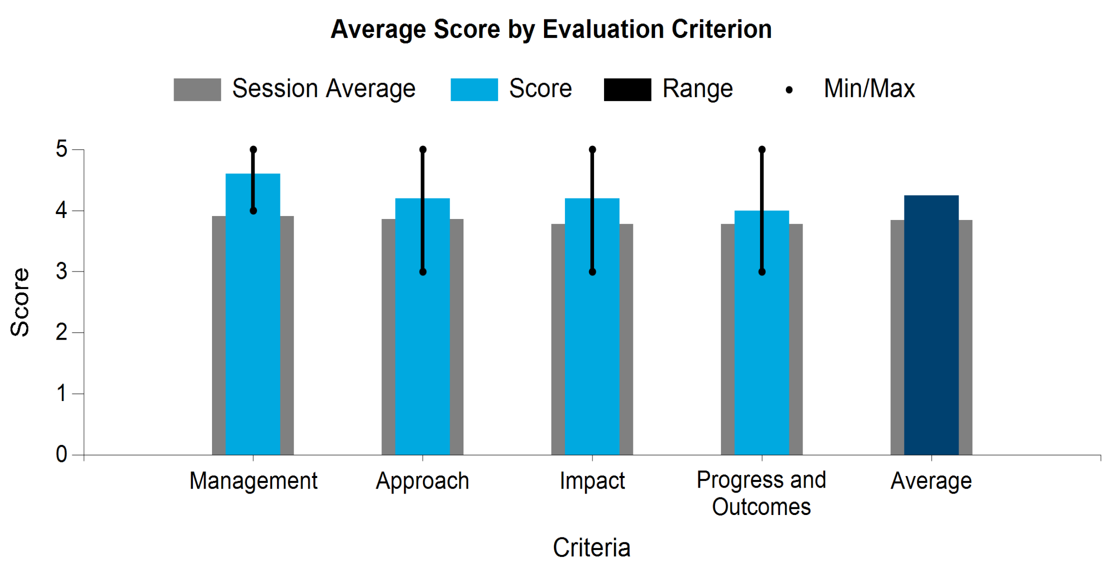
INTEGRATING AN INDUSTRIAL SOURCE AND COMMERCIAL ALGAE FARM WITH INNOVATIVE CO_2 TRANSFER MEMBRANE AND IMPROVED STRAIN TECHNOLOGIES

Colorado State University

PROJECT DESCRIPTION

WBS:	1.3.2.400
Presenter(s):	Ken Reardon
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	$2,897,618

Increasing the efficiency at which inorganic carbon can be delivered to cultivations and the rate at which algae can consume inorganic carbon are critical strategies for meeting BETO's productivity goals. The overall goal of this project is to demonstrate increased carbon utilization efficiency and areal productivity through enhanced delivery of inorganic carbon and improved strains of *Nannochloropsis oceanica* capable of higher rates of bicarbonate uptake and metabolism. Membrane modules containing immobilized, engineered carbonic anhydrase are used to transform gas-phase CO_2 into aqueous-phase bicarbonate. The effluent from this module contains high levels of inorganic carbon that are delivered to the algal pond at locations and rates guided by a detailed model of mixing, chemical speciation, and algal consumption. Since algal strains did not evolve in environments with high bicarbonate levels, we are using complementary approaches to develop *N. oceanica* strains capable of increased rates of bicarbonate uptake and metabolism. These new strains will be characterized in detail so the strategy can be developed further and transferred to other algal species. The combined strategies of improved inorganic carbon delivery will result in at least 25% higher carbon utilization efficiency, an areal productivity increase of at least 20%, and a cost savings resulting from increased carbon utilization efficiency.



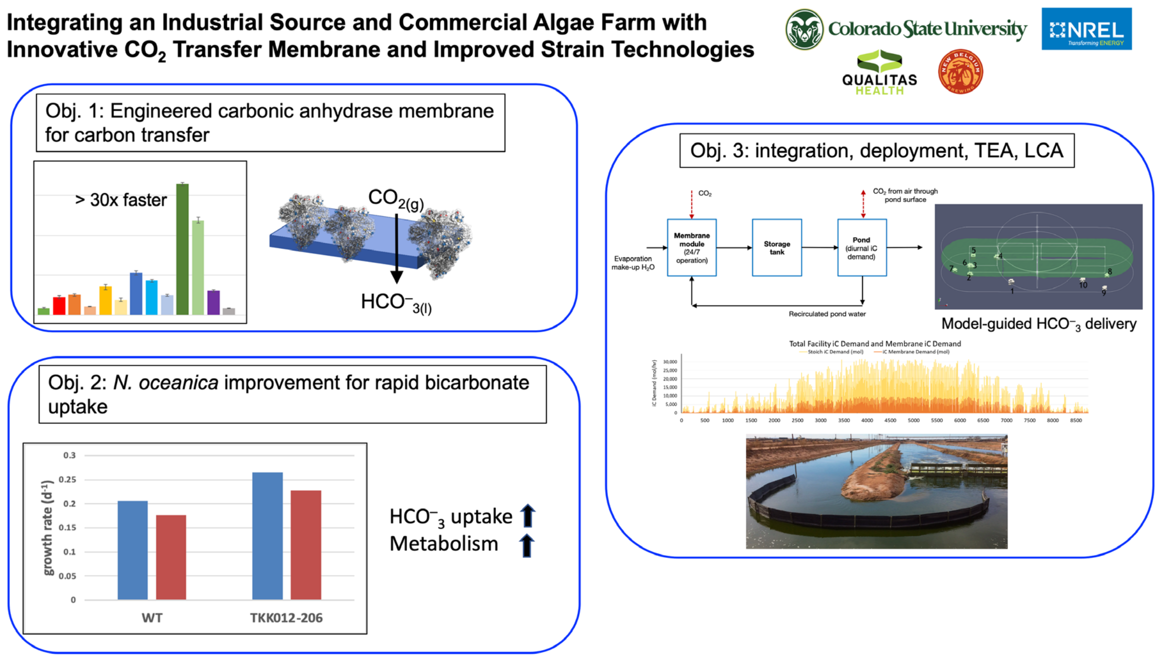


Photo courtesy of Colorado State University

COMMENTS

- Goal of novel membrane design is clearly stated. Collaborations and communications are defined. Risk management is straightforward. Task allocation is also clear. Putting a membrane on a substrate is certainly doable, and the protein engineering strategy looks promising. Trying many different carbonic anhydrases to find one that will be more stable in the conditions where it will be used is a favorable approach. This really needs to be tested outdoors. It would be good to see how the idea for membrane conjugation was developed as opposed to attaching the carbonic anhydrase to different kinds of scrubber tower packing media, ceramic beads, etc. Stable sequences were identified, attached to a membrane, and definitely seem to work. Milestones for flux and growth rates were achieved in bioreactors, and it's good to see carbon flow diagrams to figure out the best place to put the system in a raceway. Some cycling and reuse studies are definitely needed. It would be good to see if a membrane is really the way to go, and a study of the many other substrates that have more surface area per footprint, porosity, etc., that can be optimized the same way a smokestack scrubber and still packing materials are. Also, fouling and bacterial contamination need to be considered, as some critters will try to use the enzyme as a carbon source. The membrane will need to be cleaned without losing the metal coordination, which could be tricky, and is not as simple as washing it in caustic.

- The aim of the project is to increase carbon utilization efficiency by using an enzymatic membrane transfer system. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones and go/no-go decision points, providing an adequate assessment of progress for the project. The approach incorporates development of a novel carbonic anhydrase membrane that will enhance CO_2 dissolution into bicarbonate, strain improvements to enhance bicarbonate uptake, and deployment to the field with TEAs and LCAs. The approach is unique within the program and novel. Impacts of carbon losses and utilization efficiency were explained, along with impacts of higher productivities to the BETO goals. The team has identified active variants of carbonic anhydrase and a lab-scale membrane has been developed. A *Nannochloropsis oceanica* strain has been developed with increased carbon uptake capabilities at elevated CO_2 conditions. The team is modeling pond dynamics in CO_2 delivery and a carbon tracking system for the ponds has been developed. The presentation can be enhanced by more details on the molecular engineering approaches used and the carbon tracking system for ponds. The project is meeting all of its milestones to date.

- The management team seems well suited for collaboration and exchange of materials. The risk mitigation strategy is not clearly defined and therefore difficult to assess. The involvement of New Belgium as a partner allows access to real CO_2 waste streams and ensures that work products will be focused on application. The project structure based on clear, defined tasks followed by time dedicated to integration seems logical and facilitates risk mitigation well, with parallel and alternative efforts clearly defined. Integrating immobilized, engineered enzymes into a membrane seems like a sound solution for utilizing carbonic anhydrase to capture CO_2. Use of CO_2 storage demonstrates clear understanding of mechanical and biological interfaces and how to optimize that integration. Fluid dynamic models are not novel but do appear to capture critical information required to validate this system. Real profit-driven industrial partners make this work more impactful and likely to be used at a meaningful scale. Carbonic anhydrase improvements appear impactful, but more work will need to be done to achieve greater salinity and alkalinity tolerance for use as imagined. Baseline growth rates and CO_2 utilization efficiencies were not reported as defined metrics for this review, so it is difficult to compare the impact of this work to other work done in the portfolio. In the same way, improvements in enzyme activity and strain performance were not described in detail that included units, so it is difficult to measure the impact of reported progress. The stability of new proteins and constructs used seems unclear. Models seemed to be used to drive decisions in productive ways instead of simply scaling assumptions from the bench scale to the n^{th}-plant scale, and this will increase odds of project success. This project appears to be leading to an actual integrated system with commercial partners.

- This project addresses three critical areas that currently limit the viability of commercializing algae production at cost-effective, commercial scale. The first is to eliminate reliance on commercial CO_2, the second is to eliminate the requirement to co-locate the algae production alongside sources of available CO_2, and the third is allowing the use of any viable algae strain with commercial potential without worry of overly restrictive or disqualifying pH and other important media criteria. The materials presented show meaningful progress toward achieving project goals and remain aligned with the end-of-project milestone. COVID delays have been accommodated, and the balance of available schedule will afford the team the opportunity to move ahead with next steps for final evaluations and conclusions.

- This project focuses on increasing carbon utilization efficiency and biomass productivity through development and application of a novel enzymatic membrane transfer system and an engineered strain of *Nannochloropsis oceanica* that will increase carbon utilization efficiency by 25% and productivity by 20%. This project is in line with BETO milestones of developing an improved CO_2 utilization platform. The team has a clear management plan with a well-defined task structure leveraging expertise of team members. The team did not identify risk and mitigation strategies. Established channels of communication and collaboration amongst team members are outlined. The multiprong approach includes: (1) developing a membrane system for efficient transfer of CO_2 to water using engineered carbonic anhydrase with improved tolerance at $pH <7$; (2) engineering *N. oceanica* for enhanced carbon uptake; and (3) system integration, deployment, and validation using modelling, CO_2 monitoring and control, TEA, and LCA. The approach is ambitious but has a high potential for success given the team expertise, task structure, and management plan. The team has made significant progress toward their goals. They identified a highly active carbonic anhydrase variant with pH tolerance for $pH < 7$ and developed lab-scale membrane modules with milestone flux. They engineered a carbon concentrating mechanism mutant that demonstrated 29% increase in specific growth rate over the wild type at elevated CO_2 conditions. Some clarity is needed on conditions used to test the new strain, as it is critical that testing should be done at outdoor-simulated conditions. Outdoor testing of the integrated system will be critical to demonstrate success of the project. The team should address risk and cost involved in scale-up and maintenance of the membrane systems.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their thoughtful comments and suggestions. Regarding improvements in enzyme activity, data were presented on slide 22 of the presentation. Activity is shown as values of the rate parameter, k_{cat}, with larger values being better. As an example of the improvement provided by some new enzyme variants, the k_{cat} of variant E3 was >30 times that of the baseline bovine carbonic anhydrase at pH 6.3 and 35 ppt salinity. Nearly all of the suggested actions are on our road map for the final year of this project.

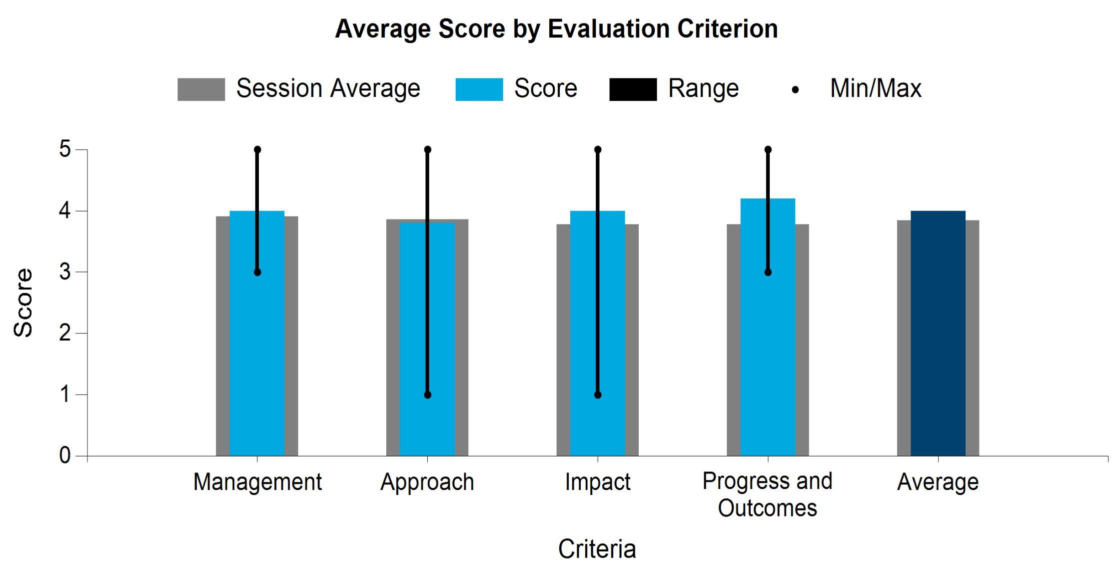
ENHANCED ALGAL PRODUCTION OF CARBONIC ANHYDRASE FOR IMPROVED ATMOSPHERIC DELIVERY OF CO_2 TO PONDS

J. Craig Venter Institute

PROJECT DESCRIPTION

WBS:	1.3.2.402
Presenter(s):	Andrew Allen
Project Start Date:	06/01/2019
Planned Project End Date:	06/30/2022
Total DOE Funding:	$2,500,175

Advanced algal cultivation and processing systems currently require access to a concentrated CO_2 source such as flue gas to support high productivity. The overall goal of this project is to develop a set of technologies that would enable high-productivity, open-pond systems to economically operate solely on CO_2 directly absorbed from the atmosphere during cultivation. This will be accomplished through integration of a suite of technologies that each accelerate the rate of absorption and associated productivity. These technologies include implementation of wave mixing to increase mass transfer, use of new strains adapted to high-pH conditions, incorporation of detailed information on carbon uptake to adjust cultivation conditions, and development of genetic tools and genetically engineered strains to decorate the surface of the algae with carbonic anhydrase or enable secretion of carbonic anhydrase by the algae. During this project, the objectives are to demonstrate 50% of the CO_2 needed for cultivation in the open raceway system can be obtained solely from the atmosphere, and to demonstrate that the genetic tools can double the absorption rate in laboratory cultivation, so that a path is identified to attain the final 50%.



COMMENTS

- Roles and responsibilities are well defined. Risk management is fairly straightforward. Communication details are great—data sharing method as well as meeting communications. It would have been helpful to see more here about how the carbonic anhydrase sequence was chosen and how it was decided to be used as-is—some sort of stability analysis. Regardless, it is an interesting strategy to advance DAC. There is a clear connection between approach and outcomes. Licensing and commercialization strategies are high quality. The milestones for productivity have been exceeded. There is concern that this cannot be done outdoors for very long as bacteria are going to use the secreted protein as a carbon source and that will be the end of it. Even at high pH there are plenty of bacterial contaminants that don't care. It will be metal limited to some extent as all carbonic anhydrases need a metal center.

- The details of the team's approach to transgenic line creation are not thoroughly explained and difficult to assess. It is difficult to tell how work done by Global Algae Innovations (GAI) developing small raceway ponds and testing mixing can be distinguished from similar work done in other projects. Media optimization work is not well described. Testing of commercially available carbonic anhydrase to accelerate CO_2 absorption also seems similar to work already done by this team. Using deflated growth rates of 6 $g/m^2/day$ as a baseline and 12 $g/m^2/day$ as a final target reduces the impact of this work when this organism is described to have a growth rate of 30 $g/m^2/day$ under optimal conditions. Exceeding this goal shows that there is potential to integrate this technology for greater overall impact in the future. Cell-driven carbonic anhydrase production has been a long-standing target for many teams. Understanding carbon capture mechanisms could be impactful to the industry. Assays for monitoring CO_2 absorption from carbonate are not novel, and this approach does not seem to improve the state of the art. Transgenic lines expressing carbonic anhydrase were developed and project goals have been achieved. The team plans to set new goals, demonstrating a commitment to project outcomes and bringing useable technology to fruition.

- The goal is to develop a system that achieves 12-$g/m^2/day$ algal biomass productivity using atmospheric carbon dioxide. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones and go/no-go decision points, providing an adequate assessment of progress for the project. The approach taken by the team is innovative, developing transgenic lines of *Picochlorum tricornutum* to produce carbonic anhydrase. These are then evaluated for productivity performance. The team has achieved the productivity targets using direct air as a sole source of CO_2. Project impacts were described from technology development and deployment and advancement of BETO goals. The team has shown that external addition of carbonic anhydrase will accelerate CO_2 dissolution from air that meets algae biomass productivity targets. The team is now working on generating transgenic lines of algae that will express carbonic anhydrase in situ. The team has advanced its goal to achieve productivities of 22 $g/m^2/day$.

- The project addresses a fundamental need for algae commercialization—the use of CO_2 captured from the atmosphere directly into the media in a predictive and controlled manner. The project elaborates on the underlying chemistry necessary in the media and algae to facilitate DAC. As with other GAI presentations, conclusions are presented, and project goals are stated as achieved but without supporting data or analysis.

- The project focuses on improving atmospheric delivery of CO_2 in algal ponds with no impact on biomass productivity using a multipronged approach—optimizing and engineering strains with carbonic anhydrase as well as optimizing conditions and mixing levels in ponds. This has clear relevance to BETO mission and MYPP goals. If successful, this project will show a decrease in the cost of CO_2 capture and delivery. The team has a clear management plan with a well-defined task structure and leads leveraging team expertise and previous experiences. The team identified risks, outlined mitigation strategies, and established channels of communication and collaboration amongst team members. Their multipronged approach in combining strain engineering, media, and process optimization is innovative and has a high potential of successfully improving DAC for algal biomass growth and productivity. The team was successful integrating the GAI strain and process at high pH on atmospheric CO_2 alone to achieve a productivity of 22 $g/m^2/day$, exceeding their target of 12 $g/m^2/day$. Testing was done in small raceways to mimic conditions of large ponds. The team should consider providing more clarity on the testing platform design and how this mini platform accounted for differences in mixing, light, and CO_2 profile in larger ponds. Overall, the team has made significant progress toward goals.

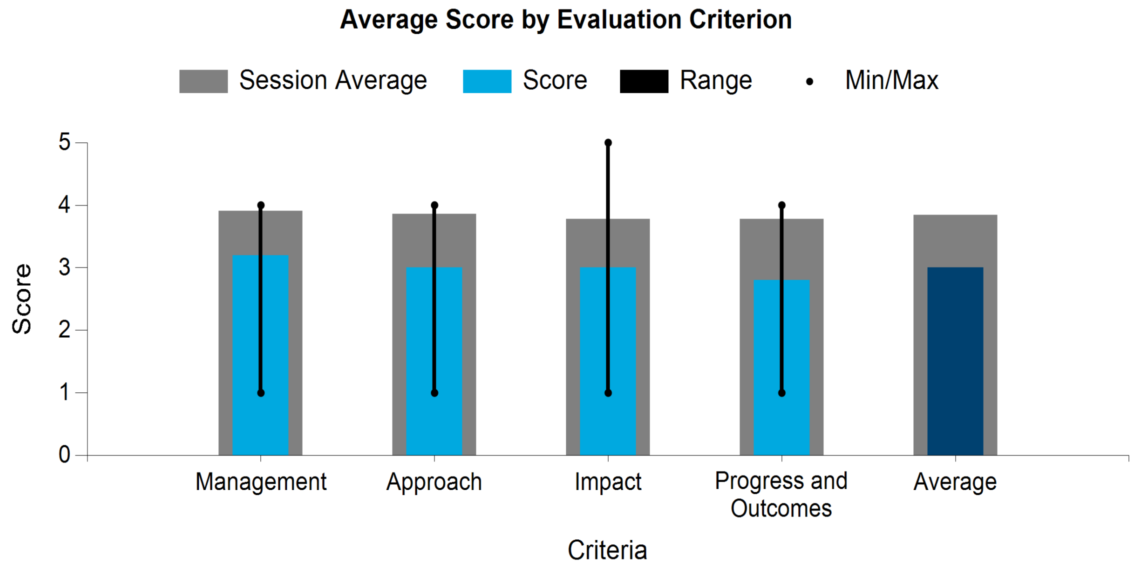
MULTI-PRONGED APPROACH OF IMPROVED BIOLOGICAL AND PHYSICOCHEMICAL SYSTEMS TO IMPROVING CARBON UTILIZATION BY CYANOBACTERIAL CULTURES

Arizona State University

PROJECT DESCRIPTION

WBS:	1.3.2.410
Presenter(s):	Wim Vermaas
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	$3,130,521

CO_2 is emitted as a waste byproduct from numerous process industries, notably including energy generation and bioethanol production. At the same time, CO_2 is also a key feedstock for advanced biofuel production by photosynthetic microbes, including genetically tractable cyanobacteria. Use of waste gas CO_2 for advanced biofuel production presents an attractive mechanism for reducing CO_2 emissions while simultaneously producing sustainable biofuels and bioproducts, examples of which are laurate (a fatty acid biofuel precursor) and methyl laurate (a drop-in biodiesel fuel). Despite the promise of this approach, poor rates and efficiencies of CO_2 absorption into the culture medium along with suboptimal biological CO_2 assimilation and fixation rates currently limit overall performance. To address this, we first increase rates of aqueous CO_2 absorption by addition of biocompatible amine solvents and a nanobubble gas delivery system. Amine solvents react with CO_2 as it enters the liquid, increasing its net solubility and thereby providing a significantly greater driving force for its absorption. Nanobubbles, meanwhile, reduce mass transfer resistance by greatly increasing the area for gas-liquid contact, as well as contact time between gases and liquids, supporting both faster and more complete transfer from gas to liquid. Rates of cellular uptake of dissolved inorganic carbon are increased through engineering of bicarbonate transport proteins for increased activity, while carbon fixation rates are increased by genetically "rewiring" cellular metabolism to increase the amount of CO_2 utilized during both day and night. Actual fermentation effluent gases derived from a brewery are tested as real CO_2 feedstock and performance in larger-scale growth systems under outdoor conditions are demonstrated. Integrated TEAs and LCAs are used to guide our approach and assess its viability. Overall, the outcomes of this research are anticipated to demonstrate at least a 50% increase in CO_2 utilization efficiency with improved advanced biofuel production under industrially relevant conditions. Outside the immediate scope of this project, the biomass may be converted to additional advanced biofuel by hydrothermal liquefaction, while valuable coproducts (natural or introduced) may also be extracted or synthesized to aid the overall economic viability of the process. This research is performed at Arizona State University by researchers on both the Tempe and Polytechnic (AzCATI) campuses, at Colorado State University, and at Nano Gas, Inc.



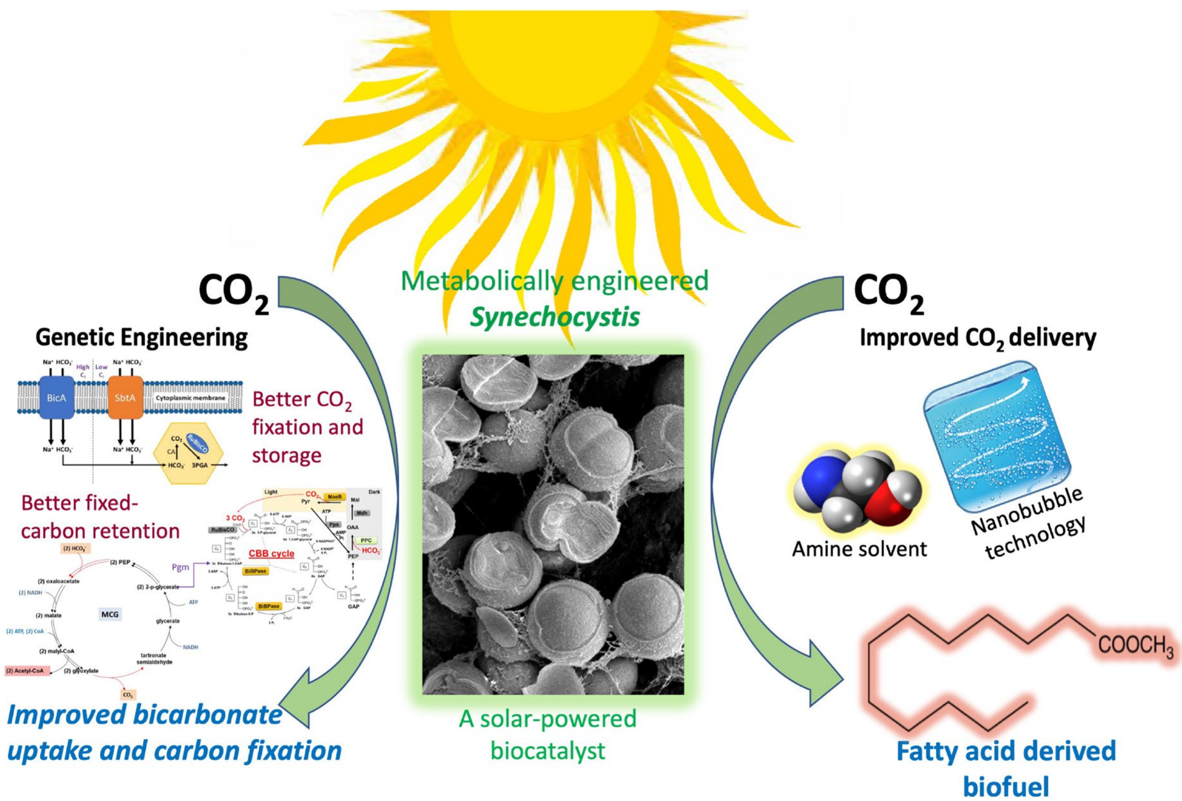


Photo courtesy of Arizona State University

COMMENTS

- It is unclear why the challenge of measuring CO_2 in the outlet was not addressed early in the project, even prior to it. This measurement is foundational to any work in this project. There appears to be no risk mitigation strategy generally in this project. The team does not seem aligned with any of the consortium partnerships that AzCATI has developed as part of the DISCOVR project and may be duplicating some efforts ongoing at Arizona State University. The inability to overcome the gas measurement challenges raises concerns about the technical viability of the effort. None of the approaches or methods used seem in any way new or novel. This project seems a bit like reinventing the wheel. The inability to accurately quantify CO_2 in the closed photobioreactor (PBR) system raises technical concerns. Baseline and target growth rates do not seem to be described in this work, but the small amount of cultivation data provided for best strains show growth rates well below the SOT. Use of amines in media may have potential to increase CO_2 utilization efficiency. Effect of amine addition to media was qualified but not quantified, so it is difficult to determine efficacy of this strategy. Use of BG-11 instead of an outdoor optimized media (such as the DISCOVR media) shows a lack of transferability or plan to scale this technology beyond the bench scale. Advances in this research seem more likely if the team is willing to partner or consult with others in this area. It is not clear how this project met its go/no-go milestone of demonstrating a 25% increase in CO_2 utilization if they could not accurately measure CO_2 off-gassing from the system. It does not appear this challenge was overcome. The reported operating pressure of 100 psi is described as a source of energy cost savings. It is not clear how those savings are calculated or achieved, and the result seems counterintuitive given the general industry drive to reduce operating pressures as an energy saving measure. Nanobubble generation did not seem to result in improved growth rate and therefore does not seem to result in greater CO_2 sequestration. Bicarbonate addition to media did not seem successful. Questions about the advantage of amine addition to media in diurnal light conditions appear to remain unanswered. The new strain does not seem more productive that the wild type. Strains were grown outdoors with reasonable productivity according to the team. All other outputs appear to be models of efforts with little application for future work in this project or for the industry generally.

- Roles and responsibilities are clearly defined. It would have been helpful to see more about risk management, with risks and their mitigation strategies listed. Sparger design is definitely useful for improving the volumetric mass transfer coefficient (k_La) of the system; multipronged approaches are good, but there doesn't seem to be a strong connection to TEA. It seems like something that should work really well in closed systems. Historically, though, when highly engineered strains are run outdoors, they don't do well. It is unclear how the bubbler's design affects the TEA. Productivity goals were not met, compared to baseline, which one would expect if sparging was the problem. There's a limit to what can be overexpressed and metabolically manipulated in the acetyl-CoA pathway before the cyanobacteria become noncompetitive with other contaminants, especially outdoors. It's unclear if sparging the media and feeding the media results in a perfusion type of culture system. It's also unclear how long this can be run before more media has to be added. It's unclear if this is a continuous process, or how this affects CapEx.

- The aim of the project is to test both physical/physiochemical and biological approaches to enhance carbon utilization, determine which one(s) are best, and then combine them. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The approach incorporates a novel system that utilizes CO_2 nanobubbles to introduce the gas into an amine bed to form carbamates, which deliver bicarbonate in solution due to their hydrolysis chemical equilibrium. The team will also improve the carbon transport and fixation mechanism in targeted cyanobacteria. They will take the best strains and integrate them with the CO_2 delivery system in outdoor PBR trials. The approach is novel and deemed viable. The amine/carbamate water chemistry and kinetics are crucial to the project and could be supported by kinetic modeling of the reactions to ensure that CO_2 dissolution and delivery as bicarbonate will be realized at an enhanced rate. Impacts of the technology development were adequately explained, along with impacts to BETO's targets and goals. The effort has shown that use of amines improves CO_2 utilization by 25%. The nanobubble generation system enhances CO_2 dissolution within the first 24 hours, but this is lost afterwards—an unexplained effect that will require modification of the nanobubble system. The addition of bicarbonate transporters to the strain was only partially effective. Adaptive evolution of a BiBPase overexpression strain that promotes carbon fixation is underway. Outdoor trial setup is underway. The team is wrestling with CO_2 uptake measurements. The team is developing Monte Carlo-based TEAs and LCAs that deal with uncertainty, which is seen as an excellent approach to remove bias from static inputs.

- The goal of this project is to isolate a biofuel-producing *Synechocystis* strain with a 15% increase in growth rate at laboratory scale outdoor-simulated conditions and supplementing with CO_2 from an industrial source. This project is of clear relevance to BETO mission and MYPP goals. The project performers seem to have a clear management plan while leveraging expertise from the different investigators. Risks involved in the execution of this project were not identified and mitigation strategies are not outlined. The team tested both physical/physicochemical and biological approaches to enhance carbon utilization in the context of scalable microalgal production. They demonstrated the use of amine solvents to improve CO_2 mass transfer into liquid and improved CO_2 utilization efficiency by 25% in biofuel-producing strain. This was done in laboratory conditions with continuous light that is not representative of outdoor testing. The team should provide some clarity for the difficulty in demonstrating amine advantage under diurnal conditions. Outdoor-simulated conditions are critical to demonstrating potential for success of this strategy outdoors. The strain engineered to have additional bicarbonate transporters did not show significant improvement over the baseline strain. Overexpression of BiBPase showed marginal growth improvement over baseline for the first 5 days. Overall, the team made progress in proposed work, but unfortunately, no significant improvement was demonstrated by the different approaches.

- The project is designed to advance some of the understanding of CO_2 utilization efficiency. This is acknowledged as challenging as well as being important for algae commercialization. It was difficult to gauge from the materials presented how the results were reflected in the LCA and TEA results, in part because baseline references were not presented. It is also difficult to gauge if any meaningful results have been achieved that can be used to advance BETO's MYPP or other goals. The project goal can be considered or otherwise accepted as being achieved as some important mechanisms have been determined. This is consistent with the complexity of the subject matter. The end-of-project milestone and some other relevant milestones remain outstanding.

PI RESPONSE TO REVIEWER COMMENTS

We thank the reviewers for their careful evaluation. Below are comments that hopefully clarify some points:

- Regarding difficulty in demonstrating the advantage of amine additions for CO_2 utilization under diurnal conditions: As indicated in the quarterly report covering January 1 through March 31, 2021, we now are able to demonstrate a >25% increase in carbon utilization with amines under diurnal growth conditions. We are continuing to improve carbon utilization with different compounds that are able to react with CO_2 to form carbamates.

- Regarding relevance to BETO's MYPP and other goals: CO_2 utilization is a key parameter for all algae cultivation. Our hope is that our work will help the entire algae community by outlining successful strategies to increase the CO_2 utilization efficiency.

- Regarding risks and mitigation: As the project evaluates the effectiveness of a number of different approaches, some individual approaches can be ineffective as long as other, independent approaches are effective. Ineffective approaches are inherent risks with a mitigation of discontinuation of that approach, after making sure that no potential/reasonable fixes have been overlooked and focus on successful ones. In this project, risks are very dispersed, and none of the risks by themselves will threaten the overall success of the project, which is to identify successful approaches toward increasing CO_2 utilization efficiency by algal cultures. Indeed, some approaches are successful or have an excellent chance of becoming successful, and we focus on those.

- TEA and LCA: TEA/LCA work thus far has focused on cost-effectiveness and greenhouse gas reduction related to CO_2 nanobubbles and fatty ester separation. Work is pivoting toward other new developments (e.g., amines) as new approaches and procedures are optimized toward the end of the project.
- Progress in terms of milestones, etc.: All go/no-go decision points thus far, including a 25% increase in CO_2 utilization efficiency under laboratory conditions, have been met. Most milestones have been met as well, with the remainder to be completed by the end of the project. As expected, some but not all approaches were successful, and the project currently focuses on those that are and those that we expect to be successful before the end of the project.
- Reliably measuring CO_2 utilization: We should emphasize that in this project we are able to reliably measure CO_2 levels in appropriate gas streams. The issue raised in the presentation simply was one of compounding error bars—CO_2 utilization is the difference between output from a culture and from a blank, and to establish CO_2 utilization improvements one has to compare CO_2 utilization from a control culture with that from an improved one. CO_2 utilization from the blank, the control culture, and the improved culture all have their experimental errors, and errors compound. We have successfully minimized standard errors and can reliably measure 25% improvements in carbon utilization. However, it is more difficult to determine whether small improvements (e.g., 10%) are significant because of the compounding error required for CO_2 utilization efficiency determinations.
- Novelty of CO_2 utilization efficiency approaches for algae cultivation: Several CO_2 utilization enhancement approaches in our project are novel. In addition to the novel application of CO_2 nanobubbles to support cyanobacterial growth, there is very sparse literature on the use of non-metabolized amines to enhance CO_2 uptake by algae. Moreover, several of the metabolic and regulatory pathways that we have engineered have not been applied to larger scale, diurnally grown cultures before.
- Providing CO_2 in nanobubble form: To the best of our knowledge, CO_2 nanobubbles have never been utilized in algae cultivation, and little is known about nanobubble properties and gas equilibration with the medium. Although a first approach of generating nanobubbles did not yield lasting improvement of biomass accumulation, CO_2 nanobubble generation with two different instruments seems promising and will be tested with cyanobacterial cultures soon.
- Integration with other BETO efforts at Arizona State University and elsewhere: AzCATI is looped in very well with ongoing BETO-supported algae cultivation efforts nationwide, and we are familiar with other algae efforts at Arizona State University, as group members are involved with these efforts; hence, synergies can be readily identified and made use of if they make sense. Note that this project is different in that it utilizes cyanobacteria rather than eukaryotic algae, and therefore there are some inherent differences, including the medium and pH used, relative to the DISCOVR project, for example.

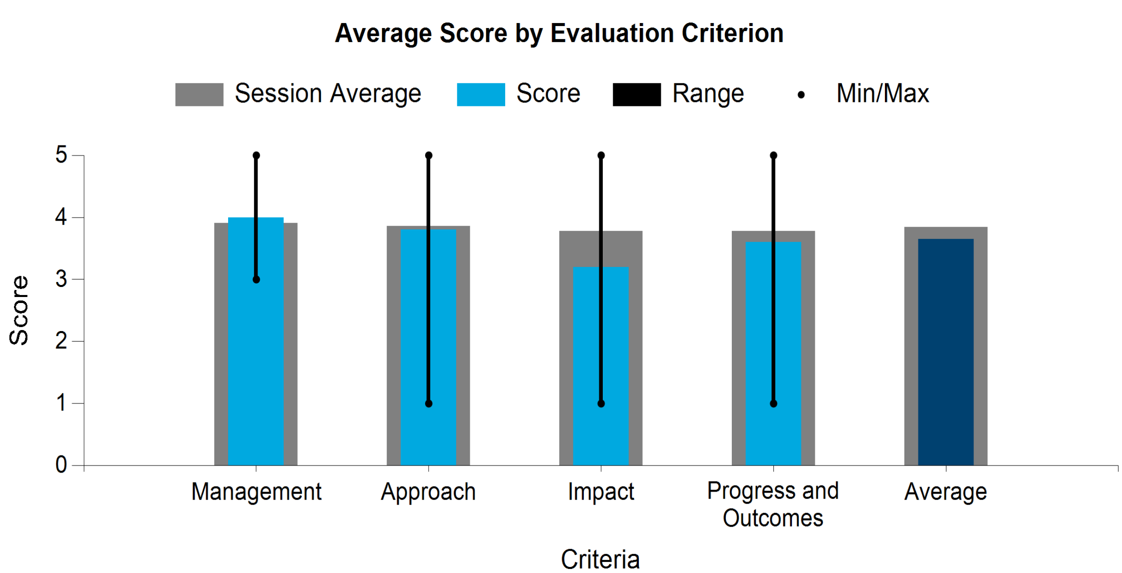
MEMBRANE CARBONATION FOR 100% EFFICIENT DELIVERY OF INDUSTRIAL CO_2 GASES

Arizona State University

PROJECT DESCRIPTION

WBS:	1.3.2.430
Presenter(s):	Bruce Rittmann
Project Start Date:	10/01/2018
Planned Project End Date:	12/31/2021
Total DOE Funding:	$2,490,971

Microalgae are a promising platform for producing renewable transportation fuels and products in place of petroleum-based products. Increasing the carbon dioxide (CO_2) concentration in gas supplied as a feedstock to grow microalgae can improve its productivity many fold over using atmospheric air. Many industrial gases contain concentrated CO_2, but with widely varying CO_2 concentrations (5%–80%) and accompanying gases. Traditional methods for delivering CO_2 to microalgae include bubbling in the CO_2, which is only 30%–50% efficient. Because collecting, cleaning, and distributing the CO_2-bearing gases incur costs, CO_2 delivery to the microalgae needs to be highly efficient. This project's goal is to develop and demonstrate strategies for using membrane carbonation (MC) technology to deliver CO_2 from industrial sources with 90% efficiency. In MC, CO_2 is delivered inside straw-like hollow fibers that are submerged in an algae pond. The CO_2 is pulled through the fibers into the pond as it is consumed and removed from the pond by the microalgae during photosynthesis, leaving behind other gases inside the fiber. For anaerobic digester-produced biogas, CO_2 is removed leaving behind concentrated methane, which can be used as natural gas for generating electricity. MC will significantly reduce CO_2 lost to the atmosphere compared to traditional bubbling, which will significantly reduce cost and emissions that contribute to climate change.



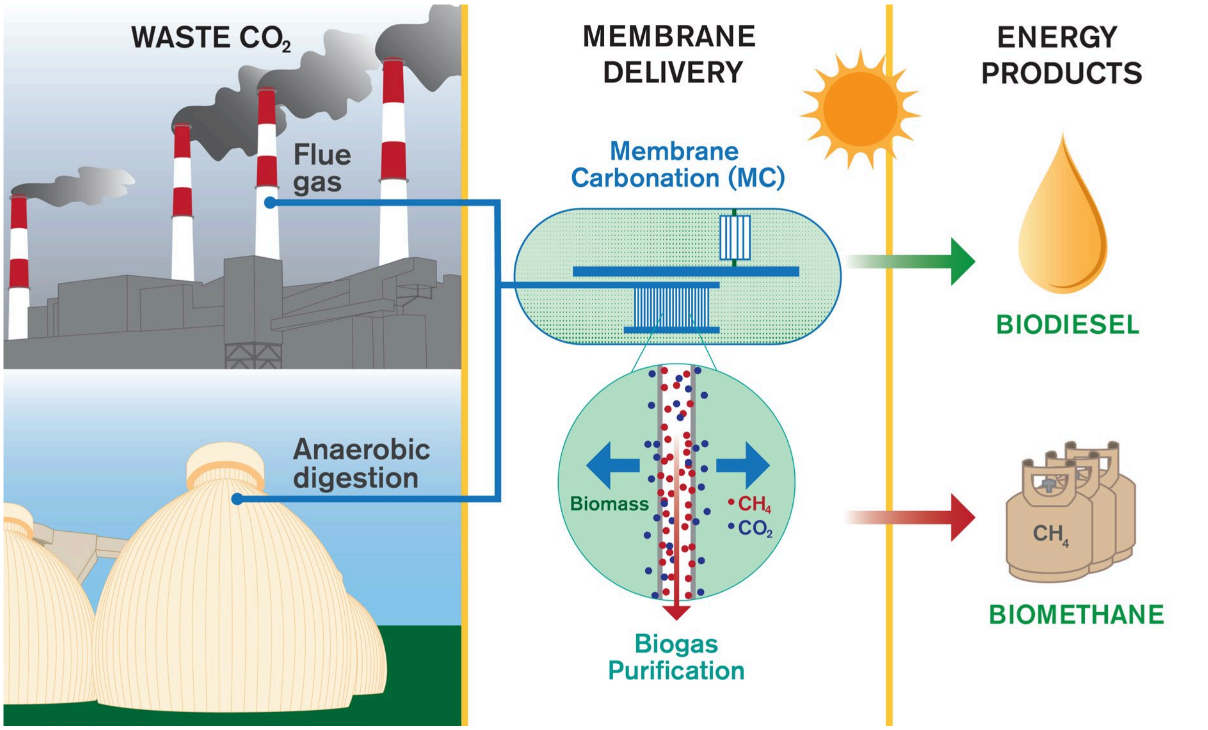


Photo courtesy of Arizona State University

COMMENTS

- Project goals are clear, and risk management is defined. It would have been good to see more about roles, responsibilities, and communication here. New sparging techniques to improve k_La are definitely needed. It is especially good that the system is modular and continuous, which makes it amenable to implementation at various scales. Connection of approach to outcomes is very good, especially due to the varying scale of different biogas implementations. The commercialization potential is obvious, as it has been implemented in a municipal facility, which for all intents and purposes really is the commercial scale expected. Congratulations on the successful implementation in the municipal digester system. The increased productivity outdoors is an impressive accomplishment. Hollow-fiber membrane biofouling was not observed and hypothesized as a result of pH diffusion gradients; fouling due to flue gas particulate may become problematic, and TEA would be affected if additional filters are needed for this. Hollow fibers are getting a bit hard to find these days, so many manufacturers are moving to flat sheets. It's easier to solvent-cast flat sheets than extrude fibers, and there are not many efficiencies of scale there. Biogas digesters are becoming more and more common, though; there are several local governments funding them and it would be great to have some economic offset. It may not be necessary to model a facility larger than an average size landfill; realistically, that's the size to be aimed for. Between landfill and farm biogas, it seems like there would be many commercial applications for this.

- The goal of the project is to demonstrate membrane carbonization using biogas and flue gas with >25% improvement in carbon utilization efficiency over sparging. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones and go/no-go decision points, providing an adequate assessment of progress for the project. The approach taken by the team includes abiotic and biotic evaluation of synthetic flue gas and biogas, outdoor cultivation, modeling to optimize experimental conditions, and TEA. The approach incorporates go/no-go at 21 months with metrics, which have been met or exceeded. Impacts were described with the technology development creating high increase in carbon utilization efficiency, purification of biogas for methane, and cost savings. If successful, the project will increase the 2018 state-of-technology productivity over 10%. The team has made progress on all aspects of the technology development and testing and will be making a demonstration using anaerobic digestors at the Mesa Wastewater Treatment Plant using 25-m^2 ponds. The discussion focused on a membrane fouling concern, which was addressed. Further concerns with sufficient availability of CO_2 from the wastewater anaerobic digesters were not addressed.

- The project is premised on addressing the very real challenges facing algae commercialization regarding the cost and quality of CO_2 and the CO_2 sources. The results presented demonstrate the technical viability of using membrane technologies to capture CO_2 from waste streams for use in algal production. The availability of raw CO_2 present in flue gases and in the off-gases generated by anaerobic digestion have been targeted as possible sources of CO_2 for algae growth and potential commercial sites. The need to rely on these sources of raw gas in practical terms requires co-location with the generating facilities, or at the very least, in very close proximity. This requirement has a practical effect of severely limiting the number of locations that may be able to have the needed site requirements to accommodate a 5,000-acre pond system and also have an existing or otherwise deployable CO_2-generating facility. In the case of municipal wastewater gases generated from anaerobic digestion, there is already a beneficial reuse for many treatment facilities that derive power from these gases to offset other plant requirements. There are existing policy and environmental practices that encourage wastewater plants to use their anaerobically generated gases for these purposes. Algae facilities may be forced to otherwise compete for these types of gas sources.

- The project team seems appropriately small and well suited to this task. Research seems focused on finding an application for the very good membrane diffuser. The most critical carbon capture component of the work seems only tangentially related to this membrane diffuser optimization. This membrane diffuser seems to be an advance over many currently used gas sparging systems. Other work done in association with this project seems to be less focused on by the project team and may result in little impact. This work does not seem to address the most challenging aspects of CO_2 utilization. It appears that an off-the-shelf membrane has been optimized to replace traditional gas sparging units in algae cultivation. This was a deliverable for past work that has already been completed. It is not clear that this advance will result in lower production costs, and distribution and storage of CO_2 are not addressed in current work. It appears that almost $2 million was spent on continuing this project with unclear distinction between past activities and current scope of work aside from demonstration at the Mesa Wastewater Treatment Plant.

- This project focuses on increasing carbon transfer and utilization efficiencies by using membrane carbonation for 100% efficient delivery of industrial CO_2 gases. This project supports the MYPP milestone of developing technologies for CO_2 utilization that increase utilization efficiency of delivered CO_2 by 25% over the 2019 baseline. If successful, the project will advance the SOT by providing an efficient way of delivering flue gas or biogas into biomass and selectively removing residual gases like methane for other applications and hence increasing the value of industrial gases. The team had a clear management plan with a well-defined task structure and leads leveraging team expertise and previous experience. They identified risk and outlined mitigation strategies. The team's approach to initially evaluate a CO_2 delivery approach for biotic and abiotic assessment at lab scale using a hollow-fiber membrane to optimize flux target followed by outdoor testing is reasonable. Hollow-fiber membranes are efficient but subject to fouling. The project did not address how fouling will be managed or the impact on cost. It's not clear from the project if indoor testing was conducted in outdoor-simulated conditions, as this will impact CO_2 utilization. The project tasks also included developing mathematical models to help guide design of the fiber module, as well as TEA and LCA to track supply and delivery cost of CO_2. Overall, the team made significant progress toward the goals mapped out. Using membrane carbonation for CO_2 delivery demonstrated an increase in carbon transfer efficiency and carbon utilization efficiency over sparging, enriched biogas effluent for CH_4, and developed models and LCA.

Outdoor cultivation was done using 4.2-m^2 raceways at AzCATI and demonstrated improved carbon transfer efficiency and carbon utilization efficiency. The team did not address the impact of their CO_2 delivery strategy on biomass productivity during outdoor testing. The team should consider addressing industrial deployment of the membrane system, as well as cleaning and maintenance of the membrane.

PI RESPONSE TO REVIEWER COMMENTS

- We kindly thank the reviewers for their time and feedback on this project.
- Response to Reviewer 1 – Scope of work: We would like to clarify that MC technology is a bubble-free gas-transfer process; thus, MC is quite different than a diffuser, which still generates bubbles that can escape to the atmosphere with high CO_2 losses. The scope of work in this project is focused on how to use MC to efficiently deliver CO_2 from a wide range of industrial gases (see Table 1 below) such that it is utilized by the microalgae and captured into the biomass. This scope significantly expands on previous work that primarily evaluated MC technology for delivering highly concentrated (90%–100%) CO_2.

Table 1. Common Sources of Concentrated CO_2

CO_2 Source	Typical CO_2 Percentage
Power-plant flue gas	4–15
Biomass combustion	15–35
Biogas from anaerobic digestion	35–40
Biogas from landfills	50
Cement kiln	50
Ethanol fermentation	80–100

Reducing biomass Cost: To date, MC technology has been able to achieve carbon transfer efficiencies across the membrane into the bioreactor of 90%–100%, which is about threefold higher than sparging-based methods and will have a significant impact on reducing costs for producing biomass, since CO_2 is the most costly nutrient. Although distribution and storage are beyond the scope of this project, they are considered in techno-economic modeling to determine the cost savings at scale. It is worth noting that some wastewater treatment plants have on-site tanks for storing raw biogas until it is utilized for peak power generation or discharged using a flare, which could be diverted for cultivating microalgae during daylight hours.

Carbon utilization: The project team has carefully conducted mass balances to determine carbon utilization efficiency in indoor (Task 3) and outdoor (Tasks 6–8) cultivation, including sparged control systems and more accurate methods for determining CO_2 off-gassing in open ponds. These methods have revealed that off-gassing occurs below pH 8, and that such losses also occur during delivery with traditional sparging methods.

- Response to Reviewer 2 – Fouling/maintenance: The hollow-fiber membranes have been operated for up to 6 weeks outdoors without requiring more than routine maintenance. They have been operated indoors for much longer times and without evidence of fouling except during a severe culture "crash." Outdoors, we have utilized two levels of routine membrane maintenance: (1) Rinsing the membranes with tap water removed nearly all culture that had settled on to the membrane; the ability to easily wash off the culture showed that fouling was minimal and that the culture was most likely settling in the small raceways. (2) Bleaching: When a culture crashed (not related to CO_2 delivery), the membranes were washed with bleach with the rest of the ponds. Bleach treatment showed no significant change in MC performance.

Membrane rupture: One concern is the potential for fibers to rupture. We have not observed membrane rupture in this project. Furthermore, when a fiber ruptures, the net flow of gas escaping the single fiber is extremely small relative to the total gas flow. Thus, many fibers would need to rupture to result in a net change in carbon utilization efficiency >1%. As part of the project, we also are evaluating the performance of the hollow-fiber membranes by tracking the flux during the culture performance by tracking the amount of CO_2 transferred and the duration that CO_2 transfer is occurring. This allows us to estimate the daily CO_2 flux, which showed no change from the beginning to end of the experiments.

Industrial deployment: This is addressed in responses to feedback from Reviewers 3 and 5. Key feasibility data will be achieved during upcoming trials at the City of Mesa's Wastewater Treatment Plant using raw anaerobic digester biogas.

Productivity: The critical element in optimizing the CO_2 delivery strategy is to improve the carbon transfer and carbon utilization efficiencies of the delivered CO_2. Optimizing the CO_2 delivery strategy will not improve biomass productivity on its own. When considering the biomass productivity as it relates to CO_2, the major driving force is the available CO_2 in the medium, which is based upon the pH and alkalinity of the media, as well as the harvesting strategy; these factors are outside of the purview of this research, which is focused on MC.

Indoor cultivation: The indoor cultivation work allows us to rapidly test various cultivation and CO_2 delivery parameters without the vicissitudes of weather and the extra costs of working at larger scale. We use the indoor results to set conditions for outdoor cultivation experiments. For example, (1) higher pressure indeed improved carbon transfer efficiency for flue gas, which was used to set pressures to greater than 15 psig for outdoor experiments; and (2) pH set points significantly influenced carbon transfer efficiency and carbon utilization efficiency, which we used to increase the pH set point (up to pH 7.9) for outdoor cultivation trials. While pH at 7 can promote growth rates, it leads to more CO_2 off-gassing.

- Response to Reviewer 3 – Locations of concentrated CO_2 suitable for on-site microalgal cultivation: While not every microalgae installation can be located near an industrial source of concentrated CO_2, the wide variety of sources indicates important potential for finding a good source. Table 1 above summarizes the many types of CO_2 sources and their typical CO_2 percentages. Further, DAC systems that capture CO_2 from air can further extend beyond these locations, which can produce a range of CO_2 concentrations, depending on cost.

  Biogas: For two reasons, particular attention should be paid to biogas. First, biogas is generated in many settings that are widespread across the United States (e.g., municipal wastewater treatment plants, concentrated animal feeding operations, dairies, and sanitary landfills). Second, biogas as a source of CO_2 provides a double benefit: concentrated CO_2 to improve microalgae productivity and biogas upgrading to biomethane. For this reason, our team is partnering with the City of Mesa to evaluate using biogas at one of the city's wastewater treatment plants. This strategy is underscored by the comments of Reviewer 5. It is worth noting that although some wastewater treatment facilities utilize existing biogas for power generation, the high concentration of CO_2 makes this process very inefficient. Utilizing the microalgae to remove the CO_2 will significantly improve the quality of the residual biomethane for power generation or delivery as a renewable fuel. Thus, biogas-generating facilities will be able to comply with existing policies and better utilize their own gases.

  Sizing microalgae cultivation systems for utilizing CO_2 from anaerobic digesters: A good question is about the size of a microalga operation that can be supplied by a source. Our team has evaluated this for the City of Mesa, with whom we are doing a field pilot at the city's Northwest Water Reclamation Plant, which has anaerobic digesters. Those digesters produce approximately 6.8×10^7 standard cubic feet of biogas per year, and its CO_2 content is 40%. That means that it produces about 2.7×10^7 standard cubic feet of CO_2 per year. Assuming a conservative 80% CO_2 capture into biomass, the algae biomass production would be about 600,000 kg dry weight/year. At an average annual productivity of 20 g dry weight/m^2/day, the required "wet area" would be about 21 acres, and the total area would be about 26 acres. Treatment plants often have open land surrounding them, and the City of Mesa has a 27-acre site adjacent to the treatment plant; hence, the city is most interested in the results of our study. The Northwest Water Reclamation Plant serves a population of about 200,000 people. Per-capita normalization of the wet-area requirement gives about 10,000 people per acre of microalgae cultivation. Anaerobic digestion of animal wastes and food-processing wastes offer even greater potential to generate the double benefits of CO_2 for microalgae and upgraded biomethane. The biogas potential from anaerobic digestion of animal wastes is approximately 55-fold larger than for anaerobic digestion at wastewater treatment facilities, and the potential is 19-fold greater for wastewater from food processing.

- Response to Reviewer 4 – The concerns of this reviewer on sufficient availability of CO_2 from wastewater anaerobic digester gas are addressed in the response to Reviewer 3.
- Response to Reviewer 5 – Roles and responsibilities: The project team meets every 2 weeks and communicates regularly by email and Dropbox document sharing. Expanding on the roles indicated on slide 5 of the presentation, Bruce Rittmann is the principal investigator and is responsible for directing the overall project and ensuring the vision and goals remain the focus of the project. Justin Flory is the project manager and will help coordinate resources and facilitate communication between the team to ensure all resources are being utilized effectively to resolve issues and reduce project risks. Everett Eustance is in charge of outdoor cultivation work in coordination with John McGowen. Yen-Yung Lai is responsible for indoor abiotic and biotic experiments with support from Zoe Frias, and for conducting microbial community analysis in coordination with Rosa Krajmalnik-Brown. Michele Young is responsible for gas transfer modeling using COMSOL, building on previous work from Eustance using Excel-based models. Robert Stirling is responsible for techno-economic modeling, and Jason Quinn is responsible for life cycle modeling.

  Particulate fouling: In evaluating coal-based flue gas, particulate matter has the potential to cause fouling to the fibers. However, all power plants have methods to remove particulate matter that would be utilized prior to delivering the flue gas to the algae. This is a common practice in current slipstream operations at coal-based power plants.

  Commercialization: The existing work on this project uses hollow-fiber membranes that span a module that can be placed near the bottom of the raceway. Commercial systems deployed for delivering hydrogen gas in membrane biofilm reactors for water treatment applications use woven sheets of hollow fibers inside cylindrical modules. Similar woven sheets would enable delivery using flat sheets in a raceway and that may be worth evaluating for large-scale ponds. Our current technology-to-market activities are focused on assessing what a "first customer" scenario would look like in terms of supply contracts. Moving beyond these types of analysis to securing or manufacturing large volumes of hollow-fiber membranes would require developing relationships with contract manufacturers and a significant entrepreneurial effort in sales, product design, installation, and support, all of which are beyond the scope of the current project.

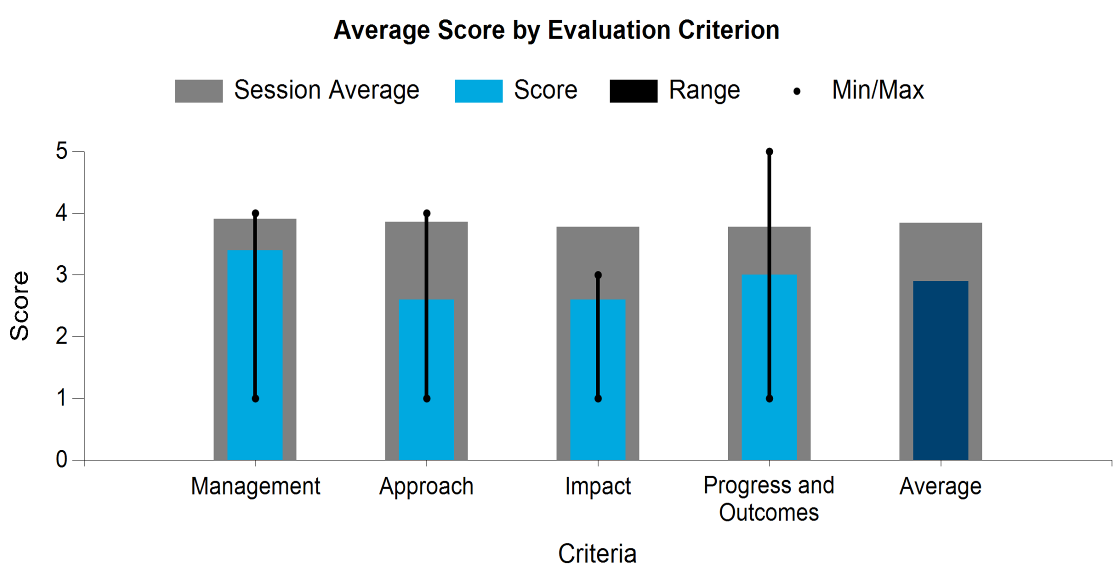
CARBON UTILIZATION EFFICIENCY IN MARINE ALGAE BIOFUEL PRODUCTION SYSTEMS THROUGH LOSS MINIMIZATION AND CARBONATE CHEMISTRY MODIFICATION

Duke University

PROJECT DESCRIPTION

WBS:	1.3.2.440
Presenter(s):	Zachary Johnson
Project Start Date:	10/01/2018
Planned Project End Date:	03/31/2023
Total DOE Funding:	$1,891,023

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



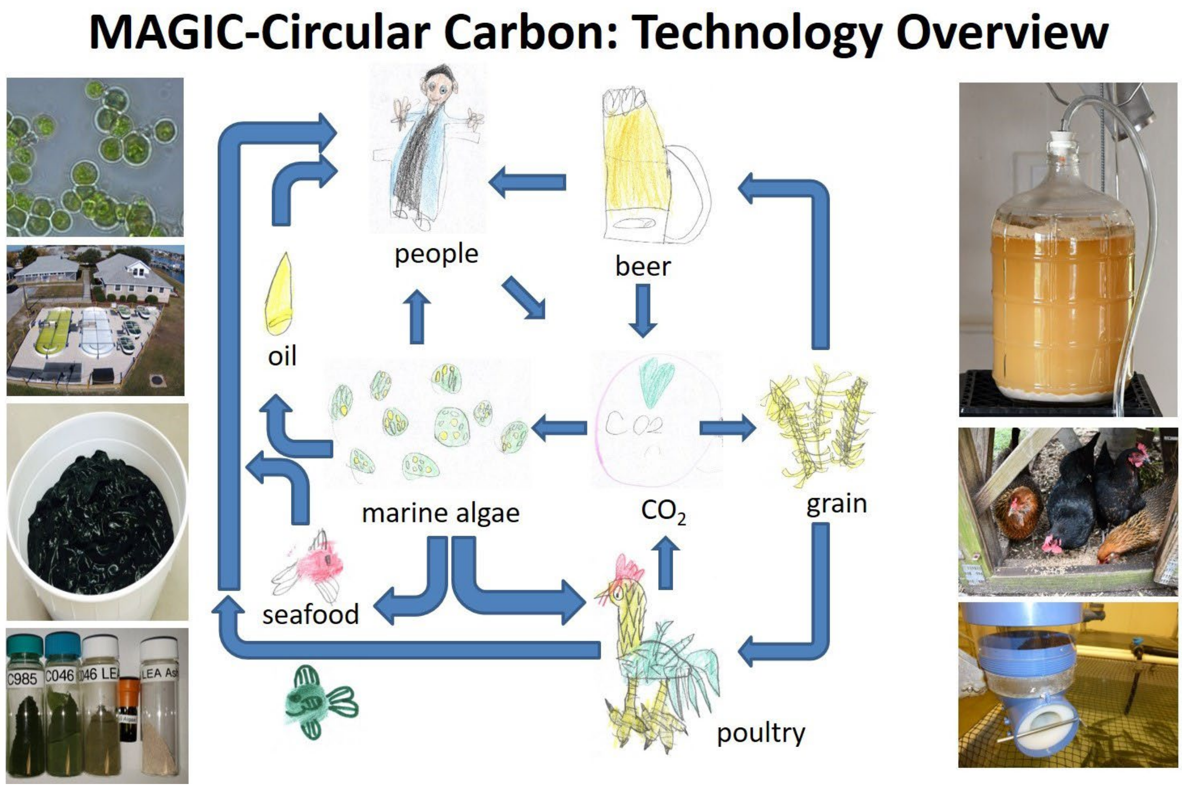


Photo courtesy of Duke University

COMMENTS

- Goals are clearly stated, and communications, including data sharing and task assignment, are well defined. It would have been helpful to see more about the project team's roles and responsibilities. The mass balance seems counter to the funding agency goals; instead of removing CO_2 from the air, this project will result in adding more CO_2 to the air. Even assuming some can be recaptured, the end process will be inefficient and result in releasing more CO_2 that is currently sequestered underground. The goal of being able to cultivate algae in areas where there is no flue gas, brewery waste carbonic acid, or other carbon capture technology in place is clearly tied to the research, but ideally this would be done via direct air capture, as opposed to feeding mined materials. This needs at least a plan to trial outdoors in open ponds; the diagram seems to indicate a closed system, which has serious consequences for the TEA if it cannot be run in raceways or open ponds. Although the need for flue gas has been decoupled, the effort is still coupled to brewery activities at the moment.

- The aim of this project is to demonstrate enhanced algal growth with reduced CO_2 requirement at scale. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The approach was described based on four major tasks for strain assessment in high-alkalinity systems, CO_2 conversion to bicarbonate using a $CaCO_3$ starting bed, demonstration of the system, and TEA/LCA modeling. The use of a carbonate bed to enhance CO_2 dissolution into bicarbonate is innovative and unique. The presentation would have benefited from a more logical approach by discussing the water chemistry right away and how the carbonate bed catalyzes the dissolution of CO_2, and then discussing what is known of strain stability under these conditions. The impacts discussion was limited to justifying the work. This can be enhanced through a broader discussion of CO_2 sources envisioned and impacts on emissions, along with impacts to the BETO and DOE goals in productivity enhancements, etc. Progress for the project is underway and appropriate, as it is at initial stages. The strain assessment is in process. The discussion would benefit from more specifics as to what strains are being looked at and extensiveness of the search. Water chemistry in a carbonate bed and a bicarbonate generation system are being developed, along with the collection of CO_2 from brewery systems. They are modeling expected losses of CO_2 in large-scale farms.

- The goal of this project is to demonstrate enhanced algal growth with overall reduced CO_2 requirement at an industrially relevant scale. If successful, the proposed work will have benefits to the algal industry and is well aligned with BETO mission and MYPP goals. The team outlined a management plan with a defined task structure and leads leveraging team expertise and previous experiences. They identified risk and outlined mitigation strategies, as well as established channels of communication and collaboration amongst team members. The team did not outline measurable goals. It's not clear if the proposed work will result in an increase in biomass productivity over baseline using their strategy since algal strains did not evolve in environments with high DIC levels. Their approach of assessing strains that require low CO_2 for growth, growth enhancement on high-DIC waters, and developing CO_2 conversion process to DIC is reasonable. However, the team should consider providing clarity on how strains will be selected and if selected strains grown on low pCO_2 will demonstrate similar or higher biomass productivity compared to current SOT. The team should address how they intend to promote CO_2-limestone contact to increase dissolution kinetics at scale using brewery wastewater, and if this will be a stand-alone system or integrated with a pond. The team should also consider outdoor testing to validate the proposed integrated process. Overall, progress has been made toward the outlined goals, but there is still some work to do to meet the end-of-project milestone.

- The need for collaboration with both B&D Engineering and Bucknell University in TEA and LCA tasks does not seem clear. The approach of splitting this work among institutions seems to present unmitigated risk of model discontinuity. The lack of commercial partnerships in the project seems to limit the work to an academic exercise. The use of limestone with high dissolved organic carbon in media seems to be an approach unique to this project. Even knowing that the team's LCA model validates this approach, it is conceptually difficult to embrace the active volatilization of sequestered carbon proposed as a component of a carbon capture and utilization strategy. An independent third-party assessment of this technology compared to other state-of-the-art design concepts may be especially useful in this case to help ameliorate this concern. Finding optimal partial pressure for CO_2 uptake may have impacts for bioreactor design and help pair strains with changeable reactor conditions. n^{th}-plant assumptions are typically less impactful than those developed with commercial production partners. TEA and LCA models developed in this project do not seem to directly integrate with models developed by the national labs or be aligned with commercial partner expectations with any product specificity. Modeling based on existing facilities or with a specific product target adds impact to these results. Baseline growth rates and CO_2 utilization efficiencies were not reported as defined metrics for this review, so it is difficult to compare the impact of this work to other work done in the portfolio. The only results of this work appear to be models based on benchtop experiments. Plans for construction of a pilot-scale converter for use in a 100-L raceway pond seem unclear. It does not appear that there is any plan to work with algae in this system during the project's period of performance. End-of-project milestones seem to lack clear metrics but do not appear close to being met by September 30, 2021.

- The project presentation provides nice detail and supporting information that documents their achievements and progress toward project goals and end-of-project milestones. It is not clear if practical strains were tested in high-DIC media. Additionally, much of the experimentation was conducted using limestone as a generator for DIC without the practicality of using limestone for the same purpose at any relevant commercial scale. The implied outcome still requires some co-located industrial source for reliance of CO_2 or CO_2 conversion. The project clearly achieves many of its goals, but additional work is likely needed to achieve the end-of-project milestone demonstrating enhanced algal growth on high-DIC water at an industrially relevant scale.

PI RESPONSE TO REVIEWER COMMENTS

- Comment 1: Dr. Beal and Professor Sills have been co-authors on seven peer-reviewed manuscripts over the span of 6 years. Although working across institutions can present challenges, this risk is managed overall. A private company is part of our team, and overall, the project is an investigation of a commercially promising system to grow algae. This project does not involve carbon capture. The use of $CaCO_3$ was proposed because it's cheaper than conventional CO_2. Every carbon source has different LCA implications. The fossil carbon contained in $CaCO_3$ that is used to grow algae and eventually released during combustion/metabolism is included in our LCA. We are currently working on TEA/LCA that will directly compare the impacts of a range of carbon sources (e.g., power plant flue gas, pure CO_2 flue gas, enhanced oil recovery pipeline CO_2, bottled chemical plant CO_2, limestone) and energy sources (e.g., grid, solar, wind), and intend to publish the results by 2022. (Note that our proposal reports the greenhouse gas impact for this system to be 3.2 kg CO_2e/kg algae, which is huge—about 10 times more than soybeans. The fossil carbon from $CaCO_3$ was the largest source of these emissions. However, we also assumed brewery CO_2 with no LCA impact for half of the carbon, and our baseline case assumed fossil carbon from a pipeline, which was even worse. Using flue gas or DAC carbon would be lower in greenhouse gases). The referenced manuscript in preparation will address concerns about transparency and be reviewed by multiple "third parties" (i.e., reviewers), similar to our publications. The national labs have developed numerous TEA/LCA models for algal biofuels, spanning a huge range of cultivation conditions, conversion methods, and upgrading processes to produce a range of fungible fuels in a variety of scenarios. However, several of the NREL algae models are actually partially based on our models, such as Clippinger and Davis's *Techno-Economic Analysis for the Production of Algal Biomass via Closed Photobioreactors* and Davis et al.'s *Process Design and Economics for the Production of Algal Biomass*. Modeling a lab facility can be useful for informing commercial models, but yields results that are not representative of commercial production. The specific product that we are targeting is high-quality algal biomass for fuels and feeds. We are on track to meet our milestones by September 2021.

- Comment 2: Both (1) identification of strains that grow on low pCO_2 and (2) enhancement of growth on high-DIC waters were identified as goals in the presentation. The presentation included preliminary results and progress on both milestones (slides 6 and 7). All strains tested were downselected from promising biofuel candidate strains identified by us and other groups (e.g., DISCOVR). Thank you for the additional comments on CO_2 conversion. We are actively pursuing approaches to optimize this system for integration with commercial-scale CO_2 sources (e.g., brewery).

- Comment 3: We agree that the bulk of our experiments to date have been at laboratory scale. This is by design—to develop and refine the process, then scale up in the second half of the project to demonstrate the process at a commercially relevant scale. This approach was taken to minimize risk.

- Comment 4: As above, milestones were specifically articulated in the presentation. We agree that we did not go into the specifics of strains or water chemistry in the presentation—there simply was not enough time to detail the list of strains, each of their specifics, and so on. We look forward to discussing the details of these results at future meetings or in publications.

- Comment 5: Thank you for the comments—team member roles were described in slide 3 under management/structure. The goal of this FOA was to utilize carbon more efficiently and grow algae with higher productivity. In this project, we are not using DAC or other approaches to minimize the carbon footprint. In another project, we are specifically addressing the use of DAC in combination with carbonate/bicarbonate conversion. Our initial results suggest that this will indeed reduce the overall use of fossil carbon. Outdoor tests will be done using raceway-style open ponds to ensure translation of results to large-scale facilities.

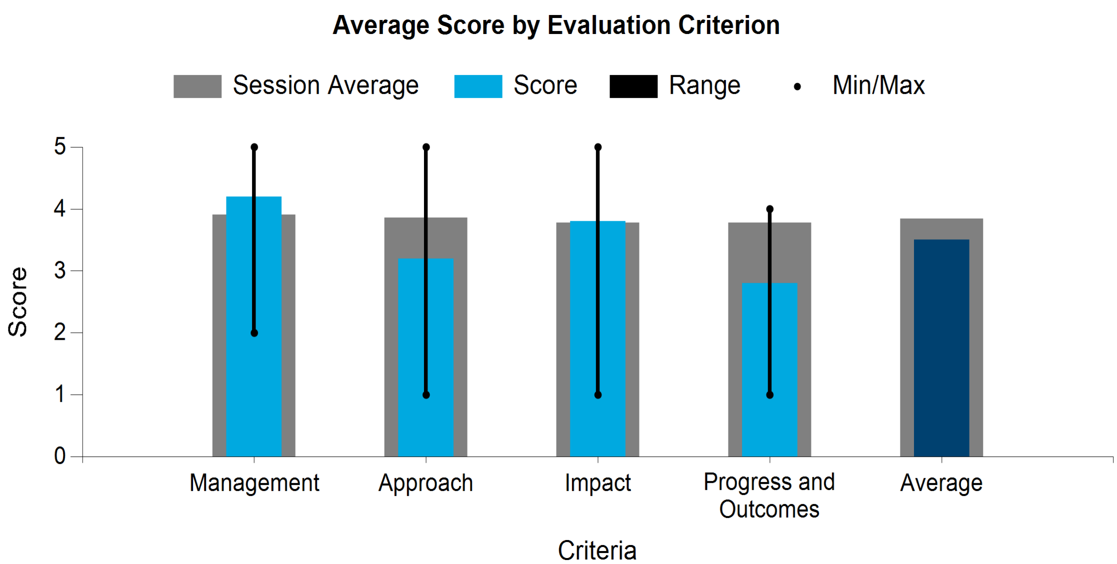
AIR CARBON FOR ALGAE PRODUCTION (AIRCAP)

MicroBio Engineering Inc.

PROJECT DESCRIPTION

WBS:	1.3.2.600
Presenter(s):	John Benemann
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	$2,899,179

The goal of this project is to eliminate the need to co-locate algal cultivation facilities with concentrated sources of CO_2, such as power plant flue gases, by identifying strains that take advantage of the increase in air-CO_2 flux at high pH, thereby increasing the resource potential for production of algal fuels over tenfold. Abiotic mass-transfer rates were measured in 1-acre and 3.4-m^2 raceway ponds to identify the pH at which the air-CO_2 in-gassing rate can support high rates of algal growth. Chemically enhanced air-CO_2 rates approached 10 g C/m^2-day at pH 12 in the brackish water tested, equivalent to a biomass productivity near 21 g AFDW/m^2-day (at 0.47 g C/g AFDW). The mass-transfer rate was found to be nearly independent of the mass-transfer coefficient, indicating that at turbulence levels achievable in typical raceway ponds, the system is reaction-rate-limited, rather than diffusion-limited. Twenty-eight culture collection strains, as well as two bioprospected isolates, were screened for growth performance under alkali conditions, with *Cyanobacterium* SSL1 selected for optimization. An alkalinity of 80–200 mEq/L was found to avoid both bicarbonate limitation and sodium stress when grown at high pH. When tested in outdoor raceway ponds in late summer conditions, biomass productivity attributed to air-CO_2 exchanged measured 11–12.5 g AFDW/m^2-day. A TEA and LCA found that alkalinity additions, required to increase bio-available inorganic carbon at elevated pH, offset the elimination of enriched CO_2 costs, although achieved a 22% reduction in greenhouse gas emissions. Future work will screen and test additional strains and identify pathways to meet DOE cost targets



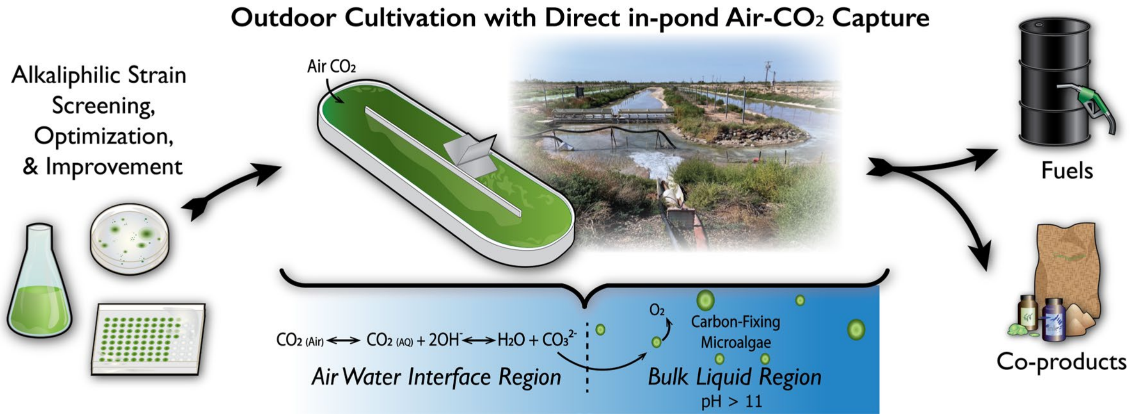


Photo courtesy of MicroBio Engineering Inc.

COMMENTS

- Project goals, roles, and responsibilities are clearly stated. Communications methods are well defined. Risk management is straightforward. Bioprospecting is always good, but the hazard is ending up with a strain that works locally and can't be used more widely. The summer productivity seems low. Mostly, the problem is that the TEA doesn't pencil out, and economic results are the only thing that will make this viable. You kind of need to know what you can reasonably make and then work backwards to figure out what you need to work on and what is the critical path to commercialization. The outcome of improving DAC by finding strains that can utilize caustic is obvious but finding a local strain might limit widespread adoption. A couple of strains were identified for development, and different alkalinity sources were screened for suitability as a supplementation source. However, cost-benefit seems fairly minimal and dependent on credits. It will be interesting to see the recovered adapted strain results.

- The goal of this project is to eliminate the need to co-locate algal cultivation facilities with concentrated sources of CO_2 by using 100% of CO_2 required from air. This is in line with the BETO MYPP milestone to develop technologies for CO_2 utilization that enable, in field research conditions, an increase in the utilization efficiency of delivered CO_2 by 25% relative to 2019 baselines by 2022. Success in this project will eliminate the constraint of co-locating algal plants with CO_2 sources and significantly reduce capital costs of CO_2 supply. The team had a clear management plan with a well-defined task structure and leads leveraging team expertise and previous experiences. They identified risks and outlined mitigation strategies, as well as established channels of communication and collaboration amongst team members. Their approach includes increasing air-CO_2 flux by chemical enhancement, identifying pond conditions that yield in-gassing rates to support high biomass productivity, identifying strains that thrive under high-pH conditions, and using TEA/LCA outcomes to guide further research. The approach is ambitious but has potential for success. Critical for success is the identification or evolution of strains that thrive at low alkalinity and high pH, which the project team has identified as a challenge. The team identified a strain with productivity of 11–12.2 g AFDW/m^2/day at high pH but required elevated alkalinity to achieve this. The reduced cost of CO_2 is offset by the cost of maintaining high alkalinity. The team should provide clarity on further strain selection and should consider strain engineering/evolution. Commercial deployment may pose a challenge due to precipitation of ammonium, Ca, and Mg from water as well as high-producing strains at pH 11.5–12.

- The goal of this project is to provide 100% of algal carbon with CO_2 from air. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The approach takes into consideration that at high pH, CO_2 mass-transfer kinetics are sufficient to provide enough dissolved CO_2 to support high biomass productivities. Effective delivery of air into a high-pH pond is necessary to maximize CO_2 transfer. The approach then will rely on finding algae strains that can effectively grow under high alkaline and pH conditions. TEA/LCA modeling will be used to assess impacts of the integrated system. The approach is deemed viable. Impacts of the technology development were adequately explained, along with impacts to BETO's targets and goals. Progress was described effectively in all areas, including understanding mass transfer for CO_2 under high pH and high alkalinity and finding and evolving strains for these conditions. A *Cyanobacterium* sp. SSL1 has been adapted to these growth conditions. TEAs/LCAs are underway to evaluate the savings when alkaline sources and costs are considered. Issues with outgassing of ammonia as the nitrogen source and precipitation of carbon as carbonate will need to be adequately addressed in future work.

- The management team seems qualified for this work, but it is not clear how tasks are divided among the team. The participation of a commercial partner in the project will help direct efforts to scalable technologies and tangible outcomes. Lack of preparation for sensors' reactions to water hardness seems to indicate lack of preliminary planning and failure to do some initial risk mitigation. It appears that the strategy is to operate at high pH with alkalophilic algal strains. This strategy is not novel, and the resource requirements required to complete validation of appropriate strains seems high. Mass-transfer models for similar systems have been developed for over 100 years. It appears that this project is duplicative of many efforts before it. The work seems impactful but not necessarily unique or extraordinary. Reported growth rates of 11–12.5 $g/m^2/day$ do not demonstrate progress even compared to this team's own internally generated data. This growth rate seems to be validated with only a single experiment conducted under ideal conditions not representative of a cultivation year. The final target of 15 $g/m^2/day$ is lower than current SOT, making this progress seem minor.

- The project's work plan specifically addresses several key elements needed to cost-efficiently produce algae and biofuels at commercial scale: (1) Eliminate dependency (and cost) on commercial CO_2, (2) eliminate dependency on location of available CO_2, (3) establish water chemistry and growth media criteria to directly capture CO_2 from the atmosphere, and (4) determine suitable high-quality strains that will grow well in high-DIC environments. The progress to date is proceeding and attaining results and milestones in support of these goals. Additionally, the materials presented provide the industry with reminders of the important and underlying mass-transfer equations, as well as determining guideline pH and other relevant criteria for achieving algae growth production targets. Another important pending conclusion presented is that a decrease in turbulence levels in the 10-acre pond is not expected to influence the air-CO_2 flux. The relatively recent start and the progress made to date position the project to hopefully achieve all goals and achieve the end-of-project milestones.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the Review Panel for their participation, constructive comments, and positive feedback. We are unsure of the first reviewer's mention of "lack of preparation for sensors' reaction to water hardness." This was identified early in the project as a risk with the 1-acre pond in-gassing studies, with a softening strategy trialed in the lab prior to implementation in the field. Even after softening, some precipitation was still observed, although this was accounted for analytically, as planned. Observed in-gassing rates in the 1-acre pond were within 20% of model predictions. Implications of source water hardness, including strain response, treatment, and disposal, were preliminarily explored in the Budget Period 2 TEA, and will be examined in more depth in Budget Period 3. We disagree with the reviewer's comment that alkaliphilic cultivation "is not novel," and "duplicative of many efforts before it." Of the algal projects within DOE BETO's portfolio, we are one of two groups exploring feasibility of this approach, with a handful of others examining this globally. The remainder are focused on cultivation using traditional methods of CO_2 supply, which, as noted in the 2016 Billion Ton Update, limits algal resource potential to less than 5 billion gallons per year. We are unaware of the "many efforts before" the reviewer refers to here—the vast majority of strain and cultivation development from 1980 on has focused on traditional

CO_2 supply, generally at pH 8 or below. The more recent ATP3 and DISCOVR field trials are no exception. Perhaps the reviewer refers to commercial *Spirulina* cultivation, with commercial operations using elevated alkalinity (generally near 200 mEq/L) and pH (~10). We note that the current approach differs from *Spirulina* cultivation in that it targets a higher pH—11.5 and above—as in-gassing rates are insufficient at pH 10 to achieve biomass productivities required to reduce feedstock production costs. Also, lower alkalinity levels than used in *Spirulina* cultivation are targeted to keep operating costs low.

- In regard to one reviewer's comment that this work is "duplicative," we were also surprised at the lack of consensus within DOE's portfolio regarding the pH required to achieve target levels of air-CO_2 flux; thus the need to re-evaluate mass-transfer models to identify the appropriate analytical assumptions for turbulence levels typical in raceway ponds, both at the pilot scale (3.4-m^2 surface area) common to research and development, as well as the large, unlined ponds required for scale-up (1-acre, tested herein, and larger). Given the uncertainty in the conditions required to achieve target air-CO_2 transfer rates, we do not see these efforts as duplicative. The observed summer biomass productivity is indeed one-third that of the 2020 summer SOT level, 11–12.5 versus 32 g AFDW/m^2-day, and the end-of-project target (15 g AFDW/m^2-day) about half the SOT value. Earlier climate-simulated trials with SSL1 demonstrated higher productivity, but only 70% of this was estimated to be from air-CO_2 exchange, with the remainder attributed to consumption of dissolved inorganic carbon within the carbonate buffering system. For a sustainable process, the air-CO_2 in-gassing rate must be high enough to support algal carbon uptake rates, without a decrease in medium-dissolved inorganic carbon throughout the cultivation cycle. Therefore, the 11–12.5 g AFDW/m^2-day measured here, under suboptimal outdoor conditions with 100% CO_2 from air, is more representative of "commercially relevant" conditions expected in a sustainable, full-scale operation relative to the earlier climate-simulated trials under idealized conditions. The end-of-project productivity target was chosen as a realistically achievable goal within the timeline of the project. Cultivation trials in Budget Period 3 aim to demonstrate further increases in the "air-only" SOT, employing similar strain screening and seasonal strain rotation approaches that enabled a 57% improvement in the CO_2-replete SOT trials. By the end of Budget Period 3, we should have a clear idea whether the chemically enhanced air-CO_2 transfer approach can eventually match the current SOT. If not, alternative approaches that can increase air-CO_2 flux at lower pH should be investigated.
- The second reviewer appropriately notes that "Mostly, the problem is that the TEA doesn't pencil out." We note that the costs shown were for a pathway that does not include high-value coproducts that have been shown to be essential to enable cost-competitive fuel production. Costs could have been more clearly presented on a dollar-per-metric ton biomass basis for a more flexible comparison—we will move toward this metric in future analyses. Note that for the TEA shown, credits associated with reductions in CO_2 emissions were small relative to the cost of the resulting fuel, lowering costs by about 5%, although they become more significant as the fuel price decreases, either through the addition of valuable coproducts, improvements in biomass productivity, or reductions in OpEx and CapEx. Two reviewers noted that ammonia outgassing and water treatment costs will need to be addressed in future work and will be incorporated into the Budget Period 3 scope. Again, we thank the reviewers for their critical assessments and positive feedback.

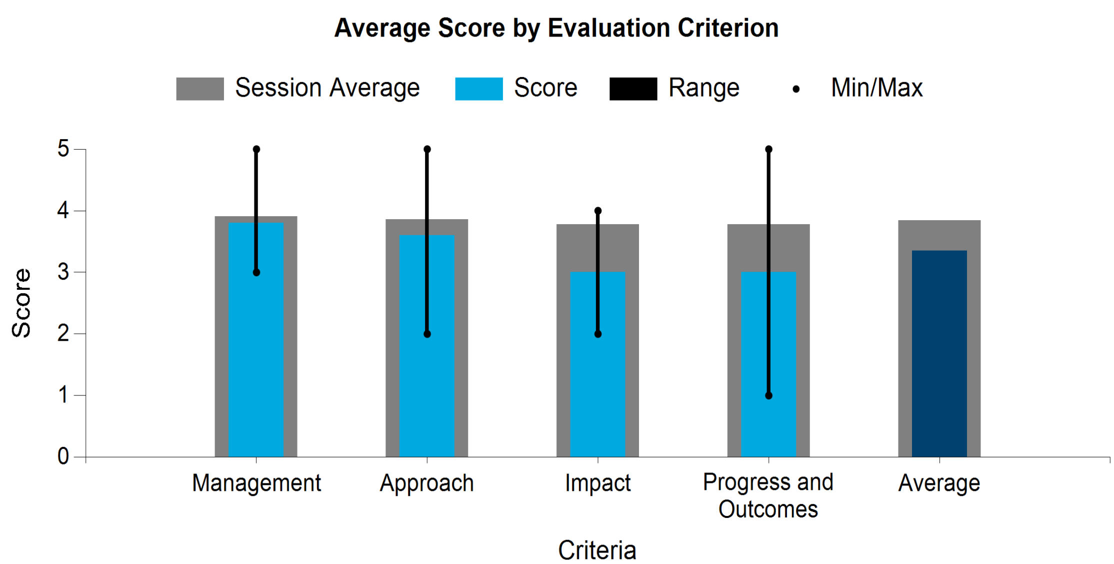
SOFAST: STREAMLINED OPTIMIZATION OF FILAMENTOUS *ARTHROSPIRA/SPIRULINA* TRAITS

Lumen Bioscience

PROJECT DESCRIPTION

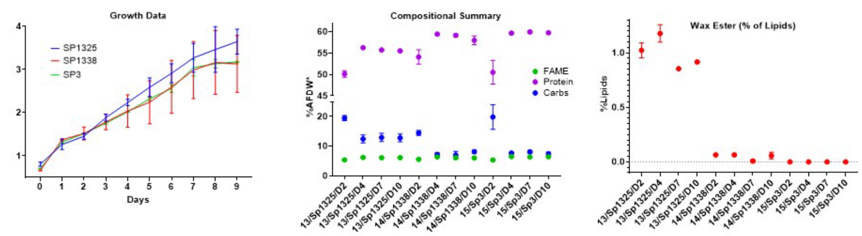
WBS:	1.3.2.601
Presenter(s):	Rachelle Lim
Project Start Date:	10/01/2017
Planned Project End Date:	06/30/2021
Total DOE Funding:	$2,450,919

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



SOFAST technology led to increases in both wax ester and carbohydrate content in *Arthrospira*

Milestone 2.3.1 Evaluate top 5 strains by indoor, artificial light testing of productivity. Demonstrate growth of wax ester strains at rates not more than 10% slower than the wild type baseline.



Sample	Fame	Protein	Carbs
17/Sp1325/D4	6.09	56.16	**11.46**
17/Sp1325/D7	6.01	55.83	**13.88**
17/Sp1325/D10	6.09	55.46	**13.71**
14/Sp1338/D4	6.4	59.35	7.6
14/Sp1338/D7	6.13	58.98	7.88
14/Sp1338/D10	6.16	57.27	8.46
18/Sp3/D4	6.54	59.82	**7.84**
18/Sp3/D7	6.36	60.18	**7.68**
18/Sp3/D10	6.41	59.63	**7.78**

LUMEN BIOSCIENCE

Photo courtesy of Lumen Bioscience

COMMENTS

- It is difficult to see how this project can provide material impact to the BETO MYPP and other program goals. The emphasis seems to center around Lumen's commercial priorities and planned or desired process systems, operations, and product profiles. As this project is reaching its completion, the presentation does not provide any materials allowing evaluation of its progress to be measured against the project goal and the end-of-project milestone.

- The aim of this project is to produce engineered strains of *Arthrospira platensis* with improved photosynthetic traits and increased lipid content, with the hope to achieve 19-g/m^2-day productivity and 14% and 16% increased lipids and carbohydrates content, respectively. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The approach is quite innovative as it is specific toward the transformation of filamentous cyanobacteria. Thus, it incorporates the use of construct libraries and transform libraries that will be used to create variants. These will then be competed to identify constructs that impart fitness or wax ester production. These will then be grown outdoors. The approach would benefit from more detailed discussion on why wax esters are targeted and specific trait modifications added to the constructs will generate high-fitness strains and/or wax ester production. Impacts were described against BETO productivity goals and barriers. The impact of the wax ester production needs to be discussed. Progress was described against deliverables. The creation of the construct libraries is quite innovative and an excellent approach to generate variants in cyanobacteria. Construct and transform libraries have been created successfully and implemented on *A. platensis*. Mutants have been competed showing mixed results in controlled experiments. Some variants with increased productivity and wax ester content have been identified. A very nice new approach, MALDI imaging (matrix-assisted laser desorption/ionization), was used to determine intrafilament population heterogeneity. Two strains will be moved to outdoor cultivation at AzCATI.

- The communication plan is well defined and data sharing methods are good—the LIMS platform for data management is good. Key productivity indicators are identified, and this has good potential for green chemistry applications. The challenges are clearly identified, and go/no-go decision points are outlined. In a closed system, whether indoors or outdoors, scale is limited by CapEx and OpEx. The stated goal of 19 g/m^2/day is lower than the BETO planned goals. It seems like the economic analysis conclusion is that it is not possible to make these strains work outdoors in an open environment, and it is not possible to meet the fuel GGE goal; therefore, the cost of fuel GGE must be offset by the highest possible profit margin product with fuel as a secondary byproduct. Typically, recombinant strains will perform excellently in bioreactors even under simulated conditions of photobleaching and temperature changes but suffer from stress and infection in open ponds outdoors; here there is minimal improvement even in bioreactors. If the TEA and project relies on therapeutic protein to be viable, this may be a problem—this requires in vivo testing at a level most academic and startup labs cannot support. Immunogenicity is a well-known problem of non-mammalian expression: Novavax even capitalizes on it, making only vaccines in baculo systems because insect proteins are notoriously immunogenic. That said, they've had little success until recently and basically specialized in expensive Phase III failures. Spray drying is also known to damage the structure of therapeutic proteins—depth filtration or disk-stack centrifuges must be used. Efforts to modify different cells to make them more humanized have largely been expensive failures (GlycoFi, for one). Over-transfection of too many sequences can also lead to all of them being only marginally expressed, and the cells become quite sensitive to general endoplasmic reticulum stress, especially if something like a chaperone to assist in folding or trafficking has been co-transfected.

- The management team seems well suited for this task. Risk mitigation strategies do not seem well described. The approach of delivering oral therapeutics and cultivation on wastewater-based media are not obviously compatible. Plans to manipulate *Arthrospira* for enhanced biofuels and bioproducts composition also seems at odds with plans for oral therapeutic products. The pathway by which wax esters will be transformed into biofuels or biofuel intermediaries seems unclear. Licensing of strains has proven to be a difficult business model to capitalize on in this small industry space so far, and there seems to be a limited market for even the best-engineered algal strains at this time. As a result, strain development work done targeting biofuels production will likely have limited impacts if Lumen's core business model is centered around drug delivery. Demonstrated production of therapeutic proteins is not unique to this work but is significant and shows the future viability of a new range of high-value products that have the potential to drive commercialization of algae. Outcomes have not yet shown significant improvements. The strategy to overcome the gap between indoor and outdoor culturing conditions seems to be planning more indoor simulation trials. The mysterious nature of carbon flux to carbohydrate synthesis represents what appears to be an unmitigated risk for planned wax ester production. Plentiful data were supplied, and this is very appreciated. It is difficult to interpret the impact of many results without understanding more details about the project than this context and format can provide. The end result demonstrates much activity, but not all of it can be interpreted for review. Outdoor growth seasons are upcoming and will be the best way to determine efficacy of this work. A tool capable of differential lipid expression seems to be an outcome of this work, and that will be a useful tool if effective.

- The team is proposing to engineer *Arthrospira platensis* (*Spirulina*) strains for improved photosynthetic traits and increased lipid content. The expected outcomes are increased areal productivity and projected biofuel yield as well as development of strain improvement toolkits and methods. Their toolkits for increased understanding and characterization of variability in novel feedstocks addresses BETO barrier Aft-E. The optimization of stress-resistance traits to increase productivity and robustness to factors such as temperature and light will address BETO barrier Aft-C, Biomass Genetics and Development. The team had a clear management plan with a well-defined task structure and leads. They identified risks and implemented mitigation strategies, as well as established channels of communication and collaboration amongst team members. The team's approach to construct libraries of traits with various coding sequences and promoter/terminator strengths is well established, and the team has the expertise to successfully deliver. Initial strain selection and characterization were done in shake flask and flat panel reactors with constant light and showed no improvement when tested under outdoor-simulated conditions. The team should consider building a high-throughput scale-down model that will further simulate outdoor conditions for strain selection and characterization. Doing so will increase chances of success during outdoor testing. The team repeated transformation under outdoor-simulated conditions and showed some improvement over the wild-type strain. Overall, the project aimed to enhance growth and lipid content of an industrially relevant organism. Success of the proposed work has not been demonstrated with outdoor raceway test beds. The project advances the state of the art by developing toolkits like MALDI imaging to assess heterogeneity, intra-filament imaging lipidemics for understanding physiology of filaments within the organism, high-throughput spectroscopy phenotyping, and spectral fingerprint for protein content.

PI RESPONSE TO REVIEWER COMMENTS

- We emphasize that Lumen Bioscience's interests in the commercialization of *Spirulina*-based therapeutics requires reliable, cost-effective manufacturing and the ability to predictably and reproducibly culture genetically engineered strains of this otherwise undomesticated cyanobacterium. Lumen's core business model is indeed centered around lowering the manufacturing cost of *Spirulina*-based therapeutics and not production of energy in the near term. However, development of cyanobacterial genetic toolkits that enhance the photosynthetic productivity of *Spirulina* are mutually beneficial to Lumen for its goals in the therapeutic market space and to a broader bioeconomy that incorporates other uses of *Spirulina* biotechnology advances. Funding from BETO for this PEAK project has enabled Lumen to better understand the growth parameters for culturing *Spirulina* and explore the genetic perturbations for improving its stress tolerance and perturbing its metabolic profile, and it has allowed us to demonstrate highly precise replication of outdoor cultivation conditions with scale-down indoor simulation reactors. These achievements directly address BETO barriers Aft-C (Biomass Genetics and Development) and parts of Aft-E (Algal Biomass Characterization, Quality and Monitoring). Likewise, our federally funded research and development center partner, NREL, established the first MALDI-based single-filament metabolic imaging method of its kind on this project with our *Spirulina* strains, identifying metabolic heterogeneity that is also very relevant to BETO barrier Aft-E. Further, as we mentioned in our presentation, harvesting and dewatering barriers (Aft-D) are innately low for *Spirulina*. Although Lumen is not an energy company, they can still significantly contribute to BETO's portfolio by lowering the cost of biomanufacturing of *Spirulina* to produce bio-based products and coproducts and resolving barriers relevant to BETO's mission.

DIRECT AIR CAPTURE OF CO_2 AND DELIVERY TO PHOTOBIOREACTORS FOR ALGAL BIOFUEL PRODUCTION

Georgia Institute of Technology

PROJECT DESCRIPTION

WBS:	1.3.2.620
Presenter(s):	Christopher W. Jones
Project Start Date:	10/01/2018
Planned Project End Date:	06/30/2022
Total DOE Funding:	$2,483,558

The objective of this project is to develop a process that extracts CO_2 from the ambient air (DAC), concentrates it, and delivers it to PBRs for the growth of algae to produce advanced biofuels. The use of CO_2 captured from the air to provide the carbon for algae growth and biofuel production offers enhanced flexibility for siting of algal biofuel installations and presents the added advantage of reduced carbon footprint. This program brings together four organizations that provide specific expertise to the project: (1) Georgia Tech for DAC materials, algae cultivation, and LCAs; (2) Global Thermostat for DAC technologies; (3) Algenol Biotech for PBR-based algal biofuel technology, and (4) NREL for process modeling and TEA. A mobile, skid-mounted DAC system capable of producing a stream of ~98.5% CO_2 at a production rate suitable for PBR integration has been constructed and demonstrated. Multiple modes of DAC/PBR integration have been modeled, and pathways to reduced carbon footprint and biofuel costs were identified. At project end, we will produce data and knowledge regarding reduction in algal biofuel costs and carbon intensity by using CO_2 from DAC fed to algal biomass, as well as innovations in DAC process design that lower the cost of CO_2 collection from air by developing a PBR-specific DAC technology that utilizes improved sorbents and less intense operating conditions.

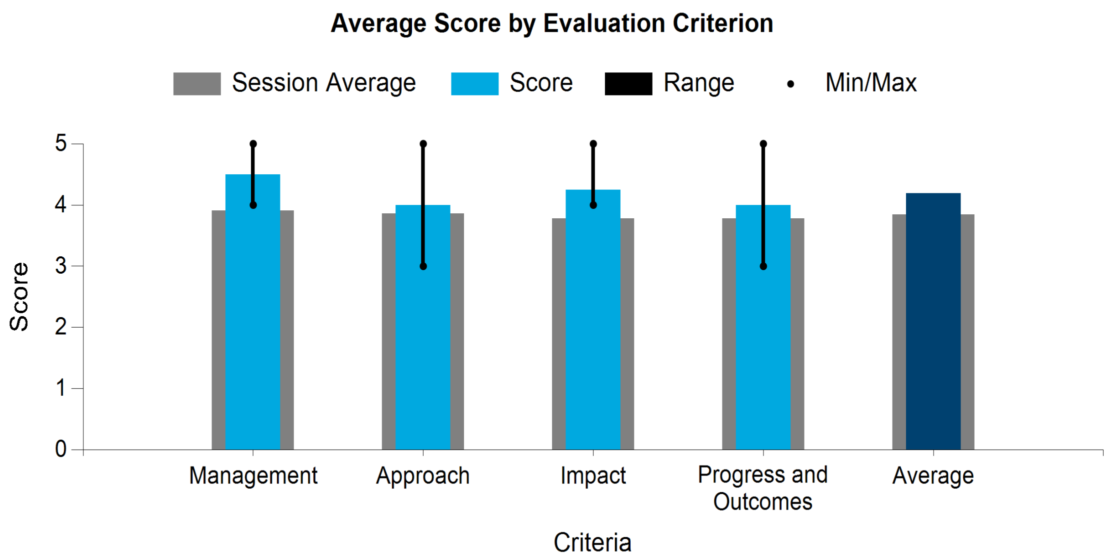


Photo courtesy of Georgia Institute of Technology

COMMENTS

- Goals are clearly stated. Roles and responsibilities are defined, and communication methods are described. It's quite impressive that the team was able to manage through not only COVID-19, but also the loss of Algenol as a partner, and recoup the institutional knowledge by hiring the collaborators directly. There have been a lot of management challenges to overcome to make this work, and they have been successfully overcome. The project is very technology- and CapEx-heavy; for this reason, it would have been helpful to see more detail on the TEA. There is a definite need for sorbent stabilizers. The CapEx-heavy nature of this method may limit what capacities in which it can be used. The partnerships developed have clear lead-ins to commercialization and scalability. The prototype is built and functioning. It would be great to see many more outdoor cycles—run it as long as possible and see how many cycles it can do! Assuming continuous operation, one can certainly stick carbon sinks in somehow as a sort of break tank.

- The goal is to demonstrate effective growth of cyanobacteria coupled with CO_2 technology for DAC. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The approach takes advantage of the Global Thermostat DAC system being integrated into PBRs growing an ethanol-producing cyanobacterium to demonstrate effective integration. Impacts of the technology development were adequately explained, along with impacts to BETO's targets and goals. The team has developed a mobile DAC that will deliver ~20 gCO_2/h to PBR culture systems and will last for months. They have also identified effective stabilizers for the amine polymer used in the DAC. A PBR system using Algenol technology has been set up for *A. spirulina* AB2293 growth outdoors. TEA/LCA of operational scenarios have been developed with two scenarios showing improvements that surpass the projects targets. Future work remains to show full integration of the technologies and demonstration of key targets.

- The goal of the project is to advance DAC technology by developing a DAC system comprising solid amine monolith adsorbents that will integrate with existing PBR technology to deliver at least 20% of the required CO_2 for algal growth. The team will be building upon a DAC system already developed by Global Thermostat by developing a strategy for extending the lifetime of DAC sorbents. This goal is of clear relevance to BETO's mission and MYPP goal, and addresses Aft-A, Aft-B, and Aft-M barriers. Successful execution will reduce cost of production and eliminate constraints of siting algal production systems close to industrial CO_2 supplies. The team has a clear management plan with a well-defined task structure and leads leveraging team expertise and previous experiences. They identified risks early on in the project and outlined mitigation strategies that were successfully executed. They also have demonstrated a well-established channel of communication and collaboration amongst team members. Their approach involves designing and building an automated DAC system, identifying heat/mass streams in both DAC and the algal cultivation technology that will allow integration of both systems, and implementing an oxidative degradation mitigation strategy to extend the lifetime of DAC sorbents. The team should provide more clarity on the flexibility of the integration strategy and if this technology can be applied to algal open ponds as well as LCA of the improved DAC sorbents. The team has made progress in building a mobile system and has identified stabilizers that limit oxidative degradation of amine species in sorbents. The team has also demonstrated cultivation of *Arthrospira platensis* outdoors using Algenol's polymeric PBRs. Critical for this project is the successful demonstration of integration of the two systems. Their TEA suggests more than a 15% cost reduction with DAC-PBR integration, which is promising.

- The management team has done a nice job adjusting to the impacts resulting from Algenol's reduced participation and the COVID-19 pandemic. The DAC technology contains interesting possibilities for future applications. The testing suggesting the long life cycles of the sorbents is very encouraging. It was not readily apparent how effective the DAC technology was at capturing atmospheric CO_2, but it nonetheless remains important given the commercial potential of DAC for decoupling algae commercial facilities from fixed sources of CO_2. No data were presented regarding algal growth rates from DAC-provided CO_2, making it difficult to truly gauge effectiveness, let alone determine how at least 20% DAC-delivered CO_2 would be established. This is important since it is a project goal.

PI RESPONSE TO REVIEWER COMMENTS

- "The team should provide more clarity on the flexibility of the integration strategy and if this technology can be applied to algal open ponds as well as LCA of the improved DAC sorbents." Answer: We anticipate that the energy/cost associated with CO_2 harvesting via DAC would not prove worthwhile for open ponds, where lower-cost ways to improving CO_2 mass transfer to reaction media would likely be preferable. Regarding integration strategy, there are many variables to consider, and we will consider a non-exhaustive subset of configurations in this project. There will remain additional DAC/PBR integration opportunities after the project.

- "No data were presented regarding algal growth rates from DAC-provided CO_2, making it difficult to truly gauge effectiveness, let alone determine how at least 20% DAC-delivered CO_2 would be established. This is important since it is a project goal." Answer: The reviewer is correct, no data were provided, because this is a Budget Period 3 objective. It is part of our current plan.

- "The project is very technology- and CapEx-heavy; for this reason, it would have been helpful to see more detail on the TEA." Answer: We will emphasize the TEA more next year.

- "It would be great to see many more outdoor cycles—run it as long as possible and see how many cycles it can do!" Answer: This is part of our plan.

- Thank you for the positive comments; no questions to answer.

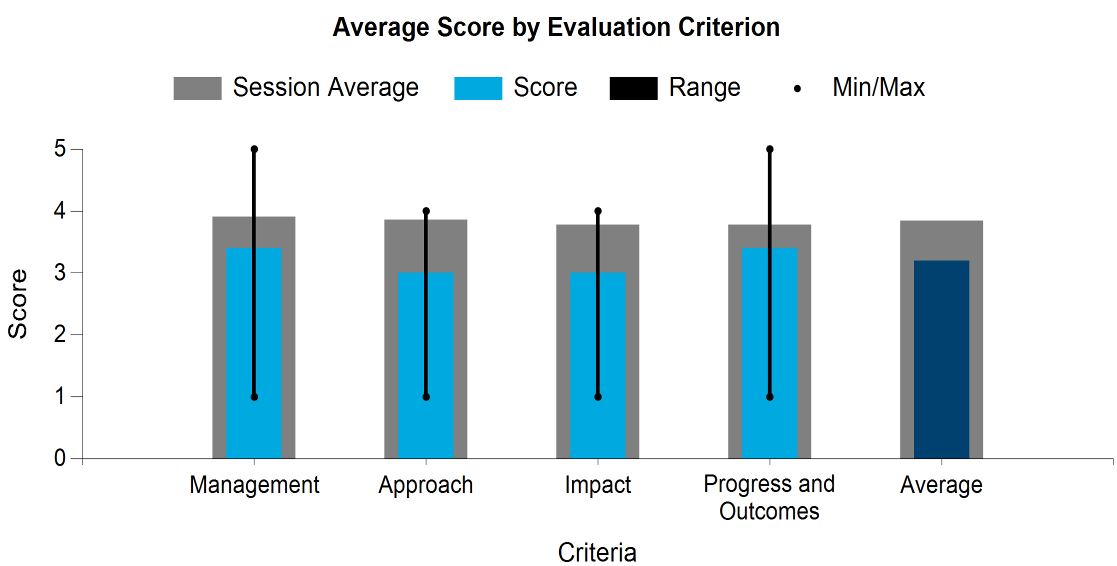
PREVENTION OF LOW-PRODUCTIVITY PERIODS IN LARGE-SCALE MICROALGAE CULTIVATION

Global Algae Innovations

PROJECT DESCRIPTION

WBS:	1.3.2.630
Presenter(s):	Aga Pinowska
Project Start Date:	10/01/2017
Planned Project End Date:	09/30/2021
Total DOE Funding:	$3,750,000

GAI is a leader in low-cost algae cultivation and has developed a high-productivity cultivation system; however, even in this advanced cultivation system, periods of unexplained low productivity are observed. We developed a database of microbes associated with algae cultivation ponds and identified taxa associated with periods of low and high productivity. Hundreds of microbes (bacteria, eukaryotes, and viruses) were isolated from our cultivation ponds. Challenge testing was conducted in laboratory conditions to identify potential pathogens and probiotics. Those tests included target algae, bacteria, and viruses. In order to separate sequences of our target algae from contaminant sequences, the genomes of two elite GAI strains were sequenced and assembled to completion, providing valuable data for other research studies. We deployed SpinDX to detect the presence of pond microbes. Further, as SpinDX quantitative detection ability was limited, we conducted initial testing of a new next-generation sequencing tool for monitoring pond microbes. The Oxford Nanopore MinION technology is affordable and does not rely on prior knowledge of pond microbes. In combination with flow cytometry, MinION provides real-time quantitative pond microbiome data and is the future of pond contamination detection for mass cultivation of algae. The next step of detection, control, and treatment of the pond microbiome is now possible.



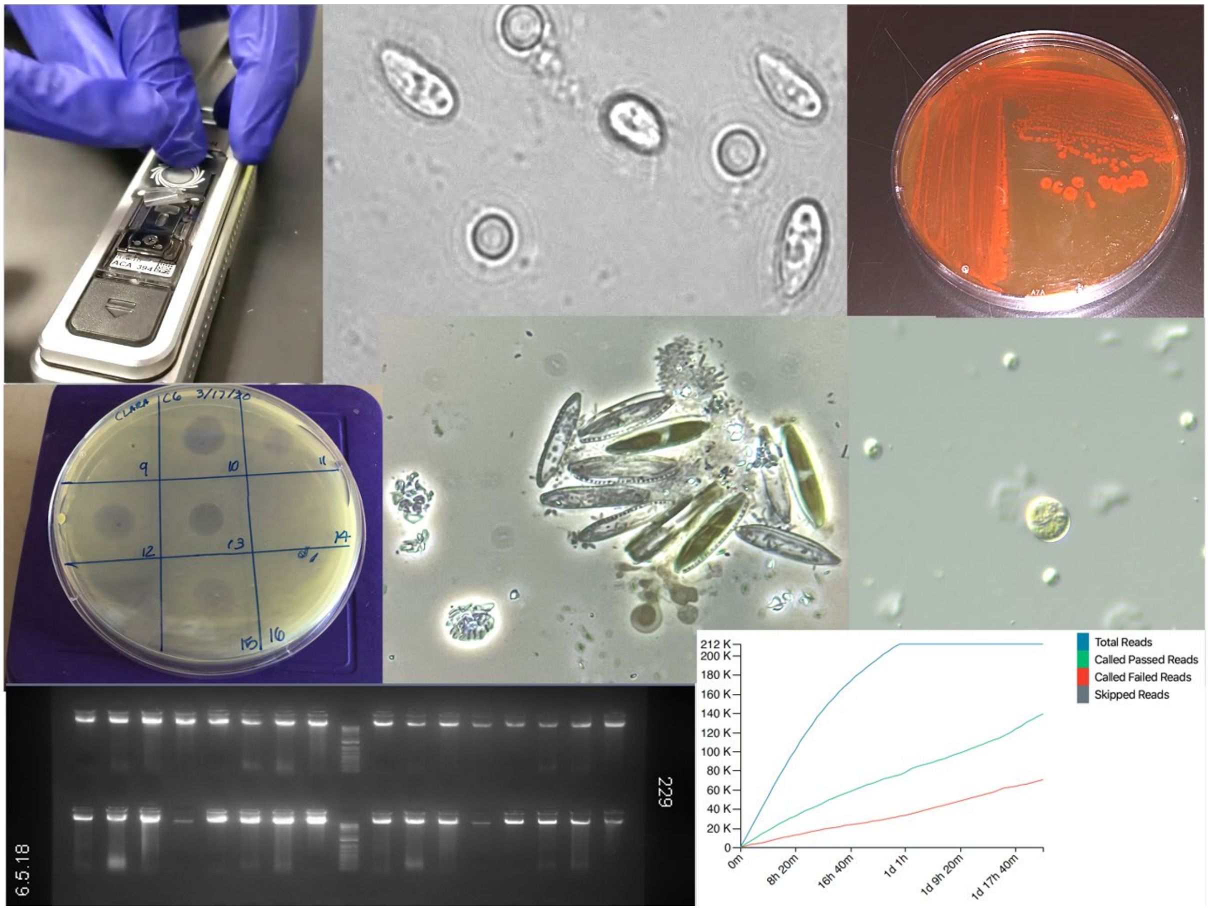


Photo courtesy of Global Algae Innovations

COMMENTS

- Communication, data sharing, and roles and responsibilities are well defined. Risk management is good and mitigation strategies are identified. A lot of the project findings depend on the SpinDX tool, which is only able to work a handful of species at a time; pond ecology and biofilms in the environment notoriously contain hundreds of species, many of them highly diverse and very location-dependent, and even more that cannot be isolated or show up in the lab as "VBNC" (viable but nonculturable). Next-generation sequencing has come down considerably in price, and the UK has made a lot more progress than the United States in that regard. If even a handful of indicator species can be consistently identified, this would be helpful and more broadly applicable. The viral identification is quite interesting—phage ecology in particular is critical work, and not merely phages that infect algae but also bacteriophages. It's also very useful that this window of intervention before the culture starts to fail observably by fluorescence has been identified, as this provides an earlier window for potential treatment.

- The aim of this project is to reduce periods of low pond productivity by identification and control of the microbiota and develop cultivation methods to achieve productivities of >25 g/m^2-d. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was not described through tasks, milestones, or go/no-go decision points, providing an inadequate assessment of progress for the project. The impact discussion lacked clear targets and goals to define the project against others, including connection to BETO long-term goals and targets. The team has made progress in method development, isolating and identifying bacterial and viral communities in algal cultivations over different periods of the year. Bacterial community variations were correlated with environmental factors and algae conditions. The team has found key correlations between bacterial community dynamics preceding changes in algal productivity. Interesting results in the genomics of *Nitzschia hildebrandi* were discussed, although how this information was relevant to the current project was not discussed. Work is also underway in viral community dynamics within the algal cultivation systems. The SpinDX system was only partially effective in community dynamics monitoring. The team is switching to monitor microbial diversity using a MinION sequencing capability. The presentation can be enhanced by providing a quantitative assessment of milestones achieved and future work. Work on control of microbiota to enhance productivities was not discussed.

- The project targets a very broad but important topic of algae pond health and the interrelationships affecting productivity thought to involve bacteria, other algae, viruses, protozoa, fungi, and many others. The project attempts to systematically address these topics and strives to develop tools for cost-effective and timely management of the ponds so as to enhance productivity to achieve a growth rate higher than 25 $g/m^2/d$. The presentation covers a significant range of material, presenting some observations and insights but often without any meaningful supporting data or analysis. No measure of improvements to growth rates are offered, nor any other measure of improvements against any baseline, making it difficult to evaluate progress against the project goal and end-of-project milestone.

- There is concern that not enough upfront planning went into budgeting for sequencing or into method development and tools to be used. Prevention of low productivity with a focus on microbiome interactions seems like an excellent target. Collection methods seem very robust and reproducible. The project approach seems to be based on large amounts of sampling and sequencing with little to no description of how to integrate these data into an actionable plan. Few of the learnings seem to be translatable to improved productivity in an outdoor setting outside of the Kauai site. Many bacteria were identified, but not based on importance of interaction in the community, only by known sequence references. Transcriptomics, metabolomics, or flux omics might have indicated critical mutualism instead of just known DNA. Any approach assessing community activity or abundance instead of just presence would have been more impactful. Data showing that environmental factors change the makeup of the community, and that duration of cultivation is directly proportional to community diversity are not surprising or directly applicable. No method to determine final product quality was described. A new cultivation methodology to increase productivity based on microbiota analysis was not described. Without this final deliverable, it is difficult to assess progress or impact in the project.

- This project focuses on reducing periods of unexplained low productivity by identifying and controlling microbiota cultivated with algae. The project goals are ambitious, as the team needs to identify not only bacteria but viruses, fungi, protozoa, and other algae, as well as develop tools for low-cost rapid analysis. The project has clear relevance to the BETO mission and MYPP goals, and if successful will progress the knowledge base on microbiota and impact on pond performance. The team had a clear management plan with a well-defined task structure and leads. They identified risks and outlined mitigation strategies. They outlined established channels of communication and collaboration amongst team members. Their approach to develop a database of microbes associated with algae cultivation ponds and identify taxa associated with periods of high and low productivity is reasonable but may require more time to build a comprehensive database and develop analytical tools required for low-cost and rapid detection. Building a database will help drive understanding on impact of microbiota on algae biomass productivity in ponds and offer avenues to controlling microbiota. However, this database is specific to a site and has to be developed for various sites to be a successful approach. The team has made significant progress toward their goal. They developed a protocol for genomic sampling from algae-associated microbiota, developed a database of microorganisms associated with algal ponds in Kauai, isolated multiple strains from all taxonomic groups, and identified microorganisms associated with low and high algal productivity. Their initial development with SpinDX was able to detect presence and absence or microorganisms in cultivation ponds, but it is not quantitative and will require further development. They proposed using next-generation sequencing technology for pond microbial detections.

PI RESPONSE TO REVIEWER COMMENTS

- We thank all the reviewers for their great comments and insight. While the project was very ambitious in goals, we were still successful in identifying previously undiscovered viruses, bacteria, and eukaryotes that are associated with low algal productivity, which was a major project objective. Though these organisms are associated with productivity, their presence does not imply causation, and we are aware of the complexities in interpretation of this type of data. Analysis of individual grow-outs showed a sequence of shifts in microbes, but there were also large differences in composition between the grow-outs, indicating strong seasonal changes. Based on data generated in this project, it is clear that tracking changes to the community is a better approach than tracking individual taxa, as was originally intended when the project started. Using Fongle, the next-generation sequencing tool developed by Oxford Nanopore, will allow us to affordably track changes in the microbial community on a regular basis. This discovery was reached late into the project, but its capability was successfully tested as part of project work. We have conducted transcriptomics analysis with the primary aim to identify and characterize viruses, but review of these data in conjunction with DNA amplicon data may reveal some intriguing functional data and is something we are thinking of doing in future work. We have discussed metabolomic analysis, but it was not within the budget or initial project objectives.

- This project was the first of its kind looking at the microbiota in the GAI cultivation system and in these algae strains, and we appreciate and agree with the suggestion to include additional data that should have been collected and analyzed. However, this project resulted in achieving a fundamental and important data set in the composition, environmental metadata, methodology, culture isolates, and characterization of the pond ecology; that has been necessary for establishing an initial understanding of a previously unknown biological system. The data generated from this project will be a launchpad for more thorough analysis (e.g., metabolomics or flux omics) in future work. We demonstrated a positive effect of *Exiguobacterium* sp. and a negative effect of *Bacillus* sp. on algal productivity in the laboratory conditions. Our next step is to test the positive effect of *Exiguobacterium* sp. outdoors. We identified a phage that can lyse *Bacillus* sp. that was associated with low algal productivity, and the phage's ability to lyse the *Bacillus* sp. was demonstrated in the lab. The very promising SpinDX capability did not work in our system and resulted in many false positives. Multiple approaches were tried, and a LAMP (loop-mediated isothermal amplification) assay was developed to successfully detect presence and absence of microbes. As indicated by the reviewers, our ability to test the effectiveness of treatment methods in outdoors ponds was limited due to an inability to regularly monitor microbiota using SpinDX. However, our goal was to demonstrate the capability to mitigate a eukaryotic, bacterial, and viral bad player in outdoor ponds, and we have a path to demonstrate this capability before the end of the project. For PEAK challenge we are planning to use a probiotic to demonstrate increased algal productivity. We currently have three probiotic bacteria that are enhancing algal growth in laboratory conditions. All three were isolated from GAI outdoor cultivation ponds during the project, and they are being tested outdoors. In just 3 years, this project work began without a single isolate or known microbe in the GAI system to now having three putative probiotic bacterial isolates to test outdoors, which is impressive given setbacks with COVID-19 and general complications with importing biological materials into Hawaii. We look forward to seeing the results for the PEAK challenge and are excited begin closing out this fruitful project.

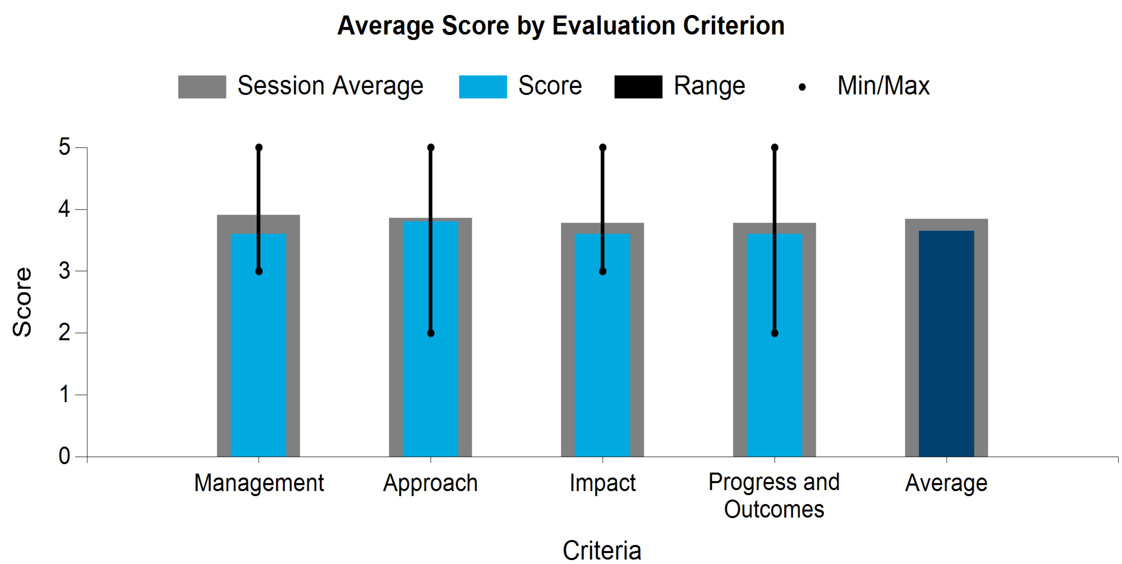
HIGH-THROUGHPUT DIRECTED EVOLUTION OF MARINE MICROALGAE AND PHOTOTROPHIC CONSORTIA FOR IMPROVED BIOMASS YIELDS

Colorado School of Mines

PROJECT DESCRIPTION

WBS:	1.3.2.640
Presenter(s):	Matthew Posewitz
Project Start Date:	09/30/2017
Planned Project End Date:	04/20/2021
Total DOE Funding:	$2,732,421

Directed evolution and targeted trait selection remain among the most powerful and successful tools available for attaining the process improvements necessary to enable commercial success in many biotechnology sectors. To develop robust and efficient algal biotechnological platforms, we are using "solar-simulating" bioreactors to select strains evolved for improved growth rates and improved tolerances to high levels of light and pH and oxidative stress. Deep sequencing (genome, transcriptome) is being used in conjunction with comparative genomics approaches to probe organismal evolution and to identify genomic alterations that enable increased biomass yields and adaptations to growth in outdoor open-pond systems. Outdoor testing is being done in collaboration with GAI using their advanced open-pond cultivation systems that are shown to minimize contamination and attain productivity increases relative to standard raceways. Despite the widespread use and proven track record of directed evolution in other bio-based applications, these tools have seen only limited utilization in the algal biofuels sector. This is primarily due to the cost, engineering, and human/fiscal resource constraints imposed by these techniques, especially when attempting to simulate the extreme photon flux and sinusoidal nature of solar light and environmental temperature fluctuations. We have built a series of low-cost, modular, and scalable growth stations that mimic algal pond growth outdoors. Importantly, these easily include the highest photon intensities of sunlight, and are capable of programmable temperature swings ranging from 5°C–60°C. These "solar-simulating" bioreactors are being used to select strains evolved for improved growth rates and improved tolerances to high levels of light and pH and oxidative stress. Data from experimental work will be used to inform sustainability assessment work, with results from modeling work used to understand impacts of the research.



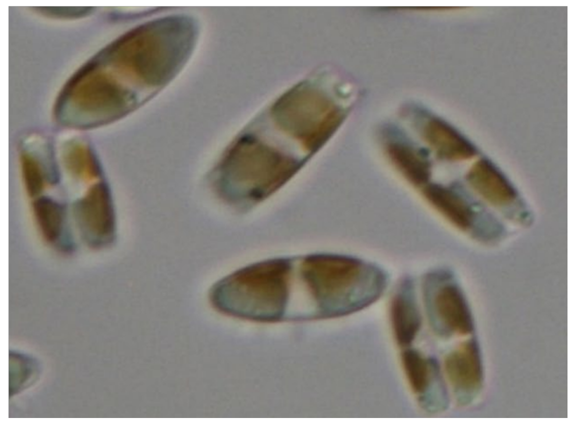


Photo courtesy of Colorado School of Mines

COMMENTS

- Roles and responsibilities are defined, communications are channeled through regular meetings, and data sharing is managed through a single point of contact. The project goals are clearly stated and aligned with BETO productivity and quality goals. Risks of moving from the lab to field trials are identified and strategies to mitigate them are stated. Pre-selecting for stress-tolerant clonal pools via directed evolution is actually really useful—especially in yeast, bacterial, and mammalian cells cultured for production scale. It's unclear why more people don't do this, because an organism that can have a generation every few hours or couple of days can develop new proteins or new metabolic workarounds a *lot* faster than a human with Schrodinger software and IDT making their sequences. This method would be highly applicable to other strains and different locations, in addition to being able to mitigate the more usual aspects of climate change—unpredictable weather. The productivity exceeded goals, redox-sensing gene clusters were identified as potential contributors to the adaptation, and it will be interesting to see what happens outdoors during the more stressful conditions.

- The goal of this project is to use directed evolution approaches to improve biomass yields, showing tolerance at high pH, O_2 concentrations, and temperature. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The main approach of the project is to systematically adapt and evolve strains under high pH, high temperature, and high O_2. The approach is sound. The impact of the project was described more as goals for the project rather than impact to the BETO program and to future efforts. The team has been able to map strain performance boundaries under different stressors and has evolved high-productivity (>60%) strains under high light and high O_2 concentrations. The team has achieved productivities in excess of 35 g/m^2-day for *Picochlorum* and 20 g/m^2-day for *Nannochloropsis* strains in simulated PBR cultivation. They are performing omic analysis of the adapted high-productivity strains. This is revealing the main gene cluster interactions for redox sensing that may be important to successful adapted strains. The team is working on field trials for adapted strains where productivities remain at 10–13 g/m^2-day, requiring further field adaptation. The project outcomes discussion would benefit from more detailed information on milestones reached and elaboration on milestones remaining to achieve success.

- The management team has several projects together and seems well suited for success in this project. It is difficult to discern how work with the *Nitzschia* diatom as part of the collaboration with GAI in an APEX project will complement these efforts. It seems likely that these strain development pipelines converge, but that synergy or overlap is not clear. Directed evolution is capable of yielding field-ready progress in a short period of time with great genetic stability and seems like a reliable approach for this strain development. It is unclear how organism consortia are being established, what goals are associated with that consortium development, or how that consortium's role in the community or overall biomass composition will be analyzed. It seems highly likely the productivity goals for this project will be met. The impact of this work will be determined by GAI's ultimate go-to market strategy and its success. The omics toolkit developed for transcriptomic networking could be useful in determining underlying mechanisms responsible for strain performance. Recent go/no-go growth rates demonstrate tangible progress, and expectation of reduced outdoor growth rates seems reasonable though perhaps somewhat speculative. The results from the first adaptation trials outdoors are not promising, and it may be appropriate to calibrate expectations of adapted outdoor strain performance to this outcome. Lab results in isolation are impressive, but with limited time left for field testing during the period of performance, it seems likely that this work will not result in an advancement over GAI's existing benchmark or the BETO SOT.

- The project seems to especially emphasize the use and results obtained using PBRs. Mention is made of a consortium that may be deployed outdoors in Kauai without corresponding presentation regarding efforts or results. PBR growth rates exceeding 30 $g/m^2/d$ are presented with outdoor growth rates between 20 and 30 $g/m^2/d$ for very short (3- and 4-day) durations, with the balance of outdoor trials in slightly higher-pH media in the 10–15-$g/m^2/d$ range. The project is approaching final tasks with the especially important summer period for field trials in Kauai that will be the final measure against the project goal and end-of-project milestone.

- The team proposes to use directed evolution to improve biomass yield of a high-performing strain grown by GAI. Their approach is to evolve a more tolerant strain to high pH, high temperature, and high dissolved oxygen concentration. The project performers seem to have a clear management plan while leveraging expertise from the different investigators. Risks involved in the execution of this project are not identified, and mitigation strategies are not outlined. Directed evolution is a well-known approach successfully used to evolve strains with improved productivity in the presence of environmental stressors. The team's approach in using directed evolution to improve a high-performing strain that, if successful, will increase biomass productivity ≥ 24 g/m^2/day. This has clear relevance to BETO's mission and MYPP goals. Successful use of directed evolution is dependent on a very good experimental design. This project's approach to first map temperature, pH, and oxygen optima and then adapt to each stressor is a great approach using the right bioreactor setup that mimics outdoor conditions. Additionally, the team leveraged transcriptomic network-based analysis in identifying key genetic and metabolic markers in adapted high-productivity strains. Indoor performance typically does not translate to outdoor performance. The team set an indoor productivity target of exceeding 30 g/m^2/day to increase the chances of reaching 24 g/m^2/day outdoors. Outdoor performance of the adapted strain was not superior to the wild-type strain in biomass productivity at normal environmental conditions but showed outdoor testing of the adapted strain in summer will be crucial to understanding productivity of the strains at high pH, temperature, and oxygen. This project has a potential to result in improved biomass productivity in the summer months. The team should consider generating indoor testing data for the high pH, temperature, and oxygen in simulated outdoor PBRs to compare performance of the adapted strain to the wild-type strain. The team has made significant progress in adapting a strain and developing an omics toolkit to understand genetic and metabolic markers in the 2 years of the project. Overall, the project seeks to address key research needs stated in BETO's MYPP with the goal of developing strain improvement toolkits and technologies for improved biomass productivity.

PI RESPONSE TO REVIEWER COMMENTS

- The PEAK project is focused on improving productivities and developing improved strain robustness under high pH, temperature, and oxidative stress conditions. The APEX project is focused on improving carbon partitioning to lipids. Ultimately, we would like to attain a high-productivity and high-lipid strain. The PEAK project is poised for multiple summer 2021 campaigns that we think are likely to meet the targeted goals of this project (24 $g/m^2/d$) for a summer growth campaign. The primary risks the project face are the stability of evolved strains during the transition from laboratory to outside culturing. To mitigate risk, we are testing three independent organisms and evolved strains in each of these lines. As advised, we are collecting extensive data sets comparing the reference strain to evolved strains in laboratory bioreactors. Lastly, omics assets are in place to explore strain stability during this summer's outdoor testing.

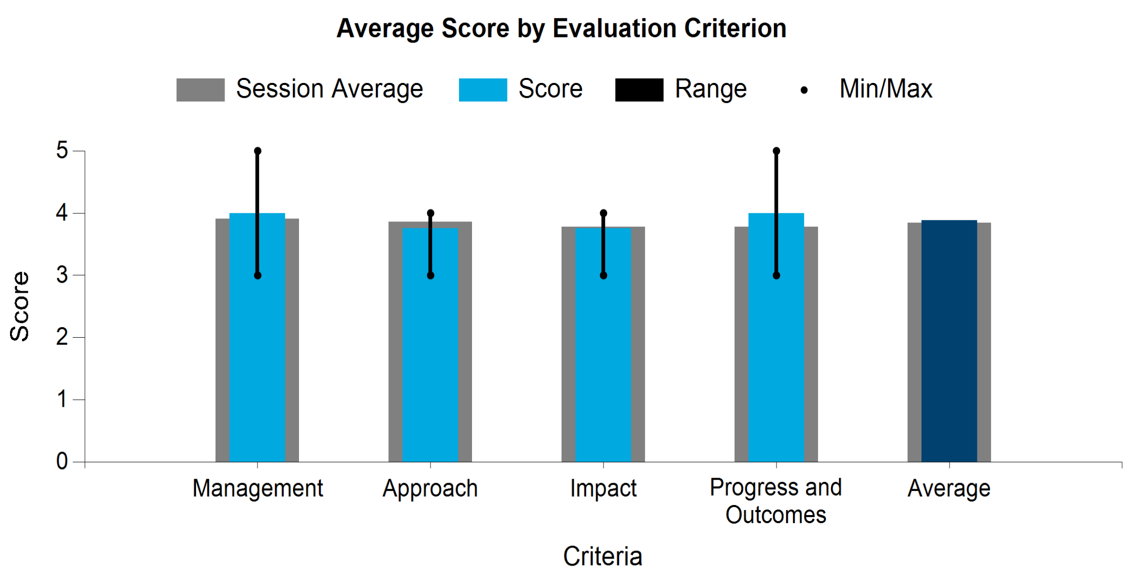
SUCCESS THROUGH SYNERGY: INCREASING CULTIVATION YIELD AND STABILITY WITH RATIONALLY DESIGNED CONSORTIA

Los Alamos National Laboratory and the New Mexico Consortium

PROJECT DESCRIPTION

WBS:	1.3.2.641
Presenter(s):	Shawn Starkenburg
Project Start Date:	10/01/2017
Planned Project End Date:	01/31/2021
Total DOE Funding:	$1,572,896

The commercialization of algal biofuels will not be realized until major technical and economic barriers are overcome, as there continues to be a need to improve culture productivity and stability, as well as better understand these metrics at commercial scales. In this project, we address these knowledge gaps by conducting research to increase yield of outdoor cultures through the rational design of consortia. Specifically, we implemented a stepwise process to biologically and ecologically engineer consortia consisting of multiple *Nannochloropsis* species and strains and growth-promoting bacteria. To construct intrageneric *Nannochloropsis* consortia, we matched species to known environmental conditions and biotic challenges at our field site to improve productivity in the spring and fall transitional periods when temperature dynamics differ significantly from summer cultivation optima. Second, we overcame the limitations of traditional bacterial screening by using a novel high-throughput tool (HiSCI: High-throughput Screening of Cell-to-cell Interactions) to isolate and select specific bacterial partners that enhance productivity. Additionally, we sequenced new *Nannochloropsis* strains (contributing to the repository of algal genomes for the scientific community) and developed molecular tracking tools for this taxon. Our implementation of technological improvements—with a focus on rationally designed cultures and consistent biomass composition—helps generate an economically viable route to improve algal productivity and biofuel yield.



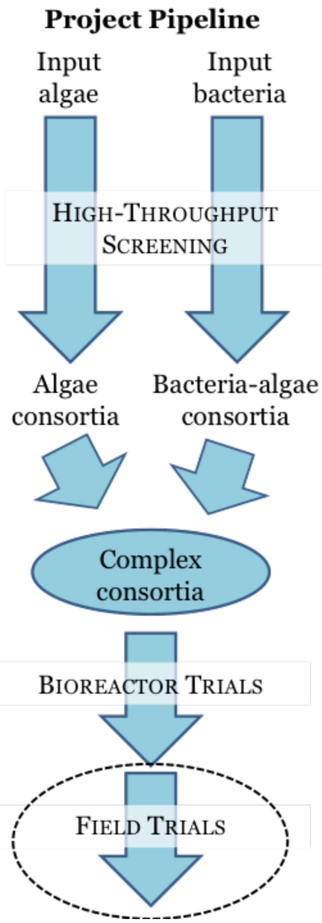


Photo courtesy of Los Alamos National Laboratory and the New Mexico Consortium

COMMENTS

- The goal of the project by Los Alamos National Laboratory and the New Mexico Consortium is to increase yield and stability of open outdoor ponds to address the current challenges of low productivity and stability due to environmental stressors. The team had a clear management plan with a well-defined task structure and leads. They identified risks and implemented mitigation strategies, as well as established channels of communication and collaboration amongst team members. The team's approach in combining strains of *Nannochloropsis* with complementary traits is innovative and critical to overcome low productivity due to light, temperature, and salinity perturbations in outdoor ponds. This approach resulted in productivities greater than average monoculture productivities. This project also implemented an algal-bacteria consortia that showed increased growth rate compared to monoculture base strain, as well as improved stability and recovery in perturbation studies. This is a significant accomplishment of this project. The team also developed toolkits like the microfluidics screening/culturing pipeline to identify probiotic bacteria, consortia libraries, and genome sequences of novel *Nannochloropsis* strains. The team should explore the impact of bacterial consortia on OD 730 as a readout for improved strain. The project had a good balance between lab-scale and outdoor testing, which is crucial to demonstrate feasibility of the proposed technology. Outdoor testing of the algal-bacterial consortia did not show significant improvement in productivity over baseline strain. The project made significant progress toward increasing productivity and stability in outdoor ponds during the 3-year period. The commercialization potential of the approach given that the algal-bacterial did not show significant improvement in productivity over the baseline strain is not clear. Overall, the project seeks to address key research needs stated in BETO's MYPP with the goal of developing strain improvement toolkits and technologies for improved biomass productivity.

- The goal of this project is to increase the stability and yield of open outdoor cultivation systems by engineering the composition of intrageneric algae and algae-bacteria consortia. The team and management plan described are appropriate for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The approach incorporates a novel high-throughput microfluidics screening method able to characterize the effect of combinations of algae-algae and algae-bacteria consorts on growth performance. The approach relies on a culture in a bead system, which is highly controlled and at the same time quite limiting, as culture stability and growth is completely dependent on efficient cell-to-cell interactions within the microfluidic bioreactor. This may not represent an ideal situation for such cultures. Nevertheless, it is a good starting point for artificial creation of a microbial consortium. The consortia are then grown under different stresses and beta trials. The impact of the work was described through the mitigation of crop productivity and stability within environmental perturbations and the development of high-throughput screening/culturing tools, consortia libraries, genome sequencing, and diagnostic assays. The team has already published some of the work. The discussion would benefit from details on the PEAK challenge goal and how it feeds into the BETO targets. The project has shown that the engineered consortia are most effective in stabilizing the cultures when there are significant environmental stresses, with gains in productivity observed. Establishing methodology to take the consortia straight to field trials is seen as a best practice. Nevertheless, challenges with competition with natural pond consortia will need to be understood and addressed. The presentation would benefit from more details on milestones achieved and remaining to be performed through the rest of the project.

- The project's plan to test and evaluate the possible opportunities of algal-bacteria consortia is novel and potentially may produce growth rates higher than monoculture. The initial results presented suggest a minor improvement may be possible. The project did not discuss the scales used in testing but emphasized the conclusion to implement testing straight to the field as a best practice. The project is on its concluding phases, and unfortunately the presentation did not present any data or supporting information in support of the project goal and the end-of-project milestone.

- The structure and organization, communication methods, and data sharing are well-defined and described. Risk mitigation methods are thought-out, especially the engineering runs prior to the shot on goal and the backup culture maintenance at multiple sites, especially during COVID. Nobody seems overstretched or tasked with too many deliverables. The approach is fairly standard, identifying optimal conditions in bioreactors prior to moving to fieldwork, but the role of high-throughput screening is not completely clear. It seems more like shotgun sequencing, as it is explained here. The amplicon tool is certainly helpful—there is improvement on consortia vs. baseline. Field results seem not closely tied to bioreactor results, but this is fairly typical for everyone. Since outdoor cultivation invariably results in some sort of bacterial consortia, it's a matter of whether the ones wanted are present or not, or if the ones that aren't wanted are present or not, and a rapid method to determine this is certainly useful. The heat/cold shock protein protection from the consortia looks very interesting. Product quality data from the consortia would be great to see, as well as more outdoor trials, as the PI noted.

PI RESPONSE TO REVIEWER COMMENTS

- Many thanks to the Review Panel for their time and thoughtful responses. The primary goal of this project was to improve the productivity and robustness of algae strains against environmental perturbations by eco-engineering of intergeneric algal consortia and algal-bacterial consortia. Our end-of-project milestone was focused on conducting research that led to productivity and yield targets of >18 $g/m^2/d$ and >117 GGE/ton, respectively, extrapolated from fall cultivation values (14 $g/m^2/d$) with consistent biomass composition. Significant effort was made to test lab phenotypes in the field prior to the PEAK challenge (two field trials were conducted early in the project to validate lab-scale results). Nevertheless, as mentioned by the reviewers, translating lab-scale improvements in robustness and productivity proved difficult. With respect to *Nannochloropsis* consortia, lab-based environmental simulations demonstrated that (1) functional diversity along axes of light, temperature, and salinity was present across *Nannochloropsis* species/strains, and (2) *Nannochloropsis* consortia productivity was greater than average monoculture productivity. We conducted these comparisons at a variety of scales from well plate (5 mL) to flask (250 mL) to bioreactor (500 mL). However, the specific environmental conditions in our short-term field trials may not have perturbed the cultivation system long enough to validate what was observed in the lab. Thus, our working hypothesis is that consortia are most likely to be advantageous over longer periods and under sustained to frequent suboptimal conditions, which was beyond the scope of this project. With respect to algal-bacterial consortia, lab-scale experiments demonstrated that bacteria can influence the productivity and/or stability of *Nannochloropsis*. We used high-throughput screening instead of a functional approach due to the resource (namely time) constraints of the project. We demonstrated improvements with metrics beyond optical density (i.e., chlorophyll fluorescence, photosynthetic yield) due to the confounding effects of bacteria in measuring biomass. As in the algal consortia, these productivity improvements were condition-specific (volume, shaking, temperature) and were easier to control and replicate in the lab. Moreover, open ponds added a level of complexity with the introduction of environmental bacteria; additional research is required to advance the application of engineered bacteria in open systems.

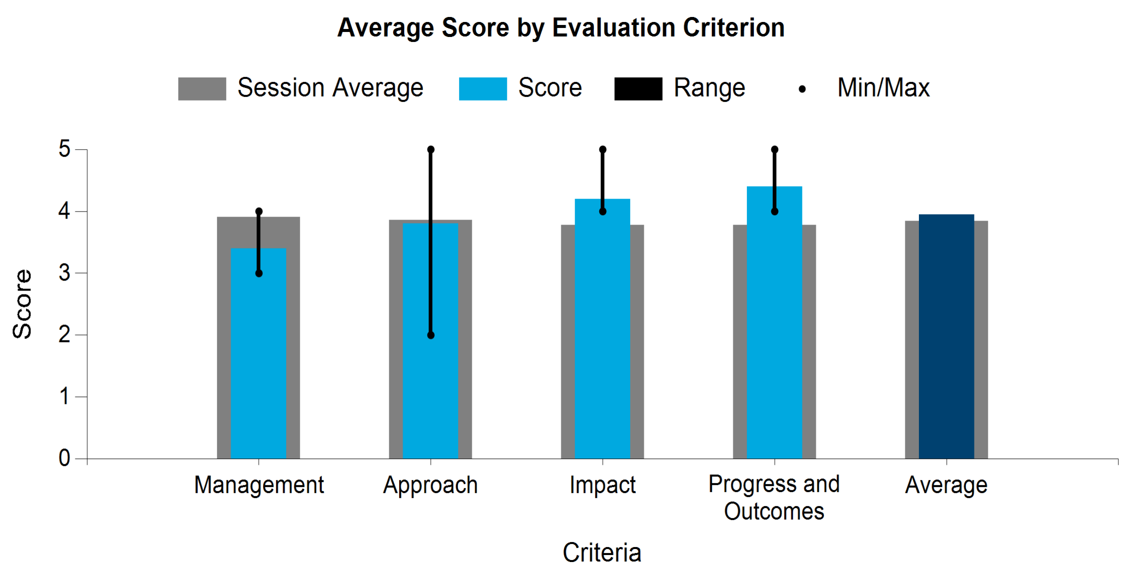
DEVELOPING ADVANCED GENETIC AND SYNTHETIC BIOLOGY TOOLS FOR IMPROVED ALGAE PRODUCTIVITY

University of California, San Diego

PROJECT DESCRIPTION

WBS:	1.3.2.650
Presenter(s):	Stephen Mayfield
Project Start Date:	09/30/2017
Planned Project End Date:	06/30/2021
Total DOE Funding:	$3,422,257

The primary goal of this project is to develop the advanced genetic tools, breeding, and high-throughput screening technologies that will advance the entire algae biotechnology field and enable economic viability of algal biofuels through production of high-value coproducts. We demonstrated the utility of this approach by applying these tools to commercial production strains of algae and cyanobacteria to advance the technology from the starting baseline state (technology readiness level 3, proof of concept) to demonstration at the pilot scale with our commercial partners, Algenesis and Reef (technology readiness level 6, prototype demonstration in a relevant environment). We have accomplished a significant portion of these goals and demonstrate this by rapidly producing the SARS-CoV-2 spike protein in algae, a critical tool for measuring antibody response in vaccinated individuals, and by demonstrating high levels of protein expression in commercially relevant algae and cyanobacteria strains. We were able to develop a process to take algae oils and convert them into two different polyurethane precursors, polyols and isocyanates. The development of bio-based isocyanate is potentially a game changer for the polyurethane industry. With Algenesis, we have turned the polyols into commercial products—to date footbeds and outsole of flip flops and shoes—that will be commercially available in spring 2021. We are now characterizing biomass productivity and lipid and protein accumulation, using TEA and LCA to evaluate the impact of coproduct production on the overall environmental and economic impact of these on biofuel production at the pilot scale.



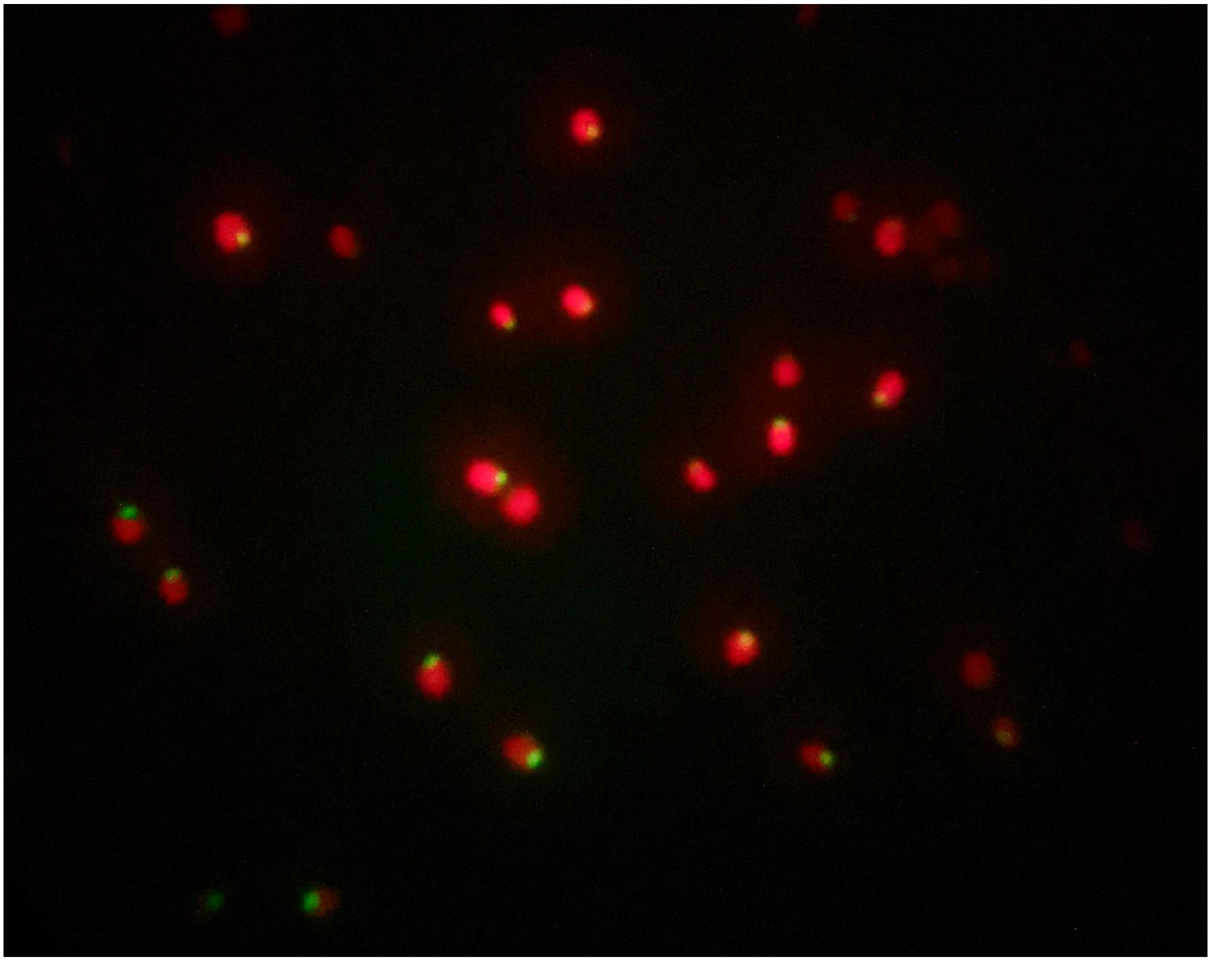


Photo courtesy of University of California, San Diego

COMMENTS

- It seems like roles and responsibilities are defined, although it would have been helpful to see more detail on communication strategies. It's great that everyone is close enough to chat frequently, but some documentation around decision-making would have been good here. It's almost a standard design now to do some synthetic biology thing, grow it up in a bioreactor, run some sort of -omics on it, and then put it outside. The empirical breeding method, though, is great and it seems like the team has come to the same conclusion that many others have with the TEA: It's not necessarily feasible to make only one product and expect that one product to yield all the profit necessary to achieve the financial goals. Excellent work on the commercialization of polymer starting reagents—targeting the fine chemicals and starting materials for semisynthesis is a great idea, as it minimizes both the economic risk of having minimal raw materials suppliers (often all the same raw materials suppliers who can be promising pie-in-the-sky, "Sure, I can make you 1,000 kg of dinitropyrazolopyrazole/ triazidomethane") and the ecological risk of making those materials from petrochemical starting materials. Be really, really careful about the protein therapeutics. They certainly make any economic analysis look great: current cost of goods of many monoclonal antibodies runs anywhere from $100–$500/g, so any way to make it cheaper is fabulous. Unfortunately, another partner even beyond the German company mentioned may be needed. Typically, MALDI-time-of-flight sequencing, circular dichroism, and nuclear magnetic resonance and X-ray to 1A resolution if possible; glycosylation and furin cleavage modification; immunogenicity in Balbc, B6, and NHP with cytokine profiles; as well as longer-term splenic beta activation and repeat exposure for sensitization are now required as part of preclinical tox studies. Also, for vaccine type of things like

COVID-19 spike, they also want epitope mapping to some degree. It's a notorious problem that Epogen in CHO cells can't even be made anymore, as people who need repeat infusions develop antibodies to the neuraminic acids. Some have resolved this with lec mutant CHO lines, and some have switched to HEK293, but it's definitely a big question to be answered before anyone could take it very seriously. Tox screening for immunogenicity in humans is expensive, lengthy, and beyond the scope of any academic lab, and it's definitely a deal breaker. The commercialization of the starting material for polymers is excellent. The flow chemistry in particular is a nice touch that makes it more readily adoptable from a health and safety standpoint—continuous processes are extremely efficient with CapEx and OpEx, and highly amenable to automated control. Growth in ponds is also great.

- The co-location of the small UC San Diego team caters well to close collaboration. Work with Algenesis will steer work toward commercial products. The role of GAI in this project is unclear. GAI may plan to commercialize strains developed in this project, but that has not been clearly communicated. Small raceway ponds and dewatering technology should be available as part of the existing UC San Diego facilities. Strain engineering and breeding strategies seem sound generally, but there is almost no description of how these critical steps or any other strain development steps will be approached. The same is true for the production process. A picture or general process flow diagram is a useful illustration to help substantiate an explanation of work, but it does not communicate approach on its own. With no details provided, it is difficult to give this approach a thorough review, even though the work seems promising based on the superficial insight reviewers have been given. Deliverables do not seem to be tied to any quantifiable metrics, and there is no indication that growth rate, biomass, or product yield are tracked or reported at all in this project. Breeding strategies seem to be a rational way to stabilize impactful traits without the stigma or regulatory challenge of GMOs, but the combined engineering and breeding strain development strategy appears to nullify the regulatory advantage of using traditional breeding strategies. This team's partnership with Algenesis has yielded tangible polyol products and offset the use of petrochemicals in the process. These products do not represent a major contribution to bioenergy markets, but this is a serious and impactful development for this project team and the industry generally. Production of a SARS COVID spike protein is not unique to this work but is significant and demonstrates the future viability of a new range of high-value biotherapeutic products that have the potential to drive commercialization of algae. GRAS (generally recognized as safe) status was granted for an algae strain during the course of the project. That deregulated status will ease use of this strain for this project and for future work. Rapid validation of COVID spike protein demonstrates rapid adaptability of this platform for recombinant protein development, making it viable for high-value product development. BASF partnership demonstrates viability of bio-based isocyanate products.

- The goal of the project is to develop advanced genetic tools to generate relevant industrial strains of algae and cyanobacteria. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, or go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The team incorporates new innovative approaches to molecular engineering, breeding, and high-throughput screening to develop strains with specific products. Targets include proteins and polyurethanes. The impact of the project was described based on the interest of relative industrial partners for each of the targets. The presentation would benefit from the addition of relevant impact to the BETO programs. The team has demonstrated synthetic, breeding, and mutagenesis and stacking of these approaches in algae. They have developed a pipeline to rapidly engineer organisms and have shown this pipeline for expression of the SARS-CoV-2 virus spike protein in *Chlamydomonas*. They have also developed new chemical conversion pathways for carboxylic acids to isocyanates. Next steps will be to take breeding experiments that stack performance traits in *Chlamydomonas* to a pilot demonstration. The presentation would benefit from discussion of quantitative yields; this should be done for the production of new mutants, generation of proteins, and even chemical conversion yields for the isocyanates and polyurethanes. This will provide a better assessment as to ability of the project to reach the goals of the PEAK FOA.

- The project team and work plan are intentionally organized to use laboratory and applied research to inform and guide commercial applications and engage commercial partner interests and resulting business opportunities. The examples of focus, clear thinking, and targeted outcomes can be used as a case study by BETO and other interested parties to showcase how opportunities of algal biomass commercial potential can be defined, developed, and delivered into the market. The project acknowledges and appreciates algae's fuel opportunity but also appreciates the scale, policy, and technology hurdles that remain. The project's development—especially of polyurethane and its many at-hand applications and ready commercial partner adopters—is clearly in support of many of BETO's primary goals. The presentation establishes the achievement of the project goal and the end-of-project milestone. The opportunity grasped, and timely relevance of producing the SARS COVID spike protein in algae and its potential application for use in an oral vaccine, may be yet another tremendous benefit of the project that could not have been envisioned at its inception.

- This project focuses on developing synthetic biology, high-throughput screening, and breeding tools to accelerate improvement in industrial strains of green algae and cyanobacteria and is of clear relevance to BETO MYPP goals. The project performers seem to have a clear management plan while leveraging expertise from the different investigators. However, the team needs to provide more clarity on channels of communication and collaboration. Risks involved in the execution of this project were not identified and mitigation strategies not outlined. Their approach to combining synthetic biology, breeding, and mutagenesis to rapidly evolve an improved strain is innovative, and if successful will be incredibly valuable across the industry. The team should consider applying this strategy to improve biomass productivity. Overall, the team has made significant progress toward the goals mapped out at the beginning of the project. They have demonstrated production of the SARS COVID spike protein in algae, as well as engineered algae to produce high-value polymer coproducts. They have developed a process to convert algae oils to polyols and isocyanates that is currently a commercial product with Algenesis. However, the team did not demonstrate an increase in biomass productivity with the engineered strain compared to wild type during the PEAK challenge.

PI RESPONSE TO REVIEWER COMMENTS

- GAI provided state-of-the-art sloped raceways ponds and harvesting equipment for the PEAK challenge. They have served as consultants to help us optimize the growth process and maximize pond productivity. Initially in the grant, we were going to use their production strains, but we were unable to develop transformation tools for their green algae strain, so alternative strains were used. Papers detailing the methods used for strain improvements have been published: Fields et al. 2019, with two additional papers under review (Berndt 2021 and Sproles 2021). Growth rate, biomass production, and product yields are measured at both lab and pilot scale and have been included in reports and publications. Because of the scope of this project and the number of tools that were being developed, there was insufficient time to detail all of the risk mitigation strategies. To address mitigation strategies in general, key participants hold biweekly Zoom calls and actively coordinate by email to address specific technical challenges. Many of the objectives are well contained within each group's expertise and abilities, so developing the different tools is clearly delegated than may have been obvious in the presentation. Additionally, monthly calls with the BETO team and quarterly reports are also used to ensure progress is tracked. All of the tools and strain improvement strategies developed in this project are being used now to improve biomass productivity and will be available to the general algae community for their use in any future projects to advance biomass productivity. Breeding work is being conducted at lab and pilot scale to further the biomass productivity and recombinant protein titers. Growth rate, biomass production, and product yields were measured at both lab and pilot scale and have been included in reports and publications. We did not mean to imply that we would ever produce an injectable therapeutic as a coproduct. We understand the complexity of making injectable drugs, as outlined above. We imagined that COVID spike protein produced in algae could be a useful reagent for monitoring antibody titers after vaccination to determine when a booster shot might be required. This project also allowed us to determine just how rapidly we could create a high-value production strain. The protein coproducts that we envision are more likely functional food and nutraceutical, which are both high value and do not need extensive purification when produced in an edible algae like ours. For those reasons, we have chosen to focus our studies on the human and animal nutraceutical osteopontin.

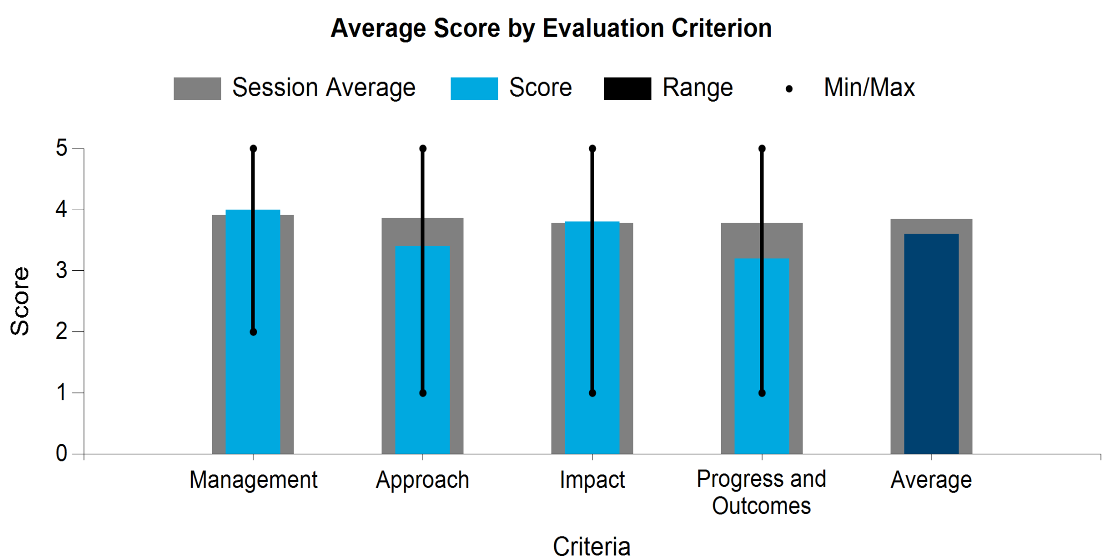
A COMPREHENSIVE STRATEGY FOR STABLE, HIGH-PRODUCTIVITY CULTIVATION OF MICROALGAE WITH CONTROLLABLE BIOMASS COMPOSITION

University of Toledo

PROJECT DESCRIPTION

WBS:	1.3.2.651
Presenter(s):	Sridhar Viamajala
Project Start Date:	09/30/2017
Planned Project End Date:	06/30/2021
Total DOE Funding:	$2,896,676

For algal biofuels to replace fossil fuels, it is imperative that cultivation systems are not constrained by (1) proximate availability of flue gas or other high concentration CO_2 sources, or (2) the energy and infrastructure burden to deliver CO_2 over long distances. Our project is investigating the cultivation of microalgae in high-pH and high-alkalinity media (pH greater than 10, alkalinity greater than 100 mEq) to achieve high biomass productivity and culture stability. Our cultivation media comprise high concentrations of DIC (greater than 60 mM) at pH values higher than 10. As alkaliphilic cultures grow, bicarbonate is taken up by the algae and CO_2 is abstracted and fixed while hydroxyl ions are released. In parallel, due to the high pH of the medium, gaseous CO_2 is dissolving into the culture medium at a rapid rate even from ambient air. The resulting high alkalinity in the growth medium ensures that sufficient bicarbonate remains available in solution for continued carbon fixation. At night, when photosynthesis is absent, the bicarbonate depleted from solution during the day is replenished via transfer of CO_2 from the atmosphere. Our results show that a fall season biomass productivity of ~16 g AFDW/m^2/d achieved with *Chlorella sorokiniana* SLA-04 can be supported on atmospheric CO_2 alone (i.e., without any inputs of concentrated CO_2). We have shown that borate is an effective rate promoter that enhances atmospheric CO_2 mass transfer and is compatible with microalgal cultures. We are also developing molecular toolkits that consist of metabolic and ecological models to be used for the prediction of optimal cultivation conditions. Lastly, CRISPR-based genome editing methods are being developed to enhance the productivity of strain SLA-04.



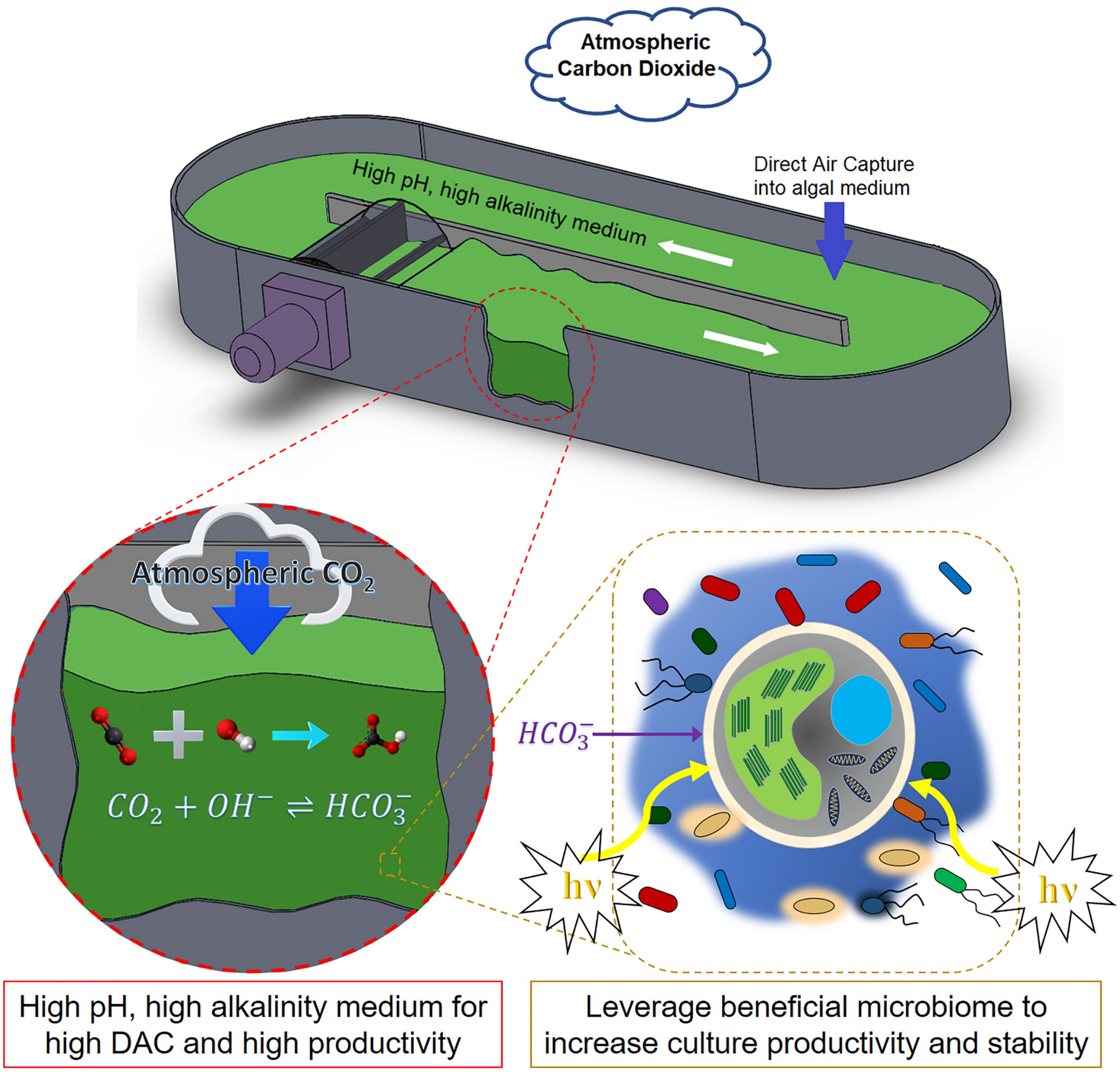


Photo courtesy of University of Toledo

COMMENTS

- The large and geographically disperse nature of the consortium creates an inherent risk for project management and communications and adds complications to reproducibility and risks that have only been superficially mitigated with a communications plan. Control of biomass composition is impactful but not novel. The same is true for the high-pH and high-alkalinity culturing conditions. Work seems to lack focus and does not appear geared toward commercial applicability now or in the long term. The amount of effort dedicated to TEA for such a small scale and modeled system makes the entire project seem like a mostly paperwork exercise with very limited verification of assumptions or conclusions in a natural environment. These models seem much more sophisticated than the ability to verify assumptions and conclusions. Decoupling lipid production from nitrogen starvation is impactful. The end-of-project goal to isolate one or more isogenic mutants for testing seems like a preliminary step instead of a final deliverable. Genome editing has not worked, and the goal or expected outcome of this work including gene target is not clear. It is not clear how labeling of active microbial community and characterization of this community in Task 3 relates to metabolic network development in Task 5. Many of the final deliverables seem like they are only intermediate steps in strain development or improved CO_2 utilization. There appear to be few tangible outcomes or metrics of progress presented for this project.

- The aim of this project is to develop cultivation approaches that use high-pH and high-alkalinity media to promote high rates of DAC capture and provide non-limiting HCO_3 concentrations for growth. The team intends to do this by optimizing media and cultivation conditions for higher carbohydrate and lipid contents, as well as developing a molecular biology toolkit to further improve productivity, composition, and robustness. The project goals are ambitious but achievable, building upon previous work in this area. The team had a clear management plan with a well-defined task structure and leads. They did not address risk and mitigation strategies but outlined established channels of communication and collaboration amongst team members. Their approach to improving CO_2 utilization using high-pH and alkaline media is well established in the field. However, a major challenge is low productivities of strains that strive in these conditions. Combining this with a process model, metabolic flux model, and diverse molecular biology toolkits to further understand alkaliphilic algal community dynamics for improved culture stability and productivity is innovative and improves the team's ability to succeed. This project supports BETO MYPP milestone of developing technologies that increase the mature modeled value of cultivated algae biomass by 25% over 2019 SOT baseline, as does developing strain improvement toolkits. Progress has been made toward establishing baseline cultivation productivity, process modeling describing inorganic carbon mass transfer, and diverse molecular toolkit development, but not in isolating a mutant strain with improved biomass productivity or in media and process condition optimization. The team identified borate as an effect promoter of CO_2 mass transfer but did not demonstrate improved biomass productivity using borate. The team should consider indoor testing of outlined strategies using outdoor-simulated conditions to gain understanding and assess performance of strains in high-pH and alkaline media before outdoor testing.

- The goal of this project is to develop algae strains that are effective growers under high pH and high alkalinity to enhance atmospheric CO_2 capture and utilization. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The description of the approach was based on an understanding of CO_2 dissolution kinetics and bicarbonate transport and utilization by algal cells. This is relevant but provides very little understanding of what technologies and methodology are being developed to overcome these limiting factors. The impact of the technology being developed was addressed both at the success of the project goals and toward the BETO overarching goals. The team has shown cultivation improvements that hit the FOA productivity targets. They have developed a process model for CO_2 mass transfer. They have shown that borate is an effective promoter for CO_2 mass transfer. They are working on microbial community characterization, including transcriptomics and metabolomics, under high- and low-alkalinity conditions. They are working on developing a metabolic network analysis for strain *Chlorella sorokiniana* SLA-04 that will be used to inform genome engineering through CRISPR/Cas9 editing. Concurrently, they showed process economics modeling that will inform impacts of cultivation changes to final targets. The project is only partially costed, and they have made very good progress. The presentation would benefit from more discussion on how the microbial community characterization will be used to help improve outcomes.

- The project is working to achieve very important progress in helping attain critical cost and strain performance improvements needed to achieve BETO's MYPP and other program goals. It has long been appreciated that the cost of CO_2 and the reliance of algae growth platforms needing to be located near available sources of CO_2 are serious impediments to viable commercialization. Similarly, it is also recognized that under proper pH and supplemental process conditions, atmospheric CO_2 can be captured into the growth media. A primary obstacle to deployment of these opportunities is the real limitations of algae strains of interest performing in the same conditions. This work presents the technical bases for atmospheric capture of CO_2 and developing and managing other important process conditions while growing productive algae strains in the high-pH and conditioned media. The resulting TEA improvements are reasonable and believable. Despite delays due to COVID, the presentation provides sufficient information to gauge that substantial progress is being made toward the project goal and end-of-project milestone.

- This is a great project. The management strategy is very solid and established. Communication methods are various and frequent. Data sharing is clear. Risk mitigation is done with lots of different sets of eyes looking for potential issues, which is exactly as it should be done. Replicates were run at different sites to validate original findings and ensure that all data are real. The approach is great. Strong theoretical background used to explain empirical observations, complete with real replicates from different sites to ensure repeatability and accuracy—this is exactly how science should be done. The goal is to eliminate a need for sparging, essentially. The connection between lab bench and commercialization requirements is very clear, and the goals are achievable, realistic, and critical to the success of algae production at commercial scale. Despite COVID-19, excellent progress has still been made with a lot of good results. It's also great that the mass balance and metabolic flux were worked both forwards and backwards to ensure they were correct ensuring that the model was representative of what was happening in the field. Some notes: CRISPR/Cas9 is a very promiscuous system; it likes to make changes here/there/everywhere. For that reason, the team may have to come up with some way to block it once it has done its job, which can be annoying. It would have been great to hear more details about the team's SLA-04 strain (understand there is limited time).

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their largely complimentary comments of the project focus, approach, management, and progress. Reviewers 1 and 4 inquired how labeling of active microbial community members and characterization of this community in Task 3 relates to metabolic network development in Task 5, and how the microbial community characterization will be used to help improve outcomes. In this project, we are determining active and inactive members of the microbiome under different growth conditions to aid in identifying the important community members. We have the capability of separating active and inactive community members from each other using either fluorescently labeled cell sorting or single-cell isolation using laser tweezers. Once the phylogenetic identity and ultimately the genome sequence is available for the active community members, metabolic models can be developed, which will be combined with the metabolic model for SLA-04 to create community-scale models. These will be used to predict optimal cultivation conditions.

- Reviewers also commented on the use of borate. In this regard, we would like to clarify that borate is a CO_2 rate promoter—i.e., it improves CO_2 mass transfer from the atmosphere into the cultivation medium such that DIC used in photosynthesis is replenished from the atmosphere more rapidly. Borate does not independently support higher growth rates in media not limited in bicarbonate.

- Additional reviewer notes relate to the mass-transfer modeling effort. Some reviewers appreciated the theoretical rigor, but there were also questions related to the utility of the model. The model serves two purposes: (1) assessment of atmospheric CO_2 capture rates for mass balance verification and (2) development of strategies to enhance these rates. In this project, we are focused on the first aspect—i.e., using the model to assess DAC rates. Improvements in DAC are outside the scope of this project.

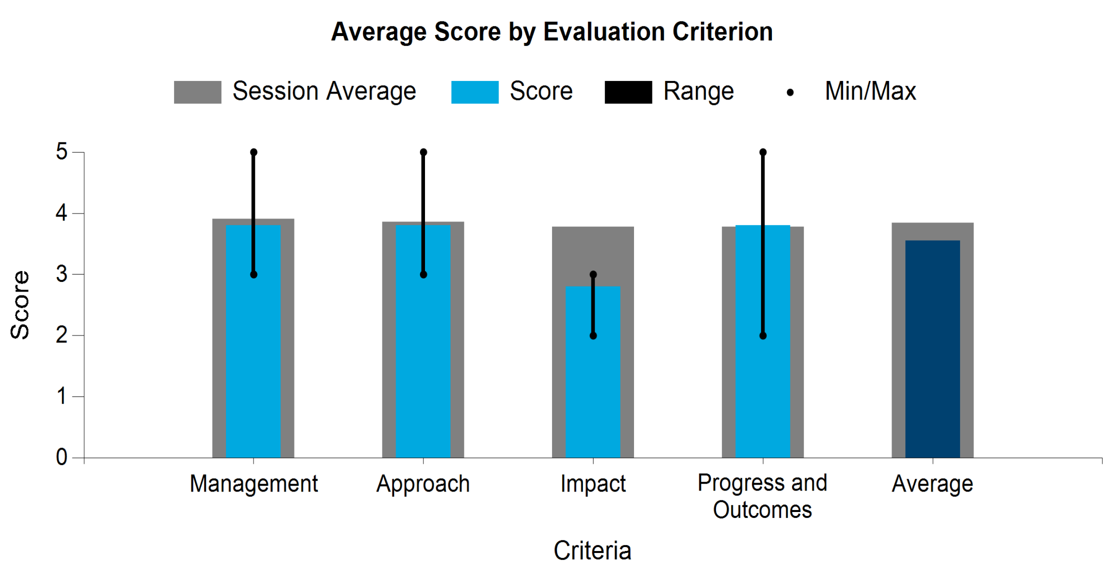
MICROBIOME ENGINEERING OF DESMODESMUS TO ALLEVIATE CARBON LIMITATION

Lawrence Livermore National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.2.652
Presenter(s):	Xavier Mayali
Project Start Date:	10/01/2017
Planned Project End Date:	09/30/2021
Total DOE Funding:	$1,410,288

Roughly half of the carbon photosynthetically fixed by microalgae is lost through extracellular exudation of dissolved organic matter. In high-biomass-density ponds, algal cells are also CO_2-limited and stressed from overproduction of O_2 from photosynthesis. In summer conditions, high light and temperature stresses further decrease algal photosynthetic efficiency. To reach the goal of 26 g/m^2/day ash-free dry weight produced during summer months, and using *Desmodesmus* strain C046 as a model system, our research aims to ecologically engineer the algal microbiome to enrich for bacteria that efficiently remineralize dissolved organic matter to CO_2 and simultaneously remove O_2. Our approach is to examine the microscale interaction between surface-attached bacteria and the *Desmodesmus* cells and use high-throughput sorting and screening with microfluidic incubation chambers developed by our industrial partner General Automation Lab Technologies. Using a combination of assays for dissolved organic matter, microbial community analysis, and algal growth quantification, we identify individual microbiome components with desired characteristics, such as high respiration rates and efficient dissolved organic matter removal. In addition to alleviating CO_2 limitation and O_2 toxicity, the outcome of this synthetic ecological engineering approach will decrease algal pond dissolved organic matter at harvest, which is a wasted resource and a supply of organic pollution if released into the environment, and which enables colonizing and possibly detrimental microorganisms to more easily invade the pond community. Our work includes scaling microbiome effects on algal growth from microwell to 100-L scale and the development of toolkits to be made available to the research community, including (1) a high-throughput assay approach for screening the effect of tens of thousands of distinct microbiomes on algal growth and (2) a medium-throughput assay for screening hundreds of microbiomes for high respiration and recycling of dissolved organic matter.



COMMENTS

- Close collaborations with other contamination control and microbiome experts seem appropriate to facilitate rapid dissemination of results and translation of new insights into actionable recommendations. Project risks seem well understood, but the mitigation strategy for translating results to outdoor settings seems inadequate. General automation technologies such as high-throughput screening paired with microbiome engineering seem to have practical potential. Targeting mutualisms beneficial to algae during heat and light stress seems like an appropriate constrained target for a potentially vast field of research. 16S rRNA sequencing may not give the detailed picture of the microbial community makeup required to engineer effective microbial consortiums. Whole-genome sequences paired with transcriptome analysis of heat and light stress challenges may give a more complete picture of community composition and activity, even without mechanistic understanding of mutualisms. Looking at inter-microbial flux and developing the tools to do so well has huge potential to illuminate mysteries about mutualisms in this field and much more generally. Presentations and posters do not seem like impactful work products, but progress toward project goals is impactful. GALT method development seems especially impactful for long-term microbiome engineering work. Discovery that these microbiomes do not have a significant effect on the chemical composition of the biomass is very significant and unexpected. This work points to the near-term feasibility of ecosystem engineering for large-scale algal cultivation. Engineered microbiomes are only marginally better than native microbiomes, though indicating a downselect for those communities most likely to participate in mutualistic relationships with algal strains could be beneficial. The team does not appear to have met its milestones and objectives at this point, but progress seems clear. The clearest picture of project outcomes will only be available once this team has completed outdoor testing as part of Task 3.

- The extensive collaborations and reporting are good; it would have been helpful to see more about project management, roles and responsibilities, and how data are shared here. It seems like the team is looking for biofilm cocultures to protect against photobleaching and heat stress by means of the nanowell system, but it isn't clear how this will be scalable or validated compared to outside pond stressors. The lack of validation and crop protection strategy means this isn't likely to be broadly generalizable. It is an interesting idea to be able to run essentially complete multi-factorial culture conditions in a series of the nanoreactors, but there is a lot there, which makes it a bad scale-down model. k_La in nanowells is definitely not going to scale. It's not clear how you deconvolute that from other factors, because the shading and k_La are so critical. The project goals were met; there was significant improvement with the designed microbiomes over baseline. Iron limitation is an interesting idea—of course, iron is usually tightly controlled to prevent a lot of random Fenton reactions from forming reactive oxygen species, iron sequestration is part of multicellular defense mechanisms against bacterial infection, and there's all the geoengineering experiments to scavenge CO_2 from the air by causing marine algae blooms.

- The project aims to alter the microbiome for *Desmodesmus intermedius* in order to increase biomass productivity under high-light and high-temperature regimes. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The team is implementing a microwell system for high-throughput cultivation of combinations of the algae with diverse bacterial biomes. Successful combinations will be tested for performance under high light and high temperature, and effective biomes will be characterized. The team will use isotope tracing to understand carbon flux between organisms. The approach is sound and was briefly described against go/no-go decision points and metrics. Rescoping of outdoor testing due to the COVID situation was discussed. Impacts were provided in a very general way. This could be improved by providing a better understanding of the state of knowledge in algae-microbial communities. The tie to the PEAK FOA can be strengthened by asserting goals for the project against the BETO goals. The team has been able to show microbiome combinatorial effects in microwell plates and has identified microbial communities that provide high algal growth under high-light and high-temperature conditions. They have also done airborne invasion and bird guano contamination studies to understand effects on microbiome and impacts on growth. Future work is planned to understand dissolved organic carbon production and utilization between organisms and outdoor mini-raceway growth. It is clear that the team understands the relevance of microbial alteration when going outdoors, as well as impacts on the cultivation process. The presentation would have benefitted from a more precise level of quantitation of organisms and variations in these when challenged with simulated contaminating environments.

- The project focuses on identifying strategies for altering the algal microbiome to increase biomass production under high-light and high-temperature conditions using *Desmodesmus intermedius* C046. Their approach of using a GALT microwell system for high-throughput cultivation under simulated outdoor conditions has a potential for success. The team had a clear management plan with a well-defined task structure and leads. They identified risks and implemented mitigation strategies, as well as established channels of communication and collaboration amongst team members. Overall, the team made progress in project goals. They demonstrated the impact of microbiome on algal biomass productivity under simulated outdoor conditions. Their optimized microbiome showed modest improvement compared to baseline. It's not clear from this project how this will be commercially deployed or how the optimized microbiome can be controlled in a raceway pond. Outdoor testing is critical to evaluate improved biomass productivity with altered microbiome.

- The project is organized and intended to deliver important tools and strategies to ultimately enhance algae performance. The project incorporates the use of GALT's technology in hopes of being able to facilitate high-throughput testing using nanoliter scale. This seems to have been able to achieve some results, suggesting improvements in algae-bacteria consortia and other performance enhancements at larger laboratory scales. The work appears to be delivering on possible tools for further work in support of BETO's PEAK and other programs, with the ever-present caveat that the work or tools must translate in larger scales and outdoor conditions to deliver real, meaningful value. The presentation provides sufficient detail to gauge that meaningful progress is being made against the project goal and end-of-project milestone.

PI RESPONSE TO REVIEWER COMMENTS

We thank the reviewers for their hard work, constructive criticism, and positive comments. Below we address criticisms point by point:

- "The mitigation strategy for translating results to outdoor settings seems inadequate." Most of the work for this project has been in the laboratory and at small volumes, and indeed the outdoor trials will occur at the end of the project, which will not leave any opportunities to change any of our cultivation parameters if the outdoor results do not show any statistically significant differences. We hope that the outdoor data, however, inform our future projects, and if the reviewer has suggestions for mitigation strategies, we would greatly appreciate constructive feedbacks and suggestions.

- "Whole-genome sequences paired with transcriptome analysis of heat and light stress challenges may give a more complete picture of community composition and activity." We completely agree but this was beyond the scope of the project.

- "Presentations and posters do not seem like impactful work products." We do not agree with this comment. We consider communicating our science to traditionally under-represented groups (Florida A&M, a historically black college) to be critical for increasing the diversity of science, technology, engineering, and mathematics (STEM) careers. Participating in BETO-organized workshops and the Algal Biomass Summit are also impactful in the field.

- “The team does not appear to have met its milestones and objectives at this point.” Indeed, our original goal of 100% biomass improvement was clearly an overly ambitious goal, and we rescoped after go/no-go milestone #2.

- “It’s not clear from this project how this will be commercially deployed and how the optimized microbiome can be controlled in a raceway pond.” Our project was low technology readiness level, and commercial development was not part of the work scope.

- “Impacts were provided in a very general way. This could be improved by providing a better understanding of the state of knowledge in algae-microbial communities. The tie to the PEAK FOA can be strengthened by asserting goals for the project against the BETO goals.” We apologize for not being clear regarding the impacts of our work to general BETO goals. At the time of the FOA, *Desmodesmus* strain C046 was identified as the SOT, and we specifically decided to work with this strain so we would not have to spend a lot of time and effort to re-establish baselines. Thus, any improvements in biomass production shown in our project can be compared to the 2016 SOT numbers already established with this strain.

- “The presentation would have benefitted from a more precise level of quantitation of organisms and variations in these when challenged with simulated contaminating environments.” Due to time limitations, indeed we did not show the relative changes in organisms’ abundance when the cultures were forcefully contaminated (note that we did not carry out microbial community analyses in the dust and guano incubations, since these were carried out in closed bottles with no new bacterial inputs from the atmosphere).

- “It would have been helpful to see more about project management, roles and responsibilities, and how data is shared here.” We apologize, and we can briefly provide more information here. During our collaboration with GALT, Lawrence Livermore National Laboratory and GALT would meet monthly via WebEx, and samples were exchanged in person by meeting halfway between Lawrence Livermore National Laboratory and GALT (in Hayward, California). Data were exchanged via Google Drive. PI Mayali was responsible for all communications with GALT and spent several full days at GALT to better understand the procedures carried out with the GALT Prospector instrument. Since the GALT subcontract ended, we no longer hold monthly meetings, and communications are on an ad hoc basis.

- “It isn’t clear how this will be scalable or validated compared to outside pond stressors.” We agree, and this is indeed a topic that deserves much further investment: How robust are microbiome interactions when the co-cultures are incubated outdoors, particularly when compared to other influences? This is precisely why this project aimed to examine the combination of high light/temperature and different microbiomes, and we are only just beginning to scratch the surface of environment-algae-bacteria interactions.

- “The lack of validation and crop protection strategy means this isn’t likely to be broadly generalizable.” We assume by “validation” the reviewer means showing that the microbiome mutualisms also work outdoors (which we have not yet carried out). We are not fully clear on what the comment about crop protection refers to, but we do think that the strategy is generalizable, because we hypothesize that the microbes that confer increased algal growth under high light and temperature have adaptations for such environments, such as pigments. Indeed, the dominant organisms in the microbiomes, generally members of the Flavobacteria, are known to have pigments, and we are working toward isolating single strains for testing with other algal strains in future projects.

- "$k_L a$ in nanowells is definitely not going to scale. It's not clear how you deconvolute that from other factors, because the shading and $k_L a$ are so critical." We agree that scaling is a problem in our project and in most other high-throughput screening projects with microalgae, but also beyond, in any area of biotechnology when living organisms are asked to make products at high scale. We unfortunately do not have the technical expertise to solve this problem.

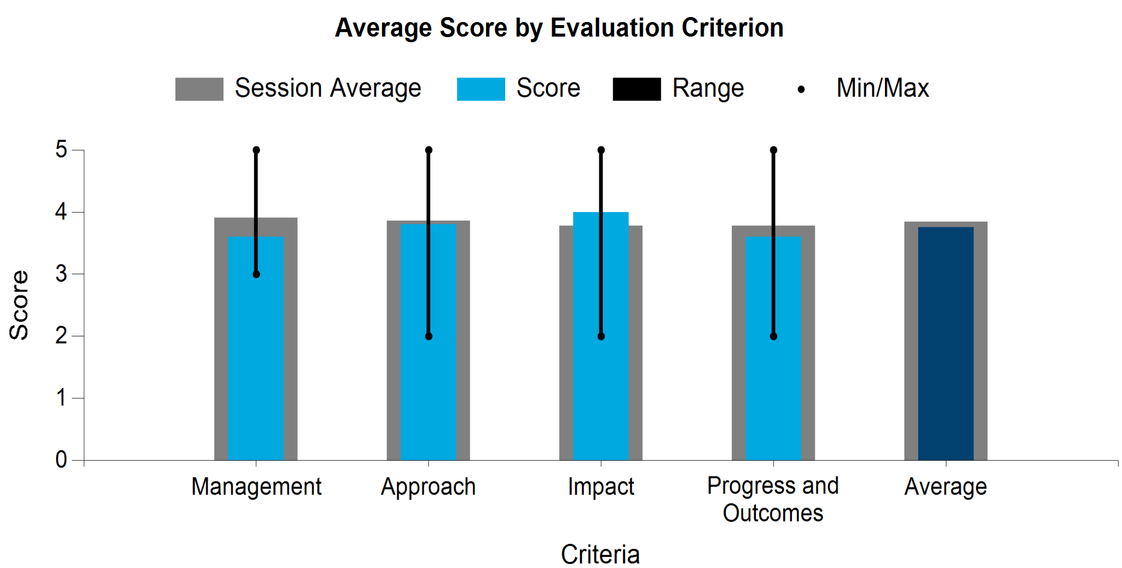
ALGAL FEEDSTOCKS LOGISTICS AND HANDLING

Idaho National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.3.100
Presenter(s):	Brad Wahlen
Project Start Date:	04/01/2015
Planned Project End Date:	09/30/2022
Total DOE Funding:	$2,160,000

This research project provides solutions to feedstock supply and logistics challenges experienced in algae biofuels to reduce the risk of feedstock loss and add value to the biomass. The project will identify degradation mechanisms of algae biomass in the moments after harvest and before conversion, and develop approaches to enhance stability, optimize storage processes that mitigate for seasonal variation in productivity to preserve outdoor-cultivated algae and to increase its value, and ensure storage processes are relevant to industrial strains and commercial scale. Stability assessments indicate that as much as 4% of algae biomass can be lost to degradation within 4 hours of harvest, but losses can be reduced to 1% with active stabilization approaches. Algae productivity varies seasonally, with summer production 3–5 times greater than in the winter, necessitating that a portion of biomass from the summer be preserved for winter conversion. This project has demonstrated that >90% of algae biomass can be preserved for 180 days using wet anaerobic storage, which when used instead of drying can reduce the cost of algae biofuels by $0.32/GGE. Storage byproducts add value, further reducing the cost of algae biofuels. The end-of-project outcome will be a long-term stabilization approach for outdoor cultures that limits losses to 10% or improves biomass value by 15%, enabling $2.50/GGE biofuels.



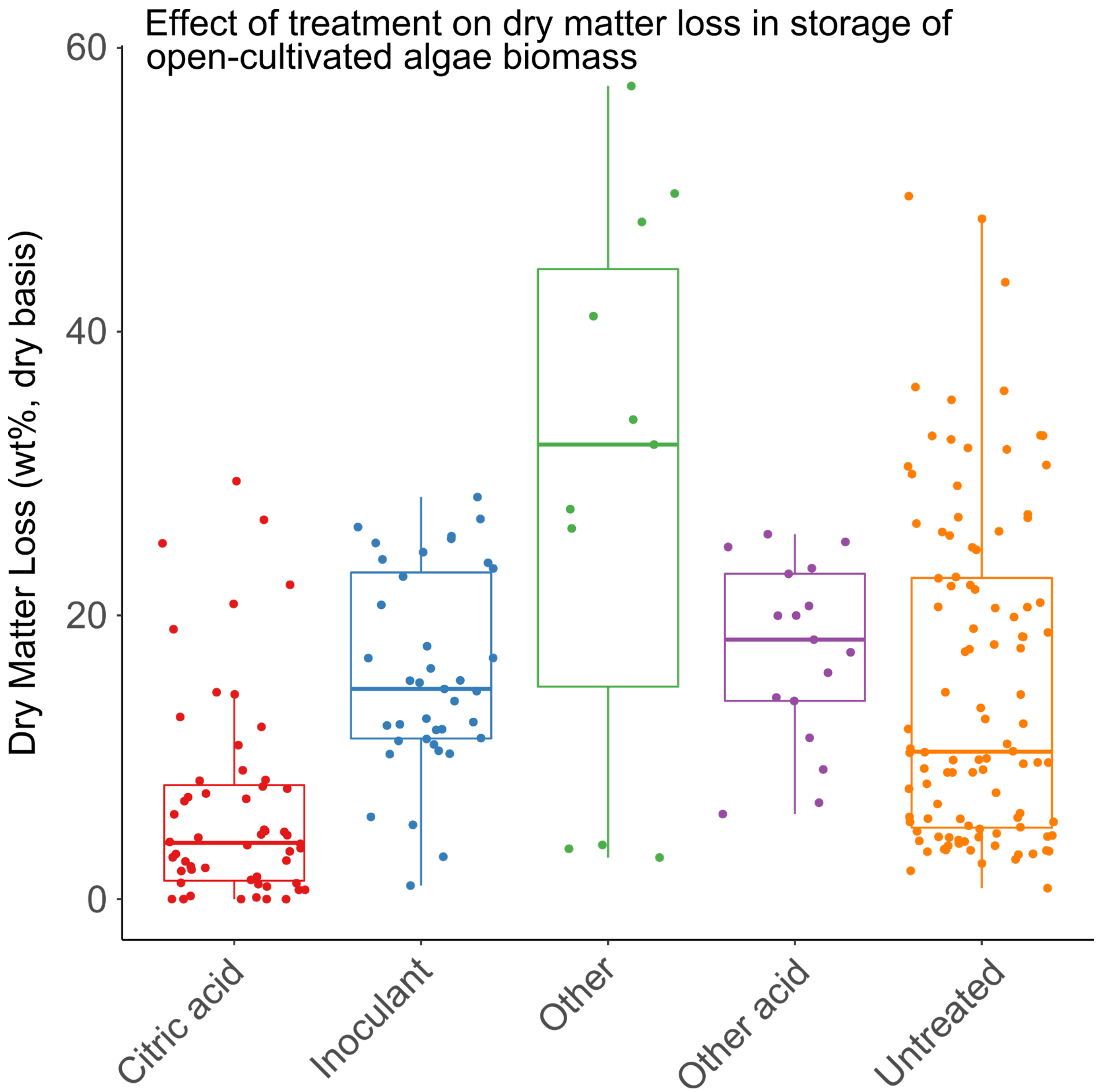


Photo courtesy of Idaho National Laboratory

COMMENTS

- Missing roles and responsibilities and risk management information. Communication methods and project goals are defined. Description of storage material stability and acidic preservation methods is clear; the difference between raceways vs. bioreactor material seems to indicate there is some bacterial degradation that led to the lactic, citric, and acetic acid methods intuitively. Primary recovery techniques are critical, especially when there is an alternative to drum drying and other more energy-intensive processing steps that would obviously affect the economic balance. Storage is certainly an issue in northern climates, and the method seems suitable for scaling. It's good the team went with the *Ball Book of Canning* method as opposed to hákarl; though there is minor concern related to the handling of the fresh material, as bruising any plant is notorious for creating endo-oxidation (i.e., tea leaf fermentation). Increase in yields is incremental, but overall keeping a processing plant running year-round and smoothing operational peaks is extremely helpful toward commercialization efforts.

- The Idaho National Lab team is well positioned to collaborate with NREL standards development efforts and contribute to the economic viability of algae products. Participation with the DISCOVR project seems well suited to positive collaboration. It is unclear that the aggressive goals described are based on clear quantifiable metrics. It appears as though most of this work is centered in modeling efforts, but the specifics of the approach seem unclear. This work seems critical but also disconnected from the realities of any current algae cultivation paradigm. The largely modeled nature of the work for n^{th} plant scenario makes it largely esoteric and hypothetical. Without market pull for this innovation, it appears to be an invention that does not answer an immediate need. Expanding the geography for cultivation by offsetting periods of low production can strengthen the reach of this industry. Go/no-go milestones appear to be based on observation of modeled success. The lack of field validation means that the impact of this work may never be realized as more than a paperwork exercise. The conclusion that community structures vary across treatments seems obvious and does not help address any challenges without more detail or actionable recommendations.

- The project has developed very important insights regarding how the stability of outdoor-cultured strains that can be used as systems and processes are designed going forward. Given the costs and overall efforts that will be a part of every commercial-scale facility, any techniques that provide queuing stability and minimize biomass losses, improve product volumes, maintain quality, and beneficially impact TCA and LCA. These directly benefit BETO MYPP and other goals. Conceptually, the application of long-term storage using wet anaerobic conditions may provide many benefits. Certainly, at scale, it can be used for process flow equalization and help optimize the average daily capacities of other unit operations so as to minimize infrastructure costs and maximize available plant capacity—all essential requirements for long-term profitable plant operations. The technical details for the design of these storage facilities will need to be carefully considered as the concept is advanced. The volume size, hermicity, safety, and operability will be important and possibly expensive to ensure the desired outcomes. Cost and scale of these facilities may be quite significant and impact commercial economics. Regardless, the concept seems ready to advance and be refined.

- The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The project aims to understand the metabolism of algae post-harvest, modes of degradation, and the fundamentals of preservation, and provide TEA assessments of impacts of storage treatments and processes. The approach is deemed appropriate for the established goal. This is a unique and critical project in the BETO portfolio. Successful preservation technologies and methodologies will have long-term impacts on the overall success of the portfolio and future BETO endeavors. The impacts across the portfolio were appropriately explained. The team has developed long-term storage capabilities that shows preservation of over 90% of the algae feedstock over 180 days. They have found that addition of citric acid enhances the stability of biomass by modifying the microbial community. They have also evaluated the recovery of valuable organic acids (e.g., succinic acid) from stored biomass. The progress and outcomes of the projects research efforts were clearly laid out, including the impacts of the results and future work still to be done.

- This project focuses on providing solutions to feedstock supply chain and logistics challenges that occur after harvest and before conversion. Previous work focused on developing long-term storage approaches that can stabilize algal biomass while reducing energy input in a cost-competitive manner. The team is building on previous work by identifying degradation mechanisms and developing approaches to enhance stability and optimize storage processes relevant to both industrial strains and commercial scale. This has clear relevance to BETO MYPP goals and addresses Aft-F and Aft-G barriers. The team outlined a management plan with a defined task structure and leads leveraging team expertise and previous experiences. They identified risks and outlined mitigation strategies, as well as established channels of communication and collaboration amongst team members. The team is well managed and has shown tremendous progress, evident in the patents and publications. Their multipronged approach of understanding algae post-harvest metabolism, mode of degradation, and fundamentals of preservation and organic acid production, as well as developing a TEA model to understand impacts on commercial-scale processes, is innovative and has a very high likelihood of success. Knowledge from this work can also be used to address nighttime loss experienced during cultivation. Significant progress has been made toward achieving goals outlined by the team. They have demonstrated stability during long-term storage using a low-cost wet anaerobic approach, queuing stability using citric acid of 15 unique strains from 34 unique cultivations. The team should provide more insight on treatment conditions and if/how biomass compositions change. The team should consider challenges of scaling wet anaerobic storage to the n^{th} plant.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewers' positive comments about the relevance of the project to BETO's MYPP goals and its unique role in helping BETO establish an economically viable algae biofuel and bioproduct industry. Effective storage is critical to establishing a thriving algae biofuels industry. One reviewer raised that point that given the current state of the industry, storage may not be an immediate need. Integrated biorefineries for lignocellulosic ethanol production did not adequately address feedstock logistics prior to building demonstration plants. These plants struggled to operate effectively due to feedstock issues. Although there is not an immediate need for algae storage, preservation and other logistical concerns are better addressed at this stage in industry development than when multimillion-dollar facilities are grappling with managing logistics and handling algae biomass. Solving this problem now could help address known concerns and reduce the risk of investing large capital in building a first-of-a-kind plant.

- The issue of scaling wet anaerobic storage to the n^{th} plant design is an important consideration for this project. We have partnered with NREL to understand how this approach to preservation might integrate with their current n^{th} plant algae farm and conversion designs, and we will continue to share our latest developments for improved integration. We are also working on developing physical models of our storage system at scale. These models will account for the effects of temperature and other variables on stability in storage, as well as help understand the optimal configuration of storage vessels that minimize cost and impact of external environmental factors. This project has made use of models to understand the impact that wet anaerobic storage could have on the economics of algae fuels and products, and the impact that environmental conditions could have on stability at scale. These models are based on data obtained from hundreds of laboratory observations of algae biomass in storage. The scale of studies has ranged from small serum vials containing 30–50 g (dry basis) of algae biomass to 5-gallon buckets containing several kilograms of algae that was then provided to partners at PNNL for conversion studies. Physical and techno-economic models will help us to address the economic viability of wet anaerobic storage. Both approaches guide our method development to ensure that wet anaerobic storage is relevant to the growing algae industry. We will continue to use TEA to effective storage treatments that can encourage preservation while having little impact on the minimum biomass selling price. We thank the reviewers for their comments regarding biomass quality in storage. It is an important consideration and one that we have sought to address. We have worked with conversion labs to assess the impact of storage on conversion of algae biomass to fuels, and so far, have been pleased to learn that stored biomass performs as well as fresh biomass in the processes evaluated to date. We recognize that each target product could have unique quality considerations, and wet anaerobic storage may need to be evaluated for impact to quality/yield for each specific product or conversion method. We welcome collaboration with producers interested in evaluating the compatibility of the wet anaerobic approach to algae preservation with their product or process. We appreciate the reviewers' feedback on the need to provide more insight on storage conditions and biomass compositional changes. We have assessed the composition of a number of strains, where we observed the carbohydrate fraction to be most affected in storage. We recognize that composition varies greatly between strains and is affected by cultivation conditions and media composition. We plan to improve our understanding of how initial composition affects stability and how composition changes in storage by assessing past samples and more consistently determining compositional changes in future samples.

- We regret that there was not adequate time to address project roles, responsibilities, risk management, and the project's progress relative to milestones and the go/no-go milestone. The project is being led by Brad Wahlen at Idaho National Laboratory. Hongqiang Hu is providing TEA for the project, and Birendra Adhikari is leading coproduct separations efforts. The physical model that will help us understand storage stability at scale is being built by Mitch Plummer, who has made similar models for herbaceous biomass storage. Cost competitiveness at scale is a risk we face in developing approaches to preservation. Our TEA and at-scale modeling efforts are being used to guide method development to ensure our preservation approaches are impactful to the algae industry as it develops. We use regular milestones to mark our progression toward the end-of-project goal, which is to reduce the minimum biomass selling price by 15%. At the end of March, we met our go/no-go milestone of reducing biomass selling price by 10%, and we are confident that we can reach the end-of-project goal. The go/no-go milestone will be based on the modeled economic impact of actual stored biomass. Specifically, we will look at the ability of storage coproducts produced in storage to be recovered to reduce the cost of algae biomass production. Achieving stability of outdoor-cultivated algae biomass is a significant risk to the success of this project given the challenge of modifying the microbial community in an open system. At the outset of this project, we were concerned that biomass loss in queuing (24 hours post-harvest) could not be reduced below 5%, and that the microbial community endemic to algae biomass could not be sufficiently manipulated/inhibited to enable preservation. We have looked at a number of different treatments to improve preservation of more difficult-to-preserve outdoor-cultivated algae cultures. We found that the use of citric acid as an additive before storage can effectively limit loss to 1% in queuing and does alter the microbial community by inhibiting the growth of *Clostridia* species, which act to degrade algae biomass. Evaluation of community structure has been an important tool that we use to understand how an individual treatment might cause a particular outcome. This has provided valuable insight into which organisms are important for preservation and those associated with degradation. This will lead to additional approaches to treatment that are targeted toward specific strains and could be less costly to employ.

THERMOCHEMICAL INTERFACE

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.4.101
Presenter(s):	Dan Anderson
Project Start Date:	04/01/2014
Planned Project End Date:	09/30/2019
Total DOE Funding:	$1,775,000

The Hydrothermal Processing for Algal Based Biofuels and Coproducts project is focused primarily on developing advanced hydrothermal liquefaction processing methods to improve process efficiency and reduce capital and operating costs to produce drop-in biofuels and coproducts from microalgae. The project is developing methods for sequential HTL processing of algae feedstocks and blends with terrestrial feedstocks. In stage 1, carbohydrates are extracted for bioconversion to coproducts. In stage 2, the residual biomass is converted to biocrude for upgrading to fuel. The waste streams from HTL are being processed to provide nutrient recycle for algae cultivation. The project is pivoting direction in FY 2021 to adapt the sequential HTL process to low-cost waste algal feedstocks. All data from these efforts directly support the algal HTL process model, TEA/LCA, and SOT. The SOT is used to determine the R&D targets to drive down fuel costs to meet DOE cost targets and demonstrate the commercial potential of the HTL conversion processes for the production of fuels and coproducts from algae and other wet feedstocks.

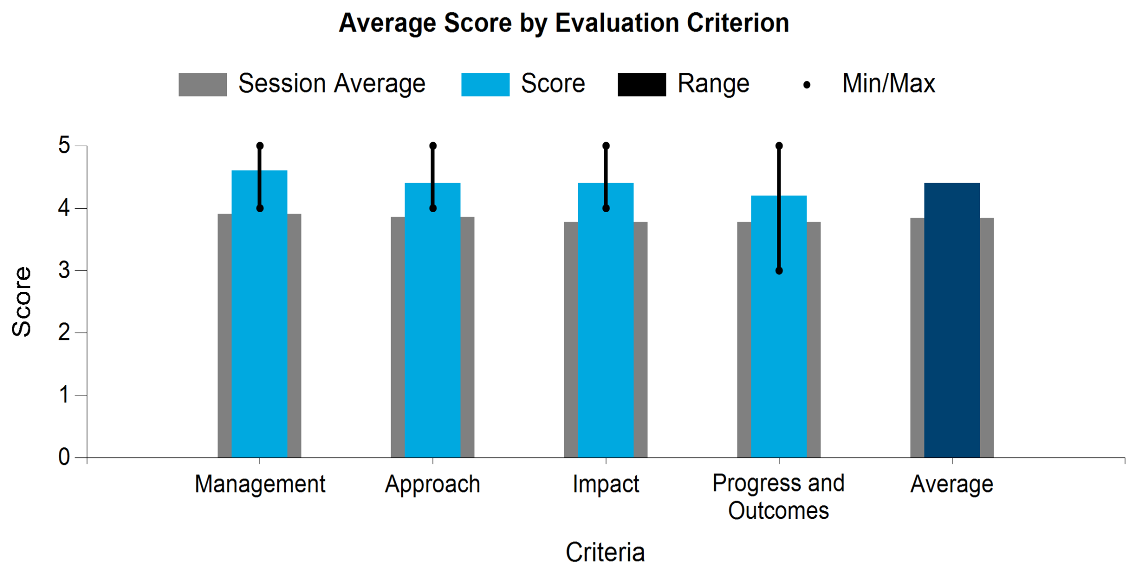


Photo courtesy of Pacific Northwest National Laboratory

COMMENTS

- This project is well integrated into other national lab efforts in a way that supports and improves all of them. Technical risks seem to be confused with tasks, and as a result, risk mitigation seems more like a work breakdown structure than a set of alternatives or parallel paths that could reduce impact of failure at any of the critical path steps. The pivot to sequential HTL for lower-value biomass seems to reflect the management team's commitment to industrially relevant solutions. The use of TEA to drive decision-making processes and informing the pivot toward lower-quality and uncultivated algae seems very sound. Incorporating lower-quality algal feedstocks into this process opens up many existing biomass sources and could further valorize existing waste-to-energy processes. Efforts of this ongoing effort have recently yielded tangible results and deployed technology. The results generated by this work have had unique impact on the development of the algae fuel industry, and historically, many teams have used assumptions about HTL to drive decisions about their commercialization plans. Real-world demonstration of this technology and integration of these data into modeling efforts will be critical for broad adoption of this technology, which seems almost certain if proven economically viable. The modeling efforts in this work are critical for optimization but still do not seem to provide potential users the information they would need to adopt technology without very detailed and resource-intensive investigations. A model that can predict HTL outputs based on easily translated feedstock composition metrics would be the most impactful work this team could do to make this technology approachable for potential users. The project team states openly and honestly that projected improvements in this process still fall shy of meeting long-term goals, but they have also offered viable solutions to overcome these shortfalls. Internal milestones appear to be met or close to target. The choice of lactic acid as a coproduct seems to lack specific market pull or customer partnership, but it does demonstrate the potential for coproduct development, providing needed adaptability for this platform.

- Roles and responsibilities are defined, and communication methods are detailed. Additional details on clearer risk management up-front and a connection to how this guided the project direction would have been helpful. Technically feasible; process optimization is critical to achieving the economic goals. The TEA and risk management strategy clearly guided the research, as opposed to being an afterthought. Clearly, the research has already had a significant impact on the biofuels community, as well as commercial impact with the Genifuel collaboration and implementation in Vancouver. This is a significant contribution to the economic feasibility of algae and agricultural waste processing into fuel and fine chemicals.

- The presentation documents the continued progress of this accomplished team of professionals and documents their interaction with other related BETO programs. They also outlined their adjustments in plan going into the new work period to maintain alignment with other programs. The work builds on previous efforts and systematically develops and evaluates unit operations in service of current best thinking regarding HTL and coproducts. While the scale of the unit operations remains at laboratory scale, there is enough basis for a professional opinion that they should be able to reasonably scale into larger and more integrated operations. It is important to appreciate they are able to evaluate the mass balances, and the finding that the only residual waste may be small amounts of ash further improves the TEA and LCA viabilities. The work is being widely disseminated via publications and presentations.

- This project focuses on developing/adapting hydrothermal process technology to enable the commercialization of algal-based biofuels and coproducts from lower-cost algae feedstocks derived from recycle of nutrients from wastewater treatment and marine macroalgal farms. This is of clear relevance to BETO mission and MYPP goals. The team has a clear management plan with a well-defined task structure and leads leveraging team expertise and previous experiences. They identified risks and outlined mitigation strategies, as well as established channels of communication and collaboration amongst team members. The team has made significant progress toward goals. They have demonstrated >65% carbohydrate extraction in stage 1 using a plug flow reactor instead of a continuous stirred tank. They have demonstrated lactic acid coproduct production and 50% biocrude mass yield for stage 2 sequential HTL (SEQHTL) processing. They have demonstrated growth of DISCOVR strains in recycled media derived from SEQHTL and have proposed multiple cycles for the recycle media. This will greatly reduce hydraulic load of large-scale algal ponds and reduce media cost. Overall, the outcome looks promising, and success in this work will reduce fuel conversion cost.

- This project is processing and providing data on SEQHTL that will inform an FY 2020 SOT report; the project will now shift to processing waste algal feedstocks. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The project is undertaking efforts to optimize each stage in SEQHTL, coproduct production, nutrient recycle, and evaluating the data from these processes for modeling and SOT updates. The effort is comprehensive and well laid out. Impacts of the technology development were adequately explained, along with impacts to BETO's targets and goals. The project team has developed a broad set of collaborative projects, industrial collaborations, patents, awards, and 20 publications. The results and outcomes of this project show excellent progress in all tasks undertaken. FY 2020 efforts focused on SEQHTL processing of corn stover/algae blends and use of carbohydrate from stage 1 in lactic acid production by fermentation. The team also demonstrated 50% biocrude yield from stage 2 processing of residual solids. Further, they showed the ability of eight DISCOVR strains to grow on recycled HTL-derived media. FY 2021–2022 efforts will focus on processing low-cost algae feedstocks. The approach for this effort was appropriately discussed and it is underway.

PI RESPONSE TO REVIEWER COMMENTS

We would like to thank the reviewers for their thoughtful and constructive comments and questions. We will address key questions and areas that need further clarification.

- "Additional details on clearer risk management up-front and a connection to how this guided the project direction would have been helpful. Technical risks seem to be confused with tasks, and as a result, risk mitigation seems more like a work breakdown structure than a set of alternatives or parallel paths that could reduce the impact of a failure at any of the critical path steps." The project has a more detailed risk mitigation plan that was included in the supplemental slides. We only had time to touch on key risk areas and how those were being addressed in our R&D strategy and associated tasks. Our risk mitigation plan does provide a set of alternatives or parallel pathways to reduce failures with each identified project risk.
- "Real-world demonstration of this technology and integration of these data into modeling efforts will be critical for broad adoption of this technology, which seems almost certain if proven economically viable." As presented in the HTL modeling project review directly following this one, the modeling team is a critical element of the overall R&D team and works side-by-side with the experimental team to integrate all testing data into the conceptual plant model as best as possible to guide the R&D and predict performance and economics at scale. PNNL is highly engaged in demonstrating performance at an engineering scale through the process development unit and is driving toward real-world demonstration through its collaborative efforts with industrial partners.
- "A model that can predict HTL outputs based on easily translated feedstock composition metrics would be the most impactful work this team could do to make this technology approachable for potential users." We agree that being able to systematically correlate incoming biomass composition with the upgraded fuel blendstock product properties is of great value. Toward this effort, we have developed reduced-order models based on PNNL's extensive library of continuous HTL processing data to predict biocrude yield and quality (https://doi.org/10.1016/j.algal.2019.101450; https://doi.org/10.1016/j.apenergy.2020.116340). This work was briefly presented in the HTL modeling project review.
- "The choice of lactic acid as a coproduct seems to lack specific market pull or customer partnership, but it does demonstrate the potential for coproduct development, providing needed adaptability for this platform." As presented in the HTL modeling project review directly following this one, the modeling team conducted a screening study to identify initial candidates for consideration with the sequential HTL configuration. Using current market size, price, and carbohydrate conversion demonstrated in the literature, lactic acid was identified as just one example, but by no means is it the only candidate coproduct. Many others are possible and moving forward we will continue to identify and hone the best options considering both coproduct market dynamics and overall process economics.

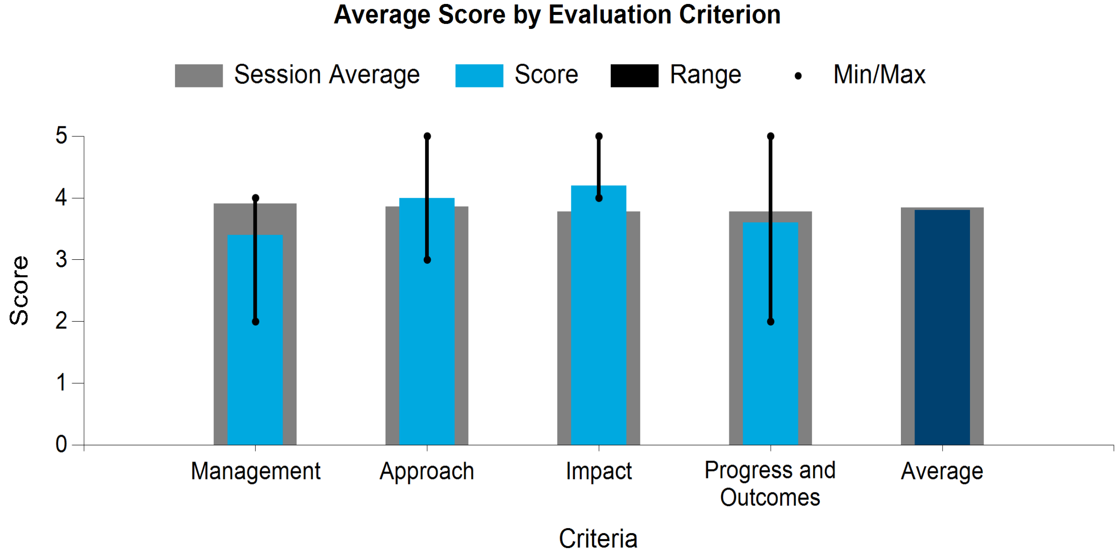
BIOCONVERSION OF ALGAL CARBOHYDRATES AND PROTEINS TO FUELS

Sandia National Laboratories

PROJECT DESCRIPTION

WBS:	1.3.4.200
Presenter(s):	Ryan W. Davis
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2021
Total DOE Funding:	$700,000

The purpose of the Bioconversion of Algae Carbohydrates and Proteins AOP project is technology development for valorization of a variety of whole-algae biomass sources for bio-based commodities, especially acknowledging the correlation between high algae productivity and high protein content. The objective of the R&D is to couple efficacious thermochemical and biochemical processing unit operations in a biorefinery context to maximize yields of fuels and bioproducts, generate value exceeding the biomass production and conversion costs, and facilitate major nutrient recycling and recovery of high-value coproducts. The conversion testing pipeline uses a diversity of algae biomass feedstocks, including various microalgae from open raceway ponds, as well as attached algae cultures arising from naturally abundant periphyton in compromised surface waters. Specific unit operations that have been investigated to date include dilute acid and flash hydrolysis pretreatments coupled to consortium-based microaerobic fermentation for targeted conversion of the biomass to chemical products. Notable successes from the work include the fusel alcohol platform, which features high titers, rates, and yields for ethanol, isobutanol, and isopentanols that have been demonstrated to be fit-for-purpose spark-ignition (gasoline-compatible) fuels, as well as a suite of fusel alcohol esters, including fatty acid fusel esters, which show improved performance over conventional biodiesel (e.g., fatty acid methyl esters). Using this foundation, we have recently begun pursuing opportunities to improve yields and process economics by utilizing the nitrogen that is abundant in proteinaceous algae biomass. To do this, we are engineering a bioprocessing consortium employing biocompatible strains of *E. coli* and *Corynebacterium glutamicum* to biologically produce caprolactam (via aminocaproate), which is a required crosslinker for enabling manufacture of fully bio-based nylon-6,6. Finally, through joint tasks with NREL, we are comparing modeled minimum selling prices for proteinaceous biomass conversion processes using projected feedstock costs, processing costs, titer/rate/yield metrics, and coproduct contributions to enable the highest probability of market penetration of algae biomass-derived chemical products.



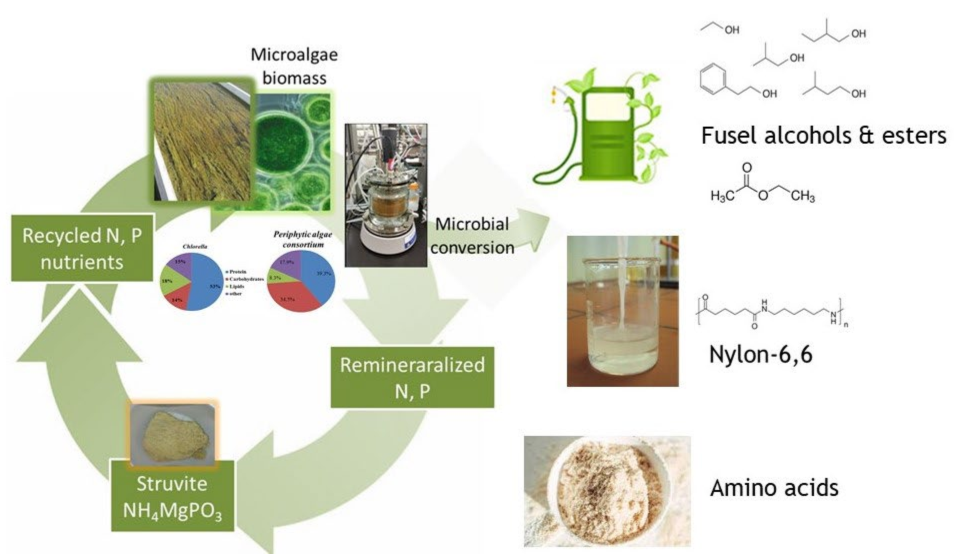


Photo courtesy of Sandia National Laboratories

COMMENTS

- Roles and responsibilities are defined, partnerships and engagement look good, communication methods are described, and goals are clearly stated, but there is not a lot of risk management information here. It's understood that the effort is going for more of a fine chemicals product with high-value amino acids, but it's a little fuzzy how this would compare to other feedstocks for alcohol and amino acid fermentation. The impact is clear: Utilization of new feedstocks and valorization of otherwise low-value material to produce coproducts has high commercial ability. The physicochemical analysis of the plastics to define their applications is impressive—this is a step many forget, for some reason. Fusel yields look pretty good, and the process is a good fit for existing manufacturing equipment, requiring minimal new CapEx investment. It's not clear that carbon black from algae can necessarily become economically competitive, but since amino acids are being made—they are typically used in animal pigment synthesis, particularly the iridescent and opalescent marine pigments, as well as eye pigmentation—it may be possible. The commercial success with polyurethanes and Zivo is great.

- The goal of this AOP project is to develop technologies for valorization of a variety of algae biomass sources for bio-based commodities using thermochemical and biochemical processing unit operations to maximize yields of fuel and bioproducts. Successful deployment of an algae biorefinery will overcome the challenge of utilizing low-value algae biomass for the production of lipids for bio-based commodities and coproducts and drive down biomass production and conversion costs. This is of clear relevance to the BETO mission and MYPP milestones. The team seems to be well managed with a good mix of research and industry partners. The team highlighted some challenges with the various approaches but did not identify mitigation strategies. The approach includes compositional determination of a variety of algal biomass, extraction of lipids, and the use of carbohydrates and proteins as substrate for microbial fermentation and co-culture. This approach is very innovative but ambitious. The team has demonstrated improvement in the utilization of protein and carbohydrate substrates to make fusel alcohol using *E. coli* co-cultures. The team is also developing a biosynthetic pathway for utilization of low-value algal amino acids for bio-based nylon-6,6 cross-linker. It's not clear from this work how the team intends to address challenges in metabolic engineering of pathways to work in *E. coli*, as well as growth and at-scale deployment of the fermentation and biosynthesis processes. Overall, the team has made significant progress in demonstrating algae biomass pretreatment using dilute acid and flash hydrolysis, achieved >10-g/L, >70% yield of algae carbohydrates and proteins to fatty acid methyl esters with nutrient recycling, demonstrated in vitro production of nylon-6,6 cross-linker, and is well on their way to develop an in vivo process.

- The management structure of the project looks like an almost overwhelming number of industry, academic, and national lab partners. It is not clear what roles and responsibilities each of these members has or how collaboration is coordinated. Conversion of complex biomolecules to fusel alcohols that can then be reformed into more complex fuel additives and chemical precursors seems like a choice made when a greater fidelity to liquid fuel products was demanded by BETO, and the shift to foams is a more appropriate direction for future research. Plans to drive flux of nitrogen into amine-containing products addresses a processing and separations risk that is often ignored or poorly ameliorated in other separation and upgrading plans. Plans to integrate learnings from traditional fermentation practices with modern bioethanol production show a practical approach to overcoming challenges. Not reinventing the wheel saves resources and eliminates risk. The presentation of rates and titers is compatible with existing bioproduct markets and represents a degree of maturity in tech transfer and commercial applicability. Drop-in-ready products have been well chosen, and alignment with the Co-Optimization of Fuels & Engines project will streamline early adoption of fuel products generated by this process. Final project goals are not clearly quantitative, making measures of success difficult. Outcomes as described seem to be largely model outputs with few quantitative metrics to ground assessment of progress of this work on its own.

- The project is designed to develop concepts in biorefining that can help optimize product production to help achieve economic viability for the algae biomass. The project presents conceptualized process diagrams showing content separation and then product end states. This is a very helpful visualization and tool for future use. The project presented interesting results and helpful guides of possible coproduct availability and their physical properties and treatment (or pretreatment) steps that would be involved. All of these are noteworthy and helpful in establishing the viability of a nice range of valuable coproducts that can help address some of BETO's MYPP and other program goals. Without mass balances and other supporting information, it was not possible to discern how much biomass could remain available for fuels or an approximate ratio of fuels to coproducts. Consequently, it was not apparent how LCA and TCA improvements were determined.

- The project's aim is to develop technologies for bioconversion of algal carbohydrates and proteins to fuels. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The team's approach is to procure biomass from various sites and perform compositional analysis, and then develop a biocatalyst by engineering pathways to *E. coli* and *Corynebacterium glutamicum* to be used in bioprocessing of algae for specific fuels. The project incorporates sustainability and economic modeling. The described approach is deemed adequate to achieve the project goal. Impacts of the technology and process development were adequately explained. Incorporation of impacts to BETO's targets and goals would enhance the presentation. The team has developed a pathway for fusel alcohol production from hydrolyzed protein and carbohydrates, along with fatty acid fusel esters from fatty acids. Further, they have developed a pathway for amino acids to nylon-6,6 cross-linker. The presentation can be enhanced by providing a quantitative assessment of milestones achieved and future work to be developed.

PI RESPONSE TO REVIEWER COMMENTS

- The presenter and project team would like to thank the reviewers for their thoughtful evaluation of this project. Specific concerns that were shared by certain reviewers were (1) lack of quantitative milestones and (2) inadequate description of risks and mitigation points. To respond to the first critique, this project includes specific milestones, including go/no-go criteria in FY 2020 and FY 2021 to address the process titer, rate, and yield metrics, which underlie the previously established TEA feasibility for this bioconversion technology (https://doi.org/10.1016/j.algal.2019.101412; 10.1016/j.algal.2019.101412). Data showing the progress toward titer, rate, and yield targets were provided on slides 8, 9, and 13. Risks were discussed in Section 2 - Approach (slide 6); however, mitigation was not adequately addressed. The overarching risk to this approach is that sufficiently high-performing enzymes can be coupled in a biochemical pathway in the chemical environment provided by the biocatalyst host (e.g., pH, osmolarity, and temperature tolerance) to support achieving the stated titer, rate, and yield metric goals. Mitigation of this risk is being pursued through use of the RetSynth (retrosynthetic analysis software) and associated databases (MetaCyc) for pathway design, in vitro enzyme testing and engineering prior to in vivo work (with support from Arizona State University academic collaborators) and testing with both model media and biomass hydrolysates. Further details about mass balances of fuels and coproducts' impact on TEA/LCA are specifically addressed in our recent BETO report with NREL and Algix, Inc. (doi:10.2172/1665822).

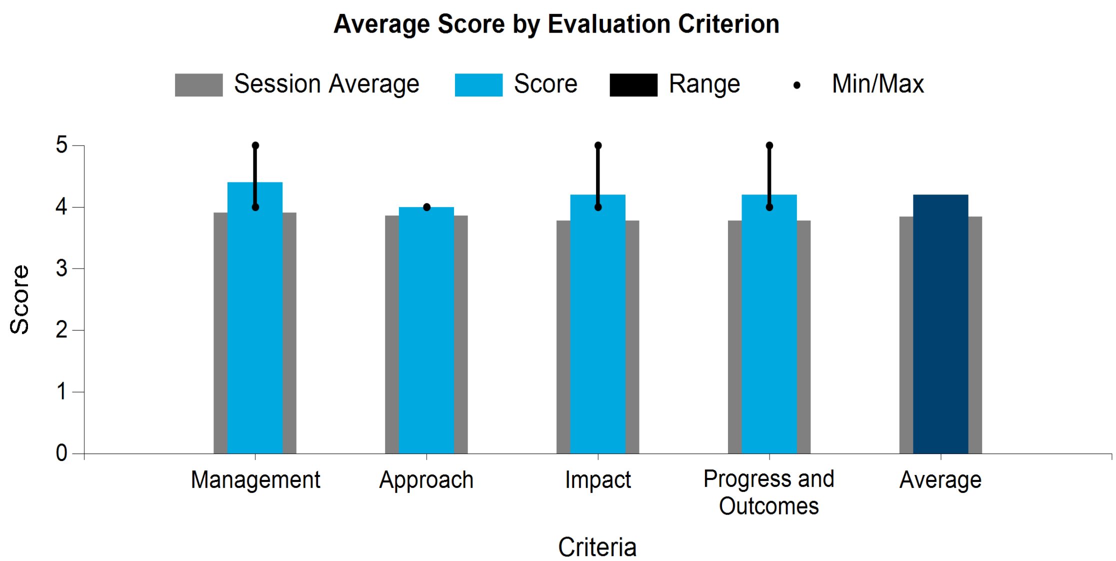
CAP PROCESS RESEARCH

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.4.201
Presenter(s):	Jacob Kruger
Project Start Date:	10/01/2014
Planned Project End Date:	09/30/2021
Total DOE Funding:	$1,812,000

Combined algal processing (CAP) is an algae biorefining approach that employs pretreatment and extraction operations to fractionate algal biomass into an organic liquid phase containing lipids, an aqueous hydrolysate phase containing carbohydrates and protein, and a residual solid phase containing insoluble matter. Each of these fractions is upgraded to fuels and/or coproducts, including non-isocyanate polyurethanes (NIPUs) from unsaturated lipids, fuels from saturated lipids, fuel precursor carboxylic acids from the hydrolysate, and conductive carbons from the solid residue. TEA of multiple CAP configurations suggests that some configurations have a viable pathway to algal biofuels at $2.50/gallon of gasoline equivalent.



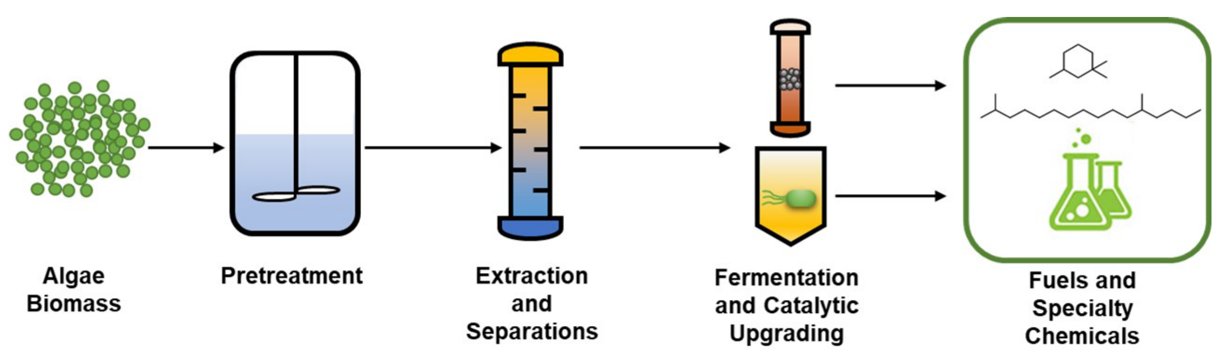


Photo courtesy of National Renewable Energy Laboratory

COMMENTS

- Combined algal processing focuses on developing biorefinery concepts that enable economically viable biofuel production that allows a path to $2.50/GGE hydrocarbon algal biofuels. Progress in the CAP research will drive down minimum fuel selling price (MFSP). The team appears to be well managed with a well-defined task structure and leveraging team expertise and previous experiences. They identified risks and outlined mitigation strategies. Their approach of developing robust technology to fractionate and valorize lowest-cost algae and then using the TEA to establish yield targets and quantify improvement is reasonable. The team has made significant progress in identifying pretreatment options that help mitigate seasonal variability in algae production, developing flexible hydrolysate fermentation that supports multiple product and fuel precursors, and demonstrating proof-of-concept for production and nutrient recycle from protein and carbohydrate-derived carboxylic acids. The team also incorporated high-value NIPU coproducts to model. The team should consider progressing the understanding on yield and losses in the different steps involved in the production of NIPU and upgrade the model to reflect this. The team should provide more clarity on CapEx required for this process.

- Communication is described, roles and responsibilities are well defined, and there is some risk management and a good array of collaborations set up to ensure multiple stakeholders' input will be considered. The approach is connected to the TEA and clearly defines process requirements. There is definitely potential for innovation in terms of semisynthesis of fine chemicals. Commercialization potential is obvious in the partnerships they've formed for polymer starting materials; financial connection between fractionation and high-value product streams is clear. The commercialization pathway is great. The jet and diesel fuel processing are also nice accomplishments. The critical role of flash hydrolysis in protein solubilization enables much higher yields of the protein-based products, which have sufficiently higher value to meet economic goals. It will be interesting to see what happens with the graphene as well.

- The aim of the project is to develop biorefinery concepts that enable economically viable biofuel production from algae of variable composition. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The team's approach is to develop and adapt technology for fractionation and use TEA to establish yield targets and show improvements in the MFSP. The fractionation technologies and incorporation of other processes should be detailed in this discussion. Impacts of the technology and process development were adequately explained. Incorporation of impacts to BETO's targets and goals would enhance the presentation. The team has shown that seasonal variation in production can be leveled through blending and storage. They have also worked on a halotolerant carbohydrate hydrolysis fermentation process to succinic acid and ethanol, and proteins to muconic acid. Other pathways being worked on include non-isocyanate polyurethane production, and conversion of carboxylic acids to ketones that will generate jet and diesel fuels. The presentation can be enhanced by providing a quantitative assessment of milestones achieved and future work to be developed.

- This project provides very interesting insights into potentially very viable applications for low-value bulk algae biomass. It presents strong evidence that a wide range of commercial applications can be simultaneously achieved, and if the biomass growth rates can be accurately calibrated against the conventional measurements used for open-pond algal growth systems, the work is clearly in service of working toward BETO goals. The opportunities involving fusel conjugates almost seems too good to be true and should be investigated to completion, including determining the types of policy support needed to benefit the existing gasoline and diesel marketplaces.

- This team's commercial and academic composition seems well suited for success; however, it is not clear what role all of these institutions play in this project. The go/no-go described uses quantifiable metrics to assess success. Risks and mitigation plans in this project do not seem well described. The challenge associated with enzyme use did seem to be resolved in a way that demonstrates good adaptability in the absence of a formal risk mitigation plan. All work seems well rooted in practical reality. The integration of this process into the NREL TEA will allow this to be more easily adopted as an industry standard. Alignment with the Co-Optimization of Fuels & Engines initiative will focus fuel development for real-world use. This work has yielded tangible results much faster than the HTL track. The CAP process was considered a long-shot parallel track to HTL. Mild oxidative treatment could be another viable pathway to fuel production. This conversion step has been a critical bottleneck for fuel development that has often been ignored under the assumption that HTL would be a conversion panacea. This research has uniquely enabled real product development. This impact is tremendous and may be the most significant update in the AAS portfolio this review cycle. Polyurethane foams are an excellent product for displacement of petrochemical use. This target and choice of feedstock is beginning to couple waste feedstocks with products that replace environmentally deleterious waste streams. This is well aligned with BETO goals and exceeds most other projects in both vision and impact. Work with Algix and interest from Patagonia demonstrates that this technology will likely be used to bring algae into the material product development arena. All goals for this project appear to have been met.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their positive and constructive comments. To clarify, fusel conjugates were not part of this project; we are focusing on drop-in hydrocarbon fuels. The lipid fractionation required for NIPU production is very similar to that required for omega-3 fatty acid production. While process losses in each step depend on algae and lipid composition, we have worked with Qualitas to understand the mass balances in their optimized commercial process and include these losses in the process models used for TEA and LCA. Similarly, the TEA models include the CapEx costs for the NIPU production, which are described in detail in our manuscript currently under review at *Green Chemistry*. Additionally, the CAP approach includes pathways to make use of these "losses," including fuel production from less-saturated lipids, and fermentation of glycerol produced during saponification. We agree that there is potential for semisynthesis of fine chemicals and are actively working with the ABC project to define new coproduct opportunities from highly functionalized classes of molecules. One class of fine chemicals we feel has significant potential is surfactants, which represent a large, diverse market with high value, and which are ideally suited to the amphiphilic molecules in algae. This opportunity is described briefly on slide 23 in the presentation. The fractionation technology is a key aspect of this project moving forward, and our FY 2021 Q4 milestone (slide 27) contains some details of what we are targeting in the fractionation step. In particular, with the transition to high-protein algae, most of the lipids are polar, necessitating saponification before upgrading to NIPU. We are targeting fractionation technology that can combine fractionation and saponification into a single step, including a longer acid pretreatment under milder conditions (analogous to the Twitchell process), alkaline pretreatment, and enzymatic hydrolysis using enzyme cocktails that include lipases. The primary BETO goal has been reductions in the MFSP (to $2.50/GGE), which was addressed on slides 3, 4, 6–8, 10, 13, 19, and 23 in the presentation. The secondary BETO target has been increasing the fuel yields (to >80 GGE/ton algae), which was not mentioned explicitly in the presentation, but was reflected in our go/no-go milestone in FY 2020 Q2, and to a lesser extent in our FY 2019 Q4 milestone (slides 25–26). A tertiary BETO goal has been a reduction in biorefinery carbon footprint, which was mentioned on slides 7 and 10 with respect to fixing CO_2 into coproducts. We have met all 17 milestones planned on this project to date (through FY 2021 Q2) in this project cycle, though a few were delayed due to COVID. Regarding future work, the MFSP graph on slide 13 of the presentation shows improvements in MFSP (to $2.50/GGE) out to 2030, mainly reflecting reductions in cultivation costs. Our remaining two milestones for FY 2021 target additional coproducts from phytol that is co-extracted with lipids but inhibits NIPU production (slide 23), and improvements in the pretreatment step that we expect will further decrease MFSP. We believe there is significant potential for graphene as well (FY 2021 Q1 milestone and slide 20). The Venn diagram on slide 5 of the presentation gives a high-level view of the roles project partners play. The industry partners are informing coproduct development (Polaris, Qualitas) and providing algal biomass to the project for CAP (Qualitas, Clearas, Gross-Wen Technologies, and AECOM). Other national lab projects are providing high-productivity biomass to the project (DISCOVR and LEAF), helping determine the robustness of CAP to ensiled or mixed biomass (Idaho National Laboratory and LEAF), helping translate fermentation technology from lignocellulose to algae (NREL Biochem platform), or helping identify new coproducts (ABC). University partners are providing algal biomass to the project for CAP (Arizona State University), co-developing fractionation technology (flash hydrolysis with Old Dominion University) or helping develop new coproducts (graphene with Rice University). The value to each of these partners from CPR is in developing CAP pathways that valorize the algae of interest to the partners, providing data on the processability of different algae streams, broadening the range of potential applications for technology developed on other projects, and providing data on the production of novel coproducts. Risks were briefly discussed on slide 4, but we agree that including the risk mitigation table developed for our AOP document in the additional slides section would have facilitated review. Risks (and mitigation) considered include:

- Potential poor performance of NIPU (which can be improved by esterifying or crosslinking with different reagents).
- Potential low yields of acrylated photopolymers (which can be improved by broadening reaction conditions or purifying the algae oil).
- Potential poor fermentation yields (which can be improved by adapting/engineering the microbes or relegating unused substrates to mild oxidative treatment or graphene).
- Potential low pretreatment yields at large scale (which can be mitigated by validating at multiple smaller scales before scaling up).
- Potential poor residue upgrading performance (which can be mitigated by considering multiple upgrading approaches, such as mild oxidative treatment and graphitization).
- Potential for none of the new pretreatment configurations outperforming the baseline, resulting in no MFSP reductions from improved pretreatments (which can be mitigated by expanding the pretreatment approaches surveyed and improving MFSP through incorporation of new coproducts). We have just begun evaluating enzymes for pretreatment on this project, but enzyme costs and usage will certainly be critical metrics for the success of that pretreatment approach.

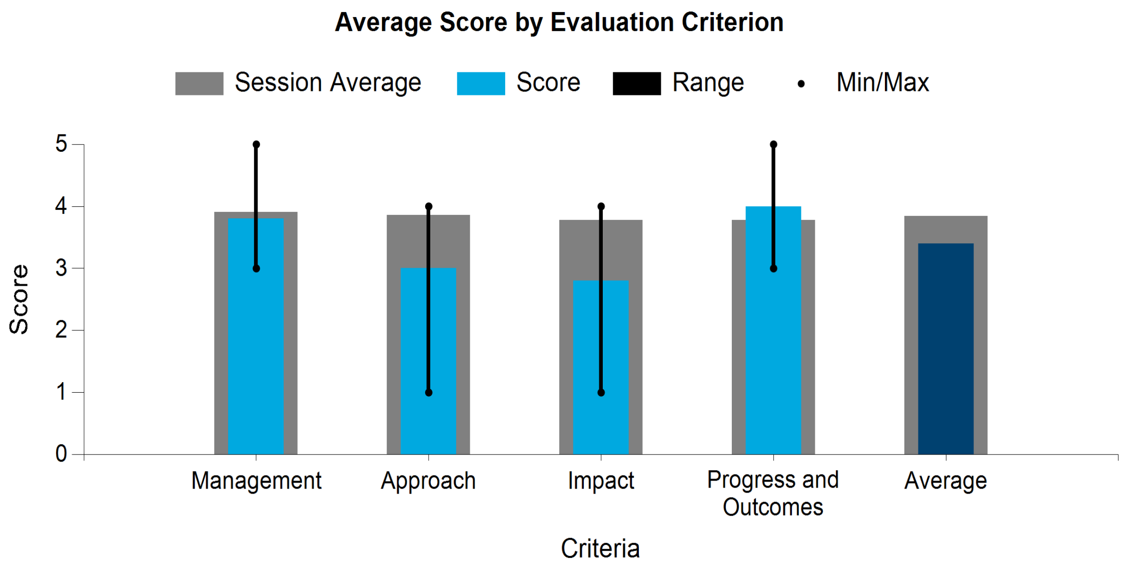
CYANOBACTERIA PHOTOSYNTHETIC ENERGY PLATFORM

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.4.301
Presenter(s):	Jianping Yu
Project Start Date:	06/24/2010
Planned Project End Date:	09/30/2021
Total DOE Funding:	$1,200,000

The primary challenge in algal technology is low energy conversion efficiency, currently up to a few percentages from photon energy to biomass energy. Most incoming solar energy is lost in the processes of photosynthesis, through mechanisms that are not fully understood. There is large room for improvement. This project develops cyanobacteria genetic tools to improve photosynthetic efficiency and biomass productivity through manipulation of energy regulation mechanisms. Increasing algal biomass productivity by 20% would translate to $0.90 per GGE reduction of MFSP. Genetic manipulation is an essential tool in understanding and improving algal productivity. While genetic manipulation is challenging for most algae, model cyanobacteria, with in-house mutant library and genetic engineering toolboxes, allow for rapid hypothesis testing and transfer of lessons to other cyanobacteria and eukaryotic algae. We developed the Energome (energy-ome) concept to guide novel engineering strategies and unleash unused potential in photosynthesis. Energome consists of cellular energy management mechanisms. For example, we found that cyanobacteria use futile cycles around glycogen and sucrose to dissipate adenosine triphosphate (ATP). By genetic modification, the model cyanobacterium *Synechocystis* 6803 showed higher energy levels, increased photosynthetic capacity, and >20% improvement in biomass yield in simulated outdoor light conditions.



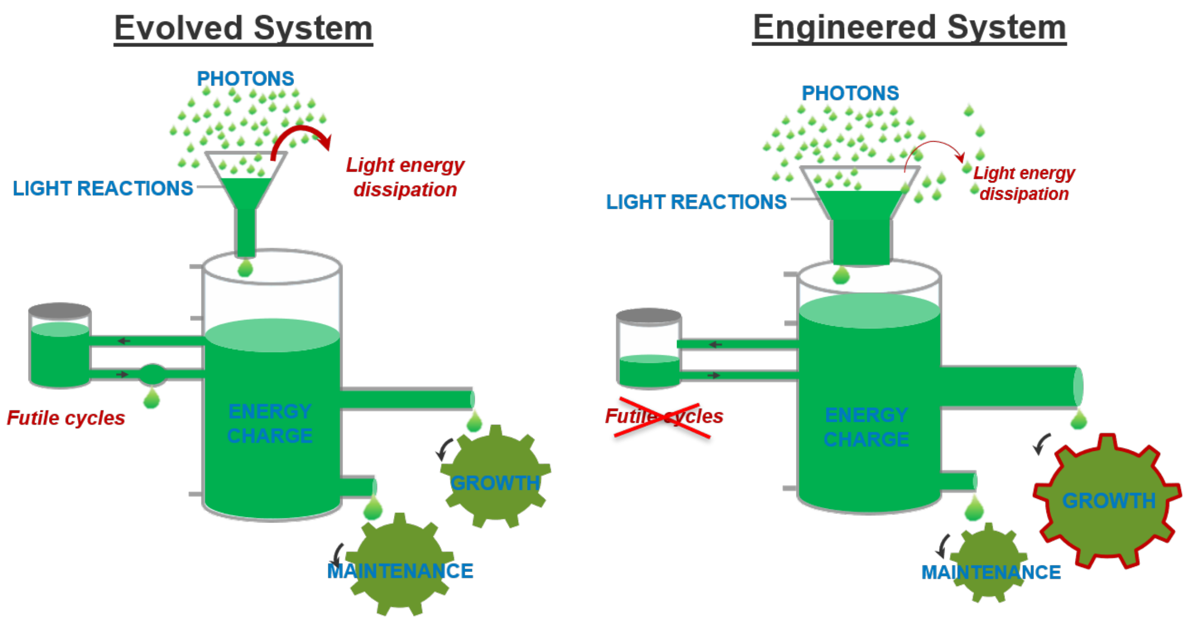


Photo courtesy of National Renewable Energy Laboratory

COMMENTS

- The team is small, so communications are straightforward. Due to the single task with the focus on only improving biomass, this project did not require a complex management strategy or role assignments. There has been considerable work done on this subject before, including scale-up to outdoor cultivation. It's not clear how this would be innovative or advance the state of the art. This strategy has already been tried at commercial scale and not been successful. Project goals were met, and the strain is more productive in a bioreactor. Manipulated strains of cyanobacteria rarely perform well in outdoor environments, particularly when subject to photobleaching stress and infection; this limits scale-up due to the increased CapEx requirements. There is no TEA impact presented.

- The concept of Energome is novel and aligns well with the synthetic biology vocabulary in wide use. Without question, an ability to improve the photosynthetic efficiency of algae remains a holy grail ambition and requires the development of the basic science and understandings that can be eventually leveraged to improve growth rates and product yields. The work here shows nice initial results at very small scale. Practical matters likely prevent any short to midterm viability to extend learnings into the field at larger scales and in outdoor ponds, making these current efforts not especially in service of BETO goals for the same periods.

- The goal of the project is to develop molecular engineering technologies in cyanobacteria that remove futile processes and redirect energy and carbon to growth and maintenance mechanisms. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was not described through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The approach is to manipulate cellular energy management controlling growth and carbon partition in *Synechocystis* through gene knockout and knockdown and extend the technology to other cyanobacteria. Describing specific steps and tasks to be undertaken would help present a logical method to achieve the project's main goal. The impacts of the project are in improved productivity. The work has generated five manuscripts in high-profile journals and a book chapter, along with a provisional patent and contributions to other efforts. The impact discussion can be strengthened by tying the project goals to BETO goals and targets. The team has demonstrated that reducing energy dissipation from futile cycles will redirect energy to cell growth. Two futile cycles were targeted: glycogen and sucrose cycles. Knockdown and knockout, respectively, of these cycles provided mutants that showed increased photosynthesis, higher energy charge, and faster growth. In environmental simulated reactors, the mutants grew 34%–40% faster than wild-type. This is an excellent outcome that will be transferred to other production strains in future work, along with finding other futile pathways that can affect energy dissipation. Moving the strains to outdoor production would be beneficial in proving the robustness of strains.

- The project team seems appropriately small for nimble decision-making and clear communications. It is not clear that rapid hypothesis testing in cyanobacteria will yield specific insight or that any lessons learned in this strain can be translated to other cyanobacteria, and it seems very unlikely that they can be applied to eukaryotic algae. Light conditions used to simulate high light stress were operated at a small fraction of average photosynthetically active radiation experienced outdoors; 200 µmol/m^2/s is low light compared to the 900–1,500-µmol/m^2/s average, or the significantly higher values that could have been checked with a photosynthetically active radiation meter simply by taking it outside. Given that the approach is built on energy dissipation under high-light conditions, the entire research approach seems fundamentally flawed. The futile cycles described in this work are not newly discovered. Increased understanding of alternative carbon fixation pathways to rubisco could be impactful if it works. The deeply flawed nature of this experimental design seems to limit any potential impact that this project may have. It appears this project was redirected to new lines of inquiry without proper rescoping to ensure that project was meritorious or that research targets and approaches are valid. The project seems to be on track to meet goals, but it is difficult to see how meeting these goals will result in progress.

- This project focuses on developing cyanobacteria genetic tools to improve photosynthetic efficiency and biomass productivity through manipulation of energy regulation mechanisms. If successful, it will lead to an increase in biomass productivity, which is of clear relevance to BETO MYPP goals. The team is leveraging many years of research at NREL in photosynthesis, carbon, and energy metabolism in *Synechocystis*. The team needs to balance indoor and outdoor testing by seeking industrial partners early on in their work. Their approach involves engineering cyanobacteria to decrease light energy dissipation and futile cycles and direct energy to biomass. Using synthetic biology to manipulate carbon sink and having very good understanding of gene targets are strengths of this approach. Glycogen and sucrose cycles were futile cycles targeted for improved energy management in cyanobacteria. Genetic tools and metabolic engineering strategies developed here can be translated to other industrially relevant strains. However, some industrially relevant strains may not be genetically tractable at this time and may have differences in biochemical pathways. This project has demonstrated that glycogen knockdown and deletion on sucrose phosphate synthase enzyme improved photosynthetic oxygen evolution and biomass productivity in outdoor-simulated conditions. It will be critical to confirm the increase in biomass productivity outdoor trials, especially under high-light conditions, as modification on photo-quenching biochemical pathways may negatively impact biomass productivity. The team should consider compositional analysis for mutant strains. Overall, success in this AOP project will provide more insight to improving biomass productivity by metabolic engineering.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewer comments. We now have composition data on the glycogen knockdown strain, which has lower carbohydrate content than wild-type, and relatively higher protein and lipid contents. The composition of the sucrose phosphate synthase deletion strain will be determined in the near future. The growth tests included simulated AzCATI outdoor light intensities as high as 1,600 µmol/m^2/s, as shown in the SAGE slide. This answers the criticism on the experimental design for not including light intensities up to 1,500 µmol/m^2/s. We are aware of industry efforts in testing glycogen deletion strategy and finding it unsuccessful. We also saw the growth deficiencies of that mutant in our lab. The current strategy of glycogen knockdown has been designed to overcome such deficiencies and has shown promise, as presented here. Regarding the comment that the futile cycles described in this work are not newly discovered, it will be helpful to show references on prior discoveries, if any. Overall, this project developed concept (Energome), approach (redirecting energy from futile cycles to growth), and tools (gene knockout and knockdown, energy charge measurement) that are successful in increasing photosynthetic productivity in a model cyanobacterium and are ready to be adopted and tested by other algae strain development efforts.

ALGAL BIOFUELS TECHNO-ECONOMIC ANALYSIS

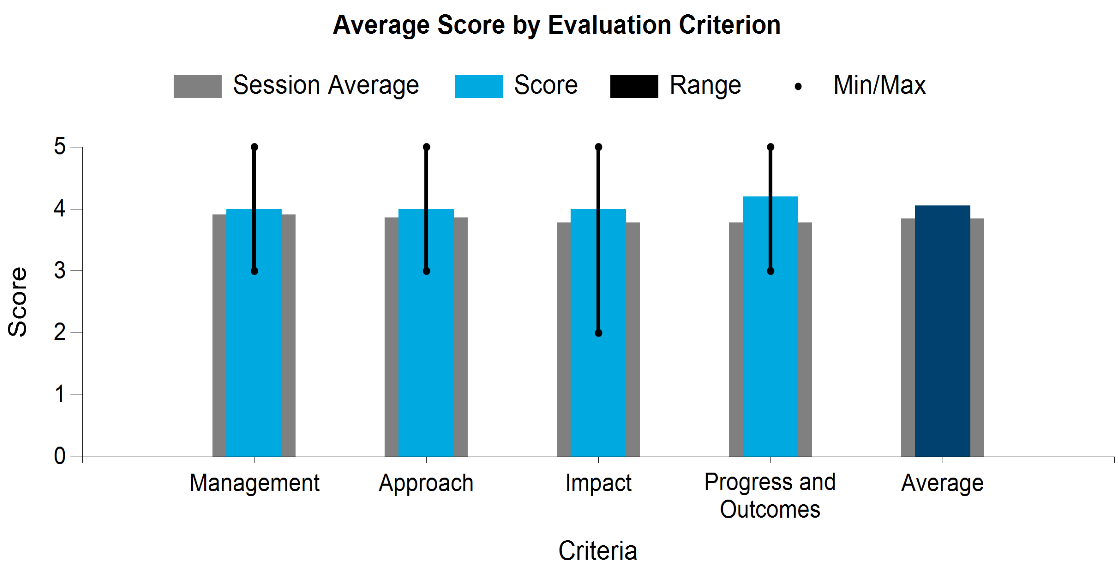
National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.200
Presenter(s):	Ryan Davis
Project Start Date:	10/01/2008
Planned Project End Date:	09/30/2021
Total DOE Funding:	$1,085,005

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

This project provides high impact and relevance though generation of critical cost data tied to funded research, with our analyses subsequently exercised by BETO to guide program plans, FOA priorities, and other directives. This includes costs for both algal biomass production and downstream conversion, most notably to support BETO's fuel cost targets below $2.5/GGE by 2030. To mitigate a key risk/challenge in constraining our work to academic analyses rooted only in future projections, our work also seeks to provide near-term value to today's algae industry through frequent industry engagement, while maintaining close ties with other BETO collaborators. This project has made numerous accomplishments since the 2019 Peer Review, including a continued focus on opportunities for value-added products, with related analyses for new pathway opportunities to achieve BETO cost goals and notable SOT improvements over prior cost benchmarks.



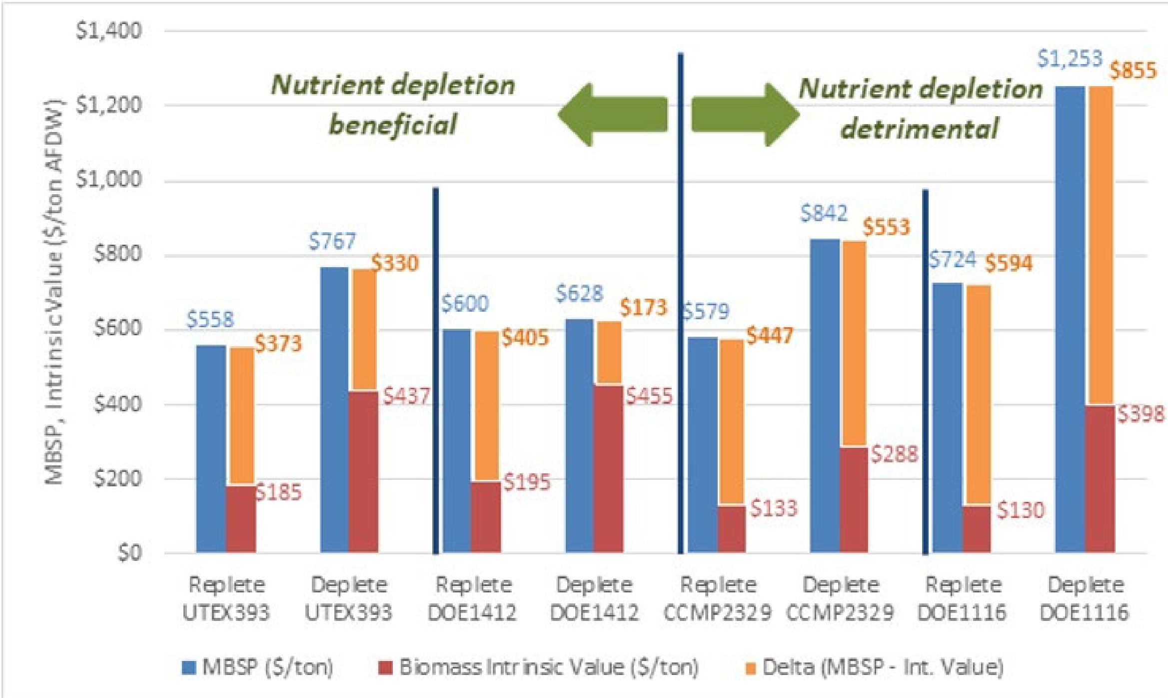


Photo courtesy of National Renewable Energy Laboratory

COMMENTS

- Goals are clearly stated, and the risk management strategy is clear; however, it would have been helpful to see more detail on roles and responsibilities, communication methods, and data sharing. More details on how costs and prices would be integrated would have been good. It's unclear if they are scraped from daily commodity market data, locally adjusted for cost of inputs and logistics, or collected for iterative recalculation. Consistent TEA methodology is needed to guide research decisions and prioritization, yet it is often sort of made up as an afterthought with little detail in terms of mass balance, market pricing, CapEx, or OpEx. In order for any research to achieve commercial viability, it needs a strong economic basis. The decisions made in response to the TEA have resulted in higher productivity, lower costs, and a refocus on fine chemicals and polymer raw materials to create additional value to bioprocessing. Again, the work on algal bloom harvesting looks especially interesting.

- The goal of the project is to provide process design and economic analysis support for the algae platform within BETO. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The team's approach incorporates Aspen Plus process modeling, discounted cash-flow calculations, and TEA modeling, and provides annual SOTs. The described approach is holistic, although adding a component of process model validation and feedback would enhance the effort. Impacts of the technology and process modeling were adequately explained, along with impacts to BETO's targets and goals. The team has progressed excellently in their modeling and reporting efforts. They developed an FY 2019 feasibility TEA for wastewater algae valorization that generates scenarios that bring biomass selling price near zero or negative. They are also working on coproduct value assessments and TEA to support SOT advancements in cultivation. The team has also worked on an update of the CAP SOT report that incorporates a polyurethane product along with catalytic upgrading. For FY 2021, they are expanding their efforts in modeling polyurethane and non-isocyanate polyurethane processes.

- The management of the project is well aligned with DISCOVR and other consortium efforts. The PI's personal ownership of this work has resulted in a tool of tremendous quality. The risk that project contributors supply hyperbolic data or data of poor quality seems misunderstood. The risk that data contributions are not in a standardized or consistent format and therefore not consistent comparisons does not seem well addressed. The approach of this project has consistently been pragmatic and methodical. Integration of partner feedback and data allows this platform to be more versatile and applicable to a broader range of potential users. The TEA seems well designed to downselect underperforming projects. This tool does not seem to be uniformly or rigorously applied for validation or verification efforts. The TEA is likely the single most important set of success metrics for the culmination of BETO work. The TEA is mature and has many new potential products and industrial contributors. The outcome of this project seems to be used only as an internal AOP metric if FOA awardees do not work with the NREL Systems Integration team to validate their work. A few simple inputs like growth rate, mass balances, and biochemical composition could be given by research teams instead of applying efforts to create their own TEAs, which are opaque and may not create consistent comparisons. This seems like it would be the most impactful outcome of this work if used that way.
- The project focuses on providing process design and economic analysis to support the algae platform and guide R&D priorities to commercialization. This team is building upon the value the TEA model has added to the algal community and is continuously evaluating new processes for impact on the cost of biomass, fuels, and other products. This project provides critical cost data to guide BETO program plans, as well as industry and research partners for research and commercialization strategies. The team appears to be well managed with a well-defined task structure and leveraging team expertise and previous experiences. They identified risks and outlined mitigation strategies. The project performers are constantly refining the TEA model to make it more relevant to the field and to have good collaboration with research and industry partners. Their interaction with the DISCOVR team and industry partners has been a great approach to calibrating and validating their model. The team has delivered several products since the last review and is well on their way to achieving the goals outlined for this project. The team has made significant progress. Their TEA model has been used to identify opportunities for algae wastewater treatment. The TEA model has been upgraded to highlight the degree of benefits or penalties moving from nutrient-replete to nutrient-deplete harvesting of biomass. The model has expanded focus on opportunities to enable algal biorefineries as well as set SOT for 2020 incorporating data from the DISCOVR project. Continuous progress of this project will guide algae research and development to an economically viable algae production platform that will lead to commercialization.
- The project is pursuing exciting opportunities for new sources of algae and new possible applications in service of helping achieve BETO goals. The ambition of using algae as a treatment or otherwise a benefiting element in/from wastewater treatment systems is long held. The opportunities to piggyback on infrastructure and systems with little to no additional investment to accommodate algae, as well as the abundance of existing nutrients and source water, simply never lose their appeal. Most previous attempts achieve limited success for a variety of reasons: as a sole unit operation, algae often does not fully meet discharge water quality requirements; the physical footprint needed for an algae-based unit operation is often impractical; treated wastewater already often has competing interests as a beneficial use element in the larger water cycle and benefits from significant policy and legal advantages; treatment optimization is often orthogonal to algae growth rate optimization; and commercial applications are limited because of co-location, land availability, and large enough volumes of daily inflow wastewater volumes. These possible risks do not seem reflected or otherwise addressed in the project, and likely will limit meaningful results in service of BETO goals.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their insightful comments and appreciate the recognition of the merits of this project in guiding NREL/BETO program directions. In response to the comment noting the potential to miss key risks in the deployment of algal systems for wastewater treatment, we are currently in discussions with two large industry players in the algal wastewater treatment space to better understand such constraints and realistic gaps/drivers in the implementation of this concept at scale, in hopes to incorporate further inputs and refine future modeling activities on the topic.

- On the comment of project interactions and data sharing, this project interacts frequently with both internal and external partners including consortia groups, FOA partners, national laboratory modelers, and industry collaborators to foster information exchange. This includes communicating data input needs, working with researchers to collect these data (often iterating several times to translate the data into the most suitable format for incorporation into the models), and sharing outputs of the TEA models to highlight key drivers and priorities versus inconsequential factors not worth experimental focus. For example, we communicate on a weekly basis with the DISCOVR consortium to communicate ongoing data needs for cultivation trials and have led the majority of the discussions with the Technical Advisory Board under that consortium based on model inputs and subsequent findings. Likewise, we communicate at least several times per month with NREL algae CAP conversion researchers to guide progress, provide TEA insights to downselect across competing research priorities, and revisit the latest performance data for use in SOT benchmarking updates.
- Finally, regarding the suggestions to further engage with industry or other groups to enhance model validation, we always value such engagements whenever they can be arranged and would continue to welcome any such opportunities. We have participated in a number of such activities across both formal and informal collaborations to “compare notes” both for validating/improving our own models, as well as vice versa to other partners with good outcomes. We would also be happy to discuss such opportunities with the Systems Integration group or others as may make sense to promote more consistent analysis of technologies and research concepts being investigated externally (though recognizing there may be constraints both regarding availability of information as well as practicality of applying our TEA models on specific technologies to other concepts that may not readily fit into the existing model framework).

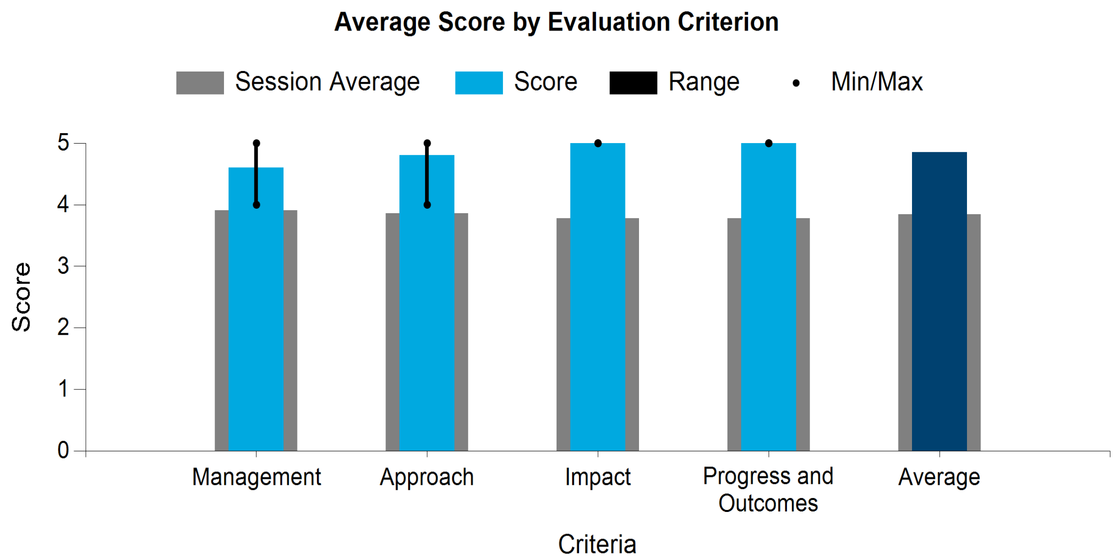
ALGAE TECHNOLOGY EDUCATIONAL CONSORTIUM

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.201
Presenter(s):	Cindy Gerk
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2022
Total DOE Funding:	$1,670,000

The Algae Technology Educational Consortium (ATEC) was created to provide algal-based educational and training programs to develop a skilled workforce supporting BETO's OPERATION BioenergizeME and its threefold mission: improve public accessibility to bioenergy production information, support formal and informal education, and engage future scientists and engineers in developing solutions. ATEC accomplishes this mission through five major foci, including community college certificate programs in algae cultivation; community college algal-based curriculum inserted into biotechnology degree programs; massive open online courses; Algae Academy, a K–12 STEM initiative; and aquaculture extension education through the Algae Cultivation Extension Short-courses (ACES). The ATEC-developed curriculum is utilized by schools in the ATEC network (>400 academic institutions) to educate and train the next generation of algal professionals filling U.S. jobs supporting algal commercialization and reduce costly and time-consuming post-hire training. ATEC was able to overcome limitations from COVID-19-related restrictions by pivoting to online curricula. Our Industrial Advisory Board, comprising industry experts, are active participants in developing and embedding the ATEC curriculum into ATEC schools, colleges, and universities. ATEC will continue to expand the curriculum to include heterotrophic cultivation and seaweed commercialization.



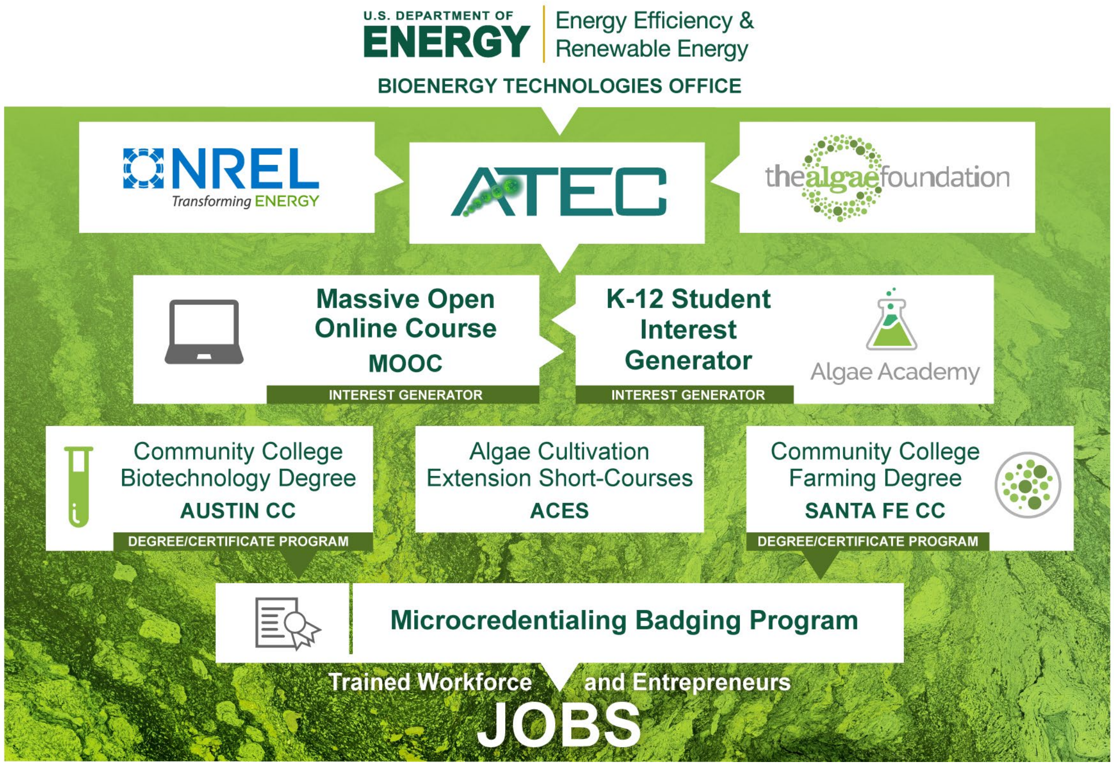


Photo courtesy of National Renewable Energy Laboratory

COMMENTS

- Acknowledging in advance that this type of project undertaking is not within my experience or training, this reviewer cannot be anything less than completely impressed by the deep thinking, organized structure, and the passion for the mission and goals conveyed by the presentation. The continued expansion of the project's success and the scale at which additional students, schools, and now universities have been engaged is exceptional. The already-achieved nationwide scope and impact is equally impressive. We can be left confident that a workforce will be ready for the algae economy.

- ATEC is operating a large, team-driven, educational consortium to support education and workforce development goals for a future algal biofuels industry. The consortium has reached an impressive number of students ranging from kindergarten up through university. ATEC is commended on their rapid pivot to online content when the COVID pandemic shut down in-person learning. Looking ahead, this ability to offer curriculum both in person and online stands to reach a wider audience, helping to meet the program's goals.

- Overall, this project seems to be doing very well. The project management plan is clear and the implementation strategy seems to be going very well. The approach involving the Algae Academy and the massive open online courses seem to be reaching thousands of students. The impact is impressive, and the project continues to add ATEC members. Most of the members seem to be coastal; more focus should be directed to recruiting schools in the center of the United States. The project met all the FY 2020 milestones and seems to be on schedule.

- This is an amazing project that shows what the passion and dedication of a few people can achieve with the proper support. The organic growth of this grassroots initiative is remarkable and is expected to reach or exceed all project goals for number of students served by the various programs. The project has developed relevant and impactful programs for all grade levels. These curriculum and training programs will equip the next generation of algal cultivation, biotechnology, and bioeconomy professionals with the skills they need to succeed in an algae bioeconomy.

- This is an exciting and very worthwhile outreach project aiming to improve education and workforce development. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The approach that the team is taking is exciting and is being implemented widely, through algae interest generators that provide curricula, standards, and outreach to K–12 along with more advanced education efforts, such as the massive open online courses. The impacts of this effort will be seen through the attraction of a new generation of scientists and well-informed public due to participation of K–12 institutions, partnerships with higher education institutions, and integrated efforts at the state and national level. The effort has met its FY 2020 milestones and has reached nearly 100,000 participants. The FY 2021 efforts are underway, having developed the training modules required for the first quarter. The team has developed an external certification and endorsement program for various technologies and methodologies and is well underway to expand its collaborative efforts with national organizations. This is an excellent project, well led, organized, and reaching a very diverse audience.

PI RESPONSE TO REVIEWER COMMENTS

- The ATEC team is very grateful to the BETO reviewers for their efforts and to BETO for continued support of algae education and bioeconomy workforce development. The ATEC curriculum and the Algae Academy have reached all 50 states and 45 countries. We continue to expand the ATEC partnering collegiate network through our collaborations with InnovateBIO and additional focused outreach. It is deeply gratifying to receive such a strong positive review. It sends us a clear message that we are on the right track and encourages us to continue to focus our energies on expanding the program to bring algae awareness to more students.

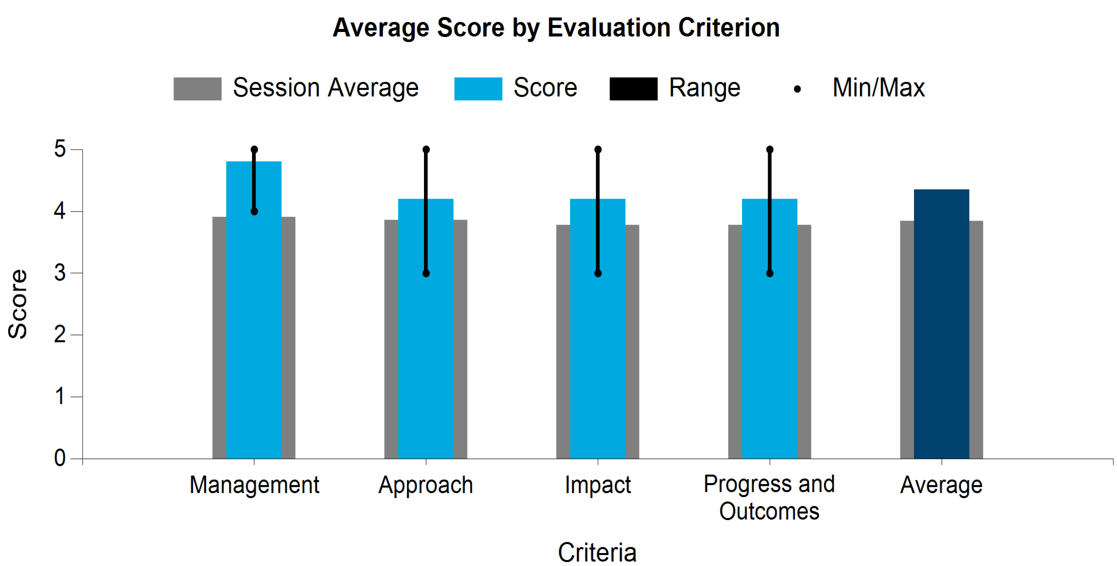
HTL MODEL DEVELOPMENT

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.202
Presenter(s):	Lesley Snowden-Swan
Project Start Date:	10/01/2017
Planned Project End Date:	09/30/2021
Total DOE Funding:	$600,000

This project provides TEA and sustainability inventory for algae conversion via HTL and upgrading to fuels and chemicals in order to direct research toward high-impact results. A target conceptual biorefinery model was first developed in 2014, and SOT assessments have been conducted annually. The purpose of the SOT assessment is to identify barriers, cost reduction strategies, and sustainability impacts, and track research progress toward the BETO 2030 cost target ($2.5/GGE). The annual SOT documents the modeled costs for that year and the associated research used in the modeling. Through the combined experimental/analysis projects, conversion costs for the pathway have been reduced from $1.22/GGE to -$0.33/GGE through the blending of terrestrial feedstocks during low-productivity seasons and a new two-stage HTL process configuration that enables high-value coproduct generation. Data availability is the main challenge of TEA, which is mitigated by frequent communication with internal and external researchers to exchange information and review cost reduction strategies. Future work will focus on using low-cost algae (e.g., seaweed, nuisance blooms) to accelerate deployment opportunities and ultimately meet the BETO 2030 goal.



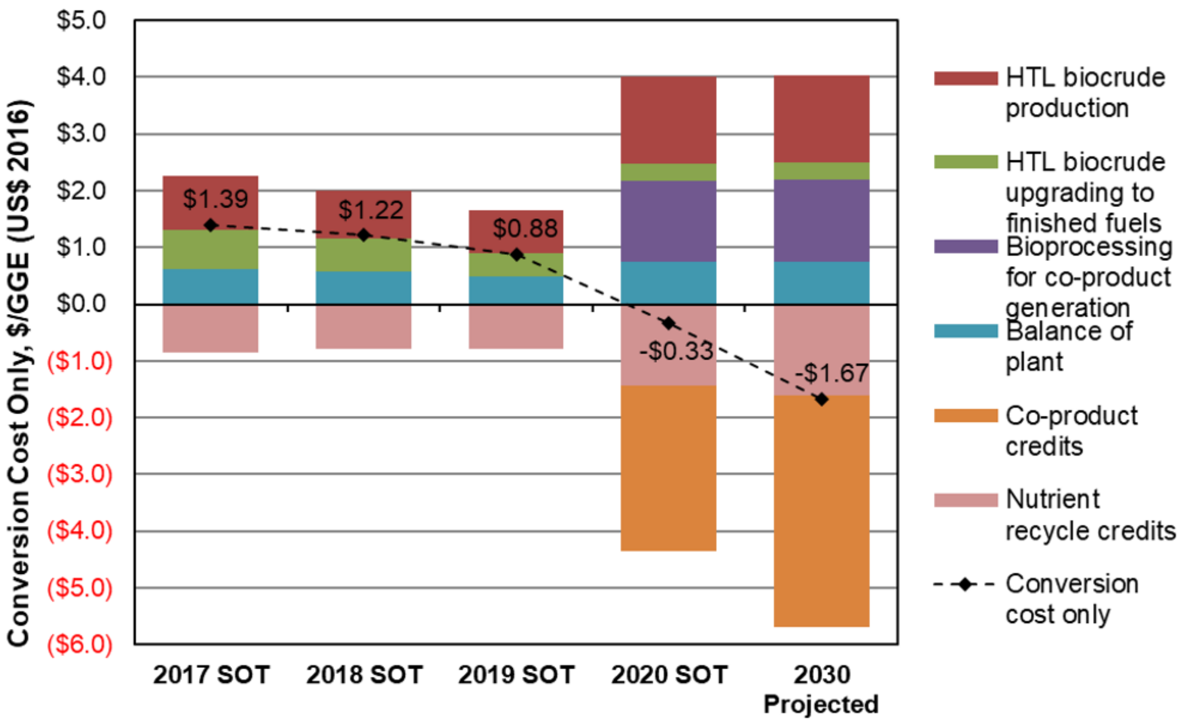


Photo courtesy of Pacific Northwest National Laboratory

COMMENTS

- Roles and responsibilities are defined across a very wide range of groups. Risk management is described in some detail. Communications methods are defined, and project goals are clear and specific. The approach is iterative and validated as well as it can be without actual automation historian data. Risk mitigation includes TEA integration with the data set to prioritize and guide specific research choices and make actionable recommendations. This research has directly led to improvements in storage and heat exchanger designs. In particular, it has also reached commercial success by launch of user licenses—well done! This effort drove the conversion cost closer to agency goals and specified high-value product choices to achieve overall economic success. There is some useful information on utilization of non-cultivated algal blooms as well.

- The aim of this project is to develop data-driven process models for TEA of algae HTL processes. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project through a thorough discussion of critical success factors, challenges, and mitigating strategies. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The team's approach incorporates close collaboration with HTL researchers for critical data to identify key cost drivers, provide feedback loops, use a defined basis in model development, engage with industry, and provide a life cycle inventory for algae HTL. The described approach is holistic and provides for frequent communication with researchers and users of the technology for validation. Impacts of the technology and process modeling were adequately explained, along with impacts to BETO's targets and goals. The team has progressed excellently in their modeling and reporting efforts. They developed an FY 2019 SOT on HTL of blended wood and algae feedstocks, and coproduct generation with SEQHTL for carbohydrate extract biocrude generation with conversion of the carbohydrate to lactic acid. In FY 2020, the team developed a model for SEQHTL that showed substantial reductions in SOT MFSP and conversion costs by adding coproduct inventories and nutrient recycle credits. The team has developed a reduced-order model and uncertainty analysis to predict HTL yield and have applied it to HTL of wet wastes. FY 2021 efforts are currently focusing on modeling HTL of biomass from algae blooms, turf algae, and macroalgae.

- This project focuses on employing a developed TEA coupled with researcher input and feedback to guide and track research toward reduction in cost of renewable fuels and coproducts from biomass via HTL processes. This project supports cost models for HTL pathways that inform BETO's annual SOT assessment and is of clear relevance to the BETO mission and MYPP. The team has previous experience with TEA, and the project appears to be well managed. They identified risks and outlined mitigation strategies, as well as established channels of communication and collaboration amongst experimental team on data needs. Technical approach as outlined by the team is reasonable and will continue to drive the TEA model to more represent a commercial process. The team has done a great job in working with researchers and industrial partners to ensure that assumptions used in the model reflect real-life processes. However, the team should consider upgrading the cost of coproducts to reflect actual costs based on the different processes as opposed to a fixed cost for coproducts. This work has provided a foundational model and TEA to many in the algal community and has helped direct design, optimization, and scaling of the HTL process.

- This project is a very good example of DOE and BETO providing industry leadership and guidance. The development and maintenance of a TEA that uses quality input data and provides for feedback and data improvements is essential to develop a high-level view of progress, trajectory, opportunities, and viability of algae-produced fuels and coproducts. This in turn helps gauge individual projects and program progress and to inform future decisions and commitments. By design, this project and its platform are poised for continuous improvement—especially as better data become available. The development of the reduced-order model for predictive algae HTL yield has tremendous potential for widespread use and may prove very helpful in managing future projects and business endeavors. The presentation illustrated the flexibility to adjust efforts to incorporate current best thinking and other novel ideas that can incorporate novel algae sources for similar evaluations and design specific applications.

- This project is well integrated into other national lab efforts in a way that supports and improves all of them. The PI is well suited for success in this effort. The claim of "780 dry tons/day based on POND model = 6,800x scale increase from data" is a tremendous leap, and even small errors in assumptions could constitute large risks to ultimate project success. This model's greatest source of weakness is likely not uncertainty about offtake markets, but lack of information about costs for maintenance and operations. Modeling Metro Vancouver or Contra Costa and tracking variance could help validate the model and make it more robust in these technical assumptions. OpEx is based on stochastic events, and feedstock variability can be derived from existing plants. Model development should be built around continuous real-world run data from partnering waste treatment operations as much as possible. The modeling efforts in this work are critical for optimization but still do not seem to provide potential users the information they would need to adopt technology without very detailed and resource-intensive investigations. A model that can predict HTL outputs based on easily translated feedstock composition metrics would be the most impactful work this team could do to make this technology approachable for potential users. Go/no-go milestones would be more impactful if they were based on some sort of real-world validation. Presenting another blend's modeled cost and demonstrating projections for improved catalysts for FY 2019 outcomes seems like it should be a rapidly obtained output of this model if the model was already mature at the beginning of the project. It also seems that adding model inputs or changing a parameter should not be an entire AOP task for a robust, mature model; even interpretation of these outputs should be somewhat routine if this model was mature at the beginning of the effort as well.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their helpful comments and feedback. The plant scale assumed is consistent with BETO's algae pond model scale. We agree that larger-scale field testing of algae production is ultimately needed to reduce uncertainties around the performance assumptions and TEA at a commercial scale. Regarding the conversion process, we expect to use our engineering scale HTL system (12x larger than bench) for sequential HTL testing in the future, which should provide insight closer to real-world operation and help identify scale-up issues. Our design case and SOT reports include detailed process design information and are made available to the public on the DOE Office of Scientific and Technical Information website. The primary purpose of our analyses is to determine feasibility early in the concept stage and guide and track progress in BETO's R&D toward reducing costs and improving the viability of the technology. A rigorous engineering design estimate by an engineering, procurement, and construction firm would be needed to support decision-making around actual project construction. We agree that being able to systematically correlate incoming feedstock composition with biocrude and fuel properties is of great value. To this end, we have developed reduced-order models based on PNNL's extensive library of continuous testing data to predict yield and quality for both algae- and waste-derived biocrudes from single-stage HTL (https://doi.org/10.1016/j.algal.2019.101450; https://doi.org/10.1016/j.apenergy.2020.116340). Our SEQHTL work is relatively new, but as more testing data sets are generated, a predictive model can be developed in the future. With regard to the project scope, analysis of the new sequential HTL process with coproduct generation for the FY 2020 SOT involved significant work, including gathering, collating, and interpreting much data and information. It also involved substantial changes to the process and cost models, including modifications to the HTL process configuration, incorporation of new feedstock blend based on most recent testing data, and addition of bioprocessing simulation based on testing data. Regarding costing of the coproducts, the analysis estimates the minimum fuel selling price using a fixed coproduct market price, consistent with the other BETO pathway analyses. Another perspective, as the reviewer points out, is to calculate the minimum coproduct selling price using a fixed fuel selling price. We appreciate the comment made by the reviewer and will work to include it in future analysis.

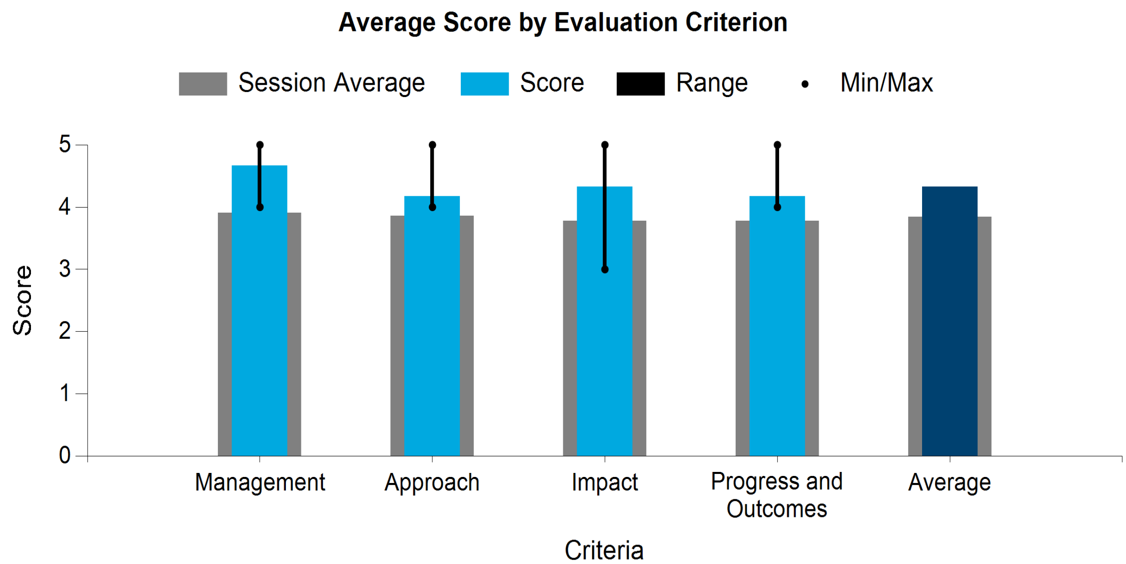
MICROALGAE ANALYSIS

Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.203
Presenter(s):	Mark Wigmosta
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2022
Total DOE Funding:	$1,725,000

In 2012, the National Research Council stated, "A national assessment of land requirements for algae cultivation that takes into account climatic conditions; freshwater, inland and coastal saline water, and wastewater resources; sources of CO_2; and land prices is needed to inform the potential amount of algal biofuels that could be produced economically in the United States." This project developed and advanced a high-resolution spatiotemporal Biomass Assessment Tool (BAT) focused on fundamental questions of where production can occur; how much nutrient, land, and water resource is required; 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 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. This project supports BETO efforts in Multi-Year Project Planning, SOT reports model harmonization, waste-to-energy, and experimental design. It has also supported industrial siting studies and outreach through the Technical Assistance Program. Results from this study have resulted in 23 peer-reviewed publications of direct benefit to industry to evaluating optimal site locations, strains, and operations.



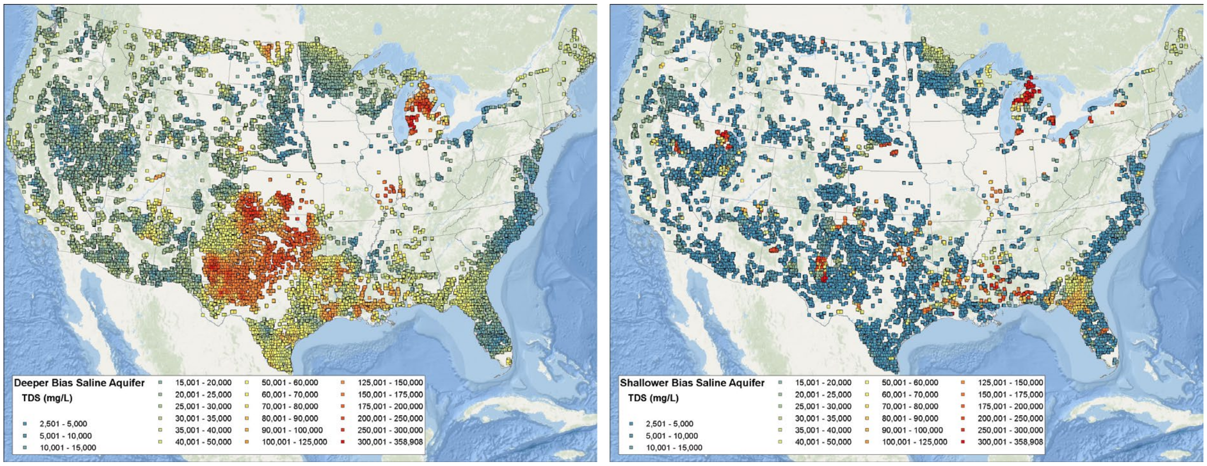


Photo courtesy of Pacific Northwest National Laboratory

COMMENTS

- Overall, this project is off to a strong start. The project management plan is strong. Communication between the project team is weekly, and risks and risk mitigation strategies are clearly detailed. The technical approach is good, and the project team is focused on identifying and assessing impacts of design and operational constraints and risks for algal biofuel feedstock production. The PNNL BAT would be a useful tool for the algae community; thus, its impact would likely be significant. The project team has made good progress addressing the project goal of developing the BAT, and the project seems to be on schedule.

- The BAT is being applied to assess alternative algal feedstock production strategies to determine which will yield the highest biofuel production at the lowest cost and resource utilization. Analyses are directly focused on BETO MYPP targets. Of interest, results for wastewater co-location are looking to meet the go/no-go target, suggesting that significant algal productivities can be achieved with all nitrogen, phosphorus, and water requirements met by co-located wastewater.

- The project provides assessments for resource availability and management nationally. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project. Progress was described through tasks, milestones and go/no-go decision points, providing an adequate assessment of progress for the project. The team uses a well-established BAT to evaluate resources nationally and provide algal production strategies. To do this, the team collaborates with other projects across the BETO portfolio (e.g., DISCOVR and BETO Waste-to-Energy) to incorporate best processing capabilities and understand resource constraints. This is a project with broad impact and wide dissemination for the BETO portfolio. The work from the project directly addresses the availability of national resources to achieve the BETO MYPP targets. It is very prolific in number of publications and major reports. Progress for this AOP includes the biomass growth assessments to guide pond operations with a 7-day forecasting tool. The team has provided an assessment of freshwater and brine water availability, and CO_2 co-location and wastewater co-location for the United States. The projects outcomes are made readily available through publications. The overall presentation would benefit from focus on understanding key inputs and how these challenge the overall assessments.

- The team seems small and contained, but also well connected for collaboration with appropriate SOT, LCA, and DISCOVR teams. Risks for this modeling effort seem low and well addressed, but the greatest risk for most of the broad modeling efforts still seems to be a lack of industry adoption and input, as the user base for the BAT model may not be growing. Designing this model for compatibility with other existing models makes it a more useful tool and can help unify metrics across the platform. Coordination with the waste-to-energy team and portfolio will broaden the scope and applicability of this work in a positive way. The methods used for verifying forecasting growth seem reasonable for very standard conditions but do not seem like they will be useful in predicting stochastic events or recovery from upsets. Integration of water stress data into this model does not promote tremendous optimism about many sites assumed to be ideal for cultivation in previous iterations of this model. This work does narrow down potential candidate sites appropriate for sustainable cultivation to a much more constrained geography, which should accurately inform potential commercial and industrial cultivators where to site future operations for the greatest positive impact. Brine water management modeling produced in this effort helps demonstrate feasible saline sources and proposes viable solutions for inland cultivation and helps align long-standing plans with updated data analysis. Greater specificity was added to an already powerful tool that will help narrow site selection for potential users by highlighting availability of critical resources.

- The work being undertaken by PNNL remains a very important tool for BETO and the larger algae industry. It continues to build on past efforts and reflects current efforts and topic areas. The methodologies and technologies used help gauge the national opportunities and leverage available resources, geographies, and regular climate patterns in evaluating the deployment potential of algae strains and production technologies, as well as offering projections into the future. In return, these efforts help inform the next set of decisions by BETO and industry, develop updated information and data that in turn is used in updated BAT modeling. This work has resulted in a significant number of publications and industry acknowledgement as a very valuable resource.

- This tool maps site selection data such as biomass growth potential, water demand, water supply, CO_2 co-location, and transport costs, providing very effective data and visuals and a key linking of resource availability to support projections of industry potential and site selection. The team demonstrated good progress on this work, which is broadly valuable to the growth of this industry and meeting program goals. The work evaluating municipal wastewater co-location can help support decision makers as algal cultivation in wastewater continues to be evaluated given the continued appeal of wastewater as a “free,” nutrient-rich water source. It could be of value within the bucket of “wastewater” to consider and differentiate between different types with interest potential (e.g., municipal, agricultural, anaerobic digestion digestate). In considering brine management options, the team may find value and efficiencies in connecting with the relatively recently formed National Alliance for Water Innovation if they are not already working together; brine management is a key focus area for them.

PI RESPONSE TO REVIEWER COMMENTS

- The insightful comments provided by the reviewers are greatly appreciated. The project team is highly motivated by the positive comments made by the panel. We appreciate the Review Panel’s view on the lack of industry adoption of broad modeling efforts; we agree with this statement as well. Moving forward, we will reengage with the industry and actively seek out engagements as part of our project outreach task. We will continue to publish project results and work closely with BETO’s SOT efforts, the Industrial Advisory Board of DISCOVR, and waste-to-energy, all of which have significant input from the industry. Field pond measurements were useful to demonstrate the concept and robustness of the short-term forecasting system under generally standard operating conditions. In the upcoming LEAPS experiment, we will include more weather variability (similar to the outdoor pond conditions in Arizona) to test the forecasting system in guiding real pond operation. For instance, if the biomass productivity is forecasted to decline in the next few days because the weather is expected to get colder, operators will know now it is the time to harvest. In operational practice, the biomass forecasting system will use an ensemble of real weather forecasts (stochastic events) to mitigate upset events (e.g., best harvesting time and dilution rate) and maximize biomass production. Of all the BAT model inputs, proper treatment of climate variability/change represents one of the greatest challenges to designing relatively stable, sustainable microalgae feedstock operations. As previous BETO model harmonization studies have demonstrated, the use of a representative location and mean climate is insufficient to properly capture climatic impacts on large-scale production. Rather than evaluating mean values (e.g., long-term mean of seasonal production) against fixed targets, we are now looking at the likelihood (probability) of achieving that target in a given year, or the persistence (number of consecutive years) of failure to meet a selected target. We feel this type of information is essential for the proper analysis of location-specific microalgae feedstock production. In coordination with the BETO Waste-to-Energy program, we have performed an assessment of national municipal wastewater treatment plants with regard to plant-specific operations and supply of water and nutrients at various treatment phases. With a biomass growth model and a water and nutrient budgeting model in place, understanding the on-premises or co-location opportunities with both high-rate algae ponds and PBRs will be a focus to provide necessary insights on physical and techno-economic boundaries, and thereby understand locations with the greatest co-location potential. A defined need in this space is to perform high-resolution land suitability analysis in concert with techno-economics to quantify actual available land area within an economically defined vicinity of a given municipal wastewater treatment plant. While the work to date has focused on municipal wastewater, we agree with reviewers that the opportunity space can be opened further with the consideration of industrial and agricultural wastewater, and we will assess available data to support this type of analysis. With regard to saline and brackish water utilization, the reviewers provide an excellent recommendation to connect with the DOE National Alliance for Water Innovation and understand possible synergies in this space, particularly around viable desalinization and brine disposal technologies that can be integrated into our modeling platform. Our team will take this recommendation to action.

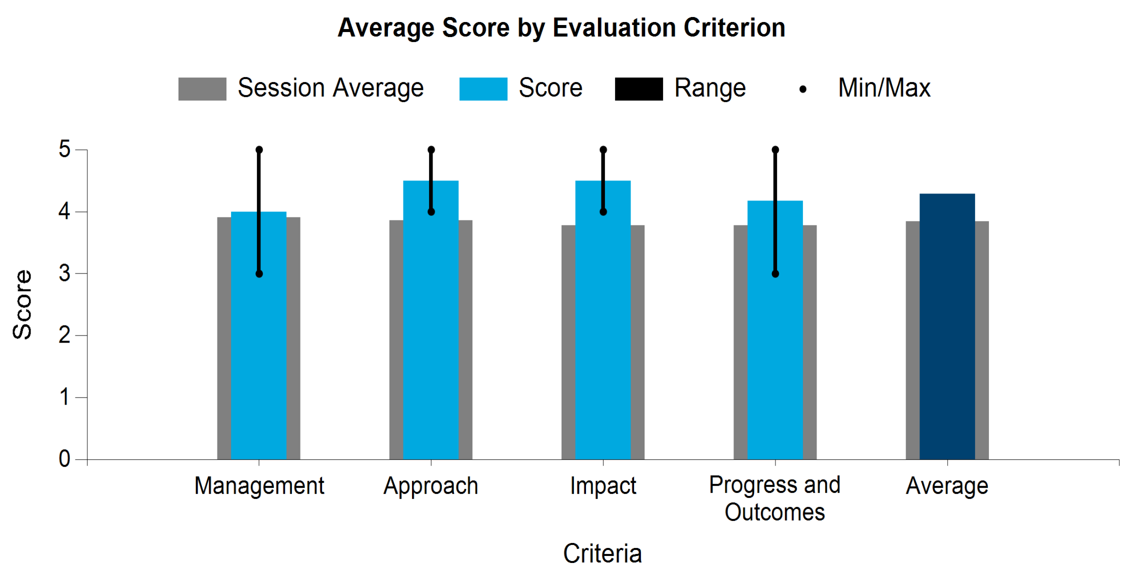
LIFE CYCLE ANALYSIS

Argonne National Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.204
Presenter(s):	Troy Hawkins
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2023
Total DOE Funding:	$275,000

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



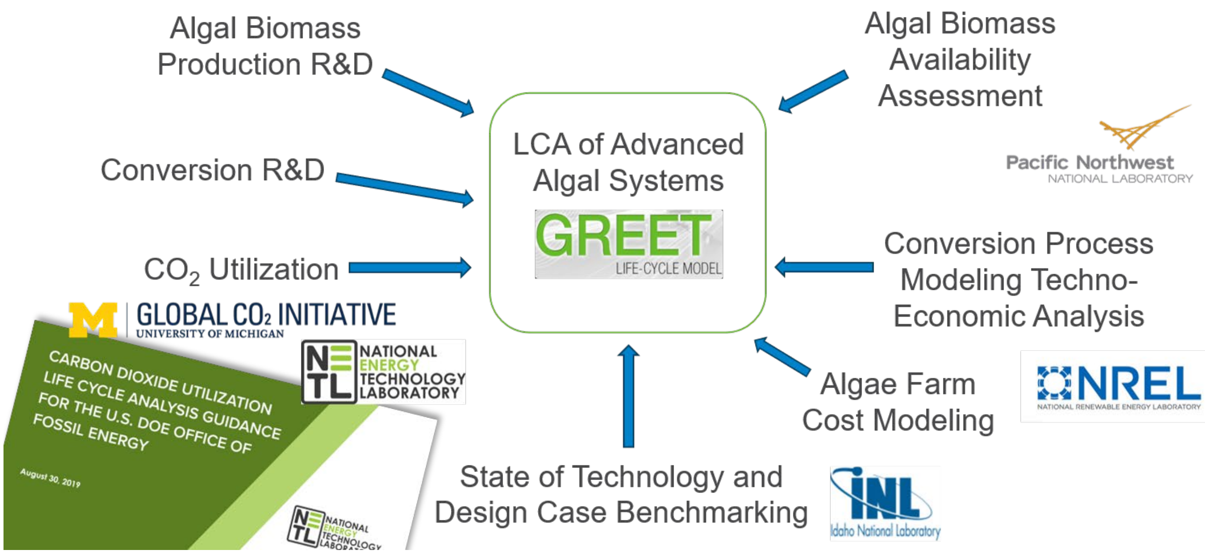


Photo courtesy of Argonne National Laboratory

COMMENTS

- The project recently started and has made good progress to date. The project management plan is clear and well described. How often the project team is meeting is not clear, and risk and mitigation strategies do not appear to be addressed. The approach seems good; four tasks are focused on key questions for understanding life cycle metrics for algal systems. LCA is important for benchmarking and tracking R&D progress of pathways to produce low-carbon, sustainable algal biomass, and thus impact is clear. Data and models will be distributed publicly in annual Greenhouse Gases, Regulated Emissions, and Energy Use in Technologies (GREET) model releases. The project team has made progress looking at the LCA of high-purity CO_2 sources and LCA of saline algae production systems.

- The five proposed project tasks, focusing largely on water considerations and CO_2 sources, align with BETO's goals for increasing sustainable algae production and reducing the resource burden of algae cultivation. LCA results are harmonized for inclusion in the GREET model and released in annual updates, allowing data to be widely available for public use. The project results will clearly support the future assessment of viable commercialization and R&D strategies. Completed and in-progress analyses are addressing the impact of water stress, high-purity CO_2 availability, saline cultivation, and on-site location feasibility for algae cultivation. The next analysis will consider impact of the CO_2 source and will incorporate DAC technology, which is timely and of significant interest to the industry.

- The GREET LCA model is an essential tool for evaluating the relative merits of differing approaches with concrete, measurable contributions to the commercialization path. The reported project is in its first of 3 years toward generating rigorous and detailed LCA addressing four critical issues: (1) marine algae systems, (2) diverse CO_2 sources, (3) algal bioproducts, and (4) integration with wastewater/manure management. The team is well organized and has made clear progress after less than 6 months. A case was made for incorporating local water availability in site selection criteria, although this may be less compelling depending on a project's specific criteria should saline or wastewater be used in place of freshwater.

- The main goal of this AOP is to provide tools to assess the sustainability of algae production systems toward biofuels and bioproducts. The approach utilizes LCA tools for case studies of interest to BETO in algae production and conversion pathways, benchmarking the state of technologies and evaluating alternative processes. The team uses new models to assess algal pathways and provides harmonization of LCA approaches across the BETO algal systems program. As such, it is an essential part of the program in modeling biomass availability and cost, sustainable production, integration of processes and platforms, and development of resource management. The impact of these tools is evident through contributions in high-profile publications and reports, along with model and analysis systems (e.g., GREET) that are widely accepted and utilized in the community. The team has created assessments for the CAP and HTL pathways and polyurethane coproducts. A new water impact assessment—AWARE-US—provides a guide to sustainable scale-up for algae farms by understanding water availability and consumption across different geographic zones. They are also developing an assessment of high-purity CO_2 availability sources across the United States. They are currently working on developing an assessment of saline water availability for algae production and impacts on water stress factors. Validation of inputs is a key risk for modeling technologies that should be discussed in more detail. There was some discussion on moving toward assessment of impacts from new direct air capture technologies being developed. The team and management plan described are appropriate for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. Discussion of potential risks and mitigation strategies would enhance the presentation of this project.

- The project team and the work product continue to provide an important reference and check on most of the individual BETO projects and programs. Its importance and effects cannot be emphasized enough. It needs to be recognized that the overall effort is limited by the available data and the ever-evolving understanding of the great many interrelated variables and the priority of events, decisions, and technology that remain in constant flux. Regardless, the team maintains a practical working knowledge regarding all the previous variables and endeavors to professionally reflect the same within the work products. The interactive nature of this approach is a good safeguard to maintain close relevance to the overall conditions involving commercialization of algae products with the ever-evolving SOT. The widespread adoption of the work products is a testament to the value and impact of these endeavors.

- The team is composed entirely of modelers, which is appropriate for this effort but makes close collaboration with end users critical for validation of assumptions and modeled results. The periodic updating of a public-facing and transparent model has clear value for a broad range of consumers. Integration of algae cultivation into this model clarifies real value of proposed pathways and allows standardization for fuel credits, making commercialization more viable. The current investigations of saline water resources, water stress modeling, and wastewater cultivation are all very practical and appropriate. The approach described seems to lack a technical validation component where assumptions can be tested, and modeled results can be verified. The stark comparison of water stress geographies to proposed ideal cultivation sites is especially impactful. Many assumptions about preferred cultivation areas seem much less desirable in light of this information. This is the very sort of information that models like this one can provide to drive critical decisions. The culmination of many dynamic variables into a single, simple, quantifiable water scarcity metric is especially impactful. The consistent use of this tool by industry and groups like the California Air Resources Board indicates that the work is impactful and far-reaching. All project milestones and deliverables appear to be met. As no risks were inherently described, it appears that there are no employed mitigation strategies to assess.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for recognizing the value of this work. It is very nice to hear that it is an essential part of the program and that the products have clear value for a broad range of consumers. The reviewer's point about better describing the potential risks and mitigation strategies is well taken, and as mentioned, validation of our models is a key risk that we have taken steps to mitigate. The team meets weekly to discuss progress and troubleshoot issues that arise. The Argonne team works closely with PNNL's algae resource assessment and hydrothermal liquefaction conversion TEA efforts and NREL's algae farm and combined algal processing TEA projects to leverage data and model outputs across the teams. The beauty of the depth of analysis happening across the BETO portfolio and the coordination amongst the projects is that values that have been deeply vetted in the context of one effort can be leveraged for another. Both PNNL and NREL have been engaged with us on the LCA of saline algae production. Outside of this project, many of the same researchers collaborate through BETO's annual SOT and target case benchmarking efforts. We also regularly review the literature to vet the values used in our analyses against those reported in outside studies. We have found that the interaction of LCA with other process modeling, TEA, and resource assessment efforts provides natural opportunities for quality assurance across projects. Finally, Task 5 in our project is specifically dedicated to harmonization across BETO's TEA, LCA, and resource assessment projects. In addition, the Argonne team has engaged with an industry partner to collaborate on the FY 2022 analysis of algae products and who will also provide field measurements. In parallel, Argonne staff are also participating in another algae technology development project led by University of Maryland and funded by the Office of Fossil Energy, which provides valuable additional perspectives to this effort. Another risk we have encountered is schedule slip, specifically associated with data hand-offs across labs. We have mitigated this risk through close communication and staged data hand-offs to allow us to have the analysis routines in place in advance. Along with schedule slip is the risk of working inefficiently, which we have mitigated by having parallel tracks in the analysis so one can always be advanced if the other is on hold. We appreciate the reviewers' positive response to our work and will continue to strive to produce rigorous analyses useful for informing research and development of advanced algal systems and, as collateral, tools that make the analyses transparent and allow others to replicate and extend the results.

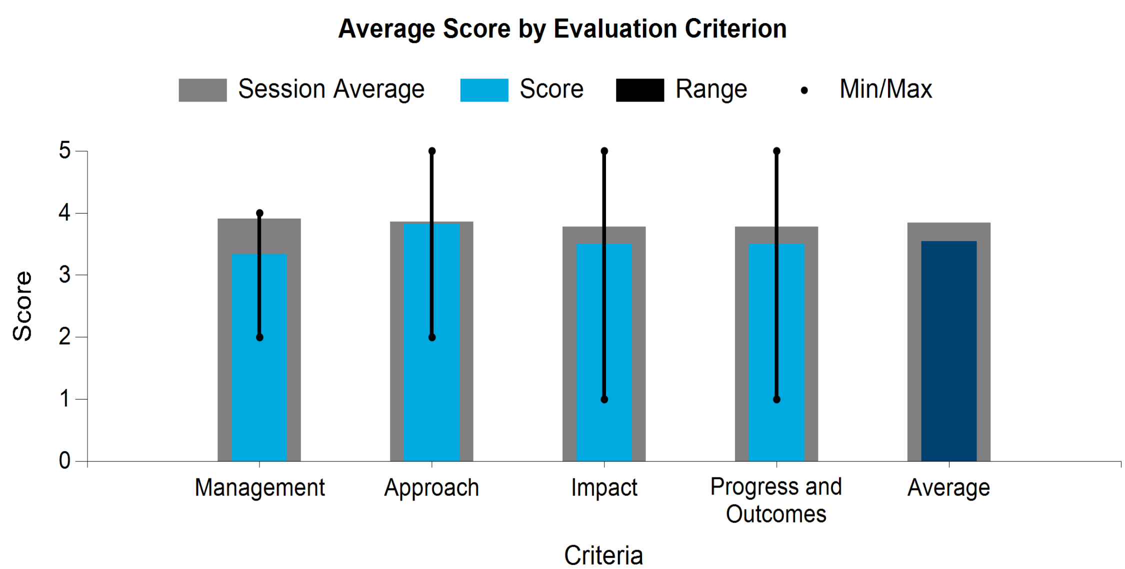
INTEGRATED LOW-COST AND HIGH-YIELD MICROALGAL BIOFUEL INTERMEDIATES PRODUCTION

MicroBio Engineering Inc.

PROJECT DESCRIPTION

WBS:	1.3.5.243
Presenter(s):	Aubrey Davis
Project Start Date:	10/01/2016
Planned Project End Date:	03/31/2021
Total DOE Funding:	$6,283,684

This project, carried out from October 2016 through March 2021, achieved algae biomass productivities exceeding the BETO MYPP goal of 3,700 gallons of biofuel intermediates per acre-year. MicroBio Engineering Inc. collaborated with the California Polytechnic State University team to screen algal strains from culture collections and new isolates for productivity, robustness, and harvestability, with the most promising strains—*Scenedesmus obliquus* (UTEX 3031) and a new *Tribonema minus*—further investigated. An *S. obliquus* strain was obtained from a strain selection protocol that was 29% more productive than the original wild-type in side-by-side comparison in outdoor raceway ponds over 8 months of cultivation. Sandia National Laboratories (Livermore) performed genomic characterization of improved strains and de novo sequencing of genomes of novel species, as well as protein and carbohydrate fermentations. Heliae Development LLC carried out mixotrophic (photoheterotrophic) cultivation of microalgae and demonstrated greatly increased biomass productivities through novel cultivation strategies. The overall process was modeled through TEA and LCA for autotrophic and photoheterotrophic pathways, with the latter achieving >60% reduction, bringing the MYPP goals to well below the $4.96/GGE goal set for 2021.



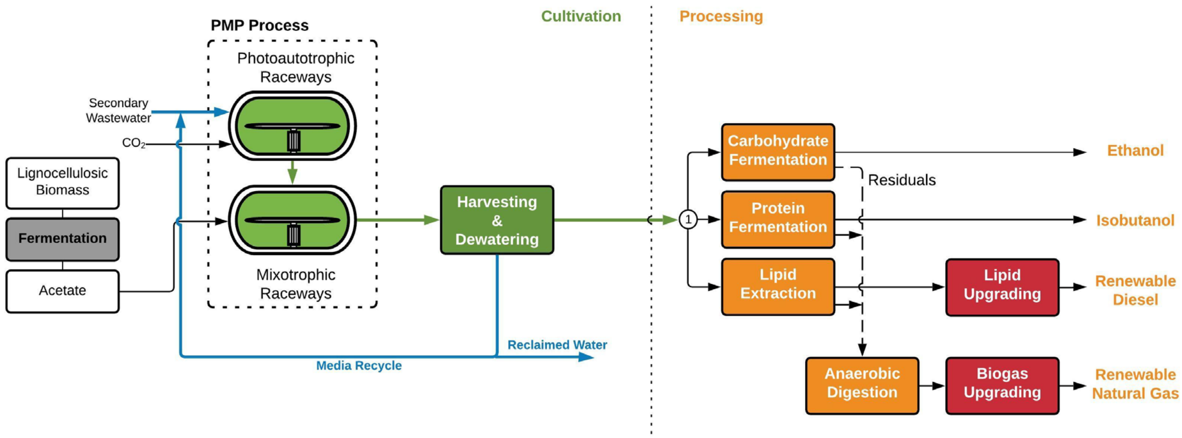


Photo courtesy of MicroBio Engineering Inc.

COMMENTS

- Overall, the project team met their project goals and demonstrated more productive *S. obliquus* strains through an adaptive laboratory evolution approach. The nine tasks for the project were well laid out with appropriate task leaders identified. Communication among the team participants was not clearly identified, and risk identification and mitigation strategies were not clearly explained. The approach to develop more productive algal strains through adaptive laboratory evolution has substantial merit and resulted in notable productivity improvements in *S. obliquus*. The impact of this project is clear. The project adds a useful tool for improving algae productivity. The project team met the final project TEA and LCA targets for the mixotrophic pathways they modeled.

- The project contains several novel concepts and approaches in pursuit of several BETO goals. A highly qualified team is assembled and was engaged throughout the project. There was no real discussion of risks anticipated or faced, and it was difficult to ascertain the data or the bases used for many of the conclusions being presented. Growth rates are presented in the mid to high teens (in g/m^2/d), whereas projected annual averages of greater than 33 g/m^2/d are listed, and 15 and 31 g/m^2/d are used for different TEA/LCA analyses, respectively. It is also unclear when reclaimed water or wastewater is used and how the individual products and corresponding coproducts are produced, at what volumes, and at what prices.

- The project is contributing annotated genomes of promising strains isolated from wastewater, which will increase the diversity of algal genomic data available for public use. It would be interesting to understand the composition of the native polyculture in order to better appreciate the differences observed in performance between the monoculture isolate (e.g., *T. minus*) and the polyculture (e.g., what percentage of the polyculture is *T. minus* across the cultivation campaign, what was the dominant algal species of the polyculture). It would also be important to show if the *T. minus* monoculture remains a monoculture during field trials, or if it reverts back to a polyculture over time when processing wastewater. From the data presented, it doesn't appear that the *S. obliquus* mutant MBR501 has the ability to outperform the native polyculture. Data for the MBE501-12 mutant is more promising, but the difference appears like it may only be significant during hot summer months, and additional long-term outdoor data will be needed to confirm this observation. Variant analysis for this strain has shown some interesting gene mutations. The team developed a mixotrophic growth strategy that has dramatically enhanced performance of *S. obliquus*, with a projected annual average of >33 g/m^2/day. According to TEA/LCA, the mixotrophic growth of *S. obliquus* for conversion to fuel products (ethanol, fuel alcohols, and renewable diesel) and fertilizer would reduce the MFSP to a projected $4.96/GGE. Overall, the project results exceeded the MYPP and project goal of producing 3,700 gallons/acre/year.

- The project is nearing the end of a 5-year project and has shown good performance in outdoor systems. Some of the planned work related to anaerobic digestion could not be completed as a result of the COVID pandemic. The reporting of work as conducted with reclaimed wastewater was somewhat confusing, as the water in question has been fully treated to tertiary standards and is of reuse quality, and therefore seems a less-attractive source water for algal farming versus a saline or untreated wastewater source. A projected 33-g/m^2/day outdoor annual average productivity with recycled nutrients and media was given but not clearly backed up as to the path to achieving this metric.

- The team and management plan described are appropriate for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. Discussion of potential risks and mitigation strategies would enhance the presentation of this project. The project is at the end of its funding period and has provided an approach through adaptive laboratory evolution and wastewater use leveraging mixotrophy to improve productivity. The algal biomass is then converted to biofuel intermediates though extraction, fermentation, and digestion for residues. The processes are then taken through TEA/LCA to assess performance and impact. The approach is deemed viable. The main impact of the project is associated with reaching BETO's goal of producing 3,700 gal/acre/yr. The team has a good record of publications, providing excellent dissemination of technologies and results. The strain screening and selection followed by evolution of *S. obliquus* via ultraviolet mutagenesis resulted in a 25% more productive strain. The mixotrophic cultivation processes developed under the project resulted in very successful *S. obliquus* cultivation with projected annual biomass productivity averages of >33 g/m^2/d. These productivities exceed the MYPP goal of 3,700 gal/acre/yr, with the TEA showing MFSP of $4.96 and LCA showing a 60% reduction in greenhouse gas emissions.

- The team's geographic co-location is favorable for easy collaboration. Involving Sandia National Labs for fuel upgrading tests seems like a wise use of resources. The roles of the participants are not well described. Outdoor cultivation seems to have happened on BG-11 media with acetate or synthetic primary clarified effluent. Extrapolating a projected annual average growth rate from cultivation on this media may not yield growth rates representative of those that will be seen in the field using wastewater or a more affordable and optimized media mix. It's unclear how taking a UTEX strain from 9.0 g/m^2/day to 13.9 g/m^2/day while introducing a mixotrophic consortium represents progress, as this benchmark falls short of the SOT and established growth rates for mixotrophic cultures. The team has said repeatedly that they use wastewater for cultivation of algae, which would be impactful from an environmental services perspective; however, during the review, it was explained that the team is using polished water that is approved for agricultural use. Making strains publicly available along with their genome sequences and associated data could have a positive impact on the industry if future researchers find value in continuing work with these strains. The TEA and LCA described seem to derive value from wastewater treatment. However, as previously noted, it does not appear that wastewater is actually being used. The final tasks associated with anaerobic digestion of fermentation broth were not completed. The project goals seem to have been met with modeled projections. The foundation of these models is somewhat in question if wastewater is the basis but is not really being used. Two genomes were assembled and annotated. A new cultivation method of mixotrophic growth was developed. Other tangible advances are unclear. Bacterial control measures may represent meaningful progress but are not described. Patents were filed as part of the project, but inventions are not described even in the supplemental materials section under patents. Modeled growth rates do not seem to have a path forward to translation into practice.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their constructive comments on this completed project. The first reviewer expressed interest in more information about the outdoor cultivation campaigns, specifically regarding the performance of the native polycultures, *T. minus*, and the improved *Scenedesmus* strains. The polycultures were routinely monitored by microscopy, and data were collected about the contribution of the major algal species found in the polyculture, which changed over the seasons. The data on polyculture composition were not presented at the Peer Review because of time constraints. *T. minus* was never a dominant species within the polyculture, although it was almost always present. Dominant algal categories included the green algae *Scenedesmus*, *Desmodesmus*, *Pediastrum*, and the filamentous *Stigeoclonium*. The monocultures tested were also routinely monitored by microscopy. When contamination by weed algae reached a threshold of 20% in either of the replicate ponds, both ponds were cleaned and restarted with fresh culture; therefore, reversion to polyculture did not occur during the trials. For a more detailed description of the methods used, please see Davis et al. 2021 (https://doi.org/10.1016/j.biortech.2021.125007). Regarding the development and performance of improved strains, we first demonstrated that mixed populations were promising prior to isolating and screening individual clones in order to improve screening efficiency. The MBE501 culture consisted of an ultraviolet induced, genetically variable population that had been selected for approximately 200 generations for improved growth. It is important to note that this was not a clonal population, but rather a mixture of genetically diverse cells with a common desirable phenotype. Because the population was diverse, results from bench-scale and outdoor trials represented the average phenotype of all cells in the population. In other words, productivity was a bulk measurement of the performance of all the individual cells in the population, with some phenotypes performing better than others. Once we established that MBE501 was promising (more productive that the wild-type), we isolated and screened numerous clones in laboratory PBRs to identify the top-performing strains. MBE 501-12, the 12th clone to be isolated from the MBE 501 population, emerged as a top performer. Once isolated and validated in bench-scale studies, outdoor trials consisting of duplicate ponds were conducted simultaneously with MBE 501, wild-type and polycultures for comparison. Although time limitations and COVID-19 restrictions prevented an annual productivity average from being obtained, 8 months of outdoor cultivation covering the major seasons (May–December) demonstrated that MBE 501-12 was 29% more productive than the wildtype *Scenedesmus*, and it outperformed the native polycultures by 13% during the same time period.

- Several reviewers requested clarification about the use of reclaimed wastewater for strain validation in outdoor trials and the integration of photoautotrophic and mixotrophic cultivation strategies to achieve project goals. Phototrophic and mixotrophic outdoor cultivations were conducted in two different locations and for two different tasks. The purpose of Task 3 was to validate monocultures in reclaimed wastewater and was conducted at the Algae Field Station in San Luis Obispo, California, through a subcontract with California Polytechnic State University. The purpose of Task 9 was to characterize the mixotrophic capabilities of strains identified as promising during the project and to optimize mixotrophic cultivation strategies for increased productivity, work that was performed through by Heliae Development LLC through a subcontract. The main objective of this project was to apply a non-genetically modified approach to generate improved strains that are more productive than the wild type. This metric would be challenging to demonstrate with cultivation in primary clarified effluent due to the amount of particulate matter, weed algae, and the high bacterial load present in primary wastewater. We validated strain improvements outdoors using filtered, fully nitrified reclaimed (secondary) wastewater effluent. With this, we successfully developed (Task 5) and validated improved strains in long-term outdoor trials (Task 3). Simultaneously, Heliae characterized the mixotrophic capabilities of *S. obliquus* in bench-scale experiments and outdoor pond trials in both BG11 and synthetic primary clarified effluent (Task 9). Cultures were supplied with organic carbon in the form of acetate, which can be derived from a waste source, such as through fermentation of lignocellulosic biomass, wastewater, or wastewater sludge. Additionally, Heliae advanced a cultivation strategy of photoautotrophy in an initial stage, followed by photomixotrophy, termed the Photo-Mixo-Partial process (see process flow diagram). The ponds were operated semi-continuously with routine dilutions and biomass harvests. During the initial photoautotrophic stage, bacterial loads remain low, allowing robust algal cultures to become established prior to boosting productivity through acetate addition. Additional bacterial control measures were developed and applied during mixotrophic cultivation by the addition of phagotrophic organisms that consume bacteria and keep their populations low. This approach has proven very successful, with projected annual average productivities exceeding 31 $g/m^2/d$, providing robust algal strains are used to rapidly establish healthy starting cultures. During the project, TEA/LCA modeling demonstrated that mixotrophy would be required to reach the ambitious BETO goals. To that end, the high yielding strains developed in Task 5 and validated in Task 3 are needed to establish healthy photoautotrophic cultures for the Photo-Mixo-Partial approach prior to trophic mode-switching. Therefore, both photoautotrophic and photomixotrophic productivities are important and were used in the modeling studies.

- In regard to using reclaimed wastewater approved for agricultural uses, that is a non-potable water source and can be used for biofuels production (https://water.es.anl.gov/documents/WaterandEnergy2015_proceedings%20paper.pdf), including microalgae. The reclaimed water used in this study was treated by the City of San Luis Obispo to levels suitable for crop irrigation. However, a vast majority of it is discharged to a creek leading to a coastal lagoon. It still contains high concentrations of nitrogen (~20 mg/L) and phosphorous (~3 mg/L), and its eutrophication potential has led the local water authorities to require more complete nutrient removal. Generally, reclaimed water has to be managed carefully when used for irrigation to avoid contaminating ground or surface waters. Nutrient removal is often proscribed even for water recycled to irrigation. Thus, reclaimed water is an apt medium for algae nutrient remediation, with expectation of treatment credits.

- Some reviewers noted that the BETO Peer Review presentation would have benefitted from a more detailed description of project participants and risks and mitigation strategies. The role of each participant in this large, multifaceted project was not emphasized due to time constraints. Briefly, MicroBio Engineering Inc. (led by Dr. John Benemann and Dr. Aubrey Davis) was responsible for project management and coordination and TEA/LCA studies. Strain selection, outdoor cultivation, biomass processing, and anaerobic digestion studies were carried out in part through a subcontract with California Polytechnic State University in San Luis Obispo (Dr. Tryg Lundquist and Ms. Ruth Spierling); genomic analysis and fermentation studies were conducted by Sandia National Labs (Dr. Todd Lane, Dr. Krissy Mahan, Dr. Ryan Davis); and the mixotrophic cultivation effort was carried out by Heliae Development LLC (Dr. Mike LaMont and Mr. Steven Pflucker). For a summary, please see “Management” on slide 4.

- Risks were identified and mitigation strategies applied throughout the project. One risk concerned the performance of the wild-type strain chosen as the focus of the project (*Scenedesmus obliquus*) and whether it would be sufficiently productive in San Luis Obispo, California. To address this, a bioprospecting effort for native isolates productive under local conditions was conducted as a mitigation strategy and resulted in the identification and characterization of a highly productive strain of *Tribonema minus* (https://doi.org/10.1016/j.biortech.2021.125007). Another mitigation strategy was that the individual tasks performed by project partners were relatively compartmentalized such that technology advancement was generally not dependent on the progress in other tasks. For example, development of novel mixotrophic cultivation strategies, the variant analysis pipeline, advanced conversion technologies, and biomass processing and optimization were independent of strain improvement success. Regarding the reviewer request for more information about patents resulting from this project, patents are in progress, and, until disclosures are filed, details cannot be provided on advice of counsel.

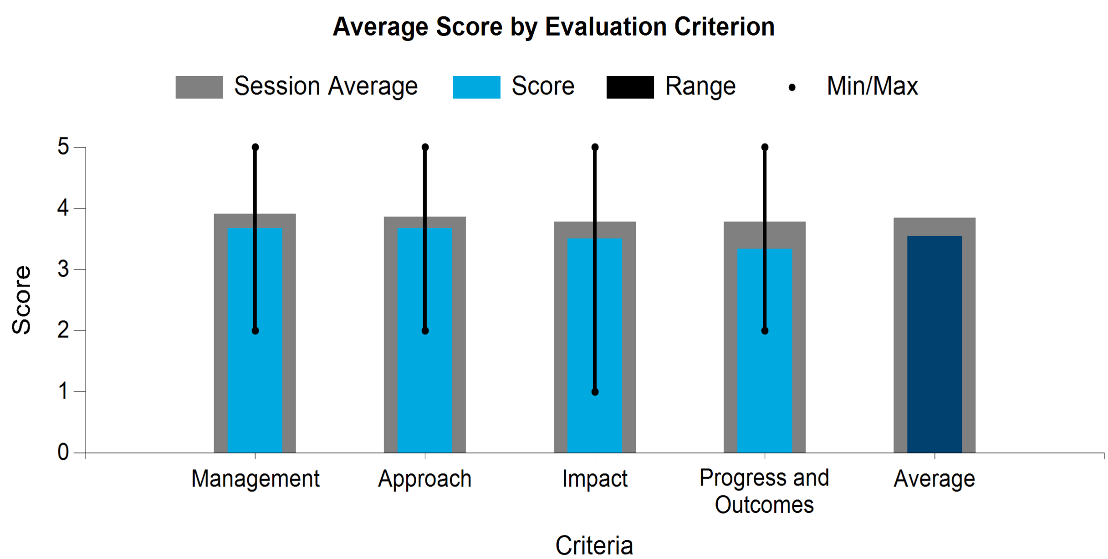
REWIRING ALGAL CARBON ENERGETICS FOR RENEWABLES

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	1.3.5.270
Presenter(s):	Lieve Laurens
Project Start Date:	08/01/2017
Planned Project End Date:	12/31/2020
Total DOE Funding:	$3,608,347

Critically needed improvements in biomass and biofuel intermediate productivity can be made by addressing fundamental inefficiencies in algal carbon conversion efficiency to biofuel intermediates. Algae photosynthesis is, at best, able to convert 5%–7% of incident light energy to biomass, while conversion to fuel intermediates falls 15%–25% short of its maximum potential due to inefficiencies along the pathways. Recent progress in the Rewiring Algal Carbon Energetics for Renewables (RACER) project consortium focused on a means to address these inefficiencies in a pathway from algal biomass to a variety of fuel intermediates, ethanol, 2,3-butane diol, lipids, and green biocrude. This project engineered a production-relevant algal species *Desmodesmus armatus* (SE 00107) to demonstrate biomass productivity improvements, with a doubling of the fuel intermediate yields. The new algae biorefinery paradigm embodied in RACER opens opportunities for algae engineering beyond efforts typically targeted solely at lipid content or improved light harvesting efficiency. Parallel approaches showed improved carbon conversion efficiency through elimination of wasted energy during photosynthesis and increased carbon flux to transitory carbohydrate storage in the cells. Outdoor operation and nutrient management strategies with improvements in pretreatment, fermentation, and extraction in a combined algal processing approach showed a 40% reduction in MFSP, with a combined biofuel productivity of >3,700 gal/acre.



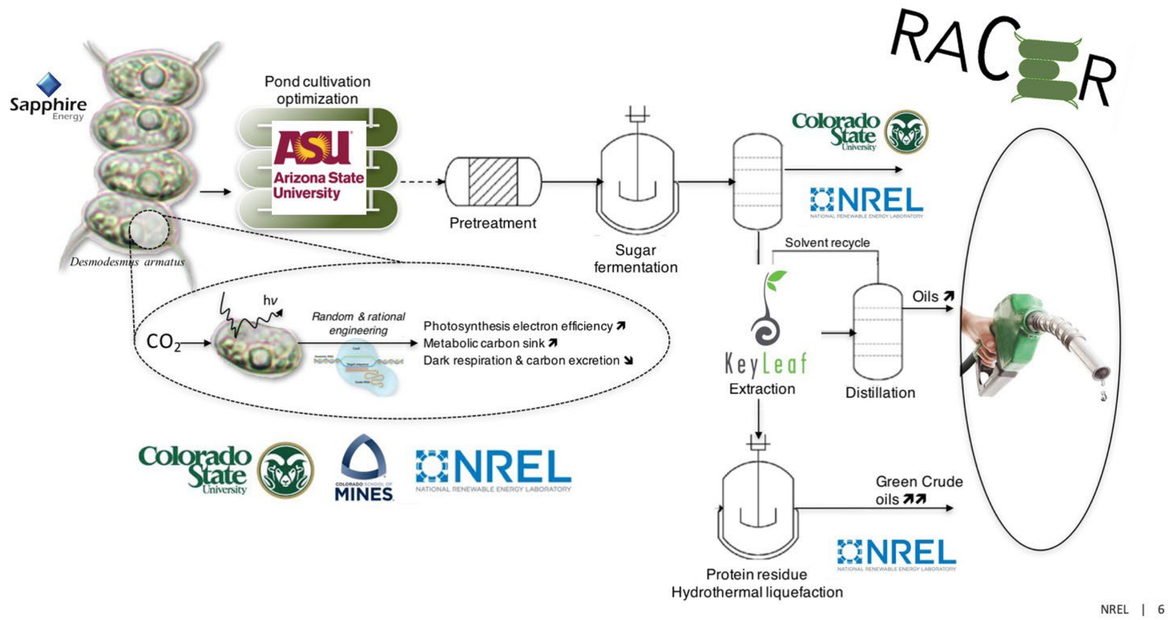


Photo courtesy of National Renewable Energy Laboratory

COMMENTS

- The overall goal of this project is to improve the intermediate productivity of *Desmodesmus armatus* through regulation of carbon conversion pathways and production of ethanol, butane diol, lipids, and HTL biocrude, toward reaching 3,700-gal/acre/yr productivity. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The overall approach for the project includes engineered strain development, which was not successful, as it has been unable to confirm genomic insertions, pond operational management, conversion to fuel intermediates, and TEA and sustainability analysis. Clarification as to why this strain was selected for these studies and level of understanding of its genome qualities and ability to engineer the strain would have been helpful. Some milestones were missed due to genotype/phenotype instability, resulting in rescoping to provide in-depth comparative genetics. A rescoping toward a comparative genomics effort is envisioned, but it is not clear how this effort will help with the main goal of the project and if it will help it achieve more stable transformants. The project has a good publication record with seven peer-reviewed publications and a patent application on genetic engineering. The project's pioneering work with the pesticide fluazinam for pond management is now being implemented in other projects for the BETO program. The enzymatic hydrolysis approach is very efficient and provides an excellent pathway toward ethanol and butane diol.

- The team draws from many teams, but Sapphire seems to be responsible for the majority of the work, and their role and responsibilities in the project seem unclear. Inclusion of a well-qualified industrial advisory board and coordination with national lab and AzCATI teams in the DISCOVR program provide excellent conduits of communication with advances to the SOT and increase the likelihood of overall project success. The reason the team dedicated so much focus and effort on a single strain, *Desmodesmus armatus*, seems unclear. If there was some reason to believe results in this strain could be translated to other strains, then this may not have been clearly understood. Random mutagenesis appears to be a risky and often fruitless strategy when compared to rational strain engineering when developing carbon conversion efficiency. This project does not seem unique in its intent or outcome. Some components of the project seem to have been completed with success, including a new contamination control strategy, but the failure to meet the most critical and challenging milestones in the project limits its impact significantly. The focus on a single strain without known transferability of any improved traits also inherently limits the impact of this work. It appears the most challenging and impactful milestones have not been met. The genetic instability of transformed organisms presents inherent risk at all scales, but this risk seems largely unmitigated by rational engineering strategies. If this one unique strain was chosen as the model organism for this project, it would stand to reason that some assurance of genetic tractability would be required before expending so much time and energy. Failing to receive TERA protocols may be indicative of the inability to stably integrate new characteristics into this strain. There is concern that the project may have fallen short of being successful toward the specific goals very early into the project, but didn't recognize or address critical path dependencies, TERA permitting requirements and timelines, or impacts of not meeting milestones and goals early on.

- The team has faced challenges with making forward progress on strain development project milestones due to genotype/phenotype instability of their engineered mutants. They are actively seeking to rescope the project to use comparative genetics to investigate carbon metabolism in this species, but it is not clear what value this will add to the overall project goals. Integrating metabolic conversion of algal sugars to butane diol by an engineered bacterium is an interesting pathway to consider, and conversion yields are impressive, but it is unclear if TEAs/LCAs support this strategy. The team was able to increase outdoor productivity by 27% with cultivation process improvements. However, the presenter acknowledged that since process improvements were exhausted early on in the project, it is unlikely that further improvements could be made during the remaining project period, which would be necessary to achieve the 17 g/m^2/day target in order to reach the final milestone goal of 40% reduction in MFSP. The team should consider focusing on butane diol conversion by testing hydrolysate from other algae species of interest to the BETO portfolio.

- This project has appeared to face many management challenges during its history, with changes to team members and shifts in thinking regarding preferred strains. Regardless, the outline and approach for RACER is directly aligned with many of BETO's MYPP goals and needs. Despite being unable to deliver a documented result, the project's ambition to submit TERA protocols should be acknowledged. It was not clear if there remains enough budget or schedule to rescope or otherwise attempt the missed milestones. While the project achieved many good outcomes, the total progress does not entirely satisfy the project goal and end-of-project milestone. The project served to transfer technology and coordinated well with other BETO programs and contributed to several SOT topics. Many project results were disseminated via publications.

- This project is nearing completion and overall results appear mixed. Project meetings occur monthly, which seems too infrequent. The risks are clearly identified, and strategies are identified. A summary of how effective these strategies were would have been helpful. Impact of the project seems good. Developed technologies (genetic engineering tools, fluazinam pest control strategy, conversion, and fermentation platforms) were transferred to other projects in the DOE algae portfolio. Progress on this project was mixed. Genome instability hampered genetic engineering efforts. Outdoor growth improvements and improved carbon conversion were realized.

- This project is nearing completion toward its goals of (1) improving photosynthetic carbon conversion efficiency, (2) advancing cultivation management techniques, and (3) tailoring and optimizing conversion processes. This project integrates upstream strain engineering with downstream biomass conversion for a more complete approach. The project successfully demonstrated a 27% increase in productivity in outdoor ponds. A key project takeaway was that pretreatment processes are critical to the conversion step and must be considered.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their complimentary feedback on this project. Several comments by the reviewers were reiterated, and we appreciate the opportunity to respond to some of the main questions. First, we acknowledge the reviewers' comments around the challenges we faced in meeting milestones that were reliant on demonstrated phenotypes. The approach of placing phenotypic improvements of algae in the critical path of such a short-duration project is inherently risky. While this project relied on transformation methodology that was proven to work in this species at Sapphire at the onset of this project, and we were able to demonstrate the respective phenotypes for a series of engineered lines in the first year of this project, we subsequently encountered significant genomic instabilities that have, to this day, been remarkably underreported in the literature. In this context, we have pivoted to a different species and are incorporating an effort on comparative genetics in the final months of this project. We built the project as a farm-to-lab-to-farm concept to expand the potential of one the most productive strains in the portfolio of our then-partner Sapphire Energy (in 2016, when this project proposal was written). *Desmodesmus armatus* was selected by Sapphire because of its robust outdoor growth in Las Cruces, and Sapphire's biotechnology and engineering team had demonstrated genetic engineering and transferred tools and background information to us. While we were able to repeat the transformation of this strain, we needed to collect many more details on genomic insertion sequence and stability data for the initiated TERA permitting process that finally elucidated a unique genomic rearrangement mechanism in *D. armatus* that may not be present in other species of algae, or not reported. This project has now started the closeout phase with the final deliverable just wrapped up on the TEA and LCA of improved productivity, composition, and fermentation/extraction and conversion metrics. There are no funds left for a rescoping effort. However, some of the reviewers mentioned the tech-transfer focus of this work and we are prioritizing this aspect, ensuring that novel technologies (and lessons learned) are transitioning to other projects. Thus, progress is not lost, but rather forms a new platform to build on to achieve some of the core carbon efficiencies that are ultimately critical for a sustainable bioenergy future.

- With respect to project management and meetings, the full team convened on a biweekly basis for the majority of the duration of this project and transitioned to monthly updates in the final year when partners focused on the demonstration aspects of this project. What was not communicated in the presentation is that the subtasks continuously had more frequent, weekly sub-group meetings. For example, the strain development and genetic engineering (Task 1) team convenes every week to report and (re)align direction and expectations.

- Finally, we want to correct the comment raised by one of the reviewers that Sapphire appears to be responsible for the majority of the work. This must be a misunderstanding; Sapphire was critical in the delivery of the strain and the initial contribution of outdoor cultivation data and know-how, but further only served in an advisory role throughout the rest of this project.

- In summary, the goal of this proposed project was to build an integrated process to test the assumption of strain selection toward achieving overall carbon conversion efficiency metrics. While each of the respective unit operations in the process flow recorded the needed improvements, an integrated outdoor demonstration of a selected mutant strain for biomass production—which then fed downstream operations—created an unpredictable and much less attractive biomass composition and recalcitrance, with significant process cost and performance penalties as a result. One mitigation strategy to this understood risk could have been to build in contingency strains with more attractive native biomass composition to achieve the conversion yield targets and start such a parallel approach earlier. In one way, this project has brought a critical challenge to the forefront of genetic engineering of algae, and hopefully through publication and dissemination of the lessons learned may shorten the path for other, similar projects. The reliance on a stable genetic transformation event and activation of an overexpressed gene of interest to yield a compositional phenotype is inherently risky, and this parallel effort should have remained separate and not be positioned in a critical path that held up the conversion tasks and deliverables. Within the constraints imposed on this project by the FOA requirements, significant progress has been made and improvements in genetic engineering, outdoor cultivation and crop control, and immobilized fermentation and HTL of protein-rich residues have all independently moved the needle and are in some form continued as part of separate projects.

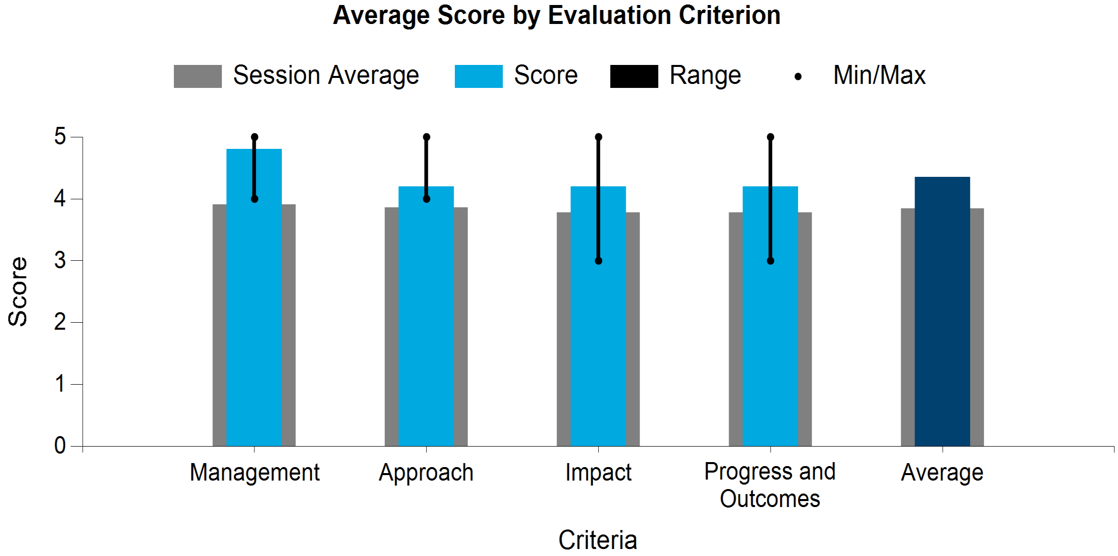
OPTIMIZING SELECTION PRESSURES AND PEST MANAGEMENT TO MAXIMIZE ALGAL BIOMASS YIELD

The New Mexico Consortium

PROJECT DESCRIPTION

WBS:	1.3.5.280
Presenter(s):	Alina Corcoran
Project Start Date:	10/01/2019
Planned Project End Date:	12/31/2023
Total DOE Funding:	$6,289,829

The OSPREY (Optimizing Selection Pressures and Pest Management to Maximize Algal Biomass Yield) project responds to a critical industry need to improve annualized productivity, stability, and quality of algal production strains for biofuels and bioproducts. We aim to generate process innovations rooted in outdoor cultivation for strain selection, maintenance, cultivation, improvement, and pest management that will result in a 50% improvement in harvest yield and robustness and 20% improvement in conversion yield. Our project's components are built on a single foundation: the year-round cultivation of a field-adapted algal biofuel strain in outdoor systems at Qualitas Health (Imperial, Texas), Cyanotech Corporation (Kona, Hawaii), the California Center for Algae Biotechnology (San Diego, California), and the Fabian Garcia Science Center (Las Cruces, New Mexico). The unique environmental selection pressures of each outdoor system will allow us to naturally develop robust cultivars with different environmental tolerances. Project components include tracking trait drift and evolution in the field and lab, using metagenomic tools to identify and track pests/pathogens, using non-genetically modified approaches to improve the baseline field-adapted strain, implementing process improvements, and assessing effects of improvements through sustainability modeling based on open raceway pond growth architectures. To date, our technical accomplishments include (1) the isolation of the field-adapted strain from Qualitas Heath ponds and establishment across the labs and additional field sites, (2) initial trait characterization of lab and field cultivars, (3) metagenome characterization across sites, and (4) application of selection and mutagenesis/selection to drive cold and hot tolerance. The project has been challenged by differential strain performance across labs and a lack of clear pests at field sites. Overall, our approach is unique because it relies on natural, outdoor selection pressures to drive fitness in strains that have already been identified as standards in the industry. Moreover, the work has broad relevance to algal biofuel and bioproduct industries, as the proposed pipelines can be applied to a variety of systems regardless of strain, location, or product.



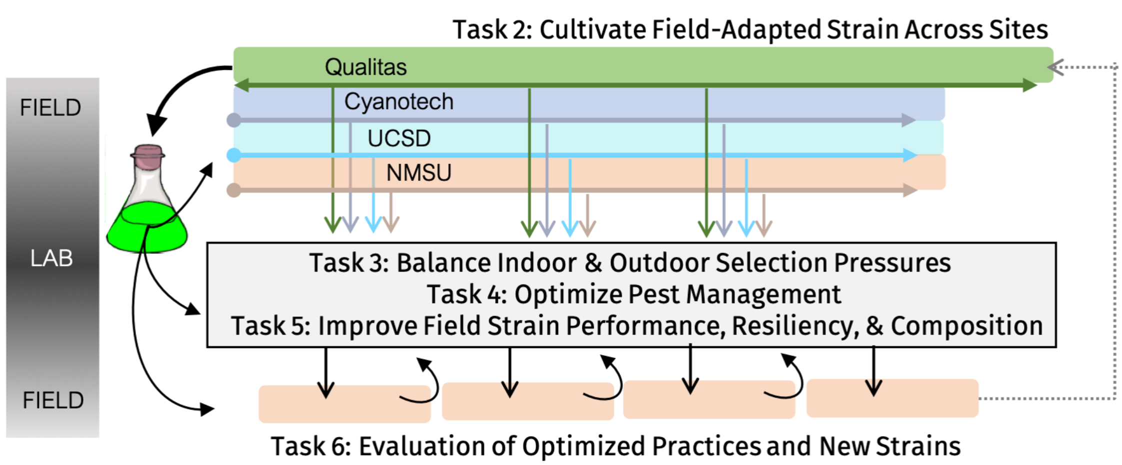


Photo courtesy of The New Mexico Consortium

COMMENTS

- Overall, this project seems to be going very well. The project management plan is strong. Communication between the project team is bimonthly and risks and risk mitigation strategies are clearly detailed. The approach of using a field-adapted industry strain and field-lab-field iterations across unique field sites has merit and seems innovative. The project demonstrates clear potential for impacting algae commercialization efforts. Multiple industrial partners are involved in the project. Task 2 has been completed and other tasks are on schedule; thus, the project has made appropriate progress toward addressing project goals.

- The project aims to develop a robust set of algae strains and cultivar systems by developing systems from the field into the lab and back into the field. The impetus is that this creates a more robust set of positive pressures for selection and cultivar management. The team and management plan described are appropriate for this project. The risks and mitigation strategies are clearly delineated for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The approach was clearly delineated against tasks, goals, and go/no-go decision points. This incorporated the development of an already field-adapted strain from one of the partners through a pipeline of indoor and outdoor selection pressures, optimization for pest management, and improvement of field strain performance, resilience, and composition. This is a complex project; therefore, the approach description would have benefited from more details on each of the steps to be undertaken, perhaps through an example. The impact of the project was described through the improvement of robustness and yields for production strains, along with the development of a metagenomic database of pests across sites and fieldable pest-tracking kits. The impact to the BETO mission could have been explained more thoroughly by providing the major goals and targets that the project is addressing. The project started with the collection of a strain that has been adapted into production by one of the partners and cultivated at other partner sites. The team then moved toward trait and microbiome characterization across sites. The team has moved the strain toward ultraviolet mutagenesis for high- and low-temperature selection and adapted evolution. The team has completed a number of its major milestones and is on track for the remaining milestones.

- The project has assembled a team and approach that can inform larger industry and program thinking regarding strains and strain development programs. The basis of using field strains to inform lab-based work and then return with the results back to the field is an important and practical step in support of near-term commercialization and reflects BETO program goals. The use of three multiple cultivation modes and four different field sites introduces complexity, but in a forward-leaning manner. The team will have an opportunity to review results against a decent profile of possible commercial locations and commercial strain candidates. COVID delays and the relatively recent project start date limits the opportunity to fully gauge progress and conclusions. It is helpful to see that initial milestones have been completed and the balance of the work remains on schedule.

- The project team is composed of a nice mix of national lab, industry, and academic partners with strong backgrounds. There has been clear and deliberate thought to project management and risk identification and mitigation. The use and study of field adapted *Nannochloropsis* strains are unique to this project. Comparing the genotypes and phenotypes of the same strain being cultivated in three labs and four field sites is an interesting approach to determine trait fluidity. The team has shown adaptability in their approach to the pest management tasks by also including untreated ponds to ensure that pest invasions can occur, as their pest management SOP was too good. It is yet to be determined if mutagenesis or cold adaptation strategies will yield mutants with favorable performance, but the project is progressing on schedule.

- This team is using a field-adapted industrially relevant strain, and outdoor-indoor-outdoor iterations across multiple, varied field sites to quantify and balance selection pressures across the lab and field with a goal toward optimizing processes with a strong focus on pest management, a critical issue for outdoor pond cultivation. The presentation was excellent and is to be commended on their strong project management, risk identification, and communication practices, which were conveyed clearly. These could be used as a model for other BETO projects. Early results have identified temperature as a key driver for pest pressures.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their evaluation of our project and the positive feedback. As pointed out by one reviewer, this is a very complex project. We welcome the opportunity here to provide additional information on the details of the project approach, with examples. Our project’s components—(1) balancing indoor and outdoor selection pressures, (2) optimizing pest management, and (3) improving field strain performance, resiliency, and composition—are built on a single foundation: the year-round cultivation of a field-adapted *Nannochloropsis* strain in four outdoor systems for approximately 3 years. The unique environmental selection pressures of each outdoor system will allow us to develop robust cultivars and will facilitate process innovations with broad geographic applicability. Throughout the project, we will track trait evolution across the field and lab sites, develop novel pest tracking tools, and improve the field strain through non-genetic modification methods. As pest management tools come online, they will be validated and deployed at the industrial field sites. In addition, we will 'beta-test' new or improved strains and process innovations (e.g., changes to laboratory strain maintenance or seeding) at the New Mexico State University test bed. Finally, we will assess improvements through techno-economic and sustainability modeling based on open raceway architectures, leveraging existing models and including fuel and coproduct production pathways. As an example, illustrating process innovations, if a particular field or lab-maintained strain (or mutant) shows greater productivity or stability than the baseline strain, we can test and/or recommend reseeding procedures to enhance field performance. Fieldable pest-tracking kits will also allow us to reach our targets. As listed in our impact slide, our targets are a 50% improvement in harvest yield based on AFDW ($g/m^2/d$), a 50% improvement in robustness based on stability metrics (e.g., high-productivity cultivation days, pond uptime), and a 20% improvement in conversion yield. Additional outputs are process innovations in strain improvement, maintenance, and cultivation; a metagenomic database of pests across diverse sites; and fieldable pest-tracking kits.

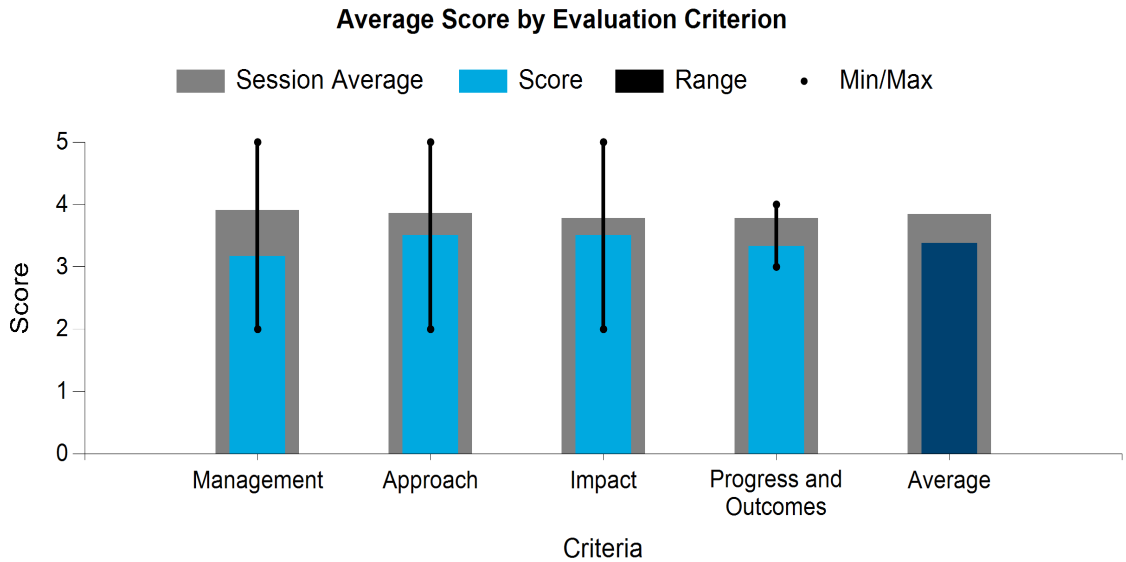
ALGAL PRODUCTIVITY ENHANCEMENTS BY RAPID SCREENING AND SELECTION OF IMPROVED BIOMASS AND LIPID PRODUCING PHOTOTROPHS (APEX)

Colorado School of Mines

PROJECT DESCRIPTION

WBS:	1.3.5.282
Presenter(s):	Matthew Posewitz
Project Start Date:	10/01/2019
Planned Project End Date:	04/30/2024
Total DOE Funding:	$4,920,378

Recent advances continue to demonstrate impressive progress toward improved algal photosynthetic productivities and biomass compositions for biofuel applications. Despite these significant strides, productivities from these photoautotrophs require further improvements in terms of biomass yields and compositions (e.g., higher oil content) for the economically successful production of algal biofuels. We propose to use a multipronged approach to improve the oil content in algae capable of sustaining high areal biomass productivities in outdoor ponds. One approach will use a suite of random mutagenesis techniques—for example, gamma radiation and atmospheric and room temperature plasma mutagenesis—to generate genetic diversity followed by targeted turbidostat/chemostat selection coupled with fluorescence-activated cell sorting to isolate higher-oil-accumulating strains of the diatom *Nitzschia* sp. GAI-229 (hereafter GAI-229), which is an exemplary, high-performance outdoor strain isolated by GAI. Diatom breeding will also be explored as a higher risk, but potentially exceptionally powerful approach to generate biomass diversity for the screening and isolation of more oleaginous strains and improve phenotype stability. The second approach will explore the disruption of storage carbohydrate synthesis using genetic engineering as a way to improve carbon partitioning to lipid biosynthesis in *Nannochloropsis gaditana*, which we recently adapted to grow in high-bicarbonate media for deployment using GAI cultivation technology. Species of *Nannochloropsis* are promising because of their endogenously high oil contents and production of eicosapentaenoic acid. Molecular engineering techniques, including CRISPR/Cas9 genome editing, are well established for species of *Nannochloropsis*, and mutants disrupted in chrysolaminarin biosynthesis have already been generated for this project and are in hand, but have not been tested for their suitability as a robust production strain. In the third approach, we will undertake a bioprospecting effort to isolate saltwater algae that are capable of thriving in GAI's high-bicarbonate, high-pH media. We propose to follow recent "survival-of-the-fittest" selection strategies that have resulted in the isolation of highly promising production strains using different media types.



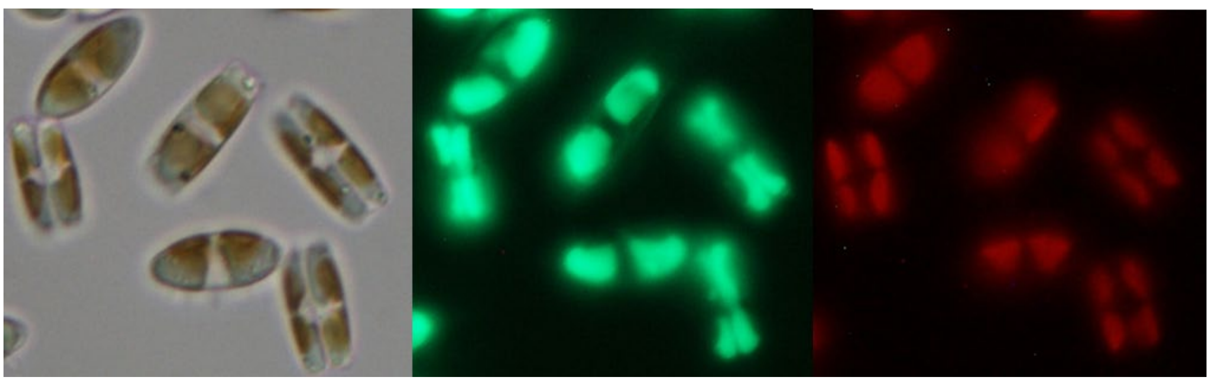


Photo courtesy of Colorado School of Mines

COMMENTS

- Bioprospecting seems to be used as a risk mitigation strategy to prepare for the chance that random mutagenesis fails to yield an optimized field strain. This effort is always worthwhile but has been done so many times by so many groups using DOE funding that it no longer seems to be a suitable risk mitigation or strain optimization approach. The unique capabilities of the Queensland University of Technology team are not clear. The project utilizes a novel plasma generation unit, but the fundamental approach is still random mutagenesis. This mutangenizing agent may generate more insertions and deletions, resulting in fewer reversions, but the strategy still seems equivalent to gambling when compared to a rational engineering strategy. The same limitations that have kept random mutagenesis strategies from yielding robust field strains still constrain the potential of this work. The flow sorting strategy seems sound and similar work has yielded positive results in the past. It appears that the team has been improving and optimizing the PBRs, and it may be they become the indoor benchtop reactors most capable of simulating outdoor conditions. Project partners are not using these PBRs, and it appears each partner in the team is using a different arrangement, which may result in poor alignment of expectations and risk that lab work will not translate to the field. The plan to use GAI field media as opposed to BG-11 or another lab mix is smart and will help transition positive results from the lab to the field. Team describes using directed evolution, mutagenesis, strain prospecting, and novel breeding strategies simultaneously to drive strain improvements. This approach would be better if new strain traits were stackable or translatable, but as described, the approach seems to be a scattered and distracted all-of-the-above approach. Unless this team is searching in unique habitats or ecosystems for specific traits or bioprospecting in its local area because of regulatory import limitations, it's unclear that this effort should be included in the scope and budget for this project. This work may result in marginal improvements in yield for GAI, but none of the learning seems very translatable to other work or the industry as a whole. The impact of this work seems limited by intellectual property protections that will shield any strains developed from broader use. This project has only recently started, and it is too early to comment on the progress and outcomes of the work.

- Given the lack of results with traditional non-GMO mutagenesis approaches, the proposal to try atmospheric and room temperature plasma mutagenesis is novel and could potentially produce more stable genotypes and phenotypes. The mutagenesis work will be conducted at the Queensland University of Technology in Australia. Breeding, selective environmental pressure, and bioprospecting efforts are also focused on increasing lipid and biomass productivities with the goal of reaching 23 g/m^2/day and >31% lipids. The novel parts of this project are very high risk but could yield significant rewards if they work. The project is in the early stages, and the team has made appropriate progress toward current milestones.

- The presentation was unfortunately not able to cover all of the materials available and fully discuss the work plan and progress to date. The discussion of the value of the unique PBRs was informative but not a part of the project. The materials presented for review seem well designed and in pursuit of strain improvements that are in support of overall BETO goals. Given the only recent start of the project, progress against project goals and end-of-project milestones are not really possible to review at this time.

- This is a new starting project initiated in October 2020. The project aims to improve lipid yields using mutagenesis and breeding of *Nitzschia* and *Nannochloropsis* strains. They also incorporate bioprospecting for new productive strains. The team and management approach described are appropriate for this project. The presentation did not provide a description of progress to be achieved through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The approach is to improve lipid yields using mutagenesis and breeding of *Nitzschia* and *Nannochloropsis* strains. Mutagenesis will be generated by the use of an atmospheric room temperature plasma that generates mutants with high levels of insertions and deletions, presumably making them more stable. High-lipid mutants will be selected using flow cytometry. The impact of the project was described through productivity gains, which, if realized, will achieve BETO targets for 2030. Other impacts were described based on the development of technologies and publication efforts that will be ensued. As this project is just getting started, the presentation focused mostly on establishing the technologies and tools that will be put in place to help achieve the goals. Unfortunately, too much time was taken in describing some basic tools, such as the PBR system being developed by the Colorado School of Mines under other efforts, critically cutting the time available for proper description of more relevant progress and/or showing how the approach will lead to projected outcomes. The plasma mutagenesis presentation would benefit by showing mutant stability tests. The goal of the bioprospecting efforts is to find *Nitzschia* strains capable of mating.

- This project aims to increase both biomass productivity and lipid content through a combination of bioprospecting and plasma mutagenesis. The best producers will be evaluated in custom laboratory PBRs and scaled to mini raceways. This is a new project that is just getting started. Initial results demonstrated higher lipids and reduced biomass growth; a trade-off that has been observed by other researchers. The project work plan includes TEA/LCA work at the end-of-project timeline for the selected strain. The team might consider a preliminary TEA/LCA earlier in the project to help drive the strain selection effort.

- This project recently started, and the project seems to be off to a good start. The management plan is not clear. How often group members communicate is unclear. Risks and mitigation strategies are also not adequately addressed. The plasma mutagenesis approach is intriguing and seems to have potential. Impacts would be significant and GAI is involved with the project, which aids commercialization efforts. Much of the presentation was dedicated to explaining how laboratory bioreactors were built and tested. Future updates should focus more on specific project tasks. Progress to date has been appropriate.

PI RESPONSE TO REVIEWER COMMENTS

- We think that bioprospecting is far from done, and specifically point to the case of new DOE SOT strains that were only recently found with new methodologies. Similar approaches are likely to lead to new and exciting strains of direct interest to DOE missions. Queensland University of Technology is a premier institution for plasma mutagenesis, which is the technique that we are focusing this project around. It far more cost-effective to leverage existing experience and equipment than to build from the ground. Each of the team's bioreactors fulfill unique aspects of the project in terms of scale. Reactors can certainly be shared across the institutions if deemed necessary and cost-effective. We are absolutely targeting our bioprospecting sites for relevance to the GAI site and media. As advised, the team will use earlier TEA/LCA to drive strain selection. The research team communicates weekly, and ad hoc meetings are initiated as necessary to focus attention on specific areas of need.

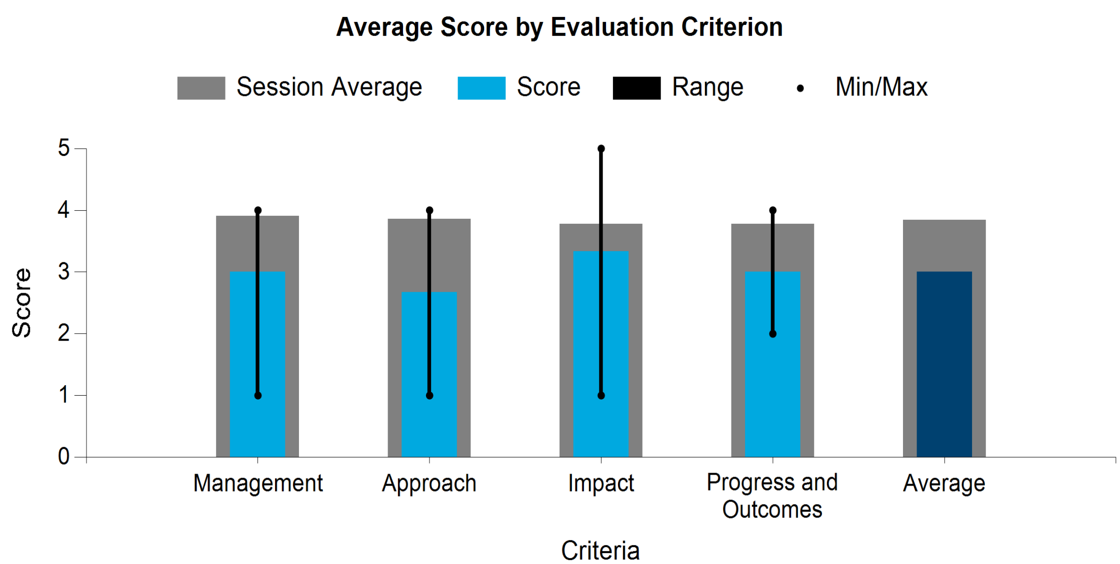
INNOVATIONS IN ALGAE CULTIVATIONS

Global Algae Innovations

PROJECT DESCRIPTION

WBS:	1.3.5.284
Presenter(s):	David Hazlebeck
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2023
Total DOE Funding:	$5,625,000

GAI has developed low-cost algae production technologies aimed at achieving commercially viable production of biofuel and high-protein meal. Radical advances have been designed and implemented throughout the entire process, resulting in many industry breakthroughs for large-scale algae cultivation, harvesting, and processing. In this project, a series of innovations in cultivation methods and innovations in cultivation monitoring tools will be developed to increase the productivity, robustness, and yield of our advanced cultivation systems. Additionally, a chain of test systems from laboratory-scale microplates through outdoor raceways producing kilograms of algae biomass will be developed and tested to accelerate the indoor/outdoor cycle rate and improve the translation of laboratory results to mass culture. After verification, the first phase includes development and validation of the test systems and initial data collection for one of the monitoring tools. The second phase is the initial R&D on the innovations to achieve the intermediate project go/no-go goals. The third phase will utilize the best combination of innovations from the second phase as the base and focus on two or three of the innovations for optimization to achieve the final project goals and commercial implementation. The goals are to overcome the challenge in translating results between laboratory and mass cultures and to increase algal productivity by 50%, cultivation robustness by 50%, and the conversion yield by 20% while achieving cost and LCA targets.



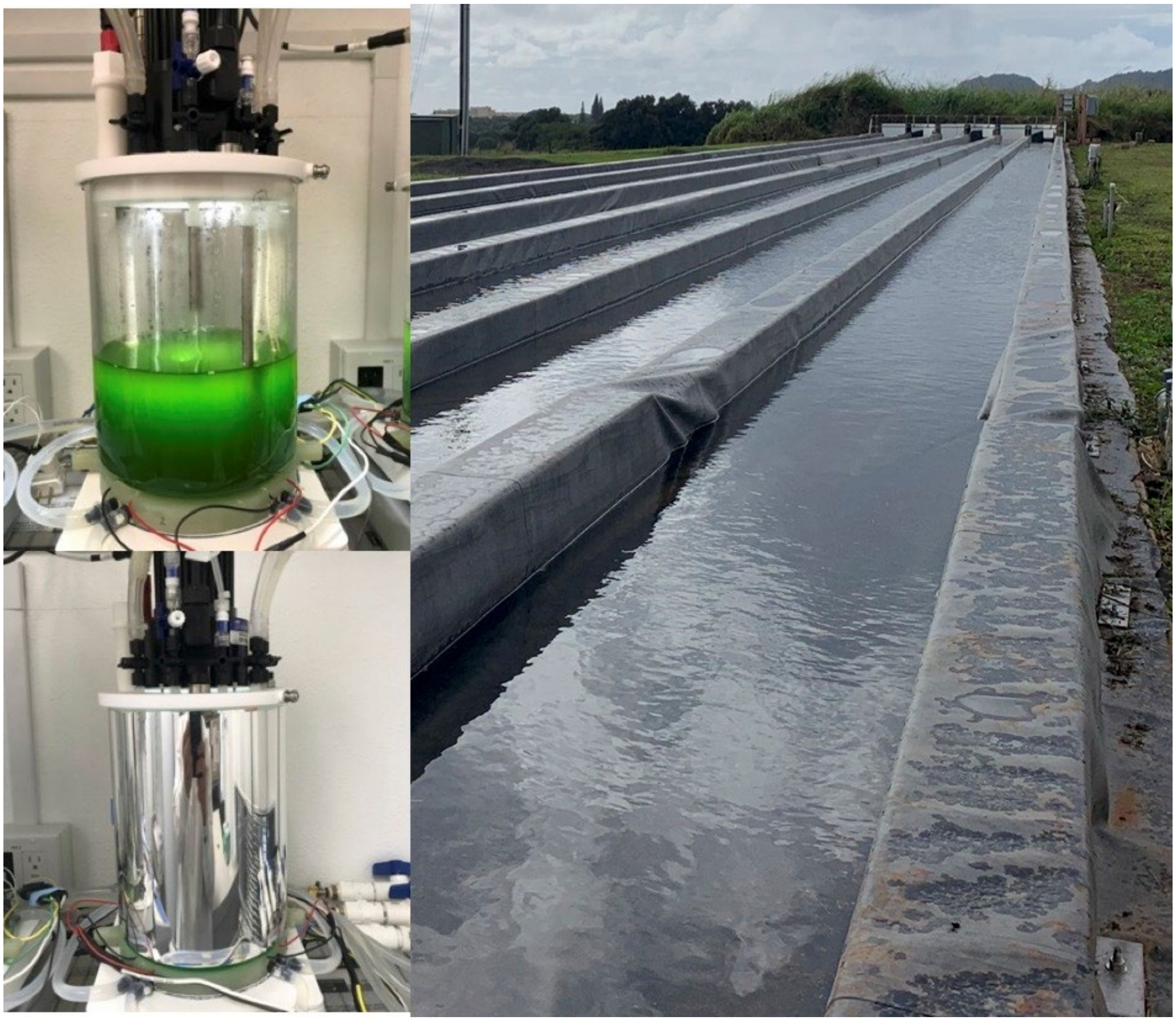


Photo courtesy of Global Algae Innovations

COMMENTS

- Overall, this project seems to be off to a good start. The project team communicates weekly via telecoms. There is a concern about how stretched GAI will be coordinating with 18 partner organizations. Specific risks and appropriate mitigation strategies should also be identified. The approach involves R&D on 12 parallel advances, which will eventually be downselected to about three for detailed development. Criteria for selecting these approaches need to be clearly defined. The project has clear commercialization potential, and GAI looks to be actively working on commercializing its technology. The project started last summer, and the results so far are encouraging. Specific improvements in each of the 12 approaches should be identified during the next review.

- The presentations showed that the team has access to cultivation equipment and many intermediate scales from bench scale to outdoor 6,000-liter ponds. In collaboration with NREL, this project proposes to develop real-time monitoring of algae cell density and health and compositional analysis by quantitative spectroscopic phenotyping. This part of the project is novel and would contribute significantly to the availability of tools for monitoring and analysis of algal cultures. This is a relatively new project, and adequate progress is being made for the spectroscopic work. However, there is no explanation of how this or any other proposed strategy will contribute to increasing algae productivity, except for the brief mention of 12 parallel approaches to improve productivity. Being that the end goal is to increase productivity by 50%, a clear description of the approach needed to achieve this very high goal is warranted. Unfortunately, the project lead did not present any data or information regarding the proposed work, making it very difficult for reviewers to assess the approach, validity, or impact of the proposed project. This was a missed opportunity for the project team.

- The small GAI team seems well poised to act quickly and communicate well. The 18 partner organizations mentioned are associated with other projects, are not named, and the nature of their collaboration is not described. The description of the technical approach used in this project is too general and vague to assess in any meaningful way. The team noted 30% improvements on each of 12 parallel advances. Any one of those advances could be impactful, but little to no information was provided to support any of the advances. The project impact described does not contain any detail and does not seem to use any metrics for describing the many possible benefits. New ponds and bench-scale PBRs were designed and built and are reported to be working well. This seems like important progress and is well suited for the high-throughput experimental work. Transitioning from larger ponds to smaller operational units appears at first a step in the wrong direction given the company's commercialization trajectory, but this will allow rapid learning and strain development with less expense without interruption of larger-scale cultivation.

- This is a new project that is just getting underway this year. The goal is to accelerate and overcome the challenge of taking production strains from the laboratory into mass culture, by increasing productivity, quality, and robustness of strains. The team and management plan described are appropriate for this project. Progress was not described through tasks, milestones, and go/no-go decision points, providing an inadequate assessment of progress for the project. Further, discussion of potential risks and mitigation strategies would enhance the presentation of this project. The approach will incorporate 12 parallel advances in cultivation control and operation, strain improvements, respiration and nighttime losses, microbiota control, and lipid accumulation rate. From these, the top three improvements will be downselected for detailed development toward 6,000-L field trials. The team has amassed a number of technologies to help with the approach, including spectroscopic phenotyping, microplate and flask testing, laboratory PBRs, small raceways, and intermediate-sized ponds within their facility in Kona, Hawaii. The approach was described at a high level and with few details on the 12 parallel advances. The impact of the project is primarily targeting the reduction of the projected selling price of algal biofuel. The impact to the BETO mission could have been explained more thoroughly by providing the major goals for MFSP and biomass production. The development of the tools and technologies will be incorporated into the GAI technology suite for franchising and licensing. GAI is a key collaborator with 18 other organizations performing for BETO. Progress was provided on the development of PBRs, raceway systems, and spectroscopy data collection. Unfortunately, it was hard to assess how these technologies will be used within the 12 parallel advances mentioned in the approach. The presentation lacked key details for proper assessment of status, progress, and outcomes.

- This project was difficult to evaluate. Much of the presentation focused on restatements of project-specific goals and necessary commercial targets. Very little data are presented, making it difficult to render opinions regarding the project and its progress toward project goals and end-of-project milestones. No details are provided of the algae strains being used, mass balances, TEA or LCA, or how 12 parallel advances will be downscaled to just three. The totaled condition may be due to the recent startup of this project or the public nature of the proceedings and the need to protect the sensitive and commercial interests of GAI. The actual detailed project status monitoring then remains entirely resident with the DOE project team.

- This project's goal is to increase algal productivity, lipid content, and process robustness by testing 12 separate approaches in parallel and then downselecting to the most impactful for further testing. The project will move between indoor microplates and PBRs and outdoor raceways. The project started recently and has successfully developed the necessary tools and deployed the new test systems in readiness for the next project phase of testing. One of these tools, a novel compositional analysis method, provides rapid, near-real-time algal cell density and health monitoring. More background and detail on the 12 improvements to be evaluated was lacking and should have been shared in the review. The presentation of more detailed data and results at the next review is anticipated.

PI RESPONSE TO REVIEWER COMMENTS

- This project just started during COVID, so there are not any significant results to present other than the design, building, and installation of the test equipment—new ponds and bench-scale PBRs. Testing with these systems just started, so there are no results to be presented yet. Extensive data collection has been completed for development of the spectroscopic compositional analysis method, but the additional data collection, especially the compositional analysis of all samples, is in progress, so there again are no results to present this early in the project. Unfortunately, the main approach—12 novel, parallel options for improving productivity or lipid content—has not been tested yet. Since they are all novel approaches, they cannot be described in an open meeting until the efficacy is determined and an appropriate intellectual property strategy is in place.

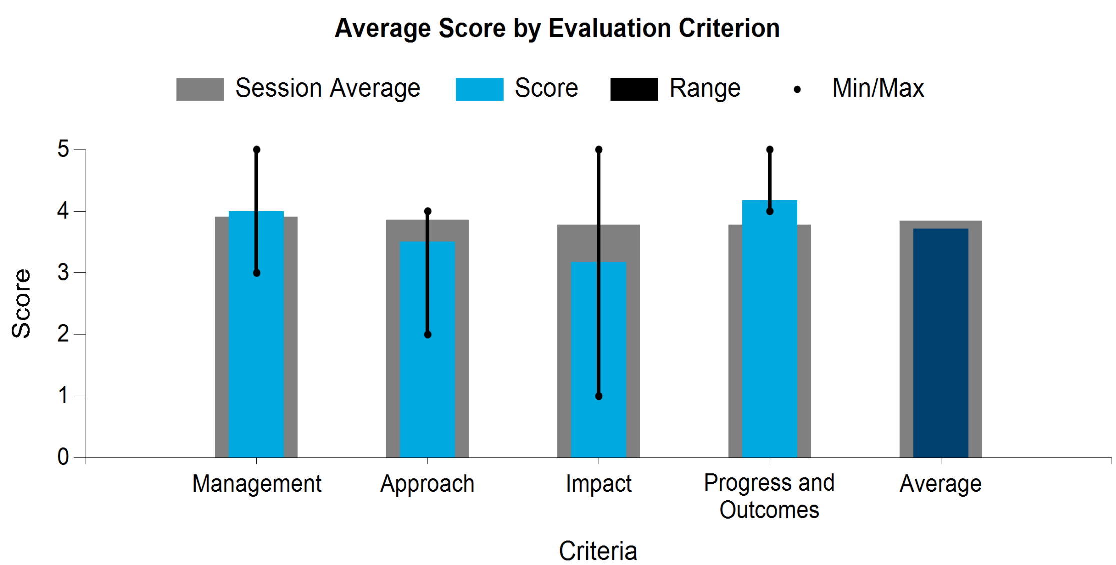
IMPROVING THE PRODUCTIVITY AND PERFORMANCE OF LARGE-SCALE INTEGRATED ALGAL SYSTEMS FOR WASTEWATER TREATMENT AND BIOFUEL PRODUCTION

University of Illinois at Urbana-Champaign

PROJECT DESCRIPTION

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

Producing biofuels from algal biomass is currently one of the most promising approaches for meeting society's need for sustainable energy because algae grows faster than other biofuel feedstocks, while using marginal land and water resources that compete less with food production. However, high biomass production costs remain a significant challenge that limits the practical use of algal biofuels. Thus, the overarching goal of this project is to develop and demonstrate an integrated system for algal biofuel production and wastewater treatment that can reduce biofuel costs below DOE's minimum fuel selling price target ($2.50/GGE). This approach is compelling because of the significant coproduct value of treated wastewater and dual-use infrastructure, and because wastewater provides a low-cost source of major algae cultivation inputs (e.g., water, nutrients). In this project, we are developing novel biological and engineering approaches to enhance the biomass productivity of a commercially available algal wastewater treatment system, called Algaewheel, and conversion of resulting mixed algal biomass to biofuels via hydrothermal liquefaction. The methods being studied to improve algae cultivation and biofuel conversion include bioaugmentation with bacteria that provide algal growth promoters, stress-induced endoreduplication to increase cell size, integration of adsorbents, dynamic control models, and nanofiltration of HTL aqueous products for improved carbon efficiency. The integration of these techniques is expected to increase areal biofuel productivity up to five times higher than the DOE techno-economic baseline at the outset of this project (900 gal/acre-yr). These improvements will be validated in bench- and pilot-scale tests, followed by demonstration in full-scale Algaewheel systems currently used for municipal wastewater treatment.



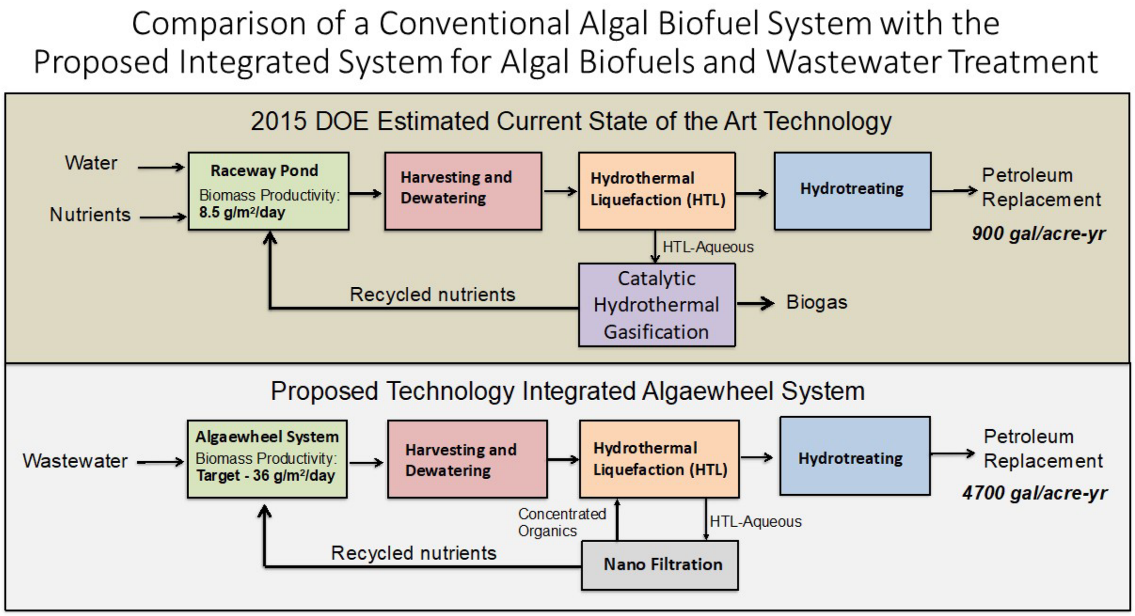


Photo courtesy of University of Illinois at Urbana-Champaign

COMMENTS

- A qualified team is assembled, and the work plan and implementation are being professionally discharged. Results are being developed and milestones completed to allow continued project progress. The technology being investigated is intriguing and likely has applications for small, remote, or difficult-to-service locations for treatment of domestic wastewaters for the benefit of public health. The small scale of these types of applications would not be able to produce any meaningful volumes of commercially viable biomass. Comparison of the measured algae production rate is 36 $g/m^2/d$ against the DOE goal of 25 $g/m^2/d$ can be misleading. The project's measurement is based on the area of the concrete structure that contains densely spaced paddlewheel units. A conventional raceway pond has only a single paddlewheel, leaving the overwhelming portion of the pond's surface area undisturbed and not in contact with mixing equipment. The type of system used by the project at a commercial scale would require the paddle wheel area as a single primary unit operation for the wastewater treatment plant to be approximately 3,470 acres (when compared to a 5,000-acre raceway pond n^{th} commercial facility). The cost and footprint of this type of system appears entirely impractical and unrealistic. The assumption of a benefitting co-location of an algae production facility as part of a wastewater treatment plant also becomes impractical, negating the envisioned availability of very low costs for ponds, CO_2, nutrients, and production costs for inputs for the TEA.

- Overall, this project seems to be off to a good start. The project management plan is clear. The project team meets monthly, which seems too infrequent; bimonthly meetings are recommended moving forward. Risks and mitigation strategies are not adequately addressed. The Algaewheel technology seems interesting, and the overall approach seems sound. OneWater, Inc. markets the Algaewheel system to the wastewater treatment industry, so the technology has commercial potential. Progress has been made in endoreduplication, pilot system startup, and biofilm development. A Gantt chart showing the process on specific tasks and milestones would have been helpful.

- The project will integrate advanced algal wastewater treatment systems to maximize biofuel production. Wastewater treatment potentially provides biomass at net-zero cost in production. The team and management plan described are appropriate for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project.

The presentation would have benefited from a discussion of risks and mitigation strategies. Although the approach for this project focuses mainly on the improvement of strains for wastewater treatment within an Algaewheel system, the team hopes to demonstrate taking the wastewater-produced biomass to biofuels through an HTL processing capability. The team's approach to enhancing biomass productivity through the inclusion of bacterial symbiotes that produce key nutrients and hormones has been shown to increase biomass productivity. The team will demo the technology in full-scale Algaewheel installations at two sites. The site discussion would benefit from better delineation of the size of the installations, type of wastewaters being treated, and effectiveness of the Algaewheel systems to produce dischargeable waters. The main impacts of the project were described based on the reduction in costs associated with wastewater treatment and utilization in algae biomass and biofuel production. The use of wastewater treatment would reduce the cost of biomass production to levels that would allow meeting DOE targets. The team also envisions publications in the peer-reviewed literature and commercialization through their current partner. The team has demonstrated endoreduplication of *C. vulgaris* under salt stress, increasing biomass productivity 50%–90%. They are currently meeting the Budget Period 2 milestone, demonstrating targeted productivities of 20 g/m^2/day, along with seasonal adjustments to 16–18 g/m^2/day. Further, 85%–90% wastewater organics and ammonia removal meet discharge limits. The project is progressing well toward goals. Demonstration of the salt stress induction within a wastewater process seems to be a key step to success that needs to be demonstrated.

- The team seems to have the right makeup of research and commercialization partners to see the project through to market potential. The team seems well equipped to manage the project over the long demonstration periods. The project approach seems well aligned with the current SOT and takes algae biomass all the way through HTL and hydrotreating processes. The integration of a novel but well-tested unit operation to replace the use of catalytic hydrothermal gasification seems like a very practical way of overcoming what has been a large technical challenge. The team has also done extensive work with recycling its aqueous stream. This work is challenging and often overlooked. Approaching this challenge directly instead of with modeling or assumptions is critical, and this team has risen to this challenge well. Looking for symbiotic organisms producing vitamins and cofactors and actively colonizing biofilms are also challenging tasks but represent practical ways to meaningfully advance the state of the art. The cultivation system optimization approaches are all very straightforward and seem promising. The approach of pairing wastewater treatment with biofuel development seems to be among the best currently under review in the portfolio and could serve as a model for future approaches to sustainable algae biofuel and bioproduct development. It is not clear how the polyploidy work relates to the rest of the project, but it seems to be yielding positive growth results, so this may be tangential but not irrelevant. The volume of water treated by this system is relatively small and this system would not scale well for large water utilities. This is, however, an impactful example of distributed wastewater treatment and dilute nutrient recovery. It may not be readily applicable to traditional urban wastewater operations but could be a very useful tool for many remote sites or agricultural point sources of nutrient runoff. Isolating eutrophication to contained reactor systems can help decouple modern agricultural practices from the environmental degradation they are often responsible for. The project team seems to have met all goals so far in the pilot system. The pilot project goals for organic and ammonia removal seem substantial and were both met. Final goals are substantial improvements from current operations, but the team seems to be making appropriate progress and should be on track to deliver despite a seeming lack of risk mitigation.

- This project aims to improve integrated performance of a wastewater-fed algae system with a mixed culture grown on the Algaewheel technology. The project is in the early stages of a 5-year project. The algal biomass generated is currently low-quality, and the reported biomass growth rates include the activated sludge biomass. The system design for successful wastewater treatment with good attachment to the wheel surface may have differing requirements versus algae harvest to support economic recovery in an integrated system. While seemingly a bigger technological leap to commercialize for algal biofuels, it will be of interest to watch this project and continue to learn and develop applied knowledge toward integrating algae with wastewater treatment. The nanofiltration unit operation was not addressed, and the integration with HTL and nutrient recycling will be an important challenge as the project progresses.

- Using wastewater to grow algae is an important avenue of research, and the project team appears committed to creating a commercially viable solution for wastewater treatment that can hopefully be coupled to production of biofuels. The project is fairly early and initial progress is acceptable. In regard to approach, it's not clear how the strain improvement experiments proposed at lab flask scale using *Chlorella* are relevant to improvements in the biomass consortia being grown in the Algaewheel system. Additionally, it would be helpful to better understand how this specific system would need to be adapted for biofuel production in order to be successful. It appears that it takes a long time to establish these biomass mats—at least 4 weeks to see colonization of a thin layer of algae. It's not clear how often the biomass mats would require reseeding, or what the impact of a single system fail (akin to a pond crash) would have on the annual average productivity of the system if restart times are so long. The specific risks that would cause the system to need to be restarted (e.g., pests, temperature, etc. for open ponds) are not clear. It's also unclear if harvesting the algae for use versus disposal will negatively affect the dynamics of the biomass mats themselves. How often the biomass (sludge) can be harvested and how that will affect downstream processes is not clear either. It's unclear how the project proposes to regulate biomass quality in this system to meet specs of downstream products. It appears that there would be extra costs associated with harvesting/handling of the biomass pre-conversion that are specific to this system; thus, it's unlikely that biofuels would be produced at conversion costs only. The interim TEAs/LCAs should carefully consider all extra personnel and harvesting/handling requirements that would be needed to adapt this system for biofuel production.

PI RESPONSE TO REVIEWER COMMENTS

- Thanks to all the reviewers for your encouragement and constructive criticism on this project. The paragraphs below provide a response to selected issues raised by the reviewers, with more of a focus on potential weaknesses, areas where some additional clarification was needed, and potential changes to the project approach. First, the issue is numbered and summarized, and then our response is provided. The sequence of issues generally follows the order of comments provided in the BETO Project Peer Review system, but some similar and related issues are grouped together at their first occurrence.

  1. Various questions related to the applicability and scalability of lab-scale polyploidy research to the Algaewheel wastewater treatment system. We agree that there is a significant amount of work to translate algal polyploidy research into practical applications with integrated systems for algal wastewater treatment and biofuel production. Even so, the stresses used to induce polyploidy in this study (salt, ultraviolet-B radiation, desiccation) were selected with consideration of their potential to be incorporated with a fixed-film algal wastewater treatment system also used for cultivation of biofuel feedstocks. For instance, with salt stress, the idea would be to drain one of the algae cultivation tanks and temporarily fill it with salt solution, then drain the salt solution and refill it with wastewater. The same salt solution would be cycled through each of the cultivation tanks gradually over time. Desiccation could be accomplished by a similar approach, except the cultivation tanks could be left empty for a period of time to cause partial drying of the algal biofilms before refilling. Ultraviolet-B lights could be attached to a traveling bridge crane and passed over the top of the algae cultivation tanks. All of these approaches require that the polyploidy-inducing stress can have its effect with intermittent and relatively short-term exposure, otherwise it will interfere with the wastewater treatment function. Most wastewater plants go through diurnal fluctuations in flow, and there are certain periods, such as nighttime, when the influent flow is low and wastewater tanks could be temporarily taken out of service for stress treatments without negatively affecting the average system capacity. Another level of translation is related to differences between the algae species used for bench-scale polyploidy research and the mixture of species in an algal wastewater system. The research will include some genera that are commonly found in algal wastewater treatment systems (e.g., *Chlorella*). We will look for advantageous polyploidy effects that occur with a variety of algae species to identify those that are most generally effective.

Finally, we can test polyploidy stress treatments with samples of the mixed algal culture harvested from the Algaewheel pilot system to assess the potential of these approaches to increase biomass productivity in the real system.

2. Various questions and comments related to cost-effective scalability of Algaewheel to large wastewater applications and to make meaningful quantities of algal biomass. The Algaewheel system is currently used in small wastewater plants, where it is competitive with other applicable technologies on a capital cost basis, and it generally has lower operating costs due to lower aeration requirements. Algaewheel would be too expensive if only used for algae biomass cultivation for biofuels, but the additional coproduct value for wastewater treatment makes it cost-effective. Algaewheel as currently designed does require more land than most other wastewater processes that use deeper tanks, and this is an important consideration that would currently limit the use of Algaewheel in many large wastewater plants. However, there are Algaewheel designs for upcoming projects with larger-diameter wheels that reduce land requirements and would make the system more cost-competitive for larger wastewater plants. Also note that the current Algaewheel wastewater plants do not use their biomass for biofuels. Thus, future applications that can garner additional revenue from biofuels will also help to grow the number and size of wastewater plants that can implement Algaewheel cost-effectively. We do not currently know the limits of wastewater plant size where Algaewheel can be cost-effective, but we will evaluate this parameter as a part of the TEA work for this project, as well as the resulting amount of algal biomass/biofuels that can be grown. The largest wastewater plants in the United States occupy hundreds of acres each and could support a significant amount of algae biomass cultivation. Also note that an algal wastewater treatment system like Algaewheel can serve as the first step in a larger algae growing operation. Since HTL conversion of biomass to biofuels can be configured to recapture most of the nutrients from the processed algae, those nutrients can be used again for growing additional rounds of algal biomass. In this case, biomass grown in the first-stage algae wastewater system will generally have very low costs due to the dual-use infrastructure paid for by wastewater treatment operations. Subsequent rounds of algae growth can then benefit from "free" recycled and sterilized nutrients derived from wastewater, but they will not receive the additional revenue associated with wastewater treatment. Thus, a lower-cost cultivation system, such as a raceway pond, will be needed for multicycle algae cultivation after an algal wastewater system.

3. Various questions related to the cost and practicality of frequent biomass harvesting and preprocessing of Algaewheel biomass for biofuels production. The time of several weeks to initiate growth of biomass in an Algaewheel system is well within the normal time to start up wastewater treatment systems and would not be a practical limitation. Once colonized, these systems continue to produce biomass continuously through routine sloughing of biofilms that happens naturally and has proven to be reliable over multiple years of operations without any need for restarting or reseeding. In current Algaewheel systems, the quality of biomass is variable and relatively high in ash content, and this is something we are working to improve in this project. Fortunately, the HTL conversion process we have selected is quite robust and can be used to produce bio-crude oil from a wide variety of biomass sources and quality. We agree that TEAs/LCAs should carefully consider all extra personnel and harvesting/handling requirements that would be needed to adapt this system for biofuel production. We would point out most wastewater plants dewater biosolids in their sludges to above 20% solids content for final disposal in land application of landfill, which is consistent with feedstock requirements needed for HTL conversion to biofuels.

4. Various comments and questions related to project risk management. Risk management was not included in the peer review presentation materials, which was sacrificed in favor of other information deemed to be more essential while still complying with the time constraints for presentation. The bulleted items below outline our current risk mitigation plan for the project, which will be updated in accordance with the peer review comments and include any previously unforeseen risks that are now recognized based on the early experience of the project.

A. Risk: Cost and longevity of the Algaewheel system and adsorbents.

   Risk Probability: Low.

   Risk Impact: Medium.

   Risk Mitigation Strategies: Pilot-scale operation showed Algaewheel system is durable over 3 years. Associate maintenance/replacing cost has been considered in economic analysis. Past study showed adsorbent can last more than a year. Regenerating adsorbent instead of replacing adsorbent to save cost.

B. Risk: HTL aqueous recycling cause toxicity to algae.

   Risk Probability: Low.

   Risk Impact: Medium.

   Risk Mitigation Strategies: The amount of post hydrothermal wastewater recycled would be significantly diluted by the influent. Provide adsorbents in the algae cultivation process to sequester problem contaminants. Activated carbon pretreatment if needed.

C. Risk: Bioaugmentation might compete with algae growth.

   Risk Probability: Low.

   Risk Impact: Medium.

   Risk Mitigation Strategies: Augmented bacteria will first be screened by the bench-scale test to confirm the symbiotic relationship.

D. Risk: Bioaugmentation at pilot scale might have different impacts at bench scale because of influent variability.

   Risk Probability: Medium.

   Risk Impact: Low.

   Risk Mitigation Strategies: Adjust bioaugmentation dosing frequency.

E. Risk: Pilot system results cannot translate to full-scale system implementation.

   Risk Probability: Medium.

   Risk Impact: Medium.

   Risk Mitigation Strategies: Implement technologies in sequence to evaluate the effect of individual technology.

F. Risk: Availability of key personnel.

   Risk Probability: Low.

   Risk Impact: Medium.

   Risk Mitigation Strategies: Previous discussions with team has worked together and has needed availability for this project. Routine communication about staff needs. Identify alternate staff to provide needed expertise.

G. Risk: Inability to obtain approval for field testing.

Risk Probability: Low.

Risk Impact: Medium.

Risk Mitigation Strategies: Maintain communication with sanitary sewer utility collaborators. Scheduled late in project to allow time for planning and approval. Perform a similar test under laboratory or pilot conditions.

DECISION-MODEL-SUPPORTED ALGAL CULTIVATION PROCESS ENHANCEMENT

Arizona State University

PROJECT DESCRIPTION

WBS:	1.3.5.287
Presenter(s):	John McGowen
Project Start Date:	10/01/2019
Planned Project End Date:	12/31/2022
Total DOE Funding:	$4,375,000

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

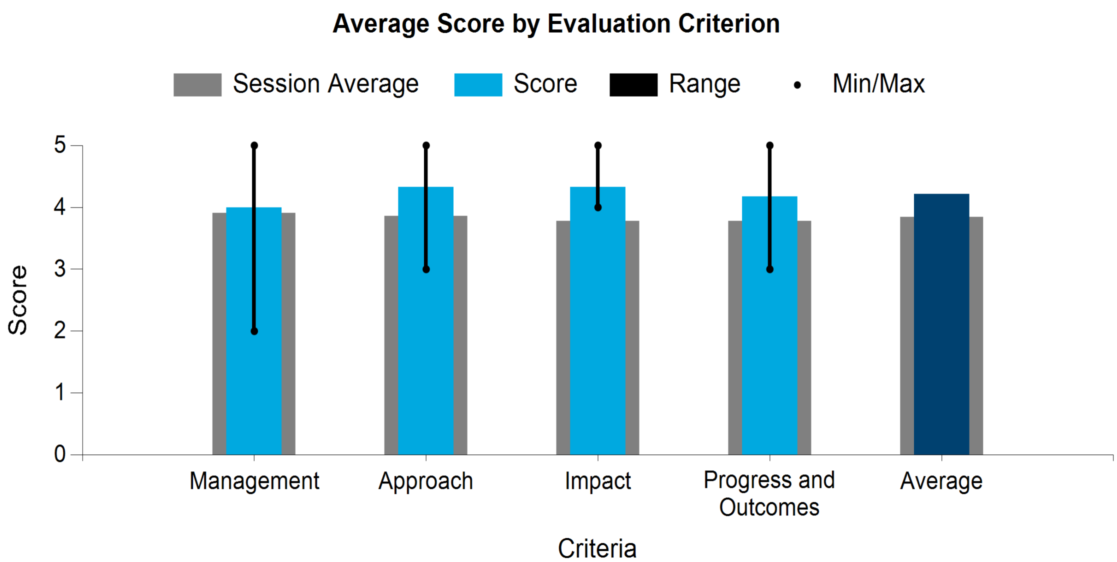


Photo courtesy of Arizona State University

COMMENTS

- The project aims to generate empirically derived culture-failure risk data for TEA/LCA modeling and quantitation of risks associated with culture failure. The team and management plan described are appropriate for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. The presentation would have benefited from a discussion of risks and mitigation strategies. The approach described to achieve the aims of this project is deemed quite reasonable and likely to achieve stated goals. The approach incorporates assessment of failure risks in cultures and quantifying impacts, optimization of cultivation performance, development of real-time monitoring technologies, and sustainability assessment. The impacts of the project were projected based on the technologies and modeling capabilities being developed, with the hope that an integrated and realistic assessment of risks will help future cultivars achieve the BETO productivity and economic cost targets. This effort will be critically affected by the generation of multi-scale and multi-parametric data that will challenge modeling efforts, a risk that could limit the impact of the project. Progress in this early effort was described in the development of a dynamic growth model that incorporates the inoculum seed train. Other efforts were described as progress to be achieved by task, some of which are well underway. The integration and demonstration of the MiProbe system to monitor water/culture quality and nutrients on a real-time basis constitutes a very innovative technological development for this project. A number of key milestones in data integration and automation have been achieved. The assessment of this data will be critical in showing how it can feed into modeling efforts and provide predictable outcomes. If these efforts can be appropriately tied together, they can have excellent ramifications in cultivar management.

- It appears that the project may be diverging away from its original project goal and end-of-project milestone. The summaries of progress that are presented seem to reflect the use of some different methods and techniques to achieve results. If so, these types of benefitting adjustments are suitable when they are made in efforts to improve the project and better reflect BETO's goals. It is important that the concept of crop insurance begins to be explored and addressed. The basic steps of assembling monitoring and decision data, the methods of its collection, and the application of predictive and probabilistic techniques is a good place to start. No doubt there will be many opportunities in the future to refine and adjust these original efforts as better technologies and more and better data become available, hopefully to a point where insurance professionals can begin to be involved. The materials presented seem well thought out and well planned. Given the recent project start, it is not really possible to gauge progress, but the trajectory looks promising.

- Overall, this project seems to be going well. The management plan is not clear. How often group members communicate is unclear. Risks and mitigation strategies are also not adequately addressed. The technical approach involving studying culture failure risks, lab to field to lab cultivation performance, process modeling, and sustainability assessments has merit and has the potential to advance the state of the art. Impacts are clear and outcomes should aid commercialization. Progress is being made in all four project tasks and the project seems to be on schedule.

- The management team seems well aware of the risks presented in the project plan and is actually driving research to quantify and mitigate some of the most substantial risks to large-scale cultivation. The work seems to be motivated by sound project management of an integrated system and the team appears well positioned to understand this system and disseminate results to the broader ecosystem. Crop failure and contamination still pose a large risk for scale-up and adoption of algal technology. This project approaches this challenge in a very practical way, trying to overcome the problem without developing new unit operations or strains, by integrating existing technology in a new process paradigm. This approach seems smart and readily translatable to the field without new inventions. Transitioning operations for batch to a semicontinuous mode would achieve many engineering and downstream efficiencies that could dramatically enhance algae cultivation at large scales. Developing robust heuristics for culture crashes and contamination requires large data sets. If or when work done by Sandia National Laboratories' team on crash ponds is integrated with this outdoor data set, it should improve the quality of predictions and make for more versatile system models. TEA outputs will be a critical measure of any contamination control strategy. Use of the NREL TEA or compatibility with it allows for the most direct comparisons of innovations made in this project to the SOT. The project addresses one of the least-well-understood risks to algae biofuel development in a way that can be a foundation for iterative improvement. Direct comparisons of different cultivation strategies are rare, and TEA outputs are most useful for comparing different strategies as opposed to determining real-world value of investments in algae cultivation. These new inputs will strengthen the TEA as a comparative tool and help highlight other work like this that can be done to reduce risk without creating new inventions. The impact of this work is not a step change advance but a very critical incremental step in the right direction for this industry. The introduction of another crop protection tool is always useful and welcome, but this work does seem tangential to the principal focus of the project. Novel sensor and inline analysis tools will need to become significantly cheaper and more robust before they will be practical components of continuously operated systems. This project makes steps toward that integration, but this work is far from complete. Open-source predictive software has the potential to be a valuable component of any large process plan, and this work takes preliminary steps to creating a robust and translatable platform. Adoption of this technology seems far from certain, and impact will be limited by consumer use. The project seems on track to meet deliverables.

- This project has multiple components that are working together to advance BETO's MYPP goals. The focus on modeling culture crashes with empirical data and assessing the risk of failure and its impact on TEA/LCA is critically important but often overlooked. These results will fill a current gap in the knowledge base that will support decisions for successful algae operations at scale. Additionally, the team is developing a continuous monitoring system based on novel sensors that will support real-time process decisions. The work and research being done around the use of biocides, pesticides, fungicides, and general pest management will be highly useful and applicable program wide. The project has made appropriate progress toward its end-of-project goals.

- This project is developing advanced cultivation methods to improve cultivation throughput, including evaluating seed strategies, pond crash modeling, integrated pest management, and development of novel sensor platforms. The project just started and is in its first year. The new sensor platforms have real potential to be impactful in managing algae farms as long as they can be proven to work reliably, and the costs and maintenance requirements do not preclude their use in a commercial setting.

PI RESPONSE TO REVIEWER COMMENTS

- Thank you for your comments. Our overall project task structure, milestones, and end-of-project goal remain as agreed upon with BETO. We are continuing to explore the regulatory space for use of different agents for crop protection, and a slide discussing this task is in the supplemental slides. Our project team has participated in two public panels—one on crop insurance as part of the Algae Biomass Summit in fall 2020, and a BETO-sponsored workshop on crop protection in spring 2021. Our project team plans to engage appropriate stakeholders and share our data on risk and cultivation failure modeling broadly, and we believe it will be a key, foundational data framework for algae crop insurance. Slide 6 of the presentation outlined the team roles and responsibilities and our communication plan, including biweekly task meetings, monthly PI meetings, and quarterly team meetings and reporting to DOE. The PI reviews risks with the team monthly and as part of the quarterly reporting, and mitigation actions are assessed and implemented as needed and draws on our team's near-decade-long experience with managing large collaborative teams on BETO-sponsored research. We have over a decade of operating sensors in the field and assessing the current state of the art—not only in terms of data generated, but also, as importantly, the cost and reliability of the platforms. The ability to deploy reliable and cost-effective sensor technology is a key metric for our project and a main driver for the choice of sensor platforms being developed. This project has multiple components that are working together to advance BETO's MYPP goals. The focus on modeling culture crashes with empirical data and assessing the risk of failure and its impact on TEA/LCA is critically important but often overlooked. These results will fill a current gap in the knowledge base that will support decisions for successful algae operations at scale. Additionally, the team is developing a continuous monitoring system based on novel sensors that will support real-time process decisions. The work and research being done around the use of biocides, pesticides, fungicides, and general pest management will be highly useful and applicable program wide. The project has made appropriate progress toward its end-of-project goals.

- Relative to the comment about integration with other projects within BETO, in particular the Sandia crash pond work and NREL TEA modeling, our project partners for this project—in particular AzCATI and Los Alamos National Laboratory—are also partners with both Sandia and NREL on DISCOVR, and thus are intimately aware and engaged with the broader BETO portfolio, in many cases directly, as we are project partners. We are using the NREL Farm Model as a key baseline reference for our own modeling work. Our sensor deployment for the MiProbe, currently on a dozen ponds and expanding to 24 ponds, including into our seed train in 2021, allows for the opportunity to look at many more cultivars and cultivation runs than are specific to this project, allowing for significant acceleration of research findings and development of the platform. While we'll have fewer physical deployments of the QBI sensor system, this will also leverage other cultivation trials ongoing at AzCATI, allowing for observation 24/7, 365 day per year, again accelerating the needed R&D on both sensor platforms. Arizona State University/AzCATI believe in open collaboration across our project portfolio, and we are sharing data, samples, pest models, etc. very broadly within the BETO stakeholder community, and specifically with our DISCOVR partners.

MARINE ALGAE INDUSTRIALIZATION CONSORTIUM (MAGIC): COMBINING BIOFUELS AND HIGH-VALUE BIOPRODUCTS TO MEET THE RENEWABLE FUEL STANDARD

Duke University

PROJECT DESCRIPTION

WBS:	1.3.5.310
Presenter(s):	Zachary Johnson
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2021
Total DOE Funding:	$6,552,939

Algae-based biofuels have been shown to be technically feasible, but widespread adoption is currently hindered by prohibitively high costs. Here we report progress on demonstrating, using a multiproduct commercialization path, an algal biofuel at a commercially relevant scale with a positive energy return that achieves the Renewable Fuel Standard with improved economics. Our approach is founded on our achievements to date in algal biofuel and coproduct development and is aimed at increasing overall algae product value (and thereby decreasing the net cost of algae biofuel). This work is primarily driven by product specifications in three markets, including (1) drop-in fuels—the foundational product, (2) an aquafeed (fish) feed ingredient equivalent to fishmeal in protein content and biochemically superior to soy and other protein meals, and (3) a poultry feed ingredient that is superior to commonly used soymeal in protein content and contains other important micronutrients. Here we show successful production of biofuel and utilization of biomass residuals (lipid-extracted algae) as an ingredient for multiple feeds. Algae coproduct valuations are based on direct replacements (e.g., lipid-extracted algae for fishmeal at $1,500/tonne), enhanced performance (e.g., improved animal health), or enhanced end product value (e.g., omega-3-containing poultry or vegan feed animals). These valuations improve the overall value of algae biomass and help lower the cost of algae-based biofuel.

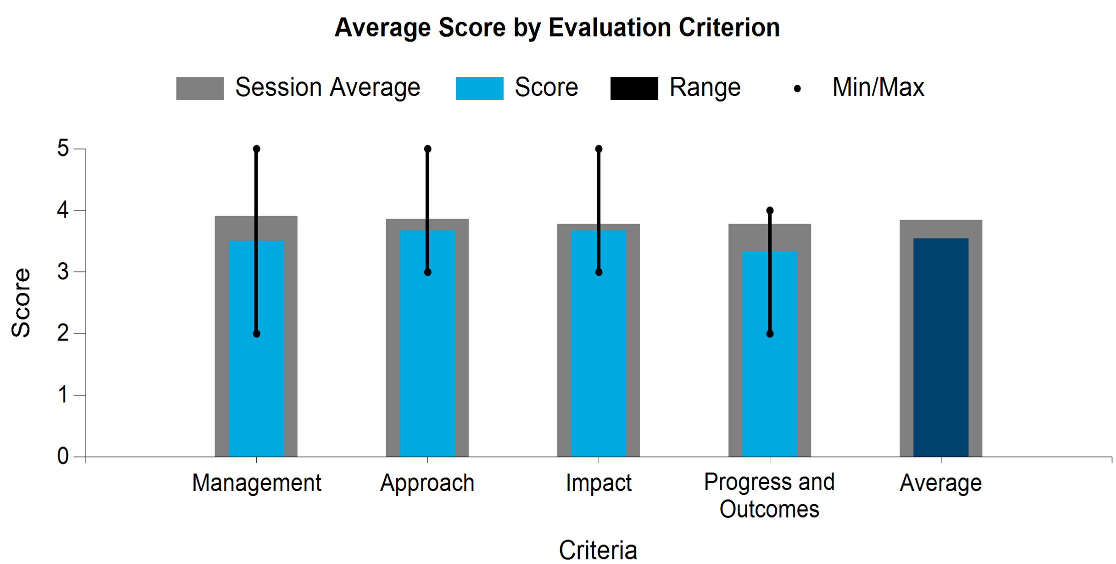


Photo courtesy of Duke University

COMMENTS

- The project management plan is vague. No specific information appears to be provided for how often the team members communicate. The risks and mitigation strategies of the project are not clearly identified. The approach seems to focus mainly on total algae produced by mass instead of focusing on algae productivity. The project produced an impressive number of publications (35), but it is not clear how much of an impact this work will have on industrial efforts. The project team claims to provide a clear pathway to economically competitive, sustainable biofuels at scale, but this pathway is not clear. Progress was made in several areas in this work, and coproducts were tested on boiler feed chicken and salmon.

- This is a large consortium project dedicated to demonstrating and validating high-value coproducts, mainly through animal feed studies. The main impact of such projects to BETO are in the assessment of possible coproducts that when produced with biofuels will increase the value of the biomass produced and hopefully decrease the cost of biofuel intermediates, making the whole process more viable financially and helping meet the MYPP goals. The team and management plan described are appropriate for this project. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. Discussion of potential risks and mitigation strategies would enhance the presentation of this project. As this is a large consortium project, the approach is holistic, incorporating strain development toward mass culture, recovery and conversion by two pathways, product demonstrations, and TEA/LCA. The approaches described are deemed viable with no inherent weaknesses. Progress was described against tasks and milestones, showing that the project is on track and has been delivering on the major efforts envisioned. The poultry and salmon feed trials performed by this team are quite relevant to the overall program success. The team is progressing very well on their assessments of the impacts of whole and lipid-extracted algae to animal and aquaculture feed. Some issues were encountered with their membrane extraction system, which are now being appropriately addressed with solvent extraction systems. The effort has been very prolific, resulting in over 35 publications in the peer literature.

- This large consortium project is nearing completion of a multiyear effort. The main focus of this project was to identify and develop high-value coproducts from the residuals after algal oil extraction to drive down the overall cost of biofuel production. The algal oil extraction methods were understood to be limited by equipment specifications not matching changing requirements (e.g., changing from freshwater to saline algal species or operating the membrane system without solvent). It is difficult to draw any conclusions as to the commercial viability of these operations as a result, and it was acknowledged that this extraction step could be optimized more on future projects. The project has had multiple successes in generating fuel products and demonstrating benefits of algal products as chicken and fish feed. The final TEA/LCA report will be of high value in understanding the impacts of these results.

- This long-running, high-cost consortium effort appears highly commercially viable because of the participation of Shell. Learning that Shell appears to have a very passive role in the management of this project and has not communicated clear specification criteria for adoption of algae fuels underscores the tremendous risk that this work will not be integrated into any commercial development despite hard work and progress. The approach of valorizing all algal biomass components and doing in-depth research on the production of each of these streams seems like a very good approach. The plans to integrate components of this project seem unclear. The use of clear benchmarking and ranking based on a small set of quantifiable metrics was a sound approach to strain development. The impact of the feed studies done by this team is especially meritorious and impactful. This work sets the stage for scalable commercial development of algae as a feed source. Given the long duration of this project, it is disappointing that the commercial partner in this project seems to have little to no interest in commercializing processed algae fuels or using the information learned in these years in any way. Descriptions of budget shortfalls resulting in unmet deliverables may indicate missed risks and risk mitigation strategies early in the effort. It appears that most of the novel or untested downstream processing components did not function quite as planned and mitigation has not been instituted. It is not clear how the fully integrated system was demonstrated.

- This longstanding project continues to be well managed and has transitioned both staff and team members during the years. The efforts have been adjusted and realigned as best as possible with BETO MYPP goals as they have evolved and been refined. A risk roster was not presented, but some examples of risks that were managed during the course of the project were discussed. The work efforts were consistent with the abstract, project goals, and end-of-project milestone. The delay in large-scale extractions and some of the lower-than-desired extraction rates from other scales and processes likely contribute to an implied project outcome that further emphasizes the need for revenue from products other than fuels. Important gaps remain—particularly larger-scale water reuse and the use of currently best available strains.

- This project seeks to reduce the cost of algal biofuels through coproduct value. The focus of the work appears to be heavily skewed toward the algae for feed component of the project, but the results of feed trials are very promising. Small-scale fuel product from oil hydroprocessing was generated as a proof of concept, but large-scale extraction and conversion testing are needed to show feasibility of the integrated model. It would have been helpful to see a timeline for milestones for this complex project, as it was difficult to understand from the presentation what tasks were completed before the previous peer review versus what new data have been generated during the current review cycle. A remarkable number of publications have come out of the work.

PI RESPONSE TO REVIEWER COMMENTS

- It is true that the extraction components did not function as planned: (1) Valicor/Trucent was able to conduct hexane extraction on one batch before leaving the project, and (2) the Texas oil extraction method was altered due to budget reductions during initial contract negotiations and the altered system did not yield sufficient extraction. However, this risk has been mitigated by establishing a new partner with MATRIC, who is very eager to get started conducting oil extraction. To date, the integrated system has been demonstrated for one strain: *Desmodesmus* sp. C046. This strain was grown at Duke (slide 8) and extracted by Trucent (slide 11), the biocrude was hydrotreated by Emerging Fuels Technologies into diesel blendstocks (slide 12), the LEA was used for feed trials in boilers (Cornell, slide 14) and salmon (Nord, slide 16), and the results of the feed trials were used as the basis of a TEA/LCA (slide 19). This is a complete demonstration of system integration. Additional demonstration of the fully integrated system for other strains is underway. Large-scale projects with integration across multiple partners have inherent risk. We have worked with Consortium members and DOE to adjust course, within time/budgetary constraints, to maximize outcomes. Our group has demonstrated a fully integrated system for one strain. We agree that there are other areas outside of the scope of this project that should be pursued. As the reviewers point out, these areas include further scale trials, water reuse, and the use of currently best available strains. We agree that larger-scale demonstration of oil extraction/testing would be desirable, but this was beyond the scope of our size of project.

Comment 4: Thank you.

Comment 5: Thank you. The management plan was presented on slide 3; however, we acknowledge that there have been changes over the lifetime of the project. This was primary due to changing consortium membership and the passing of the co-PI. The entire team communicates on a monthly basis with more frequent meetings among subgroups. Additionally, project management software and databases allow for asynchronous interactions among members, and of course, we have other discussions via email and telephone. As shown on slide 19, coproduct valuations over $1,500/t enable biocrude selling prices less the $5/GGE, which was the target of this funding opportunity and approaches economically competitive operation.

ALGAE CULTIVATION FROM FLUE GAS WITH HIGH CO_2 UTILIZATION EFFICIENCY

Global Algae Innovations

PROJECT DESCRIPTION

WBS:	1.3.5.610
Presenter(s):	David Hazlebeck
Project Start Date:	10/01/2018
Planned Project End Date:	12/31/2021
Total DOE Funding:	$3,125,000

The objective of this project is to obtain high CO_2 utilization in algae cultivation by increasing the capture efficiency from flue gas and increasing overall utilization efficiency and productivity in the cultivation system, as well as to build on GAI's many advances and breakthroughs in prior DOE projects to integrate and test all of the components for the full process from flue gas through biofuel intermediate product. The project includes two budget periods, a technology development phase, and then a 1-year integrated test. The technology development phase is complete. The goals for the technology development were exceeded with 98% capture efficiency in a single-pass absorber on simulated coal flue gas. In cultivation on this flue gas CO_2, over 100% carbon utilization efficiency was attained. In processing, the energy use for drying and extraction was reduced to 0.36 MJ/kg, and a much cleaner oil was extracted than can be obtained with conventional solvent extraction. Because greater than 100% carbon efficiency was observed with the improved cultivation conditions, some CO_2 was being absorbed directly from the atmosphere. Therefore, the cultivation system was further adjusted, and supply of all CO_2 from direct air capture into the raceways was achieved at a cost of $8/ton of CO_2. These technologies will be integrated in a 1-year test to measure the key performance parameters and robustness of the integrated process.

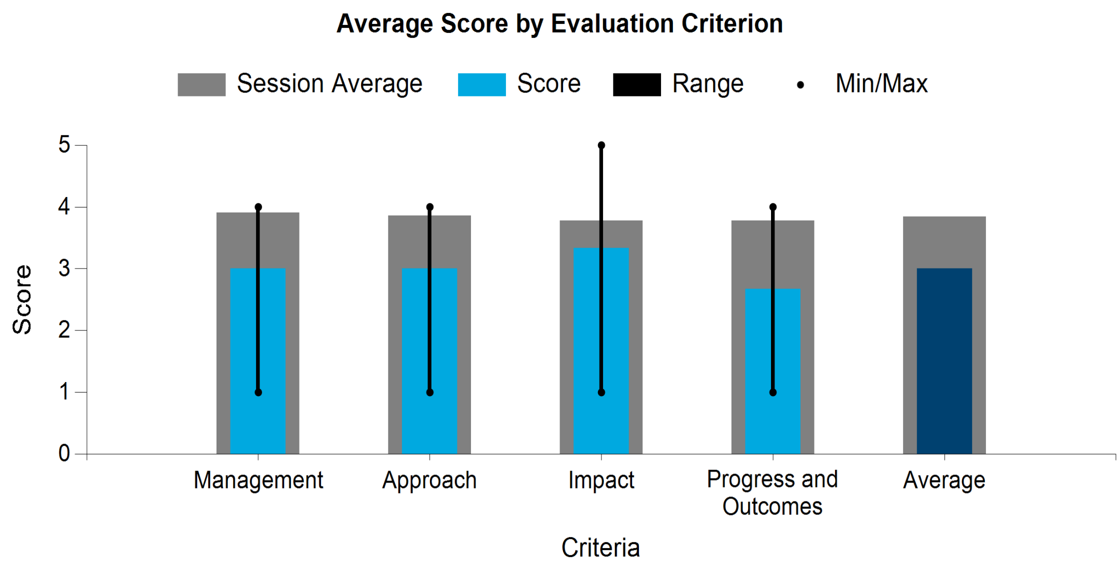


Photo courtesy of Global Algae Innovations

COMMENTS

- Overall, this project has been successful. Almost all of the project milestones have been achieved and their impact will likely be significant. The data management for this project seems good. GAI meets with the project team weekly and has ongoing projects with 18 other organizations. There is mention of a formal risk management table, but no details were discussed in the presentation. The approach has the potential to advance the state of the art and is well laid out in the block flow diagram. Impact of the project seems significant. Cost reductions for flue gas capture and direct air capture are significant and should aid commercialization efforts. Overall, this project seems to have completed all of the project goals except one, which is impressive. The presentation focused mainly on the big picture, whereas more time should have been devoted to presenting data related to the project.

- The goal of the project is to obtain high CO_2 utilization in algae through increased capture efficiency from flue gas and increased utilization efficiency and productivity in the cultivation system. The team and management plan described are appropriate for this project. Progress was not described through tasks, milestones, and go/no go decision points, providing an inadequate assessment of progress for the project. Further, discussion of potential risks and mitigation strategies would enhance the presentation of this project. The approach incorporates carbon capture and utilization and processing but moved to direct air capture from its initial efforts with flue gas, presumably due to the efficiency of the air capture system developed under the project. The technology incorporates direct air CO_2 capture into a carbonate-laden aqueous solution, where it becomes available to algae in the form of bicarbonate. The use of algae species able to grow efficiently in the high-alkalinity solutions is a key component of the technology. The goal is to show large-scale demonstration of the technology and cultivation within this final year of funding. The approach was described at a high level and with few details. The impact of increased carbon capture and utilization efficiencies is materialized through projected cost of CO_2 delivery at $8/ton, associated with the direct air capture system developed under this project. This represents over a

20-fold decrease in the cost of supplying CO_2, which is huge. Carbon capture efficiency and utilization gains claimed provide a large impact for the project, and the technology is viewed quite favorably. Unfortunately, it was hard to assess how these excellent results were met due to the lack of details.

- The goal of this project was to increase flue gas capture efficiency and cultivation carbon dioxide utilization efficiency. The process decouples the flue gas from the cultivation operations by using a carbon dioxide absorber coupled to media ponds. The project has shifted to a direct air capture approach based on managing the pond chemistry, which works for certain algal strains. The project is operating at an 8-acre scale and entering a final phase of an integrated 1-year operation. While it is understood that aspects of the technology are considered proprietary, insufficient process data or supporting project details were shared to support an evaluation of the project approach, impact, or progress and outcomes of this investment toward furthering the commercialization of algal production.

- The small GAI team seems well poised to act quickly and communicate well. The 18 partner organizations mentioned are associated with other projects, are not named, and the nature of their collaboration is not described. The description of the technical approach used in this project is too general and vague to assess in any meaningful way. The team describes outstanding advances in carbon capture and utilization efficiency, as well as improvements in drying and oil quality; however, little to no data or details were provided to support these advances. Consequently, the lack of detail makes it very difficult to assess the true impact of this technology relative to the industry or BETO goals. The team plans to receive eight or more patents for work done in this project, but none of those inventions are described. All milestones are reported to be met or exceeded, but data or information to support this success is lacking. No technical detail is given about how any milestones are achieved or what technologies have been developed.

- This is one of the few projects operating as a test-scale algae farm, and GAI is attempting to scale up to a 160-acre site. This is an important contribution to gathering much-needed real-world data outside of the academic algae test bed sites that are available. The project lead made the claim that this project increased carbon capture and utilization efficiency to >90% through improvements to the CO_2 absorber unit, which resulted in a projected cost of $25/ton. Furthermore, the project lead claimed that direct air capture technology was shown to achieve the same productivity as flue-gas-supplied CO_2 and resulted in a projected cost of $8/ton. Similar claims were made regarding reducing energy used for drying by 97% and extraction by 91%. Unfortunately, the project lead did not present any data to substantiate these claims, making it very difficult for reviewers to assess their validity or impact. This was a missed opportunity for the project team. The limited data that were presented showing productivity of the two carbon capture and utilization strategies was confusing, in that it appears the culture/strain used is very volatile, showing wide swings in productivity (10–30 g/m^2/day) and some suggestions of pond crashes over very short periods of time (weeks). From this graph, it also seems plausible that these cultures were carbon-limited for both processes, which would account for the lack of difference observed between the two cultivations strategies. It is not clear from the presentation if the team has adequate support for algal biology in their management structure or their scope of work. There was no mention of current interim average annual productivities that have been reached, or if the team is on track to meet the end-of-project target of 20 g/m^2/day.

- This project was difficult to evaluate. Much of the presentation reflected philosophical opinions regarding algae's immense possibilities and the restatement of project specific goals and necessary commercial targets. Very little data are presented in support of the results and conclusions being presented, making it difficult to render opinions regarding the project and its progress toward project goals and end-of-project milestone. No mention is made of the algae strains used, mass balances, TEA, or LCA. The totaled condition may be due to the public nature of the proceedings and the need to protect the sensitive and commercial interests of GAI, and the actual detailed project status monitoring remains entirely resident with the DOE project monitors.

PI RESPONSE TO REVIEWER COMMENTS

- The project has gone through a go/no-go review in which the DOE verification team confirmed the results presented. This was a multi-hour review, so repeating it in the few minutes allotted during the Peer Review for technical progress would be futile, which is why summary-level data and results were presented. The actual data values for each milestone achieved were presented (e.g., 98% carbon capture efficiency with simulated coal flue gas, over 100% utilization efficiency, 0.20-MJ/kg energy use for drying, and 0.16-MJ/kg energy use for extraction). The daily productivity for a 38-day test with direct air capture was presented, and the test substantiated the claim of high productivity with direct air capture. The test showed that an average of over 23 $g/m^2/d$ was achieved with direct air capture, and this was the same productivity as achieved for a raceway with externally supplied CO_2. There were no pond crashes, but since this is actual outdoor data, including days with very high rainfall, the productivity does vary over a broad range. Importantly, the variation was consistent between the raceway with externally supplied CO_2 and the raceway operating with direct air capture. The extraction and drying unit operations are the subject of multiple invention disclosures, which are being converted to patents, so of course we cannot describe the approach in an open meeting. Overall, the results presented are extremely positive for the development of algae for biofuels and commodities, especially high productivity with direct air capture, low-energy drying, and low-energy extraction.

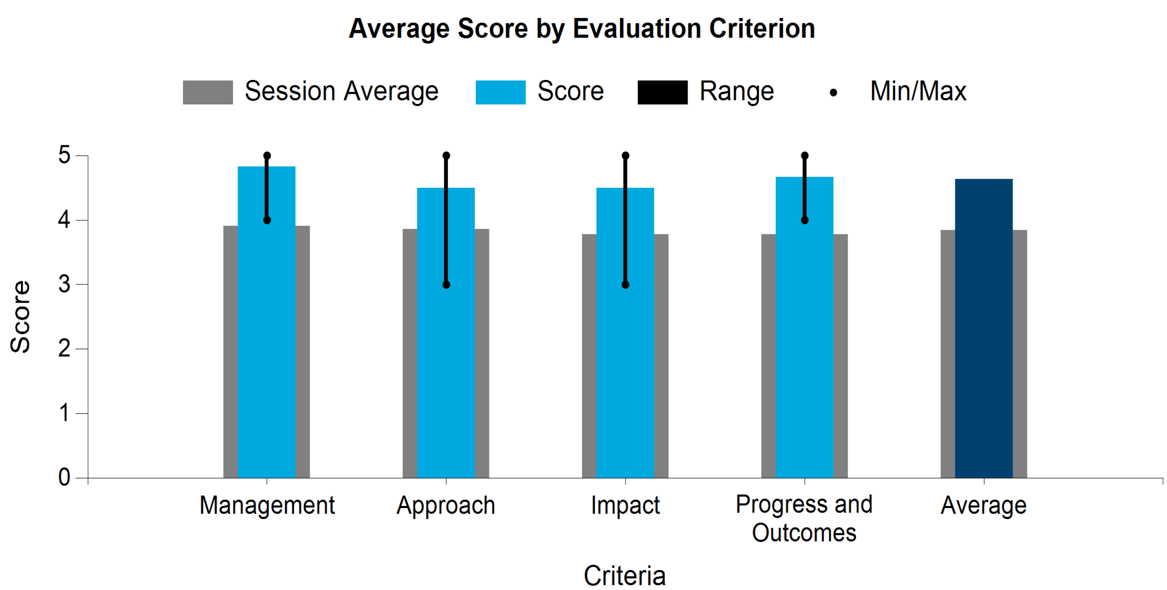
DISCOVR

DISCOVR Consortium

PROJECT DESCRIPTION

WBS:	DISCOVR
Presenter(s):	Michael Huesemann
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2022
Total DOE Funding:	$1,799,127

To meet the 2030 annual productivity target (25 $g/m^2/day$), the DISCOVR consortium—a collaboration between PNNL, Los Alamos National Laboratory, NREL, Sandia National Laboratories, and the algae test bed at Arizona State University—was tasked with identifying and testing new high-productivity algae strains and finding ways to further improve biomass productivity during cultivation, enhance biomass value, and increase crop protection and culture stability. Major accomplishments include successful execution of the DISCOVR strain downselection pipeline, culminating with the top seven strains deployed in outdoor growth trials at AzCATI. These were selected from a comparative study of temperature and salinity tolerance of 42 strains, followed by productivity assessment of 24 strains under climate-simulated conditions, leading to 13 strains cultivated in outdoor ponds. Addition of growth promoting molecules, reducing oxygen inhibition, and luminostat control each increased productivity by >20% relative to baseline productivity. Targeted compositional shifts in response to nutrient depletion resulted in >20% increase in value productivity (in $\$/m^2/day$). New methods were developed to predict and prevent pond crashes using spectroradiometric monitoring, machine learning, and integrated pest management. A concerted effort in year-round cultivation trials led to a 57% increase in annual biomass productivity in 2 years (11.7 to 18.4 $g/m^2/day$), equivalent to a reduction in biomass selling price of 27%, from $824/ton to $603/ton.



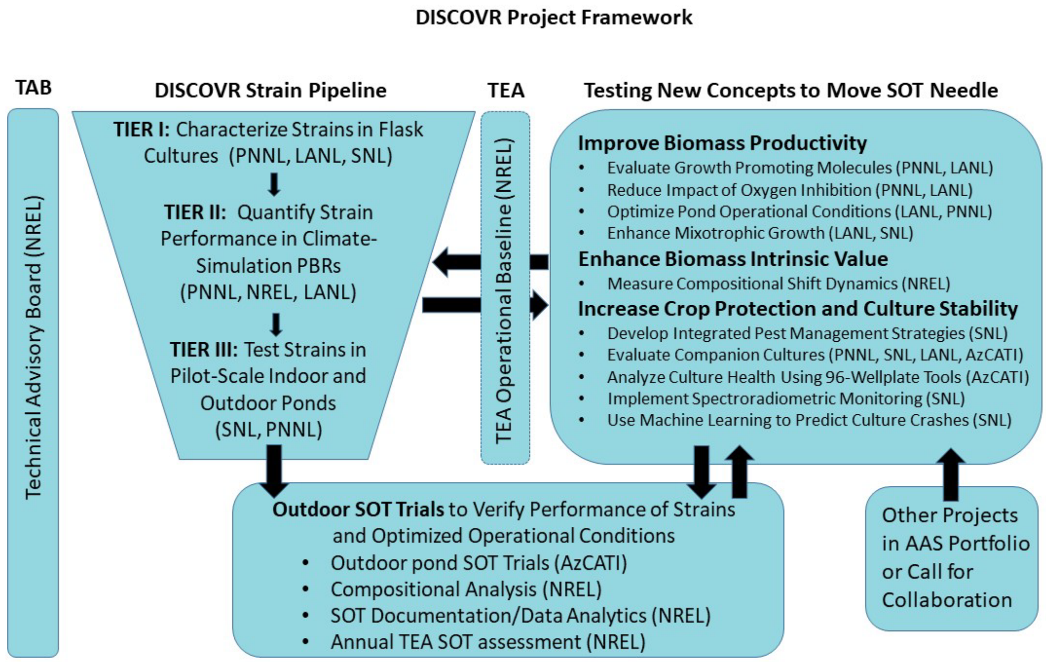


Photo courtesy of DISCOVR Consortium

COMMENTS

- DISCOVR is a large, coordinated research effort that aims to improve biomass productivity and value by downselecting strains via the DISCOVR pipeline and validating lab-scale studies in SOT cultivation campaigns at the AzCATI outdoor test bed site. A formal technical advisory board comprising experts from academia and industry provides quarterly feedback on approach and results. Downselection of strains through the pipeline, along with the application of strategic cultivation and crop protection approaches, has yielded significant results increasing annual average productivity to >18 g/m^2/day with a summer season average in excess of 31 g/m^2/day. Improvement in biomass productivity >20% compared to baseline was met for two of the seven strains tested outdoors. The addition of growth-promoting molecules also stimulated productivity of *M. minutum* at lab scale by 30% during simulation of winter weather conditions, but further work will be needed to establish the feasibility of this approach at scale. Taken together, these results support BETO's MYPP objectives and show continual progress toward meeting and exceeding the project's end goals. It may be of interest to the team to consider further refining the Tier I pipeline protocol for incoming strains of interest, and to consider if initial pipeline cultivation conditions are introducing bias that may favor performance of some species over others (e.g., adapting all strains to the same DISCOVR media versus using a strain's optimal media, observed variability in bacterial load of seed cultures). For example, it was noted that nitrogen and phosphorous requirements for optimal growth of *P. celeri* were not present in the lab medium and were later amended in outdoor trials, leading to significantly higher growth of this strain outdoors than was simulated in the lab. This observation suggests that some other strains may not have performed at their peak in the earlier Tier I studies, and high performers could have potentially been missed due to cultivation bias. This is worth addressing in future work.

- Overall, this large project seems to be going very well and progress is being made toward improving algae productivity during outdoor growing conditions. The DISCOVR team has weekly meetings and is guided by a technical advisory board. Risk and mitigation strategies are identified. Updates on how risks are being addressed would be helpful. The coordinated approach to identify stable and highly productive strains, increase productivity, improve culture stability, and test these strains in outdoor ponds is likely to advance the state of the art. Significant gains have been made in biomass productivity and biomass cost per ton, which likely will have a positive impact on commercializing industrial algae efforts. Larger productivity gains will likely be necessary to justify the addition of the growth-promoting molecules. The project team seems to be making appropriate progress toward project goals.

- The DISCOVR program is a large consortium effort focused on reducing algae biofuels production costs by increasing cultivation productivities via strain screening and testing of new operational strategies across 15 separate tasks. The program is using clear, measurable target milestones, making their progress against these evident. The strain characterization program has evaluated 42 strains to date, including cold and warm weather strains and a range of salinity conditions before scaling to indoor PBR operations followed by outdoor pond operations in Arizona. A number of the initiatives evaluated were successful in improving productivity and run times in outdoor conditions. TEA modeling was added to the program based on previous Peer Review comments. This modeling is providing valuable feedback to support driving the research areas of focus and has identified the importance of focusing on seawater strains and strains that can be gravity-settled. An extension of this could be a consideration to expand the DISCOVR program to focus beyond target productivity rates to set metrics to lead to the most optimal strains for both cultivation and downstream processing based on integrated process considerations. It was understood that crop rotations are used to operate at maximum productivity during each season, and these crop changeovers and associated inoculum support systems are not fully modeled in the TEA model results. This would have some throughput impact when translating to a commercial-scale system.

- The project provides an excellent example for organizing, assembling, and managing the aggregation of very diverse and extended technical programs into a consolidated, practical, and productive endeavor. The management team has undertaken a risk management and mitigation program that reflects many of the earlier lessons learned, proactively coordinates with all the adjacent and related BETO projects and programs and integrates many of their findings and progress toward advancing many of BETO's major stated goals. Just as importantly, the project helps identify new or revised topics that, in turn, help inform many of the adjacent projects and programs. Consideration should be given the maximum practical extent to enhance the DISCOVR platform into being able to test with genetically modified strains in open-pond environments. This can help inform the laboratory and toolkit development programs, as well as help define possible timelines and expectations regarding the impact of these technologies.

- This appears to be one of the largest and most geographically dispersed teams under review this year. That large team separated over different labs creates an inherent risk that seems to have been well mitigated in this case. As a result, this project does appear to truly gain from each of the sub-team's strengths, expertise, and resources in contribution to a collaborative effort that is greater than the sum of its parts. This team comprises the most highly regarded researchers in the field, and the management of the project is a reflection of this excellent team. The amount of work and its sweeping scope makes it difficult to compare to most other projects in the portfolio. In fact, the high degree of synergy between this effort and other AOP efforts is so high that it is difficult to separate many national lab projects from their contribution to this consortium. The DISCOVR project appears to be the flagship of BETO-guided efforts and seems as closely aligned with BETO priorities and goals as can be imagined. The funneled downselection of strains seems well planned and logical, but it also seems redundant to the National Alliance for Advanced Biofuels and Bioproducts consortium effort that launched the BETO AAS Program. This work is well targeted at impactful efforts and has demonstrated impactful technical achievements. The call for collaboration is a step in the right direction for ensuring lasting impact from this effort. The team has engaged an appropriate advisory board and looked for commercial stakeholders to participate. The involvement of Aequor in the DISCOVR project is positive, but the fact that only two private companies applied to participate in the project and the one private company now working with the team is not an algae company may indicate tepid private sector response and very low likelihood of any commercialization impact occurring as a result of this work. It appears that the team has achieved all of its goals on schedule. The end-of-project milestone of achieving a 10% year-over-year improvement in productivity from 11.7 $g/m^2/day$ seems like a very difficult goal to fall short of achieving before September 2022 given that one of the strains used in the study had already proven a sustained growth rate of almost three times that amount in an outdoor open pond. All the work done by this team is of the highest quality. All internal milestones have been achieved with very small change in the commercial landscape or progress toward BETO's ultimate goals.

- This is a flagship project for BETO taking an approach to reduce total microalgae biofuels production costs by applying an integrated screening platform for the identification of high-productivity strains with cellular composition suitable for biofuels and bioproducts for resilient, year-round outdoor cultivation, and testing new concepts to increase annual SOT productivities and reduce the minimum biomass selling price. The main goals include improving biomass productivity, enhancing biomass intrinsic value and value productivity, and increasing crop protection and culture stability. A new spectroradiometric pond monitoring system is also being developed through this project. The project uses TEAs to screen economic feasibility of new concepts prior to pond trials. The final goal is for new strains and optimized conditions to be tested in year-round seasonal SOT trials at the BETO test bed at AzCATI. The project has a call for collaboration with industry and academia to develop strains through the DISCOVR pipeline and is a key collaborator in a large number of other BETO projects. The project's main impact will be in increasing the annual SOT productivity with concomitant decreases in minimum biomass selling price, with the hope to reach BETO 2030 target of $488/ton years ahead of schedule. This is a very high-profile project for the BETO platform with multiple collaborations and inputs into other projects within the BETO platform. The project has tested 42 strains for temperature and salinity tolerance under their Tier I screen. Twenty-four strains passed to their Tier II screen to be grown under climate-simulated conditions in PBRs. Thirteen of the strains passed into the Tier III screen for test bed cultivation. Of these, seven strains have been evaluated in seasonal SOT trials at AzCATI. Some strains have shown the ability to grow close to 20 $g/m^2/day$, and cultivation improvement processes, along with controls, monitoring, and production improvements, are projected to be able to hit the BETO 2030 targets. The spectroradiometric tool and cultivation practices being developed are seen as key innovations for this platform. The progress is resulting in very high-impact publications in the peer literature. Management and communication team and strategies are well described and excellent. A unique set of critical success factors have been identified for the project. Risks and mitigation strategies were identified and described by task. Go/no-go milestones based on measurable productivity and failure modes provide a basis for strain selection into the pipeline. Progress was described through tasks, milestones, and go/no-go decision points, providing an adequate assessment of progress for the project. A technical advisory board with excellent consultants and a discussion process is included.

PI RESPONSE TO REVIEWER COMMENTS

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

  COMMENT: It may be of interest to the team to consider further refining the Tier I pipeline protocol for incoming strains of interest, and to consider if initial pipeline cultivation conditions are introducing bias that may favor performance of some species over others (e.g., adapting all strains to the same DISCOVR media versus using a strain's optimal media, observed variability in bacterial load of seed cultures).

  RESPONSE: The reviewer brings up a reasonable point about reviewing and potentially refining the initial stages of the DISCOVR screening pipeline. We will consider further refinement and broadening of our approach, both to streamline the process for incoming strains and to address potential biases in the screening effort. Unfortunately, the potential for biases in any screening effort is unavoidable. False positives will get weeded out in further testing, but false negatives early on, as the reviewer notes, can lead to missing high-productivity strains due to simple cultivation biases. In our approach, we chose to reduce the number of media formulations tested and increased the number of strains screened. The screening medium was chosen with input and feedback from the Technical Advisory Board as a reasonable approximation for an industrially relevant cultivation medium, using major elements that would be present in future nutrient recycle streams (e.g., ammonia-nitrogen). This medium will certainly not be optimized for every strain tested, but for strains that pass the initial screening efforts and graduate to indoor pond simulators and outdoor cultivation, medium optimization is a potential avenue for increased productivity. In our reexamination of biases, we will consider exploring variations in medium composition on top DISCOVR strains, as well as rescreening poor-performing strains in alternate test media.

COMMENT: Larger productivity gains will likely be necessary to justify the addition of the growth-promoting molecules.

RESPONSE: Growth-promoting molecules or plant hormones are cost-effective and inexpensive, and they are actively being used in agriculture at scale. An initial cost analysis performed by Bruno Klein and Ryan Davis at NREL suggests that if we supplement algal strains with indole acetic acid, we only need a very small increment in growth—as little as 2% for adding 1 µM indole acetic acid—to achieve a reduction in minimum biomass selling price. Assuming a 30% increase in outdoor productivity in response to 1 µM indole acetic acid addition (as was observed in our ePBR experiments) for all 12 months of the year, the overall reduction in minimum biomass selling price would be 16%, and thus more than make up for the additional cost of adding growth-promoting molecules.

COMMENT: Consideration should be given the maximum practical extent to enhance the DISCOVR platform into being able to test with genetically modified strains in open-pond environments. This can help inform the laboratory and toolkit development programs, as well as help define possible timelines and expectations regarding the impact of these technologies.

RESPONSE: We agree that it is anticipated that at least a fraction of the future-needed improvements in biomass productivity and composition may require the deployment of genetically modified strains, and that the DISCOVR consortium is ideally positioned to lead the way to streamline the deployment of such strains in an outdoor test bed in a coordinated manner. So far, there have been only limited reports of genetically modified strain candidates exhibiting higher biomass productivity with sufficient long-term genomic stability, and therefore pursuit of outdoor deployment has not been an immediate priority for DISCOVR. However, at laboratory scale, GMOs (e.g., *Nannochloropsis gaditana*) have been shown to alter biomass composition, particularly lipid content. The AzCATI test bed site has successfully obtained approval and permits for outdoor deployment of multiple genetically modified algae strains (for affiliated projects in the DOE BETO competitive portfolio, such as PACE and RACER) via the required TERA by the U.S. Environmental Protection Agency. The AzCATI test bed site has a designated six-pond separate location that is encompassed by secondary containment and has incorporated necessary field safety precautions. It is anticipated that this TERA permitting process could be streamlined and adapted, translating to an ultimately much shorter approval for deployment of novel strains made available by the community, with demonstrated performance (and compositional or resilience) improvements demonstrated at the laboratory scale under relevant and simulated conditions. We believe that the DISCOVR team is ideally positioned to enable and accelerate the deployment of genetically modified strains to relevant outdoor environments.

COMMENT: An extension of this could be a consideration to expand the DISCOVR program to focus beyond target productivity rates to set metrics to lead to the most optimal strains for both cultivation and downstream processing based on integrated process considerations. It was understood that crop rotations are used to operate at maximum productivity during each season, and these crop changeovers and associated inoculum support systems are not fully modeled in the TEA model results. This would have some throughput impact when translating to a commercial-scale system.

RESPONSE: TEA efforts in the DISCOVR consortium are continuously being revised and updated to better reflect the design of algae farms using "n^{th} plant" assumptions and to encompass new metrics that aid in demonstrating the viability of deploying the strains identified under DISCOVR in large-scale cultivations. A recent addition of a comprehensive modeling effort to establish and iterate over an “operational baseline” TEA seeks to include more granular design details for the inoculum train, incorporating the ramifications of pond downtime metrics, and does cover the cost impacts of dynamic strain rotation throughout the seasons. While DISCOVR is, through close collaboration, involved with and guides experimental conversion research, the project scope does not currently include conversion research. Rather than expanding the focus to downstream processing, we are committing to broadening the interactions and knowledge base of an integrated system inclusive of fuel/product yields. An example of a close interface with a conversion path and the cost and yield implications of algae productivity and composition is DISCOVR’s recent work toward establishing an objective basis for biomass “intrinsic value” assessments. This approach allows for quantifying economic trade-offs between productivity and improved compositional quality for downstream conversion and thereby identifying the most optimal strains to support this trade-off while minimizing the cost penalty associated with possible growth rate reductions.

COMMENT: The funneled downselection of strains seems well planned and logical, but it also seems redundant to the National Alliance for Advanced Biofuels and Bioproducts consortium effort that launched the BETO AAS Program.

RESPONSE: We believe that the DISCOVR project stands on the shoulders of many great achievements in the algae R&D community and is uniquely positioned to make the improvements and advances needed to achieve the aggressive productivity, fuel, and product cost and sustainability targets. The funneled downselection of strains in the DISCOVR project is an extension and refinement of prior strain characterization efforts that were carried out in the National Alliance for Advanced Biofuels and Bioproducts and Regional Algae Feedstock Testbed consortia, while the outdoor deployment infrastructure and processes at the AzCATI test bed site are building on an established knowledge base from the ATP3 consortium. Relative to the National Alliance for Advanced Biofuels and Bioproducts project, two innovative strain characterizations are carried out in the DISCOVR project to objectively downselect strains. First, at Tier I, the maximum specific growth rates of strains are measured as a function of temperature and salinity to identify the optimum growing season and the optimum medium salinity. Only strains with the highest maximum specific growth rates are downselected for subsequent testing in PNNL’s laboratory environmental algae pond simulators (LEAPS) climate-simulation PBRs. Second, at Tier II, strains are cultured in the LEAPS PBRs under identical climate-simulated winter and summer season (for Arizona) conditions to allow for the objective comparison of all strains under similar seasonal cultivation conditions. This has never been done before in any other BETO-funded project. The strains with the highest seasonal (winter/summer) areal biomass productivity are then tested in outdoor ponds. In summary, such a rigorous and objective strain downselection process is unique to DISCOVR and brings together the most promising strategies in an innovative and outdoor-relevant deployment pipeline, underpinned by rigorous data collection and dissemination and TEA in the form of annual SOT reports.