

Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVER): A Coordinated Research-Driven Approach to Improve Microalgal Productivity, Composition, and Stability for Commercially Viable Biofuels Production

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Abstract

To meet the U.S. Department of Energy (DOE) 2030 annual algal biomass productivity projection of 25 g/m²-day, the DISCOVER consortium, a collaboration between Pacific Northwest National Laboratory (PNNL), Los Alamos National Laboratory (LANL), the National Renewable Energy Laboratory (NREL), Sandia National Laboratories (SNL) and the DOE State of Technology (SOT) algae testbed at Arizona State University (ASU), 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. The recent research efforts of the DISCOVER consortium have already had a significant impact on improving annual algae biomass productivity and reducing the minimum biomass selling price. Following the introduction, in 2018, of new DISCOVER strains for testing at the SOT testbed at ASU, and simultaneously applying innovative concepts to improve biomass productivity and culture stability, the annual biomass productivity has increased from 11.7 to 18.4 g/m²-day, a 57% gain in just two years, equivalent to a reduction in biomass selling price of 27%, from 824 to 603 \$/ton.

Keywords: microalgal biofuels, climate-simulation bioreactors, raceway ponds, biomass composition, crop protection.

Bioenergy from microalgae has the potential to contribute substantially to the nation's renewable energy and carbon capture missions, but significant challenges remain for transitioning from research at the lab and pilot scale to commercial algae production in large-scale farms [1,2]. The lack of publicly available, long-term, pilot-scale, outdoor agronomic data on algae cultivation makes it difficult, even unreliable and risky, to predict algae yield and quality performance at large scale. Increased research and development, as well as pilot-scale demonstrations, are needed to improve areal productivity and overall system operational reliability and performance, reduce uncertainty and economic risk in commercial deployment of an microalgal biofuels process, and address and validate data gaps between actual and assumed values [3,4].

Knowledge gaps identified by the algae research and development community are related to four main barriers to commercial deployment. The first barrier is the selection of microalgae species. This selection is often based on a singular or small set of characteristics, such as fast growth or the accumulation of a high value product [5]. In the context of long-term algae farming for bioenergy or carbon capture, however, the focus must be on a combination of high and highly consistent seasonal (and annual) areal productivity, attractive biomass composition, and resilience to pests. By meeting these primary characteristics in selected algae species with thermal tolerances for outdoor pond cultivation in specific seasons, a flexible seasonal crop rotation strategy is likely to ensure consistent yearly biomass production by minimizing pest pressures [6].

The second barrier is understanding and developing strategies to predict and subsequently act to prevent or mitigate crop failures that prohibit long term cultivation success. Currently, one of the most impactful causes for crop failures is biotic contamination. Harmful organisms include

viruses, bacteria, fungi, predatory and competing algae, rotifers, amoebae, and other grazers, and aquatic insects and their larvae [7-9]. These organisms can significantly reduce the reliability of large-scale biomass production by reducing outdoor pond cultures' mean time between failure [10]. Consequently, it is imperative to rapidly identify the causal microbial agents prior to and during a crash, and better understand the mechanisms involved in biotic contamination events so that mitigation strategies can be applied to maintain crop value and viability [10].

The third barrier is the absence of a reliable and effective path for implementing and validating strategies that work at the laboratory scale to outdoor, larger-scale production. It is well known that typical shake flask and photobioreactor experiments that demonstrate a certain success outcome in a laboratory setting often do not achieve the same results in an outdoor pond cultivation setting. Understanding those scale-up issues that prevent translation from laboratory to outdoor scale will allow the rapid testing of hypotheses and provide confidence that laboratory results will translate effectively to outdoor trials, ultimately resulting in more rapid increases in outdoor productivity.

The fourth barrier is the current cost of biomass production based on the State of Technology (SOT) defined by the associated technical and economic factors involved in algae farming [11]. To help drive down the cost of biomass production, there is a need to establish an objective framework to evaluate hypotheses and strategies that are expected to show promise for cost-savings by conducting a rigorous technoeconomic analysis (TEA) in the context of an algae farming operation. The ability to then rapidly validate those hypotheses and strategies will result in cost-savings for algae farming operations.

To address these critical barriers to large-scale, commercially viable algal biomass and biofuels production, a collaborative consortium, entitled *Development of Integrated Screening, Cultivar Optimization, and Verification Research (DISCOVER)*, was established. This consortium is funded by the Bioenergy Technologies Office (BETO) of the U.S. Department of Energy (DOE) and constitutes a partnership between four DOE national laboratories - Pacific Northwest National

Laboratory (PNNL), Los Alamos National Laboratory (LANL), National Renewable Energy Laboratory (NREL), and Sandia National Laboratories (SNL) - and the Arizona Center for Algae Technology and Innovation (AzCATI) at Arizona State University. To address the knowledge gaps described in the four barriers above, the DISCOVR consortium has established a state-of-the-art, highly coordinated research strategy (Figure 1) with an overarching goal to answer critical questions about outdoor algae performance, including the magnitude of seasonal, species-dependent, areal biomass productivities, the impact of operational strategies (e.g., harvest frequency, timing, and dilution rate), the influence of the pond culture environment (e.g., night temperatures, pH, water chemistry), and the responses to biotic and abiotic stressors.

The overall approach consists of a strain down-selection pipeline approach which is guided by successive benchmarks that embody characteristics necessary for an algae species to be successful in outdoor cultivation. A strain down-selection approach culminates in outdoor validation trials. Feeding into the strain down-selection pipeline in an independent (i.e., at any Tier depending on the technology) and back-and-forth fashion, are new laboratory-validated concepts to increase productivity, biomass value, and resiliency. This coordinated research framework also aims to apply lessons-learned from outdoor cultivation to laboratory-scale studies to validate and further improve the predictive power of laboratory experiments, with the goal of improving outdoor productivity, biomass value, and resiliency. In this way, a robust and validated approach for a laboratory-to-farm research and development strategy is created.

To assess the potential impact on costs associated with employing a new promising strain or testing various operational improvements, our research approach filters potential low technical readiness level research (top right box, Figure 1) through a TEA model based on a 5,000 acre outdoor algae farm [1]. This allows us to evaluate potential research hypotheses in the context of a farm design, to understand if significant progress on cost metrics will be met *prior to* conducting research at the laboratory scale and subsequent deployment and testing in the outdoor testbed. Similarly, the TEA also provides target metrics for observed improvements based on input and operational costs, thereby bounding experimental conditions to those that, if successful, will also be cost effective. This TEA-driven approach imparts an important focus to the testing of new concepts. Ultimately, promising approaches are then assessed at the AzCATI

outdoor testbed site in DOE State of Technology (SOT) trials, with those data then feeding into NREL's algae TEA farm model to quantify the minimum biomass selling price, using associated productivity and harvest metrics [11].

To ensure industrial relevance and stakeholder engagement, DISCOVER presents their activities quarterly to an external Technical Advisory Board (TAB) consisting of algae experts from academia and industry. The TAB offers input and advice with respect to experimental approaches and results, and this feedback is taken into consideration by the DISCOVER team for planning future research activities. In addition, a rolling, open solicitation has been issued to the public entitled the "Call for Collaboration", which provides a framework for interested parties to formally interact with DISCOVER. Collaborators may ask to have algae strains or innovative technologies objectively tested and validated using the DISCOVER research approach or as part of an upcoming outdoor SOT trial. Thus, by incorporating outdoor demonstrations vetted by TEA and soliciting input from an industry-guided Technical Advisory Board and the broader algal research community, the DISCOVER project aims to accelerate the development of large-scale microalgae biomass production for economically viable biofuels production.

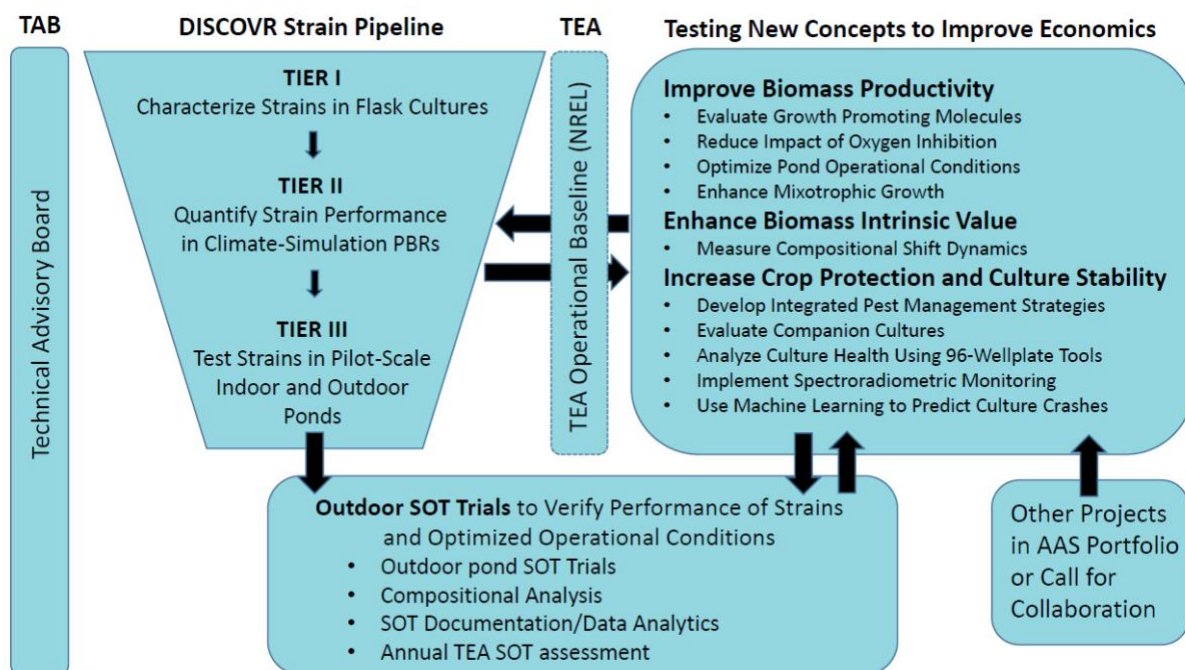


Figure 1. The highly coordinated DISCOVER experimental strategy to address and overcome barriers to agronomic-relevant algae cultivation. AAS = Advanced Algal Systems program (BETO).

Specific DISCOVER Research Areas

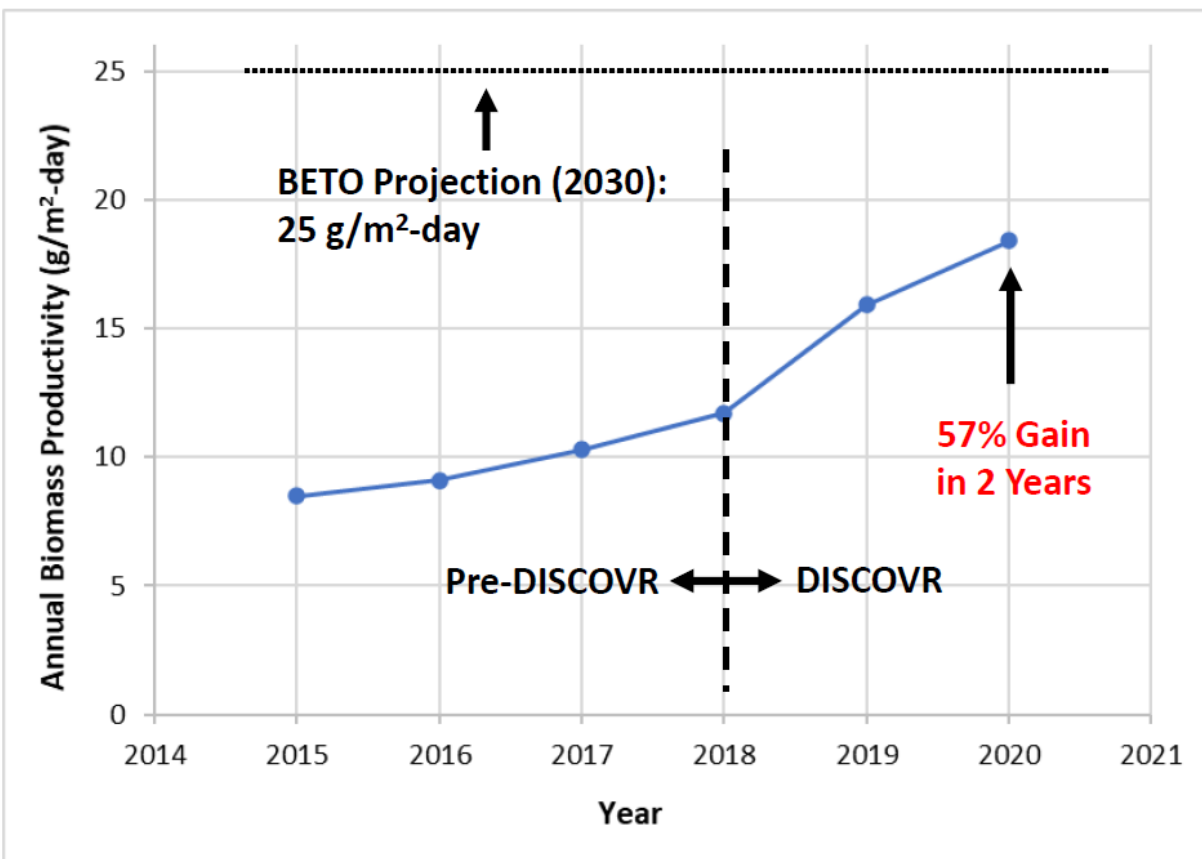
To address the barriers of strain selection for achieving high seasonal productivities and for ensuring culture resilience, the DISCOVER algae characterization strategy applies a tiered pipeline to quantify critical characteristics of a broad range of algae species and strains. Initially, at Tier I, batches of strains obtained from culture collections or industrial partners are screened in thermal and salinity gradient incubators specifically designed by PNNL with respect to their maximum specific growth rate as a function of temperature and salinity, respectively. A subset of the fastest growing strains with suitable temperature and salinity tolerances are then subjected to indoor cultivation tests at Tier II that are concurrently carried out at PNNL, LANL, NREL, and SNL. The indoor cultivation tests at Tier II seek to quantify areal biomass productivity under conditions simulating outdoor Arizona ponds under winter and summer climate conditions across several platforms: PNNL's recently developed and validated Laboratory Environmental Algae Pond Simulator (LEAPS) photobioreactors [12], NREL's Simulated Algal Growth Environment (SAGE) reactors, and LANL ePBR systems [13]. Biomass is analyzed for basic composition (lipids, protein, carbohydrates) to understand the seasonal compositional makeup of the biomass, to ultimately calculate the biomass intrinsic value and determine its effect on minimum biofuels selling price [14]. The resistance of algae strains to other deleterious algae, bacterial communities, or predation is assessed in laboratory scale stress tests at SNL [15]. Strains that exhibit the highest winter and summer season productivity at Tier II move on to Tier III where they are tested in small raceway ponds at the PNNL Algae Testbed (PAT) in Arizona as a final step in validating a strain's outdoor performance in terms of biomass productivity and culture stability, before conducting official SOT trials at the AzCATI testbed. Tiers II and III leverage our rigorously established and previously validated correlation between indoor laboratory studies and outdoor cultivation trials, allowing for rapid laboratory hypothesis testing in addressing barrier 3. Finally, the down-selected strains having the highest biomass productivity and culture stability identified during Tier III combined with potential new operational concepts, after vetting with TEA, move

forward to long-term outdoor testing at the SOT testbed site at AzCATI. The data from these outdoor trials at AzCATI are used on a monthly and seasonal basis as inputs to NREL's annual SOT report [11].

Concurrent to the strain characterization and down-selection in the DISCOVER pipeline, hypotheses for improving strains by increasing biomass productivity, shifting biomass composition to enhance intrinsic value, or improving crop stability and resistance to pests (top right box, Figure 1) are also tested in a laboratory setting. These results are then filtered through our operational TEA model (middle box, Figure 1), and those that show promise in reducing costs are shuttled to either the strain down-selection pipeline or used as strategies to increase productivity at the AzCATI outdoor testbeds.

The recent research efforts of the DISCOVER consortium have already had a significant impact on improving annual algae biomass productivity and reducing the minimum biomass selling price (MBSP) [16]. Following the introduction, in 2018, of new DISCOVER strains for testing at the SOT testbed at AzCATI, and simultaneously applying innovative concepts to improve biomass productivity and culture stability, the annual biomass productivity has increased from 11.7 to 18.4 g/m²-day, a 57% gain in just two years (Figure 2A). The concomitant decrease in minimum biomass selling price, as determined by NREL's Algae Farm Model [11], was from 824 to 603 \$/ton, a 27% decline in two years (Figure 2B). The goal of the DISCOVER consortium is to meet the U.S. Department of Energy 2030 projections for annual biomass productivity (25 g/m²-day) and minimum biomass selling price (\$488/ton) significantly ahead of schedule.

A



B

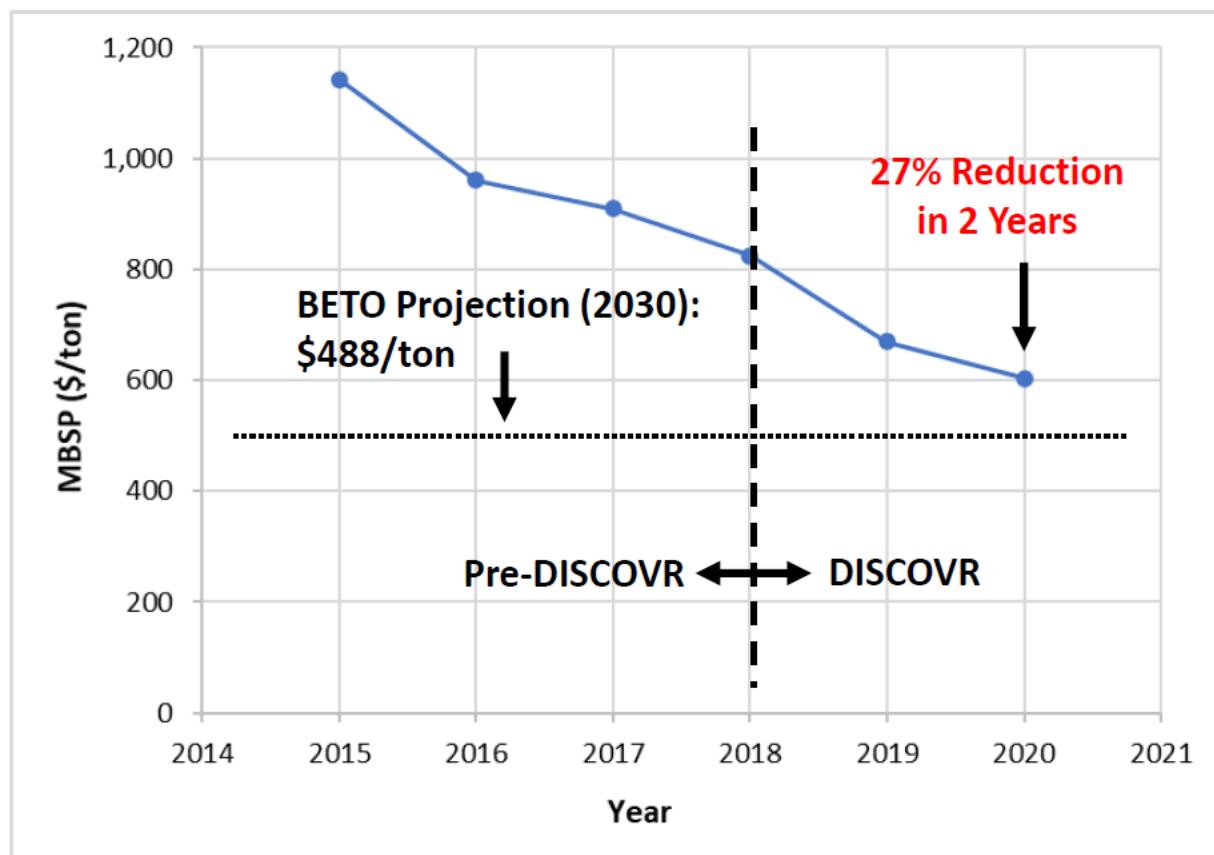


Figure 2. Increases in SOT annual biomass productivity (A) measured at the SOT testbed at AzCATI from 2015 to 2020, with concomitant decreases in SOT minimum biomass selling price (B). The vertical dashed line in 2018 marks the time point where new DISCOVER strains and innovative concepts were started being tested in AzCATI SOT trials.

In conclusion, research performed by the DISCOVER consortium provides critical cultivation data to facilitate commercial algae farming operations geared towards economically viable biofuels through testing of validated algae species and pond operational strategies. By using our innovative approach in addressing critical barriers, we have demonstrated a 57% gain in annual SOT biomass productivity, translating to an almost 30% reduction in SOT minimum biomass selling price, firmly establishing this consortium to make rapid progress towards achievement of DOE's ambitious productivity and cost projections. The DISCOVER team will deliver high-

performing strains that will be made available to the algae community, with a robust data set that will broaden the scientific understanding of the economics and sustainability of the algae biofuels across a range of strains, thereby enabling commercial algae cultivation for biofuels and bioproducts.

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Declaration of authors contribution

Michael Huesemann: Conceptualization, Writing – Review & Editing, Supervision, Project Supervision; Eric Knoshaug: Conceptualization, Writing – Original Draft; Lieve Laurens: Writing – Review & Editing; Taraka Dale: Writing – Review & Editing; Todd Lane: Writing – Review & Editing; John McGowen: Writing – Review & Editing.

Informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this project.

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