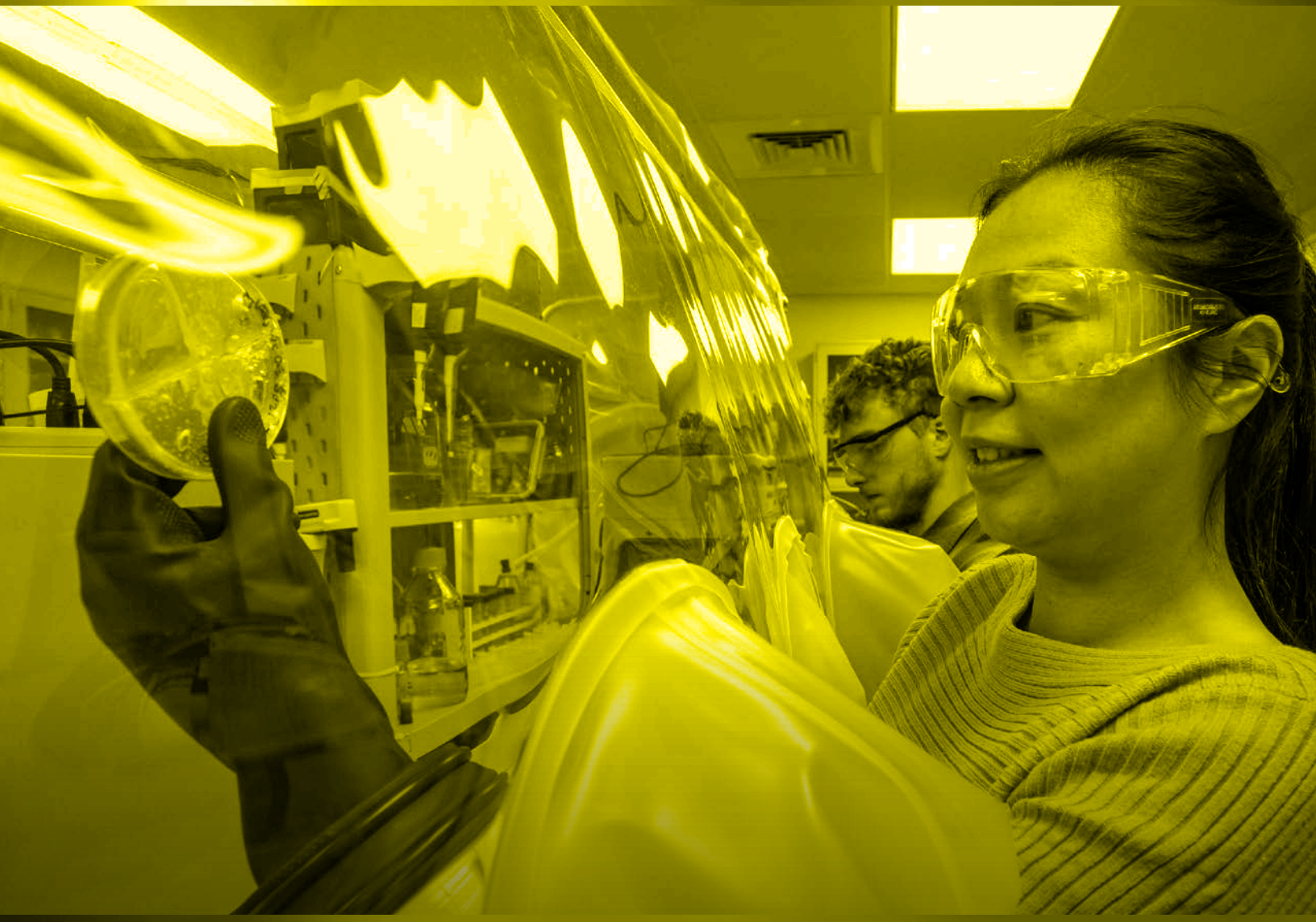


AGILE BIOFOUNDRY CONSORTIUM

TECHNOLOGY AREA

CONTENTS

INTRODUCTION

The Agile BioFoundry (ABF) Technology Area is one of 11 technology areas reviewed during the 2023 Bioenergy Technologies Office (BETO) Project Peer Review, which took place April 3–7, 2023, in Denver, Colorado. A total of 31 presentations were reviewed in the ABF session by six 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/sites/default/files/2023-04/beto-00-project-peer-review-abf-apr-2023-bentley.pdf.

This review addressed a total U.S. Department of Energy (DOE) investment value of approximately $86,928,379.00, which represents approximately 12% of the BETO portfolio reviewed during the 2023 Peer Review. During the Project Peer Review meeting, the presenter for each project was given 30 minutes to deliver a presentation and respond to questions from the review panel.

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

BETO designated Gayle Bentley as the ABF Technology Area review lead, with contractor support from Frank Fields (Boston Government Services LLC). In this capacity, Gayle Bentley was responsible for all aspects of review planning and implementation.

AGILE BIOFOUNDRY REVIEW PANEL

Name	Affiliation
Karen Draths*	Michigan State University
Brentan Alexander	Synonym
Doug Friedman	BioMADE
Hanny Rivera	Ginkgo Bioworks
Gale Wichmann	Amyris
Fuzhong Zhang	Washington University in St. Louis

* Lead Reviewer

AGILE BIOFOUNDRY REVIEW PANEL SUMMARY REPORT

Prepared by the Agile BioFoundry Review Panel

INTRODUCTION

The Agile BioFoundry was established by BETO in 2016 and currently exists as a distributed consortium of seven national laboratories: Lawrence Berkeley National Laboratory (LBNL), Argonne National Laboratory (ANL), Sandia National Laboratories (SNL), Pacific Northwest National Laboratory (PNNL), Los Alamos National Laboratory (LANL), Oak Ridge National Laboratory (ORNL), and the National Renewable Energy Laboratory (NREL). A ***biofoundry*** provides an integrated infrastructure that combines biological, chemical, and engineering disciplines and tools that enable automation, high-throughput measurement, integrated data acquisition/analysis, artificial intelligence, and machine learning (ML) to enable rapid design, construction, and testing of genetically reprogrammed organisms for biotechnology applications. The ABF exists to support BETO's decarbonization mission of the energy-intensive chemicals industry and transportation sector by accelerating development of optimized routes to direct replacement chemicals, performance-advantaged bioproducts, and CO_2 utilization for chemicals and by leveraging design-build-test-learn (DBTL) infrastructure to meet opportunities and challenges related to sustainable aviation fuels (SAFs).

Thus, the ABF is a public infrastructure investment that is intended to expedite advancement of the bioeconomy by accelerating innovation and encouraging adoption of new biomanufacturing methods. The specific objective of the ABF is to develop and deploy state-of-the-art tools and technologies that enable commercially relevant biomanufacturing of products. ABF partnerships with industry and academia enable adoption and utilization of advanced tools, infrastructure, and expertise to facilitate growth of the bioeconomy. The ABF's stated goal is to reduce bioprocess scale-up time for its partners by 50% from a current average of 10 years.

In this review, the ABF presented progress reports for the following activities: (1) DBTL infrastructure, demonstration products, and beachheads; (2) host onboarding and development; (3) process integration and scale-up; (4) techno-economic analysis (TEA) and life cycle analysis (LCA); and (5) industry engagement, outreach, and management. Internal ABF activities within these areas are funded via annual operating plan (AOP) funds provided by BETO. Outward-facing, partnership-related research is funded via funding opportunity announcements (FOAs) or directed funding opportunities (DFOs). Five FOA projects and 10 DFO projects were reviewed.

The ABF recently presented a plan for the 2023–2025 funding cycle, and subsequent reviews indicated that although the ABF has completed significant and meaningful work, quantifying reductions in development time and costs remains challenging. This analysis echoes the 2021 Peer Review, which cautioned that the current ABF approach might not be well suited to achieve its high-level goals. For reference, the ABF has received more than $124 million in BETO funds since its inception. As a result, BETO program management directed the ABF to suspend research activities for two quarters and to dedicate all efforts to devising a new strategic plan with defined implementation tasks. The new strategic plan and corresponding budget allocation were described by ABF leadership and extensively discussed with the review panel.

STRATEGY

During the recent strategic planning process, the ABF defined its mission as follows: Develop biomanufacturing tools, processes, and partnerships that enable sustainable industrial production of renewable fuels and chemicals for the nation. To advance its mission, the ABF is now organizing itself in goal-oriented units in place of the previous task-oriented units (e.g., DBTL, host onboarding). The new strategic plan calls for advancing pathways, strains, and processes for production of two types of SAF (thermophilic ethanol and microbial alkanes) and two biochemicals (muconate and 3-hydroxypropionic acid [3-HP]). Tools development

will continue within the framework of advancing strains for sustainable fuels and chemical production, and an invigorated emphasis on partnerships will target funds-in relationships with industry in addition to the standard FOA and DFO partnership mechanisms. The review panel agrees that the revised mission and goals align better with BETO's mission. There is concern that the ABF vision of "sustainable biomanufacturing of affordable fuels and chemicals" overstates ABF's bioeconomy role beyond infrastructure to include production. The panel advises caution regarding writing performance metrics specifically tied to bioproduction. Furthermore, CO_2 reduction metrics were not contextualized (e.g., actual versus theoretical CO_2 reduction), which may introduce new challenges when ascertaining whether ABF has achieved its goals. Although the panel agrees in principle that internal research should focus on a small number of specific targets, no rationale was provided for selection of muconate and 3-HP over other bioproducts ABF has previously targeted. SAF is admittedly well aligned with the BETO mission; however, its economic viability remains uncertain. The panel recommends ABF revise their newly stated goals. For example, details surrounding tool benchmarking to "equivalent industry-accessible, state-of-the-art baselines" are not well defined, which makes categorizing tools for further development or sunsetting particularly challenging. Panel members would have appreciated more details describing the mapping of specific capabilities, tools, and personnel onto the strategic goals to ensure efficient expenditure of resources.

To fulfill its role as a biofoundry and achieve its high-level goals, the panel agrees that the ABF must interact extensively with both industrial and academic partners. Both the FOA and DFO funding mechanisms were deemed appropriate. Overall, the panel was impressed by the quantity, quality, and research progress for the reviewed ABF partnerships. One goal of the new strategic plan is to direct greater than 50% of ABF funding to partnership-related research. This is viewed favorably by the panel and is expected to strengthen ABF skill sets and provide companies, regardless of size or resources, access to cutting-edge technologies. The ABF acknowledges that oversubscription to ABF funding opportunities is critical to maintaining a competitive process, which the panel agrees will provide the best opportunity to develop impactful research programs. Echoing sentiments of the 2021 Peer Review, current panel members are concerned that ABF efforts to disseminate information regarding technological advances fall short of expectations. Panel members perceived an emphasis on journal publications as a measure of accomplishment and productivity, although this was not explicitly stated by ABF leadership. Because journals are not universally available to potential industrial partners, publications may not be the most effective means to reach the appropriate audiences. The panel recommends more extensive description of advances via other means, including the ABF website (e.g., text, videos, presentation slides) and newsletters. The Advanced Biofuels and Bioproducts Process Development Unit (ABPDU), which is fully under the ABF umbrella but maintains its own website, provides an effective model for information dissemination. Among the strongest concerns raised by the panel was the lack of a consistent, coordinated strategy for the ABF to engage industry stakeholders, despite various outreach and engagement activities. The absence of engagement with the industry advisory board (IAB) during the recent strategic planning process provides evidence that this is the case. The panel recognizes that industry stakeholders and their corresponding needs vary widely, which likely results in conflicting messages. The panel encourages the ABF to develop a consistent strategy to gather and catalog stakeholder feedback and then determine methods to provide maximum benefit to the industry.

Reviewer attention tightly focused on the new strategic plan, leaving less time to thoroughly evaluate the inward-facing ABF technological portfolio. Review of the task-oriented units of (1) DBTL infrastructure, demonstration products, and beachheads; (2) host onboarding and development; (3) process integration and scale-up; and (4) TEA and LCA indicates that ABF research is generally of high quality, and that unique synthetic biology (synbio) and bioprocessing capabilities are being developed. The ABF team has developed many core technologies, including pathways, products, strains, sensors, multi-omics tools, computational tools, and modeling tools. Multiple challenges remain before biomanufacturing processes will be economically competitive, particularly at commodity scale, which is required for SAF and biochemicals. The ABF plan to advance a small number of targets in these spaces may highlight the technological gaps, which can then be addressed. Panel members have concerns related to reducing resources to enable host onboarding and genetic

tool expansion for nonstandard organisms, which falls squarely within the ABF mission. Reviewers recommend judicious, continued investment in host onboarding/genetic tool expansion. Conversion of the lauded Host Onboarding Tool (HObT) web portal to an outward-facing, publicly accessible tool in a timely fashion is imperative.

As stated above, panelists are pleased that the new strategic plan includes a strong emphasis on partnerships and a commitment to invest at least half of BETO funds on partner-related research. Funding partnerships with the National Science Foundation (NSF) and BioMADE and a funding opportunity specific for minority-serving institutions broaden access to ABF tools and capabilities, which is viewed positively. Current and future partners are encouraged to clearly highlight how the partnership benefits the company/academic effort, the bioeconomy, and the ABF. The 2021 Peer Review recommended exploration of a fee-for-service mechanism for ABF engagement. Reviewers support the new effort to initiate strategic partnership projects, which are expected to increase engagement with industrial stakeholders. Details relating to recruitment of industrial partners, execution of partnership agreements, and intellectual property (IP) management were lacking, however. Reviewers encourage the ABF to work closely with their national lab partners to streamline and standardize these processes.

STRATEGY IMPLEMENTATION AND PROGRESS

The ABF continues research on a range of projects closely aligned to its mission. This includes internal projects focusing on tool development, capabilities, and demonstration projects, as well as outward-facing partner-related research. The ABF is in active partnerships with organizations ranging from small biotechnology startups to large, mature corporations. The ABF also has multiple academic partners. All task-related units are involved in a mix of internal and outward-facing research projects. Host onboarding/genetic tool development is engaged by many partner organizations, while TEA/LCA has performed a large body of work focused on internal projects related to beachheads and demonstration projects. This TEA/LCA work provides a foundation of research that enables prediction of the strongest drivers of both economic and environmental impacts. Reviewers note that the strength of the portfolio would be positively impacted by increased competition for research funds within the FOA and DFO selection processes. More widespread and thorough dissemination of ABF tools, capabilities, and advances through all possible means must be a focus for ABF management going forward. Similarly, a consistent approach for interacting with industrial stakeholders is an important need.

Reviewers characterized many of the partner-related projects as on the leading edge of the field. For several projects, even if characterized as on the leading edge, the combination of research status and product requirements make it difficult to envision a path to economic viability over the next decade. Across the board, reported progress indicated that assigned researchers are working diligently. Outward-facing projects to be discussed at future reviews should include a single slide aligning project status with all proposed milestones, tasks, and timelines. Reviewing projects before completion of five quarters of funded research is not recommended. Communication, technology transfer, and data sharing between the ABF and partner organizations appears to be adequate.

Past accomplishments for technology-related, task-oriented activities justify an optimistic attitude regarding achievement of near-term and midterm goals described under the previous strategic plan. For example, several beachhead molecules were produced in multiple host strains with impressive titer, rate, and yield (TRY) metrics. This progress was enabled by contributions across the ABF infrastructure, including DBTL, biosensors, host onboarding/genetic tools development, multi-omics analysis, and process integration/scale-up. Under the new strategic plan, the ABF is well positioned to make significant progress on SAF and biochemicals. The panel notes that deliverables for fiscal year (FY) 2025 were provided, but in many cases, these lacked context or specific details. Midterm ABF goals were not included.

Panelists agree that the ABF management team provides an appropriate level of project management to ensure beneficial outcomes for both partners and the government.

RECOMMENDATIONS

Modify ABF Management Structure

The panel unanimously recommends the ABF adopt a management structure whereby one individual oversees all ABF activities, including allocation of research funding. The new ABF director should be in alignment with BETO's vision for the ABF and should devote their full professional effort to ABF management. The panel recommends the following changes to the ABF organizational chart proposed in the new strategic plan. The director would report to the BETO technology manager. Direct reports to the director would include (1) engagement and outreach, (2) business development and partnering agreements, and (3) strategic implementation. The director would seek advice and counsel from the executive committee and the IAB. At least one reviewer advocated for selection of the director from candidates outside the current ABF management structure. The committee arrives at this recommendation following observations of protracted high-level decision-making and failure to address review recommendations adequately and in a timely fashion.

Standardize Research Agreement Templates

Protracted timelines for negotiation and finalization of research agreements are a powerful disincentive to work with a national laboratory consortium such as the ABF. The panel unanimously recommends that the ABF prioritize development of standard cooperative research and development agreement (CRADA) templates that outline IP management plans, licensing, and royalty rates. Templates from other national laboratory consortia such as Bio-Optimized Technologies to keep Thermoplastics out of Landfills and the Environment (BOTTLE™) may provide a starting point. The review panel believes the quality and quantity of ABF partnerships with industry will determine whether ABF is successful in its mission to expedite advancement of the bioeconomy. Reducing barriers to establishing strong partnerships must be a top priority.

Allocate BETO Funding to Partner-Driven Projects

The panel unanimously recommends that the ABF set a more ambitious goal of allocating 75% of BETO funds to partner-driven research projects. Following 6 years of allocating much of its funding to internal research projects, ABF has developed an impressive list of tools, capabilities, and infrastructure. The panel suggests the ABF is now in a strong position to assume its originally envisioned role as a service provider. The panel reminds ABF management that more effective dissemination of ABF capabilities and consistent engagement with industrial stakeholders are recommended to increase competition for ABF funding and services. Ultimately, the level of interest to form productive research partnerships and the extent of industry use of ABF capabilities will provide ABF with validation and proof of impact.

Map Partner-Driven Projects Onto Specific ABF Tools, Capabilities, and Infrastructure

The panel unanimously recommends that ABF management map all partnerships (past, current, and future) onto the specific tools, capabilities, and infrastructure being utilized, and at the conclusion of a project, clearly indicate for each all meaningful contributions to research progress. This exercise will highlight the extent of interest/utilization for each tool, capability, or infrastructure component and is expected to be useful to ABF management, BETO, and future panel reviewers.

AGILE BIOFOUNDRY PROGRAMMATIC RESPONSE

INTRODUCTION

BETO would like to thank the reviewers for the thoughtful and careful review of the ABF research portfolio, particularly in light of ABF's ongoing implementation of its revised mission and goals. This feedback is

extremely helpful as BETO seeks to ensure the ABF is successful in achieving their newly defined objectives. Many of these comments provide clear direction that BETO will use to inform directives to ABF moving forward. This program response will address several of these comments directly, but BETO appreciates and will consider all of the provided comments in the ABF's management moving forward.

The reviewers note that the new vision to support "sustainable biomanufacturing of affordable fuels and chemicals" may potentially overstate the ABF's bioeconomy role. While this comment is well taken, BETO believes that the ABF can indeed meet this new mission by clearly understanding and overcoming barriers to production of the strategically chosen bioproducts and biofuels. While ABF may not develop the entire bioprocess and lead the full-scale demonstration, ABF can contribute to overcoming critical barriers to scale-up and thereby enable biomanufacturing at large.

The program agrees that additional, clearer metrics around the goal to reach CO_2 reduction targets and other critical objectives are needed. These comments will be taken into consideration specifically regarding the noted benchmarking task and other critical milestones.

The program agrees that improved dissemination of the ABF's tools, capabilities, and advances will improve awareness of ABF's value to the community. External communications are also related to the reviewers' comments regarding industry engagement. The program appreciates this feedback and agrees that the ABF's industry relationships can be strengthened and partnership agreements can be streamlined along with improved IP management.

Recommendation 1: Modify Management Structure

The unanimous recommendation for a dedicated ABF director is appreciated. BETO recognizes the need for more efficient decision-making processes. Implementing a streamlined management structure will enable the ABF to progress their research objectives and improve timeliness. The program also acknowledges that this consortium is led by researchers at national laboratories, where the structure of those laboratories often prevents investigators from being able to focus on a single project.

Recommendation 2: Standardize Research Agreement Templates

The program appreciates the reviewers' assessment of the research agreement process. The program fully supports the improvement of the research agreement process in order to facilitate collaborative projects and interactions with industry.

Recommendation 3: Allocate BETO Funding to Partner-Driven Projects

The program notes the recommendation to set a more ambitious goal to dedicate 75% of BETO funds to partner-driven research projects. This suggested goal and the currently proposed target of 50% partnership projects both reflect a commitment to intensified industry collaborations. While BETO may not recommend that the ABF reach the 75% budget target, the program is committed to ensuring that the spirit of the partnership metric is met as the ABF proceeds. The reviewers noted that the ABF's originally envisioned role was as a service provider. While the ABF can indeed provide valuable services to the bioeconomy, BETO still sees great value in investing in the catalytic infrastructure that develops the scientific tools and capabilities that will ideally enable transformative improvements in microbial strain engineering and bioprocess design. As the ABF matures, it will be important to ensure that the scientific engine driving the ABF's value to the community is running strong, in addition to working hand in hand to solve specific technical challenges.

Recommendation 4: Map Partner-Driven Projects Onto Specific ABF Tools, Capabilities, and Infrastructure

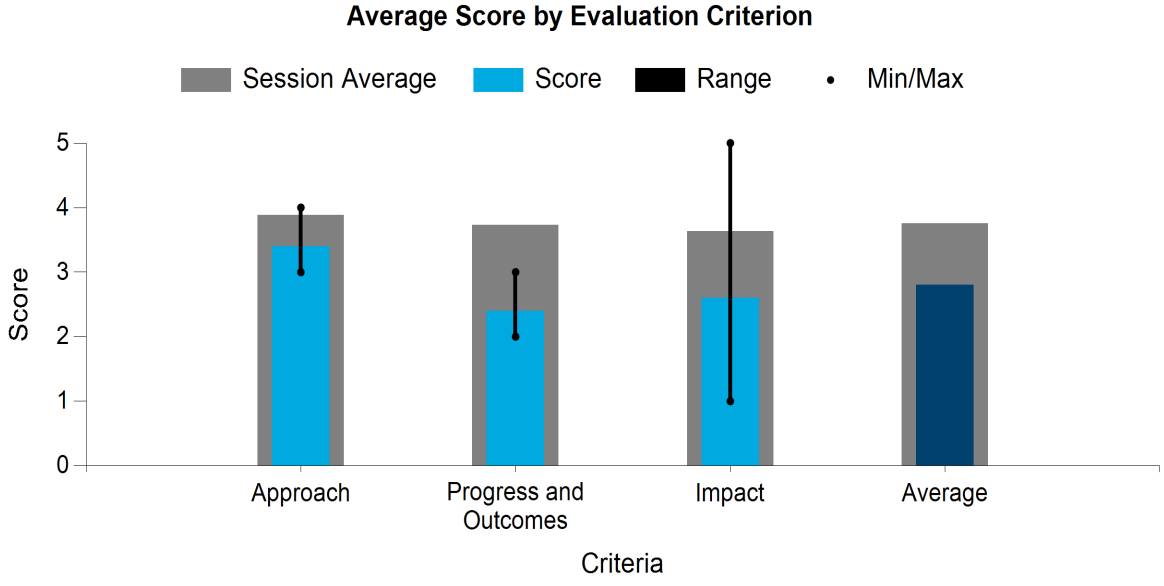
Furthermore, the recommendation to map all partnerships onto specific tools, capabilities, and infrastructure is appreciated. The program agrees that it is important to provide clear indications of meaningful contributions to research progress.

In conclusion, the review panel has provided valuable guidance for the ABF as they embark on a new strategic direction. BETO is committed to addressing the recommendations provided, and seeks to reflect on these recommendations in the management of the ABF moving forward. The program would like to again thank the reviewers for their time and expertise.

ACCELERATING ENGINEERED MICROBE OPTIMIZATION THROUGH MACHINE LEARNING AND MULTI-OMICS DATASETS

Lygos Inc.

WBS:	2.3.2.209
Presenter(s):	Bryce Dille; Nick Ohler; Rebecca Lennen
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2022
Total Funding:	$2,857,142.00



COMMENTS

- This project began with some ambitious goals to generate significant quantities of data to use to drive an ML tool to rapidly accelerate performance of the target strain using multiple DBTL cycles. The experimental design appears to be aimed at capturing as much data at as many points as possible in order to train the ML model. This approach is a strong one in both demonstrating ML capabilities and validating the ability of ML models to find useful insights in the noise that drive strain development.

- Unfortunately, both pandemic and partner financial issues appear to have taken a major toll on the progress of this award. Only one DBTL cycle was completed, and as such, the learnings from that cycle were not returned to the ML model for further cycles. The success of the first cycle is muted: Although improvements were seen in a number of strains, the improvement did not reach targets (although such success was not expected after a single cycle). Salvaging some learnings from this study would be important. Passing data back to the ML model to see how its predictions change based on the first cycle could provide interesting insights into what has been learned thus far and what new pathways may yet emerge. Review of the weights in the ML model may give guidance to which data (from the 80,000 points collected) were most useful and impactful, providing guidance and direction on where to focus

limited data-gathering resources in future activities. Without this closing work, the impact of this study is muted.

- Strength: This project benefits from the ABF's multi-omics capabilities, which helped Lygos generate a large proteomic data set.
- Weaknesses/areas for improvement:
    - The team proposed to complete six DBTL cycles, which is not a lot considering a total budget of $2 million. Even more concerning, only one cycle was completed, representing a major failure of the project.
    - Although the team generated a large number of omics data sets, the data set was not used to inform engineering targets, as a continued round of engineering was not performed. The data set also failed to provide any useful knowledge. So, it seems nothing can be learned from the large data set obtained. It is not clear what useful output this project has produced.
    - The impact of the project is quite limited. No improvement on TRY was mentioned. Although new strains were created and were claimed to be Lygos's highest producers, it is not clear how much improvement was obtained and to what degree these strains helped the commercialization process.
- I'm not sure what happened between 2021 and 2023 for this project. Back in 2021, they were nearly done, or significantly done, with the first DBTL cycle, and here 2 years later it's just finishing with the data. As the presenters stated, it was overly ambitious from the start and didn't need so many samples for the first DBTL cycle. Still, the project clearly advanced learning for both the ABF and the company. The first DBTL cycle was ultimately successful in generating useful data and improvements, but it seems it came too late, and it's not clear these learnings will be incorporated. The work merits more cycles if the partner is still developing this strain, although I recommend fewer samples and getting through it more quickly.
- This project really needed a table of all metrics and tasks and percent completion toward them. This project fits well with the mission of both the ABF and BETO. The value proposition and impact to the U.S. bioeconomy is clear.
- It's not clear how many key performance indicators were in the process/milestones.
- Given the really low ability to actually build the recommended interventions, I would like to better understand why that was the case—whether that was a big deficiency of the ML model or the ability of Lygos or the ABF to synthesize constructs.
- The project focused on malonic acid production using the acid-tolerant, nontraditional host *P. kudriavzevii*. Project goals include leveraging multi-omic data sets to populate ML networks, making predictions for strain modifications to increase product formation, and iterating the DBTL cycle a total of six times. As described, the project was a good selection for the solicitation. Management plans were sparsely addressed, and ABF partners were not specified. Although complex, interdependent workflows were a recognized risk; communication plans were not provided. An initial set of 24 strains were cultured in fermenters, and omics data were obtained; however, the project was discontinued early. The overall impact is minimal due to failure to complete the project. Project strengths are as follows. Selection of malonic acid as the targeted product was well justified. Focus on Key Performance Indicator 2 over Key Performance Indicator 1 was appropriate and justified. The initial strains analyzed indicated the possibility of good advancement in strain development if it had been completed as described. Project weaknesses are as follows. No improvements in ML algorithms were realized. A description of the

>80,000 data points per cycle was not included (e.g., number of strains, time points examined, multi-omic methods). Strain engineering was a skill assigned to Lygos but appeared to be not as expected given that only a single gene deletion was completed. Whether reliable workflows and data collection schemes were developed remains unclear.

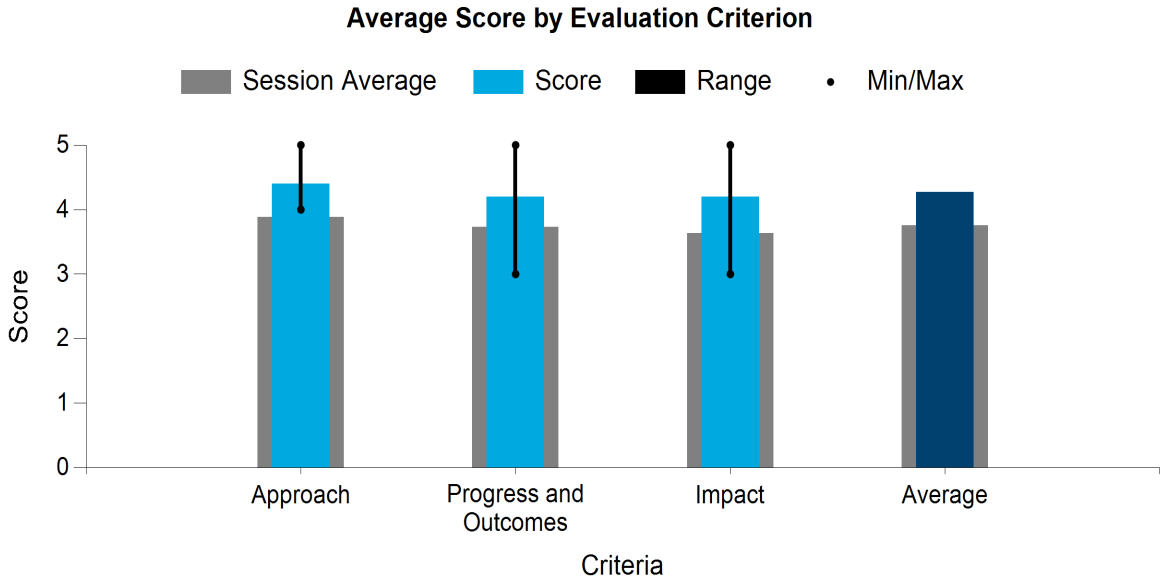
DEVELOPMENT OF *BACILLUS* AS AN INDUSTRIAL HOST FOR THE MICROBIAL PRODUCTION OF BIOPOLYMERS

ZymoChem

PROJECT DESCRIPTION

WBS:	2.3.2.213
Presenter(s):	Harshal Chokhawala; Thomas Mand
Project Start Date:	10/01/2018
Planned Project End Date:	03/31/2023
Total Funding:	$1,666,908.00

Biotechnologies that target high-value products hold immense long-term promise, yet efforts to bring these technologies to market are often hampered by low titers, high cost of feedstock, and organisms that are difficult to engineer. Thus, it would be beneficial to develop microbes, bio-based processes, and related process technologies that are tailored for utilizing sugar-rich feedstocks derived from low-cost lignocellulosic biomass. Biopolymers are an example of high-value bioproducts due to their ability to replace petroleum-based polymers used in many industries. *Bacillus* strains are currently used as industrial producers of some biopolymers. However, these fermentations generally require feedstocks supplemented with expensive components, which limits economic viability. To address this challenge, ZymoChem partnered with the ABF to develop genetic tools in a non-model strain of *Bacillus*, utilize the tools to construct a carbon-conserving strain of *Bacillus*, optimize biopolymer production in minimal media containing C5 or C6 sugars, and demonstrate biopolymer production at the 300-L scale. We demonstrated success in each of these endeavors and surpassed the final milestone titer by 3.6-fold through a combination of strain engineering and process development. With this successful partnership with the ABF, we were able to significantly decrease the overall risk to our commercialization efforts by lowering the cost of biopolymer production.



COMMENTS

- The carbon-conserving C5 pathway developed by ZymoChem represents an interesting production pathway that would improve the carbon efficiency of molecule production. The research work was designed to implement this pathway in a new organism while increasing overall strain performance. The

result of this work is a bit of a mixed bag: A host with the C5 carbon pathway was created, but its performance was poor, and the strain does not appear to be near commercial viability at current TRY levels. Further process optimization work and piloting appear to have been done on C6 sugars, and so do not use the carbon-conserving pathway. The benchmark used in the study also appears to be essentially a starved wild-type strain, so it is unclear why this serves as a useful benchmark instead of the wild-type alone, which is much closer to the performance levels of the engineered strain. The overall impact is therefore a bit muted; however, the toolkit developed within the *Bacillus* host may find use in further studies with ZymoChem or other users.

- Strengths:
    - The ZymoChem team has clearly benefited from strains and tools (promoter library) developed by the ABF.
    - The ABPDU helped to scale up the production and purification.
    - Clear improvement on titer and rate metrics, exceeding the project milestone. High titer (75 g/L) and high rate (2 g/L/h) were achieved. These values are impressive, although details on how it worked were not provided.
    - A majority of the proposed tasks were completed.
- Weakness/area for improvement: Elimination of sporulation was identified to improve product titers. It is not clear why removing sporulation would help. The final strain that produced high titer and rate seems to be from another mechanism. It is difficult to evaluate its scientific impacts.
- Thank you presenters for Slides 6 and 7 stating progress on all milestones and tasks! This is needed in all slide decks. There were also clear impact slides and value proposition to BETO and the U.S. economy. Overall for the ABF this is a great project. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. The work in this proposal clearly advances the capabilities of the ABF, and at the same time, the partner gains knowledge and access to capabilities it does not have in-house.
- This has been a highly successful project in which all the milestones and goals were reached. The overall project timeline seems a bit long to me for the improvements achieved, though it is not entirely clear what drove the timeline or if there were delays in contract negotiation, etc. The presentation of the data here was rather confusing—for instance, the scale of "titer" changes in each of the slides, at first being 10 g/L in the engineered strain (Slide 8), but then 45 g/L in the wild-type (Slide 14). It was not clear exactly which conditions the final improvement was under and whether that was the end result/goal that ZymoChem originally desired.
- An unidentified strain of *Bacillus* was engineered for production of an unidentified biopolymer. Project goals included implementation of ZymoChem's carbon conservation technology in a non-model organism, which required genetic engineering tool development. Fermentation and process development was also included. The project was a good selection for the BioEnergy Engineering for Products Synthesis FOA, whose areas of interest include development of non-model organisms and production of data sets to enable ABF learn methodologies and the metric of demonstrated titers exceeding 20 g/L. The project achieved good overall progress and has future commercialization potential. Project strengths are as follows. Project milestones and tasks were articulated for all participants. Strong progress was made in the development of genetic engineering tools for the non-model organism, which enabled forward progress on strain development. Transcriptomic data, which will have utility for future stain development strategies, enabled elimination of sporulation without a negative impact on biopolymer production.

Fermentation and process development for strains growing on glucose enabled significant improvement in product formation by the wild-type organism (45 g/L), while combined strain and process development afforded products (72 g/L) at 300-L scale. Project weaknesses are as follows. It is unclear whether the *Bacillus* strain is fully onboarded by the ABF for use by the community at large. The strain is not listed on the ABF website of onboarded organisms. Implementation of carbon conservation technology was not described beyond initial demonstration, which is disappointing given the 4-year project timeline. Strain development will be necessary before carbon conservation technology bears fruit: The carbon-conservation-enabled strain grew approximately twofold slower and produced fourfold less product relative to the wild-type. I'm curious about the long-term strategy for the carbon conservation pathway given the need for C5 feedstock streams. Ultimately it will be necessary to engineer the strain to co-utilize C5 and C6 feedstocks.

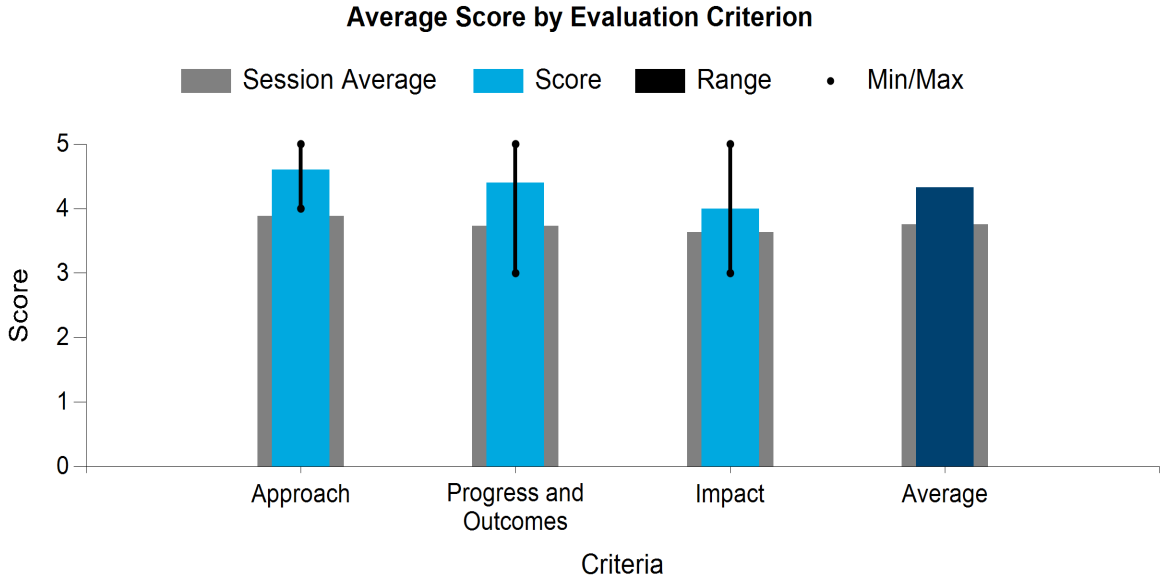
ACCELERATING POLYKETIDE SYNTHASE ENGINEERING FOR HIGH-TRY PRODUCTION OF BIOFUELS AND BIOPRODUCTS

University of California, Berkeley

PROJECT DESCRIPTION

WBS:	2.5.3.207
Presenter(s):	Jay Keasling
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2023
Total Funding:	$3,125,741.00

Polyketide synthase (PKS) enzymes have a modular, deterministic logic that holds the potential to act as a flexible chemical factory for the biological production of a huge diversity of valuable small-molecule compounds. However, engineering a custom PKS to produce a specific desired product currently requires years of trial and error, for reasons that remain poorly understood. In this project, we have developed a rapid, high-throughput DBTL cycle for PKSs and demonstrate its utility for production of material precursors. The objectives are to (1) develop a rapid, high-throughput DBTL cycle for PKSs that will enable production of a large number of unnatural, organic molecules on demand at high TRY; (2) demonstrate the utility of the PKS DBTL cycle to produce three molecules: one commodity chemical (caprolactam or valerolactam) and two novel material precursors (caprolactam or valerolactam derivatives); and (3) demonstrate the utility of the PKS DBTL cycle to increase the TRY of one molecule (caprolactam or valerolactam). In this project, we have successfully demonstrated our high-throughput PKS DBTL pipeline and have biologically produced valerolactam and several other novel nylon monomers.



COMMENTS

- The use of the well-understood PKS pathway to enable production of billions of potential molecules is an interesting approach with considerable potential. This research aims to improve the ability to create PKS pathways to targets without relying on extensive trial and error, the current state of the art. By utilizing ML and simulation tools, the thinking is that functional PKS pathways can be found more quickly. This research has demonstrated the ability to rapidly produce a wide range of pathways for

testing and validation, allowing a faster development process. However, the process still feels a bit "brute force," and it is unclear whether the learnings from the experimental setups are being passed back to ML models to improve their performance and efficacy. Ideally with more runs and data, the number of PKS candidate pathways that fail would be reduced, while the performance of pathways would also increase. Folding in tools like AlphaFold may be helpful in advancing the research. The impact of this approach is still limited by low overall performance (<1-g/L titers at lab scale), but this research may show a path to generating significantly more data to better train ML models and rapidly improve learnings to increase performance.

- Strengths:
    - Engineering PKSs offers enormous potential for bioproduction of chemicals with diverse but precisely defined structures. However, PKSs are notoriously difficult to engineer due to their complicated structures and folding issues. This team has obtained substantial success in expressing novel polyketides. The ability to build and test 100 PKSs in 9 months is impressive, considering the previous state of the art (one PKS for one Ph.D. in 5 years to complete).
    - The team has made excellent progress, achieving most milestones.
    - Production of four lactams, including three novel nylon precursors, was achieved. This result proved the possibility of building and testing novel polymer precursors from PKS libraries.
- Weaknesses/areas for improvement: N/A.
- This project was started in 2020 and is nearly completed. The goal of the project, essentially "develop a high-throughput DBTL cycle for PKS engineering, and test it by building a PKS to make the nylon monomers caprolactam, valerolactam, and novel derivatives," fits perfectly with the ABF mission to further synbio to produce industrially relevant molecules, and in this case has the bonus of harnessing the diversity of enzyme function to create new molecules. The project is making an array of valuable products, including several novel plastic (nylon) monomers with potentially better properties. They are using a much better host than the organisms that have PKS enzymes in them for faster growth and faster engineering.
- The project is ambitious to build an entire pipeline for high-throughput engineering and screening, which will be useful for many projects for a long time. The project will be very successful and meet its milestones and goals. It was very refreshing to see all the milestones listed and their state of completion. This needs to be required for all projects and AOP funding. They were honest in early struggles and failures. Industry is going to be more reluctant here to show struggles and failures, making the milestone list important. There was some legitimate concern about the difficulties and lack of progress midway through the project, but the project team overcame them and was ultimately successful.
- Also, all FOA and DFO reviews going forward should have an impact slide clearly stating benefits to the ABF and the U.S. bioeconomy.
- The project has been very successful based on meeting the ABF's and BETO's missions: "We have confirmed production of valerolactam and three additional novel nylon precursors with PKSs with titers from 1 to 10 mg/L … We have demonstrated that our build pipeline can build hundreds of PKSs at once in high throughput."
- This is overall a very strong project. The presentation did a great job at showcasing the project goals, milestones, and how milestones have been met. I appreciated their details on the diversity, equity, and inclusion (DEI) components of the work. I would also recommend that all presentations be required to

show milestones and their achievement of them. I would suggest that they should show all milestones at least in the appendix. While the eventual impact of developing PKSs as a biosynthetic pathway tool is large, the current project titers are still rather low (despite improvements from the state of the art), and further development will be needed.

- This projects seeks to design ML tools that enable successful prediction of PKS design, which would ultimately lower the barriers to PKS engineering and improve the efficiency of the DBTL cycles for those biosynthetic pathways that use a PKS. This approach represents a significant improvement relative to trial-and-error methods that are currently in use for novel PKS design. The management plan provides for regular meeting between all teams, and individual teams meet as needed. However, there is no indication of specific ABF personnel participating in the research program. Risk and mitigation plans are provided. One risk that should also be considered includes successful PKS gene synthesis and protein folding, but insufficient substrate throughput to detect and accurately identify the resulting product. It is also unclear how novel PKSs are being evaluated from the standpoint of product formation. For example, is product formation assessed for an *in vivo* process, or are enzymes purified and challenged with a variety of starter units? A commendable plan is in place to increase the diversity of project participants and to improve the development of young scientists by allowing them to take ownership of their assigned tasks. The project has made good progress. ClusterCAD has been shown to successfully demonstrate an automated approach to PKS design. Results have been disseminated in one peer-reviewed paper, and additional manuscripts are planned. ClusterCAD is available to the ABF and to the community at large. Quantitative end-of-project milestones are not likely to be met by the project end date. The impact of the project is jeopardized by modest PKS substrate throughput, which would limit applicability in the performance-advantaged materials space due to low TRY values. The impact is likely to be more significant when applied to identification of complex novel structures, which would include higher-value specialty chemicals and the pharmaceutical space. Methods for improvement of the catalytic properties of PKS enzymes should also be considered.

PI RESPONSE TO REVIEWER COMMENTS

- Comments: The use of the well-understood PKS pathway to enable production of billions of potential molecules is an interesting approach with considerable potential. This research aims to improve the ability to create PKS pathways to targets without relying on extensive trial and error, the current state of the art. By utilizing ML and simulation tools, the thinking is that functional PKS pathways can be found more quickly. This research has demonstrated the ability to rapidly produce a wide range of pathways for testing and validation, allowing a faster development process. However, the process still feels a bit “brute force,” and it is unclear whether the learnings from the experimental setups are being passed back to ML models to improve their performance and efficacy. Ideally with more runs and data, the number of PKS candidate pathways that fail would be reduced, while the performance of pathways would also increase. Folding in tools like AlphaFold may be helpful in advancing the research. The impact of this approach is still limited by low overall performance (<1-g/L titers at lab scale), but this research may show a path to generating significantly more data to better train ML models and rapidly improve learnings to increase performance.

- Response to Reviewer 1: Thank you for your helpful comments and suggestions. As you mentioned, this project was initially “brute force,” but with a goal of collecting enough PKS data to make future projects possible with much less trial and error. As our successfully producing strains/designs were only achieved recently, we did not yet have enough diverse examples of working designs to train our ML models until now. At present, we are just completing a large “build” round, which will provide enough positive working examples to inform ML models that require on the order of hundreds of examples. We are using a wide array of “features” to describe each design, including features computed from AlphaFold structures as you suggest. Now that we have working strains, we are also working to optimize

metabolism and growth conditions to improve TRY and have already identified several bottlenecks that will allow us to increase production titer substantially.

- Comments: Strengths: (1) Engineering PKSs offers enormous potential for bioproduction of chemicals with diverse but precisely defined structures. However, PKSs are notoriously difficult to engineer due to their complicated structures and folding issues. This team has obtained substantial success in expressing novel polyketides. The ability to build and test 100 PKSs in 9 months is impressive, considering the previous state of the art (one PKS for one Ph.D. in 5 years to complete). (2) The team has made excellent progress, achieving most milestones. (3) Production of four lactams, including three novel nylon precursors, was achieved. This result proved the possibility of building and testing novel polymer precursors from PKS libraries. Weaknesses/areas for improvement: N/A.

- Response to Reviewer 2: Thank you for your supportive comments.

- Comments: This project was started in 2020 and is nearly completed. The goal of the project, essentially "develop a high-throughput DBTL cycle for PKS engineering, and test it by building a PKS to make the nylon monomers caprolactam, valerolactam, and novel derivatives," fits perfectly with the ABF mission to further synbio to produce industrially relevant molecules, and in this case has the bonus of harnessing the diversity of enzyme function to create new molecules. The project is making an array of valuable products, including several novel plastic (nylon) monomers with potentially better properties. They are using a much better host than the organisms that have PKS enzymes in them for faster growth and faster engineering. The project is ambitious to build an entire pipeline for high-throughput engineering and screening, which will be useful for many projects for a long time. The project will be very successful and meet its milestones and goals. It was very refreshing to see all the milestones listed and their state of completion. This needs to be required for all projects and AOP funding. They were honest in early struggles and failures. Industry is going to be more reluctant here to show struggles and failures, making the milestone list important. There was some legitimate concern about the difficulties and lack of progress midway through the project, but the project team overcame them and was ultimately successful. Also, all FOA and DFO reviews going forward should have an impact slide clearly stating benefits to the ABF and the U.S. bioeconomy. The project has been very successful based on meeting the ABF's and BETO's missions: "We have confirmed production of valerolactam and three additional novel nylon precursors with PKSs with titers from 1 to 10 mg/L … We have demonstrated that our build pipeline can build hundreds of PKSs at once in high throughput."

- Response to Reviewer 3: Thank you for your supportive comments. We are glad that our presentation framework was effective in communicating both the challenges and accomplishments of this project.

- Comments: This is overall a very strong project. The presentation did a great job at showcasing the project goals, milestones, and how milestones have been met. I appreciated their details on the DEI components of the work. I would also recommend that all presentations be required to show milestones and their achievement of them. I would suggest that they should show all milestones at least in the appendix. While the eventual impact of developing PKSs as a biosynthetic pathway tool is large, the current project titers are still rather low (despite improvements from the state of the art), and further development will be needed.

- Response to Reviewer 4: Thank you for the positive comments and feedback. At the time of our presentation, our success in producing valerolactam and three additional novel nylon precursors was relatively recent. As described in our comment to Reviewer 1, after successful proof-of-concept production, we shifted much of the project to optimizing our PKS designs, growth conditions, and host metabolism. We have recently discovered that a key pathway precursor is being rapidly diverted to other metabolic pathways, and we are optimistic that our current efforts to divert this flux toward our nylon

precursor will result in substantial titer improvement. We have made substantial progress in improving TRY and have a clear path forward for further improvement. We believe the learnings from this project will also enable accelerated TRY improvements for other PKS projects in the future.

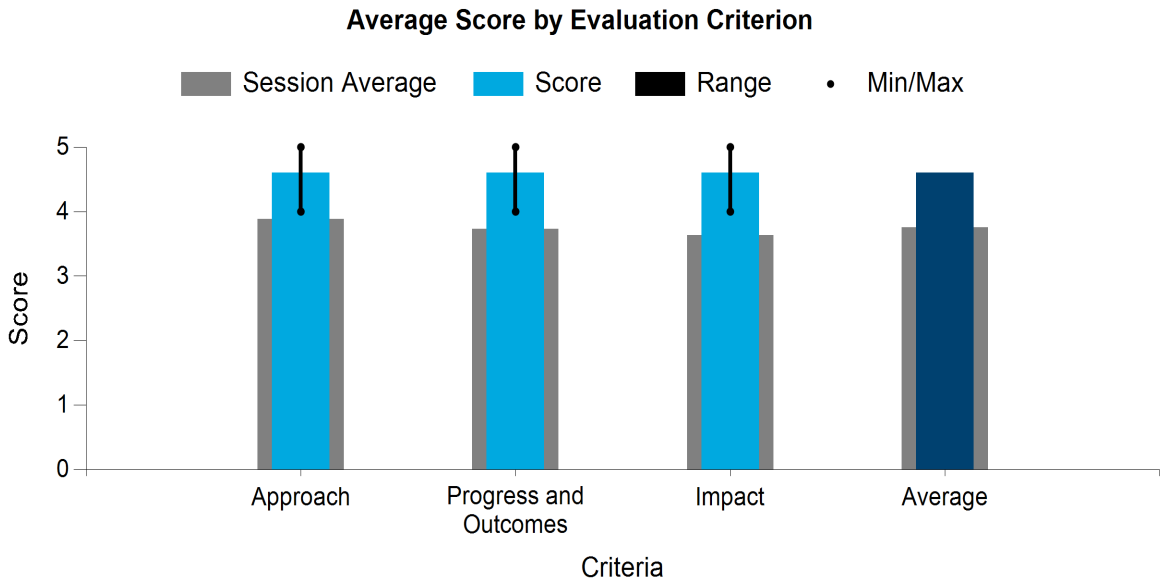
- Comments: This project seeks to design ML tools that enable successful prediction of PKS design, which would ultimately lower the barriers to PKS engineering and improve the efficiency of the DBTL cycles for those biosynthetic pathways that use a PKS. This approach represents a significant improvement relative to trial-and-error methods that are currently in use for novel PKS design. The management plan provides for regular meetings between all teams, and individual teams meet as needed. However, there is no indication of specific ABF personnel participating in the research program. Risk and mitigation plans are provided. One risk that should also be considered includes successful PKS gene synthesis and protein folding, but insufficient substrate throughput to detect and accurately identify the resulting product. It is also unclear how novel PKSs are being evaluated from the standpoint of product formation. For example, is product formation assessed for an *in vivo* process, or are enzymes purified and challenged with a variety of starter units? A commendable plan is in place to increase the diversity of project participants and to improve the development of young scientists by allowing them to take ownership of their assigned tasks. The project has made good progress. ClusterCAD has been shown to successfully demonstrate an automated approach to PKS design. Results have been disseminated in one peer-reviewed paper, and additional manuscripts are planned. ClusterCAD is available to the ABF and to the community at large. Quantitative end-of-project milestones are not likely to be met by the project end date. The impact of the project is jeopardized by modest PKS substrate throughput, which would limit applicability in the performance-advantaged materials space due to low TRY values. The impact is likely to be more significant when applied to identification of complex novel structures, which would include higher-value specialty chemicals and the pharmaceutical space. Methods for improvement of the catalytic properties of PKS enzymes should also be considered.

- Response to Reviewer 5: Thank you for your supportive comments and constructive feedback. We agree that it would have been useful for us to include a slide outlining which ABF personnel were involved in this project. Briefly, we have a host construction (build) team lead by Dr. Chris Johnson at NREL, an ML (learn) team led by Dr. Phil Laible at ANL, an ML (learn) team led by Dr. Hector Garcia Martin at LBNL, a proteomics (test) team led by Dr. Chris Petzold at LBNL, and a metabolomics (test) team led by Dr. Jon Magnuson at PNNL. Our PKSs have been evaluated for product formation *in vivo* using liquid chromatography/mass spectrometry. Thus far, we have detectable levels of product production *in vivo* for a number of our PKS designs, but it is indeed possible that some strains are catalytically active yet remain below limits of detection. For purposes of this project (optimizing these strains and creating ML models), we believe it would still be appropriate to categorize these PKS designs as expressed/folded proteins that are effectively catalytically inactive. Our Peer Review presentation was presented relatively shortly after producing our first successful designs, but we have subsequently identified and corrected some significant metabolic bottlenecks that allowed us to achieve large improvements in TRY, as well as some changes to our PKS designs themselves that improve catalytic activity, and we are optimistic that we will continue to improve TRY in the remaining months of the project. We agree that further research into optimizing PKS catalytic activity would be valuable here, especially in light of the extremely high TRY obtained in laboratory conditions with some natural PKSs.

DEVELOPING MULTI-GENE CRISPRA/I PROGRAMS TO ACCELERATE DBTL CYCLES IN ABF HOSTS ENGINEERED FOR CHEMICAL PRODUCTION

University of Washington

WBS:	2.5.3.212
Presenter(s):	Carol Rhodes; James Carothers
Project Start Date:	10/01/2019
Planned Project End Date:	12/31/2023
Total Funding:	$2,269,966.00



COMMENTS

- This is a core project that builds capabilities for the ABF that can be useful and applicable to a potentially wide range of industrial users. By developing new CRISPR activation (CRISPRa) and interference (CRISPRi) tools in two ABF host strains (CRISPRa to activate/maximize the production pathway, and CRISPRi to inhibit side pathways to other products), the team is building up the ABF library of information and tool set to apply similar approaches to other organisms in the future if needed for a different engagement. The work in this study validated the ability to build complex, multi-gene programs using CRISPR and applied this knowledge to 4-aminocinnamic acid production. The feedback loop, particularly on the ML side, is a further interesting factor in this research. However, the degree to which the feedback loop was closed and the level of enhancement achieved through the use of the ML tool was not well validated. Further cycles and improvements driven by the ML tool would be helpful in validating the ability of ML to shorten development times by reducing required cycle counts.

- Strengths:

  - Engineering multi-gene activation in non-model organisms to guide metabolic flux is an important tool to industrial biomanufacturing but has not been well developed. This team demonstrated successes in developing these tools.
  - The team used a combination of mechanistic modeling and an ML model to address the large combinatorial space of multi-gene programing; the approach is highly innovative and effective.
  - The PI developed a straightforward method to measure DBTL cycle efficiency. The method makes it very clear to quantify their progress and impact. More than 30% DBTL cycle time reduction was achieved. This can be a good model for the other ABF teams to follow—developing similar methods to quantify their DBTL efficiencies.
  - The team has achieved all the proposed milestones on time and demonstrated the build of six guides in 5 days, which is impressive.
  - Multiple high-impact publications resulted from this project.

- Weaknesses/areas for improvement: N/A.
- This is overall an academic and research and development (R&D) program that has large potential to help the field of synthetic biology and therefore U.S. biomanufacturing. For this reason it fits with BETO's mission. This work clearly fits well with the mission of the ABF. Thank you for Slide 14 showing all tasks and progress toward them. The project is clearly making good progress and has a high likelihood of being successful.
- This is a very relevant project that leverages the ABF's skills and expertise. The development of CRISPRa/i tools is useful for the synbio field and can open up new methods for gene expression tuning. The project has proceeded mostly on schedule and met several main milestones and deliverables, though from the later slides, it appears that less progress has been made on the second host and on the production of 4-aminocinnamic acid in *P. putida*. The current production levels are also very small (in the micromolar range), hence my lower score on the impact category. There is likely still a lot of development needed before there is strong impact for the production of the molecule of interest.
- Funded in response to the 2019 multi-topic FOA, work completed under this project appropriately responds to the area of interest that includes development of tools for use in the ABF that show increase in DBTL efficiency and production of data sets that enable the ABF learn methodology. The key metric was a 30% increase in efficiency in DBTL cycle time relative to the state of the art in the same organism. An ambitious plan was devised to create CRISPR-Cas expression tools capable of bacterial gene activation and inhibition followed by demonstration in two ABF hosts. The initial plan was to implement these tools in *P. putida* and *A. baylyi*, although a verification team seems to have recommended maximizing learning in the *P. putida* system at the expense of work in *A. baylyi*. This was a sound recommendation. The project has achieved good progress in creating and evaluating efficient new activation/inhibition tools for *P. putida* that should be applicable to other ABF organisms possessing basic genetic engineering tools. Publication of several manuscripts in high-impact journals ensures good visibility, particularly for an academic audience. Project strengths are as follows. Although it was frustrating that no information was provided in advance, the presentation slides are clear and informative. Project roles and milestones were well articulated. Definitions for gene activation and DBTL efficiency are provided, which enables measurement of progress toward quantitative milestones. Plasmid-based genes were activated in the range of 8–32 times, and genome-based genes were activated 1–29 times. Variability in the same gene activation is not a problem given the ability to quickly assess multiple constructs. Gene repression varied from 1–25 times. DBTL efficiency increased more than 30%. Project weaknesses are as follows. Although it was stated that the number of multi-gene CRISPRa/i

assessed simultaneously does not impact product formation, insufficient evidence was provided to support this conclusion. The choice of 4-aminocinnamic acid as the model system for assessment of their tool relegated product formation to exceedingly low concentrations (maximum 0.6 mM, about 0.1 mg/L). I would prefer to see the tool assessed on a well-defined pathway that affords higher product concentrations. This would enable a larger product range and rationalization of the impact of higher and lower gene expression. Future experiments in bioreactors should be reconsidered given the time frame remaining and the modest concentrations of 4-aminocinnamic acid produced.

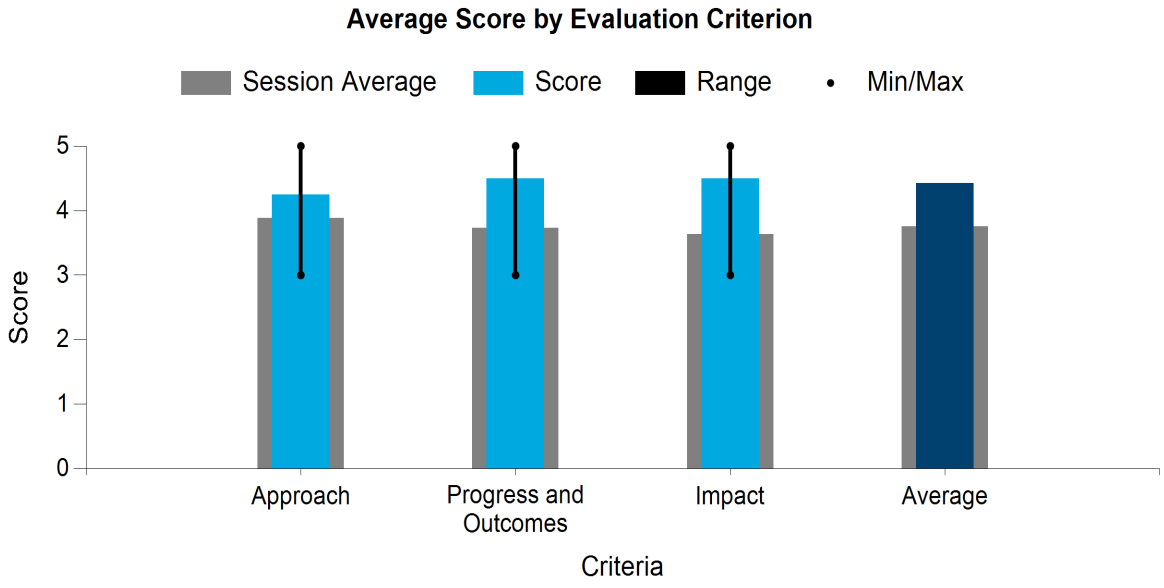
ABF DFO WITH C16 BIOSCIENCES

Sandia National Laboratories and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.707
Presenter(s):	John Gladden; Karthikeyan Ramasamy; Katarina Younkin; Michele R Jensen; Di Liu
Project Start Date:	02/20/2020
Planned Project End Date:	02/01/2024
Total Funding:	$1,449,000.00

Palm oil is the most widely used vegetable oil in the world; however, its production causes a variety of issues, including environmental damage and increased greenhouse gas (GHG) emissions. The motivation of the project is to overcome these issues by developing alternative palm-oil-producing approaches by engineering microbial-derived oils with equivalent properties. In this project, C16 Biosciences partners with the ABF to engineer *R. toruloides*, an ABF host, to produce mid-chain fatty acids and fatty alcohols as an alternative to products derived from palm kernel oil. The main challenge is that these chain lengths are particularly rare in nature and are not a naturally abundant fraction of microbial lipids. Here we leverage tools, knowledge, and capabilities previously developed in the ABF on *R. toruloides* and design a number of strategies to (1) engineer the fatty acid synthase, (2) bridge bioconversion gaps, and (3) address pathway bottlenecks. We further implemented functional genomics and multi-omic analysis to address these challenges. We have achieved our go/no-go milestone by reaching production of over 5% mid-chain fatty acids and fatty alcohols as a proportion of overall lipids or fatty alcohol profile. This project would enable C16 Biosciences to establish *R. toruloides* as a platform to produce a wide range of oleochemicals as a sustainable alternative to palm kernel oil, as well as to promote CO_2 emissions reduction and sustainable biomanufacturing.



COMMENTS

- C16 is developing a palm oil alternative using synbio processes. ABF tools are helpful to C16 in identifying strains and pathways to produce mid-chain fatty acids that can be optimized for its product and process. The ABF was successful in generating strains capable of producing the target lipids, and is currently in the process of working to better understand the bioconversion gaps that limit further

production. Decreased degradation of product is also targeted through further gene identification and strain design. The current results appear to require further optimization in order to produce commercially viable results, and it is not clear how ongoing work to optimize the current strain will yield the performance increases needed, although some detail was likely not provided in these slides due to IP concerns.

- Overall, this is a great project for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. The work in this proposal clearly advances the capabilities of the ABF, national labs, and BETO.

- The presentation lists several milestones that were achieved and go/no-go criteria in FY 2022. Presumably the project is going well, but next time, put all milestones and status (and percent complete if not complete). It's hard to judge without this info. Very good impact slide clearly stating the benefit to the ABF and the U.S. bioeconomy.

- This project is an excellent example of how core ABF work can be leveraged for commercial applications. ABF's prior work with *R. toruloides* helped enable the success of this project. The question posed leveraged ABF strengths in difficult strain engineering and metabolic-based discovery. The impact of the work on C16 and the overall palm oil industry is well articulated.

- The project between C16 Biosciences and the ABF seeks to engineer *R. toruloides* to produce mid-chain fatty acids and fatty alcohols as an alternative to palm kernel oil. The project leverages ABF *R. toruloides* expertise and multi-omic capabilities. The three-pronged experimental approach includes engineering Type II fatty acid synthase, identifying bioconversion gaps, understanding the mechanism, and eventually alleviating product degradation. This is a challenging project, but the plan is sound and the project timeline fortunately extends for 3 years. Functional genomics and metabolomics are in place as a mitigation strategy for unknown biochemical gaps that may be identified in the biosynthetic pathway and to guide strain development to reduce product degradation. Specific DBTL tasks were assigned, although communication plans were not provided. DEI was not addressed. Significant progress has already been achieved. Several iterations of fatty acid synthase engineering were evaluated, one of which provided an approximate tenfold increase in the relative level of mid-chain fatty acids compared to the control. Product concentrations were not reported, however. Although experimental details are limited, it seems that heterologous expression of one protein has increased mid-chain fatty acid product at least sixfold. Feeding studies and functional genomics have identified potential gene candidates that may be involved in product degradation. Given the additional 1.5 years on the project timeline, the prospects for additional advancements are high. As mentioned, product concentrations have not been indicated, so it is difficult to assess commercialization potential. It seems that the technical advances already achieved would justify venture investment in C16 Biosciences, should that be their goal.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for the positive evaluation and feedback of the project. As the reviewer suggested, we did not provide some technical details due to IP concerns. Our ongoing work focuses on (1) further engineering the fatty acid synthase based on our first two rounds of design and testing results, (2) identifying and bridging bioconversion gaps to enable and improve mid-chain product synthesis, and (3) addressing product degradation. We have clear research plans to optimize yields of current strains, with milestones and objectives to address these areas. We thank the reviewer for the suggestion, and we will be sure to put all milestones and their completion status next time. Regarding communication plans, we hold monthly meetings to discuss progress and next steps and have action items clearly laid out for each team. In addition, the project uses a shared team drive to share data, protocols, quarterly reports, presentation slides, and meeting follow-up discussions. We also hold meetings in smaller groups to discuss focused topic areas as needed. DEI is very important and integrated to the project, including team

composition, creating an environment for discussion to be inclusive for feedback from team members and leadership. We did not report product concentrations due to potential IP concerns. In that regard, C16 Biosciences has clear objectives on product concentrations to meet their commercialization plans.

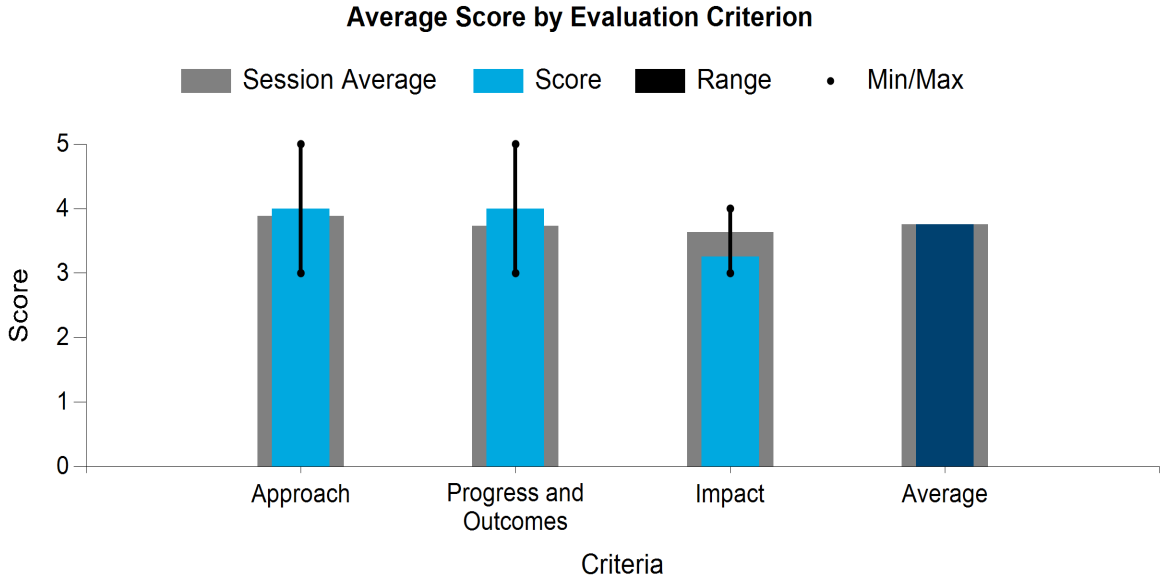
ABF DFO WITH THE UNIVERSITY OF DELAWARE AND WASHINGTON UNIVERSITY IN ST. LOUIS

Lawrence Berkeley National Laboratory and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.708
Presenter(s):	Deepti Tanjore; Katy Christiansen
Project Start Date:	02/26/2020
Planned Project End Date:	09/30/2023
Total Funding:	$500,000.00

The nonconventional yeast *Yarrowia lipolytica* is a promising biomanufacturing chassis well suited for production of oleochemicals and terpenoids, including biofuels such as limonene and bisabaline and other valuable chemicals of the carotenoid family. Scale-up of these processes, however, has been marred by phenotypic changes, such as loss of titer (or titer instability). This problem is exemplified by an engineered ß-carotene-producing strain that was developed as a platform for the production of b-ionone (https://doi.org/10.1186/s12934-018-0984-x) as part of a collaboration between co-PI Tang and Arch Innotek, a St. Louis biotech startup. In brief, the mevalonate pathway was optimized by enhancing flux from acetyl coenzyme A (CoA) to terpene precursors by overexpressing several upstream mevalonate pathway enzymes (push) and by reducing flux (block) toward squalene synthesis. Then, we overexpressed carB and carRP via genome integration to pull flux from geranylgeranyl diphosphate to ß-carotene, achieving approximately 4 g/L using benchtop bioreactors. The ß-carotene strain was further engineered by overexpression of a novel carotenoid cleavage dioxygenase, resulting in ß-ionone fermentation (approximately 1 g/L). However, when moving those engineered strains to larger bioreactors, cell performance significantly dropped. This problem has catalyzed the current collaboration between PI Blenner and co-PI Tang to investigate the nature of titer instability in this b-carotene strain as a model to understand more broadly the factor that led to cellular heterogeneity during cell line development and scale-up.



COMMENTS

- Understanding performance loss during organism scale-up is an important topic that can help enable future work by helping strain designers and process engineers optimize organisms and conditions for performance at scale. In this research, multiple hypotheses for performance loss during scale-up are presented and tested experimentally. The design of experiments utilizes amber systems to enable testing of a variety of growth conditions quickly. As of the time of the presentation, early results had demonstrated titer loss as a function of generation and culture conditions, providing useful data to further analyze (ongoing) and better understand mechanisms associated with performance loss. The applicability of this research may be limited to a set of conditions and a single organism, limiting impact. However, further experimentation with other hosts or comparison against other academic data may help draw learnings applicable more broadly.

- This is a great project overall for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. The work advances the capabilities of the ABF, and at the same time, the partner gains knowledge and access to capabilities it does not have in-house.

- The execution of the work plan appears to be going well from what was on the slide. Next time, put all milestones and status (and percent complete if not complete). It's hard to judge without this info. Also, all FOA and DFO reviews going forward should have an impact slide clearly stating benefits to the ABF and the U.S. bioeconomy.

- The overall work plan and approach is not the best approach to solve the problem. This is advice to the partner, not the ABF. The ABF is doing what the partner is asking them to do. Looking at Slide 11, I'd say there is, practically speaking, no difference in stability (instability). The relatively small shifts in instability aren't going to matter in a real-world manufacturing setting. Clearly the population is going to zero production pretty quickly, with a cliff at Day 2. No amount of media tweaking is going to solve that problem. There are other fundamental issues at play. The genomic sequencing is the obvious place to start. You probably could have just focused on one media condition. Even if the genetic rate of mutation might change in different conditions, that knowledge is not helpful or applicable to solve the massive instability. Clearly any media condition is leading quickly to zero production.

- Focus on the genetic mutations, and think about other fundamental issues that could be leading to such strong selection to shut down production. Also, try to add a kill switch for anything that shuts down production (i.e., addiction circuit that the ABF has developed for other projects). Sometimes you don't need to understand a problem to fix it.

- This project addresses important questions for biomanufacturing around the causes of cell heterogeneity and declines in performance across fermentation scales. The scientific approach presented is reasonable and leverages ABF capabilities. There have been several fermentation rounds completed. However, given that the project started in 2021 and several fermentation rounds have been done, I find the lack of data around any potential gene candidates concerning at this stage (the project is slated to end in September). As a result, the overall impact of the work is not as easy to gauge; however, elucidating the mechanism of decline in performance under larger-scale fermentation is a key problem for the synbio field and worthy of resources.

- The partnership between Washington University in St. Louis, the University of Delaware, and the ABF seeks to link multiple macroscopic causes (O_2, mixing, and shear stress) to the genetic instability and metabolism (i.e., product formation) of *Y. lipolytica* in a concerted fashion. The project leverages ABF omics expertise (proteomics and metabolomics) to understand the root causes of strain instability and production instability for this promising biomanufacturing chassis. Several hypothetical causes are presented. The ambitious experimental plan called for implementation of fermentation at an ABF site and

included several variations in what appear to be challenging culture conditions. Problems encountered in fermentations (drained reactors, foaming issues, and unusual mixing dynamics) were overcome but likely slowed progress. Risks and mitigation strategies were not provided. Responsibility for various data collection was assigned without indicating what communication plans were in place. DEI plans were not described. Loss of productivity was observed, as expected. Omics analysis has commenced on various samples. The project has two additional quarters before completion. The end-of-project milestones were overly ambitious and are not likely to be met. It is not clear from the presentation if multiple strains with varying gene expression were cultured, making it difficult to understand how optimal levels of gene expression will be determined. Whether strain engineering strategies will be evident from omics data remains to be seen. This project would have benefited from a tighter experimental plan—for example, selecting a specific culture condition that brings about a change in productivity/strain stability (pH, dilution, and O_2) and analyzing replicates at two values.

PI RESPONSE TO REVIEWER COMMENTS

- RESPONSE: Thanks for the comments. We agree that the study on other hosts can be useful. However, due to limited resources and challenges in this study, we cannot investigate other hosts. We look forward to continued investment from the ABF (and NSF) to support the importance of the work noted.

- RESPONSE: We thank the reviewer for their comments. We want to clarify that the goal of this project was not to solve the problem of instability, but to study it. It would be a company's justified approach to solve instability; however, we are not a company, and our interest is to understand *how* instability arises. We strongly feel that the ABF's capabilities are most useful for generating fundamental knowledge rather than solving low-hanging problems. We are still awaiting further data, but sequencing data suggest that something other than point mutations are at play. We are still analyzing the omics data and genome sequences. We hope to get more insights this summer. Moreover, we have some interesting results (e.g., use oil extractive fermentation process) that may improve strain stability by alleviating burdens and stresses. We'd never know this if we didn't focus on understanding the problem. We will be much more successful in fixing the problem once we develop the understanding.

- RESPONSE: Our project has been significantly delayed due to COVID. In 2021, the bioreactor facility was not fully operational, and all omics analyses and services are very slow. Another issue is the supply chain that delayed the purchases of chemicals and lab supplies. To continue this work, we have applied for an NSF-ABF grant to continue this project and resolve strain stability problems.

- RESPONSE: In this study, we had various bioreactor operation problems because long-term chemostat culture is always challenging. Additional repeated tests have been performed to overcome this problem. We had pre-meetings to coordinate project work and have monthly meetings to update on progress and make any necessary adjustments. The University of Delaware, Washington University in St. Louis, and ABF teams are meeting monthly to discuss the work plans and risk mitigation strategy. Also, we have collected various samples and are waiting for the omics results from ABF labs. We hope to get more understanding once these samples are analyzed. DEI plans, while valuable, are not a requirement of this project; nevertheless, we still demonstrated a commitment to DEI. A Ph.D. student, Alyssa Worland, has worked with the ABF lab for 9 months. She has accumulated rich experimental skills and data analysis experience. Moreover, an undergraduate student (Millie Savage) from Lincoln University (a historically Black university) is working at co-PI Tang's lab at the Washington University in St. Louis to help with yeast culture and data analysis this summer. Such student training is a part of workforce development for female and minority students. We have proposed to build strains with varying levels of gene expression and stability for the NSF/ABF project, a mechanism that better (at all) resources the academic partners. We hope this reviewer remains enthusiastic about this direction. We agree that the research plan could have been tighter. We did eventually come to this position, and finished with a tight set of conditions on the last runs.

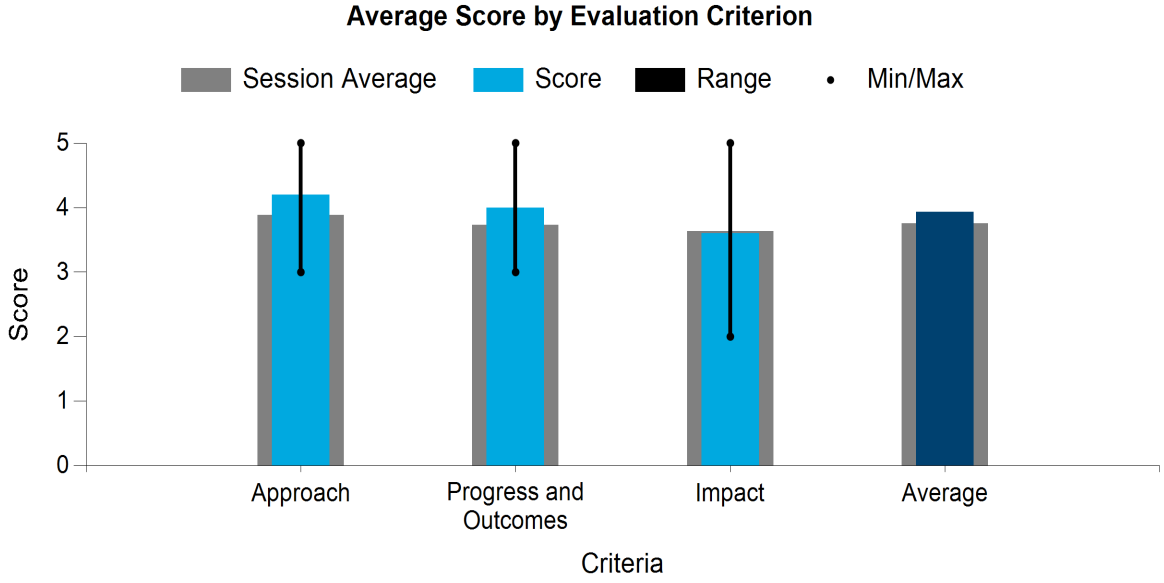
ABF DFO WITH ENDURO GENETICS

Los Alamos National Laboratory, Lawrence Berkeley National Laboratory, and National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.709
Presenter(s):	Deepti Tanjore; Katy Christiansen
Project Start Date:	03/05/2020
Planned Project End Date:	07/29/2023
Total Funding:	$500,000.00

A major challenge to industrial development of new bioprocesses is scale-up. Engineered production strains experience significant selective pressures caused by metabolic burden and toxicity of heterologous overexpression. This selective pressure promotes growth of low- or non-producing cell variants, and thus leads to production decline at scale. Enduro Genetics is a startup that uniquely and directly addresses the challenge of cellular culture heterogeneity. Enduro has pioneered an analytical algorithm based on next-generation sequencing to uncover and quantify genetic heterogeneity throughout the different stages of a fermentation (https://doi.org/10.1038/s41467-018-03232-w) and is working with industrial companies to investigate the culture heterogeneity that arises in their processes. In this DFO project, the goal is to understand the causes of heterogeneity, especially in large bioreactor runs. We will also apply customized gene circuits to prevent loss in productivity in the ABF strains.



COMMENTS

- Scaling is a particular challenge in biological systems, as larger reactors have a tendency to select for organisms that are nonproductive. In order to overcome this limitation, this research looks to couple the production gene with a key survival/growth gene in the organism. The approach is potentially applicable to a wider range of organisms and targets. Preliminary results show that engineered strains are outperforming wild-type strains by a significant margin at 300-L scale. Fermentation studies will validate the approach further. Learnings from this study can be used by the ABF for other hosts and targets that are facing scaling challenges.

- Strengths:
    - The team has identified essential genes that can be used in *P. putida* for selection of stable producing strains.
    - Early evidence was obtained and showed that Enduro's strain outperformed the wild-type strain at 300 L.
- Weaknesses/areas for improvement:
    - Because Enduro is a Europe-based company, it is not clear how this project can benefit the U.S. bioeconomy.
    - The connection between work done in *P. putida* at NREL and *B. subtilis* at ABPDU is unclear.
- This is a great project overall for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. Enduro's value proposition to the bioeconomy and biological manufacturing through fermentation is very large. Strain instability is a major problem all synbio companies have to overcome, and having off-the-shelf tools to fix it would be enormously helpful. The work advances the capabilities of the ABF, and at the same time, the partner gains knowledge and access to capabilities it does not have in-house.
- The execution of the work plan appears to be going well from what was on the slide. Next time, put all milestones and status (and percent complete if not complete). It's hard to judge without this info. Also, all FOA and DFO reviews going forward should have an impact slide clearly stating benefits to the ABF and the U.S. bioeconomy.
- This project addresses a highly relevant problem in bioprocessing. The lessons learned and tools developed for this will have impact beyond the specific project. The project demonstrated a successful increase in the production of the target molecule at 250 mL. It was not clear why the 300-L results were still pending if three out of the four campaigns at that scale have been completed. The project is slated to end in September 2023, but no work on *P. putida* was presented or appears to have been done yet, which raises some questions on the feasibility of achieving the full project goals.
- The project between Enduro and ABF aims to address culture heterogeneity in large-scale fermentation by coupling cell growth to production. The approach includes concept demonstration with Enduro-prepared *Bacillus* strains and porting the technology into *P. putida*. The project leverages ABF expertise in *P. putida* engineering, fermentation process development and scale-up, and genomics. Seven essential genes in *P. putida* have been targeted, for which three constructs are ready for insertion and four of which remain under construction. Three *Bacillus* strains have been cultured at 250-mL scale at ABPDU. Risk management, communication plans, and DEI were not provided. For the *Bacillus* strains analyzed, product signal is amplified in the range of 9- to 14-fold over wild-type at 250-mL scale. Product identity and concentrations are not provided. Deep sequencing results are not provided. Two quarters remain before project completion. It is not possible to determine commercial potential currently. It was reasonable to select as many as seven *P. putida* targets to evaluate the concept, but extending the project to 3 years would have enabled a more thorough evaluation of the technology in *P. putida*.

PI RESPONSE TO REVIEWER COMMENTS

- RESPONSE: We thank the reviewer for taking the time to review us and provide feedback. We look forward to contributing our learnings for ABF to use in the future. We plan to publish at least one paper from this work.

- RESPONSE: The project will benefit the U.S. bioeconomy by creating joint learnings in a strong background within fermentation scale-up—an important field to the U.S. bioeconomy. By integrating work from both sides of the Atlantic, it is expected that the U.S. bioeconomy will be able to leverage advances in Europe. For the work done in *P. putida*, Enduro's technology will be applied to a U.S. strain development project. The work in *P. putida* at NREL uses the same underlying technology for product addiction as in *B. subtilis* at ABPDU, thus testing the technology in two different systems and stages of development.
- RESPONSE: Thank you for the comments and suggestions. We will make sure to follow them going forward.
- RESPONSE: For *Bacillus*, at the time of submission, there were some questions on whether minor medium differences between the runs at 300 L precluded direct 1:1 comparison. Further, it appeared that one run at 300 L might have been subject to at least partial contamination. This is currently being investigated. For *P. putida*, as reported in our presentation, strain construction is nearly complete, and we anticipate initial strain evaluation to commence soon. Following that, downselected strains will be scaled up to evaluate strain stability, which we expect to be able to complete by the end of the project. This project start was delayed due to COVID, so we expect to extend until December, if necessary.
- RESPONSE: Due to the very limited amount of time allotted to presenting these projects, we were not able to include risk management. Communication and DEI are broadly relevant to the ABF, and as such, were presented elsewhere. We agree that the project may have benefited from a longer time frame, but budgetary constraints favor shorter collaborations, and we believe we are on track to complete this project in the time we have.

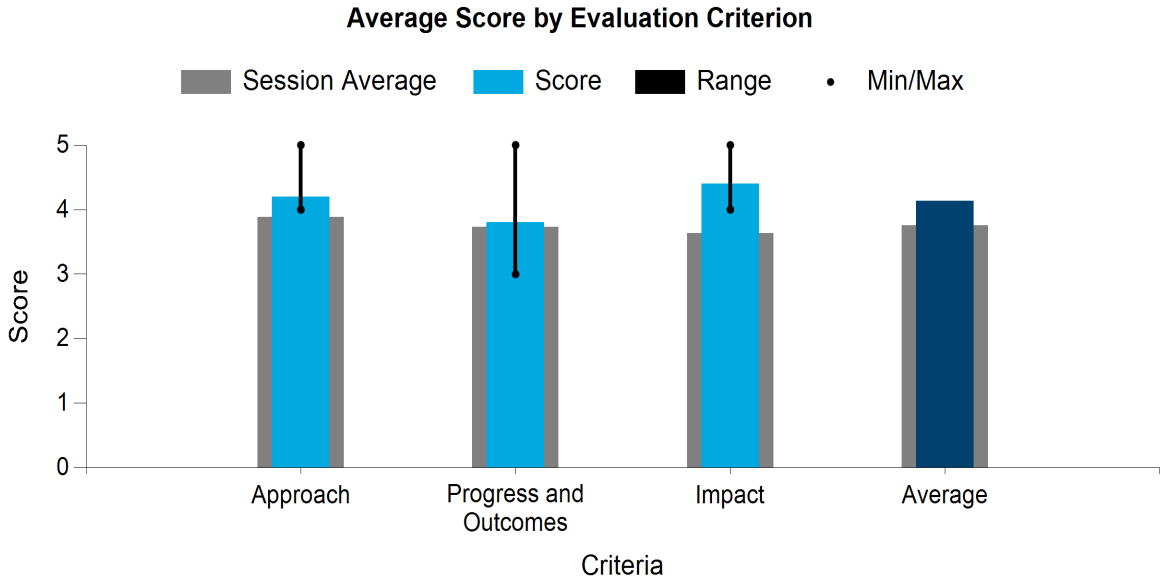
ABF DFO WITH SUPERBREWED FOOD

Los Alamos National Laboratory, Oak Ridge National Laboratory, and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.711
Presenter(s):	Adam Guss; Karthikeyan Ramasamy; Michele R Jensen; Tim Theiss
Project Start Date:	02/24/2020
Planned Project End Date:	05/05/2024
Total Funding:	$1,400,000.00

A major limitation of traditional anaerobic fermentation processes is the low mass yields of products due to the decarboxylation of pyruvate to acetyl-CoA. Most products have a maximum theoretical carbon yield of 66%. To help overcome this inefficiency, Superbrewed Food has developed a novel fermentation platform called MixoFerm, combining sugar fermentation with the addition of additional electrons in the form of H_2. This allows theoretical yields to approach 100% carbon yield. Strains have been engineered to produce isopropanol using this approach, but the resulting strains are unstable. In this project, we are investigating the mechanism(s) of strain instability and decreasing performance to help understand the phenomenon and devise strategies to overcome it. We are also developing biosensors that can detect the presence of isopropanol and control the expression of an essential gene, enabling the selection of strains with improved isopropanol production. Together, these approaches will result in the generation of enhanced isopropanol-producing bacterial strains and provide insight into mechanisms of strain instability.



COMMENTS

- This research is aimed at using ABF expertise to further optimize the performance of a strain for Superbrewed Food that makes isopropanol. The approach uses a biosensor circuit in order to detect strains producing the product of interest, with a goal of using the sensor to identify strains with higher performance, which can then be isolated. A further effort to link the biosensor to survival should drive evolution toward strains of higher performance. This project is now in flight, with fermentation runs completed and results sent for further analysis at the national labs. Results are pending for whether

targets can be identified that improve performance. If successful, this research will point toward a potential optimization pathway that would be helpful for other users as well.

- Strengths:
    - This project aims to address strain instability during isopropanol production, which is a common problem in microbial fermentation that leads to diminished performance. Knowledge learned from this project may be applicable to other bioproduction systems.
    - The combination of sequencing and omics analysis may reveal insights into the mutation mechanism.
- Weaknesses/areas for improvement:
    - This project seems like it is just getting started; not much progress was reported.
    - The project aims to address cell-to-cell variations in fermentation, but no single-cell-based analysis technique was proposed.
- This is overall an excellent use of ABF technology and expertise to advance the bioeconomy and help a smaller company with infrastructure it does not have. It advances both the ABF and the company. It's great to see the impact slide for projects clearly stating benefits to the ABF and the U.S. bioeconomy. This should be required on all FOA and DFO slides going forward.
- An addiction switch is a very good idea and is likely to work. The only criticism is that sometimes you don't need to fully understand the problem if you have a way to fix it, like in the addiction switch. Some of this might be overkill; just try the addiction switch first and see if it fixes your problem sufficiently.
- Also, next time, put all milestones and status (and percent complete if not complete).
- This is an interesting project that leverages ABF's skill and capabilities. The development of biosensors to track production outputs is likely to be useful in other projects and applications, particularly for anaerobes. Though the project only started last year, I was surprised that there was not any substantial data presented; it would appear the project may be behind schedule.
- The partnership between the ABF and Superbrewed Food seeks to understand the cause of and identify solutions to strain instability that occurs over time in the anaerobic Superbrewed Food process for isopropanol production. The approach leverages ABF genomics, proteomics, metabolomics, and biosensor development expertise. Anticipated challenges/risks are the difficulty of working with anaerobic organisms and the fact that omics data obtained from bulk culture samples or from individual colonies may not reveal the cause of instability. One risk mitigation strategy to be pursued includes construction of a biosensor to enable selection of improved strains. This work has not yet been initiated. Communication between team members occurs monthly. DEI plans were not specifically addressed. The project is in Q3 of a 2-year project, so progress is difficult to assess, although strains have already been cultured and omics work is underway. The project impacts for all partners, including Superbrewed Food, ABF, and the U.S. bioeconomy, were clearly and accurately stated. Assessment of commercialization potential is inappropriate at this point in the project timeline.

PI RESPONSE TO REVIEWER COMMENTS

- Thank you for the helpful comments. Initial work was slowed due to a contamination event in the bioreactor, but that has now been fixed and work is proceeding. For single-cell work, molecular methods typically rely on extensive polymerase chain reaction amplification, which is known to skew some

results. Therefore, we are initially focusing on (1) bulk culture sequencing and measurements to understand general trends, and (2) sequencing of colonies derived from single cells in the bioreactor population to look at genomic changes that occurred in individual members of the population. If need be, we will consider trying single-cell approaches to gain more information.

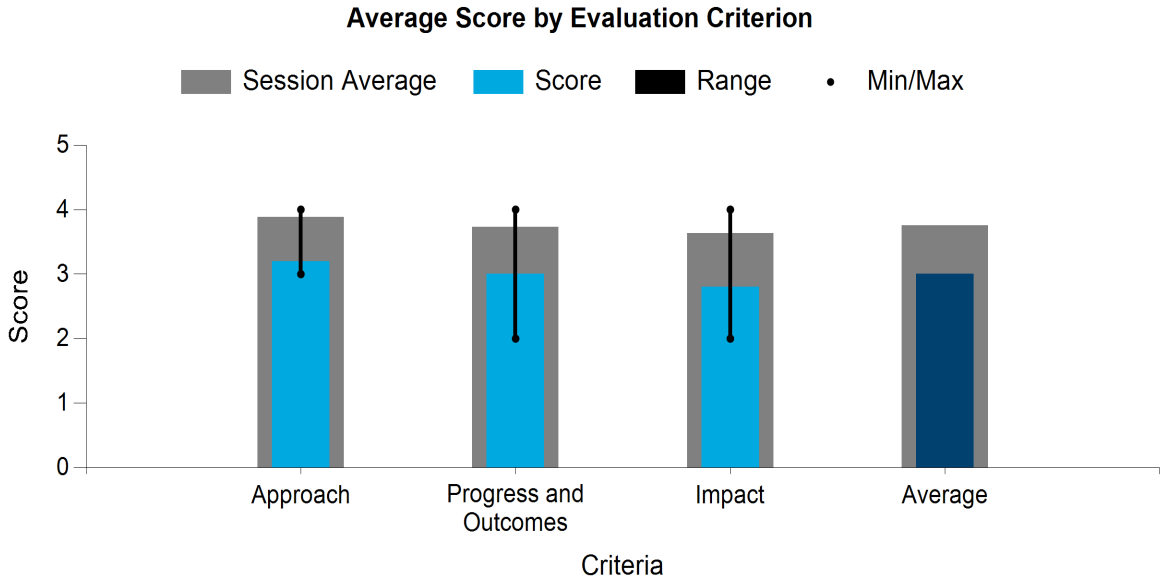
ABF DFO WITH KALION INC.

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.712
Presenter(s):	Laura Hollingsworth; Megan Krysiak; Michelle Reed; Violeta Sanchez i Nogue
Project Start Date:	04/02/2020
Planned Project End Date:	10/22/2022
Total Funding:	$425,000.00

Kalion Inc. is an industrial biotechnology manufacturer of high-purity chemicals from biomass, including glucaric acid, to be used in the industrial, material, and pharmaceutical markets. The project goal is the application of ML in conjunction with high-throughput cultivations and metabolomics to understand and overcome two different challenges: (1) improve glucaric acid productivity (the rate of production slows considerably after 48 hours, which limits the overall productivity that can be achieved in the process); and (2) decrease its production costs (components from complex media are necessary to achieve robust production). Through a series of experiments, a set of metabolites contained in rich media that improved performance were identified using metabolomics, high-throughput cultivations in bioreactors, and ML-friendly experimental designs. It was found that the substrate feeding strategy was the potential major driver to enhance bacterial performance of glucaric acid production. The major industrial impacts from this project are improving the production process technology, as well as advancing infrastructures and workflows (where ML approaches in conjunction with high-throughput cultivation and metabolomics are used) to improve the analyses by making them more rapid and comprehensive.



COMMENTS

- The ABF worked with Kalion to improve strain performance of glucaric acid production, primarily through changes to media. Given the complexity of the media and difficulties untangling the impacts of various media components, a structure experimental design was used to perform rapid media screens, with the intent to use data as part of ML training. The results of the experiments identified some key media components, primarily sugar concentration, that impact final titer. The experiments did not seem

to produce any surprising results, and the ML feedback loop was not clearly closed, so it is unclear how the ML tool was utilized in the experimental process. The use of this ML tool to predict applicable media was not discussed.

- Strength: The team benefited from the high-throughput cultivation and metabolomics analysis tools to analyze glucaric acid production in Kalion.
- Weaknesses/areas for improvement:
    - The discovered results—higher glucose concentration leads to higher product titer—are mostly expected, and thus have limited scientific impact.
    - Only different media compositions were analyzed; the project can further benefit from ABF's strain engineering capabilities.
    - It is not clear what the proposed milestones are and how well the team has achieved these milestones.
- This is a great project overall for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. It's not clear from this slide deck what the value proposition is to the bioeconomy of glucaric acid; the assumption is that a company would not be going after it if there was not good TEA. It is not clear what the demand from the chemical industry is.
- Next time, put all milestones and status (and percent complete if not complete). It's hard to judge without this info. Also, all FOA and DFO reviews going forward should have an impact slide clearly stating benefits to the ABF and the U.S. bioeconomy. This project is especially hard to evaluate as there was no mention of milestones or progress toward them. There has been progress and learning on media optimization and metabolomics work.
- The work in this proposal clearly advances the capabilities of the ABF, and at the same time, the partner gains knowledge and access to capabilities it does not have in-house.
- The ABF implied that the BioLector high-throughput fermentations have a lot of variation, which makes it hard to conclude data and use for artificial intelligence. After years of trying to prove the usefulness of these instruments, the company I work for has officially shut down all work on these systems for that reason. They are too unreliable and can't get good replications, among a lot of other technical issues. The ABF should consider getting rid of them and investing in more 250-mL Ambr tanks for moderate-throughput fermentations.
- The presentation of this project was rather confusing. There are two stated goals: improving productivity after 48 hours and finding less complex media that can achieve similar results. There are no results or lessons learned presented for the first goal. On the second goal, the slides put forth confusing information. In Slide 4, it is not clear whether myo-inositol was being added to the media. Also, glucose is not identified in the list of 14 relevant metabolites, so it is not clear why that is the focus of later work as opposed to other sugar sources such as mannose, which is identified. The graph on Slide 5 does not have a legend for colors, and as such is basically impossible to interpret. The title states that only the 2a sugar group saw an increase in titer, though the results appear nearly identical to those from glucose (and other treatments). The increase in titer from increasing glucose shown on Slide 5 is very mild: A tenfold increase in glucose leads to less than a twofold increase in titer. It is not clear whether having a media so rich in glucose would be any better than the current media that Kalion is using. The impact of the project on Kalion's practices or any future work they might do in line with project goals is not discussed.

Concluding that a microbe will make more product when fed more sugar seems like a very obvious result. Overall, this project was pursuing a very basic question and delivered meek results.

- The project between Kalion and the ABF seeks to apply ML and metabolomics to improve glucaric acid productivity and decrease production costs. One challenge/risk provided by the project team is the possibility that scaling to 2-mL bioreactors for media screening could limit productivity and product profile resolution. No mitigation strategy was provided, and there was no indication that product profiles between 2 mL and 0.5 L were compared. No information was provided regarding project management, communication, or DEI. I have several reservations about the experimental plan and data analysis. Glucaric acid production was demonstrated in 0.5-L bioreactors in several rich media. Presumably these media were not supplemented with myo-inositol. Metabolomic results are reported (volume not specified) for media supplemented with myo-inositol, which is two steps removed from glucaric acid. Decreased concentrations of metabolites when myo-inositol is converted to product are not necessarily representative of metabolites when glucaric acid is synthesized from glucose. In a different experiment, it was concluded that glucaric acid production improved when a group of sugars was added to the media; the data shown do not support this conclusion. A training set was generated from a particular experimental campaign to increase learning, but I see no evidence of ML to alter media, culture conditions, etc. It seems that insufficient resources were available to complete the experiments originally planned. Although project continuation may yield useful additional data, at this time commercialization potential is not evident. The impact of this project for ABF tools/resource development was not evident.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewers' comments. Because the project ended in February, we did not include the completed milestones, but we recognize that would have helped the reviewers. Over the course of this project, some research questions tangential to the main project goals arose, and given the time and budget constraints, we decided to prioritize efforts toward those that could be of immediate benefit for Kalion while advancing ABF's infrastructure and workflows.

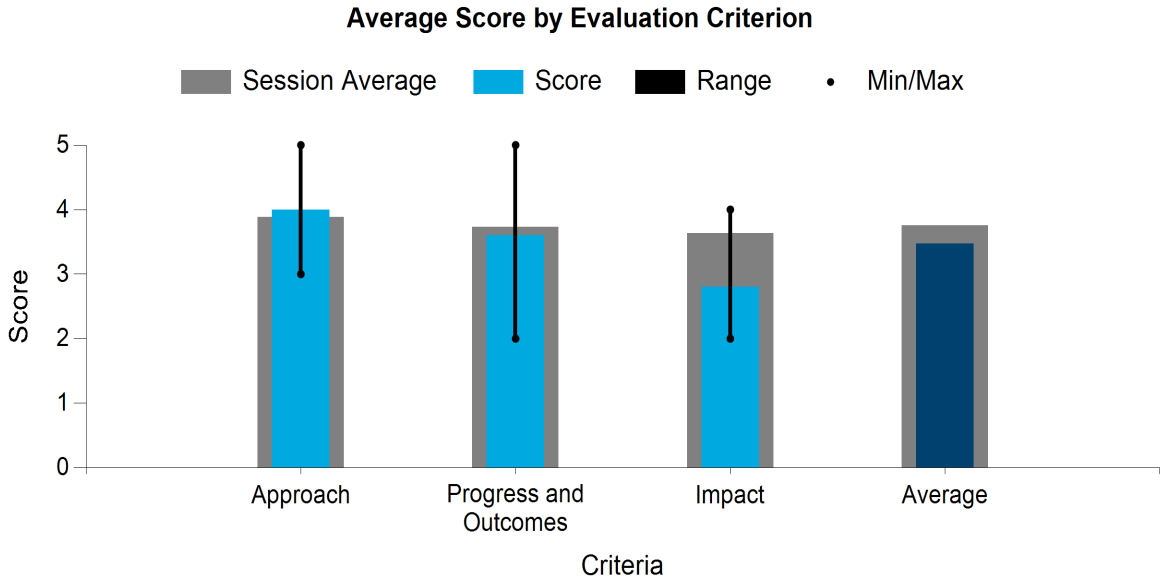
ABF DFO WITH INVAIO

National Renewable Energy Laboratory and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.713
Presenter(s):	Jon Magnuson; Katarina Younkin
Project Start Date:	03/23/2020
Planned Project End Date:	01/01/2022
Total Funding:	$410,000.00

This project is a partnership between Invaio, with their pioneering platforms for identification and development of active biologics, and two national labs (NREL and PNNL) in the ABF who are applying their strengths in fungal genetic engineering and fermentation scale-up to the expression of Invaio's valuable antimicrobial peptide (AMP) against a bacterial pathogen of citrus. Currently, the widespread use of biologics in crop protection is severely hampered by the lack of available tools for their mass production. This project is focused on developing an efficient bioprocess to produce and secrete the AMP in an ABF protein/peptide production host. A few viable hosts were examined for tolerance to the AMP. *Aspergillus niger*, which produces products in the generally regarded as safe category, was selected for expression of the heterologous AMP. AMP production has been verified by targeted proteomics using internal peptide standards, and culture optimization efforts continue to maximize titers. Developing the precursor to a scalable production process for this AMP is beneficial for Invaio as a foundation for further development and commercialization of this AMP, and is beneficial for the ABF by building a platform strain for production of other peptides and proteins, as well as procedures to approach the development of other protein production hosts.



COMMENTS

- This project represents a good use of ABF resources, wherein a target product/molecule is identified and the ABF is brought in to help develop the biologic tools to generate the molecule of interest. In this case, the ABF used its expertise to find a suitable strain, engineer the strain for molecule production, and then begin optimizing that strain. Progress is ongoing, with performance targets below desired levels. The muted performance of the strain at current scales puts the strain far from commercial viability and limits

impact. Further positioning by the ABF to demonstrate pathways to commercially relevant TRY levels is recommended.

- Strength: This project benefits from the fungal production strains developed at the ABF. *Aspergillus niger* was shown to be the best AMP tolerance strain.
- Weaknesses/areas for improvement:
    - Current AMP titers and yields are way too low. AMP is less than 0.1% of total proteins produced by the best host. Its impact to commercial AMP production is very limited.
    - Only media optimization was discussed to improve AMP production. There was no mention of the use of any genetic strategies to improve AMP titer.
    - The project lacks preliminary TEA. It is unclear whether bioproduction of AMP is economically viable given the measured AMP toxicity.
    - The use of a large peptide fusion may result in a low carbon yield.
    - This project does not seem to align with BETO's mission on GHG emissions reduction or bulk chemical/SAF production.
- Objective: Develop a high-yield, cost-effective, large-scale fermentation bioprocess to produce an AMP with Invaio; collaborate with the team at Invaio to develop an expression/secretion host for peptides with antimicrobial action.
- Very good alignment with the goals of the ABF to advance industry and the bioeconomy. Not so much aligned with BETO on SAF and decarbonization, and that is OK. ABF should be supporting the U.S. bioeconomy to advance new products for a more sustainable planet as its mission.
- Very good; the project showed all milestones and reported on progress. They said there were tight timelines and a small budget, so it's impressive what they were able to accomplish. Also, all FOA and DFO reviews going forward should have an impact slide clearly stating benefits to the ABF and the U.S. bioeconomy.
- The path to commercialization is on track, but it's still pretty early stage (technology readiness level [TRL] 4). This is why "Impact" got a 4.
- This was a straightforward project that leveraged ABF capabilities; however, the alignment to BETO priorities and goals is not very clear. It also did not have as much of a potential impact of technology development as other ABF DFOs. The project end is set as April 27, 2023, and several tasks were still rather uncomplete at the time of Peer Review (Milestones 3 and 4). The impact of the work was not well explained. What is the application of this peptide? The intended market size? What is it replacing? Only agriculture is mentioned in an early slide, but no details are provided. As such, judging the impact is difficult.
- The partnership between the ABF and Invaio developed a sound approach to use ABF fungal hosts for expression of an AMP. This approach leveraged ABF fungal genetic engineering for heterologous protein expression, proteomics, and process development/scale-up expertise. The team appropriately mitigated anticipated risks by assessing host sensitivity to AMP and constructing multiple (eight) transgenic strains in two different fungal species. Communication mechanisms and frequency between the ABF and Invaio were not addressed. DEI approaches were not addressed. Good progress was made on generating and evaluating strains, modifying media, and detecting product. Unfortunately, product concentrations were

quite modest (0.33 mg/L). Whether the detected product was unadulterated gla1-AMP was not addressed, although identical sequence to AMP was listed as a project must-have. The end-of-project milestone to conduct 100-L bioreactor cultivation is not warranted given current product levels. Significant improvement (103-fold) in AMP expression must be realized before meeting the low end of product concentration stated in the end-of-project milestones. Without these improvements, commercialization prospects cannot be assessed.

PI RESPONSE TO REVIEWER COMMENTS

- We would like to thank all the reviewers for their constructive comments and feedback. One reviewer recommended that all ABF "funding opportunity" project reviews have an impact slide clearly stating benefits to the ABF and the U.S. bioeconomy. We appreciate the comment and will emphasize such impacts more in the future. The potential impact to the U.S. bioeconomy from the Invaio project stems from the need for additional methods to target plant pathogens and protect crops. Biological antimicrobials and pesticides have the potential to decrease the use of more toxic chemical agents and pathogen resistance that often accompanies the extensive use of such agents. The initial development of a fungal process for expression of the AMP in this project provides a foundation for further strain and process development and future commercialization. For the ABF, the expression vector and genetically modified fungal strain developed within this project has the potential to be a chassis for production of other peptides and proteins (including enzymes) of high relevance to biofuel, bioproduct, and biomaterial production. As noted by many of the reviewers, this project is not aligned with BETO's current focus on SAF. The year that this project was proposed predated that emphasis, but admittedly, AMPs are not products that fit into the commodity chemical realm. The economic and sustainability impact of biologicals would be more indirect in avoidance of waste and monetary losses due to crop failures, repeated applications of pesticides to kill the insect carriers of the bacterial plant pathogen, destruction of diseased trees, etc. Both TEA and LCA would be instructive regarding those impacts in the case where AMPs were successfully employed for citrus greening disease prevention and treatment.

- The project is aligned with the ABF's goal to reduce the time and cost to market of bioproducts, and to accelerate microbial strain and process development for industrial deployment. Production of a soluble and secreted AMP was a significant step forward from previous biotechnology development for this AMP. The scientific challenges encountered were very instructive for future peptide/protein bioprocess development. We appreciate the reviewers' comments noting the significant challenges and progress in this project. Producing an AMP in a microbe was certainly a high-risk task, and that was noted in the proposal. To mitigate that risk, Task 1 focused on addressing that immediately by selecting the best host available, with a decision point in that regard. The ABF has received positive feedback from Invaio, which noted the progress made in peptide expression vector and strain development, detection of the AMP, and titer increases through genetic modification and bioprocess optimization. The reviewers noted that despite the progress, there is still a need for further improvement. The ABF and the Invaio team recognize the need for significant increases in AMP titer before moving forward with significant scale-up and commercialization. The smaller-scale (0.5–2-L) bioreactor work was used to assess performance gains that could be achieved by moving from small tube and flask cultures to a more realistic bioprocess environment without the expense of a large bioreactor run. Multiple approaches were discussed with Invaio on paths forward, including additional genetic modification strategies and bioprocess development. Those could be the foundation of future work, but the limited project resources remaining will be focused on peptide purification and efficacy testing to demonstrate proof of principle for the initial process developed in this project. IP protection will be further discussed with the company, and then we intend to publish results in a peer-reviewed journal.

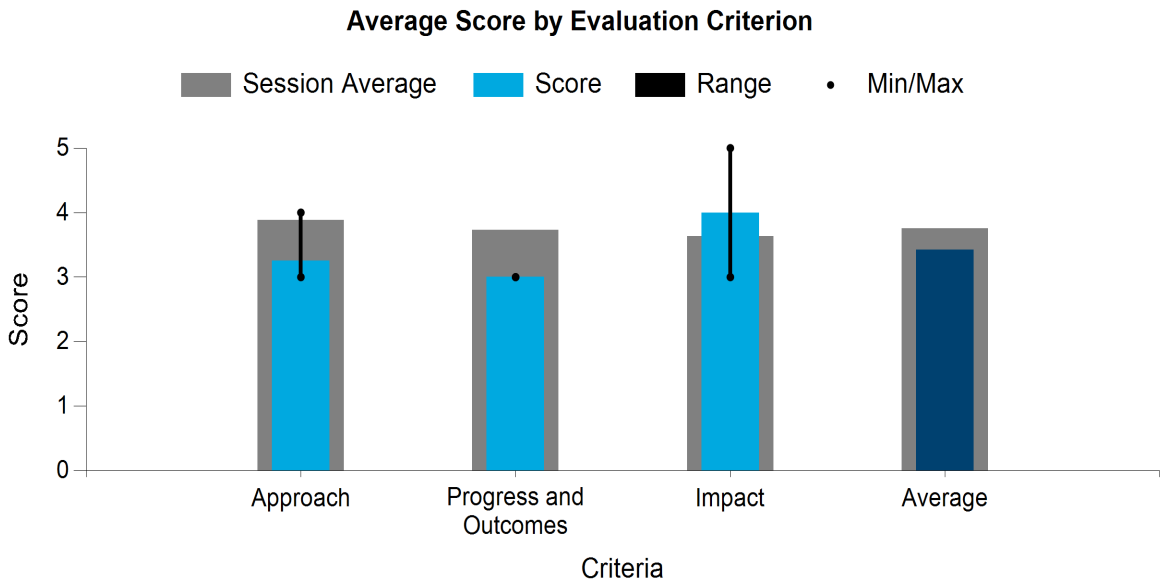
ABF DFO WITH DANIMER

National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.714
Presenter(s):	Gregg Beckham; Michelle Reed
Project Start Date:	10/01/2021
Planned Project End Date:	09/30/2023
Total Funding:	$500,000.00

This ABF DFO project with Danimer Scientific focuses on the development of a strain and a corresponding bioprocess to convert bio-based feedstocks to mixed-composition polyhydroxyalkanoates (PHAs). Danimer Scientific produces PHAs today at industrial scale in proprietary strains and for many applications where bio-based, biodegradable materials are advantaged. The project team consists of NREL to lead the strain engineering efforts, PNNL to conduct systems biology experiments that will inform further strain engineering, and Danimer to conduct bioprocess development and materials development. To date, we have onboarded Danimer strains and demonstrated successful engineering thereof. We anticipate conducting systems biology studies in spring 2023. Overall, the impact of this DFO project could be improved material properties accessed through the DBTL cycle for designer PHA production.



COMMENTS

- Although this project is nearing the end of its award window, there is very little information provided in the presentation to inform the review. IP concerns are limiting disclosure for this project. In general, the production of PHA formulations with new and interesting material properties is an impactful goal that can lead to new products and materials with superior characteristics to legacy alternatives. The ABF's familiarity with the technology suite should enable learnings to the benefit of Danimer. The progress on milestones is unclear, as is the impact of the PHA formulations identified and synthesized.

- This is a great project overall for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. The value proposition of PHAs is assumed but not stated clearly. A lot of PHAs are not great for plastic replacement. The goal says, "Working to develop new PHA

formulations that have not been reported in the literature and that could lead to new material properties for PHAs," presumably to improve the ability of PHAs to be useful as a real replacement for plastics. Again, assumed but not clearly stated. A clearer impact slide as it relates to BETO and the bioeconomy goals should be included.

- Next time, put all milestones and status (and percent complete if not complete). It's hard to judge without this info. This project is especially hard to evaluate as there was no mention of milestones and progress toward them. There has been progress and learning listed, but I'm not sure how much of the work is on track. I understand a company needs to keep their work more secret. You can list milestones just as numbers and give a percent completion and on-track status.

- Overall, PHAs are an important market, and the brief description of the approach appears straightforward. However, there was very limited substance in the information shared about this project. The project began in 2021 and it's a few months out from completion, yet no real data or results were shared. It is difficult to evaluate the progress and impact in this case. Even if this lack of information is from company proprietary information reasons, at least the overall milestones and progress should be shared.

- The project between Danimer and the ABF seeks to develop designer PHAs in an industrially relevant host to enable scale-up. Based on end-of-project milestones, the goal is to achieve PHA composition to within 10% of a targeted composition of 75% C4 and 25% C8, C10, and/or C12. Strain engineering is taking place in Danimer strains, which were successfully onboarded. Parallel pathways are being implemented in *P. putida* KT2440, which is extensively used by ABF. Very few details are provided to enable assessment of the experimental approach or progress. Scores given for approach and progress/outcomes will be relatively low, but this is out of a desire for consistency across project review. The impact of the project assisting in development of a strain that will ultimately be used in a commercial process is what ABF should be striving to routinely achieve. Danimer's leveraging of ABF expertise in iterative strain engineering (design/build) combined with omics characterization (test/learn) is an appropriate use of national lab resources to accelerate development of bioeconomy products. Knowledge of Danimer's strain capabilities and quantitative milestones expands ABF expertise, which will be applicable to future projects.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the understanding from the reviewers that we cannot disclose the full findings of this project. As the reviewers note, this is work toward improving PHA material properties, and we are on track to complete most of the project deliverables, but there will be outstanding questions remaining to be addressed in follow-up work.

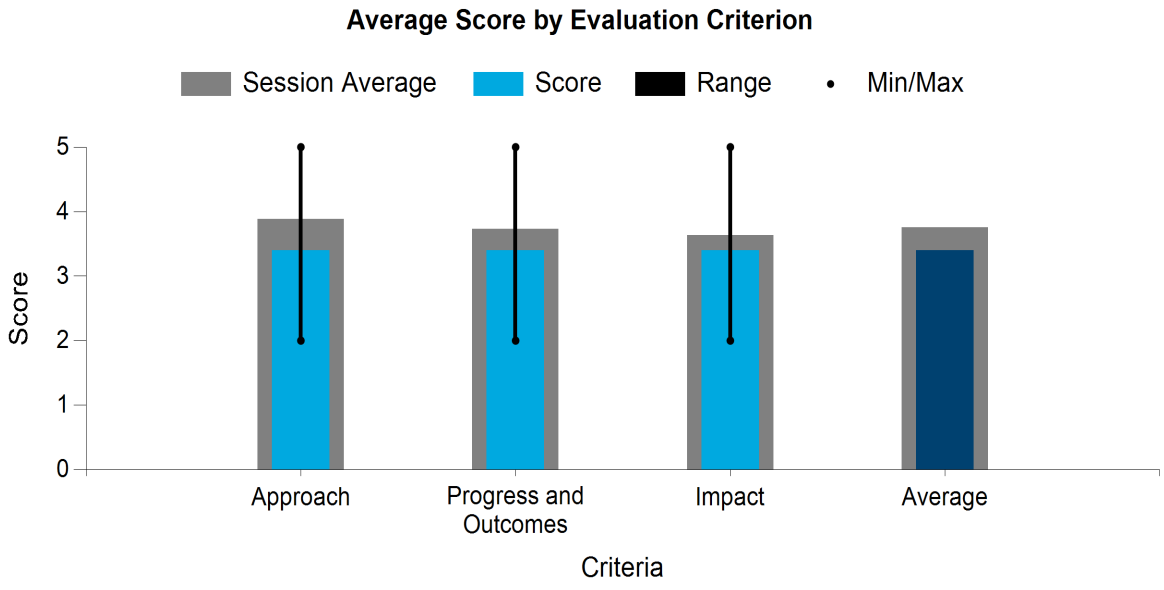
ABF DFO WITH PYRONE

Sandia National Laboratories and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.718
Presenter(s):	John Gladden; Karthikeyan Ramasamy; Katarina Younkin; Michele R Jensen; Alberto Rodriguez
Project Start Date:	01/27/2021
Planned Project End Date:	12/01/2023
Total Funding:	$600,000.00

The use of fatty acids as substrates for microbial fermentation can be an alternative to valorize lipid-containing waste streams by converting them to bioprivileged compounds such as triacetic acid lactone (TAL). Pyrone Systems Inc. partnered with ABF members at SNL and PNNL to build and optimize engineered yeast strains for biosynthesis of TAL from fatty acids. Fatty acids represent a direct source of the polyketide metabolic precursor, acetyl-CoA, and the yeast *Candida viswanathii* has the ability to metabolize different fatty acids by beta-oxidation. We engineered *C. viswanathii* to promote the compartmentalization of fatty acid degradation and TAL biosynthesis pathways in the peroxisome, as part of a strategy to increase product yields. TAL-producing strains were subjected to adaptive laboratory evolution to improve growth and production rates in the presence of oleic acid, and isolates with faster growth than the parental strain were identified. A multi-omic characterization of the generated strains will enable the identification of potential bottlenecks and new targets for process optimization.



COMMENTS

- This project seeks to create TAL from fatty acids using a yeast host. The project is in its early phases, and has started with a focus on evolving the host to improve performance characteristics. At the current stage of research, a pathway was identified and utilized in a host, and some comparison studies were run to understand media impacts on performance. Most open questions remain, with work ongoing to better understand the impacts on performance and to optimize the strain for further rounds of optimization.
- Strength: TAL seems to be a high-value platform chemical that has multiple applications.

- Weaknesses/areas for improvement:
    - The team used adaptive lab evolution for strain engineering, which is too old a technology and does not reflect the state-of-the-art strain engineering capability of the ABF. The method is not targeted and thus not effective, as seen from the results.
    - The current TAL titer is only 6 mg/L, which is too far away from the team's final goal of >5 g/L. It is hard to imagine the proposed approach will lead to a thousandfold enhancement during the second half of their project. The impact on commercial production of TAL from this project is very limited.
    - The current TAL titer is also too low compared to previous published results.
- This is a great project overall for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. The value proposition was stated and had impact slides clearly stating benefits to the ABF and the U.S. bioeconomy. The work in this proposal clearly advances the capabilities of the ABF, and at the same time, the partner gains knowledge and access to capabilities it does not have in-house.
- Next time, put all milestones and status (and percent complete if not complete). It's hard to judge without this info. This project is especially hard to evaluate as there was no mention of milestones and progress toward them. The slides do show a lot of technical progress and learnings; it's just not possible to judge if they are on track or not.
- This project addresses a straightforward but difficult challenge and adequately leverages ABF capabilities to deliver promising results with direct impact to industry and advance bioproduction processes generally. While the project seems on track to meet the 5-g/L target, having already seen 5 mg/mL titers in their strains, there may be limitations to reproducing those titers at higher fermentation volumes. This risk is not mentioned explicitly and may limit the eventual impact of the project for Pyrone.
- Pyrone Systems is partnering with ABF to build and optimize engineered *Candida viswanathii* strains to synthesize TAL from renewable fatty acids. The approach calls for three experimental activities: (1) engineering the yeast strain to preserve acetyl-CoA and malonyl-CoA in the peroxisome to enable TAL synthesis; (2) adaptive laboratory evolution experiments revolving around high growth on fatty acids and exploration of supplemental carbon sources; and (3) characterizing the evolved strain by genome sequencing and multi-omics to discover new targets for strain improvement. Tasks have been assigned to the various partner organizations, but no indication of communication plans is provided. No risk assessment with corresponding mitigation plans is provided. The project is in the third quarter of a 2-year timeline. Some progress has been made. Two strains were generated that are incapable of growth on oleic acid as a sole carbon source and synthesize TAL (maximum concentration reported 10 mg/L). Two approaches to adaptive laboratory evolution were initiated, and some media assessment has been performed to maximize product formation. However, I have strong reservations and questions about the experimental approach. What genetic modification tools are in place for the selected organism? Is this strain on the list of onboarded ABF strains? Does ABF or Pyrone Systems have expertise in engineering this organism? Although adaptive laboratory evolution is emerging as an important strain engineering tool, it is best applied on organisms for which there is a foundation of knowledge, engineering tools, etc., which enable the combination of random and rational engineering. Also, if the TAL-synthesizing strain does not grow on fatty acids, as indicated, why would adaptive laboratory evolution for a strain with superior growth on fatty acids improve TAL production? It would seem this means acetyl-CoA is exiting the peroxisome to enable cell growth. The end-of-project milestone calls for a strain that produces 5–10 g/L of TAL, which requires a thousandfold increase relative to current TAL production. Finally, is there a

reason why fatty acids are selected as feedstock rather than sugars? Strains that synthesize more than 1 g/L of TAL from glucose have been reported. Would improving glucose to TAL be a better approach?

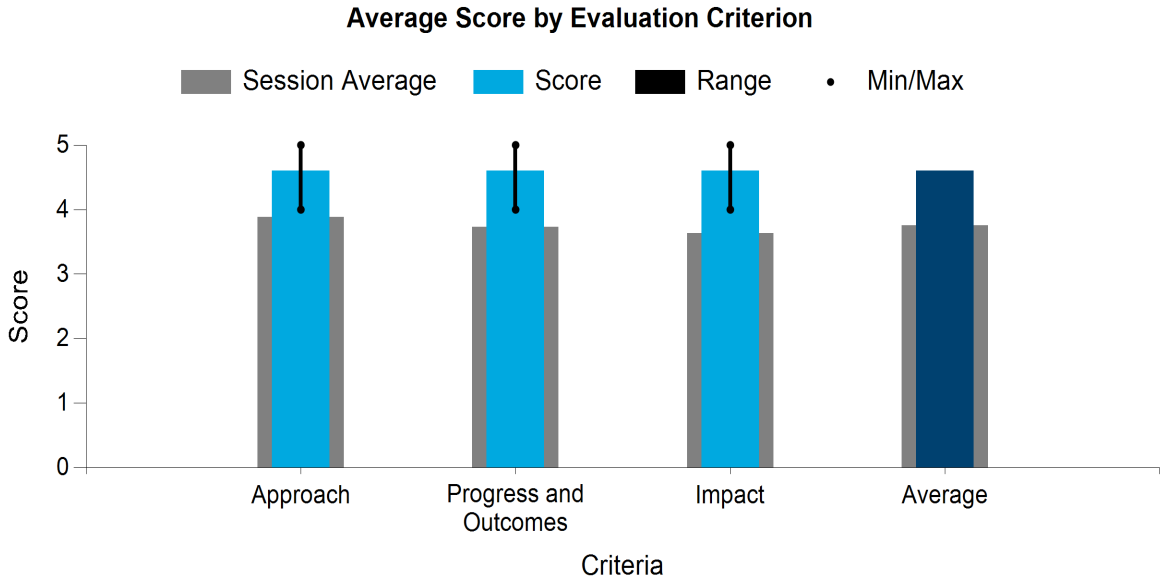
ABF DFO WITH TECHNOLOGY HOLDING INC.

Los Alamos National Laboratory, National Renewable Energy Laboratory, and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.719
Presenter(s):	Gregg Beckham; Karthikeyan Ramasamy; Michele R Jensen; Michelle Reed
Project Start Date:	03/11/2021
Planned Project End Date:	12/01/2023
Total Funding:	$1,400,000.00

This ABF DFO project with Technology Holding and partners focuses on the development of both a strain of *Pseudomonas putida* KT2440 and a corresponding bioprocess to convert cellulosic sugars to beta-ketoadipic acid, which can be used in performance nylons and polyesters. Our approach follows the DBTL cycle, wherein we have transferred learnings from muconic acid production in *P. putida* to develop a glucose- and xylose-utilizing beta-ketoadipic acid production strain. This strain achieves 65 g/L of beta-ketoadipic acid at 0.7 g/L/h and a Carbon mol yield of 0.40. We are currently onboarding arabinose utilization as well. To identify nonintuitive strain modifications as well, we are deploying a beta-ketoadipic acid biosensor and building randomly barcoded transposon insertion sequencing (RB-TnSeq) libraries and gene overexpression libraries in beta-ketoadipic acid production strains. Moreover, we are using global metabolomics and other systems biology tools to identify off-target pathways. Lastly, we are scaling up beta-ketoadipic acid production to kilogram-scale production for Technology Holding for evaluation in performance polymers with their partners.



COMMENTS

- This project aims to develop a new strain for the production of a chemical precursor, beta-ketoadipate. This molecule can be used to produce bio-based fibers like nylon and polyester. The ABF was well suited to perform this work given prior work on strains with similar metabolic pathways, and utilized that knowledge to develop a functional strain with apparently commercial-relevant performance metrics. Small quantities of product have been produced, and further scaling is ongoing to generate more product for testing by end users.

- Strengths:
    - The team used a combination of the ABF's most recent tools (e.g., product sensors, RB-TnSeq) and strains (*P. putida*) to produce b-ketoadipate from hydrolysate, representing a good leverage of ABF capabilities.
    - Substantial progress has been made in a short period of time, indicating the team is highly efficient in delivering results and reaching milestones.
    - Extremely high titer and yield were reported, demonstrating both the impact of ABF technology and the process commercialization potentials.
- Weaknesses/areas for improvement: N/A.
- This is a great project overall for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. The value proposition was stated and had impact slides clearly stating benefits to the ABF and the U.S. bioeconomy. The work in this proposal clearly advances the capabilities of the ABF, and at the same time, the partner gains knowledge and access to capabilities it does not have in-house.
- Next time, put all milestones and status (and percent complete if not complete). It's hard to judge without this info. This project is especially hard to evaluate, as there was no mention of milestones and progress toward them. There clearly has been progress and technical advancements, but it's impossible to say how well overall the project is on track.
- This project is another great example of appropriately leveraging the ABF's prior work and expertise to address an industry-relevant problem. The ABF's prior *P. putida* and muconic acid work has enabled faster progress and more ambitious goals for this project. The combination of TnSeq, adaptive laboratory evolution, and biosensor development helps ensure project success and pulls across ABF expertise. The project has already delivered test material and appears on track to meet the final 1-kg goals.
- This project leverages an extensive database of knowledge and expertise that the ABF has accumulated related to a particular biosynthetic pathway in *P. putida* to produce a new performance-advantaged bioproduct, beta-ketoadipate. Multiple ABF tools will be brought to bear to improve the TRY. Because ABF personnel are literally the global leaders in developing this pathway, I have no doubt the experimental approach is well considered. Given the challenges of demonstrating applications for new materials, Technology Holding will benefit from strain development efforts at the ABF.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for the positive feedback. In terms of milestones, this project is on track, and we have successfully met all of our milestones.

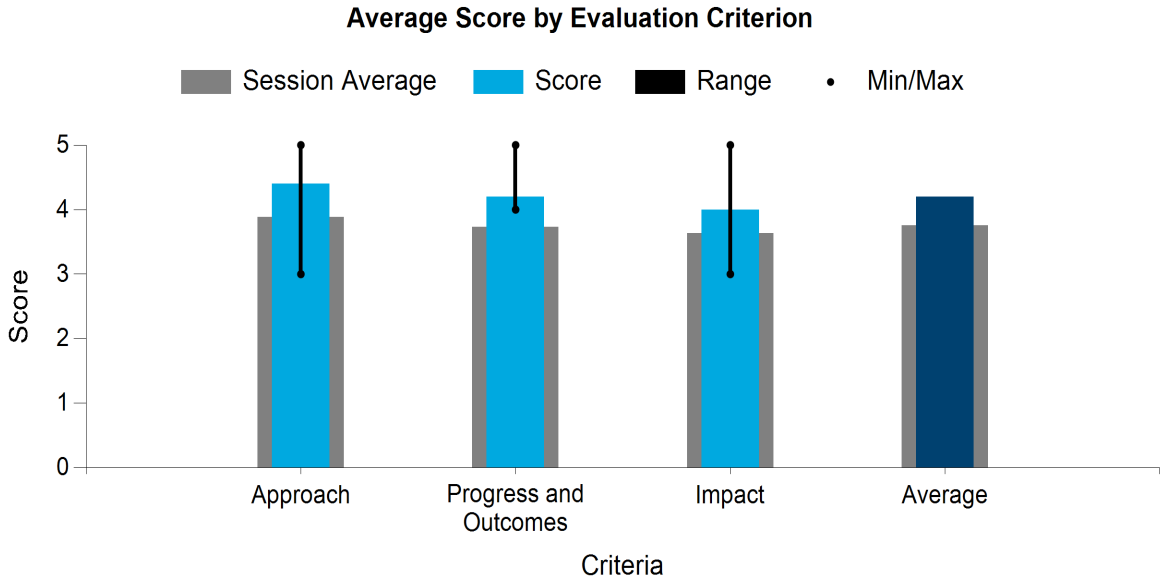
ABF DFO WITH IMICROBES

Los Alamos National Laboratory, National Renewable Energy Laboratory, and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	2.5.3.720
Presenter(s):	Eric Sundstrom; Karthikeyan Ramasamy; Katarina Younkin; Michele R Jensen
Project Start Date:	03/11/2021
Planned Project End Date:	12/01/2023
Total Funding:	$1,000,000.00

Bioconversion of gaseous feedstocks enables efficient production of fuels, chemicals, and materials from a variety of waste feedstocks, including electrochemical intermediates, syngas, landfill gas, biogas, and flared natural gas. At present, metabolic engineering, laboratory bioreactor equipment, commercial manufacturing facilities, and professional expertise within the biomanufacturing industry are heavily focused on heterotrophic processes, with minimal support available for the growing number of companies exploring gaseous feedstocks. This limitation is particularly acute for aerobic gas fermentations, which require stringent attention to process safety and dynamic control of multiple gas feeds. This project seeks to address these challenges by leveraging ABF capabilities in bench-scale gas fermentation, process scale-up, high-throughput proteomics, and deep learning to accelerate strain and process development for aerobic gas fermentations. In particular, we will work closely with our industry partner, Industrial Microbes, to improve production of the renewable polyethylene substitute poly(3-hydroxy)propionate (P3-HP) from ethane gas, targeting a threefold improvement in TRY over the initial project baseline. Initial laboratory results have established a performance baseline and harmonized TRY and proteomics data for P3-HP production between the Industrial Microbes and LBNL fermentation laboratories. This research effort will accelerate deployment of renewable polymer production from waste gas feedstocks via direct deployment with Industrial Microbes, while building generalizable capabilities within the ABF to rapidly improve performance and predict process scalability for aerobic gas fermentations.



COMMENTS

- The primary impact of this project is developing capabilities at the ABF and ABPDU to perform gas fermentation at lab and pilot (300-L) scales using flammable gases that create process complexities. Such a capability is likely to be useful to any innovator planning to explore gas fermentation technology, as the complexity of the system (from a design and operations perspective) makes a third-party test platform particularly attractive. This project has a clear and logical approach to execution, with an initial focus on tech transfer and reproducing results demonstrated by iMicrobes, and later stages aimed at scaling up and improving TRY over baseline. The project is nearly on schedule, achieving close to its ±15% target on performance versus the iMicrobes baseline; however, most of the heavy lifting lies ahead in scaling this process up to 300 L with 3× improvement on TRY.

- The impact of this project is unknown at the current review point, as the larger pilot reactor has yet to be validated against the baseline performance. Demonstration of performance at pilot scale will validate the ability of the ABF to scale gas fermentation processes, and demonstrated follow-on interest from iMicrobes or other gas fermentation innovators will validate the impact of this new capability.

- Strengths:

    - The combination of multiscale process engineering, high-throughput proteomics, and deep learning is a powerful tool. The team leverages this unique capability from ABF.

    - Conversion of ethane to P3-HP is challenging in its process. This team will leverage the capability of ABPDU to address this issue.

    - The team consists of highly capable PIs with complementary expertise.

- Weaknesses/areas for improvement:

    - (Not a weakness) this project has recently started; there are not many results to review.

    - *E. coli* does not seem to be a good host for this conversion. Methane monooxygenases are notoriously difficult for engineering. There have been many failed attempts to functionally express these enzymes in *E. coli*. The team is suggested to consider alternative methylotrophic hosts.

- This is a great project overall for the ABF. It fits very well into the mission of the ABF to support industry and advance the bioeconomy. The value proposition was stated and had impact slides clearly stating benefits to the ABF and the U.S. bioeconomy. The work in this proposal clearly advances the capabilities of the ABF, and at the same time, the partner gains knowledge and access to capabilities it does not have in-house.

- The project is new, and first goals are not due yet. The slides do list project goals. Still, a table of milestones and progress toward them should be included. They did state 80% achievement of the first goal.

- This project leverages ABF gas fermentation capabilities and prior genetic engineering work in *P. putida* to address a relevant challenge for an industry partner. The project approach is straightforward and rational. The goal of using ML to better understand and predict scale-up performance will have implications and impact beyond the project and is a good synergy between the ABF's expertise and industry needs. The project appears to be on track and has seen successful progress thus far.

- This collaboration seeks to leverage ABF bioprocess development, proteomics, and ML to create a predictive model for bioconversion of ethane into P3-HP. Project strengths include clearly defined tasks

assigned to the project participants. The project was initiated in October 2022, but good progress has been made with regard to technology transfer from iMicrobes to ABPDU. This project will enable ABPDU to advances its safety protocols, equipment, and foundational knowledge for utilization of gaseous feedstocks. There are multiple weaknesses in the project plan. The project does not appear to include a strain development component, and no information was provided regarding gene/pathway expression, induction, etc. With that in mind, it is not clear how proteomic data based solely on bioprocess changes will be utilized to develop predictive models and, more importantly, to improve the process to obtain higher TRY. No risks were included in the presentation, which makes me question whether ample thought was given to how the project plan might be disrupted and what strategies will be followed to keep the project on track. The connection between the stated final product, P3-HP, and the final project goal to demonstrate 300-L ethanol scale-up is also not clear.

PI RESPONSE TO REVIEWER COMMENTS

- We would like to thank the reviewers for their thoughtful comments. We heartily share their enthusiasm for the impact this project can provide, both specifically for production of P3-HP from ethane, and more broadly for predictive scale-up of aerobic gas fermentation processes. The reviewers are correct that heterologous expression of the methane monooxygenase has been notoriously difficult to achieve! Industrial Microbes has solved this problem as part of their core technology suite and has already demonstrated methane monooxygenase rates in *E. coli* sufficient to support commercial production of P3-HP. The benefits of working in *E. coli* as opposed to native methanotrophs include acceleration of DBTL cycles, utilization of mixotrophic metabolism, and reduction of scale-up risk. Use of *E. coli* enables rapid deployment of processes that would be extremely challenging in methanotrophs, including conversion of ethane and carbon dioxide to P3-HP. We note that the project does include a strain development component at Industrial Microbes, which will iteratively leverage fermentation data to target scale-up-relevant improvements in the production strain. Microbial response to bioprocess changes is specifically targeted for this project because of the complex biosynthetic pathway, which requires high flux of oxygen, CO_2, and ethane to the conversion host; management of ethanol concentrations as a key intermediate; continuous pH control; and carefully managed fed-batch supply of glucose for cell growth. Given these parameters, evaluation of strain performance under scale-up-relevant conditions is impossible to replicate outside of bioreactor trials.

- ABF funding provides a unique opportunity for Industrial Microbes to access key national laboratory capabilities in gas fermentation, allowing them to assess bioprocess robustness and response to perturbation at an early stage of development. The resulting proteomics data will identify changes to the strains, including increases in stress response proteins, pathway protein degradation, relative expression of pathway proteins, and increases in insoluble protein diagnostic during the culturing under variable conditions. These results will be utilized alongside standard fermentation metrics to inform strain development efforts. While not explicitly described due to the brief presentation format, we have conducted extensive risk analysis and mitigation as part of the project scoping and execution. We have deployed parallel development of the higher-risk ethane-to-P3-HP conversion process alongside the lower-risk ethanol-to-P3-HP conversion process to ensure progress is not halted if the higher-risk pathway encounters technical challenges early in the research effort. We note that the 300-L scale-up effort will target P3-HP production from ethanol feedstock, as ethanol is a key intermediate generated from biological ethane oxidation. This scale-up result will be used by Industrial Microbes alongside bench-scale ethane fermentation data to achieve pilot- and commercial-scale P3-HP production from ethane. In addition to mitigation of feedstock risk, extensive technology transfer and harmonization of fermentation and analytical protocols were conducted to minimize risk associated with site-to-site variability. We are excited that the project is on track, and we look forward to sharing more comprehensive results at the next BETO Peer Review.

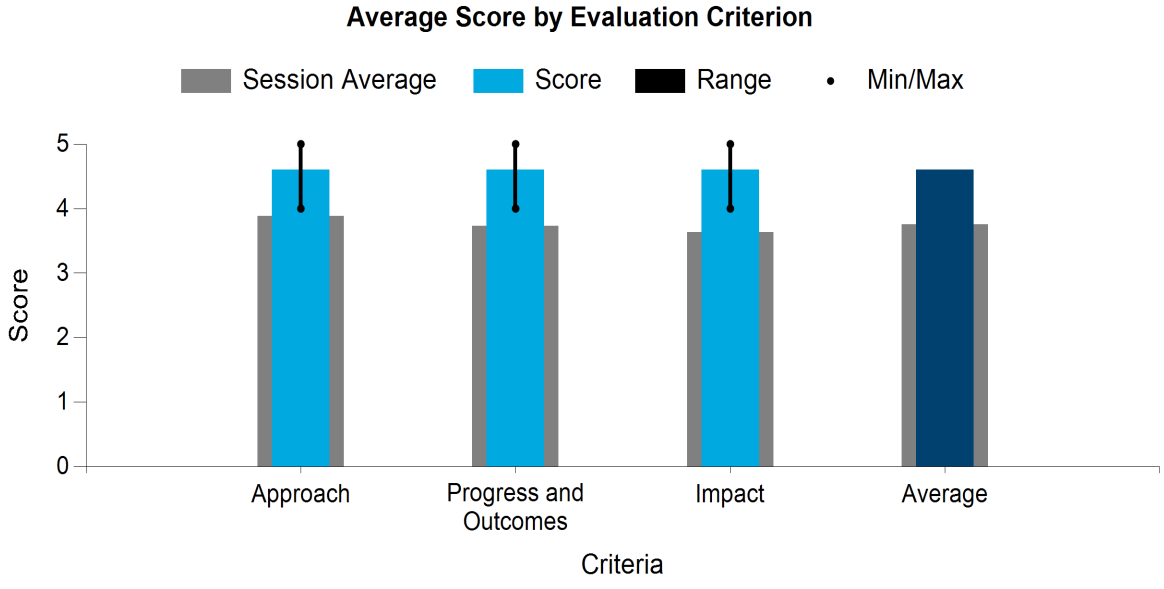
A TWO-CHAMBER GROWTH AND PRODUCTION SYSTEM FOR ROBUST CONTINUOUS BIOPROCESSING

Pow Genetic Solutions Inc.

PROJECT DESCRIPTION

WBS:	2.5.6.201
Presenter(s):	Ouwei Wang; Shannon Hall; Maggie Stoeva
Project Start Date:	10/01/2019
Planned Project End Date:	06/30/2023
Total Funding:	$3,108,303.00

The current bioproduction process, (fed-)batch fermentation, is repetitive and serial in nature, with each batch having to regenerate the biocatalyst, which has fundamentally limited the effectiveness of biomanufacturing. Replacing this system with a continuous process would minimize equipment downtime, increase volumetric productivity, and drastically reduce operating and capital expenses, paving the way for "high-yield, low-cost" biomanufacturing. The project team has developed a process that overcomes genetic drift and contamination, the major challenges of continuous biomanufacturing. We solve these two problems by combining three emerging technologies: (1) the introduction of a highly controllable and economical genetic switch technology that minimizes genetic drift and enables efficient decoupling of growth from production, and (2) an economical biocide/biocide-resistant system to prevent biological contamination during prolonged continuous fermentation, using (3) a two-chamber fermentation process to physically separate growth from production. This process has been implemented in Visolis's patented mevalonolactone production system at the 2-L scale, with contamination-free run times >500 h and ~3× increases in productivity over fed-batch. The process has been scaled to 30 L and is being optimized for maximum productivity. Our TEA confirms the "capital-light" promise of the platform and informs on future scale-up and deployment strategy.



COMMENTS

- Pow.Bio has developed a novel continuous fermentation approach that utilizes some biological tricks to separate growth from production while enabling a straightforward contamination control system.

Progress in this research is aimed at scaling the process to larger reactors and tech transfer to the ABPDU to run the two-stage continuous fermentation process. Overall goals for performance at various scales have been reached, and the continued scaling of this technology will enable higher performance for a variety of product targets. Further TEA work is recommended to better position this technology against other continuous fermentation approaches, and further discussion on the limitations of this technology, particularly around the products and molecules that can allow for a separation of growth and production in the host, would better clarify the potential impact of this technology.

- Strengths:
    - The use of cheaper inducer and contamination control is innovative and can improve process economics.
    - The developed two-chamber fermentation technology showed a substantial improvement in accumulated product amount and productivity (but not yield). The team obtained a high productivity of 2 g/L/h for 72 hours at 30-L scale. This result is very impressive.
    - All proposed milestones were achieved with satisfactory progress.
    - TEA showed that continuous fermentation has substantial benefits compared to fed-batch fermentation; thus, the developed technology may have a major impact on improving biomanufacturing economics.
- Weaknesses/areas for improvement:
    - Tech transfer of the two-chamber fermentation system to ABPDU failed. It is not clear how to address this issue. Scale-up fermentation seems to be the only connection of this project with the ABF.
    - The contamination control system may not work if the production pathway is mutated while the selection gene (chlorite dismutase) is not. This can happen at a high probability because the pathway is often much larger (easier for mutation) than the selection gene.
- Continuous fermentation is clearly a benefit to U.S. bio-based manufacturing. The value proposition here is clearly laid out. Impacts to the bioeconomy are clearly stated (that was very nice to see). Their approach overall is good. I just don't know how universal it will be to many different types of microbial hosts. Also, many other companies and facilities do run continuously, or close to continuously. For many systems, you need some cell growth to keep the cells healthy and generate intermediates for catabolic processes. Overall, this technology could be very helpful and useful, and it is definitely in line with BETO's and ABF's missions, although their uniqueness seems overstated.
- Chlorite and chlorite dismutase is a very novel and potentially very useful tool to control contamination in industrial manufacturing fermentations, which is a big problem. I am dubious of their claims that there are no regulatory issues around chlorite usage. Having residual chlorite could mean very substantial changes and added cost to waste disposal of fermentation broth—for example, the practice in Brazil of spraying vinasse (fermentation broth) on cane fields as fertilizer. The tool could still be very useful, but again, the universality of the approach might be limited.
- The slides show all three go/no-go decisions, which is very good to see laid out. Still, a table of all milestones and progress toward them is needed for an accurate review, and going forward this should be requested for all projects from BETO. Industry can just list them numerically if they can't give details.

- Clearly this project has made a lot of progress and is advancing biomanufacturing in the United States. They should consider now working with BioMADE to increase the TRL.

- This was a highly successful project (all milestones met) with a high relevance and impact for the overall bioeconomy. Developments that enable the use of continuous fermentation will be highly needed in order to scale the bioeconomy. This project leveraged ABF and ABPDU expertise well and was able to advance their technology.

- Project participants devised a multitiered approach to demonstrate a robust, contamination-resistant continuous production system. This process will be used to synthesize mevalonolactone, a platform molecule of one of the partners. The approach addresses several issues that increase biomolecule production costs by combining an inexpensive and tightly regulated genetic switch, an economical method to address reactor contamination, and a two-chamber design that separates bacterial growth and biomolecule production. Project participants and their corresponding expertise are provided, but defined tasks are neither listed nor assigned to participants. Communication and technical transfer between Pow.Bio and ABPDU are facilitated by their physical proximity, but communication frequency is not indicated. Several risks are provided, although the mitigation strategies were not necessary given the successful execution of the project. No plan was provided to address DEI on the project. Quantitative production goals (rate and duration of productivity) from cellulosic sugars have been achieved on a 2-liter scale. Additional experiments confirmed successful contamination control, co-consumption of C5 and C6 sugars, and good plasmid stability. Additional experiments are in progress to achieve the quantitative goals for product formation from cellulosic sugars on a 30-liter scale. This approach has a high potential for successful scale-up, potentially to commercial scale. The product is described to have the potential to address a market size totaling $10 billion, although the status of catalysis required to obtain end products is unknown. The potential impact (8-fold increase in product, 4.4-fold decrease in production cost) is striking. Utilization of this technology on other biomolecule products targeted by the ABF is worthy of exploration.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their comments and agree that further expanding the scale and use cases of our continuous technology will greatly benefit the U.S. bioeconomy. Several comments are addressed in more detail below.

- “Tech transfer of the two-chamber fermentation system to ABPDU failed. It is not clear how to address this issue. Scale-up fermentation seems to be the only connection of this project with ABF. The contamination control system may not work if the production pathway is mutated while the selection gene (chlorite dismutase) is not. This can happen at a high probability because the pathway is often much larger (easier for mutation) than the selection gene.” We would like to reiterate that the tech transfer of the two-chamber system to ABPDU has not failed. On the contrary, we have initiated the process, and ABPDU has already successfully replicated the growth chamber, run as a turbidostat. We have developed a tech transfer package and will be initiating a run at ABPDU in the coming weeks. We anticipate no real risk in their ability to successfully execute the project (as we have done so in-house multiple times already), and we are located close to the ABPDU and will be available on-site for troubleshooting. In regard to the contamination control system (i.e., chlorite and chlorite dismutase), it is decoupled from mutations that may occur in the production pathway. To clarify, chlorite/chlorite dismutase may be used to prevent or treat contamination in either chamber. Separately, in order to prevent mutation/loss of the production pathway, we have taken a two-pronged approach: (1) growth occurs in the absence of production, and hence there is no selective pressure on the pathway to mutate, and (2) growth is limited in the production chamber, and hence, even if a single microbe mutates the production pathway, this will not affect productivity of the system as a whole since outgrowth of this microbe is prevented.

- "Their approach overall is good. I just don't know how universal it will be to many different types of microbial hosts. Also, many other companies and facilities do run continuously, or close to continuously. For many systems, you need some cell growth to keep the cells healthy and generate intermediates for catabolic processes. Overall, this technology could be very helpful and useful, and it is definitely in line with BETO's and ABF's missions, although their uniqueness seems overstated." We agree with the reviewer that it is critical to determine the translatability of our platform, and have already initiated industry partnerships to develop a two-chamber continuous system for protein production in yeast. We plan to further expand our host repertoire in the coming months. In regard to other facilities running continuous fermentation, we would like to make the distinction between our process and theirs; we are running a mesophilic *E. coli* process, where a high-value molecule has been engineered into the host background. In terms of what we have seen in industry, continuous fermentations (1) rely on extreme conditions (pH, gas fermentation, and ethanol production), (2) are for the production of ethanol or mixed-culture biomass (naturally contamination-resistant, and no danger of mutation/production biomass), or (3) rely on expensive, single-use materials (i.e., pharma). In these cases, the risks of contamination and pathway loss are minimal (there is no engineered pathway). Hence, we uniquely solve the barriers to the adoption of continuous processes for synbio more broadly (i.e., loss/mutation of the production pathway, and contamination of non-extreme processes). Finally, we agree that a certain flux through central metabolism is needed for robust production. However, to date, our team has demonstrated this continuous platform process with bacterial and yeast hosts, with product classes from organic acid to a food protein (growth-coupled).

- "I am dubious of their claims that there are no regulatory issues around chlorite usage. Having residual chlorite could mean very substantial changes and added cost to waste disposal of fermentation broth—for example, the practice in Brazil of spraying vinasse (fermentation broth) on cane fields as fertilizer. The tool could still be very useful, but again, the universality of the approach might be limited." Chlorite is generated naturally by perchlorate-reducing bacteria in nature, and we have an enzymatic system in place to remove chlorite. The challenge we are facing is actually how to maintain a non-zero chlorite concentration in the tank.

- "Further TEA work is recommended to better position this technology against other continuous fermentation approaches, and further discussion on the limitations of this technology, particularly around the products and molecules that can allow for a separation of growth and production in the host, would better clarify the potential impact of this technology." We thank the reviewer for this comment and plan to expand our TEA alongside our planned further scale-up. As mentioned above, we are also expanding into additional hosts and processes to confirm the wide applicability of our platform.

- "Project participants and their corresponding expertise are provided, but defined tasks are neither listed nor assigned to participants. Communication and technical transfer between Pow.Bio and ABPDU are facilitated by their physical proximity, but communication frequency is not indicated. Several risks are provided, although the mitigation strategies were not necessary given the successful execution of the project." The full proposal, intermediate validation, and quarterly reports include task assignment; we will make sure to include the full list of tasks, milestones, and assignees for future presentations at BETO Peer Review. Pow.Bio and ABPDU communicate regularly as part of BETO progress update meetings, and during tech transfer have been in close, weekly communication. The main outstanding risk mentioned is tech transfer to the ABPDU. To mitigate this risk, we (1) scaled up successfully in-house and (2) initiated the process several quarters prior to the milestone run. The final risk is maintaining our productivity using cellulosic hydrolysate as a feedstock; we have confirmed that fed-batch tanks result in productivity comparable to that with glucose.

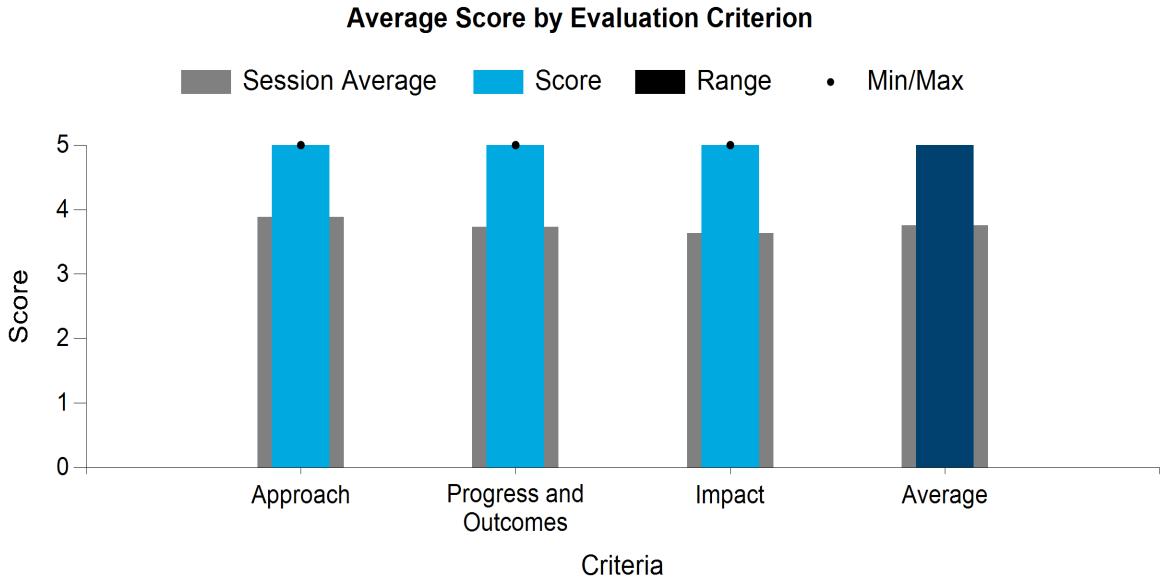
LBNL ABPDU SUPPORT

Lawrence Berkeley National Laboratory

PROJECT DESCRIPTION

WBS:	2.6.1.101
Presenter(s):	James Gardner; Deepti Tanjore; Katy Christiansen
Project Start Date:	07/13/2010
Planned Project End Date:	09/30/2022
Total Funding:	$6,216,319.00

The ABPDU, as part of LBNL, was authorized in 2009–2010 and commissioned in late 2011 as a shared community resource to provide process optimization, prototyping, development, and piloting and scale-up services to the biofuels and bioproducts research and development community, including industry, academia, and the national labs. Over the past 10 years, the ABPDU has performed exemplary process science research, leading to multiple publications in high-impact-factor journals (see "Research" at abpdu.lbl.gov). Through collaborations with a diverse set of partners (see "Collaborations" at abpdu.lbl.gov), the ABPDU staff has identified key areas for process development research that will benefit the biofuels and bioproducts community and accelerate commercialization of bioprocesses. This AOP details four tasks that not only continue ABPDU operations, but also build on previous experience and generate public data in areas that directly align with BETO's program priorities: (1) ABPDU Operations, (2) Learning from Data for Predictive Scale-up of Biofuel Technologies, (3) Biomanufacturing Using Gaseous Feedstocks (supporting the development of SAF, renewable diesel, and coproducts), and (4) Capital Upgrades. The process research and optimization work that the ABPDU conducts brings value to the entire biofuels and bioproducts community and provides high-visibility examples relevant to the BETO mission.



COMMENTS

- The ABPDU is an impressive organization that makes an outsized impact on the synthetic biology space vis-à-vis its funding levels and staffing. The organization provides a critical service to startups and established companies alike looking to advance their ideas beyond TRL 1 and test new theories or approaches. The leadership at the ABPDU has built a unique and hard-to-replicate capability that is well tuned to the needs of the industry. It is clear that ABPDU leadership (1) listens to the "voice of the

customer" to ensure that their offerings meet the demands of industry and (2) is committed to continually updating and evolving their offerings to remain relevant in a rapidly evolving field. The ABPDU is especially impressive for the ways in which it makes impact outside its own walls: Their commitment to workforce development through seeding trained laboratory experts throughout the industry is commendable, as are their digital products and collateral to share learnings, tools, and tricks with the community at large.

- The ABPDU has proven through its impact on follow-on funds the level of industry growth enabled by the asset, and this proof point confirms the appropriateness of the approach and strategy.
- The challenge for the ABPDU moving forward will be in supporting further innovation in the industry and evolving their tool set and capabilities within a constrained staffing and budgetary picture. Further direct governmental support would very much be welcome to allow the ABPDU more flexibility to invest in staff, operations, and capabilities to broaden its functionalities for the industry. Downstream processing, in particular, is an often overlooked area of process development early in technology scale-up, and further capabilities in this space would further enhance the ABPDU portfolio. Much of the ABPDU demand prior to the last 12 months came from word-of-mouth, and although ABPDU has begun to invest in more advertising and outreach, measurement of the impact and reach of those strategies will be needed to properly refine and target the message. This work should be expanded: The ABPDU has a unique capability to serve as a funnel to other organizations (governmental or otherwise) that can take the next step in the scale-up journey with ABPDU clients. Forming relationships with these organizations and developing a scale-up pipeline will further drive impact at ABPDU, enabling their client companies to access scale-up support and complementary tools (such as TEAs) to further their technical innovations.
- Strengths:
    - The ABPDU team has made impressive achievements over the past 2 years. They have been working with a large number of partners from both industry and academia on a wide range of strains and processes.
    - The ABPDU team has highly efficient management under the leadership of Deepti Tanjore. This has made the ABPDU highly productive during the past few years. This is reflected not only by delivering impressive results in various projects, but also in interacting with and servicing different partners, outreach activities, and communications to the public, as well as collecting feedback.
    - The team has made efforts to address previous panels' comments on promoting public awareness of the ABPDU.
    - The ABPDU managed to keep a short contracting time; this should be set as a good model for the other ABF teams to learn from and shorten their CRADA times.
- Weaknesses: None.
- The ABPDU was really a shining example of a BETO-run organization after a really challenging set of days reviewing the ABF. The ABPDU has brought in many outside partners and is a sought-after capability. The leaders are doing an excellent job bringing in partners and money and continuing to increase awareness in industry of the ABPDU and its capabilities. They said they could contract in a matter of a few weeks, which is impressive, and ABF needs to follow that example. The website is very informative (if a bit wordy, and could use a little improvement there), but overall has great videos, standard operating procedures, and content. The ABPDU continues to expand its technical capabilities and expertise. It's also doing a great job in DEI.

- Really, there isn't much else to say here. Don't mess with what is going well. The leadership team should be highly recognized for their work and excellence and retained at all costs. Don't mess with what is clearly working.
- In the next review, don't leave this for the last session on the last day, but put it with the ABF reviews.
- Overall, the ABPDU serves its mission effectively. The management of the ABPDU works well and can deliver on a variety of industry projects in a timely and reasonable manner. Communication and outreach by the ABPDU are standardized and coordinated and also include a variety of mediums. Industry perceptions of the ABPDU are favorable. The ability to execute strategic partnership project contract vehicles instead of relying solely on CRADAs greatly increases the effectiveness and appeal of the ABPDU for industry. The ABPDU website is straightforward and accurately conveys their capabilities. The use of case studies highlights their success, capabilities, and diversity of industry partners served. Training programs address both workforce development in the bioeconomy and recruitment of minorities into STEM fields.
- While the industry listening day appears to have been successful and generated useful feedback, it would have been good to know how many of the attendants had worked with ABPDU in the past and how many were new ABPDU engagements. I would recommend targeting at least a 50/50 breakdown for future listening sessions to better understand what capabilities and resources could bring more industry partners to the ABPDU as opposed to just serving current partners better. I love that the ABPDU makes short, useful bits of code available for use on their website. This is a great example of working in good faith with industry partners and facilitating technology for the bioeconomy. The ABPDU has also done a great job at considering the overall impact of their work and tracking those outcomes (e.g., the information shared on Slide 33).
- Internally, it is evident that the ABPDU is carefully considering how to improve their equipment management and safety, as well as the retention and satisfaction of their staff. Hiring a program manager to alleviate administrative burden and allow for more mentorship time by PIs is a great example of an insightful intervention that has both scientific and DEI benefits.
- The two tasks that were presented, microbial image analysis and gas fermentation, are relevant and will have a high impact for industry. Tracking culture health and response during fermentation processes is challenging, and the imaging platform could be a very useful tool for addressing this. This is a worthwhile endeavor and a good example of challenges that should be tackled by the ABPDU and national labs, as it may be too risky/costly for industry to do. If this effort does yield reliable methods for correlating cell phenotypes with culture performance, the impact would be substantial. However, the likelihood of success is perhaps rather low given the range of biological variability; any insights gained may be limited to specific species/conditions and not generalizable. This is not a reason to not pursue the work, but simply something to keep in mind for realistic evaluation of the potential project impact. The development of gas fermentation capabilities and development of strains that can use C1 feedstocks for growth is well in line with BETO priorities around decarbonization and the use of waste feedstocks. The development of these resources will undoubtedly have a positive impact on industry partners and on the bioeconomy more generally.
- The industry projects presented showed a wide breadth of use of ABPDU resources and had substantial progress and success. Most projects also address key BETO priorities such as lignocellulosic biomass conversion, SAF production, and bioplastics.
- In summary, the ABPDU appears to be functioning well and is conducting internal projects that leverage national lab strengths and that will have wider applications for the bioeconomy. External partner projects are well aligned with BETO and DOE priorities and have shown appropriate success and progress.

ABPDU management appears strong, external engagement mechanisms have been successful, and expansions of outreach are well planned.

- The ABPDU is a valuable component of the ABF, and investments in its operation add a positive multiplier to the bioeconomy. The ABPDU began operations in 2012, which is approximately 5 years earlier than the ABF *in toto*. If the new strategic plan results in an ABF that is as productive and service-oriented as the ABPDU, which I am confident is possible, the bioeconomy will be well served by this public investment. In addition to providing standard scale-up services to produce product, the ABPDU interfaces with strain improvement (i.e., via metabolic samples produced at scale) and downstream processing evaluation. These are valuable de-risking activities. ABPDU staff also participate in tool development (predictive scale-up, microbial imaging, and use of gaseous feedstocks) for use on future projects. It would be easy to attribute ABPDU success to a high barrier to entry for bioprocessing needs, which is not really the case for strain development. However, engagement with 75 organizations more likely indicates the ABPDU is doing many things well, both for its partners and for the bioeconomy. They are customer- and potential-customer-oriented. They provide extensive information about their capabilities and educational materials on their website, in quarterly newsletters, and on social media. Their industry days are well attended, which means their customers are invested in ABPDU improvement. Their training of interns and inexperienced scientists is valuable from a DEI perspective and for expanding the bioeconomy talent pool.

PI RESPONSE TO REVIEWER COMMENTS

- RESPONSE: We thank the reviewer for taking the time to review and provide comprehensive feedback. We appreciate their effort. We do agree that, to evolve further, the ABPDU has to provide unique cutting-edge tools that will advance state-of-the-art capabilities available to enable novel synbio and other fermentation-based technologies. Based on feedback from industry, for the past couple of years, we have been keen on developing capabilities such as the self-driving bioreactor and gas fermentation. With substantial internal LBNL funds (approximately $1 million), we were able to get the gas fermentation capability to a stage where we started to collaborate with industry partners on process development. With BETO funding, we are working toward delivering these capabilities to the industry. We look forward to pursuing similar projects in downstream processing, as suggested by the reviewer. We agree with the reviewer that we need to develop metrics to measure the impact of our communication and outreach activities. We are working on this topic.

- RESPONSE: We thank the reviewer for their valuable time. We very much appreciate the feedback and look forward to responding to any further questions or comments.

- RESPONSE: We thank the reviewer for their effort in reviewing us and providing valuable feedback. We agree with the reviewer that our website could use fewer words. We are working on this topic and hope to have new content on our website by the end of FY 2023.

- RESPONSE: We thank the reviewer for their generosity in spending time reviewing us and providing comprehensive feedback. We agree with the reviewer that the imaging project is low TRL and thereby tricky. We are looking at this problem in a few different ways, and this particular project is helping us define the problem much better. For example, realizing that real-time sampling of live cells from a bioreactor is unavailable as an off-the-shelf capability was a significant development for us. Similarly, we want to understand the limitations of artificial intelligence/ML applications in this space. We plan to develop a more robust project from these initial studies.

- RESPONSE: We thank the reviewer for sharing their limited time in reviewing us and providing comments. We agree that strain engineering poses a different contracting challenge than bioprocessing needs. As we generate IP through development of novel fermentation equipment and pursue

collaborative research work with industry, the ABPDU will face similar challenges. We have been relatively successful in executing a few funds-in CRADA projects in the past couple of years and are hoping to take this approach for future early-stage projects as well.

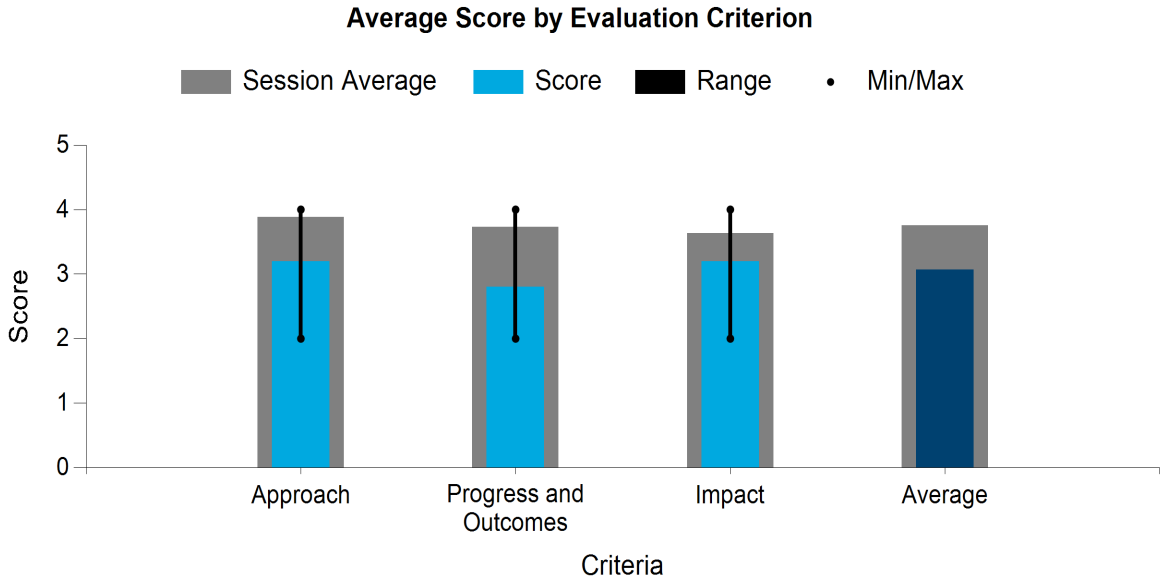
ABF INTRODUCTION AND OVERVIEW

Lawrence Berkeley National Laboratory

PROJECT DESCRIPTION

WBS:	ABF1
Presenter(s):	Katy Christiansen; Nathan Hillson
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022
Total Funding:	$20,000,000.00

The ABF, a consortium of seven national labs, has operated since 2016, with the goal to enable biorefineries to achieve 50% reductions in time to bioprocess scale-up. The ABF's relevance is that it has been a $20-million/year public infrastructure investment that increases U.S. industrial competitiveness, with impacts including the reduction of technical barriers for industrial and academic partners; increased access to broadly enabling engineering biology infrastructure, precluding the need for industry to reestablish metabolic routes and hosts; and a greater diversity of publicly available microbial hosts. Top challenges for the ABF have been leveraging past collaboration learnings with future collaborators, predictive scale-up and method transferability, and the intellectual framing of strategic beachheads (metabolic intermediates). The ABF's outcomes have included the development and deployment of technologies enabling commercially relevant biomanufacturing, with technical accomplishments spanning demonstrations of bioprocess improvements across microbial hosts and metabolic targets, the establishment of strategic beachheads and the development of TEA/LCA approaches to assess them, the onboarding and development of microbial hosts, and bioprocess scale-up. This presentation focuses on the ABF up until September 2022, with a subsequent set of presentations covering the ABF from October 2022 to date.



COMMENTS

- The ABF utilized multiple presentations to document their past performance and journey in developing a new strategic plan for the next funding cycle. These presentations grew off each other, and as a result, it is difficult to separate them as required in this review process. Instead, I have used the "Strategic Plan" presentation to provide feedback on the future plan, and have used the presentations related to specific past activities to review past performance. Please look there for my comments.

- Strengths:
    - Overall, the ABF team has been making satisfactory progress on their proposed projects. They have met the milestones and demonstrated the transfer of the 3-HP pathway and tools between different hosts (proposed five molecules/tools, achieved seven). These successes have proved the value of ABF technologies in potentially reducing DBTL cycles and bioprocess scale-up time for industrial partners.
    - Several ABF core teams demonstrated high titer/rate bioproduction of chemicals, such as muconate and 3-HP.
    - Collaborations with industry clearly demonstrated that ABF has empowered U.S. industry in biomanufacturing development.
- Weaknesses/areas for improvement:
    - Although efforts were made to improve the dissemination of ABF's capability, further improvements are needed to better serve the biomanufacturing industry. This includes updating the strains and tools information on HObT and publicizing the website soon. Right now, the database is still not available to the public.
    - The wide adoption of ABF tools (mostly omics, ML, and the ABPDU) by industrial partners and success on the malonyl-CoA/3-HP pathway are exciting. However, it is somewhat disappointing that the only beachhead used by industry is malonyl-CoA. Given the size of past support, multiple beachhead molecules are expected to benefit society.
- Most scores were moderate for judging impact on previous goals because there was a balance of very low scores and some high scores. Low scores because (1) nearly all DBTL goals were not met, (2) management structure was not sufficient to lead the ABF to success, and (3) there was poor industry outreach/engagement, as well as not engaging the IAB effectively. High scores for overall a lot of solid work advancing synthetic biology, delivering on several projects that advance U.S. capabilities, and advancing industry to be impactful. Also good plans for addressing DEI.
- Good:
    - Better tools for tracking DBTL, and showed DBTL cycle data for results of transferring one molecule pathway to a different host. Hit the Q4 2021 milestone: "2X efficiency improvement in automated DBTL engineering cycle unit operations compared to FY22Q2_DBTLI_R1 non-automated baseline efficiencies demonstrated."
    - Key milestones (Slide 25) and impact (Slide 27) show a lot of good progress was made in the last 3 years developing the tools and infrastructure to advance synthetic biology in the United States and help BETO's goals to decarbonize the chemical industry.
    - Hit the metric set earlier to "demonstrate transferability of ABF technologies and ability to accelerate bioprocess development."
    - ABF metabolic map depicting beachheads was added to the website (it should not have taken until after the 2021 Peer Review to do this).
    - Challenge noted: "Our lack of ability to predict how a process will scale, or how well a method can be transferred across facilities, may limit the impact of our research and development efforts." It's true that tech transfer and replication of process/results between sites is challenging and often hard.

This is part of scale-up and can take a lot of time and effort to work through. It's good that the ABF is recognizing this as a challenge, and it's not insurmountable. It takes time (money) and people dedicated on both sides to work through the differences. Usually it's best to send people from one facility to another to understand firsthand and help each other solve the problems.

- The ABF's interaction with different consortia is good and noted. They should always be looking to partner with and benefit from other consortia.
- The new emphasis on DEI is very good.

- Better, and could still use improvement:
  - It's good that the ABF is starting to capture some DBTL. I think it's a mistake to abandon all DBTL metrics going forward. Revise the DBTL goals to be more realistic and relevant. Still have a goal to reduce the DBTL cycle or even parts of DBTL that make the most sense, are the most commonly used, or are the biggest bottleneck. It doesn't have to be every capability and every tool. A metric could be time needed to finish one complete experiment and learn from it (per tool or per instrument), and focus on making those individual unit ops more efficient. Drop wall time versus clock time. What matters is how long it takes you to learn from an experiment. If there is some large downtime (let's say you need to ship samples from one site to another, and that takes weeks), then that is part of your cycle time and something to target to be more efficient. Focus on gaining efficiencies and improving the infrastructure already in place.
  - The ABF states that the "Capabilities section of ABF website was updated, and ABF began providing capabilities webinars," but the website could still be improved to be clearer in what the ABF can offer (e.g., services, tools, HObT) with examples and videos. The ABPDU should be the gold standard for websites. Sit down with the ABPDU and learn from what they have done.
  - "Beachhead development/selection was informed by industry, and one example is a collaboration project that leverages acetyl-CoA/malonyl-CoA beachheads." It's good that the beachheads got some traction, but overall it seems to have either not been of interest to much of industry, or it was not advertised to industry well. It's good the ABF is not pursuing beachhead strategy anymore.
  - I don't understand the challenge that says, "Only portions of past collaborative data or learning methods that do not reveal the underlying primary data may be available." The ABF should reserve the right to learn from all data and projects from all work done in the ABF. Learning is different from using propriety information. If the ABF is saying they don't have access to past data (data are not stored properly), that is a big issue that needs to be addressed going forward.
  - Slide 28 (how impact is disseminated): It's definitely an improvement over 2021. However, publications is not a good metric for impact. It's what academics see, but it doesn't correlate with progressing real-world changes or what BETO wants to see. What matters are the number of licenses, offtakes, manufacturing or pilot samples produced, or companies spun out from technology.

- Bad:
  - Slide 7 says "ABF's HObT now publicly provides information about onboarded host organisms." The host onboarding done in the ABF is one of the greatest benefits the ABF has provided (or could provide) to industry. First, the HObT website is literally inaccessible from the ABF website. This is puzzling and disappointing. Also, if you know how to find the HObT website, it has no useful information other than the species and publications listed. There is no explanation of what

the different tiers mean, what tools are available, or the general status of the strain. The explanation for why this was not listed on the website was that this was not ready for the public yet. Work (money) needs to be allocated to getting this website up to date (parts, tier explanation, and protocols) to make sure the money already spent is not wasted, and this incredibly useful work done by ABF can be accessed and used by the public. This should have been done years ago (let alone by the end of 2022).

  - Overall DBTL cycles were not met, and for reasons that are still not clear, were said to be hard to measure/quantify. It really isn't that hard to quantify how long it takes to go from one step to another, or from the start of an experiment to when data are returned.

- Approach: The ABF's approach has advanced the state of the art and has produced innovative results in the past 2 years. However, the cohesiveness of the work in advancing toward a common goal was not evident. The presentation mentioned a gaps analysis resulting from industry engagement but provided no learnings from that effort (in this or any of the later presentations). It would be highly beneficial to map the ABF's efforts onto a comprehensive overview of the bioeconomy and more strategically target where the ABF can have an undue impact for advancing the bioeconomy relative to academia, industry, etc. That kind of strategic oversight should be what the management plan is focused on, and I'm not sure I see sufficient evidence that this is the approach that the ABF has taken. This concern was also mentioned in the 2021 Peer Review report. In that context, the management of the ABF appears too disjointed, with individual PIs pursuing work of interest to them as opposed to collectively driving toward a common vision that integrates into a larger road map of bioeconomy technology gaps. The slide on DEI efforts lacks specific metrics (e.g., number of speakers, number of target poll participants, number of presentations).

- Progress and outcomes: Some of the projects presented show solid progress and outcomes. For instance, fungal and yeast demonstrations showed production of a variety of molecules and much higher than the 1-g/L targets. Serine recombinase-assisted genomic engineering for host onboarding is a promising new method for enhanced genetic transformations, though it is not clear from the presentation the time frame corresponding to those accomplishments. The partnership with NSF to allow more minority-serving institutions to leverage the ABF is an exciting and positive accomplishment. A few milestones were labeled as complete but upon digging did not exactly meet the stated descriptions. For instance, the ABF listed as completed: "Bring a total of at least 15 microbial hosts to at least Tier 1, and provide corresponding information, resources, and tools via publicly accessible ABF HObT website." The resources described are not in fact available on the HObT website, as was pointed out during the Peer Review. This website was discussed in the 2021 Peer Review and does not appear to have been made more accessible since that time. It was also evident from discussions during the Peer Review that there is a lack of industry engagement and that the IAB is not properly utilized by the ABF.

- Impact: Overall, the ABF met their end-of-project milestones for the 2021–2023 AOP period (those listed in the key milestones and quad chart slide).

- The ABF has published a lot of scientific papers on the conducted work; however, they should diversify the dissemination of information to other mediums. For instance, blog posts and videos should be available on the ABF website to share fundamental insights gained from ABF work that may not be publication-ready, or negative results that may not be of interest to journals but can nevertheless advance knowledge about synbio and biomanufacturing processes.

- The number of licensed technologies seems quite low relative to the number of inventions, with only two licenses versus 36 patents, records of inventions, and software disclosures. One aspect that may be limiting licensing of ABF technologies is the degree of applicability of the work, as well as the

presence/absence of continued support for the tools, especially in the case of software technologies. The bandwidth/feasibility of software support should be considered in the development on any new software tools intended for licensed use.

- ABF leadership provides five activities that encompass the technical approach: (1) demonstration of ABF capabilities via DBTL infrastructure, demonstration projects, and metabolic beachheads; (2) use of TEA and LCA; (3) onboarding of industrially relevant host organisms; (4) integration of bioprocess scale-up as a test capability; and (5) industry engagement and outreach. Identification of these activities is indicative of sound scientific merit; however, this summary will not specifically review whether these activities were adequately executed. Each activity will be judged individually in the reviews that follow. No management plan for successful implementation of these activities was provided, which leads me to believe the current management structure is not as strong as it should be. Who takes ownership, provides ultimate oversight, and possesses final decision-making authority for each activity? A multi-site biofoundry funded with public resources requires a defined management structure with strong leadership willing to set priorities and distribute resources (financial, technical, and human). Leadership by consensus is ineffective and results in delayed decision-making. Multiple risks were identified with appropriate mitigation strategies; however, failure to describe a management structure is itself a risk that needs to be addressed. Communication and collaboration mechanisms between the different activities were not provided in this specific presentation, but extensive interactions with lab leaders over the course of the review revealed strong professional relationships among these principals, which appears to provide adequate internal communication. Twelve DEI-related activities were listed as completed, in development, and/or ongoing. These activities were spread over current staff, future workforce development, minority-serving institutions, and compliance with the Americans with Disabilities Act (ADA). Specific metrics related to DEI should be developed and reported on in future peer reviews to monitor progress in this regard. Both progress/outcomes and impact for each activity will be reviewed on an individual basis in the reviews that follow.

PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the extensive, thoughtful, and helpful comments that the reviewers have provided (and the time and effort that went into them), both for this particular presentation and for all following ABF-related presentations. As the reviewers have noted, our presentations flowed from one to another, which made it difficult for the reviewers to fully compartmentalize their feedback on a per-presentation basis. Several reviewer comments were provided verbatim across multiple presentations. Our approach in responding has been to address comments as they arise, and rather than replicating our (same) responses across presentations, to instead refer the reader to our previous responses. While more detailed responses can be found below (and in our responses for subsequent presentations), we thought it would be helpful to make several high-level responses here. The ABF and BETO teams worked intensively and extensively together during the recent ABF strategic and implementation planning activities, and the ABF is committed and fully bought-in to its new direction and is excited to support BETO in achieving its ambitious goals for 2030.

- Host onboarding and development activities will continue within the ABF, as directed by industry and other partners in collaboration projects. We will continue to make improvements to the ABF website and specifically to our HObT web application to better depict our capabilities. As part of our capability benchmarking activities (which will help prioritize ABF development efforts and enable business development and technology transfer), the ABF will continue to consider DBTL-related metrics.

- We have changed from a consensus-driven management approach to the lead PI (with BETO support) having and exercising decision-making authority. A new strategic implementation lead will help ensure that all work in the ABF remains aligned with its strategic and implementation plans, and that work is not disjointed. The ABF is continuing on its path to becoming more of a “customer”-facing entity, with

the establishment of new business development co-leads, upcoming changes to how the ABF interfaces with its advisory board, and increasing amounts (>50% by the end of FY 2025) of its resources dedicated to collaboration projects.

- The ABF has contributed (and is contributing) to multiple product/process commercialization efforts, several of which are building off of established ABF beachhead molecules. Regarding the HObT website, we agree that HObT will be useful to the public and further the ABF's impact and dissemination of its accomplishments and capabilities. HObT itself has not been published, nor has the ABF Host Tier system, and the functionality that permits only public sharing of publicly disclosed (and not prepublication) information regarding experiment data, strain, and sequence information has not been implemented yet. For these reasons, as discussed at the Peer Review, we have not yet promoted HObT (e.g., linking from the ABF website). Once these issues are resolved (target date Sept. 30, 2023), we will more prominently promote HObT. The capabilities associated with each tier, and the progress made within each tier for each organism, is (in fact) publicly accessible now. We are excited to make the rest of the public information accessible through HObT as quickly as possible.

- Regarding the observation that only beachhead (malonyl-CoA to lipid products) has been used by industry thus far in collaboration projects, we note that ß-ketoadipic acid is being worked on with an industry partner in a DFO project (protocatechuic acid beachhead), as are PHAs (acetyl-CoA beachhead). We have also had several companies express interest in 3-HP through both aspartate and malonyl-CoA beachheads via their proposals into the competitive ABF funding opportunity. As discussed in the Peer Review, challenges regarding DBTL metrics (and the reasons behind them) were part of the reason for the strategic redirection of the ABF toward more measurable goals. We agree with the reviewers that we should not abandon all DBTL metrics going forward. DBTL metrics will be used internally within the ABF when prioritizing opportunities for improvement (including new capability development), and as part of our capability benchmarking activities.

- Regarding the management of the ABF, until 2023, decisions across the seven national labs in the ABF consortium were predominantly made through consensus-driven approaches and processes, which, as discussed, have their limitations. Starting in 2023, the ABF's lead PI (with support from BETO) will have authoritative decision-making control, in line with reviewer suggestions, along with updates to the org chart that show clearer lines of hierarchy. Regarding the ABF not using its IAB as effectively as possible, a prominent component of the restructuring exercise—and ongoing discussions between industry engagement efforts, BETO management, and, in the future, ABF business development—is to better define the roles and goals of having an advisory board and tailoring interactions and utilization efforts of the board for these outcomes. We welcome these changes and improved interactions, as they will be needed as industrial collaborations intensify.

- Regarding the ABF's ability to learn from all projects (internal and collaborative), this is not a data management issue, but rather a concern that models trained on data could be reverse-engineered to reveal the underlying proprietary training data. There are a number of means of mitigating this risk, but it remains an important risk to be cognizant of and work with collaborators to address. Regarding individual PIs within the ABF pursuing disjointed work, we now have an ABF strategic implementation lead, who will monitor activities for continued unified alignment with the ABF strategic plan. Regarding the need for the ongoing support of ABF software tools to drive licensing efforts, it is expensive (i.e., a high opportunity cost) to maintain software. The ABF seeks commercial solutions where possible. For essential software for which there is no commercial solution, the ABF continues development, support, and maintenance. The ABF prefers to license software to companies that will themselves take on the support and maintenance of the software for its customers. The ABF agrees that a lack of future obligated support for its (nonessential or where a commercial solution is available) software could

negatively impact licensing, but in these instances, the costs to the ABF would exceed the benefits of the licensing.

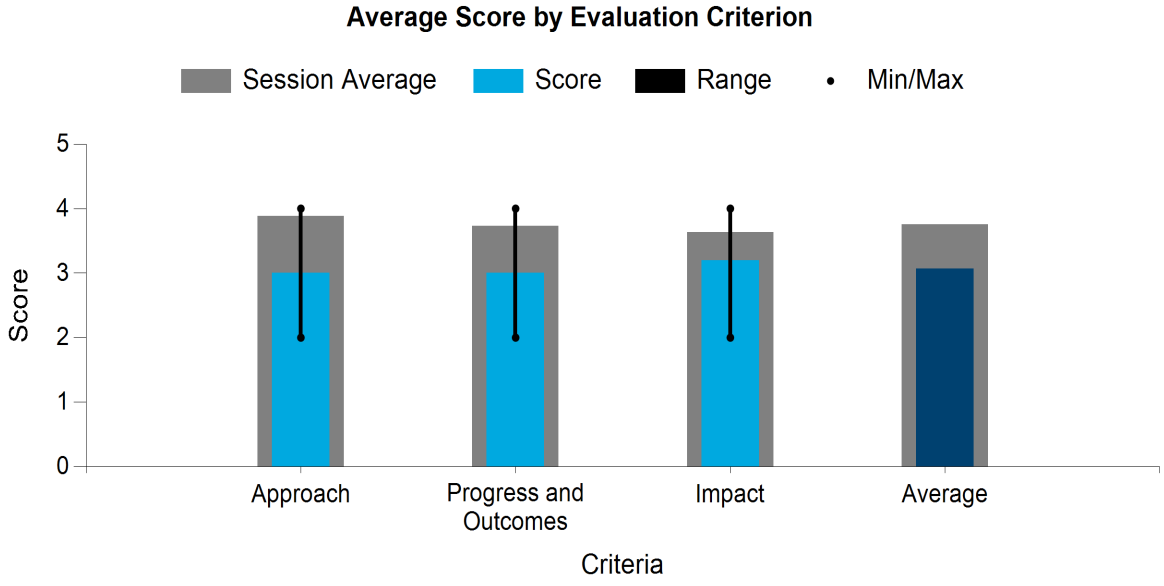
ABF FUTURE STRATEGY – IMPLEMENTATION PLANS

Lawrence Berkeley National Laboratory, National Renewable Energy Laboratory, Sandia National Laboratories, and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	ABF10
Presenter(s):	Gregg Beckham; John Gladden; Jon Magnuson; Katy Christiansen; Nathan Hillson
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

In January 2023, the ABF began to develop the implementation for its recently completed strategic plan. Implementation planning was completed in March 2023, including sets of milestones to go along with the goals and deliverables described in the strategic plan. The relevance of developing the implementation plan is that it ensures that ABF planned activities for FY 2023–2025 meet BETO's budget allocation and are consistent with a path likely to accomplish the deliverables established in the BETO-approved revised ABF strategic plan. The impact of establishing the implementation plan is primarily the resumed operations of a reimagined DOE ABF, with consequential impacts including industry-partnered commercialization paths to SAF and renewable biochemicals with significant carbon dioxide equivalent (CO_2e) reducts, as well as benchmarked ABF capabilities demonstrating substantial operational performance gains. The top challenges of completing the implementation planning process are maintaining team morale and interpersonal relationships; developing a transparent, inclusive, and equitable activity prioritization process; and supporting broadly enabling tools. The outcome of this activity is to develop a budget-constrained implementation plan that largely accomplishes the deliverables established in the ABF strategic plan and gracefully breaks from the status quo to preserve capabilities and relationships as best as possible.



COMMENTS

- The ABF utilized multiple presentations to document their journey in developing a new strategic plan for the next funding cycle. These presentations grew off each other, and as a result, it is difficult to separate

them as required in this review process. Instead, I have used the "Strategic Plan" presentation to provide feedback on this presentation as well. Please look there for my comments.

- Strengths:
    - Use of go/no-go to downselect bioconversion systems in SAF and biochemicals is an effective approach to allow the ABF to focus their resources.
    - It is critical for the ABF to continue developing new tools/capabilities to keep them as the leader in biofoundries. Several proposed new capabilities are innovative and have a lot of scientific merits.
    - The use of genome-scale screening tools and artificial intelligence/ML-guided approaches can effectively enhance TRY metrics of target biochemicals.
- Weaknesses/areas for improvement:
    - There is a list of tools proposed to be developed. It is not clear whether these tools were selected by their impacts to industrial biomanufacturing or because these are ongoing projects in ABF PI labs. It is also not clear what criteria were used to select these tools as targets. Some of these tools are more specific to individual projects rather than general tools that can benefit the broader biomanufacturing industry. For example, protein structural modeling to change substrate specificity and improve thermostability seems to be specific to the thermophilic ethanol production project.
    - The FY 2024 milestone for alkane SAF is to measure baseline TRY. It is unreasonable to take a year to just complete baseline measurement. The FY 2025 milestone is set to achieve the final target of 75% theoretical yield. It is unlikely to achieve such a big transition from baseline TRY to final deliverable in just 1 year. If it is possible, it would suggest that the FY 2025 milestone was set too low. Additionally, specific approaches to reach 75% theoretical yield are lacking.
    - It is not clear how the 500-kiloton CO_2e reduction will be measured. This number is associated with titer, yield, and scale of each conversion system. Will this number be demonstrated through scalable production? If not, this number is meaningless, because any bioconversion system multiplied by a huge imaginary scale value can potentially reach 500-kiloton CO_2e reduction. It is not clear how this milestone can be evaluated by FY 2025.
- These comments are the same for the ABF Future Strategy (1) Strategic Plan; (2) Goals, Milestones, and Deliverables; and (3) Implementation Plans:
    - The ABF and BETO have both struggled for years with how the ABF fits in with BETO's mission. This struggle is still ongoing. While it's clear the ABF needs new direction, management, and goals, at the same time, BETO needs to decide whether or not it's going to support an outwardly facing biofoundry focused on supporting synthetic biology. BETO was absolutely correct in calling out the ABF's failures to deliver on previous goals and forcing a reorganization of the ABF. That being said, the ABF was founded with a vision to support and advance synthetic biology in the United States, both for industry and at the national labs. This has frankly never seemed to fit well with BETO's overall mission statement in the past. However, with the Biden administration's call to decarbonize the chemical industry, ABF finds itself more aligned with BETO than in its previous history. My recommendation is for BETO to support the ABF consortium as it was intended—namely, to be outwardly facing, heavily engaged with industry, and providing services to all U.S. research efforts (industry and academia) to advance synbio goals and develop America as a leader in bioproduction of the molecules our world needs. This means changing the current

focus of the ABF away from some of their core work and putting funds back to supporting external partners and ABF infrastructure.

- I recommend the ABF revise their goals in the current strategic plan. This is not going to be something the ABF (and maybe BETO) wants to do. However, the current strategic plan is not well thought out in some areas, and seems to overcorrect and try to appease perceived BETO unhappiness. It's much better to spend another few months rethinking and replanning than waste $45–$60 million over the next 3 years.

- Specifically, the core SAF targets seem out of scope with the ABF's mission and put in to appease BETO. First, the economics of SAF from lignocellulosic sugars is never going to work. Not unless BETO is OK relying on massive government subsidies for SAF, far more than the current subsidies. It's not possible to be economically viable to make SAF currently from cheap sugar cane, using a terpene strain that has near-maximum theoretical yield and a very simple and cheap purification process. The extra costs of lignocellulosic sugars only make the economics worse. The ABF should get clarity from BETO on what level of subsidy is OK to assume if they are going to pursue this path. Second, instead of the ABF trying to guess what would be the best SAF molecule for them to develop, they should be engaging with the big industry players to understand what they want, if there is any overlap between what is needed/wanted, and what the ABF/synbio can help develop. The ABF and BETO need to be brutally honest here, and if the economics of hydrocarbon production from cellulosic sugars is not going to be the most economically viable route to SAF, then don't work on this in the ABF. With regard to the consolidated bioprocessing (CBP) work proposed by the ABF, the CBP is very high risk and high reward. I don't know enough about the challenges of CBP to know if the ABF is adequately addressing the risks, and therefore the probability of success. I do think the CBP work overall is aligned with BETO and is in the national interest to fund at the national labs. It is this kind of "blue sky" visionary work that industry won't do because of the risk, cost, and timelines involved. This is where government funding of basic science can push development of new technologies. That being said, should this work be done in the ABF? Again, I cannot judge that because little detail was given on why the ABF thinks they are best suited to do this work.

- Assuming some or all of the proposed SAF work is dropped from the ABF strategic plan, what should they do with the money? Some recommendations (in rank order): (1) Continue to host onboarding work, which we learned was cut completely. This is my number 1 recommendation for the money. The host onboarding is one area that ABF and BETO funds can really make a difference to advance new technologies and develop new strains to enable new chemicals and pathways at better costs. This is expensive and time-consuming, and often industry is reluctant to take it on. If they do develop a unique microbe, they will keep it strictly proprietary. Host onboarding should pick a small number of microbes that have very different properties, would access very different feedstocks or fermentation abilities, and are likely to be tractable genetically. Then, develop them to a high TRL rating to make them really useful. I have a lot to say about the apparent lack of progress on the HObT website since 2021 and the lack of helpful information to the U.S. research community from this work when reviewing this part of the ABF presentation. Regardless, if further work on host onboarding is done, there must be funds allocated to bring the website up to date and make the tools developed useful to the public, or BETO can consider a lot of that work and money wasted. (2) Develop another chemical target, if there is another one as developed and with clear TEA and CO_2e reduction benefits. (3) Put more money to partner-facing projects (FOAs) that support BETO's goals and will also support industry and commercial realization of BETO's goals.

- Several aspects of the technical targets had not yet been worked out by the time of the BETO review in April. Given that the ABF had 4–5 months already to replan, this is surprising and disappointing. The tools section of the strategic plan is not well thought out at all. They have not come up with criteria to even choose which tools to benchmark, or how to conduct benchmarking. It seems like no one wanted to give up their pet project, and with no one really in charge, they are having a hard time figuring out what to pursue further. It is good to not spend time and effort trying to build a capability that is already publicly available that people can access. DOE/BETO/national labs should focus on offering capabilities or research tools that are not available or that industry is not going to do. However, I caution that the ABF should not throw out a capability or tool even if it is measured to be not as good as something already out there. It might be that the ABF provides a different benefit that makes the tool/capability useful to researchers and industry. For example, an ABF capability might be almost as good as something already commercially available, but ABF might be cheaper, more accessible, or offer more capacity to a limited service. The ABF needs to figure out their criteria and how they are going to benchmark tools immediately and complete the assessments as soon as possible. In the current plan, it looks like this will not be done until the end of 2025.

- Lastly, I think it's a mistake to abandon all DBTL metrics going forward. Revise DBTL to be more realistic, but still have a goal to reduce the DBTL cycle (or even parts of DBTL that make the most sense, are the most commonly used, or are the biggest bottleneck). It doesn't have to be every capability and every tool. The team can measure time needed to finish one complete experiment and learn from it (per tool or per instrument), and focus on making those individual unit ops more efficient. Drop wall time versus clock time. What matters is how long it takes you to learn from an experiment. If there is some large downtime (let's say you need to ship samples from one site to another, and that takes weeks), then that is part of your cycle time and something to target to be more efficient. Focus on gaining efficiencies and improving the infrastructure already in place. There is some acknowledgement of this in the strategic plan; under tool milestones it says, "Operational performance gains of significant impact demonstrated for at least 2 ABF benchmarked technologies."

- The ABF said there was large interest from chemical companies for the proposed 3-HP and muconic acid targets. (I know from my own company that industry is very interested in muconic acid to make nylon.) These two core projects are much more aligned with the capabilities the ABF has built, have a high chance of success, and align with BETO's goals. These two projects should definitely continue and are a good change from the beachhead approach, which for whatever reason did not appear to get much industry traction.

- The shift in emphasis to bring in more external money (FOA and DFO) and have >50% of the ABF's budget committed to external partnerships by 2025 is a good change of direction and furthers the goals of the ABF to support industry and other research to translate lab work to real-world changes in decarbonization and reducing CO_2. It's going to be very challenging to achieve a significant increase in industry money into the ABF in the near future, especially if contracting takes a year. It will take time to get >50% of the ABF's budget committed to external partnerships and reach an average of more than fivefold oversubscription for the ABF's funding opportunities. The 2024 milestone to have two funds-in projects means the ABF needs to land those partnerships in a few months if contracting is going to take a year. Also, the length of time to contract must be improved. The ABF needs to redo its approach to IP and how it contracts projects to de-risk bringing in more industry partners and money. BOTTLE has worked out a very clear IP management plan that allows different levels of industry access to any IP developed. There is an option for industry to keep all the IP (and the national labs then get a higher percent royalty fee). The ABPDU also has worked out smooth contracting and IP understandings that allow them to

contract in a few weeks, and industry does not have to share IP if it does not want to. The ABF should have developed something like this years ago.

  - There was no mention of how the ABF will measure reduction of CO_2e in their partner projects. Given the ABF's track record regarding an inability to quantify metrics, this does raise concern.

  - The ABF revised management plan is significantly better than the old management structure. The one area that still needs to be changed is for the lead PI to be solely accountable and responsible for running the ABF. There should not be any decision by committee. The ABF needs a 100% dedicated full-time employee running it. This person should be an "outsider" with no emotional connection to the past 6 years of the ABF. The executive committee reports to and advises the ABF PI but does not make decisions. It's clear that the ABF has really suffered from decision by committee and a lack of someone whose job is 100% running the ABF.

- Approach: The ABF has strong capabilities and expertise to target the proposed targets.

- Progress and Outcomes: Go/no-go industry interest gates for the four molecules will be highly important. Beyond just go/no-go, incorporating industry feedback into what alternative molecules, pathways, and approaches should be pursued by the ABF will be critical. It will also be extremely important to request feedback from diverse industry sectors, not just synbio or synbio-adjacent players. There are a lot of new capabilities listed under new development (Slide 12). It is not clear how these capabilities will be developed given the focus of core funding on the four molecules. During previous sessions it was stated that new software tools will not be developed, yet several are listed here, and the plan is not clear. If the plan is to develop these as part of funds-in projects, this is highly risky since the funds-in projects may not be interested in developing or paying for specific tools that are not directly related to the work.

- Progress/outcomes and impact are given neutral scores of 3 because this is a review of plans rather than progress. Implementation plans to achieve milestones related to tools, SAF, and biochemicals are sound, with acknowledgement that details are necessarily sparing. Technical excellence demonstrated in previous performance periods lends confidence that progress will be made in these areas, assuming management takes an active role in reorienting research objectives across the ABF scientific enterprise. Going forward, partnerships carry the greatest risk to the new strategic plan, but few details were provided as to how the stated milestones will be achieved. Three activities are provided to achieve milestones: actively engage industry to develop and promote adoption of ABF processes/technologies, develop new IP management plans to enable efficient licensing, and demonstrate/measure ABF's impact on and contribution to biomanufacturing. Without specific actions to be taken and without demonstrated prior success in this area, it is difficult to feel confident that partnership goals will be achieved. As a reminder, expediting partnership agreements and formulating IP management are complicated by the ABF distributed model.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback. We responded above for the "ABF Introduction and Overview"; "ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads"; "ABF Past Accomplishments – Industry Engagement, Outreach, and Management"; "ABF Past Accomplishments – TEA/LCA"; "ABF Past Accomplishments – Host Onboarding and Development"; "ABF Past Accomplishments – Process Integration and Scale-Up"; "ABF – Lessons Learned and Introduction to Future Plans"; "ABF Future Strategy – Strategic Plan"; and "ABF Future Strategy – Goals, Milestones, and Deliverables" presentations. As stated above for the "ABF Introduction and Overview" presentation, we reiterate our appreciation for the extensive, thoughtful, and helpful comments that the reviewers have provided (and the time and effort that went into them), for this particular presentation and for all previous ABF-related presentations. While more detailed responses can

be found in our responses for previous presentations, we thought it would be helpful to repeat several high-level responses here. The ABF and BETO teams worked intensively and extensively together during the recent ABF strategic and implementation planning activities, and the ABF is committed and fully bought-in to its new direction and excited to support BETO in achieving its ambitious goals for 2030. Host onboarding and development activities will continue within the ABF, as directed by industry and other partners in collaboration projects. We will continue to make improvements to the ABF website and specifically to our HObT web application to better depict our capabilities. As part of our capability benchmarking activities (which will help prioritize ABF development efforts and enable business development and technology transfer), the ABF will continue to consider DBTL-related metrics. We have changed from a consensus-driven management approach to the lead PI (with BETO support) having and exercising decision-making authority. A new strategic implementation lead will help ensure that all work in the ABF remains aligned with its strategic and implementation plans, and that work is not disjointed. The ABF is continuing on its path to becoming more of a "customer"-facing entity, with the establishment of new business development co-leads, upcoming changes to how the ABF interfaces with its advisory board, and increasing amounts (>50% by the end of FY 2025) of its resources dedicated to collaboration projects. The ABF has contributed (and is contributing) to multiple product/process commercialization efforts, several of which are building off of established ABF beachhead molecules.

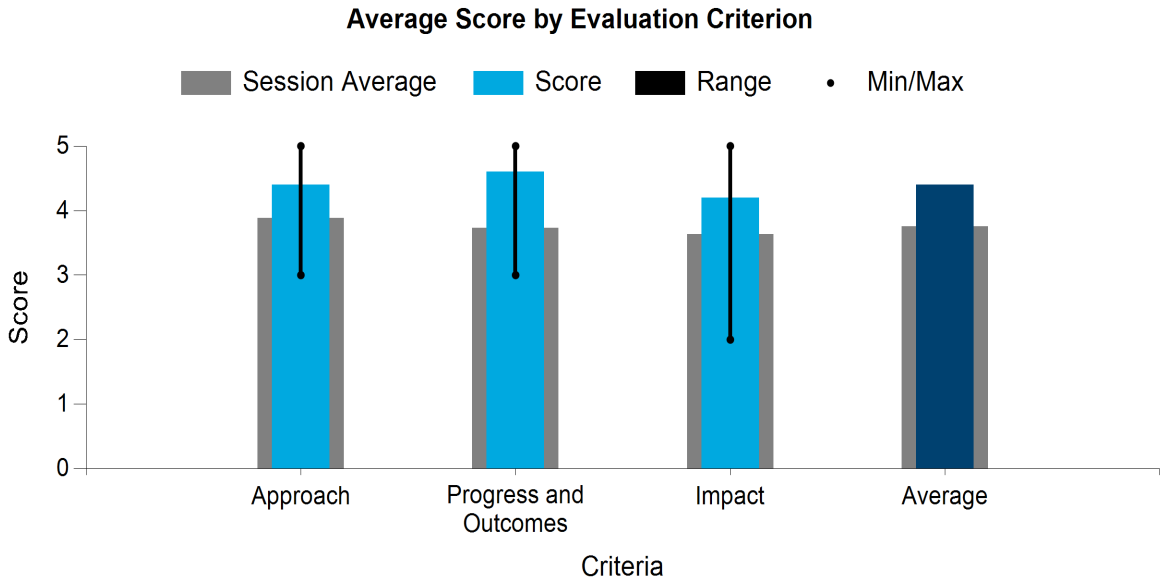
ABF PAST ACCOMPLISHMENTS – PROCESS INTEGRATION AND SCALE-UP

Lawrence Berkeley National Laboratory and National Renewable Energy Laboratory

PROJECT DESCRIPTION

WBS:	ABF11
Presenter(s):	Davinia Salvachua; Deepti Tanjore; Katy Christiansen
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

The main objectives of the Process Integration and Scale-Up (PISU) task were to produce lignocellulosic hydrolysates, screen microbial strains, and develop bioreactor cultivation processes to improve product TRYs. In the past 2 years, we developed bioprocesses for (1) muconate production by engineered *Pseudomonas putida* and (2) terpene production by *Rhodotorula toruloides*. In addition, we evaluated *P. putida* as a host for the production of various value-added compounds. The PISU task has also been involved in the production of targeted compounds at larger scales for industry and other DOE-BETO-funded projects to test downstream product recovery processes and analyze material properties. We also demonstrated better utilization of traditional real-time data for in-line process control, and we are now working toward using imaging as an approach to better understand bioreactor performance.



COMMENTS

- Scale-up is a critical step in bringing new products and strains to market. Understanding the challenges in scale-up, and in particular understanding what issues are likely to appear at larger scale that are not manifested in lab-scale experiments, is critical to ensuring R&D efforts in the lab will have applicability at commercially relevant scales. It is therefore important for BETO to have scale-up of technologies as a goal, and for the various research groups and consortiums under BETO to communicate and share knowledge and best practices. It is less obvious that these activities should live at the ABF. Where scale-up is most useful and applicable at the ABF is in understanding the performance of the various strains in

its catalog at a variety of scales. By understanding the various trends of different organisms as they are grown in progressively larger reactors, the ABF can better guide industrial partners on the best strains to utilize for commercial applicability; they may not be the best-performing strains at the lab scale.

- This context informs the approach and impact scoring for this activity. The approach presented is centered around the idea of developing this type of knowledge by understanding experimental results at different scales and optimizing processes for different hosts at larger scales to improve TRY. The demonstrated successes and activities appear to be more one-off, however, and there doesn't seem to be a coordinated scale-up strategy for the various hosts in the library. Impact is low for this reason, and because there does not seem to be a way for scaling issues at still larger scales (beyond those offered by the ABF) to be fed back to the team at the ABF. It is important for the ABF to follow partners after they outgrow ABF capabilities in order to close the feedback loop from outside the ABF on scale-up learnings (e.g., 100-L to 100,000-L reactor sizes) that can further help optimize the lab-scale testing and experimental work done by the ABF.
- As a result, the scale-up activity at the ABF is at a crossroads. One path forward focuses on more scale-up testing across hosts using AOP funds to better understand the ABF strain library and the behavior of each host at various scales. This work would then inform host selection for new product development. The other path limits scale-up activities to DFO or funds-in uses where scale-up is a critical part of the research opportunity. This pathway may begin to overlap with efforts at ABPDU or BioMADE, and so coordination with other groups in orbit with the ABF is important to ensure ABF funds are spent on the tools and capabilities where the ABF has the most specialized knowledge and potential for impact. Being a scale-up partner may not be the best use of ABF funds if other organizations that operate at the 100–5,000-L scale are better placed to take hosts and products identified at the ABF to the next TRL stage.
- Strengths:
    - The PISU team has made excellent progress. All proposed milestones were delivered on time.
    - Extremely high TRY was obtained for multiple conversion systems, including production of muconate, ß-ketoadipate, and 3-HP from various carbon sources. Muconate production was scaled up to 150 L, and high titer was achieved at this scale. These results are very impressive.
    - The PISU team has also demonstrated collaborations with industry and academia, proving their values in facilitating the U.S. bioeconomy and the scientific society.
- Weaknesses/areas for improvement: N/A.
- Overall, the work done in the PISU is highly relevant to many aspects of the ABF. Scale-up is hard; ask anyone who's done it. It's imperative to do scale-up work to advance the TRL of any project, whether industry or national labs. As the PISU team states, the work is also necessary to give better, more realistic parameters to the TEA/LCA teams for their modeling. The most important aspect of any synbio project is to generate material to have for actual application testing and understanding the impurity profile. ABF is encouraged to generate representative samples as soon as possible (in PISU) to accelerate downstream purification and applications testing.
- The work on oxygen uptake rate in fermentation is very important, and it's good to see that being implemented. That should be considered standard on all fermentations for the ABF. This is routine in industry.
- Several milestones were reached successfully. It's not clear if this is all the milestones (feedback to BETO for all projects to list all milestones and percent completion on them).

- Scale-up is one of the biggest challenges for the bioeconomy. As such, the work done under this task is highly relevant for advancing the U.S. bioeconomy. The work that was presented does address many important challenges, such as understanding cell heterogeneity and its impact on fermentation performance across scales. The media development tools are also a highly relevant and potentially impactful contribution and could be expanded to better account for the expected performance under lower-carbon-intensity media feedstocks. One shortcoming I see in the approach is that the experiments and data from fermentation runs do not appear well integrated into other aspects of the DBTL cycle. For instance, how might design or build considerations impact eventual performance across scales? This could be a highly impactful question that the ABF is well suited to solve. The dissemination of the work done could use improvement. For instance, the media optimization tool could/should be accessible through more than just publications (e.g., through a web app or other interactive mechanism).

- The PISU team continues to perform well, meeting a diverse number of ABF needs. It takes strong technical and organization skills to successfully operate over the range of bench scale (2 mL) through pilot scale (9,000 L) for various organisms. Their Pilot City Internship Program for high school students is a creative addition to DEI initiatives that exposes students to real-world STEM professional opportunities at a point early enough to influence selection of an educational path.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback, some of which was responded to above for the "ABF Introduction and Overview"; "ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads"; "ABF Past Accomplishments – Industry Engagement, Outreach, and Management"; "ABF Past Accomplishments – TEA/LCA"; and "ABF Past Accomplishments – Host Onboarding and Development" presentations. In terms of interactions with BioMADE for scale-up, we note that we are planning to have a joint funding call that will be pursued with BioMADE in the future. We also note that the ABPDU is not the only scale-up facility in the ABF—NREL's Integrated Biorefinery Research Facility can scale to 9,000 L. In terms of feedback loops with industrial partners, we are certainly interested in feedback from industrial partners, but this is not our decision to make; stated differently, we rely on connections to DFO partners after the award to provide this feedback, and we will consider a mechanism in the funding opportunity process going forward where this can be included. In terms of generating samples for separations, purification, and downstream testing, we fully agree, and we are doing this again for several target compounds in the FY 2024 time frame in collaboration with the performance-advantaged bioproducts projects in the BETO portfolio. In terms of milestones, all PISU milestones were met and delivered in a timely fashion. In terms of integrating scale-up into the DBTL cycle, we agree that this is an excellent opportunity that we are not fully taking advantage of, and we will work to improve this going forward. With regard to a coordinated scale-up strategy for hosts, the PISU task produces hydrolysate for fermentation and conducts bioprocess development of ABF hosts in bioreactors at multiple scales. To this end, we tested multiple ABF hosts at bench and pilot scale. In the past 2 years, we demonstrated multiple target-host combinations across bench to pilot scales, delivered lignocellulosic hydrolysate, developed several new bioprocess tools, and enabled DFOs and other consortia.

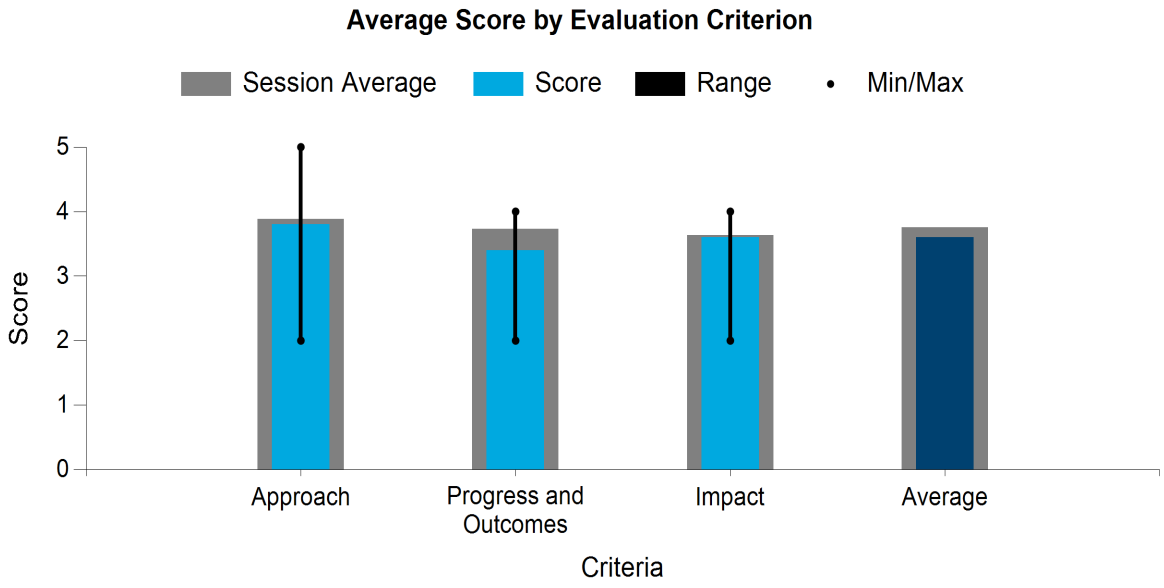
FUNDING AND PARTNERING MECHANISMS

Lawrence Berkeley National Laboratory

PROJECT DESCRIPTION

WBS:	ABF12
Presenter(s):	James Gardner
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

Since its inception, the ABF has partnered directly with industry and academia to accelerate biologically produced fuels and chemicals toward commercialization and to develop new tools and capabilities that increase the ABF's ability to better support the synthetic biology and biomanufacturing communities, in its effort to support the SAF and decarbonization mission of BETO. The ABF's partnering mechanisms have since grown, matured, and diversified, and they continue to do so. With 37 partnerships either complete, in active R&D, or in contracting, the ABF's support of its external partners is strong, as are the relationships the ABF has developed with its external reviewers and partnering organizations, including the NSF. The ABF's ongoing partnering work with minority-serving institutions will continue its support of important DOE objectives in DEI in science. The positive impacts that the ABF has made through its partnerships, as well as the resulting tools and knowledge that the ABF can reapply, are lowering barriers to entry for small organizations and startups and offering rare expertise and capabilities to larger organizations as well. This presentation outlines these activities and achievements.



COMMENTS

- The ABF utilized multiple presentations to document their journey in developing a new strategic plan for the next funding cycle. These presentations grew off each other, and as a result, it is difficult to separate them as required in this review process. Instead, I have used the "Strategic Plan" presentation to provide feedback on this presentation as well. Please look there for my comments.
- Strengths:
    - DFOs provide a unique funding mechanism for industry to directly collaborate with the ABF and utilize the ABF's capabilities. This funding mechanism effectively motivates both industry and

ABF personnel to be deeply involved in the projects. Most projects are also highly aligned with BETO's goals for producing bio-based chemicals.

- A large number of industry partners have been involved with and directly benefited from the DFO funding mechanism. Excellent feedback was received from these industrial partners.
- The DFO projects also utilize a wide range of ABF technologies and showed a balanced distribution among different technical areas in DBTL, hosts, process, TEA/LCA, and software.

- Weaknesses/areas for improvement:
  - It is surprising that no DFO team worked on SAF projects, which is a central mission for BETO.
  - Although great overall progress has been made from DFO projects, some individual DFO teams have unsatisfactory performance. They either failed to deliver the proposed milestones or created little impact to industrial biomanufacturing or BETO's goal in producing sustainable bio-based chemicals/GHG reduction. Some monitoring mechanism is needed to ensure the funded teams perform their proposed tasks.

- The goals of the funding for the new strategic plan are overall very good. As mentioned many times in previous reviews, there is a high risk to being able to hit a high level of industry funding, mostly due to the long contracting period and complicated IP approval process. The ABF needs to redo its approach to IP and how it contracts projects to de-risk bringing in more industry partners and money. BOTTLE has worked out a very clear IP management plan that allows different levels of industry access to any IP developed. There is an option for industry to keep all the IP (and the national labs then get a higher percent royalty fee). The ABPDU also has worked out smooth contracting and IP understandings that allow them to contract in a few weeks, and industry does not have to share IP if it does not want to. The ABF should have developed something like this years ago. Again, this is another example of the ABF management not being focused, with a single person responsible for making the ABF as successful as possible.

- Overall, the ABF has demonstrated productive partnerships with impact to industry. Strengths: Projects have largely been successful and achieved relevant milestones. There are a few "return customers" such as Lygos, indicating that the work the ABF provided was seen as valuable. The new partnership strategies with NSF and minority-serving institutions will increase the impact of the ABF to advance research and technologies led by minority researchers. Weaknesses: Long contracting timelines are such that there is even risk of the original project scope becoming out of date/irrelevant. The ABF's projects with partners are quite wide and diverse; while this is in some ways a strength, I also see that it may limit the ability of the ABF to leverage industry projects as a way to advance the bioeconomy forward in a more strategic/coherent manner. Having projects with more related end goals may facilitate advances in the difficult sectors more easily.

- The greatest strength of the funding and partnering approaches has been the ability to evolve and improve based on prior experience, feedback, etc. Evolution of partnering mechanisms will be critical to implementation of the revised ABF strategy. The greatest weakness and most significant risk to ABF partnering is delays in formalizing agreements to get projects underway. If the ABF wants to increase funds-in partnering mechanisms, 12-month delays to complete agreements will be unacceptable to organizations working with performance milestones and timelines. In essence, there will always be organizations willing to accept 12-month delays prior to project initiation, but those are not the organizations capable of moving the needle on bioeconomy growth. Additional strengths include continued expansion of partnership funding organizations (now to include DOE, NSF, and BioMADE) and a DEI plan that includes funding opportunities specific for minority-serving institutions. During the

competition for a limited number of partnerships (DFOs and FOAs), minimal attention is given to commercialization potential. Evaluation of partnerships to date reveals a very wide range of commercial potential, from high to vanishingly low. Low-commercial-potential projects may still be worthy of funding, but the benefit to the ABF and bioeconomy should be made explicitly clear in all applications. Benefits to the partner (e.g., company, academic institution), the ABF, and the bioeconomy should be provided in all future Peer Review materials. If the ABF wants to secure more funds-in agreements and improve oversubscription levels of DFOs and FOAs, improving both the quantity and quality of data provided on the ABF website is imperative. The ABF should look to the ABPDU as a model for elevating its information dissemination. Inclusion of case studies that provide information such as tools used, fold improvement in product, etc. would be very valuable. At the very least, these case studies provide specific examples of ABF capabilities.

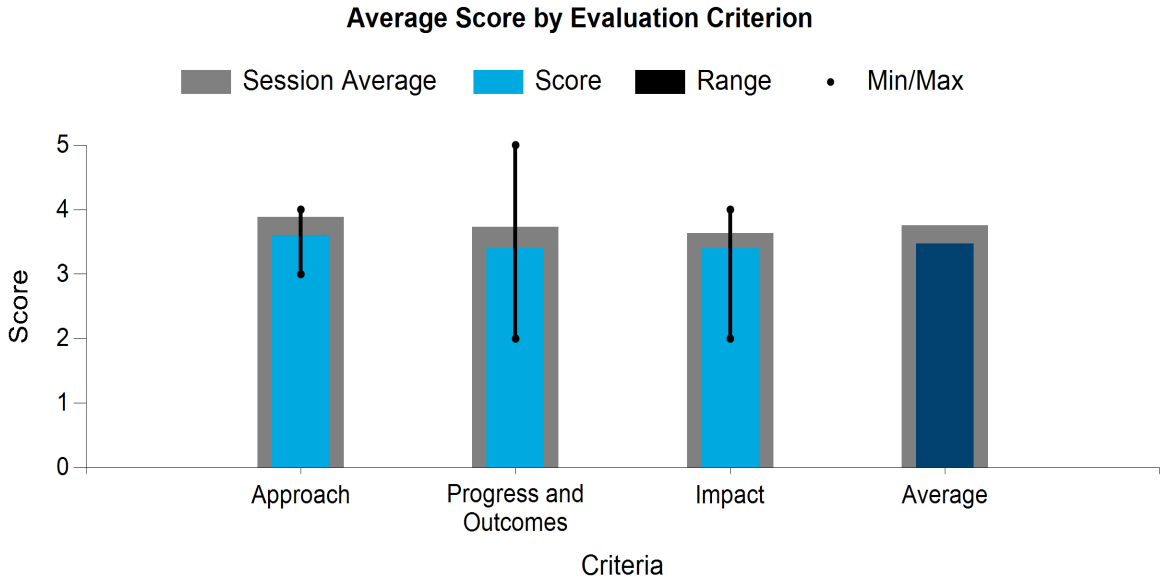
ABF PAST ACCOMPLISHMENTS – DBTL INFRASTRUCTURE, DEMONSTRATION PROJECTS, AND BEACHHEADS

Lawrence Berkeley National Laboratory, National Renewable Energy Laboratory, Sandia National Laboratories, and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	ABF2
Presenter(s):	Gregg Beckham; John Gladden; Jon Magnuson; Katy Christiansen; Nathan Hillson; Di Liu
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

Since 2016, DBTL infrastructure and demonstration projects have been core ABF activities. In 2020, beachheads were added along with the demonstrations as a strategic activity, to make more of the biochemical and bioprocess space accessible. The relevance of these tasks are that they (DBTL infrastructure) support the ABF and other BETO projects and can be leveraged by industry; and (demonstration projects and beachheads) assess BETO investments in ABF capabilities and are key drivers toward industry collaboration projects. Top challenges for the ABF have been leveraging past collaboration learnings with future collaborators, predictive scale-up and method transferability, and the intellectual framing of strategic beachheads (metabolic intermediates). Outcomes and technical accomplishments are highlighted in this presentation and span DBTL infrastructure, demonstration projects, and beachheads across bacteria, fungal, and yeast organisms.



COMMENTS

- The overall goals of the DBTL project are sound: enable a 10× improvement in DBTL cycles. Accelerating the development of strains and technologies for new target products and molecules is an important goal that is aligned with the ABF's mission. It allows a focus on building the tools and capabilities to enable these DBTL improvements. The approach, however, relies on "demonstrating projects," primarily through a focus on measuring the unmeasurable: It is not clear how the ABF can

demonstrate a 10× improvement in DBTL, the baseline is not well defined, and it's unclear what technical metric would demonstrate that the ABF is succeeding. This lack of clarity undermines a focus on impactful tools and capabilities, as it's difficult to determine which tools and capabilities have impact.

- Progress is therefore difficult to measure and quantify. The ABF has surely built a capable suite of tools and performed sound science. Commercial applicability, however, is much less certain. The ABF's toolkit, from its analytical capabilities to ML integrations, should present an impactful narrative. Unfortunately, the ABF has not been able to tell that narrative cohesively. There are only anecdotal examples of the DBTL cycle performing, and the applicability of the "learn" portion of the DBTL cycle is particularly unproven. A focus shift toward industry feedback as an indicator of success and impact is recommended. If the ABF is truly accelerating the DBTL cycle beyond the state of the art, industry will seek out ABF support in further projects. Industry interest in only a portion of the ABF toolkit will provide evidence of which ABF innovations are commercially applicable. Follow-on funds and product commercializations after ABF collaboration will cement the impact of the ABF on the field.

- Current impact is therefore hard to quantify as well. The toolkit that the ABF has built, the hosts onboarded, and the learnings from multiple projects employing DBTL cycles appear to have value, but the lack of commercial success points is apparent. The ABF is not able to point to any one commercially mature product or molecule that they were instrumental in creating or accelerating, a troublesome sign for the applicability of the capabilities built at the ABF. A narrow focus on publications as the primary outcome of projects is misguided; publications may show scientific development, but they are a poor proxy for commercial applicability and overall impact. Further commercial validation, as noted above, will provide considerably more evidence of ABF impact.

- Strengths:

    - Excellent technical achievements have been made on the bacteria, yeast, and fungi projects. Transfer of the malonyl-CoA beachhead between multiple strains for 3-HP production was successfully demonstrated. Satisfactory TRY metrics have been obtained.

    - The presented work involved several research teams from multiple national labs and a large number of researchers. Coordinating and managing such a large team is a big challenge, but the ABF was able to demonstrate high efficiency managing such a large team and delivered good results.

    - The multi-omics analytical tools were proven to be useful, and have helped multiple industrial partners generate large data sets.

- Weakness/area for improvement: Challenges in quantifying the DBTL cycles were discussed. These challenges are well received by the review panel. However, as the major project goal, the ABF is still expected to provide some quantitative measures on the time and resources reduced when industry adopts ABF technologies.

- This review is focused more specifically on DBTL infrastructure, demonstration projects, and beachheads. A general review of the past goals and accomplishments of the ABF was given in the "ABF Introduction and Overview" session. I will reiterate one point that is highly relevant here. I think it's a mistake to abandon all DBTL metrics going forward. Revise the DBTL goals to be more realistic and relevant. Still, have a goal to reduce the DBTL cycle or even parts of DBTL that make the most sense, are the most commonly used, or the biggest bottleneck. It doesn't have to be every capability and every tool. The metric could be time needed to finish one complete experiment and learn from it (per tool or per instrument), and focus on making those individual unit ops more efficient. Drop wall time versus clock time. What matters is how long it takes you to learn from an experiment. If there is some large

downtime (let's say you need to ship samples from one site to another, and that takes weeks), then that is part of your cycle time and something to target to be more efficient. Focus on gaining efficiencies and improving the infrastructure already in place.

- Most scores were moderate or low for judging impact on previous goals because there was a balance of very low scores and some high scores. Low scores because (1) nearly all DBTL goals were not met and (2) management structure was not sufficient to lead the ABF to success. High scores for overall a lot of solid work advancing synthetic biology and delivering on several projects that advance U.S. capabilities and advance industry to be impactful. Also good plans for addressing DEI.
- Good:
    - Overall, so many amazing tools and capabilities have been built in the ABF (Slides 15–19). The ABF really is a fantastic resource to advance BETO's goals and support industry to be a leader in synbio in the United States.
    - I don't remember hearing about the semiautomated combinatorial media pipeline in 2021. This seems like something really useful to industry, academics, and national labs. This is a good service that should be aggressively marketed, and this slide needs to be on the ABF website. See comments below on how to improve the website.
    - Better tools for tracking DBTL, and showed DBTL cycle data for results of transferring one molecule pathway to a different host. Hit the Q4 2021 milestone: "2X efficiency improvement in automated DBTL engineering cycle unit operations compared to FY22Q2_DBTLI_R1 non-automated baseline efficiencies demonstrated."
    - Impact and metrics slides show that a lot of good progress was made in last 3 years developing the tools and infrastructure to advance synthetic biology in the United States and help BETO's goals to decarbonize the chemical industry. Slide 28 is especially impressive, with muconic acid and 3-HP especially standing out as good examples of beachhead and exemplar to advance decarbonization of the chemical industry.
- Better, and could still use improvement:
    - A lot of these slides are very clear and show all the tools and capabilities of the ABF and what they have to offer (Slides 15, 16, 18, and 19). Why are these slides and images not being used on the ABF website? Slide 15 does a much better job at communicating all the amazing technical capabilities the ABF has to help partners than all the words currently on the ABF page. I would not worry if some of these activities are not being actively supported in the new strategic plan. The ABF said if a company wanted to use some of that infrastructure, then those capabilities would be used and further developed, so there should be no issue with advertising them. It's good that the beachhead slide is finally up on the website, but it's really buried, hard to find, and not at all obvious to industry or someone looking at the site to know the ABF has developed these beachheads. I only found it by clicking through the host onboarding capability. Is the semiautomated combinatorial media pipeline even mentioned on the ABF website anywhere? I tried clicking around and couldn't find it.
    - It's good that the beachheads got some traction, and the demonstration of muconic acid and 3-HP as exemplars is excellent work to further decarbonization of the chemical industry. However, overall it seems to have either not been of interest to much of industry, or it was not advertised to industry well. It's good the ABF is not pursuing beachhead strategy anymore.

- Bad: Overall, DBTL cycles were not met, and for reasons that are still not clear, were said to be hard to measure/quantify. It really isn't that hard to quantify how long it takes to go from one step to another, or from the start of an experiment to when data are returned.

- It is clear that the ABF has done a lot of really fascinating and novel work. The beachhead molecules were a hard task, and the ABF demonstrated significant progress toward those molecules. Past and future work on 3-HP is likely to have substantial impact across various industries. One of the other strengths of the ABF has been their improvements to the learn part of the cycle. The national labs have a dramatic advantage when it comes to access to computing power. It would be wonderful to see the ABF fully leverage that access to develop and support software that can accelerate synbio design. One drawback on that end, given the national lab structure, is that developing the software would need to be an ongoing project to enable the software to be updated, supported, and evolved according to industry needs. The current focus of the national labs on publications and more academia-like dissemination methods actually hurts the potential impact of the software and models that the ABF has the ability to create.

- This summary provides an assessment of progress/outcomes and impact. Approach was assessed in a previous review. The DBTL infrastructure goal was to design, operationalize, and maintain DBTL infrastructure as a core component of the ABF to support other ABF tasks. Substantial progress has been made in high-throughput screening and multi-omic analysis, resulting in multiple publications. Whether these advances obtain the 5–10× improvement in cycle efficiency is unclear because metrics were not provided. A semiautomated media optimization pipeline was also developed, and software was made more accessible to the visually impaired. The demonstration projects and beachheads goal was to develop bacteria, yeast, and fungi hosts to efficiently, cost-effectively, and sustainably produce beachhead and exemplar pairs to aid commercialization. As documented in the presentation slides, substantial progress was made in preparing organisms capable of producing high titers of multiple important molecules, although no indication was provided whether these processes are cost-effective. Tool development is expected to have a positive impact, as these can be applied to future research objectives. The impact of achieving milestones related to beachheads is difficult to assess. While certainly providing a demonstration of ABF competency and capabilities, whether a partner organization is interested in using these specific strains is not known at this time.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback, some of which was responded to above for the "ABF Introduction and Overview" presentation. Regarding the ABF not yet being able to point to a commercialized product that it has enabled, the early TRL nature of the ABF's value proposition does go hand in hand with significant lead times before a product may come to market. That said, Lygos saw a very significant leap toward a commercially viable process for producing isobutyric acid as a function of its collaboration with the ABF. Similarly, the strong collaborative relationship with ZymoChem has resulted in technology for producing polyglutamic acid that has garnered interest from large offtake partners and is nearing commercial viability. Further, Visolis has been able to raise very significant private funds by virtue of its technological advances, some of which arose from its successful ABF collaboration for mevalonate production. These are just a few examples of the impacts that ABF-enabled research is having in the community. With at least 20 distinct products and fuels to its credit, the breadth of biomanufacturing research to which the ABF has contributed is significant. However, relative to the length and vagaries of product development timelines, and the foundational role that ABF research plays for its collaborators, one could conclude that it is early days, yet. We acknowledge that the ABF team needs to do a better job of measuring and reporting the entirety of its impact. The recent expansion of the management and business development teams, along with the acquisition of grant management software, is affording us the time to more carefully monitor the ongoing impact that ABF R&D offers its partners and industry. Regarding the ABF website capabilities section, we agree that we can continue to make

improvements to these pages. We will evaluate what graphics or images we can add to these pages to better communicate the ABF's capabilities.

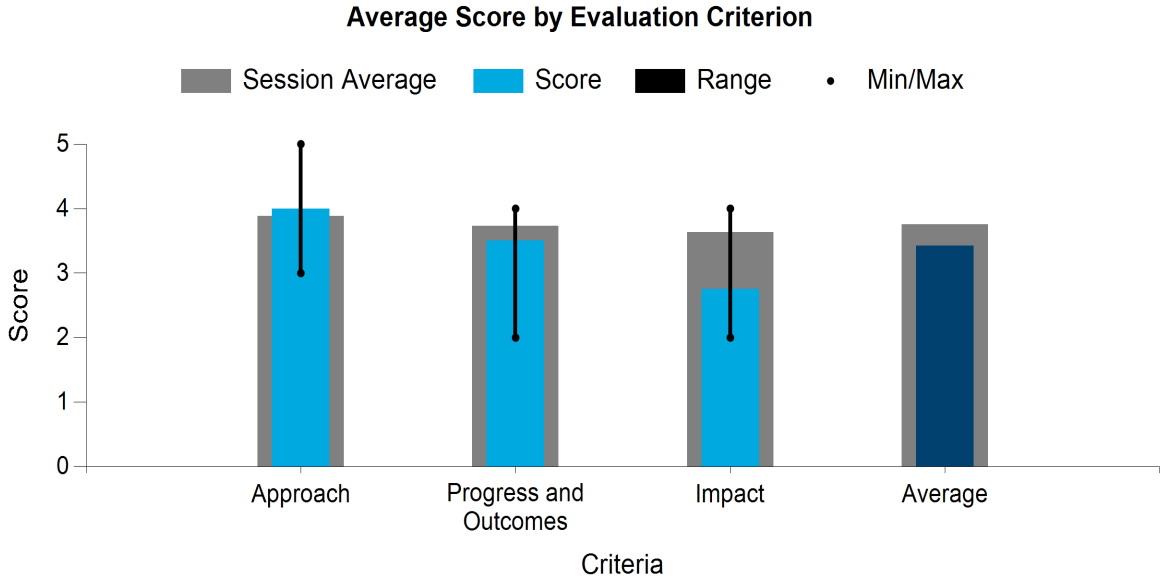
ABF PAST ACCOMPLISHMENTS – HOST ONBOARDING AND DEVELOPMENT

Oak Ridge National Laboratory and Los Alamos National Laboratory

PROJECT DESCRIPTION

WBS:	ABF3
Presenter(s):	Adam Guss; Katy Christiansen; Taraka Dale; Tim Theiss
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

Non-model microorganisms often have advantageous physiological traits that could be leveraged for advanced bioprocessing, such as the ability to thrive at low pH or to utilize uncommon feedstocks such as syngas or mixtures of oligomeric sugars. However, a lack of genetic tools and fundamental knowledge about these organisms hinders strain development. The role of the Host Onboarding & Development (HOD) team is fourfold: (1) evaluate and select hosts for use within the ABF, (2) develop genetic tools and data sets that allow new hosts to be used for DBTL cycles within the ABF and by outside stakeholders, (3) improve genetic tools for BETO "state of technology" organisms to increase DBTL cycle efficiency across the BETO portfolio, and (4) develop a web interface with information and data on each ABF organism for internal and external stakeholders. In this talk, the HOD team will discuss how we use our "tier system" framework for evaluating the readiness and guiding the development of an organism for DBTL cycles. We will discuss progress in host development, detailing advances in the development of genetic tools, synthetic biology parts, and biosensors for multiple organisms. Finally, we describe progress on the web portal, named HObT, for sharing host-specific information (e.g., development status, strain attributes, protocols/parts) within the ABF and with the community at large.



COMMENTS

- Building up the strain library at the ABF is a key activity that separates ABF capabilities from others in industry. By serving a potentially wide range of companies across a diverse host set, the ABF can maximize its applicability to industry and ensure its continued relevance. The goals and approach of the ABF in this space are therefore built on a sound theory, and this activity in particular demonstrates the

need for governmental support to build the infrastructure and capabilities that industry is unlikely to develop alone and on its own. The knowledge built at the ABF on multiple strains, the benefits and trade-offs of each, and the process of working with each is unique knowledge that can separate the ABF from competitors.

- It is less clear, however, that the particular strains that the ABF has onboarded, or the decisions about which hosts to focus on, are the most important commercially. The ABF has fallen short in disseminating to industry their capabilities around hosts. HObT was presented as a solution to provide a standardized way of comparing hosts; however, the website is broken, information is missing, and its development and maintenance appears to be a low priority for the ABF. It is further unclear if HObT was designed with industry input to ensure that the parameters and information shown are of interest and use. This lack of focus on HObT is unfortunate and also indicative of the problems surrounding host onboarding at the ABF: the host library is a key differentiator for the ABF, and the ABF needs market feedback to both validate the importance of the strains that have been onboarded and help prioritize the next strains to bring on. The ability of the ABF to onboard strains and work with new hosts is not in doubt, although the velocity of these activities is uncertain. It is the commercial applicability of the hosts that is in question. Measuring impact based on publication count is a poor proxy for impact, especially as many journals are not open access, limiting the knowledge transfer to industry. More direct industry outreach is needed to better demonstrate the impact of the ABF's library and the particular strains with the most commercial potential. The strains that are utilized the most in industry engagement will help the ABF better understand which hosts to invest further resources into and what parameters are most important to industry, guiding the onboarding of new hosts that better meet industry needs.
- Strengths:
    - The use of the tier system to categorize hosts allowed the HOD team to better focus on a smaller group of strains with higher depth.
    - The HOD team has made the efforts to develop the HObT website that summarizes the most important information about microbial hosts and their tools.
    - The HOD team continued to make excellent progress on onboarding more strains, improving their tier levels, and developing genetic and sensor tools.
- Weakness/area for improvement: It is disappointing that the HObT website is not available to the public. As a result, it is also not possible to review the value of the website and the impact of their work.
- I think the HOD work is one of the best assets of the ABF. Slide 21 sums up the benefits and impact to industry very well. The team took feedback from 2021 very well and adjusted its approach to have a clear plan for how to balance breadth versus depth by developing the appraisal framework (Slide 14). This is a good process to evaluate how to use resources for greatest impact in HOD. The technical advances are significant, and it's clear this part of the ABF has brought real value to industry and work being done at the national labs. It's wrong to defund this work in the new strategic plan. The potential benefits of this work toward making a strong U.S. bioeconomy are huge. I can't stress enough that this work should continue. It might not be possible to continue the work at the same scope, but some work in HOD should continue. Below I offer ideas and comments on where to find the money and what to focus on.
- Before that, though, there is one large problem with the HOD, which is access and documentation of the HObT website. If it wasn't for the lack of public access to the information on the HObT website, I would have given the HOD work 5s in every category. However, the lack of public access to the data and advances in the HOD program have to be taken into account. The presentation makes it sound like the

HObT website is available to the public, but it is not. First, there is no link anywhere to HObT on the ABF website (if there is, it's so buried as to be practically not available). Due to reviewer access, I was able to find the website. However, there is no discussion of what the different tiers are, and I was not able to see anything expect the publications. None of the protocols or parts or any other aspect of the HOD work was accessible. From Slide 13 it looks like more data is there, but not available to the public. I tried registering but couldn't. It's imperative that the HObT website be made accessible to the public, to encourage industry to understand what the ABF has to offer. It's a big barrier to have to contact the team to inquire. That drives away interest. Also, as mentioned in pretty much every other review on the ABF, the website overall needs a massive overhaul and updating. There are so many great images and visualizations that explain the ABF, and in this case the HOD work, that should be on the website but are not—for example, Slides 10, 12, 15, and 16 (although the tier system needs a bit more explanation on Slide 10). These images should all be on the website to engage industry. At the very least (and I strongly recommend more than just this), funds need to be allocated to bring the HObT website to the general public and make it functional. When asked about this at the review, the answer was that "the website wasn't ready yet" for the public. I don't understand this, because the HObT website existed in 2021, and it doesn't look like much has changed since then. The website should have been made available to the public back in 2021. It's basically a waste of all the great work and success in HOD not to have the HObT website available to the public.

- It was said at the review that at the last minute, to balance the budget, the HOD work was being defunded going forward. That is a wrong overcorrection in response to the call for an updated strategic plan from BETO. As I said to BETO, and elsewhere in my reviews, I think the emphasis on SAF work in the ABF is misguided and an overcorrection. That SAF work should be reduced or eliminated and the funds allocated elsewhere. My first recommendation was to refund HOD. Here is what I said to BETO: The host onboarding is one area that the ABF and BETO funds can really make a difference to advance new technologies and develop new strains to enable new chemicals and pathways at better costs. This is expensive and time-consuming, and often industry is reluctant to take it on. If they do develop a unique microbe, they will keep it strictly proprietary. Host onboarding should pick a small number of microbes that have very different properties, would access very different feedstocks or fermentation abilities, and are likely to be tractable genetically. Then develop them to a high TRL rating to make them really useful. The apparent lack of progress on the HObT website since 2021 and the lack of helpful information to the U.S. research community from HOD is a problem that must be addressed. Regardless, if further work on host onboarding is done, there must be funds allocated to bring the website up to date and make the tools developed useful to the public, or BETO can consider a lot of that work and money wasted.

- The HOD team has done a superb job of developing new tools for numerous nontraditional organisms. I view the decision to focus on improving the utility of a smaller number of strains (raise tier classification) at the expense of expanding the list of organisms as a positive. Unfortunately, dissemination of information related to these developments has been abysmal. Publications are a useful mechanism to disseminate knowledge and new capabilities; however, the ever-increasing number of journals and limited availability to private-sector scientists demands the ABF website be the principal means by which all ABF capabilities are advertised. The HOB team indicated that 15 hosts have been successfully onboarded, but the ABF website provides a list of 11 strains. The team presented the HObT database as a public-facing tool, indicating that the major Version 3.5.0 was recently released. This reviewer was unable to find a link to HObT on the ABF website. After an extended internet search that was only justified because I was aware it existed and was intent on finding it, I was not able to register to log into HObT. During the Peer Review, ABF management acknowledged that HObT was not released for public availability but would be made available soon. (As of May 6, 2023, registration to the site remains unavailable.) The lack of communication between ABF management and a task lead is disappointing. A low score for impact reflects the lack of availability of this tool.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback, some of which was responded to above for the "ABF Introduction and Overview"; "ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads"; "ABF Past Accomplishments – Industry Engagement, Outreach, and Management"; and "ABF Past Accomplishments – TEA/LCA" presentations. To clarify, the HOD team is not completely sunsetting work, but rather moving to an externally facing activity, where collaborative funding opportunities will be used as needed to onboard and develop hosts that are specifically needed for industrial or other partners. This will help to ensure that ABF resources are put toward the direct requests and needs of industry. The point is well taken that continued host development is important, and we will also strongly consider reinitiating internally driven host development to supplement the HOD efforts that are collaborative with industry.

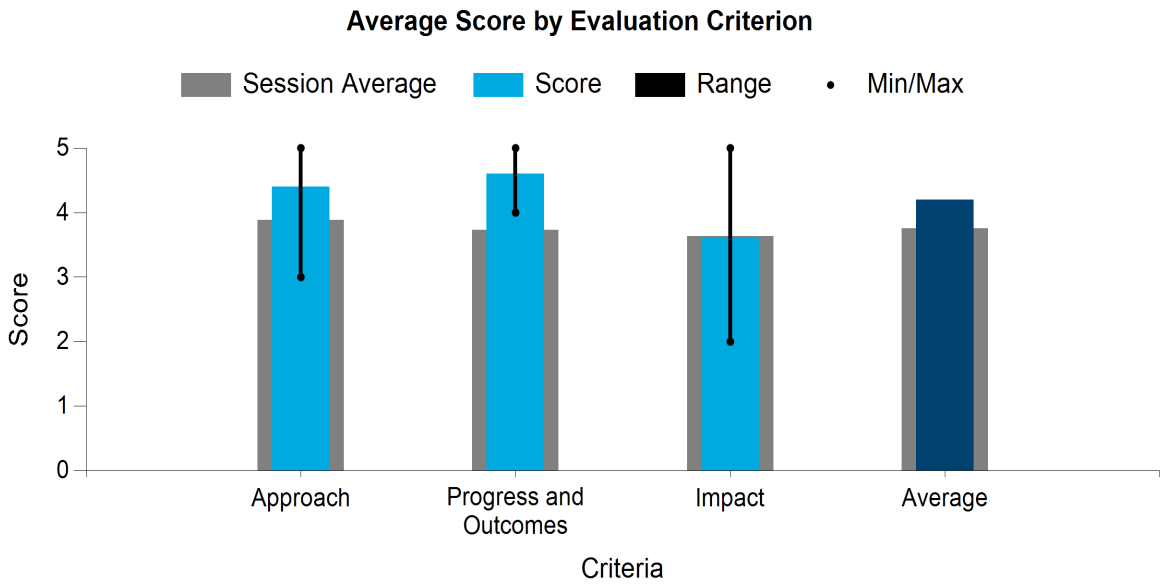
ABF PAST ACCOMPLISHMENTS – TEA/LCA

National Renewable Energy Laboratory and Argonne National Laboratory

PROJECT DESCRIPTION

WBS:	ABF4
Presenter(s):	Bruno Klein; Megan Krysiak; Thathiana Benavides
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

The Integrated Analysis task in the ABF conducts TEA and LCA to quantify the economics and environmental impacts of bioprocesses under development. The team from ANL and NREL develops TEA and LCA models of selected compound targets and hosts of interest to the ABF in an effort to provide an analysis-based foundation to the R&D. To date, the Integrated Analysis task has performed process modeling and analyses on multiple pairings of bioproducts and hosts, centered around major metabolic "beachheads" and "exemplar" molecules chosen for each of the metabolic pathways of interest. This presentation provides an overview of the approach used for TEA and LCA in the ABF and of the progress, outcomes, and impacts of TEA and LCA to support ABF goals, with a focus toward developing bio-based products that are both environmentally and economically viable.



COMMENTS

- TEA and LCA modeling is a core strength of the national labs and is of critical importance to the growth of the bioeconomy. There is a lack of information and data in the space that enables companies, from startups to Fortune 100s, to understand the likely costs of production for disparate products at a variety of production scales. As such, the ABF has a key role to play in sharing its expertise in LCA and TEA work to the industry to enable better research and commercialization decisions based on fundamental unit economics at scale. Unfortunately, the approach the ABF has taken with the TEA and LCA work limits its overall impact and undercuts its ability to be a driver for ABF outreach and success.

- The approach used by the ABF appears to champion model complexity and rigor over broader impact and applicability. Given the limited resources available at the ABF to support TEA and LCA work, it is

logical to ask whether a more simplistic modeling paradigm that is nevertheless applicable to a wider set of molecules, and therefore impactful to a broader set of companies, would better serve industry. It appears that the current modeling approach suffers from false precision, wherein considerable model complexity provides a false belief in model accuracy and precision. In actuality, the TEA and LCA results have large uncertainty bounds. This is not a criticism of the results; TEA and LCA at TRL 2 to 4 require a broad set of assumptions, each with its own uncertainty, and results are always going to be approximate. The results presented appear to back this up: The contour plots for productivity and yield versus GHG emissions and cost are relatively simplistic, appearing as essentially a 1D reciprocal in the case of the GHG and a 2D reciprocal in the case of costs. This implies that a few key parameters govern the results, opening the door to simplifications. The question should thus be whether a significantly simpler model would provide similar precision in results, allowing for more output and impact with the same funding support. The use of AspenTech, for example, may not be necessary to calculate process variables and conditions when a simpler fundamentals-based technical model would suffice. If model simplifications and parameter stripping would have minimal impact on result quality, that would open the door to the ABF providing significantly more TEA and LCA for a variety of molecules and technologies with similar staffing and spend as today. This simplification may then allow further activities to demonstrate the validity of the model, including baselining off known and scaled production processes to determine model accuracy and precision in a more meaningful way.

- The TEA and LCA work would be more valuable if available to more companies, particularly in a stand-alone fashion. The ability to engage the ABF on a small-dollar, funds-in basis to perform a rapid LCA and TEA for a customer (with appropriate understanding of model uncertainties and potential errors) could provide the ABF with a beachhead market to approach industry, rapidly build credibility, establish relationships and trust, and enable follow-on projects to develop molecules and products when TEA results indicate a likely commercially viable product.

- Strengths:

    - Carrying out TEA/LCA for common beachhead/exemplar molecules is useful. The Integrated Analysis team reduced the scale of analyses by downselecting one exemplar per beachhead due to similarity between exemplars derived from the same beachhead. The approach has merits in covering a large number of molecules.

    - The Integrated Analysis team has made substantial progress on TEA/LCA. A total of eight different pathways were analyzed.

    - Analyzing the bioproduction from alternative pathways or in different microbial hosts can help industry choose the best bioprocess. These results are impactful.

- Weakness: It is not clear whether any TEA/LCA result from the Integrated Analysis team has been actually used by industry when developing their bioprocesses.

- Overall, the TEA and LCA work in the ABF, and offering these capabilities to the public and partners, is incredibly impactful. Most smaller companies or academic labs don't have access or experience in these models, and it's crucial to understand if a project is likely to succeed or fail (TEA), as well as the value to BETO and their goals for decarbonization (LCA). Beyond BETO, most companies in the synbio space are driven by a desire to improve their plant via some benefit like reduced energy or resource usage or less CO_2 emitted. The ability to partner with ABF to gain access to TEA and LCA capabilities is a powerful incentive. In addition, it's important for more projects to have these analyses done to help inform BETO of projects more likely to succeed in advancing their goals.

- Often the trade-offs between yield and productivity are not understood and sometimes surprising. Having this information for the development teams to use is crucial to a successful project.

- Why the beachheads were not more adopted by industry partners is not clear, and could be due to a number of reasons (discussed elsewhere). However, the beachhead strategy in the old ABF model was well thought out from a TEA and LCA standpoint, and the targets made sense.

- The work in the presentation is well thought out, and it appears the TEA and LCA teams are well integrated into supporting the work of the ABF.

- LCAs/TEAs are a great way for the ABF to help advance the synbio industry. The national labs can conduct more thorough, well-informed, and robust analyses than many industry organizations have the bandwidth or in-house expertise to do. Examining the beachhead molecules in this manner was an insightful and productive endeavor. I think, however, that the impact of the TEA/LCA work by the ABF could be greatly increased if these models, instead of being put into an academic publication, were disseminated as an interactive web app that could be used by industry. This would allow users to change parameters based on their own supply chain costs and better understand when it may make sense to pursue biological alternatives for exemplar molecules that may be in their products. I think it also makes a lot of sense for the ABF to offer TEAs/LCAs as part of the work/services available for industry FOAs/DFOs.

- The TEA/LCA team provides strong support to ABF activities. Utilization of sensitivity analysis is a plus for situations where experimental data are not available. Is it possible for TEA/LCA to be proactively marketed to bioeconomy startups to provide critical economic assessment information for project planning, fundraising, etc.? After clicking through the Capabilities portion of the ABF website, I was never directed to a page that described TEA/LCA capabilities under Design, Build, Test, or Learn. When I searched "life cycle assessment," I found that if I scrolled down further on the Capabilities page, there was a link for Analysis Capabilities. It wasn't easy to find. Is it possible for the team to partner with IAB member organizations to refine and validate the ABF's TEA/LCA methodologies?

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback, some of which was responded to above for the "ABF Introduction and Overview"; "ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads"; "ABF Past Accomplishments – Industry Engagement, Outreach, and Management"; and "ABF Past Accomplishments – TEA/LCA" presentations. We agree there could be opportunities to apply a more broad-based approach toward process simulation in the future, while also recognizing the need of starting the effort with robust and well-informed models based on technical depth and rigor to establish a self-consistent foundation in the framework. In general, we have received feedback from the IAB and from other external stakeholders that reports and models/tools published by our teams have been well received and found good traction in guiding their own decisions and directions, particularly for longer-established projects that have had more opportunity for dissemination by length of time in the public domain (from which key pieces of model frameworks have been leveraged for Integrated Analysis activities in the ABF). One example is the Greenhouse gases, Regulated Emissions, and Energy use in Technologies (GREET) model (a publicly available LCA tool that is used to provide a transparent and harmonized framework across feedstocks, pretreatment options, and conversion technologies to products), which has been leveraged in the consortium to provide environmental impacts of the bioconversion routes of interest.

- The rigorous approach toward TEA/LCA by the Integrated Analysis task has been acknowledged by the board of reviewers, as well as the fact that this approach has been successfully applied to set the stage in covering a broad space across eight different biomolecules. This feedback will be taken into

consideration in working to create reduced and more flexible models leveraging the work we have conducted in prior years. These models can allow for an in-depth probing of more process-relevant metrics for the downselected fuels and products of interest to the consortium, as they will focus on the aerobic production of different molecules and their respective downstream processes (although preliminary assessments point to the main economic and environmental drivers of the process being the same in either reduced or larger models). In the upcoming project cycle, the aspect of using TEA/LCA results as guidelines will be intensified, as the consortium will aim at transferring ABF technologies to the industry. The TEA/LCA team plans to further engage with partners and adapt our analysis to the needs of each technology/pathway, aiming at both providing recommendations toward goals for emissions reductions and cost viability and validating process simulation results.

- Regarding the visibility of TEA/LCA on the website, we will evaluate how we can rearrange our menus to make this information easier to find and accessible from multiple locations on the website. ABF's TEA and LCA capabilities have not been widely called upon by the DOE-funded projects that have moved forward in DFOs. While multiple groups have referenced TEA and LCA as part of the proposal's activities, they have more often than not chosen to perform these assessments internally, as part of their cost share, rather than including related tasks in the CRADA scope of work. One successful DFO proposal is utilizing TEA/LCA capabilities as part of the ABF's scope. But perhaps the reviewer is pointing to a need for the ABF to develop a broader and more thorough understanding of how ABF collaborative research is informing its partners' decision-making processes, both in the near term and long term. Be it through formal assessments like TEA and LCA or through other types of analyses, the ABF is keen to work with its partners and its advisory board to collect and use informative, impact-related data, and based on those data, augment the ABF's value proposition.

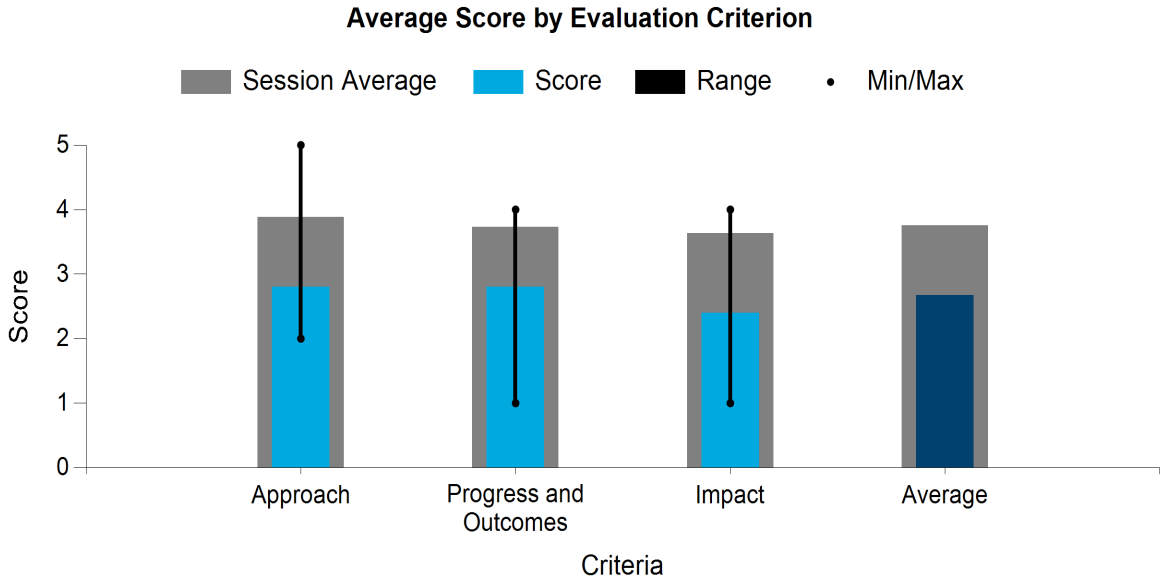
ABF PAST ACCOMPLISHMENTS – INDUSTRY ENGAGEMENT, OUTREACH, AND MANAGEMENT

National Renewable Energy Laboratory, Argonne National Laboratory, and Lawrence Berkeley National Laboratory

PROJECT DESCRIPTION

WBS:	ABF5
Presenter(s):	Christopher Johnson; Emily Nelson; Megan Krysiak; Phil Laible
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

To develop infrastructure to support industrial biotechnology, an understanding of the needs of the industry is critical. As such, the ABF Industry Engagement and Outreach (IEO) team organizes and facilitates interactions with industry, providing feedback from the stakeholders that supports decision-making and project planning. Their activities also aim to increase the visibility of the ABF and attract collaborators from academic and industrial communities. These goals are accomplished through the workings of three highly interwoven, strategic focus areas: Assessment, Outreach, and Interactions. Overall, the IEO task contributes to the alignment of ABF activities with BETO's milestones and facilitates communication of the ABF value proposition to key stakeholders in industry, R&D organizations, and the public. The ABF management team's objective is to organize project management and project management infrastructure, develop internal and external communications, and provide deliverables to BETO. These activities apply to both the core project, with specific scopes of work described for each lab, and the collaboration projects, for which scopes of work are agreed with successful applicants to the ABF funding opportunity program. Management will coordinate with BETO leadership (to the extent possible) on new opportunities (e.g., DFOs, FOAs) and interactions with other federal agencies (e.g., NSF) to access ABF capabilities.



COMMENTS

- Though the overview and goals related to industry outreach that were articulated by the ABF have resonance, including facilitating tech transfer to industry, educating partners on ABF capabilities, and

generating data on industry needs and shortcomings, the approach that the ABF has taken to achieving these goals is lacking. ABF's work in industry engagement and outreach appears poorly organized and lacking in coordination. An IAB meets irregularly, with poor meeting planning and goal setting for the group. The "Annual Industry Days" do not appear to provide the ABF with significant inbound funds-in project interest or useful feedback on the direction of the ABF, capability needs, or strategy. The ABF could not document successful examples of tech transfer out of the ABF to industry that resulted in commercial success.

- The root of the failures in industrial outreach may stem from a lack of interest on the part of the ABF to truly engage with industry, learn their needs, and adopt programs and tools for industry use. This is evidenced by the fact that many of the touted ABF outreach "successes" of the last funding cycle are nonfunctional: Its HObT host database is not open to outside users and isn't listed on the ABF website, the website itself lacks detailed information on capabilities, the ABF only appears to track social media "follows" instead of more useful indicators such as interactions or clickthroughs, etc. The ABF was unable to articulate a clear definition for what "industry" means and did not appear to have any organized industry segments. The needs of startups and Fortune 100s are different and likely need different outreach strategies. Another approach still is needed for peripheral industry participants, such as feedstock suppliers or consumer packaged goods companies. At Year 8 of the program, it is disappointing that there are not well-defined outreach strategies for each group. The overall impression is that the ABF appears more focused on developing its capabilities based on the research interests and specialties of the various PIs that make up the ABF consortium. While it's possible that these interests may in fact align with industry needs, the lack of a functional feedback loop with industry leaves the ABF at considerable risk of drifting from core industry needs, undermining its stated mission.

- The future hire of a dedicated business development manager is a welcome sign that the ABF understands many of these shortcomings; however, this hire alone will be insufficient to improve industrial outreach activities without buy-in from the various PIs at the ABF. To achieve success, the ABF needs to fully define its role; as of now, the ABF talks of serving industry but primarily functions as a science-doing enterprise. The ABF, however, is not just another FOA recipient, and therefore needs to reimagine itself as an enabling platform for industry. As such, its industry outreach activities should be driving decisions on budgets, capability development, and which tools to be retired due to lack of interest. Outreach should be occurring in some form on a daily basis, with regular contact and readouts with key decision makers at the ABF. IAB(s) should be engaged and involved in setting goals and strategy.

- I am sympathetic to the challenge of creating an industry-centric organization within the confines of the national labs. Difficulties in aligning the IP goals of the various national labs with industry requirements and streamlining contracting processes hamper industry engagements and will continue to act as headwinds on the ABF in the future without change and reform. Happily, the success of BOTTLE and the ABPDU to engage industrial parties with pre-negotiated IP arrangements provides a workable template for the ABF to emulate.

- Strengths:

    - The IEO team has made multiple efforts to improve the awareness of ABF technologies to industry and the broader society.

    - There are rapid increases in the report of ABF activities in social media that promote ABF's presence and technology.

    - Having the IAB is beneficial to the ABF.

  - The team has been updating the ABF's website with its newest technologies and capabilities.

- Weakness/area for improvement: The assessments from one-to-one interactions have provided valuable information from industry's perspective. It is not clear how the ABF used the assessment results to improve their activities.

- This review is focused specifically on "Industry Engagement, Outreach, and Management." A general review of the past goals and accomplishments of the ABF was given in the "ABF Introduction and Overview" session. This part of the ABF acknowledged that "efforts delocalized in early years of the foundry" and "activities refocused and expanded with increased budget in rescoping exercise." It's good to see the honest reflection at the start. Despite renewed focus and money midway through, it's clear the strategy to really connect with industry can still be much improved. Beachheads were not widely adopted by industry. Overall it seems to have either not been of interest to much of industry, or it was not advertised to industry well, or both. It's not clear which is the case.

- The new plan to adopt a professional business outreach person for the ABF is an excellent idea, based on the BOTTLE consortium model. Also, the website for the ABF needs significant upgrading. This will help attract industry awareness and help them understand what capabilities are offered. Many of the slides in various presentations have really clear graphics and easy-to-understand visualizations of the tools and capabilities offered by the ABF. These slides are way better at communicating what the ABF has to offer than all the text on the ABF website. I would not worry if some of these activities are not being actively supported in the new strategic plan. The ABF said that if a company wanted to use some of that infrastructure, then those capabilities would be used and further developed, so there should be no issue with advertising them. It's good that the beachhead slide is finally up on the website, but it's really buried, hard to find, and not at all obvious to industry or someone looking at the site to know that the ABF has developed these beachheads. I only found it by clicking through host onboarding capability. Is the semiautomated combinatorial media pipeline even mentioned on the ABF website anywhere? I tried clicking around and couldn't find it.

- There was a lot of discussion during the review days and internally among the reviewers about the structure of IP and how the way IP, CRADAs, and contracting is a barrier to industry engagement. The ABPDU said they can contract in a few weeks. The BOTTLE consortium has worked out streamlined IP for faster contracting (not needing every national lab to review and sign off on it) and clear IP levels for different amounts of industry funding versus exclusive rights. Clearly these are things the ABF can learn from and copy. For the current plan, it's going to be very challenging to achieve a significant increase in industry money into the ABF in the near future, especially if contracting takes a year. It will take time to get to >50% of the ABF's budget committed to external partnerships and reach an average of more than fivefold oversubscription for the ABF's funding opportunities.

- The management structure of the ABF probably significantly contributed to the lack of a cohesive IP plan. It lacked a single point of accountability (someone full time in charge) who would take on streamlining IP, or making sure it got done. One of my recommendations to BETO (shared by the review panel) is to change the proposed management structure to have a lead PI be solely accountable and responsible for running the ABF. There should not be any decision by committee. The current plan has a lead PI and executive committee in the same box. That is still a committee. The ABF needs a 100% dedicated full-time employee running it. This person should be an "outsider" with no emotional connection to the past 6 years of the ABF. The executive committee reports to and advises the ABF PI, but does not make decisions. It's clear that the ABF has really suffered from decision by committee and a lack of someone whose job is 100% running the ABF.

- Most scores were moderate or low for judging impact because there was a balance of very low scores and some high scores. Low scores because (1) industry outreach and engagement could be significantly better for the ABF and (2) management structure was not sufficient to lead the ABF to success. High scores for overall a lot of solid work advancing synthetic biology and delivering on several projects that advance U.S. capabilities and advancing industry to be impactful. Also good plans for addressing DEI.

- The industry engagement component of the ABF overall appears ad hoc and infrequent. The IAB does not appear to be well engaged (several of the listed companies do not have active members in the IAB currently). There was no summary provided of the kind of interactions that the ABF has with the IAB, what support have they provided, the lessons learned, and how the ABF has incorporated feedback. I also find it odd that the ABF does not share or consolidate the information from industry interviews and engagements into some kind of road map that outlines the challenges, pain points, gaps, etc. in the synthetic biology space. That kind of product could actually be quite helpful for the industry and would also help the ABF guide its effort in a more strategic manner. A gaps analysis is mentioned in the milestone table, but there were no details on this in the slide decks or during Peer Review. Going forward, I do agree that the IAB should be diversified quite a bit to include industries that may not use synbio much currently but that could benefit from transitioning toward biological solutions. The ABF could play a key role in understanding and addressing the needs and wants of those companies and helping to move them toward biological alternatives.

- The management of the ABF seems okay. However, given that the management structure is changing, I would and did recommend that the ABF have a full-time or nearly full-time lead to coordinate ABF efforts and ensure that changes are implemented efficiently and that industry is routinely engaged.

- IEO efforts are falling short of what is needed for the ABF to thrive in the role of sought-after partner to industry. A lead individual is assigned to each goal. Outreach, interaction, assessment, and an appropriate list of activities for each are provided. Lack of business development and marketing experience is recognized as a risk, but management should be more aggressive about remedying the situation quickly if ABF partnership goals are to be achieved. Outreach materials do not provide the level of information and detail needed to attract the number or quality of partnerships that are the goal. For example, the website categories of “Capabilities” and “News” should provide information and examples so that bioeconomy organizations immediately recognize the ways in which the ABF can assist them in solving their own challenges. Direct feedback from current and former IAB members to peer reviewers would be beneficial for future reviews. Two risks related to management inefficiencies caused by the distributed model and slow execution resulting from consensus-style management are not given the appropriate level of attention. These risks are not being managed effectively. Regarding the distributed model, the question is not “Is all the work getting done?” but “Could all the work be completed with fewer resources to make some available for additional activities?” Consensus-style management adds a second layer of inefficiency to the current ABF model. The executive committee consists of a group of talented, creative, and effective scientists. The goal should be a management team of effective, decisive managers.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback, some of which was responded to above for the “ABF Introduction and Overview” and “ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads” presentations. Regarding ABF contracting timelines and IP licensing and how these activities compare across the ABF, ABPDU, and BOTTLE: First, there are multiple strategies and process improvements we are pursuing to improve the ABF’s contracting timeline. Among them are automated document production, designated workflow ownership for document processing, possible elimination of CRADA usage requirements for non-practicing institutions, greater oversight from ABF management as a function of expansion of management full-time employees, and perhaps more effective relationships with the DOE site offices at each of the ABF labs. However, the reviewer offers both a false

comparison between ABF and ABPDU contracting mechanisms and a slightly incorrect timeline for ABPDU contracting. Many of the ABPDU's external collaborations with industry utilize the funds-in (self-paid), strategic partnership project mechanism for contracting, which takes about 3 months to complete. The ABPDU can often turn to the strategic partnership project because there is less expectation of new IP to arise from the research. This stands in contrast to the ABF's research, where companies and institutions are relying on federal dollars to fuel new innovations. The funding source and scope of work usually results in the requirement for a multi-lab CRADA. The BOTTLE model is also quite different and is serving as an example for the ABF moving forward, as the ABF seeks to adopt more activities similar to BOTTLE, such as moving to the all-funds-in, large-company focus, as well as the nature of the materials, technologies, and objectives of the consortium. All that said, however, the ABF does acknowledge that there is likely much that can be learned from both the ABPDU and BOTTLE. As the ABF's business development activities continue to expand and diversify, the group is evaluating all of the available contracting models and all of the ways in which to collapse the contracting timeline. IP licensing outcomes and increased contracting speed will be key performance indicators for these processes. Due to time constraints, we were unable to present many details that might have been helpful to the reviewers related to how industry engagement, outreach, and management interact to respond to feedback received in one-on-one interviews, industry days, meetings with the IAB, and forums for industry interaction.

- We agree that the ABF needs to be more responsive to industrial needs in order to have impact and are working toward strategies to address this need. As such, and as mentioned in this presentation and elsewhere, the ABF plans to hire a dedicated business development co-lead, who we envision will work closely with the IEO and management teams to increase and improve our ability to identify and respond to industrial needs and partnering opportunities. We believe this could represent a step change with respect to the ABF's industrial impact and overall direction. Another important pivot on the horizon is a movement toward 50% of the ABF's funding being allocated for industrial partnerships in the form of funding opportunity projects and other partnerships. Industrial responsiveness is intrinsic to the projects, and an increase in their funding should promote greater industrial impact.

- Regarding the ABF website, we have continued to update the website each year and agree that more work can be done to improve it, particularly to make it more relevant to industry in ways the ABF can help them and to provide more detailed information on the ABF's capabilities. We will continue to evaluate how the website can be improved and to make sure all relevant information and capabilities are represented. The IEO team does track detailed analytics on social media interactions, impressions, link clicks, comments, shares, and more on a monthly basis. However, given the time constraint, we were unfortunately unable to present this level of detailed information. In response to comments and concerns about using the IAB of the ABF as effectively as possible, a prominent component of the restructuring exercise and ongoing discussions between industry engagement efforts and BETO management (and, in the future, ABF business development) is to better define the roles and goals of having an advisory board for the ABF and tailoring interactions and utilization efforts of the board for these outcomes. We welcome these changes and improved interactions, as they will be needed as industrial collaborations and new types of partnerships intensify. As discussed above, moving forward, the ABF PI will have more authority (backed by BETO) and decision-making ability. While this change will be beneficial in many ways, even an omnipotent ABF dictator would not singly be capable of addressing the contracting challenges faced by the ABF, an issue much larger than the consortium itself. The ABF disagrees that the PI should be an "outsider" with no past connection to the ABF. The ABF, including its PI, is prepared for and supportive of making substantial changes, as illustrated by the strategic re-envisioning of the ABF and its implementation thereof.

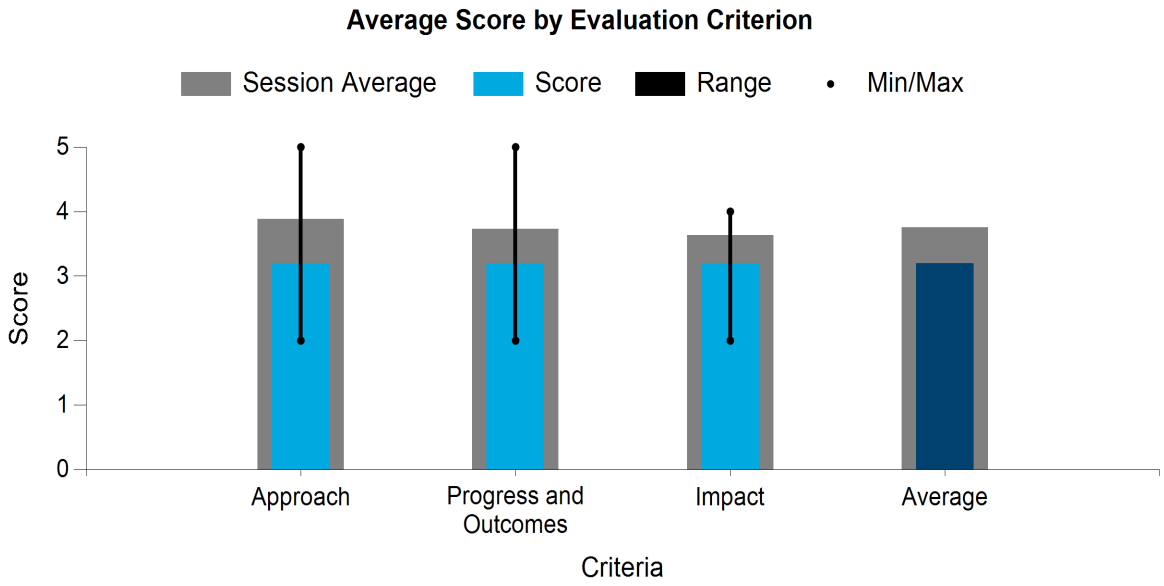
ABF – LESSONS LEARNED AND INTRODUCTION TO FUTURE PLANS

Lawrence Berkeley National Laboratory

PROJECT DESCRIPTION

WBS:	ABF6
Presenter(s):	Katy Christiansen; Nathan Hillson
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

In June 2022, the ABF presented to BETO its concept for operations in FY 2023–2025. This presentation included a section dedicated to "Lessons Learned from Past Years." In September 2022, BETO instructed the ABF to do strategic planning, including directives to create a "Reviewing History" working group to assess what worked well and what did not work well historically. The relevance of this activity is that it ensures that the ABF's future plans are made wisely. Top potential challenges include applying lessons learned in a nuanced fashion, overcoming the temptation to maintain the status quo, and applying consensus-driven lessons learned equitably. The outcomes of this activity include the gathering of lessons learned over the past 7 years of ABF operations, along with the outcomes of the "Reviewing History" working group, which for example includes ranked lists of what did and what did not work well historically for the ABF. These lessons learned have been applied to the ABF's strategic and implementation planning processes. At the end of the presentation, a brief introduction to the ABF's future plans is provided.



COMMENTS

- The ABF utilized multiple presentations to document their journey in developing a new strategic plan for the next funding cycle. These presentations grew off each other, and as a result, it is difficult to separate them as required in this review process. Instead, I will use this space to specifically speak to the "lessons learned" portion of the presentation and the process through which the ABF conducted its strategic planning process. A review of the new strategic plan can be found in the review of later presentation materials.

- The ABF has found itself after its initial 8 years facing an identity crisis, stuck between an apparent desire on the part of the ABF to essentially maintain status quo activities and the desires of BETO to drive to activities that more clearly demonstrate impact and results. This is not to say that ABF personnel do not understand or recognize the failures of the organization in the past or the difficulties with which impact can be measured based on its prior goals and strategies. However, the implicit message throughout the lessons learned presentation was that BETO was imposing a new strategic direction upon the ABF, and that the ABF did not wholly agree with the new approach. This is unfortunate. Whether the ABF is at fault for past activities failing to align with BETO priorities or whether BETO has changed its priorities on the ABF and unfairly moved goalposts is not particularly relevant at this stage; what matters is that the success of the ABF and the ABF's position in BETO's success is undermined when trust between the organizations is damaged by a misalignment between the goals and ideas of leadership. The apparent breakdown of trust that has occurred in this instance is concerning and appears to have negatively impacted the ability of ABF leadership and BETO to collectively work together to find a strategic direction of interest to BETO and ABF PIs.

- The level of engagement between the ABF and BETO during the strategic review process is not clear; however, the indications from the presentation provided paint a picture wherein the ABF circled the wagons during their strategic review process, to the detriment of that process and the resulting plan. It is clear from the materials BETO prepared that their goal was to ensure that the funds spent through the ABF were impactful and could be readily connected back to commercial deployments and successes. The ABF's approach through the strategic review therefore should have been to focus on the components of its portfolio most likely to achieve such impact. Instead, the ABF appears to have taken a narrower approach, focusing on strategies to respond to the details of the criticisms (measurability of goals and demonstration of commercially relevant molecules) instead of engaging in a more thorough examination of the activities likely to drive impact. There are certainly important questions that the ABF sought to work through during their strategic planning exercise: How to protect the "core" that has been built over 6 years from atrophying? How to demonstrate the value of their tools? How best to demonstrate commercial interest and traction? The problem is that the ABF seems to have failed to recognize that industry is a key partner in answering those questions and ensuring the resulting plan was most likely to achieve impact; their voice should have been instrumental in guiding strategy for a future ABF more responsive to industry needs.

- The way the ABF is managed and run is part of the issue. The ABF is primarily governed by a set of national lab PIs, and no one PI appears to have the buy-in of the others to make critical decisions on behalf of the whole. None of these PIs spends a majority of their work hours on the ABF, resulting in a distracted and misaligned governing group that makes a focused strategic planning process that much more difficult. The ABF would benefit from a senior leader who spends a large majority of their time on the ABF specifically and who has the trust of BETO and the other PIs to make key strategic decisions on behalf of the whole. Similar to a CEO acting on behalf of a board, this person should oversee the day-to-day operations of the ABF and set strategy and goals, for eventual approval by the governing body (PIs, BETO, or some combination thereof) at regular intervals. A streamlined governance structure could do much to help the ABF overcome some of the institutional inertias and blind spots that seem to have tripped up the ABF in this most recent strategic planning cycle.

- Strengths:

    - The ABF's past lessons learned is an effective approach that has allowed the ABF to learn from multiple issues associated with previous technical goals, methods, and management. Changes were proposed to address these issues.

  - The shift of metrics from TRY improvements to focus on TEA and GHG emissions makes sense. TRY metrics alone cannot inform industrial success and societal impact. Using TEA and GHG emissions as metrics will allow the ABF to better focus their efforts on promoting the biomanufacturing industry and better align them with BETO's missions.

- Weakness/area for improvement: Accurately tracking DBTL cycles and comparing DBTL times with and without ABF technologies is indeed challenging. For the past 7 years, the ABF has been proposing to use these metrics to evaluate their success. It is unfortunate that such a challenge was only realized after 7 years of ABF activities. So far, the new metric makes sense. This also serves as a warning to the ABF to constantly practice the "past lessons learned" activity to evaluate the new metrics.

- The numbers reflect a review of the past ABF goals, metrics, accomplishments, and impacts. Same as given in the ABF introduction and overview.

- Some specific comments on the slide deck in this presentation are below.

- It's clear that the DBTL cycle time metrics were not going to be met and were not being captured in the ABF in order for them to meet their milestone goals. On Slide 10, the team lays out the arguments for why DBTL was hard to capture and why it was not as relevant to tracking progress. The latter argument has merit, and focusing on impact factors such as TEA and GHG reduction are more aligned with BETO's mission. That being said, it really should not have been impossible to come up with a way to track DBTL. Slide 10 says DBTL failed to capture other useful metrics showing progress like sample throughput, resource intensity, and number of cycles needed [to learn]. That is all part of DBTL, and improvements on those metrics should lead to a reduction in DBTL cycle time. Moving forward, the metrics in the new strategic plan are mostly easier to quantify, and the ABF should be able to track progress toward them.

- Moving away from beachheads to only molecules "directly of interest to industry via industry partnerships" [except the molecules being developed in the core activities] is a good idea. The beachheads were not engaging industry, and the TEA/LCA for them was difficult to assess.

- The new appraisal framework for host selection and prioritization is a good compromise to breadth and depth and should continue in the next 3 years (we were told in the meetings that all HOD work had been cut in the final budget).

- Bioprocess round robins: Tech transfer is difficult, and "everything matters" when trying to reproduce a fermentation or manufacturing campaign at a different site. The process of doing the tech transfer is often very helpful to understand the baseline system. Seems like the ABF is learning that. This will be helpful when transferring processes from a national lab back to an industrial partner. However, I also caution the ABF to do the minimal piloting and scale-up work required to meet their goals or partner goals, and to work with BioMADE to do more work on scale-up.

- Slides from the ABF seem to indicate good engagement with the IAB. Feedback from the advisory board is that they feel like an afterthought and the ABF does not come fully prepared to discuss matters, and the fact that the industry board was only asked to participate in the new strategic plan at the very end to review once all the decisions had been made shows the ABF does not engage effectively with the IAB. This needs to be addressed in the next 3 years.

- Ideas on how to improve the IP and CRADA negotiations are mentioned many times in other places in this review.

- Some of the lessons/analyses they presented seemed poorly suited for having learned the "right lessons." For instance, their lessons learned regarding IAB use largely focused on meeting format as opposed to actually requesting feedback from the IAB on the work that the ABF had done and how to do it better. I think better engaging with the IAB is important, but I'm concerned that this is the lesson that was shared here, especially when it was also clear that the IAB was not engaged in the strategic replanning that occurred. Listing a future function of the IAB to give postdoc industry panels is also a poor use of the IAB. The slide on host onboarding decisions is not at all clear. What was actually learned about which host should be elevated, as well as which should not be? Are there any clear signals from industry on promising hosts that could unlock new frontiers if upgraded to Tier 4? Or completely novel hosts that should be onboarded, at least at Tier 1 or 2, because then industry could take it from there? There is not any "lesson" presented on how to prioritize organism onboarding in a strategic manner. Shifting from purely TRY metrics to parameters that are informed by TEAs/LCAs is a good lesson learned in my opinion. It was also great to see that they were able to improve their CRADA processing time, though it is still very long. The purpose and selection criteria behind the Miro poll results presented were not at all clear. Were votes limited by person? Where things that rated low in the "didn't work well" category deemed successes or failures? Why were none of the "things that worked well" deemed worthy of discussion? The approach here is quite messy and lacks actionability.

- Scores for this presentation were not meaningful, resulting in a selection of 3 on all counts. The ABF's willingness to undertake a strategic re-envisioning process reflects positively on the organization's desire to be a forward-moving, relevant participant in the growth of the bioeconomy. There is no doubt significant progress on all scientific fronts has been made over the history of the ABF. Two acknowledged challenges will be highlighted. Regarding the temptation to maintain the status quo, I fear the ABF may fall prey to avoiding meaningful, though perhaps painful, decisions. Second, applying consensus-driven lessons learned equitably highlights the danger of consensus-driven management. Whether a strategy/activity/tool is effective should not be subject to agreement of all parties. The strategy/activity/tool is effective if it provides value. Did it result in meaningful improvements in ABF research (rate of discovery, TRY improvements, etc.)? Were partners enthusiastic to use it? Did it draw potential partners to the ABF? I also find the following comment troublesome: "without disproportionately adversely affecting a small demographic." Nobody has an established right to participate in the ABF. If some demographic big or small is not contributing to the ABF mission, then the unfortunate reality is that piece must be removed for the good of the organization. The Miro-assisted self-assessment provided some curious information. When asked "What is working well?" top performers (tool development, multi-omic capabilities, multiscale processes, and pathway development) received 8 or 9 votes, but organizational culture (3), teamwork (0), and strong collaborative relationships within the ABF (0) scored very low. When questioned, lab leaders expressed their fealty to the distributed model. Is it possible that lab leaders, with more time available for inter-lab communication, feel a strong sense of collaboration and teamwork, but that task-oriented scientists/engineers/administrative staff lack a similar sense of teamwork?

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback, some of which was responded to above for the "ABF Introduction and Overview"; "ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads"; "ABF Past Accomplishments – Industry Engagement, Outreach, and Management"; "ABF Past Accomplishments – TEA/LCA"; "ABF Past Accomplishments – Host Onboarding and Development"; and "ABF Past Accomplishments – Process Integration and Scale-Up" presentations. The ABF and BETO had extensive and intensive interactions during the ABF's recent strategic planning and implementation planning activities. The ABF team is committed to its new direction, which will support BETO in achieving its ambitious 2030 decarbonization goals. We agree that industry, as a key stakeholder and beneficiary, should inform how the ABF evolves and prioritizes its

activities. We did provide our strategic plan to our IAB for their feedback, and we did receive some responses. Our choices of SAF and biochemical targets will be informed by industry feedback (as part of go/no-go milestones at the end of FY 2023).

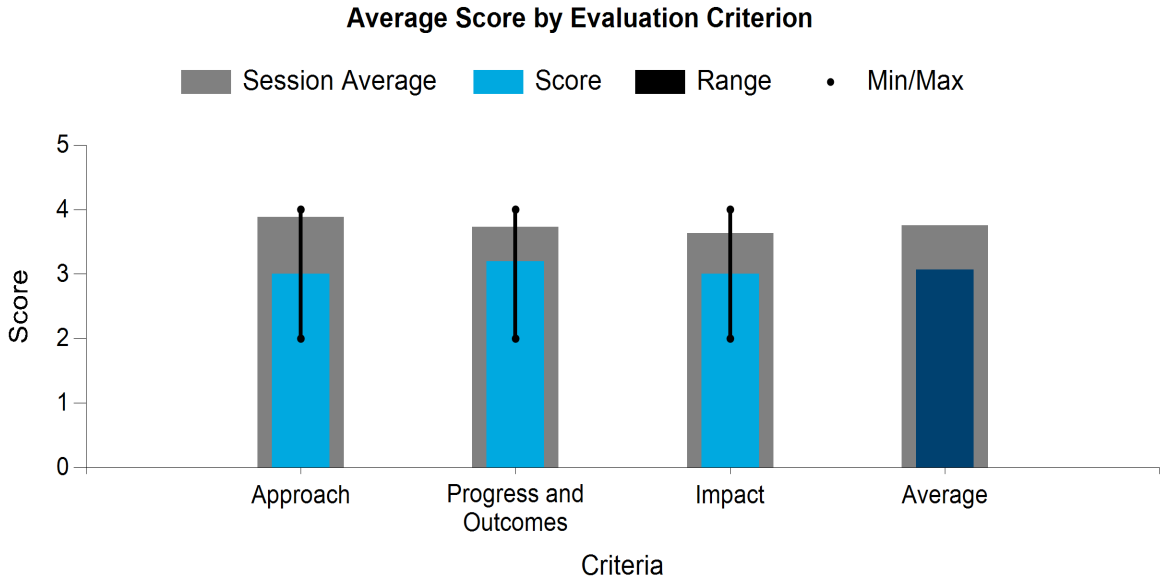
ABF FUTURE STRATEGY – STRATEGIC PLAN

Lawrence Berkeley National Laboratory

PROJECT DESCRIPTION

WBS:	ABF8
Presenter(s):	Katy Christiansen; Nathan Hillson
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

In September 2022, BETO instructed the ABF to do strategic planning, including guidance regarding the process. In December 2022, the ABF completed its strategic plan. The relevance of this activity is that it ensures that the new ABF strategic plan is timely and well aligned with BETO's goals. The impacts of this activity include the genesis of a reimagined DOE ABF and the progression into the ABF implementation planning process. Top challenges included contrasting planning process preferences across the ABF national labs, overcoming the temptation to maintain the status quo, and that any reorganization can jeopardize an individual or team's sense of belonging. The outcome is that the ABF developed a strategic plan, using an inclusive and transparent process, that follows BETO's guidance and provides a compelling and implementable path forward. This presentation focuses on the new vision and mission statements, identified strategies, and updated organization chart. The next presentation will focus on the selected goals and FY 2025 deliverables for each strategy.



COMMENTS

- The ABF has found itself after its initial 8 years facing an identity crisis, stuck between an apparent desire on the part of the ABF to essentially maintain status quo activities and the desires of BETO to drive to activities that more clearly demonstrate impact and results. This is not to say that ABF personnel do not understand or recognize the failures of the organization in the past or the difficulties with which impact can be measured based on its prior goals and strategies. However, the implicit message throughout the strategic planning presentations was that BETO was imposing a new strategic direction upon the ABF, and that the ABF did not wholly agree in the new approach. This is unfortunate. Whether the ABF is at fault for past activities failing to align with BETO priorities or whether BETO has changed its priorities on the ABF and unfairly moved goalposts is not particularly relevant at this stage. What

matters is that the success of the ABF and the ABF's position in BETO's success is undermined when trust between the organizations is damaged by a misalignment between the goals and ideas of leadership. The apparent breakdown of trust that has occurred in this instance is concerning and appears to have negatively impacted the ability of ABF leadership and BETO to collectively work together to find a strategic direction of interest to BETO and ABF PIs.

- The ABF's updated strategic plan suffers as a result. The plan falls short in identifying relevant goals and measurable targets to demonstrate achievement of its mission. The ABF appears to be inhibited by an unworkable governance structure that lacks empowered leadership or individual accountability. As presently constructed, the ABF appears to operate as a consensus coalition of PIs at national labs, each with individual research interests and budgetary needs. Prior to the most recent budgetary cycle, no one PI within the ABF had visibility into the actual spend at the other partner labs, a worrying indicator that no one person is empowered to ensure the success of the ABF's mission. Further, the PIs that drive ABF work at each lab are only able to allocate a minority subset of their time to ABF activities. Although this is standard practice within the national labs, the result is that no one person wakes up every morning and goes to sleep every night thinking about the ABF. At best, this seems to have resulted in a sort of institutional inertia where no one individual is fully empowered to take responsibility for the organization as a whole. At worst, the sometimes competing research interests and budgetary needs of each lab have led to a lack of institutional direction. ABF leadership seems to have an understanding of this dynamic; the continued references during the review that BETO oversight was driving changes to strategy implied that the changes were unwanted and that self-reflection at the ABF was not possible without an external forcing function. In developing this new strategic plan, the ABF betrayed a lack of ownership of their future direction by at times seeking to shift responsibility for potential future shortcomings to BETO.

- The ABF is filled with considerable potential, has done and continues to do good science, and has built unique capabilities. At issue is the tension between a view that the ABF should be primarily a research organization driving science in response to the research interests of the various scientists who work and interact with the ABF, and a view that the ABF is meant to be a platform upon which government funds can be best leveraged to enable industry to innovate faster and better. This tension is apparent in both ABF talking points and BETO talking points as well, and the identity crisis resulting from the inconsistent messaging is holding the ABF back. To best achieve the impact that BETO seems to be driving toward, the ABF needs to reimagine its role in the ecosystem as one of service provider, talking in the language of clients and customers. Validation and proof of impact will come from the level of interest received by industry in ABF services, as well as the level of cash-in support that the ABF can achieve. Focusing on tool benchmarking or internal research projects risks missing the market. In order to successfully pivot to a customer-centric role, the ABF will likely require a change in organizational structure to one that revolves around a single PI who is aligned in vision with BETO, empowered to make decisions, and lacks distractions: They should spend a majority of their time on ABF activities. This will free the ABF to better confront its operational challenges and engage in self-reflection based on industry feedback. Perhaps seven labs aren't needed to drive the ABF's vision, or perhaps some labs don't have tools and capabilities valued by the market. The ABF must be in a position to engage in this difficult self-reflection and make hard choices around the use of its limited funds.

- A key first task for the ABF should be seeking a reset in industry engagement. It is unclear to this reviewer whether the goals and approaches articulated by the ABF for this new strategic plan have any connection with the needs of industry, or are tethered to capabilities that are in need within the market. The ABF has a difficult task as an organization that focuses on TRL 2–4; in general, such early-stage work is often too risky and commercially disconnected for industry to pay major attention, and yet the needs and wants of industry are critical in deciding which platform technologies and capabilities to develop to support the technologies that will have industrial applications in a few years' time. In

response to this tension, the ABF currently engages in internal need-finding to make decisions around which areas to focus funding, but without industrial feedback and engagement, there is significant risk that these choices are academic in nature and lack commercial viability or interest. Tools to pull in industry feedback are currently unused or underused. The ABF has an IAB that it engages with on an irregular basis and does not seek material input from on key decisions or strategies, including this latest strategic plan. The support of large industrials, such as Boeing, was championed at points; however, that outreach did not appear to be part of any coordinated, deliberate outreach strategy to engage industrial players, gather feedback, and respond to the industry needs. A dramatic rethinking of the ABF's engagement with industry will set the stage for further changes and realignments that will best position the ABF for impact.

- A rethinking of the budget mix in the latest strategic plan should go hand in hand with this new focus on industrial engagement. It made sense for the ABF to spend 75% of its budget on AOP activities while it grew its toolbox and capabilities. At this point in its life cycle, that split no longer makes sense, and a majority of funds should be directed toward industry through DFOs to ensure that the tools developed by the ABF are of use to industry. Some internal capabilities may not garner industrial interest; that may be a sign that they are not industrially relevant or competitive against alternatives. This engagement will help drive further decisions on where to focus limited AOP cash, which capabilities to support and which to discard, and which innovations to invest in. The ABPDU provides a guide for the type of industry outreach and service that the ABF can achieve.

- Similarly, it is reasonable for the ABF to try to identify likely molecular targets of commercial interest, including SAF and the specific biochemicals of interest (muconate and 3-HP). However, the goal of driving significant progress on four targets should be built around commercial engagement. A key refrain heard during the presentation was that "industry engagement is based on capabilities, not products," a statement that does not match with the "four core products" focus of the new strategic plan. Although it appears the idea is to use the four products as a mechanism to show the value of the ABF, the described goals in the "go/no-go" reviews are qualitative at best and risk becoming a confirmation of the PIs' priors. Industry engagement can once again help ensure the best utilization of resources and avoid confirmation biases within the team. By pushing for four targets developed through DFOs instead of using AOP funds, the ABF can seek industrial partners for its four target molecules and further solicit proposals for other products and pathways. Through a competitive process with industry, the ABF will be better positioned to identify the four targets with the highest probability of commercial applicability and likelihood of success, multiplying impact. SAF may prove to be a poor target for ABF activities given the long history of failures utilizing synbio techniques for fuels production. Four might not even be the right number. If four targets are desired, it is highly unlikely that the ABF will see success in all targets chosen, and other potential molecules of interest should be considered to provide for attrition of targets and eventual downselect at a later time as progress is made.

- Benchmarking, which was brought up through the strategic plan as a mechanism to prove impact, should be deprioritized or removed altogether, as it likely does not represent a good use of resources. Other metrics, primarily commercial interest and funds-in support, will implicitly demonstrate the value of the ABF vis-à-vis alternatives. The tool capabilities doesn't mention TEA; however, it was noted that TEA drove decisions presented elsewhere in the presentations, a core disconnect. TEA and LCA work is something the ABF has and can continue to build a strong capability in, one that is readily applicable to industry and can be rapidly deployed through funds-in contracts on a small-dollars basis to gain initial traction with industrial partners and establish working relationships. IP issues related to contracting and speeding time for CRADA approvals and contract reviews was mentioned as a challenge, but no serious proposal was presented to move contracting time frames to a commercially relevant time scale (as BOTTLE and ABPDU have done).

- As noted above, the issues with the current strategic plan do not appear to be caused by any single person or problem, but rather result from a collection of issues that limit the flexibility and creativity of the ABF as presently constructed. The ability for the ABF to maximize impact will require structural changes to overcome these challenges and enable the organization moving forward.
- Strengths:
    - The new strategic plans are better aligned with BETO missions on promoting GHG reduction, SAF, and renewable bulk chemicals.
    - The new goals and metrics are adjusted to focus more on transfer of ABF technologies to industry. These adjustments are appropriate. Higher impacts to industrial biomanufacturing can be expected.
    - The strategic implementation unit has substantial merits. To avoid issues as learned from the past (e.g., realizing challenges in measuring DBTL cycles after 7 years of efforts), having the strategic implementation unit constantly monitor and provide feedback to the executive committee of the ABF would be beneficial.
    - The business development and partnering agreements unit will also be helpful. The review meeting discussed multiple cases where collaborations with industry were delayed by establishing a CRADA. The business development and partnering agreements unit may help to shorten this process.
- Weaknesses/areas for improvement:
    - Building industrial partnerships was listed as one of the four strategies to pursue, and a milestone of achieving at least two funds-in projects was described. This can be a risky strategy because a successful industry partnership relies on appealing technology/success from the other strategies. Considering the current large gap in economically viable SAF manufacturing, it is extremely challenging to have funds-in projects on SAF within the next 2 years. Similarly, having funds-in projects on tools can be challenging because most companies (particularly small companies) focus on developing products rather than tools. Thus, most likely the funds-in projects will come from industrial interests in commercializing one of the two proposed chemicals, muconic acid and 3-HP. Both of these chemicals are good targets; however, there are only two of them. So it might be challenging to attract two funds-in projects in 2 years.
- These comments are the same for the ABF Future Strategy (1) Strategic Plan; (2) Goals, Milestones, and Deliverables; and (3) Implementation Plans:
    - The ABF and BETO have both struggled for years with how the ABF fits in with BETO's mission. This struggle is still ongoing. While it's clear the ABF needs new direction, management, and goals, at the same time, BETO needs to decide whether or not it's going to support an outwardly facing biofoundry focused on supporting synthetic biology. BETO was absolutely correct in calling out the ABF's failures to deliver on previous goals, and to force a reorganization of the ABF. That being said, the ABF was founded with a vision to support and advance synthetic biology in the United States, both for industry and at the national labs. This has frankly never seemed to fit well with BETO's overall mission statement in the past. However, with the Biden administration's call to decarbonize the chemical industry, the ABF finds itself more aligned with BETO than in its previous history. My recommendation is for BETO to support the ABF consortium as it was intended—namely, to be outwardly facing, heavily engaged with industry, and provide services to all U.S. research efforts (industry and academia) to advance synbio goals and develop America as a leader in bioproduction of molecules our world needs. This means changing the current focus of

the ABF away from some of their core work and putting funds back to supporting external partners and ABF infrastructure.

- I recommend the ABF revise their goals in the current strategic plan. This is not going to be something the ABF (and maybe BETO) wants to do. However, the current strategic plan is not well thought out in some areas, and seems to overcorrect and try to appease perceived BETO unhappiness. It's much better to spend another few months rethinking and replanning than waste $45–$60 million over the next 3 years.

- Specifically, the core SAF targets seem out of scope with the ABF's mission and put in to appease BETO. First, the economics of SAF from lignocellulosic sugars is never going to work. Not unless BETO is OK relying on massive government subsidies for SAF, far more than the current subsidies. It's not possible to be economically viable to make SAF currently from cheap sugar cane, using a terpene strain that has near-maximum theoretical yield and a very simple and cheap purification process. The extra costs of lignocellulosic sugars only make the economics worse. The ABF should get clarity from BETO on what level of subsidy is OK to assume if they are going to pursue this path. Second, instead of the ABF trying to guess what would be the best SAF molecule for them to develop, they should be engaging with the big industry players to understand what they want, if there is any overlap between what is needed/wanted, and what the ABF/synbio can help develop. The ABF and BETO need to be brutally honest here, and if the economics of hydrocarbon production from cellulosic sugars is not going to be the most economically viable route to SAF, then don't work on this in the ABF. With regard to the CBP work proposed by the ABF, the CBP is very high risk and high reward. I don't know enough about the challenges of CBP to know if the ABF is adequately addressing the risks, and therefore the probability of success. I do think the CBP work overall is aligned with BETO and is in the national interest to fund at the national labs. It is this kind of "blue sky" visionary work that industry won't do because of the risk, cost, and timelines involved. This is where government funding of basic science can push development of new technologies. That being said, should this work be done in the ABF? Again, I cannot judge that because little detail was given on why the ABF thinks they are best suited to do this work.

- Assuming some or all of the proposed SAF work is dropped from the ABF strategic plan, what should they do with the money? Some recommendations (in rank order): (1) Continue to host onboarding work, which we learned was cut completely. This is my number 1 recommendation for the money. The host onboarding is one area that ABF and BETO funds can really make a difference to advance new technologies and develop new strains to enable new chemicals and pathways at better costs. This is expensive and time-consuming, and often industry is reluctant to take it on. If they do develop a unique microbe, they will keep it strictly proprietary. Host onboarding should pick a small number of microbes that have very different properties, would access very different feedstocks or fermentation abilities, and are likely to be tractable genetically. Then develop them to a high TRL rating to make them really useful. I have a lot to say about the apparent lack of progress on the HObT website since 2021 and the lack of helpful information to the U.S. research community from this work when reviewing this part of the ABF presentation. Regardless, if further work on host onboarding is done, there must be funds allocated to bring the website up to date and make the tools developed useful to the public, or BETO can consider a lot of that work and money wasted. (2) Develop another chemical target, if there is another one as developed and with clear TEA and CO_2e reduction benefits. (3) Put more money to partner-facing projects (FOAs) that support BETO's goals and will also support industry and commercial realization of BETO's goals.

- Several aspects of the technical targets had not yet been worked out by the time of the BETO review in April. Given that the ABF had 4–5 months already to replan, this is surprising and

disappointing. The tools section of the strategic plan is not well thought out at all. They have not come up with criteria to even choose which tools to benchmark, or how to conduct benchmarking. It seems like no one wanted to give up their pet project, and with no one really in charge, they are having a hard time figuring out what to pursue further. It is good to not spend time and effort trying to build a capability that is already publicly available that people can access. DOE/BETO/national labs should focus on offering capabilities or research tools that are not available or that industry is not going to do. However, I caution that the ABF should not throw out a capability or tool even if it is measured to be not as good as something already out there. It might be that the ABF provides a different benefit that makes the tool/capability useful to researchers and industry. For example, an ABF capability might be almost as good as something already commercially available, but ABF's might be cheaper, more accessible, or offer more capacity to a limited service. The ABF needs to figure out their criteria and how they are going to benchmark tools immediately and complete the assessments as soon as possible. In the current plan, it looks like this will not be done until the end of 2025.

- Lastly, I think it's a mistake to abandon all DBTL metrics going forward. Revise DBTL to be more realistic, but still have a goal to reduce the DBTL cycle (or even parts of DBTL that make the most sense, are the most commonly used, or the biggest bottleneck). It doesn't have to be every capability and every tool. A metric could be time needed to finish one complete experiment and learn from it (per tool or per instrument) and focus on making those individual unit ops more efficient. Drop wall time versus clock time. What matters is how long it takes you to learn from an experiment. If there is some large downtime (let's say you need to ship samples from one site to another, and that takes weeks), then that is part of your cycle time and something to target to be more efficient. Focus on gaining efficiencies and improving the infrastructure already in place. There is some acknowledgement of this in the strategic plan; under tool milestones it says, "Operational performance gains of significant impact demonstrated for at least 2 ABF benchmarked technologies."

- The ABF said there was large interest from chemical companies for the proposed 3-HP and muconic acid targets. (I know from my own company that industry is very interested in muconic acid to make nylon.) These two core projects are much more aligned with the capabilities the ABF has built, have a high chance of success, and align with BETO's goals. These two projects should definitely continue and are a good change from the beachhead approach, which for whatever reason did not appear to get much industry traction.

- The shift in emphasis to bring in more external money (FOA and DFO) and have >50% of the ABF's budget committed to external partnerships by 2025 is a good change of direction and furthers the goals of the ABF to support industry and other research to translate lab work to real-world changes in decarbonization and reducing CO_2. It's going to be very challenging to achieve a significant increase in industry money into the ABF in the near future, especially if contracting takes a year. It will take time to get to >50% of the ABF's budget committed to external partnerships and reach an average of more than fivefold oversubscription for the ABF's funding opportunities. The 2024 milestone to have two funds-in projects means the ABF needs to land those partnerships in a few months if contracting is going to take a year. Also, the length of time to contract must be improved. The ABF needs to redo its approach to IP and how it contracts projects to de-risk bringing in more industry partners and money. BOTTLE has worked out a very clear IP management plan that allows different levels of industry access to any IP developed. There is an option for industry to keep all the IP (and the national labs then get a higher percent royalty fee). ABPDU also has worked out smooth contracting and IP understandings that allow them to contract in a few weeks, and industry does not have to share IP if it does not want to. The ABF should have developed something like this years ago.

  - There was no mention of how the ABF will measure reduction of CO_2e in their partner projects. Given the ABF's track record regarding an inability to quantify metrics, this does raise concern.

  - The ABF revised management plan is significantly better than the old management structure. The one area that still needs to be changed is the lead PI being solely accountable and responsible for running the ABF. There should not be any decision by committee. The ABF needs a 100% dedicated full-time employee running it. This person should be an "outsider" with no emotional connection to the past 6 years of the ABF. The executive committee reports to and advises the ABF PI, but does not make decisions. It's clear that the ABF has really suffered from decision by committee and a lack of someone whose job is 100% running the ABF.

- Approach: Given the focus on industry partnerships and funds-in projects under the new strategic plan, I cannot reconcile the lack of industry stakeholder outreach. At minimum, current and past industry partners should have been engaged to provide feedback in an iterative fashion. The lack of industry feedback on the new strategic plan makes me worry that the goals and structure they created may not be well received by industry. Though they mentioned during Peer Review that they will engage with industry to validate the choice of those molecules in the coming months, I again question why this was not already done as part of the strategic planning.

- Progress and outcomes: Given that the core activities will now only focus on four molecules, it may be worthwhile to reduce the number of labs that receive core funding to ensure that progress can be made to achieve the desired benchmarks. Additional labs can receive funding for industry-directed projects. However, I do understand and appreciate the need to balance maintaining consistent staffing and having dedicated funding to support researchers across the ABF. While I see the new org chart as better suited to address the ABF's new goals, it may also be helpful to increase the full-time employee allocation of the ABF lead PI, given that past organizational structure has been slow or unable to address the recommendations of prior Peer Review reports.

- Impact: While the new metrics that the ABF has set forth are relevant to BETO initiatives, some of them are not explained in sufficient context to evaluate appropriately. For instance, is the 500-kiloton reduction for each project? Over all the projects that the ABF does? Over what time frame? Is the metric achieved when the CO_2 reduction occurs (e.g., when the product replaces a fossil fuel alternative)? Or is this based on the theoretical, predicted CO_2 reduction measured in initial TEAs? In aiming to increase the proportion of funds-in work as part of their portfolio, there are several operational constraints, discussed during the Peer Review, such as long contracting times and IP models that will likely limit their ability to meet those targets. Though the ABF and BETO can attempt to alleviate some of the bureaucratic hurdles, much of the power to change those systems lies outside ABF and BETO control (e.g., DOE side offices, IP standards of federally funded research and development centers).

- Progress/Outcomes and Impact were given scores of 3, but these are not meaningful for a forward-looking plan. Approach was also given a score of 3, but in this case reflects shortcomings in the approach to developing a new strategic plan and the plan itself. The revised ABF vision and mission aligns with the BETO mission and research priorities. Four strategies will be pursued: partnerships, tools, SAF, and biochemicals. Focusing on SAF and biochemicals is directly relevant to BETO research priorities. Continued focus on tools development is anticipated to advance the field of synthetic biology, which, when applied to SAF and biochemical projects, will advance commercial potential. Partnerships enable public utilization of ABF tools and expertise, which was the original motivation for establishing the ABF. Going forward, the quantity and quality of funds-in partnerships will provide a measure of the ABF's role in growing the bioeconomy. A lack of outside organizations eager to partner with the ABF will be indicative of challenges (e.g., inadequate marketing/self-promotion of ABF capabilities, slow contracting process, unmanageable IP scenarios, or identical tools available at same/lower cost from other

providers). This will provide an incentive for the ABF to remediate deficiencies identified in the current review process. Deficiency remediation is expected to improve the oversubscription and quality of partnerships (commercial potential) resulting from DFO and FOA mechanisms. If the ABF wants to increase partnerships with private organizations, a stronger emphasis must be placed on identifying and remediating challenges in establishing partnerships. Direct, specific feedback from current and former IAB members should have been integrated at the initiation of strategic planning rather than after the strategic plan was in place, as indicated in the presentation. Different ABF national labs and teams having contrasting planning process preferences was presented as a challenge to arriving at a new strategic plan. This is an acknowledgement that the distributed model adds a significant level of complexity and inefficiency to the ABF structure. Reorganization jeopardizing an individual or team's sense of belonging was also presented as a challenge, which indicates a mindset whereby labs and research programs have higher priority than the ABF. That is not correct. ABF structure, operations, and achievement of milestones have priority over any individual or team. Regarding the new organization chart, the lead PI resides in the same box as the executive committee, and this unit interacts directly with the ABF advisory board and provides oversight to strategic implementation. Based on this structure, the lead PI has no additional decision-making or responsibility. The ABF's consensus-driven decision-making in the past has proven to be inefficient. Creation of a new strategic plan provides an opportunity to change this model. Engagement/outreach and business development/partnering agreements are shown as not reporting to anyone. If engagement/outreach and business development/partnering are not performing, who has the responsibility to provide that feedback and force changes to be made? A different approach might be to have the lead PI answerable to the BETO technology manager, and engagement/outreach, business development/partnering, and strategic implementation answerable to the lead PI. The executive committee could be on the same level as the lead PI to provide advice, but no component answers directly to the executive committee. This model would improve efficiency, provide decision-making authority to the lead PI, and make the lead PI answerable to the BETO technology manager.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback, some of which was responded to above for the "ABF Introduction and Overview"; "ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads"; "ABF Past Accomplishments – Industry Engagement, Outreach, and Management"; "ABF Past Accomplishments – TEA/LCA"; "ABF Past Accomplishments – Host Onboarding and Development"; "ABF Past Accomplishments – Process Integration and Scale-Up"; and "ABF – Lessons Learned and Introduction to Future Plans" presentations. The ABF and BETO have set a goal that by the end of FY 2025, 50% of ABF resources should be associated with collaboration projects, and in that sense, the ABF is on a path toward more of a "customer"-facing entity. While industry (or other collaborator) proposals (whether funds-in or funding opportunity) are clearly directed by the "customer," core industry projects—in which internally devised ABF projects receive industry cost share (demonstrating their interest)—can also be influenced by and benefit from collaborator partner interactions. Should the ABF not receive sufficient interest in core industry project opportunities, the ABF has contingency plans (e.g., redirecting resources from internal projects to funding opportunities) to achieve the target 50% resource allocation target. Benchmarking is very related to the ABF becoming more of a "customer"-facing entity as well, as it helps the ABF prioritize its tool development activities and demonstrate the value of ABF capabilities to prospective customers.

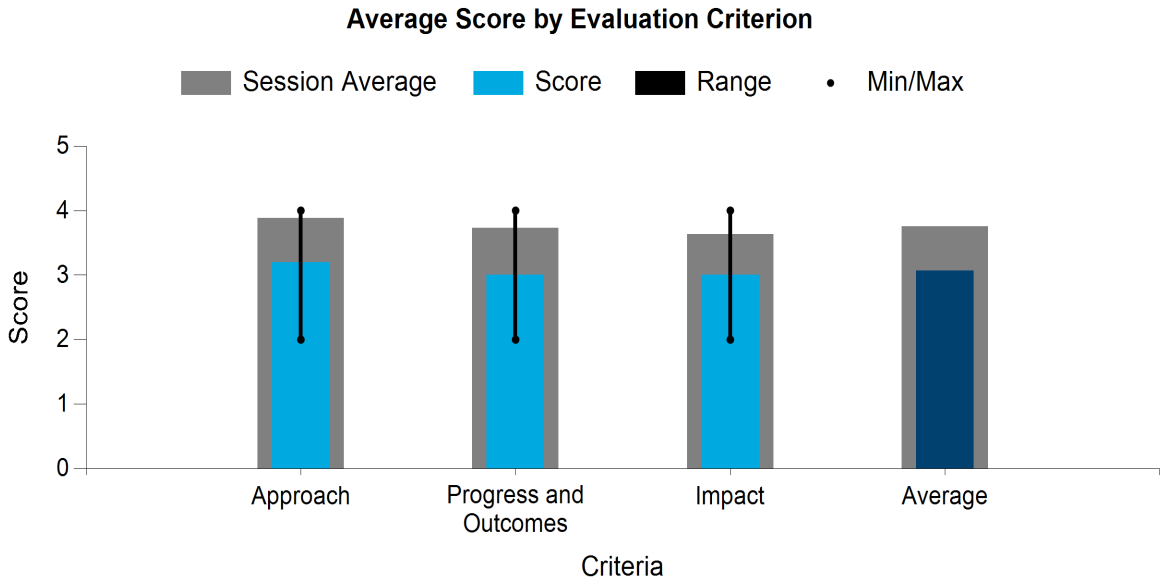
ABF FUTURE STRATEGY – GOALS, MILESTONES, AND DELIVERABLES

Lawrence Berkeley National Laboratory, National Renewable Energy Laboratory, Sandia National Laboratories, and Pacific Northwest National Laboratory

PROJECT DESCRIPTION

WBS:	ABF9
Presenter(s):	Gregg Beckham; John Gladden; Jon Magnuson; Katy Christiansen; Nathan Hillson
Project Start Date:	10/01/2015
Planned Project End Date:	09/30/2022

In December 2022, the ABF completed its strategic plan, which included goals and deliverables for FY 2023–2025. The relevance of setting these goals and deliverables is that they ensure that they are timely and well aligned with BETO's goals. Top challenges include developing goals that serve the ABF and BETO beyond FY 2025, developing deliverables that do not dictate their implementation, and developing deliverables that are likely accomplishable (even without knowing precise budget constraints or implementation details). The outcomes of these activities are a set of goals for the ABF, including partnerships, tools, SAF, and biochemicals, along with FY 2025 deliverables for each goal. This presentation covers in detail the deliverables for each goal. Milestones will be covered in the subsequent Implementation Plans presentation.



COMMENTS

- The ABF utilized multiple presentations to document their journey in developing a new strategic plan for the next funding cycle. These presentations grew off each other, and as a result, it is difficult to separate them as required in this review process. Instead, I have used the "Strategic Plan" presentation to provide feedback on this presentation as well. Please look there for my comments.

- Strengths:

  - The overall goals and milestones for the four targeted areas are appropriate. Achieving these proposed milestones will bring substantial impacts to industrial biomanufacturing.
  - Muconic acid and 3-HP are reasonable biochemical targets given the ABF's previous achievements and experience in producing these chemicals. Previous TEAs/LCAs also showed potential for economically viable biomanufacturing, and highlighted areas for further technology development.

- Weaknesses/areas for improvement:
  - A quantitative milestone is needed to describe the gains of significant impact for two ABF tools. It is not clear how much impact is significant and how to quantitatively measure the impact by FY 2025.
  - The rationale to select CBP of ethanol as a SAF target is unclear. Commercial benefits of CBP are well received. However, it is not clear whether ethanol is a right choice for SAF, given its low energy density and low heating value. Innovations for CBP ethanol production are also limited.
  - The 500-kiloton CO_2e reduction should not only be a deliverable for the partnerships team. It should be the target of the entire ABF team. Failure to achieve this milestone can be caused by insufficient technology transfer to industry, or by ABF technologies not being of interest to industry.

- These comments are the same for the ABF Future Strategy (1) Strategic Plan; (2) Goals, Milestones, and Deliverables; and (3) Implementation Plans:
  - The ABF and BETO have both struggled for years with how the ABF fits in with BETO's mission. This struggle is still ongoing. While it's clear the ABF needs new direction, management, and goals, at the same time, BETO needs to decide whether or not it's going to support an outwardly facing biofoundry focused on supporting synthetic biology. BETO was absolutely correct in calling out the ABF's failures to deliver on previous goals, and to force a reorganization of the ABF. That being said, the ABF was founded with a vision to support and advance synthetic biology in the United States, both for industry and at the national labs. This has frankly never seemed to fit well with BETO's overall mission statement in the past. However, with the Biden administration's call to decarbonize the chemical industry, the ABF finds itself more aligned with BETO than in its previous history. My recommendation is for BETO to support the ABF consortium as it was intended; namely, to be outwardly facing, heavily engaged with industry, and provide services to all U.S. research efforts (industry and academia) to advance synbio goals and develop America as a leader in bioproduction of molecules our world needs. This means changing the current focus of the ABF away from some of their core work and putting funds back to supporting external partners and ABF infrastructure.
  - I recommend the ABF revise their goals in the current strategic plan. This is not going to be something the ABF (and maybe BETO) wants to do. However, the current strategic plan is not well thought out in some areas, and seems to overcorrect and try to appease perceived BETO unhappiness. It's much better to spend another few months rethinking and replanning than waste $45–$60 million over the next 3 years.
  - Specifically, the core SAF targets seem out of scope with the ABF's mission and put in to appease BETO. First, the economics of SAF from lignocellulosic sugars is never going to work. Not unless BETO is OK relying on massive government subsidies for SAF, far more than the current subsidies. It's not possible to be economically viable to make SAF currently from cheap sugar cane, using a terpene strain that has near-maximum theoretical yield and a very simple and cheap

purification process. The extra costs of lignocellulosic sugars only make the economics worse. The ABF should get clarity from BETO on what level of subsidy is OK to assume if they are going to pursue this path. Second, instead of the ABF trying to guess what would be the best SAF molecule for them to develop, they should be engaging with the big industry players to understand what they want, if there is any overlap between what is needed/wanted, and what the ABF/synbio can help develop. The ABF and BETO need to be brutally honest here, and if the economics of hydrocarbon production from cellulosic sugars is not going to be the most economically viable route to SAF, then don't work on this in the ABF. With regard to the CBP work proposed by the ABF, the CBP is very high risk and high reward. I don't know enough about the challenges of CBP to know if the ABF is adequately addressing the risks, and therefore the probability of success. I do think the CBP work overall is aligned with BETO and is in the national interest to fund at the national labs. It is this kind of "blue sky" visionary work that industry won't do because of the risk, cost, and timelines involved. This is where government funding of basic science can push development of new technologies. That being said, should this work be done in the ABF? Again, I cannot judge that because little detail was given on why the ABF thinks they are best suited to do this work.

- Assuming some or all of the proposed SAF work is dropped from the ABF strategic plan, what should they do with the money? Some recommendations (in rank order): (1) Continue to host onboarding work, which we learned was cut completely. This is my number 1 recommendation for the money. The host onboarding is one area that ABF and BETO funds can really make a difference to advance new technologies and develop new strains to enable new chemicals and pathways at better costs. This is expensive and time-consuming, and often industry is reluctant to take it on. If they do develop a unique microbe, they will keep it strictly proprietary. Host onboarding should pick a small number of microbes that have very different properties, would access very different feedstocks or fermentation abilities, and are likely to be tractable genetically. Then, develop them to a high TRL rating to make them really useful. I have a lot to say about the apparent lack of progress on the HObT website since 2021 and the lack of helpful information to the U.S. research community from this work when reviewing this part of the ABF presentation. Regardless, if further work on host onboarding is done, there must be funds allocated to bring the website up to date and make the tools developed useful to the public, or BETO can consider a lot of that work and money wasted. (2) Develop another chemical target, if there is another one as developed and with clear TEA and CO_2e reduction benefits. (3) Put more money to partner-facing projects (FOAs) that support BETO's goals and will also support industry and commercial realization of BETO's goals.

- Several aspects of the technical targets had not yet been worked out by the time of the BETO review in April. Given that the ABF had 4–5 months already to replan, this is surprising and disappointing. The tools section of the strategic plan is not well thought out at all. They have not come up with criteria to even choose which tools to benchmark, or how to conduct benchmarking. It seems like no one wanted to give up their pet project, and with no one really in charge, they are having a hard time figuring out what to pursue further. It is good to not spend time and effort trying to build a capability that is already publicly available that people can access. DOE/BETO/national labs should focus on offering capabilities or research tools that are not available or that industry is not going to do. However, I caution that the ABF should not throw out a capability or tool even if it is measured to be not as good as something already out there. It might be that the ABF provides a different benefit that makes the tool/capability useful to researchers and industry. For example, an ABF capability might be almost as good as something already commercially available, but ABF's might be cheaper, more accessible, or offer more capacity to a limited service. The ABF needs to figure out their criteria and how they are going to benchmark tools immediately and complete the assessments as soon as possible. In the current plan, it looks like this will not be done until the end of 2025.

- Lastly, I think it's a mistake to abandon all DBTL metrics going forward. Revise DBTL to be more realistic, but still have a goal to reduce the DBTL cycle (or even parts of DBTL that make the most sense, are the most commonly used, or the biggest bottleneck). It doesn't have to be every capability and every tool. A metric could be time needed to finish one complete experiment and learn from it (per tool or per instrument) and focus on making those individual unit ops more efficient. Drop wall time versus clock time. What matters is how long it takes you to learn from an experiment. If there is some large downtime (let's say you need to ship samples from one site to another, and that takes weeks), then that is part of your cycle time and something to target to be more efficient. Focus on gaining efficiencies and improving the infrastructure already in place. There is some acknowledgement of this in the strategic plan; under tool milestones it says, "Operational performance gains of significant impact demonstrated for at least 2 ABF benchmarked technologies."

- The ABF said there was large interest from chemical companies for the proposed 3-HP and muconic acid targets. (I know from my own company that industry is very interested in muconic acid to make nylon.) These two core projects are much more aligned with the capabilities the ABF has built, have a high chance of success, and align with BETO's goals. These two projects should definitely continue and are a good change from the beachhead approach, which for whatever reason did not appear to get much industry traction.

- The shift in emphasis to bring in more external money (FOAs and DFOs) and have >50% of ABF's budget committed to external partnerships by 2025 is a good change of direction and furthers the goals of the ABF to support industry and other research to translate lab work to real-world changes in decarbonization and reducing CO_2. It's going to be very challenging to achieve a significant increase in industry money into the ABF in the near future, especially if contracting takes a year. It will take time to get to >50% of the ABF's budget committed to external partnerships and reach an average of more than fivefold oversubscription for the ABF's funding opportunities. The 2024 milestone to have two funds-in projects means the ABF needs to land those partnerships in a few months if contracting is going to take a year. Also, the length of time to contract must be improved. The ABF needs to redo its approach to IP and how it contracts projects to de-risk bringing in more industry partners and money. BOTTLE has worked out a very clear IP management plan that allows different levels of industry access to any IP developed. There is an option for industry to keep all the IP (and the national labs then get a higher percent royalty fee). The ABPDU also has worked out smooth contracting and IP understandings that allow them to contract in a few weeks, and industry does not have to share IP if it does not want to. The ABF should have developed something like this years ago.

- There was no mention of how the ABF will measure reduction of CO_2e in their partner projects. Given the ABF's track record regarding an inability to quantify metrics, this does raise concern.

- The ABF revised management plan is significantly better than the old management structure. The one area that still needs to be changed is the lead PI being solely accountable and responsible for running the ABF. There should not be any decision by committee. The ABF needs a 100% dedicated full-time employee running it. This person should be an "outsider" with no emotional connection to the past 6 years of the ABF. The executive committee reports to and advises the ABF PI, but does not make decisions. It's clear that the ABF has really suffered from decision by committee and a lack of someone whose job is 100% running the ABF.

- Approach: My comments on the approach to strategic planning have already been covered in responses to other presentations.

- Progress and outcomes: Regarding benchmarking ABF processes to the equivalent industry-accessible state-of-the-art baselines, when asked to further clarify this during the Peer Review, they stated that any proprietary industry methods would not be appropriate comparisons. As such, it is unclear what these benchmarks will be or what purpose they will truly serve if the goals are not specified beforehand. For instance, is this benchmarking metric for the development of tools? If so, which tools? What parts of the process are important to measure? Time, scale of project, output, etc.? For projects that don't have direct TRY outputs (e.g., modeling approaches), what is the benchmark? I also see a discrepancy with the goal to develop at least six new tools while reducing the core function to develop only the four stated molecules. There is also some tension in the goal to increase oversubscription while also increasing the number of funds-in projects, as having more cost share or 100% funds-in will make it less appealing to industry.

- Impact: The planned increase in industry-funded work will require the labs to safeguard more of the work that they do and keep it from public knowledge (e.g., through exclusive licenses). With the four-molecule focus of the core work, the new plan may limit the ABF's eventual public impact. It also bifurcates the identity of the ABF in two; one side as a research entity advancing government priorities and the other as a contract research organization for industry.

- Scores for Progress/Outcomes and Impact are not relevant for project planning; hence, neutral scores of 3 were given. Approach was given a score of 4 because there is substantial merit to the approach developed. Goals and deliverables have been set that align with the revised strategic plan.

PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their feedback. We responded above for the "ABF Introduction and Overview"; "ABF Past Accomplishments – DBTL Infrastructure, Demonstration Projects, and Beachheads"; "ABF Past Accomplishments – Industry Engagement, Outreach, and Management"; "ABF Past Accomplishments – TEA/LCA"; "ABF Past Accomplishments – Host Onboarding and Development"; "ABF Past Accomplishments – Process Integration and Scale-Up"; "ABF – Lessons Learned and Introduction to Future Plans"; and "ABF Future Strategy – Strategic Plan" presentations.