BIOCHEMICAL CONVERSION R&D

TECHNOLOGY AREA

CONTENTS

INTRODUCTION

In the Biochemical Conversion Research and Development (R&D) session, five external experts from industry and academia reviewed a total of 39 presentations (representing more than 39 projects, as a few presentations were collaborations of different projects across multiple national laboratories).

This review addressed a total U.S. Department of Energy (DOE) investment value of approximately $140,331,161, representing approximately 19% of the Bioenergy Technologies Office (BETO or the Office) portfolio reviewed during the 2017 Project Peer Review. During the Project Peer Review meeting, the principal investigator (PI) for each project was given 20–60 minutes (depending on the project's funding level and relative importance to achieving BETO goals) to deliver a presentation and respond to questions from the Review Panel.

The Review Panel evaluated and scored projects for their project approach, technical progress and accomplishments, relevance to BETO goals, and future plans. This section of the report contains the results of the project review, including full scoring information for each project, summary comments from each reviewer, and any public response provided by the PI. Overview information on the Biochemical Conversion R&D Program, full scoring results and analysis, the Review Panel's summary report, and BETO's programmatic response are also included in this section.

BETO designated Jay Fitzgerald as the Biochemical Conversion R&D Technology Area Review Lead. In this capacity, Dr. Fitzgerald was responsible for all aspects of review planning and implementation.

BIOCHEMICAL CONVERSION R&D OVERVIEW

he Biochemical Conversion R&D Technology Area focuses on R&D of biological processes that convert biomass to biofuels, chemicals, and power. Biochemical processes also complement thermochemical conversion by providing residual materials for further processing.

Projects presented in the Biochemical Conversion session include a broad range of efforts, generally targeted for one or more of three purposes: (1) to deconstruct lignocellulose into biochemical intermediates, such as cellulosic sugars (both five-carbon (C5) and six-carbon (C6)) and lignin; (2) to biologically upgrade those biochemical intermediates into fungible liquid transportation fuels and bioproducts; and (3) to biologically upgrade thermochemical intermediates (gaseous or aqueous). Prior to fiscal year (FY) 2012, BETO focused on converting cellulose to ethanol and utilizing lignin for power. In 2012, based on integrated pilot-scale runs, BETO estimated a minimum cellulosic ethanol selling price at an nth plant of $2.15/gallon (in 2007 dollars, with a $58.50/ton feedstock cost and a conversion contribution cost of $1.32/gallon).[29]

After reaching this cost target, BETO shifted research from cellulosic ethanol to cellulosic hydrocarbon fuels. Prior to FY 2016, BETO's state-of-technology (SOT) model assumed that any C5 sugars generated after deconstruction would be valorized to bioproducts, and any lignin would be combusted onsite for process energy.[30]

[29] L. Tao, D. Schell, R. Davis, E. Tan, R. Elander, A. Bratis, *NREL 2012 Achievement of Ethanol Cost Targets: Biochemical Ethanol Fermentation via Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover* (Golden, CO: National Renewable Energy Laboratory, April 2014), NREL/TP-5100-61563, https://www.nrel.gov/docs/fy14osti/61563.pdf.

[30] U.S. Department of Energy, Bioenergy Technologies Office (BETO), *Multi-Year Program Plan* (BETO, March 2015), https://www.energy.gov/sites/prod/files/2015/04/f22/mypp_beto_march2015.pdf.

Based on advances in research, in FY 2016 BETO shifted to the assumption that all C5 and C6 sugars would be converted to hydrocarbon fuels, and the lignin would be valorized to bioproducts.[31] Because this shift took place in the middle of the review period, a number of project descriptions and reviewer comments recognize this change in the project-specific sections below.

Regardless of which of the above two pathways a project follows, the resulting sugar-rich stream (hydrolysate) can then be fed to organisms that ferment the sugars to fuel precursor molecules. Projects that use low-temperature catalytic and mechanical systems to produce sugars (and/or other intermediates from biomass) and/or upgrade those sugars and intermediates to create finished fuel blendstocks were also presented in this session.

The SOT assumptions are mission-critical for certain national laboratory annual operating plan (AOP) projects that seek to verify BETO SOT assumptions. For competitive funding opportunity announcement (FOA) projects, there is, dependent on the FOA, much more latitude.

One of BETO's priorities is to make the biochemical conversion process more cost-effective. The process breaks down the cell wall of plant matter by introducing enzymes or acid to extract the sugars, which are then converted to biofuels using microorganisms. The process is costly due to the complex nature of the cell wall. Lignocellulose (mainly lignin, cellulose, and hemicellulose) is the primary component of plant residues, woody materials, and grasses, and the cell wall structure of this plant matter is partially comprised of long chain sugars (carbohydrates), which can be converted into biofuels. Due to its complex structure, lignocellulose is more difficult to break down into sugars, making this material more expensive to convert into biofuels.

A key to developing cost-competitive cellulosic biofuels is reducing the processing and capital cost and improving the efficiency of separating and converting cellulosic biomass into fermentable sugars. Current R&D focuses on high-yield feedstocks, more-efficient enzymes, and more-robust microorganisms to advance biochemical conversion processes. The resulting advanced biochemical conversion technologies will increase fuel yields in integrated biorefineries—facilities that combine conversion capabilities with heat and power efficiencies to produce fuel and products.

BIOCHEMICAL CONVERSION R&D REVIEW PANEL

Name	Affiliation
Suzanne Lantz*	DuPont
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[31] U.S. Department of Energy, Bioenergy Technologies Office (BETO), *Multi-Year Program Plan* (BETO, March 2016), https://energy.gov/sites/prod/files/2016/07/f33/mypp_march2016.pdf.

TECHNOLOGY AREA SCORE RESULTS

BIOCHEMICAL CONVERSION R&D REVIEW PANEL SUMMARY REPORT

Prepared by the Biochemical Conversion R&D Review Panel

Impact

In its 2016 Multi-Year Program Plan (MYPP), BETO outlined approaches and targets for "enabling deployment of increasing amounts of biofuels, bioproducts, and biopower" and recognized the need for value-added co-products, from lignin or upgrading of sugars, to meet their stated goal of $3 minimum fuel selling price (MFSP)/gasoline gallon equivalent (gge). A strength of the Biochemical Conversion Technology Area is the development of clear price and performance targets. BETO put a stake in the ground and challenged the projects to demonstrate (verify) production of hydrocarbon fuels at $3.00/gge by 2022. The entire BETO research team in the field understands and acknowledges this goal, which is important as part of generating a cohesive program. The Review Panel was particularly impressed with how the National Renewable Energy Laboratory (NREL) team was working together and fully engaged with this target. It was interesting to observe that presentations from other national laboratories were well done, but did not display the same integration within the larger program.

The 2015 Review Panel expressed a concern that the singular focus on relatively near-term (2020/2022) goals might limit project diversity. With that constraint, the 2017 Panel felt that projects were undertaking a diverse array of approaches to achieve BETO goals. A variety of carbon sources, organisms, fuel molecules, and chemical co-products were covered by the various projects. Thus, BETO is not placing all of its bets on one process at this stage. There will necessarily be process down-selections to take those with the highest likelihood of success to the validation stage. Some projects, particularly at the national laboratories, may need to converge and pool efforts to deliver one or two processes to validation. There may be new FOAs in the future, pending appropriations; the Review Panel is not privy to the goals of those opportunities, but they may be more future-focused to refill the pipeline or to improve upon promising technologies that will not meet the 2020/2022 timeframe.

Strengths

The projects that ranked highest were generally core and consortia projects that have broad impact across BETO, and indeed, the industry at large. The impact of the core projects (i.e., facilities/resources including process integration, bench- and pilot-scale fermentation, and analytical and computational support) is program-wide. These projects develop and implement new standard procedures, making relevant benchmarking and comparison possible. In the case of Analytical Methods Development and Support, the impact extends industry-wide. The laboratory analytical procedures (LAPs) have been downloaded and quoted tens of thousands of times and provide a uniform basis for comparison of results. They support projects with reliable and reproducible data, which is not a trivial task. The entry of this team into the D3 Renewable Identification Number "pathway" arena with the U.S. Environmental Protection Agency is a good example of an area that desperately needs standardization. Analytical Methods Development and Support has the respect and reputation needed to deliver a solution that industry, government, and all concerned will accept. Biochemical Process Modeling and Simulation has specialized expertise in modeling and simulation, which supports multiple protein engineering and process development projects by targeting and guiding laboratory work to the areas with the highest probability of success.

The consortia are relatively new, and the Review Panel anticipates significant impact will be in the future (see further discussion in the "Synergies" section).

The renewed focus on making chemical products in parallel to fuel is critical and will play a major role in developing a strong biorefining industry. The Panel recognized the progress made in lignin conversion research, which is addressing BETO's focus on co-products as an economic necessity. The two most relevant lignin valorization projects were Lignin Utilization (see further discussion under "Innovation") and Synthetic Microorganisms To Enable Lignin-to-Fuel Conversion (see further discussion under "Commercialization"). The ultimate value will be determined by industry participation and acceptance, but it is entirely appropriate for the BETO portfolio to include and drive this research in these early stages. At least one panelist questioned the focus on adipic acid from lignin and encouraged flexibility, or even a multi-product stream (biorefinery model more like a petrochemical refinery).

The highest-ranking industrial presentation was Novozymes' enzyme engineering effort (SynTec—Synthetic Biology for Tailored Enzyme Cocktails), and Lygos' technology development (Design and Optimization of a Biochemical Production Platform with Biosensor-Guided Synthetic Evolution) for malonic acid production (see further discussion under "Commercialization"). These are good examples of BETO encouraging innovation and supporting pre-commercial development, respectively.

Several projects did a good job of presenting metrics, including titer, rate, and yield (TRY) targets and achievements—NREL's Targeted Microbial Development, Ohio State University's Engineering Clostridia for n-Butanol Production from Lignocellulosic Biomass and Carbon Dioxide (CO2), NREL's Lignin Utilization, and American Process Inc.'s Continuous Membrane Assisted IBE Fermentation from American Value-Added Pulping Cellulosic Sugars.

Weaknesses

The projects developing fungal strains, particularly filamentous fungi, could benefit from a working group, or a consortia-type organization, to share knowledge and experience. A more consolidated effort with a critical mass of expertise could help to move these efforts along more efficiently. This was also identified as a weakness by the 2015 Review Panel. The objectives of the filamentous fungi projects are different but the tools, strains, and know-how are highly related. The same may be said for the yeast projects. Both groups should keep in touch with the Agile Biomanufacturing Foundry (Agile BioFoundry) as they evaluate candidate host strains.

The Kiverdi project (Engineering Thermophiles To Produce Drop-in Fuels from Syngas) was organized as a nice collaboration with NREL, but the false starts related to host selection and the overall challenges of the project make it unlikely to deliver on BETO metrics. It was unclear to the Review Panel whether incubator projects such as this were subject to validation and held to the BETO $3/gge metric. The Panel expressed concern about the techno-economic suitability of the project, and some level of pre-project validation might have caught the initial issues related to unsuitability of the host strain. No target, revised target, or current metrics were presented, and the presenter was unable to state targets when queried. Some nice methods were ultimately developed, but this project is at a basic research stage.

The J. Craig Venter Institute project (Maximizing Multi-Enzyme Synergy in Biomass Degradation in Yeast) presented limited progress in a sun-setting project. The 2015 Review Panel recommended a departure from the planned consolidated bioprocessing (CBP) approach, which may have slowed progress. There were nice technological achievements, but the performance target was not met and the project is far from BETO metrics. The technology approach is similar to what Novozymes successfully demonstrated for screening in their BETO project reviewed in this same session. The project team is looking for future industrial partnership, which could help to validate the approach and provide needed feedback related to techno-economics.

Innovation

Although there is a long history of lignin research, the projects using lignin as a substrate for co-products are targeting the biggest challenge and opportunity in the lignocellulosic value chain. The 2015 Review Panel identified lignolytic enzyme research as an area requiring greater emphasis, and BETO responded. Some projects have taken more successful approaches than others, but this is still an exploratory stage in lignin upgrading R&D. The Lignin Utilization project was ranked highly and takes advantage of oxidative depolymerization, followed by biological conversion to a chemical intermediate. Reasonable progress is being made on mono-lignin compounds. Respecting the integrated nature of this work, the team is well-organized and collaborating with pretreatment, microbial development, and separations projects. There is also a significant analytical effort to characterize the milieu that is lignin. The Panel had some concerns about the choice of alkaline pretreated lignin (basically deacetylated lignin) as a new NREL standard substrate (compared with the historical diluted-acid pretreated corn stover lignin industry standard), but as there is no industry convergence at this stage, this material is acceptable for demonstration of possible opportunities.

The Renewable Carbon Fiber Consortium was highly ranked and considered to be an exciting new product opportunity in sugar upgrading, to bio-acrylonitrile. The product choice, techno-economic analysis (TEA), challenges, and approach were well-presented, and a balanced team of national laboratories, academics, and industry are participating in the project. The use of real biomass-derived sugars from Biochemtex and NREL hydrolysates is a strength of this program and demonstrates the use of actual biorefinery material. The Panel expressed some concerns regarding whether this ambitious project fits the BETO 2022 metric, but the Panel is very supportive of the work continuing.

More new project concepts might be developed with increased use of laboratory-directed R&D—congressionally authorized funds that the national laboratories have discretion to use on high-risk seed projects (sometimes BETO AOPs have evolved from laboratory-directed R&D projects), creating a path to develop sufficient data for some projects to enter the BETO Biochemical Conversion portfolio.

Synergies

The assembly of new subject-centered consortia is effectively taking advantage of technological synergies across the platform. The organization into consortia helps the national laboratories be more efficient in their research, pooling expertise and avoiding redundancy, while also encouraging a broader perspective on problem-solving across different processes and identification of common problems (particularly for the Separations Consortium). It also provides a central point of contact for industry, allowing companies to more easily find expertise across the national laboratories. The Review Panel expressed some concerns about coordination between geographically distant research groups, and the various consortia are taking appropriate steps to manage this challenge.

Some of the national laboratories clearly have core skills that can be used by industry—particularly the process modeling group and the analytical group at NREL, and the supervisory control and data acquisition (SCADA) effort at Pacific Northwest National Laboratory (PNNL). For these, outreach and dissemination are critical, with the Analytical Methods Development and Support project at NREL providing an excellent model for this in making standard methods available online.

The BETO program also has a number of groups well-equipped to handle the transition from laboratory work to several levels of bench- and large-scale operation. This is another strength and a valuable resource for demonstrating industrial utility. The program would be further strengthened if the coordination between the Advanced Biofuels Process Demonstration Unit (ABPDU) and Biochemical Process Pilot-Scale Integration

(NREL) was improved, as they seem to be operating independently. Impact could be enhanced by identifying a central point of contact to help define where a scale-up project might best be located.

This review included three filamentous fungi platform development projects at three national laboratories. Communication and coordination between these groups in a working group or consortium was identified as a potential area for improvement.

Focus

Technology Gaps

The 2015 Review Panel identified reactor design and aeration design as technological gaps that could use focused attention. These gaps remain in 2017, are still highly relevant, and would impact projects across the portfolio.

Standard Materials

Perhaps there could be more emphasis on the national laboratories (or even contract research organizations) producing generally applicable material like platform strains or software, with an emphasis on providing "open-source" material for the public domain. Examples include Analytical Methods Development and Support's LAPs and NREL's acid-pretreated corn stover, which became industry standards. Explicitly structuring some future FOAs to develop open-source materials might be a way to address this gap. The Agile BioFoundry is currently pursuing a licensing model, but the Review Panel would encourage BETO and the national laboratories to consider making the expression pipeline open source. For national laboratory projects, perhaps there should be a requirement for the project plan to explicitly identify deliverables that will be released for public use (strains, tools, reports, etc.).

Biorefinery Scenario

The renewed focus on making chemical products in parallel to fuel is critical and will play a major role in developing a strong biorefining industry. Within the program, however, the choice of adipic acid from lignin seems arbitrary. While it may be an excellent target, there are many structures that meet the selection criteria. It may be counterproductive and premature to pre-identify a specific structure before there is an understanding of the selective transformation of something as complex as lignin. It might make more sense to focus on lignin conversion as a broad technology. There has been excellent progress, but placing all bets on one structure seems limiting. It would be useful to see how the economics change if lignin is taken to compounds X and Y, more like a petrochemical refinery scenario.

Technology Communication

The BETO portfolio encompasses technology development that should be communicated beyond peer-reviewed publications and conferences. It could be useful for a national laboratory to give a full picture of successes and failures in a report, webinar, or newsletter rather than publish a journal article covering only positive results. Perhaps a plan for dissemination of information should be a requirement at the beginning of the project (with an opt-out for companies with confidentiality requirements).

Commercialization

Strengths

The sun-setting Lygos project, Design and Optimization of a Biochemical Production Platform with Biosensor-Guided Synthetic Evolution, is a good example of a combination of valid commercial target and development of core technology. This is the type of effort that is essential for BETO to reach its goal of fostering the

development of new technology capable of making a material difference to reliance on foreign oil and petrochemicals. The project was built around bio-malonic acid as a target (a DOE top-30 molecule) and enabled development of a novel biosensor for rapid screening. The approach was a combination of computational design, high-throughput screening, and bench-top fermentation, transitioning to scale-up at the ABPDU. Overall, this was a nicely organized project from bench-top to small-pilot operation using hydrolysate and capitalization of the ABPDU to demonstrate higher-volume malonic production. This small company is progressing towards commercialization and it seems to have made excellent use of BETO funds to develop a high-performing platform for strain generation and screening. The Review Panel did question the market size and whether TEAs were available but understands that cost data for a commercial venture are confidential.

The ABPDU provides scale-up and commercialization support for a variety of projects relevant to the bioeconomy. As a bioprocess research incubator, it is a one-stop shop that includes pretreatment, fermentation, recovery, catalysis, and analysis with a stated goal of one commercial outcome per year. One customer has commercialized a product, and three are in pre-commercial prototyping. The NREL bench- and pilot-scale laboratories also partner with companies and provide scale-up support and expertise. Communication and coordination between these facilities was identified as an area for improvement.

The Texas AgriLife project—Synthetic Microorganisms to Enable Lignin-to-Fuel Conversion—has very relevant alignment with BETO's mission of valorizing lignin. It is a nicely integrated process that offers routes to convert lignin. This sun-setting project achieved technical titer targets with real biorefinery waste, and it is a nice demonstration of omics-guided strain design for both laccase production and polyhydroxyalkanoate (PHA) production by Rhodococcus opacus. The work has attracted potential licensees.

Weaknesses

Quantitative targets, and progress toward those targets, were often difficult to ascertain from the presentations. Although the use of TEAs was mentioned in nearly every presentation, the results and implications were rarely shown. Commercial partners such as J. Craig Venter Institute, Kiverdi, and Amyris presented few TRY targets or SOT. A suggestion for commercial entities would be to present relative improvement targets, as Novozymes did. The Texas AgriLife project is one example in which the Review Panel struggled to get a good sense of where the project stands with regard to original goals, as well as how much of a gap lies between the current process and economic viability. Although it has attracted potential licensees and met titer targets, the relevance of the targets and economic viability of the project were not clear in the review. To date, the Panel is unaware of much PHA production by fermentation because of the cost (e.g., Metabolix efforts). The project team is aware of the history, and a commercialization partner, ICM, will likely enforce diligence in the TEA.

Recommendations

Recommendation 1: Increase Project Management Rigor

Improve Consistent Use of TEAs and Quantitative Tracking of Progress. The extent to which the projects used project management tools was variable. The Review Panel recommends more critical project management based on TEA-guided milestones and measurable progress toward goals. A more uniform tracking of SOT progress over time and against milestones would benefit both the reviewers and the project. There may be opportunities for BETO to strengthen the projects themselves by increasing the requirements for timelines, risk registers, responsibility assignment matrix (e.g., RACI – responsible, accountable, consulted, informed) charts, key performance indicators, milestones, stringent go/no-go decision points, or similar tools, to be shown clearly in the pre-

sentations and referenced in the regular check-in meetings. There is also an opportunity to be more explicit in dividing milestones from goals. For example, a milestone could be "complete testing of 15 new cellulase enzyme cocktails by Quarter 4," and then once could attach it to the broader goal, "achieve 100 g/L of glucose."

TEA usage has increased since previous reviews but was inconsistent. TEA should be seen as an essential tool for feeding back to the technology development team (and BETO) and modeling the impact that process changes have on the overall economics—but respecting that the rigor will be different for a technology readiness level (TRL) 2 project versus TRL 6. It seemed that some of the project TEAs were made to fit the target, rather than judged against it. It should also be useful to show how success in one or more of the TRY levers (increasing titer, rate, or yield) affects the TEA. Some projects were waiting for this or that accomplishment before conducting a TEA, but assumptions must have been included in the FOA proposal to show that the $3/gge target was achievable. BETO might provide a standard TEA table, for example, for TRY-related projects that could be tracked throughout the project. The Review Panel recognizes that one table will not fit all projects and all stages. Consistent use of TEAs and a desire to see progress tracked throughout the project cycle were also recommendations of the 2015 Review Panel.

The Review Panel expressed a concern that a few PIs are leading a lot of projects or sub-projects, and that even the most talented scientist may struggle to give sufficient time to each project unless there are management levels that allow this to work efficiently. Perhaps a check could be made at the funding time and periodically afterwards on the percentage of the project manager's time devoted to the project.

Consider Alternative Evaluation Process for Core Operations Teams at National Laboratories. Critical support groups (e.g., analytics, pilot plant, bench-scale validation, modeling, separation, etc.) should not have the same cycle as research projects for evaluation. The efforts of these core technology groups are extremely valuable to the BETO activities, as reflected in many high rankings. However, they are dependent on the efforts and research of "customer projects" to define and carry out their tasks, and they seem to have a limited number of projects of their own. Project start and end dates (e.g., 3-year project cycles) are irrelevant for core services. Using the same criteria for these groups as for research projects may not accurately represent their important contributions, and may indeed be an unnecessary distraction.

Explain Multiple FOAs Represented in Peer Review. There was some confusion amongst the Review Panel regarding which projects were funded by which FOA and whether they should be held to the MYPP 2022 MFSP metrics. An example was the Amyris project for farnesene production from lignocellulosic sugars, which has a very high cost target relative to BETO fuel goals for 2022, but is relevant for non-fuel markets. Further, although it is a logical next step to evolve the process from Brazilian cane sugar to domestic lignocellulosic sugar, the targets did not seem very rigorous given Amyris' experience producing farnesene from cane sugar and Renmatix delivering clean sugars. The project received some low relevance scores in a fuel context and an overall low ranking, even though the probability of success on the stated goals is high.

Recommendation 2: Continue To Support Consortia Organization in Specific Technology Areas

The potential of the consortia should be realized in the near future. The Separations Consortium and SCADA projects are addressing practical current industrial challenges. The Renewable Carbon Fibers Consortium is developing new routes to useful chemicals. The Agile BioFoundry seeks to deliver licensable host strains and design-build-test-learn pipeline enhancements. Such organization helps the national laboratories be more efficient in their research, pooling expertise and avoiding redundancy, while also encouraging a broader perspective on problem-solving across different processes and

identification of common problems. It also provides a central point of contact for industry, allowing companies to more easily find expertise across the national laboratories. Other research areas that could benefit from central steering and a critical mass of expertise are fungal strain development and lignin depolymerization.

Recommendation 3: Encourage Use of Industrial Advisory Boards and Partnerships

The consortia formed industry advisory boards, and a few of the individual national laboratory projects did as well. Some notable projects could have benefitted from guidance from someone with specific domain expertise, including Enzyme Engineering and Optimization (directed evolution of a cellulase in yeast), Biological Lignin Depolymerization, and the J. Craig Venter Institute project. When potential industrial partners are identified but decline to participate in funding opportunities as full partners, they may be receptive to an advisory role to help the project set realistic targets, obtain relevant materials, gain valuable insight, and envision practical process application.

BIOCHEMICAL CONVERSION R&D PROGRAMMATIC RESPONSE

Introduction/Overview

The program would like to thank the reviewers for their time and effort on the Panel as well as for their thoughtful recommendations. The reviewers identified the focus of all of the Biochemical Conversion projects on a $3/gge cost goal as a strength of the portfolio. BETO agrees that cost targets help to focus our R&D efforts on meaningful pathways with near-term impact. Additionally, the Panel noted that the 2015 reviewers identified the potential for a lack of project diversity as an area of concern if efforts were too focused on the 2022 cost target, but they felt that despite focus on the cost goal, project diversity was adequately maintained.

The Panel identified reactor design and aeration design as potential technology gaps. BETO has funded work on aeration design in the past but is moving the focus of the Biochemical Conversion Technology Area beyond aerated reactors, so this work will likely slow in the future. The reviewers also identified a need for standardized materials, including feedstocks, strains, and software, to be used by other laboratories as well as industry in a publicly available manner. BETO hopes to make strains available through the Agile BioFoundry in as freely available a manner as possible. The national laboratories already provide resources like cellulosic sugars to industrial and laboratory partners, but BETO will look into expanding this effort. The Review Panel commented that, although pursuing a strategy that relies on chemical production is the right path, it is critical to not get locked into one product like adipic acid too early. We agree with this recommendation and are pursuing a variety of example products.

The Panel's recommendation to continue pursuing lignin valorization as a core program strategy is appreciated, and BETO is expanding its lignin valorization work in FY 2018. BETO is also developing plans to work on a variety of lignin substrates, including acid pre-treated substrates, in addition to deacetylated and mechanically refined – enzymatically hydrolyzed substrates. The Panel also felt that renewed focus on making chemical products in parallel to fuels was the right direction for the program, particularly noting the lignin valorization projects. BETO plans to expand this work in the future given the critical role of lignin valorization in cost-competitive fuel production from biochemical pathways.

The following sections specifically address the top three recommendations from the Review Panel.

Recommendation 1: Increase Project Management Rigor

The program is in agreement with the reviewers that stronger metrics are needed to track progress across projects. We have made this an active area of focus since the 2015 Project Peer Review and, as the reviewers noted in the first section, we have made progress in this area, especially at the national laboratories. We will continue to work to create clear, quantitative goals across all projects, including competitive projects.

The reviewers recommended that BETO consider the commitment level of project PIs in the portfolio. BETO will monitor this and ask junior scientists to serve as PIs where appropriate in the portfolio to ensure adequate effort is given to all national laboratory projects by the PI of record. The national laboratory projects that serve as enabling capabilities and provide core services will also be considered for alternative evaluation, including the expanded use of joint milestones as recommended.

We also share the reviewers' concerns about a lack of consistent TEA methodology across projects and will work with the validation team to help standardize these to the extent it makes sense for a given product or pathway.

In addition, the Panel recommended validations and better alignment of project goals for all competitive projects, including incubator projects. BETO will work to address this in future funding opportunities and will work with the validation team on a tiered approach to validations of projects of different sizes. A concerted effort will also be made at the next Project Peer Review to allow the reviewers easy access to explanations of the goals of the different FOAs the Panel will evaluate.

Recommendation 2: Continue To Support Consortia Organization in Specific Technology Areas

The reviewers recommended continued support for the consortia structure pioneered after the 2015 Project Peer Review. Active consortia will continue to be refined and strengthened, and new consortia will be planned in areas such as fungal genomics, where appropriate given subject matter and resources. In addition, BETO intends to better coordinate management structures of consortia and encourage the sharing of best practices amongst the various efforts.

Recommendation 3: Encourage Use of Industrial Advisory Boards and Partnerships

Industrial advisory boards are being emphasized in all current and future consortia. Larger project areas will work to develop plans to better engage industry in FY 2018. We cannot force competitive projects to include industry advisory board input, but we will emphasize industrially-relevant reviewers in the review process.

The Panel also noted that the scale-up facilities that BETO operates are a valuable part of the portfolio and suggested that a coordinated effort for these facilities to engage with industry would be helpful. We agree that the ABPDU is an excellent resource, and we will continue to encourage them to partner with industry. Additional industrial engagement through the Agile BioFoundry with the ABPDU and other scale-up facilities is underway, and BETO will attempt to better highlight these capabilities through a request for information or other means going forward.

PRODUCTION OF HIGH-OIL, TRANSGENE FREE CAMELINA SATIVA PLANTS

(WBS #: 1.1.1.104)

Recipient:	Yield10 Bioscience
Principal Investigator:	Kristi Snell
Project Dates:	10/1/2015–9/30/2017
Project Category:	New
Project Type:	FY 2013—Incubator: DE-FOA-0000974
Total DOE Funding:	$1,996,598

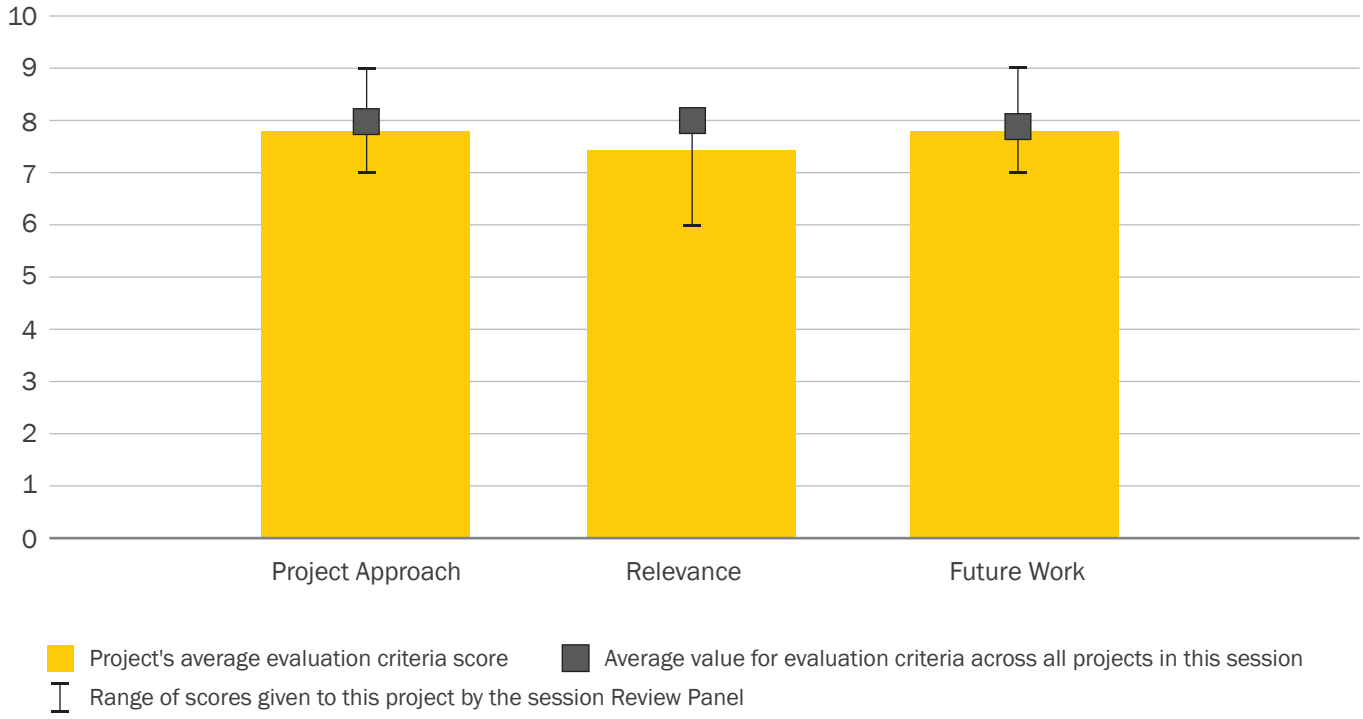
Project Description

The objective of this project is to develop a Camelina sativa feedstock with significantly increased seed yield and oil content to maximize oil yields per acre. Camelina is an oilseed crop with high potential as a bioenergy feedstock due to its high oil content and low inputs for cultivation. To accelerate market entry, a next-generation technology is being used to develop genetically modified organism–free plants that are expected to provide an expedited path through regulatory approval.

The program has two oil production benchmarks: (1) increase production of seed to 2,500 pounds/acre with seed containing 45% oil, and (2) increase production of seed to 3,500 pounds/acre with seed containing 60% oil. Hitting the first benchmark will significantly expand the potential of Camelina as a crop and will provide the profitability necessary to incentivize farmers to grow Camelina, resulting in the production of renewable feedstock for the biobased energy and chemical industries. The second benchmark (3,500 pounds of seed/acre with 60% oil content) will have a significant impact on the availability of renewable Camelina oil for the biodiesel and aviation fuel markets. Because the final plants produced in this program will not contain transgenic DNA, they are expected to have an expedited approval path

Weighted Project Score: 7.7

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



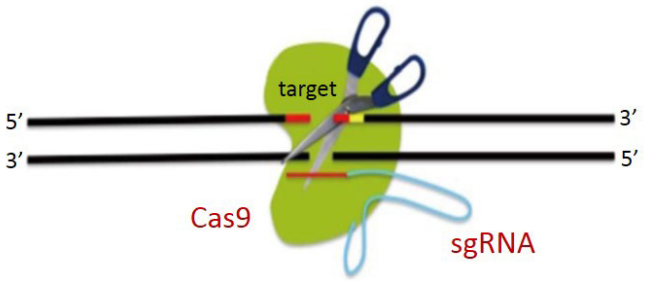


Photo Courtesy of Yield10 Bioscience

through regulatory agencies, significantly reducing costs and time to commercialization. The technology developed in this program will also be relevant to increasing seed and oil yield in related oilseed crops, such as canola.

Overall Impressions

- In a general sense, there would seem to be value in developing an oilseed crop specifically for biofuels, removing concerns about edible oil characteristics and focusing purely on lipid yields. Also, removing the concern about lines of bioenergy oilseed crops crossing into lines for oilseed crops for food production seems significant. It would have been good to see the agronomic benefits of Camelina over other crops explained better in the presentation—it may not be a food crop, but if it competes for the same land as a food crop, there is still a food versus fuel debate. Does it show higher yields than competing oilseed crops or other crops on marginal or drought-prone land? How do the inputs compare?

  The approach to identification of specific genetic targets was not shown the presentation. This may be due to the proprietary nature of the project, but it makes it difficult to assess how likely the project is to succeed. All we may glean from the presentation is that the project team has made some genetic changes using CRISPR/Cas9 and the agrobacterium system. We are left with the question of how far can targeted gene disruption alone go to improve oil yields in the plant?

- The PIs are carrying out a straightforward effort to develop Camelina as a new bioenergy crop and supply for the biorefinery. The scientific approaches are solid, but the project faces a considerable challenge in convincing farmers to grow a crop at levels sufficient to make a difference in the fuel market. Much more detail about the path to commercialization would strengthen this project: where will the 13 million acres be located? What proof is there that farmers would actually switch to Camelina?

- The goal is to develop Camelina sativa plants with higher oil yield, with no foreign genes so it could be easier to get through regulatory process. Better oil yield will make the cost low enough to provide enough farmer profit to make it worth planting. Plant engineering is slow, but the team has made good progress so far and appears to have a solid metabolic engineering approach. They have also advanced the CRISPR/Cas9 approach in plants, which could impact the larger plant genetics community. Targets are reasonably set. Bigger than the technical challenge may be getting the improved seed into the market. It is not clear if the team has good channels to the market and distribution.

- Camelina oil and seed yield improvements should be valuable to current Camelina growers and will potentially expand the feedstock supply for bioenergy to new growers. Camelina has the advantage of being a low-input, non-food-competing crop.

- The project is well-structured and managed. There is good collaboration between North Carolina State and Yield10 to bring this novel genome-editing, technology-driven improvement of Camelina as an energy crop. Yield10 will continue this project after the incubation period to get the expected 3,500 pounds/acre seeds with high oil content, which is needed to enable the farmer to grow camelina with more profit in a dual season approach where applicable. The crop doesn't compete on arable land or divert resources from the food chain and, as such, fits very well with BETO's mission; if successful, it will have overall positive societal impact.

PI Response to Reviewer Comments

- Commercialization path: Recognizing the highly-concentrated nature of the seed business, Yield10 does not plan to become a seed company. Instead, the company plans to fill an innovation gap in the agricultural biotechnology space due to (1) reduced investment in basic R&D from ongoing agricultural sector consolidation and restructuring, and (2) the urgent need for new technology approaches. Yield10's role is to discover, optimize, and translate its yield trait innovations into crops to demonstrate their economic value to the growers and seed companies. We are essentially in the business of creating high-value assets in the form of proprietary yield technologies and de-risking them by progressing along the commercial development process to complete multi-year field trials. A third-party agricultural company would then either license or acquire rights to Yield10's technology for commercialization.

  We have, however, outlined a three-phase commercialization plan that could be implemented at a low capital cost to commercialize the BETO lines. This plan would be used to demonstrate the value proposition of the improved Camelina seed lines necessary for investment in larger-scale seed-crushing infrastructure.

  - In Phase 1, seed from 10,000 acres from contract growth arrangements with farmers would be generated to supply feedstock for an entry point to a U.S. commercial business (see table below). Contract growth on 10,000 acres with a line producing the program intermediate target yield (2,500 pounds/acre and 45% oil) can be initiated with 100 farmers, who will each dedicate 100 acres (see table below). Interested crushing, biodiesel, and feed companies will be identified and off-take agreements negotiated for sale of the resulting oil and meal. This will allow a quick, practical start to the business with proper distribution channels already in place.
  - In Phase 2, Camelina production would be expanded to 50,000 acres, with off-take agreements negotiated for sale of the resulting oil and meal. The results from the Phase 2 operations would be used to attract financing for a U.S.-based facility.
  - In Phase 3, a dedicated oilseed-crushing plant would be constructed with the capacity to process seed grown on 100,000 acres. This is enough oil to produce approximately 12,418,830 gallons of biodiesel (conversion factor of 7.7 pounds of oil per gallon of biodiesel, based on generalized information for canola oil). Money for constructing this initial plant could be obtained through financing or through a partnership or joint development agreement with a larger company.

Please refer to table below, which illustrates the production potential of this three-phase commercialization plan.

	Base Case (40% oil, 1,500 pounds/acre)	Intermediate Target (45% oil, 2,500 pounds/acre)	Final Program Target (60% oil, 3,500 pounds/acre)
Acres	13,100,000	13,100,000	13,100,000
Seed harvested (tons)	9,825,000	16,375,000	22,925,000
Oil (tons)	3,340,500	6,263,440	11,691,750
Biodiesel (gallons)*	867,662,340	1,626,866,880	3,036,818,180
Fold increase	1	1.87	3.50

Calculations assume use of the intermediate target line (45% seed oil; 2,500 pounds of seed per acre).

- Crushing capacity assumes 300 days/year plant operating schedule.
- Yields assume 85% recovery of oil and meal from crushing and extraction.
- Assumptions for Camelina oil, meal, and seed prices are based on March 2014 canola prices: Camelina oil $0.40/pound (canola oil $0.43/pound); Camelina meal $0.10/pound (canola meal, $0.18/pound).
- Farmgate price for Camelina seed is $0.15/pound (canola farmgate $0.22/pound).
- Crushing and operating costs of crushing facility are $40/ton of seed.

BIOCHEMICAL PLATFORM ANALYSIS PROJECT

(WBS#: 2.1.0.100)

Project Description

This project performs TEA to support and guide Biochemical Conversion Technology Area's R&D efforts through using process and economic models. These models translate key process parameters into overall economics to set future R&D targets and track performance progress against those targets. BETO uses the outcomes of integrated TEA modeling to guide program plans, as do other NREL partner projects to quantify the impact of research on key technology barriers.

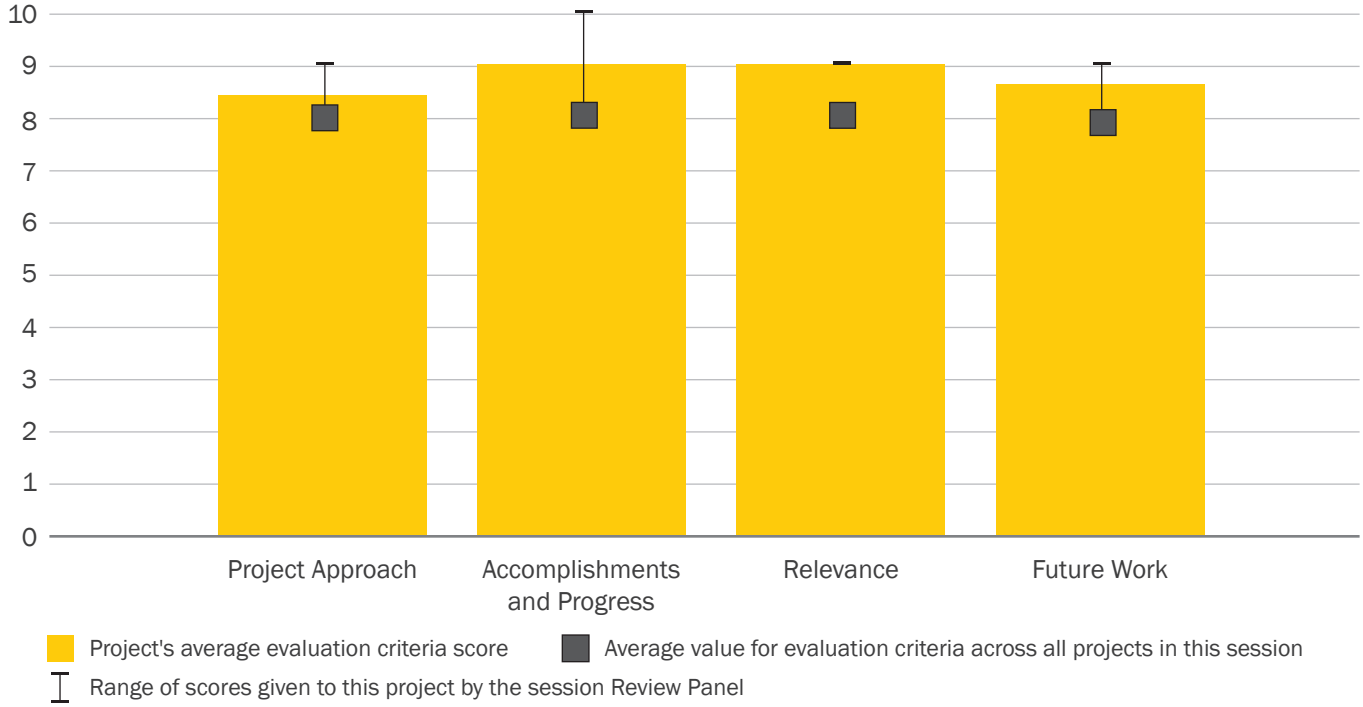
This work is highly relevant to BETO's program goals in that "bottom-up" conceptual modeling conducted under the project serves as a basis for understanding the technical feasibility to meet "top-down" program cost targets. Our TEA models may be leveraged to direct R&D towards the most economically impactful priorities by providing a framework to translate technical performance to cost reductions within a biorefinery. These efforts help to maximize efficiency of research funding and ultimately support the goal of demonstrating $3/gge fuel cost targets by 2022.

This analysis project has made significant achievements since the 2015 Project Peer Review, including creating new TEA models to highlight the economic potential and R&D challenges for new pathway concepts to

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Ryan Davis
Project Dates:	7/22/2002–9/30/2017
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$750,000
DOE Funding FY 2015:	$750,000
DOE Funding FY 2016:	$750,000
DOE Funding FY 2017:	$750,000

Weighted Project Score: 8.8

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



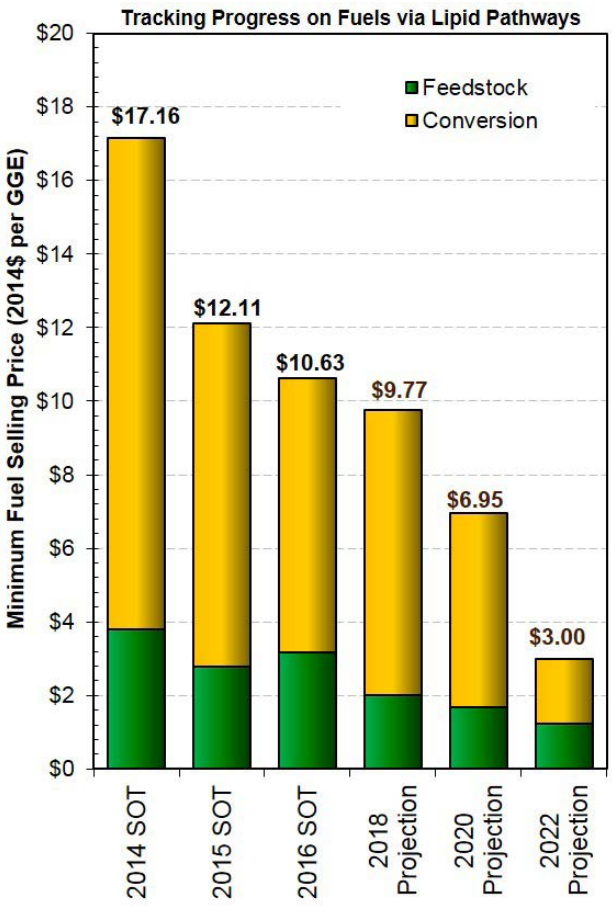


Photo Courtesy of National Renewable Energy Laboratory

ultimately achieve 2022 program targets. This work demonstrated the ability for a range of pathway options to achieve $3/gge, contingent upon the ability to utilize lignin for conversion to value-added co-products.

Overall Impressions

- The project is crucial to the success of the other NREL projects. Cost is often the final determinant in whether particular processes will be developed further. Providing good-quality models for processes early on will make the development work of those projects more relevant and help guide choices in where to invest time and effort experimentally.

  The presentation was well put together and logical, and it came through strongly that the modeling team is well-versed in the required methodologies and is paying attention to the requirements of the other projects. Two important aspects of the project will be (1) the interaction with the individual NREL projects (to support ongoing decision making, such as tornado plots to identify higher-value targets for cost reduction), and (2) the value provided by the project to a wider audience of stakeholders. Beyond publishing models, it may be worthwhile for the team to consider ways to help companies or other national laboratories improve their own modeling capabilities, through workshops and provision of tools. Another aspect to consider could be increasing the bandwidth of the team to allow it to provide a fee-for-service offering on similar modeling methods to other BETO funding recipients or outside companies, in a similar way to ABPDU.

  A further way to help the NREL groups would be to provide a simplified version of the process cost model (e.g., a "ready reckoner" in Excel) that allows process parameters and results from experiments to be input, and then final costs to be extracted. This offers the benefit of allowing researchers to play with the numbers without requiring time from the modeling team, and it even allows researchers to use cost as the metric for modeling a response surface when varying process conditions in design of experiments.

- TEA and platform analysis is a strength of the program, as it adds credibility to BETO's stated goal of transferring the program's work to industrial stakeholders. Partnerships with industry will not develop without a compelling economic justification, and the program is doing an excellent job of providing these data and making sure that the team members understand the goals as research directions are chosen. Presentation of this activity could be clarified with a better description of the interplay between the many moving parts of the program (MYPP, SOT, AOP, FOA, researchers, and process engineers). They all interact and are all important, but clarification would give the outsider a better idea of program organization.

- The Biochemical Platform Analysis project is an essential component to meeting the objectives in the MYPP and essentially serves as the cornerstone to all other BETO projects. It is intended to provide high-level guidance on BETO's project portfolio, selecting the best fuel/co-product scenario, process design, and technical target metrics. The team has done a good job at this, using rigorous models and thorough data analysis. It would be helpful to understand exactly how the co-bioproduct target molecules were chosen. It seems that products with higher value and/or larger market could be identified.
- The use of TEA analysis in project management increased dramatically from 2013 to 2015 to 2017. Modeling and design cases are now key tools in tracking progress, assessing feasibility, scalability, sustainability, and economics. This platform is closely integrated with the R&D projects, particularly NREL. Communication, public transparent models, and design cases with external stakeholders are also an important role of this team, providing a common language and reality checks with industry. This team should also be involved in the continual analysis of $3/gge reduction as a relevant target.
- Overall, this is a great project with the right infrastructure to accomplish its goals. The project can benefit from benchmarking its assumptions more critically with industry-relevant metrics where possible—perhaps through joint projects where better understanding of the cost structure can be shared.

PI Response to Reviewer Comments

- We thank the reviewers for their positive feedback in recognizing the impact of this project for BETO and the utility in guiding R&D priorities for NREL and the community. We do offer a number of different collaboration/"fee-for-service" mechanisms for partners seeking to leverage our TEA capabilities, and we have worked with numerous industry and academic groups over recent years to provide TEA/life-cycle analysis/process modeling support. We also participate in various partnership outreach functions and have hosted visitors from industry, academia, and other national laboratories seeking to work with our TEA modeling group to better understand TEA practice. Additionally, we have made a number of our models publicly available and are working to publish others once they have been properly refined, vetted, and automated for usability.

  We support the notion of exploring more simplified TEA approaches for less-developed concepts and have recently begun to act on this feedback through several mechanisms. In terms of "tools" for quicker analysis, within NREL's TEA group (with support and input from this project) we have developed a high-level qualitative framework tool to help guide R&D thinking and work prioritization. The tool is focused on identifying potential benefits and challenges for a particular concept with respect to process complexity and expected yields (primary drivers on MFSP), as well as knowns/unknowns required to run a more detailed TEA. Additionally, (also with collaboration from this project), NREL's TEA team has begun to develop a "quick turn-around analysis" tool, which takes this a step further to provide cash-flow and MFSP estimates for a process of interest, given inputs for processing costs and yields, without necessitating the use of a full Aspen Plus process simulation (although we stress the latter is still important in tracking mass and energy balances to reasonably quantify those metrics for new concepts). For novel concepts, which do not have precedent from a similar TEA pathway, our group has the capability to perform preliminary "back of the envelope" calculations, even with Aspen Plus, relatively quickly, given our proficiency in that software and preference to maintain thermodynamic rigor, which can have a large influence on overall yield/cost results.

  Regarding the comment on selection of specific co-products, the primary intent of our TEA work in that respect has been to quantitatively demon-

strate the benefits that may be gained by introducing co-products as a means to reduce fuel costs and ultimately enable economic viability in a conceptual biorefinery. To date, we have approached this by reflecting co-product molecules that have been the subject of internal NREL research focus (previously succinic acid from sugars and more recently adipic acid from lignin) as representative examples to demonstrate proof-of-concept for commercially relevant high-value bioproducts, which do generally have high market volumes or potential to produce derivative products with high market volumes. This forms a basis upon which industry may build in the future for similar multi-fuel/product biorefinery concepts, recognizing that biorefineries on a national scale would target many different co-product opportunities based on the market drivers at the time.

DEVELOPING THERMOASCUS AURANTIACUS AS A THERMOPHILIC FUNGAL PLATFORM FOR INDUSTRIAL PRODUCTION OF CELLULASES

(WBS#: 2.2.3.102)

Recipient:	Lawrence Berkeley National Laboratory
Principal Investigator:	Steve Singer
Project Dates:	10/1/2014–9/30/2016
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$150,000
DOE Funding FY 2016:	$540,000
DOE Funding FY 2017:	$540,000

Project Description

The project objective is to develop a thermophilic fungal platform for cellulase production. Cellulases remain a significant portion of the projected cost of producing sugars from plant biomass. Reducing the cost of enzymatic hydrolysis depends on identifying more efficient enzyme preparations and hydrolysis parameters that enable cost-effective release of sugars. Thermostable cellulase mixtures that perform at higher temperatures will enable the use of high temperatures and shorter reaction times for saccharification, allowing for utilization of waste heat, lowering viscosity, and overcoming end-product inhibition.

In this project, the thermophilic fungus, Thermoascus aurantiacus, has been developed as a platform for cellulase production. Cultivations up to 20 L at ABP-DU produced high titers of cellulases using xylose and hemicellulosic hydrolysate as inducers. These cellulases demonstrated comparable performance in saccharifications of dilute acid and base-pretreated corn stover compared to commercial enzymatic mixtures. The T. aurantiacus cellulases were capable of maintaining that performance at higher temperatures than commercial

Weighted Project Score: 7.6

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

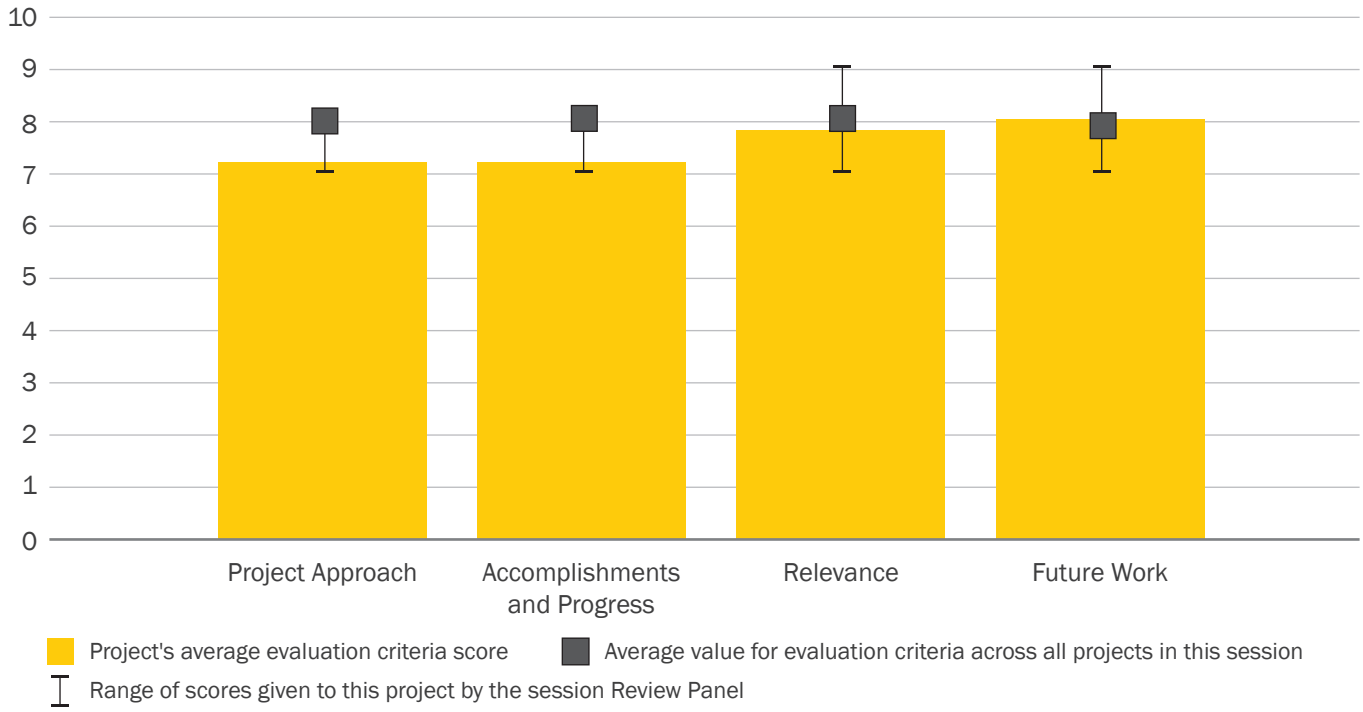


Photo Courtesy of Lawrence Berkeley National Laboratory

enzymes. A genetic system for targeted gene deletions was developed using Agrobacterium-mediated transformations. These gene deletions will serve as the basis for a hyperproduction strains that may be deployed in biorefineries where the byproducts of biomass pretreatment will be used to produce cellulases.

Overall Impressions

- Overall, I like the scope of the project a lot. Providing a thermostable enzyme mixture as a further option to industry, a higher-temperature protein production system, and the possibility of producing a full cocktail on a C5 hydrolysate stream all represent significant benefits to the cost of cellulosic processes.

  There is a lot for the team to do to move this project forward, but it has a lot of potential. I would ask the BETO team to look again at the decision to pause funding for the project. Although this represents only the start of a long-term project, it does have the potential to make a step-change in the process economics. The long-term nature of developing a novel fungal system from scratch means that this is often hard to push this forward in industrial companies, and proving out the first part of the technology and perhaps releasing platform strains to industry would allow the technology to bridge that gap.

- The project appears to have identified an organism that operates at higher temperatures and is able to carry out hydrolysis of actual biorefinery hydrolysate. The protein production levels appear to the lower than needed, but the team has a plan in place to address that deficiency. However, the project is paused, so future work is not yet certain.

- The objective of this program is to develop a cellulase production organism that can leverage the C5 stream for onsite production and also enable high-temperature hydrolysis. The team has identified a promising host strain that accomplishes both these objectives. This impacts two important aspects of the MYPP: improved enzymes and utilization of C5 stream. However, more thorough TEA is needed to demonstrate that this use of C5 has a significant cost impact, as well as what the enzyme titer has to be. Also, I don't have a good feeling as to how close this is to being technically ready for commercialization.

- The discovery of C5 induction of cellulose production is very interesting. The high-temperature growth (and protein production?) of T. aurantiacus and the high-temperature hydrolysis performance of the enzymes have potential use. Developing this new strain for commercially relevant, scalable protein production has numerous technical challenges, including a robust fermentation process and high protein titer. A common challenge with high-temperature enzyme cocktails is that even at their optimum, they yield lower sugar titers than CTec2 (Novozymes Cellic CTec2, a commercial enzymes package for cellulosic ethanol production, launched in 2010) at its optimum, and the state-of-the-art has moved well beyond CTec2.

- The project is aiming at a needed BETO goal of developing a fungal expression system to lower enzymes production costs. Technically, the project manager is encouraged to partner even more with other fungal development projects in NREL as this can accelerate the team's R&D achievements. I would also

encourage BETO to consider a consolidated effort on fungal strains development programs. The project team has good overall understanding of the biology and developed good tools for this unique thermophilic fungal strain. It is hard to evaluate, though, how economically viable this will be concerning the short-term objectives of BETO's 2022 goals.

A thorough TEA and realistic timeframe to translate this to industrial partners should be considered. The interest from enzyme companies is understood and appreciated, but it is not clear if they will be willing to switch to such a production host for onsite production as suggested. This angle, if it can be strengthened through better economic justification, can help the project with more momentum and funding in the future. The use of C5 sugars to produce enzymes versus use for product production is good idea if the TEA merits such direction.

PI Response to Reviewer Comments

- TWe thank the reviewers for the positive comments about the progress on the project and potential for this work in the future. We are confident this early-stage work has provided the tools to rapidly increase protein titer, bioreactor performance, and genetic tractability of T. aurantiacus. Preliminary saccharification tests have established that the T. aurantiacus enzymes are competitive with enzymes from commercial mixtures on biofuel-industry relevant substrates, and this is before any optimization has been performed on the T. aurantiacus enzymatic mixture. The observation of C5 induction provides a unique way to make T. aurantiacus a platform for onsite enzyme production and will also be a driver of fundamental research to understand regulatory mechanisms for protein production in a thermophilic fungal model organism.

LIGNOCELLULOSE CONVERSION TO HYDROCARBON FUELS - DECONSTRUCTION

(WBS#: 2.2.3.105)

Recipient:	Pacific Northwest National Laboratory
Principal Investigator:	Mike Lilga
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$0
DOE Funding FY 2017:	$200,000

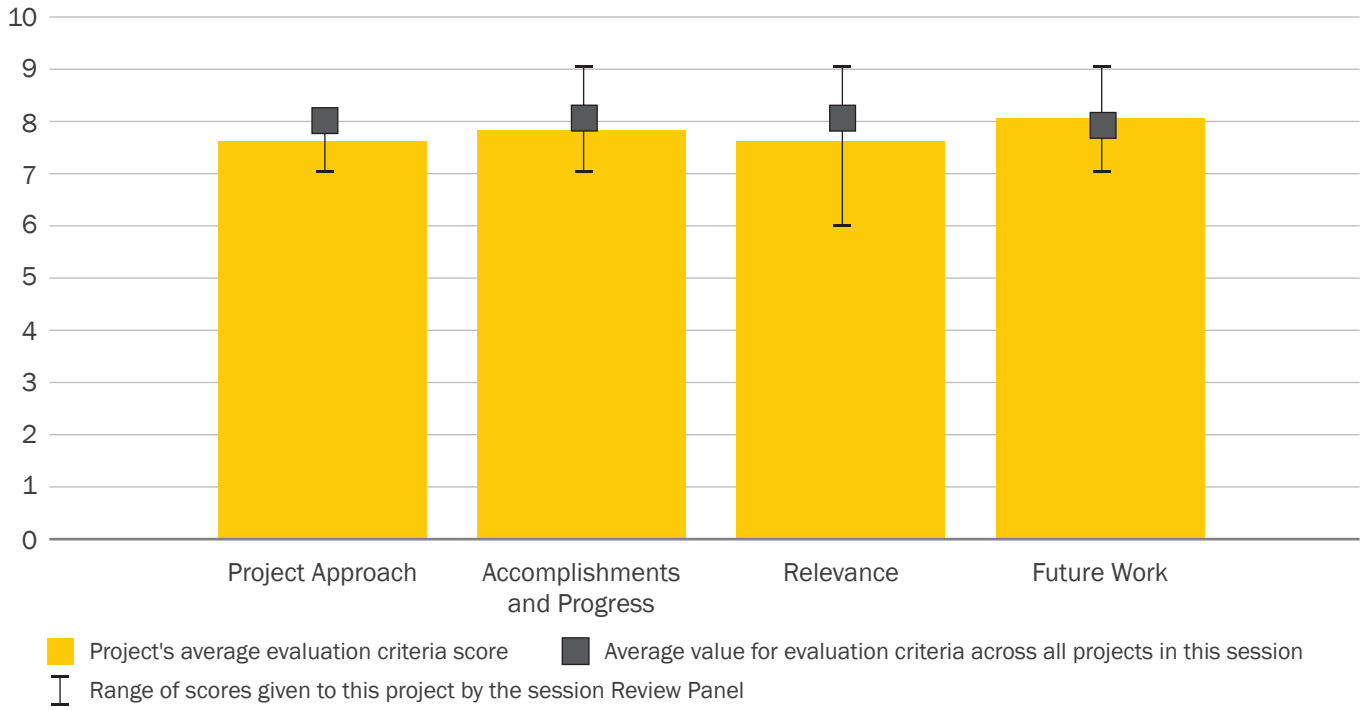
Project Description

Catalytic and biochemical processing of lignocellulosic sugars and sugar-derived intermediates potentially offer advantages over high-temperature pathways by allowing milder processing and by providing routes to open-chain fuel components not readily available by other means. Current deconstruction technologies, however, do not reduce ash content. Ash fouls catalysts and scales reactors and is one of the major issues that still needs resolution. Organic impurities inhibit fermentations.

The objective of this project is to develop transferable technologies to produce low-ash sugars and sugar-derived intermediates from woody, herbaceous, or other lignocellulosic biomass that can be used in biologic or catalytic processes for producing distillate-range hydrocarbon fuels, fuel blendstocks, and chemical products. Development of a new technology using biphasic deconstruction media in batch or flow reactors provides options for producing sugars and sugar oligomers or sugar-derived furan intermediates. The deconstruction technology does not require neutralization, minimizing waste, and can be recovered for reuse. Significantly, low-ash products, possibly suitable for catalytic processing, are produced. This work addresses pretreatment and deconstruction barriers by providing clean streams for

Weighted Project Score: 7.8

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



upgrading. Diverse downstream processing options are enabled, increasing the probability that new economical routes to fuels will be developed.

Overall Impressions

- The project looks interesting in producing some novel routes to deconstruction and generation of intermediates that would provide an alternative to current dilute acid/enzyme hydrolysis/fermentation to product routes.
- In such cases where there are solvents and catalysts to be recycled in the presence of biomass (e.g., the xylene/nonanoic acid combination), it would be good to have working TEA models set up, where targets for percentage recovery of these components can be assessed.
- The project is a straightforward and novel approach to biomass separation that gives good yields of sugars with low impurities. This makes the process a potential solvent-based catalyst for biomass deconstruction in the biorefinery. More details on the ability to recover and reuse solvent in these systems will strengthen understanding of this project as it is considered for larger-scale operation and testing.
- This program intends to develop two new biomass deconstruction methods that result in cleaner sugar (or sugar derivative) streams than the current dilute acid approach. Both methods have shown promising progress, but it is not clear where performance stands relative to where it needs to be. Will it require $1 million, $10 million, or $100 million worth of R&D before it becomes commercial? More rigorous quantitative metrics should be set for the future. Also, the team should partner with downstream projects (like fermentation) to see how the resulting sugars perform.
- Developing a new deconstruction technology to provide low ash and clean sugars is potentially transformative, expanding downstream processing and product opportunities. The team has made good progress demonstrating that different process designs favor either sugar or furan-type products. Reasonable cost is critical to implementation, so scaling and TEA are important future work. This may be a platform technology, but for BETO, it's important to identify a target process and demonstrate integration and <$3/gge.
- The project made some nice progress demonstrating how to potentially reach and align the TEA-guided solutions to reality checks in the laboratory. TEA results suggest that the project's ability to reach commercial reality should be evaluated. The project team is advised to use SMART (specific, measurable, achievable, relevant, timely) goals with critical metrics to measure progress toward goals to judge where the project is benchmarked to the TEA goals. Technically the program made good findings regarding the particle size being ineffective, direct one pot reaction to furans, no need for neutralization, and ability to separate oligomers. These are all good achievements in the right direction.

PI Response to Reviewer Comments

- Thank you for the supportive comments. We also believe the technology is novel with many potential benefits, including solvent recyclability, ash removal, and the ability to choose operation conditions to produce either sugar oligomers or sugar-derived intermediates, such as furans.

  TEA will be conducted throughout process development to guide research and benchmark against BETO goals and other technologies. To date, the only TEA comparison we've made is to the NREL case, which was shown in the presentation. The overall yield is most important to process economics. Sugar yield in the deconstruction portion of the process can be increased by a de-lignification pretreatment step, which we hope to conduct this fiscal year (FY 2017). The efficiency of solvent recovery

is also an important input to future TEAs. At this time, we have not evaluated solvent recovery and the degree of make-up that might be needed.

In addition, the amount of water used in deconstruction is an important factor in process economics. While we typically run at about a 1:1 H_2O: organic volume ratio, we have also explored ratios as low as 0.14:1 and 0.018:1. At 160°C, 21.5 mL H_2O/150 mL xylene (0.14 vol. ratio) gave 27% weight reduction—the same as when using 100 mL H2O/150 mL xylene (0.67 vol. ratio), which gave a 24% weight reduction. Process and economic improvements using less water will be investigated in future work.

Future efforts will be directed at improving economics, as assessed by TEA, and moving the process to market. Tech-to-market is certainly a critical element of moving this technology forward. The deconstruction process being developed seeks to generate polysaccharide or sugar-derived intermediate streams that have low ash. Such feeds enable diverse downstream processing options, increasing the probability that new economical routes to fuels will be developed.

BIOMASS CONVERSION TO ACRYLONITRILE MONOMER-PRECURSOR FOR PRODUCTION OF CARBON FIBERS

(WBS#: 2.3.1.200)

Recipient:	Southern Research Institute
Principal Investigator:	Amit Goyal
Project Dates:	1/1/2015–4/30/2018
Project Category:	Ongoing
Project Type:	FY 2013 - Carbon Fiber: DE-FOA-0000996
Total DOE Funding:	$5,981,713

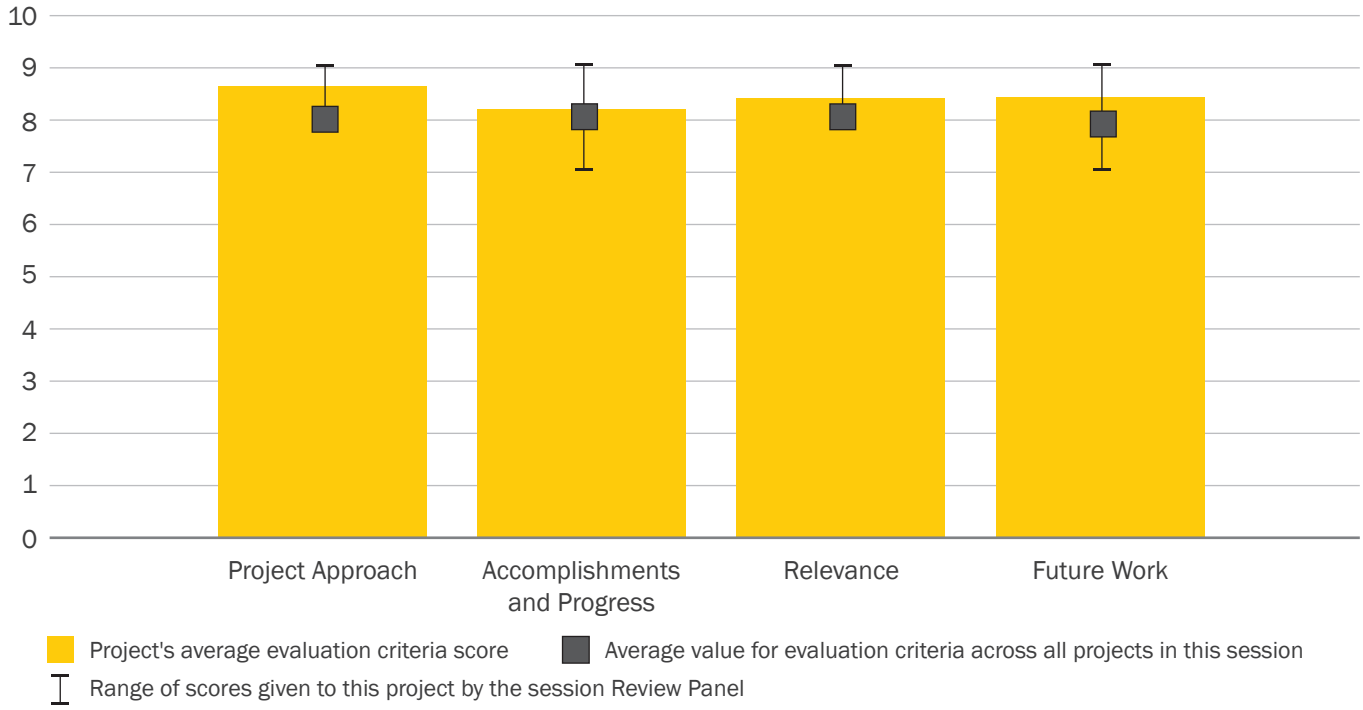
Project Description

Polyacrylonitrile-based, lightweight, high-strength carbon fibers are receiving great interest from THE automotive industry, particularly in their bid to improve fuel efficiency of vehicles (car weight reduced by 50% improves fuel efficiency by 35%). However, widespread application of carbon fibers is presently deterred by THE high manufacturing cost (>$10/pound). Ninety percent of the world's carbon fibers are polyacrylonitrile-based, derived from acrylonitrile (ACN) monomers—commercially produced from petroleum-based feedstock (e.g., propylene). Propylene prices are volatile, and its production is decreasing in the United States.

Alternative feedstocks that are available at scale, commercially viable, have a sustainable conversion process, and produce a high-purity product are desired in order to effectively reduce the cost of ACN to reach less than $1/pound. Southern Research Institute, in cooperation with BETO, is using widely available non-food, biomass-derived sugars as raw materials at mild conditions to produce ACN (the resulting product is referred to as B2ACN). The process consists of multiple catalytic reaction steps, including hydrocracking, dehydration, and ammoxidation. In the Phase I study, several novel, high-performance catalysts were developed with an overall recoverable product yield of 35%–40% and

Weighted Project Score: 8.4

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



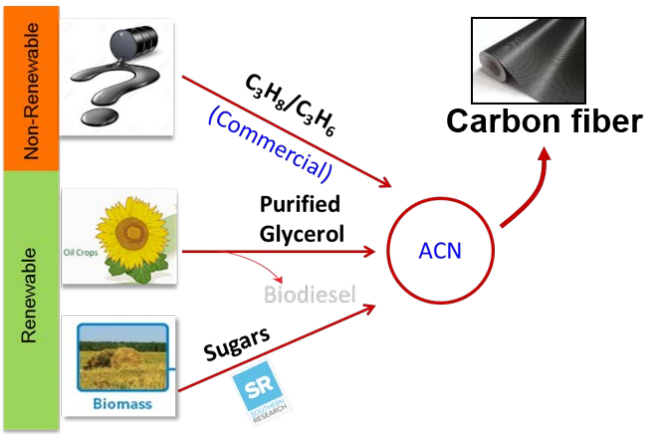


Photo Courtesy of Southern Research Institute

carbon recovery of 60%–80%. Based on experimental results and preliminary TEA/life-cycle analysis, we project significantly reduced cost (15%–22%) and greenhouse gas emissions (~37%) compared to conventional ACN processes.

Overall Impressions

- The project team appears to have produced a commercially attractive catalytic system to generate ACN from biomass sugars. The team looks to be poised to go forward and test out the system at larger scale and work through issues that may appear during continuous operation. Obviously, there will be a lot more work to do, but the organized approach has paid off so far, and the project looks to be on track.
- The team has developed an interesting catalytic process for the conversion of sugars to acrolein through three catalytic steps. The production costs for ACN are currently projected to meet BETO goals, but the overall process is burdened with a number of side products that will reduce industrial utility and interest. A better plan for dealing with these materials, or improvement in process selectivity, will be necessary before this process can be used for ACN production. The PIs and BETO may want to consider merging this project with the Renewable Carbon Fiber Consortium since new partnerships and collaborations may result in a suite of useful ACN approaches that might not be developed separately.
- The current ACN production from propylene is a complex and hard-to-control reaction, with a toxic byproduct. This program will provide a renewable route using a three-step catalytic process. The team has developed a novel catalyst for the first step, production of glycerol from mixed sugars, and solved some challenges around feedstock purity and a catalytic byproduct. They have achieved all metrics for Phase I, and TEA indicates favorable economics if ultimate targets are met. Critical issues that still need to be addressed are scale-up and the effect of feedstock variability. Also, it would be good to address how the economics and feasibility of this project compare to alternate approaches to renewable ACN (e.g., the biological catalytic route of the Renewable Carbon Fiber Consortium).
- This project has nicely outlined project management and metrics. The developed catalysts and process appear to be on track to meet the ACN production cost of $1/pound. The product still needs to be tested for quality. There are interested commercial ACN manufacturers. Phase II approval is pending, but it looks promising. Note that this chemical conversion project was reviewed in the bioconversion session.
- This is a very good demonstration of dedicated team effort that is well-aligned with industrial partners to meet project goals. This is a highly skilled chemical engineering group with a can-do attitude and good collaboration for achievement mentality. The project is a good demonstration of biomass conversion potential to carbon fiber with good economical and sustainability impacts. The project team is advised to try out other lignocellulosic sugar streams that may be more advanced in their commercialization road to de-risk this end.

PI Response to Reviewer Comments

- We are very much looking forward to addressing the side streams more rigorously in Phase II.

  We will definitely try out more lignocellulosic sugar streams if scope and budget permit.

UPGRADING LIGNIN-CONTAINING BIOREFINERY RESIDUES FOR BIOPLASTICS

(WBS#: 2.3.1.206)

Recipient:	Texas A&M University
Principal Investigator:	Joshua Yuan
Project Dates:	7/1/2016–6/30/2019
Project Category:	New
Project Type:	FY 2013—Incubator: DE-FOA-0000974
Total DOE Funding:	$2,499,993

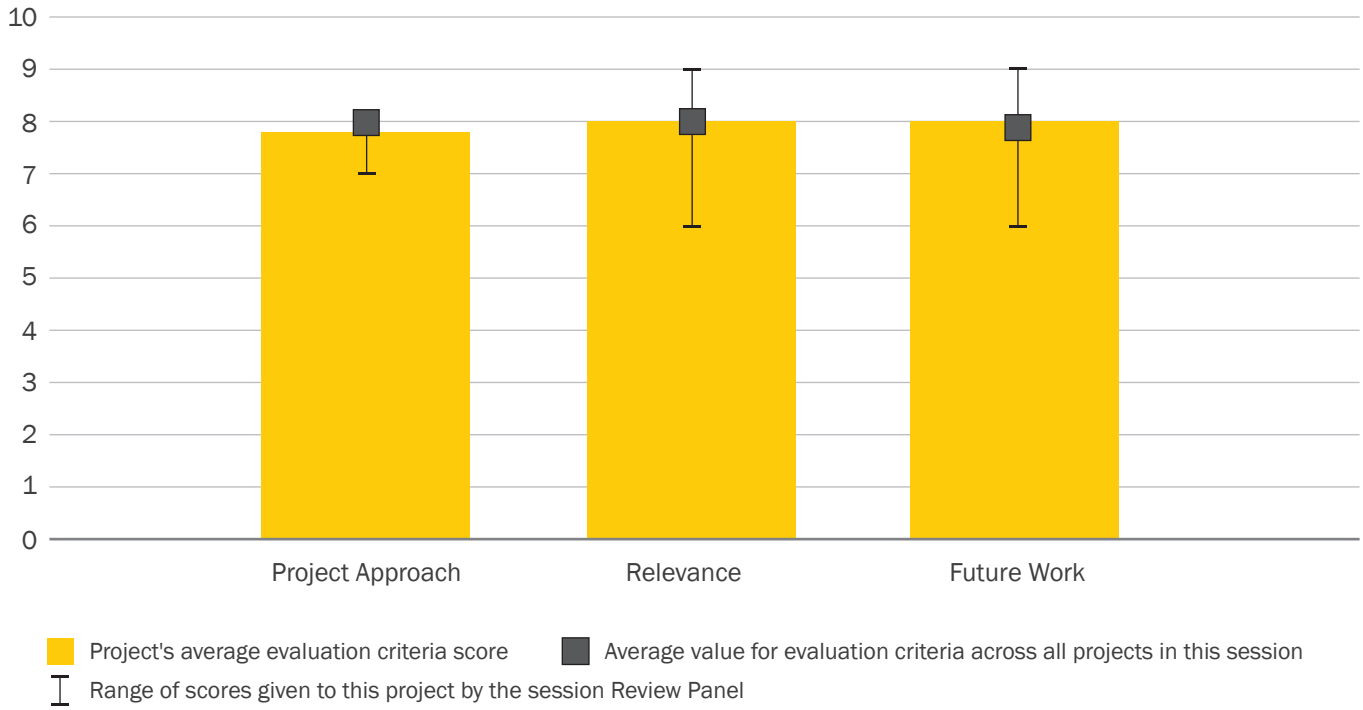
Project Description

This project uniquely addresses BETO's mission and goals for "process development and optimization of a single-unit operation for the upgrading of chemically or biologically derived intermediates to fuels and products." The project has the following three objectives: (1) process enablement by engineering and optimizing microorganisms to convert biorefinery waste streams to PHA for bioplastics; (2) process development by characterizing biorefinery residues, optimizing lignin treatment and fermentation, and designing the novel bioprocess; (3) process integration and optimization by conducting biorefinery onsite scale-up, as well as TEA and life-cycle analysis for the lignin-to-PHA upgrading process.

The project is structured into two budget periods. The first budget period is 24 months, and the second budget period is 12 months. All three tasks will be carried out in both budget periods. The technical targets for the two budget periods are as follows. In Budget Period 1, we aim to achieve 2.4 g/L PHA titer and 30% utilization of lignin. In Budget Period 2, we aim to achieve 50% utilization of lignin and 8 g/L PHA titer. The ultimate targeted performance, therefore, is 50% lignin utilization and around 8 g/L PHA titer. Achieving the research objectives will allow us to leapfrog the technology to address an important challenge in modern biorefinery development.

Weighted Project Score: 8.0

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



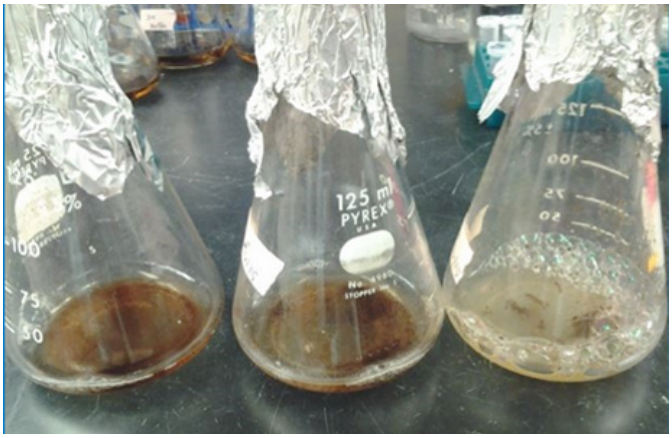


Photo Courtesy of Texas A&M University

Overall Impressions

- Overall, the presentation indicates a depth of expertise in the subject area. These are clearly areas in which the Texas A&M team is actively engaged.

  As feedback, the presentation gave a very brief idea of the overall goals and structure of the effort (broken out into fairly logical steps), but then it dived right into detailed graphics that look to be taken directly from manuscripts drafts, rather than providing clear explanations of the strategies and methods that will be used on the project, significantly clouding the issue. Some idea of the timelines of activities and how tasks inter-relate would have been much more useful than such detailed figures.

  Complete TEA models will be essential to finally understand whether the chemical treatments under examination here to generate lignin streams make economic sense. Pulling out the PHA portion may be useful at some point for the detailed assessment of process parameters, but understanding the effect of the process scheme, solid liquid separations, dilution of biomass material, washing steps, etc. on overall economics is central.

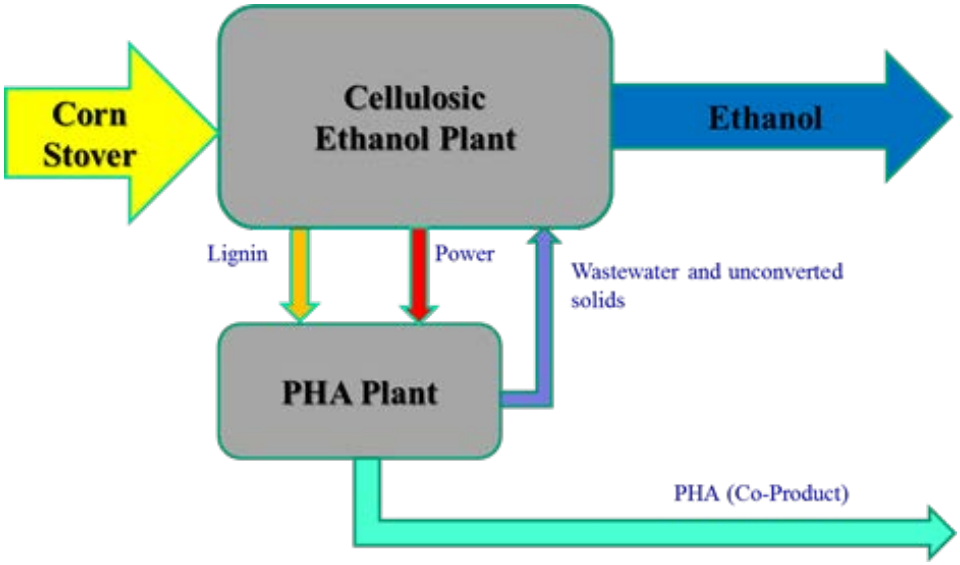
- The PI appears to be on an interesting path for upgrading a biorefinery waste stream through a nice combination of genetic engineering and biomass fractionation processes. Targeting PHAs as the product would seem to be a high-risk approach, given that PHAs have a long history of interest, but an equally long history of failing as a large scale commercial product. A stronger justification of the product choice would strengthen the project.
- Lignin utilization is an essential component of the MYPP, and this project aims to convert this stream to a value-added product. The team has a good metabolic engineering approach to develop a P. putida strain to produce PHA from the lignin monomers, and it has some promising initial results that indicate the interim project targets set are achievable. However, PHA is a challenging market to enter, and so far, there has been limited success. There is also the technical challenge of controlling monomer chain length distribution. The uniformity of the product may also relate to market potential. Finally, it would be useful for this team to collaborate with the NREL lignin conversion work since there appears to be some redundancy.
- This project has identified good starting strains for a lignin-to-PHA development project. It is ambitious to ask a strain to depolymerize and degrade lignin, and produce PHA at a commercially relevant $5/kg, in the toxic milieu of biorefinery hydrolysate. The project is considering pretreatment modifications to optimize both the biofuel and bioproduct yields. This presentation contained a very good use of TEA data for examining process options and setting reasonable throughput and performance goals.
- This is a good team with high energy and vast knowledge in the field. The challenges are well-understood, and the de-risking approach of all levels—from pretreatment, to strain engineering, through classical metabolic engineering and system biology approaches, to fermentation process engineering and process engineering—are well factored in. I am not sure if the TEA of PHA at $5/kg is economically viable, but I trust the PI to follow up on this and drive the cost even to the lower $2/kg suggested in one of the scenarios. BETO is highly encouraged to consolidate the critical parallel efforts across academic and national laboratories on similar targets (i.e., PHA ex P. putida from lignin) into one bigger project that will focus on several focused layers of pretreatment, strain engineering, and process development/engineering.

PI Response to Reviewer Comments

- We are engaging industrial contacts regarding both our gasoline-like main product and chemical (BTEX) co-product values and how they can be incorporated into existing refining infrastructure. Our main product still is a fuel, and the BTEX is intended as a co-product to offset operating expenses.

  The ethanol producers will have the flexibility to partially or fully produce Vertimass products (gasoline-like fuel and concentrated BTEX) or continue to make ethanol to take advantage of market conditions and maximize their revenue. We anticipate the ethanol RINs will transfer into our fuel product, supporting higher prices. However, the ethanol RINs do not currently transfer into the BTEX product, but these BTEX products command a price premium over fuels, so this is partially hedged.

Task	Year 1				Year 2				Year 3				Milestones
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
Project Management and Commercilization	●												Project management plan finalized
Objective 1 Process Enablement													
Subtask 1.1, Additional strain screening			●										At least one strain grows 1.5 times better on lignin
Subtask 1.2, System biology analysis				●									Provide 5 expression system, 3 for secretion
Subtask 1.3, Systems biology-guided microbial engineering to maximize PHA								●					>1 engineered strain to depolymerize >40% lignin
Subtask 1.4, Reiterative strain improvement								●					>1 engineered strain with >25% conversion rate for PH
Subtask 1.5, Develop stabilized strain for bioifermentation										●			1 stabilized strain with aforementioned performance
Objective 2 Process Development													
Subtask 2.1, Lignin and pretreatment residue characterization					●								Determine 1 best type of feedstock for lignin conversion
Subtask 2.2, Pretreatment conditions and lignin reactivity						●							Determine a proper pretreatment condition
Subtask 2.3, Chemical pre-processing development											●		One process to decrease Mn by half
Subtask 2.4, Fermentation optimization									●				One process to convert 50% lignin at >25% conversion
Subtask 2.5, Bench scale process development												●	One process to convert 90% lignin at >30% conversion
Objective 3 Process Optimization and Scale-up													
Subtask 3.1, Scale up on site of biorefinery												●	One process to convert 90% lignin at >30% conversion
Subtask 3.2, TEA and LCA							●						Initial report on TEA and LCA to guide strain improvement
Program-wise Go/NoGo point: At the end of year 1, we will set the Go.NoGo milestone to be 1 engineered microbial strain converting 40% of lignin; and HDO converting rest of lignin at higher than 50%. At the end of Year 2, the Go/No Go Milestone will be 150g/L butanol with a 20% conversion efficiency for lignin to butanol using biological route and over 70% HDO lignin efficiency.													



Block diagram illustrating the relationship between an existing cellulosic ethanol plant and a bolt-on PHA production plant proposed by this project

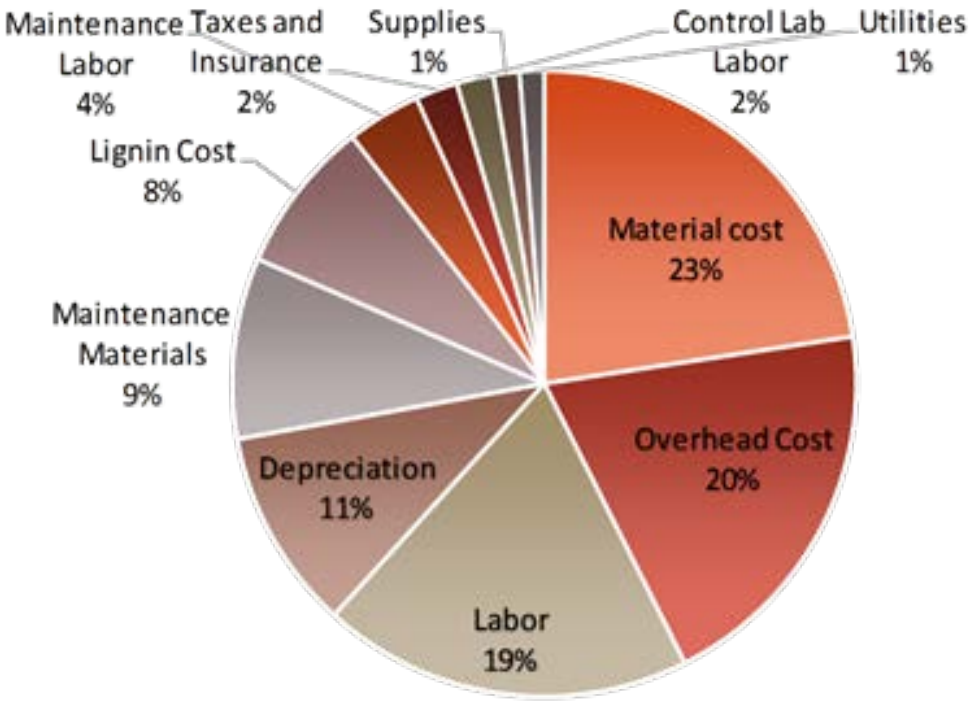
Third, regarding PHA as a product, the bioplastics market is increasing exponentially. According to some estimates, by the end of the project at 2019, global bioplastics production will reach 7.8 million tons. PHA is one of the major bioplastics with biodegradable capacity and also has a significantly increasing market. In spite of the failed joint venture between Metabolix and ADM, the global PHA production capacity is actually increasing, primarily in Asia. Many factors contributed to the Metabolix exit. The proposed approach clearly has two major advantages. On one side, the feedstock is the low-cost biorefinery waste. On the other side, the process integrates with the lignocellulosic biorefinery, which will reduce the overall capital expenditure and operation cost for the bioplastics units. The multi-stream integrated biorefinery will allow us to further reduce the cost for bioplastics production, as indicated in the TEA analysis.

Fourth, regarding the economic competitiveness of PHA at $5, the price of PHAs produced by current manufacturers is estimated at $1.66–45.54/kg, while the minimum PHA selling price calculated in this initial TEA is $2.84/kg with the proposed strategy. The technology is therefore competitive with current platforms.

Fifth, regarding engineering multiple activities within the same strain, we appreciate and agree with the reviewer that strain engineering alone may not achieve the technical and economic targets of the project. This is why we have included fractionation and pretreatment optimization, along with fermentation improvement, to mitigate risks. The choice of the strain and the bioprocess configuration will eventually depend on the integration of these different layers of technologies, as guided by TEA and technology performance.

Sixth, regarding the collaboration with other NREL projects for lignin conversion, we have already discussed the synergy and collaboration with the relevant PIs. Importantly, based on our discussion and project review, the efforts between different lignin utilization projects are very complementary to one another, and there is no significant redundancy. These multiple projects enable the development a complementary and complete portfolio of technologies for multi-stream integrated biorefineries. It will also help to de-risk the technology development and improve the productivity and accountability.

The figure below shows a breakdown of the relative cost contributions to the minimum PHA selling price. Material costs represent the largest cost contribution and are primarily driven by solvent costs for PHA extraction and purification. Thus, project work around optimization of solvent selection, recovery, and recycling represents a major opportunity for reducing the minimum PHA selling price and improving the techno-economics of the project.



Cost contributions to minimum PHA selling price

BIOLOGICAL LIGNIN DEPOLYMERIZATION

(WBS#: 2.3.2.100)

Project Description

The joint Biological Lignin Depolymerization project between NREL and Sandia National Laboratories aims to develop biological solutions for the depolymerization of lignin polymers and oligomers. Overall, this project will contribute to lignin valorization efforts in the biorefinery, which are essential for cost-effective hydrocarbon fuel production. Specifically, this project has examined potential approaches to depolymerize both (1) solid residual lignin resulting from process-relevant, BETO-funded polysaccharide deconstruction approaches, such as the Deacetylation, Mechanical Refining, and Enzymatic Hydrolysis project, and (2) solubilized lignin from process-relevant catalytic treatments of lignin, streams that both contain polymeric and oligomeric lignin.

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Gregg Beckham
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$125,000
DOE Funding FY 2015:	$500,000
DOE Funding FY 2016:	$600,000
DOE Funding FY 2017:	$550,000

The Biological Lignin Depolymerization project has identified effective basidiomycetes for producing laccase- and peroxidase-rich cocktails and has combined these cocktails with microbes to demonstrate higher extents of lignin depolymerization via a "microbial sink" for catabolism of the low-molecular-weight aromatic species. Moreover, we have demonstrated the conversion of solubilized lignins by multiple aromatic-catabolic microbes using native enzymatic machinery. Going forward, this project will focus on elucidating the full enzyme suites employed by microbes for breaking down

Weighted Project Score: 6.9

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

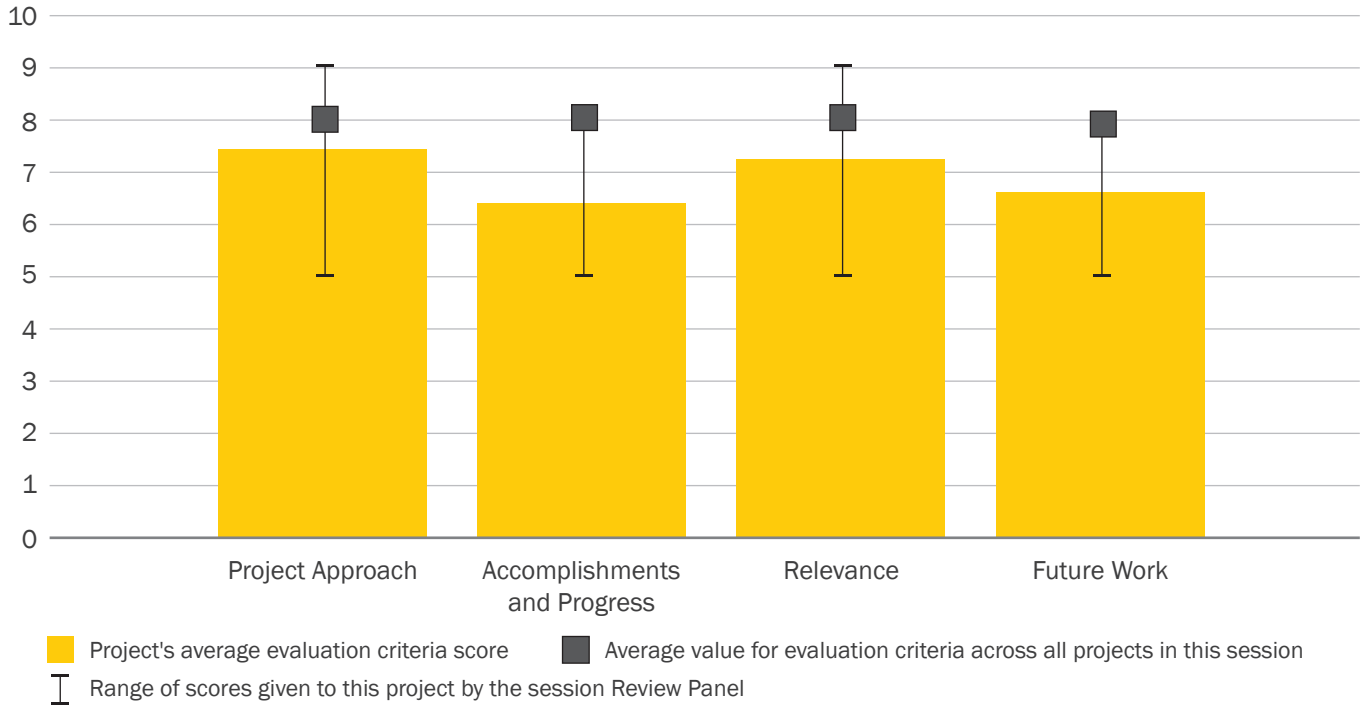


Photo Courtesy of National Renewable Energy Laboratory

solubilized lignin polymers—to make lignin oligomers more readily available for aromatic-catabolic microbes that are being engineered to produce co-products in other BETO projects.

Overall Impressions

- The idea of a microbial sink to drive forward lignin degradation by pulling in low-molecular-weight compounds was a good one. This could have presented the opportunity to make a thorough survey of ligninases in the presence of a production organism. Given the source of many ligninase sequences in the public domain, heterologous expression in a fungal host, like T. reesei, would have perhaps allowed a more comprehensive survey.

  The project has identified issues and developed improved techniques, but it doesn't feel like the CBP idea of expressing ligninases in a Gram-negative host is great direction to go in. Certainly, there are multiple options for mixing chemical catalysis and depolymerization conditions with biological solutions, but I think these would be more complete with a more robust approach to enzyme diversity.

- The project presents a novel idea of combining fungal enzymes with microbial systems to realize both lignin deconstruction and simultaneous inhibition of repolymerization. However, the current process runs the risk of becoming overly complex, as additional treatments of lignin are necessary for its solubilization, while a large amount of organism development still appears to be necessary. Further, a better understanding of the lignin composition and the amounts actually converted will strengthen the project. The project would benefit from settling on one or two routes for deeper examination and optimization.

- This program takes an alternate approach to lignin depolymerization, which the team has shown to be extremely challenging. After challenges getting enzymes to robustly solubilize lignin, the team decided to refocus on using chemical solubilization followed by microbial depolymerization of the soluble lignin. This was a good strategic decision, and it makes the project more viable and synergistic with other work.

- There were many encouraging reviews in previous years. Utilizing the lignin cake has high industrial relevance. The concept of biological depolymerization for lignin conversion—and even as a cleanup for chemical oxidative depolymerization—still has a very long way to go. The enhanced understanding of lignin structure and bonds that this and related lignin projects contribute is valuable. Linking this knowledge to key enzymes with the specificity to break those bonds is also valuable. Producing a CBP organism that can depolymerize/solubilize/monomerize lignin and "bio-funnel" the myriad aromatics to a product like muconic acid would be a home run, if it were possible. It's a really long stretch goal because the proof-of-concept hasn't worked, and maybe this is not the best use of these talented resources.

- The project is very relevant to BETO goals, the management plan is sound, and the technical plan (given the challenges of this topic) is reasonable. The starting from scratch approach, given the poor reproducibility of existing knowledge from prior academic laboratory work, puts and additional hurdle

before the project team. This is very low-budgeted project for the task at hand, and it is impressive to see how much work to de-convolute challenges and identify opportunities was carried. Capitalizing on the Environmental Molecular Sciences Laboratory at PNNL and the experts at the Biological Research Center of the Spanish National Research Council is highly encouraged for prospecting of good candidates. It might be useful to partner with enzyme providers to identify good starting points to enzymes expressed by P. putida, as the CBP approach, while it has potential and value in some aspects, is still not technologically ready. The project team and BETO should evaluate this project in the bigger project context of lignin de-polymerization and conversion to products.

PI Response to Reviewer Comments

- We thank the reviewers overall for the constructive feedback and comments. Expression of heterologous enzymes in filamentous fungi is one route to produce ligninolytic enzymes, but this would require a very significant amount of time and resources, which we do not currently have bandwidth for in this project. This is an interesting approach, perhaps meriting its own AOP. In addition, this approach has been tried extensively in the peer-reviewed literature with known enzymes, and it has not yielded tangible results for the depolymerization of insoluble lignin, to our knowledge. More work on discovering novel lignolytic enzymes (primarily nucleophilic enzymes) that cleave specific lignin linkages is needed. As such, we are shifting focus to identify and engineer nucleophilic enzymes that are able to cleave dimers and small oligomers that result from chemical catalysis.

We also completely agree with the reviewer that secretion of oxidoreductases will be challenging; as such, we have stopped work on expressing oxidoreductases to be secreted in bacteria and are focused solely on nucleophilic enzymes that are able to break down dimers and oligomers.

In terms of process complexity, we stress that this project going forward will be solely focused on identifying and engineering enzymes that are able to cleave dimers and oligomers in tandem with detailed lignin analytics, directly in line with the reviewer feedback. We are also focusing on the catalytic streams being produced in the Lignin Utilization project and by industrial conversion processes.

Regarding the CBP concept, this was simply a proof-of-concept study. In this study, we identified that many aromatic-catabolic microbes are able to depolymerize oligomers. This finding in itself is valuable, when taken with the analytical and proteomics work that is ongoing, to understand what linkages in oligomers are being broken by which enzymes and—just as importantly—what linkages are not being broken. This will enable us to understand the interplay between chemical catalysis (what linkages remain in dimers and oligomers) and the microbial engineering going forward.

In terms of the larger lignin portfolio and the collaborative efforts, we thank the reviewer for the positive comments. We also note that this project closely collaborates with the Lignin Utilization and Targeted Microbial Development projects, and indeed, in many cases, the same staff members are working between these projects. The Biological Lignin Depolymerization project keeps the “big picture” in mind throughout the development of biological lignin depolymerization strategies.

FUNGAL GENOMICS— GENETICS (FORMERLY: FUNGAL GENOMICS)

(WBS#: 2.3.2.103)

Recipient:	Pacific Northwest National Laboratory
Principal Investigator:	Jon Magnuson
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$1,650,000
DOE Funding FY 2016:	$1,650,000
DOE Funding FY 2017:	$1,500,000

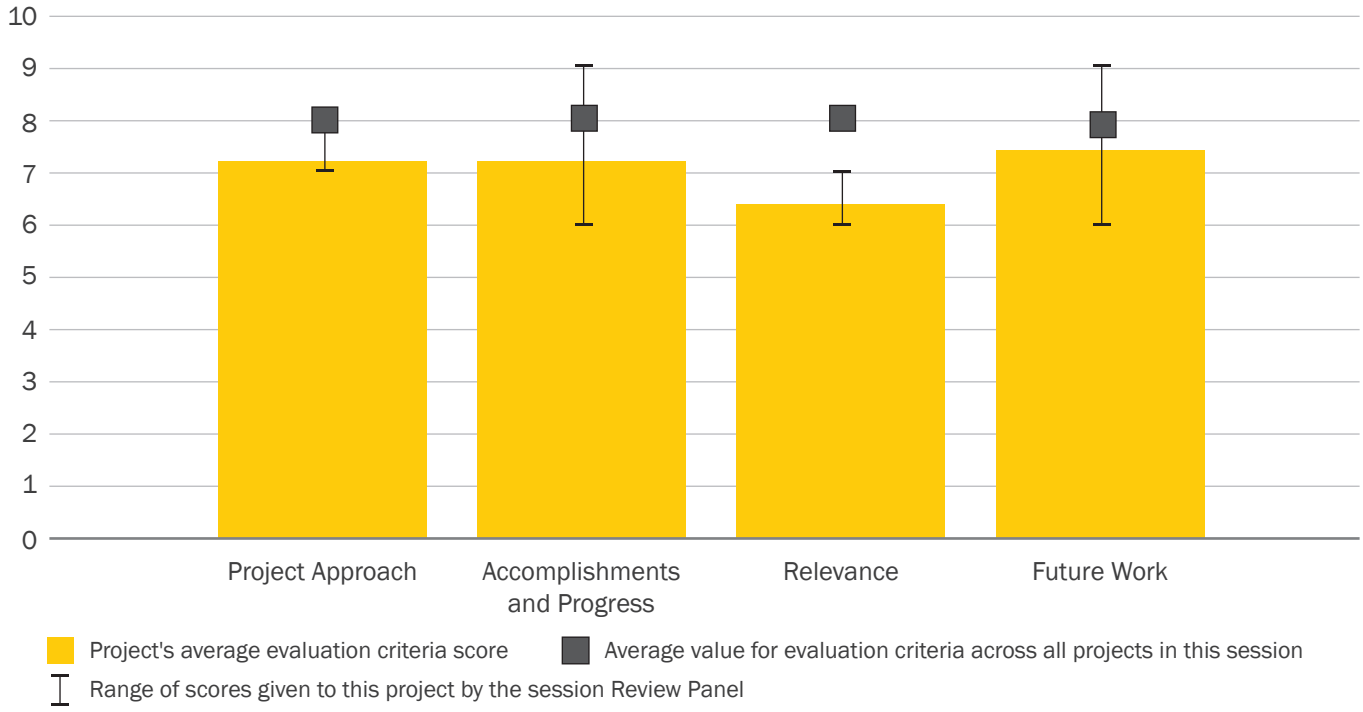
Project Description

In the Fungal Genomics project, we focus on the development of non-traditional but industrially relevant fungal platforms with desirable attributes for producing advanced biofuels and bioproducts or their precursors at a scale that can be translated to industrial processes. Desirable attributes include the ability to utilize a wide variety of sugars from lignocellulose, robustness with regards to growth in the presence of inhibitors from biomass pretreatment, and the ability to produce a variety of compounds at TRY that drive toward techno-economic feasibility.

We utilize a parallel approach of manipulating the organism through genetic engineering and bioprocess engineering to develop and optimize the overall bioprocess. With regards to TRY in oleaginous yeast Lipomyces, we have achieved 95% of theoretical yields of paraffinic biofuel precursors in the form of triacylglycerides at up to 56 g/L titers at 1 g/L/hour. The challenge is to approach all of these high values at once using impure sugars. We have genetically engineered Aspergillus to produce two polyketides at more than 0.5 g/L and terpenes at tens of mg/L. This workhorse fungus provides a platform for a variety of biofuels/bioproducts precursors to add value to the biorefinery and to help lower the selling price of the biofuel.

Weighted Project Score: 7.1

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



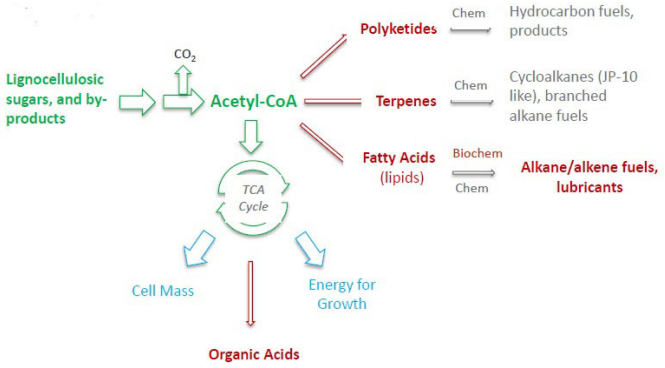


Photo Courtesy of Pacific Northwest National Laboratory

Overall Impressions

- The project offers potential to expand the suite of organisms available to industry as platform hosts, with advantages in process tolerance and developed genetic tools.

  The execution of the project and prioritization of tasks probably needs some attention. In a few areas, it feels like a more systematic approach would help out, and perhaps direct collaboration with outside groups (mostly here I'm thinking about the results from the NREL team on predicting mutations for metabolic engineering).

- While the overall concept is solid and uses a well-established procedure for choosing and engineering organisms for bioproduction of chemicals and fuels, the specific experimental work has been less successful. By focusing on a highly reactive chemical (octatrienoic acid [OTA]) as a product, the PIs have chosen a material that may be difficult to isolate and test as an intermediate for other materials. It is likely that OTA is not the best choice in this program and that production of other materials may benefit the experimental effort.

- The Fungal Genomics project aims to exploit the unique capabilities of fungal organisms for bioconversion. Non-Saccharomyces fungal species have traditionally been used in a variety of fermentation processes, from antibiotics to citric acid. Oleaginous yeast represents an opportunity for free fatty acid production, and the team has identified a species that may be more suitable than Yarrowia and put in a lot of effort to develop good genetic tools. As oleaginous yeast is a major focus of NREL projects, there should be more collaboration between NREL and PNNL to ensure work is not redundant. Aspergillus is also a good host with already established tools, but the titers obtained for the target product (OTA) are still very low. It will be challenging to get increases of two to three orders of magnitude because polyketide synthases are hard to manipulate. In addition, the compound will likely be very reactive, both in vivo and in subsequent processing. The 3-hydroxypropionate work may be more fruitful in the short term.

- The team is making good progress in developing genetic tools to facilitate development of Lipomyces for lipid production. Development of new, robust, industrially relevant bioproduct and biofuel production hosts has the potential to accelerate new product introductions.

- The team shows good progress in developing molecular tools and understanding of the challenges. There is great collaboration with the SCADA team to enable process development with a real-time, data-driven approach. The current focus on Lipomyces is the right choice, although the economics are questionable overall (not in the project team's hands) and need better guidance based on rigorous TEA analysis. A slide with metrics of progress toward goals, including metrics of TEA in regards to the technical accomplishments, would be nice and should be applied across all projects. I recommended you have a joint umbrella with a critical mass of expertise to gain momentum and focus all the fungal development projects for enzymes and fuels on the biggest gain for the buck approach.

PI Response to Reviewer Comments

- We thank the Review Panel for their positive comments and helpful critiques. We appreciate the gen-

erally positive comments about the significance of the development of genetic tools in Lipomyces starkeyi and its promising potential as a lipid producer for biofuels applications. We are working on selecting an even more robust biomass hydrolysate-utilizing strain through use of a turbidostat, and we appreciate the emphasis the Panel placed on this research thrust. In addition, we will soon obtain less-inhibitory hydrolysates available from new industrial partners, which we will test in the near future to maximize the chance of increasing TRY in Lipomyces.

The collaboration with the NREL team (regarding Lipomyces) is active but could use additional emphasis and communication. We initiated the transfer of additional materials and knowledge between the laboratories at the 2017 Project Peer Review meeting in March and plan to visit one another in the near future.

The comments and concerns regarding the relatively low OTA titers obtained in Aspergillus to date (concerns that included meeting our March 31 milestone), degradation issues, and utility as a biofuel or bioproduct intermediate are well-taken. Since the Project Peer Review meeting, we have exceeded our March 31, 2017, go/no-go target of 1 g/L in shake flask studies—where, in contrast to the bioreactors, the degradation issue is not observed. Thus, we have demonstrated a greater than 50% improvement over titers reported at the Project Peer Review meeting.

We have hypotheses regarding prevention of degradation of OTA in the bioreactor environment, based on prior evidence in our laboratory or precedence in the literature, which we will test in the near future. These include restricting oxygen late in the culture and increasing nitrogen concentration to prevent expression of accessory enzyme in the OTA biosynthetic cluster from modifying OTA to another chemical compound. Regarding the utility of OTA as an intermediate for biofuels or bioproducts, since the Project Peer Review meeting, we have demonstrated that it hydrogenates readily. We are now very excited about ketonization to dimerize the resulting octanoic acid for a biofuel precursor, as well as another process using the monomer that could lead to an extremely useful bioproduct in two well-understood catalytic steps.

We are certainly open to alternative target selections, but with the encouraging results on OTA since the Project Peer Review—meeting our milestone and promising developments on the catalytic conversion front—we are planning to push hard on this molecule through the remainder of FY 2017 towards the very ambitious year-end 2.5 g/L milestone; we will then revisit this target selection, in cooperation with our BETO technology manager and our industrial advisory panel, before proceeding with work on OTA or an alternative target in FY 2018.

SYNTHETIC METABOLIC PATHWAYS FOR BIOCONVERSION OF LIGNIN DERIVATIVES TO BIOFUELS

(WBS#: 2.3.2.104)

Recipient:	Oak Ridge National Laboratory
Principal Investigator:	Adam Guss
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$230,000
DOE Funding FY 2015:	$350,000
DOE Funding FY 2016:	$350,000
DOE Funding FY 2017:	$350,000

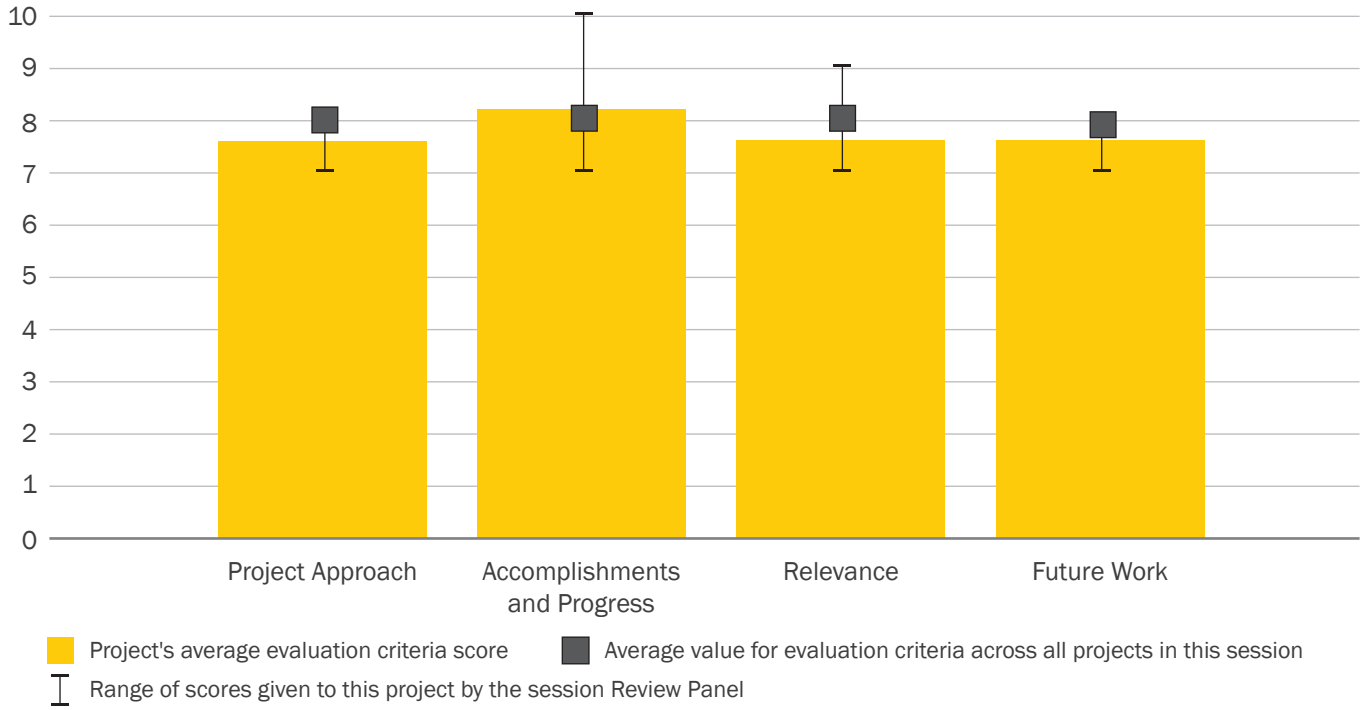
Project Description

A vital component to meeting domestic sustainability and energy independence goals in the United States is the economic production of liquid transportation fuels and chemical building blocks from lignocellulosic biomass in a biorefinery. While the majority of biofuels research has focused on the conversion of sugars into fuels and products, adding value to the lignin fraction of biomass (for instance, by bioconversion to high-value products) is essential to meeting BETO's 2022 goal of $3/gge. Therefore, the goal of this project is to develop biological routes to convert lignin-derived aromatic compounds into value-added fuels and chemicals. To this end, we are engineering Pseudomonas putida to accomplish the following objectives: (1) increase conversion of aromatic compounds derived from lignin to medium-chain-length PHAs, and (2) produce medium-chain-length alcohols (e.g., C8–C12 alcohols) and other molecules.

Engineered strains of P. putida had a 100% increase in PHA abundance per dry cell weight and a PHA titer increase of 200% during growth on depolymerized lignin. For alcohol production, we used a new DNA integration system to test 20 initial pathway designs, which allowed

Weighted Project Score: 7.8

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



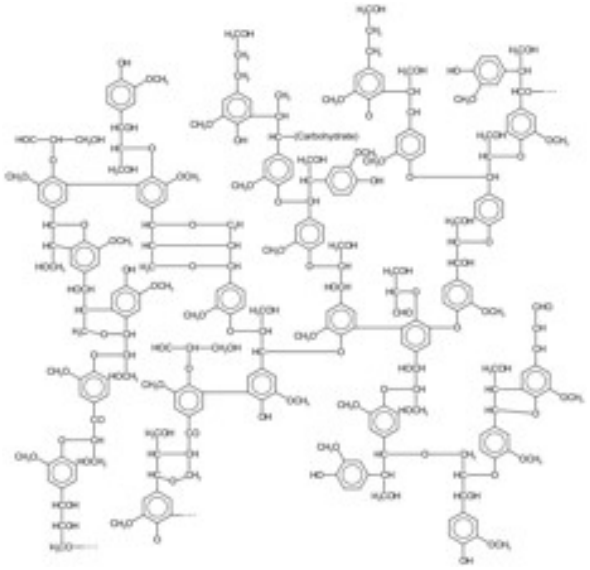


Photo Courtesy of Oak Ridge National Laboratory

for conversion of coumaric acid to approximately 12 mg/L decanol and dodecanol. Further strain design and pathway optimization is expected to dramatically improve alcohol yield and titer, providing proof of concept for lignin valorization toward enabling a bioeconomy.

Overall Impressions

- Although the funding level for the Oak Ridge National Laboratory project looks lower compared to the efforts at NREL, it looks like a very valuable addition to the technology package. In particular, the phage integrase system for integrating multigene cassettes at a single site at high efficiency looks like a great tool to enable higher-throughput synthetic biology strategies. In a short slide deck, it isn't possible to cover every aspect, but it would have been good to see how the team will make full use of the technique (e.g., whether Oak Ridge has the automation capability, or whether the team will tap into the Agile BioFoundry effort).

  The medium-chain-length alcohols look like a good target for an output molecule. Getting an early read on the toxicity of these products to the P. putida system would be a good idea, to make sure there isn't another large technical challenge out there that will limit progress towards economic levels of production, in addition to the metabolic engineering required for use of lignin derivatives.

  Delivering the TEA will be a key step in identifying performance targets for the team.

- Efforts to increase the potential portfolio of lignin products are a strength and will expand opportunities for industry to scale up lignin conversion processes. It is unclear whether the medium chain alcohols are reasonable targets as their markets will be much more fragmented than adipic acid. The PIs should work to better define the reasonable market share that could be realized with medium-chain alcohols and determine whether their materials would be produced at a high enough volume to affect the BETO 2022 milestone.

- This project aims to convert lignin depolymerization streams to PHA or medium-chain alcohols. The need for lignin valorization to enable BETO to reach the economic targets in the MYPP is well-established, and it is important enough that various approaches to utilizing lignin monomers should be considered. However, titers and yields for this program are much lower than that for conversion to adipic acid. Also, the metabolic pathways are much longer due to the need for integration into central metabolism (acetyl-CoA) rather than a direct bioconversion pathway to muconate. This program has shown some promising results, and thus should be continued for now, but eventually a decision has to be made to focus P. putida efforts to a single product. Thus, the program manager and PI should keep this in mind, especially when considering continuing the effort beyond the end of this project period.

- The project is making progress producing PHA and medium-chain-length alcohols in P. putida. The project is cognizant of the current selling price for medium-chain-length alcohol, and comparison to the adipic acid base case. They are currently conducting a TEA (FY 2017 milestone), which should

provide a useful reality check on the significance of the titer milestones. Development of a high-throughput, screen-amenable, site-specific recombination system for transforming P. putida as efficiently as a replicating plasmid is a nice accomplishment that can be leveraged.

- The project team developed an array of metabolic engineering tools and an understanding of how to work with P. putida that will likely accelerate other future programs in this direction.

PI Response to Reviewer Comments

- We would like to thank the reviewers for their insightful comments and questions.

  The phage integrase system is simple enough that automation is not required for a throughput in the range of dozens to a few hundred pathway variants, and the bottleneck then often becomes strain characterization. While automation would make strain construction less tedious, automation and/or high-throughput methods will be even more critical for strain characterization. For much higher throughputs, a robust screen (e.g., fluorescence-activated cell sorting) or selection will be critical for rapid strain improvement. To enable higher-throughput methodologies, we and our collaborators are working to adapt the CRISPR-based method "CREATE" in P. putida, which, for instance, could be combined with the integrase system to screen or select protein or pathway variants that have improved performance.

  Regarding product toxicity, we have already evaluated toxicity of some medium-chain alcohols, and P. putida is tolerant to at least 1% volume/volume (~8 g/L) octanol and decanol, which is well beyond the solubility limit for these compounds. Determining the tolerance to additional alcohols and mixtures of alcohols will be important going forward.

We agree that TEA is critical as a point of reference to evaluate strain performance, identify target metrics, and model economic feasibility. We have completed the initial TEAs for each of these routes, and there are promising strategies to help meet the $3/gge BETO goal. Work is ongoing between the R&D team and the TEA team to outline specific process metrics and R&D strategies to meet yield targets and address any outstanding data gaps, with a plan to re-examine this TEA with this additional data by the end of FY 2018.

We agree about the need to demonstrate substantial progress in producing an exemplar molecule. Muconate has high carbon efficiency from aromatics, making it a promising target, but it is challenging to make from other substrates. Molecules like medium-chain-length alcohols, on the other hand, are derived from central carbon metabolism and can be generated from more than just aromatics, including acetate and hemicellulose-derived breakdown products that are often present in lignin streams. Therefore, we feel there is value in having a portfolio of products that can be made from lignin, including ones derived from central metabolic intermediates, such as medium-chain-length alcohols.

This project allows BETO to diversify the product slates for upgrading waste carbon, including from lignin or carbon lost from other sources, like acetate or hemicellulose derivatives. Expanding the range of potential products and markets available from these waste carbon streams will further ensure that one product will not completely saturate a market. We plan to use a combination of TEA and discussion with BETO technology managers to strike the best balance between progress on exemplar molecules and product diversity.

BIOLOGICAL UPGRADING OF SUGARS

(WBS#: 2.3.2.105)

Project Description

The Biological Upgrading of Sugars project develops robust microbial strains to produce fuel precursors at the required TRY for the BETO 2022 hydrocarbon fuel target of $3/gge. From FY 2015–FY 2017, the Biological Upgrading of Sugars project has conducted a large screen of oleaginous yeast strains to produce fatty acid–derived products, resulting in the down-selection of a particular yeast strain (Rhodosporidium toruloides 4444) for producing diesel precursors. This strain has been engineered to produce secreted fatty acid–derived products. Moreover, we have screened a yeast genome knockout collection to identify genes that render yeast more susceptible for cell lysis—one of the most expensive steps in intracellular fatty acid recovery.

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Gregg Beckham
Project Dates:	10/1/2014–9/30/2017
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$1,800,000
DOE Funding FY 2016:	$1,800,000
DOE Funding FY 2017:	$1,800,000

Moving forward, the Biological Upgrading of Sugars project has shifted focus to anaerobic secreted products, namely short-chain carboxylic acids, given the significantly lower production costs, the ability to reach larger scale than aerobic processing, and the ability to convert these acids to jet and diesel fuels. We are currently evaluating both a bacterial (Clostridia butyricum) and a low-pH yeast platform for an eventual down-selection towards a 2022 pilot-scale demonstration. By providing strains to integration efforts, this project is directly relevant to meeting BETO cost target goals, and we closely

Weighted Project Score: 8.4

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

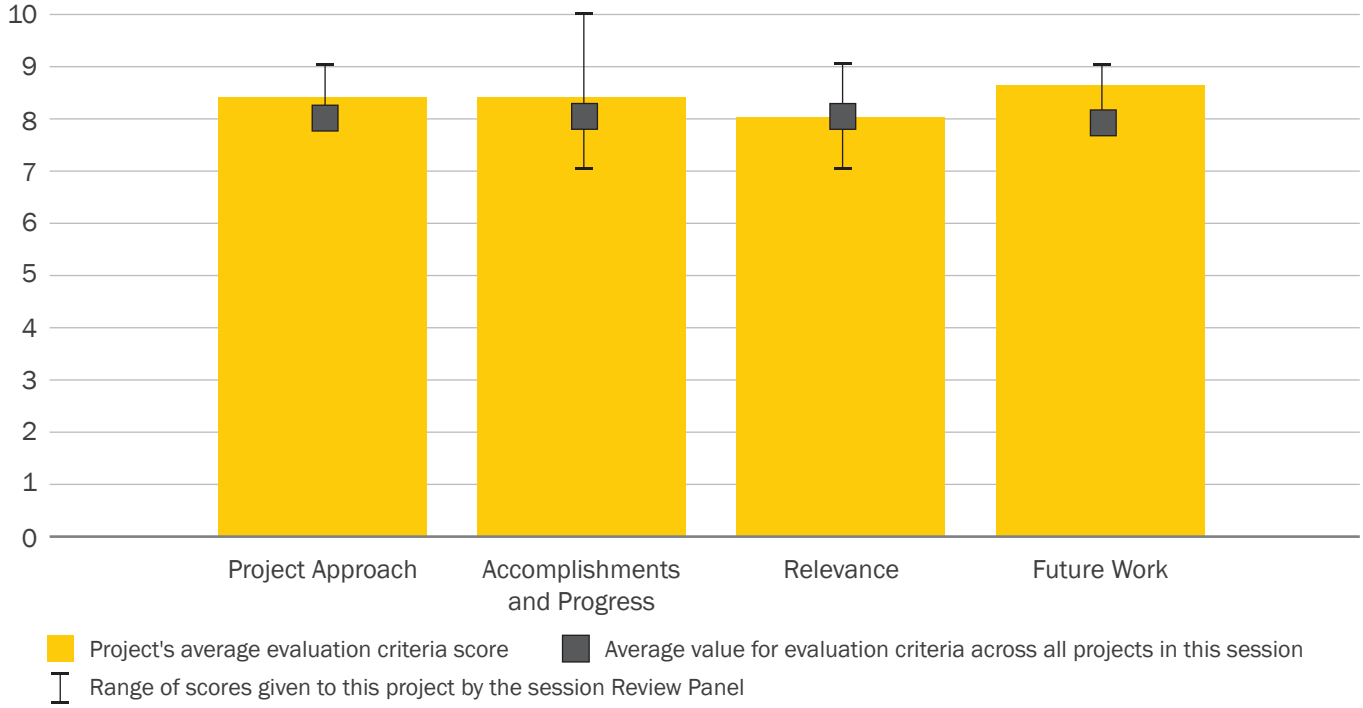


Photo Courtesy of National Renewable Energy Laboratory

collaborate with other BETO projects on co-designing strains within the context of a fully integrated conversion process to produce renewable hydrocarbon fuels.

Overall Impressions

- Overall, the project looks well-run and has been positioned properly to take a fresh look at host strain selection for economic production of advantageous products from the sugar streams generated in the NREL process. What comes through strongly is that the team has taken a logical, coherent approach, with the addition of some areas of innovation (yeast strain lysis sensitivity and continuous extraction). The project looks to be well-connected to other NREL projects and has formed connections to industrial partners for key technology areas.

  A useful output may be a more detailed report of the strains and technologies surveyed than may perhaps be considered for a peer-reviewed publication. The utility here is to save companies from covering the same ground, and so a detailed report of the options explored would be very useful.

- The targets for biological upgrading of sugars are reasonable approaches for producing hydrocarbon fuels from biomass and offer routes that fit within current BETO TEAs. Good progress has been made on both of the research aims of the program (biological lipid and acid production), and the future work has identified the key challenges that should be addressed. The project overview would be strengthened by more detail regarding the catalytic upgrading of short-chain acids to fuel-length hydrocarbons, both in a technical and economic context.

- The Biological Upgrading of Sugars project is a critical piece of the biorefinery, responsible for the conversion of sugars to fuels and co-products. The team is developing two product/host combinations to mitigate risk and is even looking into different possibilities within each aim. Excellent progress has been made toward the targets, and it seems likely that all will be reached by the end of the project. However, some of the most challenging aspects of strain and process development still remain (possibly before or after the interim targets), after all the more obvious and straightforward strain improvements have been done. Future plans include the use of omics and coordination with the bench-scale integration group, which could help elucidate the more non-obvious routes to improvement.

- The Biological Upgrading of Sugars project has taken several routes to sugar upgrading to make fuel precursors. They have done a nice job of making down-selections to accomplish the following objectives: (1) deliver an oleaginous yeast strain to an integration team for longer-term process development, and (2) turn their strain engineering attention to C2–C4 produced in anaerobic fermentation. Yeast and bacterial candidates are still being considered for C2–C4 production, and that down-selection is the future focus. The presentation was well-organized, and decision points were clearly explained. Add SMART (specific, measurable, achievable, relevant, timely) goals.

- The project objectives are well-aligned with BETO's objectives of finding molecules (other than ethanol, diesel, and jet fuels precursor molecules) to meet the $3/gge equivalent. The choice of strains,

molecular tools development, and testing strategies are sound. The management approach is well-structured, and the team has established much good collaboration to accelerate this R&D effort. The PI, together with the TEA team, needs to evaluate the production cost of C16–18 fatty alcohols as fuel. I doubt if hydrolysate feed of C6/5 stream will cut it compared to alternatives in the market (i.e., plant oil and petro-derived long-chain alcohols). The PI already recognized this, and future work takes this into account. Another recommendation will be to try to find know-how (which exists) in industry in the United States as way to accelerate this R&D effort. As for the short-chain carboxylate program, a good risk-mitigation strategy was taken with evaluating both Clostridia and acid tolerance yeast. It would have been useful, and it's possible, to direct choice of routes based on thorough TEA, which appears to be the team's direction.

PI Response to Reviewer Comments

- Overall, we thank the Review Panel for the positive comments and constructive feedback to improve the quality and output of the Biological Upgrading of Sugars project. We agree that a detailed report on the history of the project would be a useful output for industrial entities, and we will make this a target of the project output going forward.

  In addition, we apologize for omitting details on the catalytic upgrading routes due to time constraints (this work was presented in detail in the Thermochemical Conversion session), but the catalytic work is being done in very close concert with the biological and separations components to ensure success in process integration. Briefly, we are approaching near-theoretical acid coupling yields to generate mixed ketones from C2/C4 and C4/C6 mixed acids during continuous processing with stable catalyst performance for over 24 hours. Near quantitative yields were also achieved when converting methyl ketones to branched cyclic compounds via condensation pathways. Lastly, quantitative hydrodeoxygenation of mixed ketones was demonstrated with over 24 hours of catalyst stability. Further work is ongoing to evaluate these upgrading routes with biologically derived mixed acid streams.

  Lastly, we also note that SMART goals are a core component of our management strategy, even though these were not listed explicitly in the presentation.

CONTINUOUS MEMBRANE-ASSISTED IBE FERMENTATION FROM AVAP CELLULOSIC SUGARS

(WBS#: 2.3.2.202)

Project Description

This project utilizes diverse lignocellulosic sugars from pine, stover, and straw using the American Value-Added Pulping (AVAP) process in Thomaston, Georgia. We produce isopropanol, n-butanol, and ethanol (IBE) by fermenting genetically modified Clostridia Acetobutylicum, AVAPClo. Fermentation using continuous membrane-assisted fermenters targets productivity of 12 g/L/hour over 0.5 g/L/hour in batch and cuts capital costs in half. We also use solvent recovery using non-toxic liquid/liquid extraction in place of stripping targets to reduce thermal energy in half. Water, unused sugars, nutrients and intermediates are efficiently recycled.

Recipient:	American Process Inc.
Principal Investigator:	Dr. Vesa Pylkkanen
Project Dates:	7/1/2015–11/30/2017
Project Category:	Ongoing
Project Type:	FY 2014—Biological and Chemical Upgrading: DE-FOA-0001085
Total DOE Funding:	$3,088,632

The project has successfully completed the intermediate validation stage. The integrated run was performed at average 10 g/L/hour productivity using pine C5 and C6 feedstocks. The second budget period focuses on optimizing process parameters in a 500-hour run. We will apply value engineering and process integration to reach an IBE production cost of $2/gallon from the benchmark $3.20/gallon and to determine lifetime greenhouse gas emissions. Proper R&D scale-up will be followed by potential commercialization efforts. The expected outcome is a robust process to broaden feedstocks and intensify sugar-based upgrading to higher alcohols at below the DOE target MFSP of $3/gge. IBE alcohols

Weighted Project Score: 8.2

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

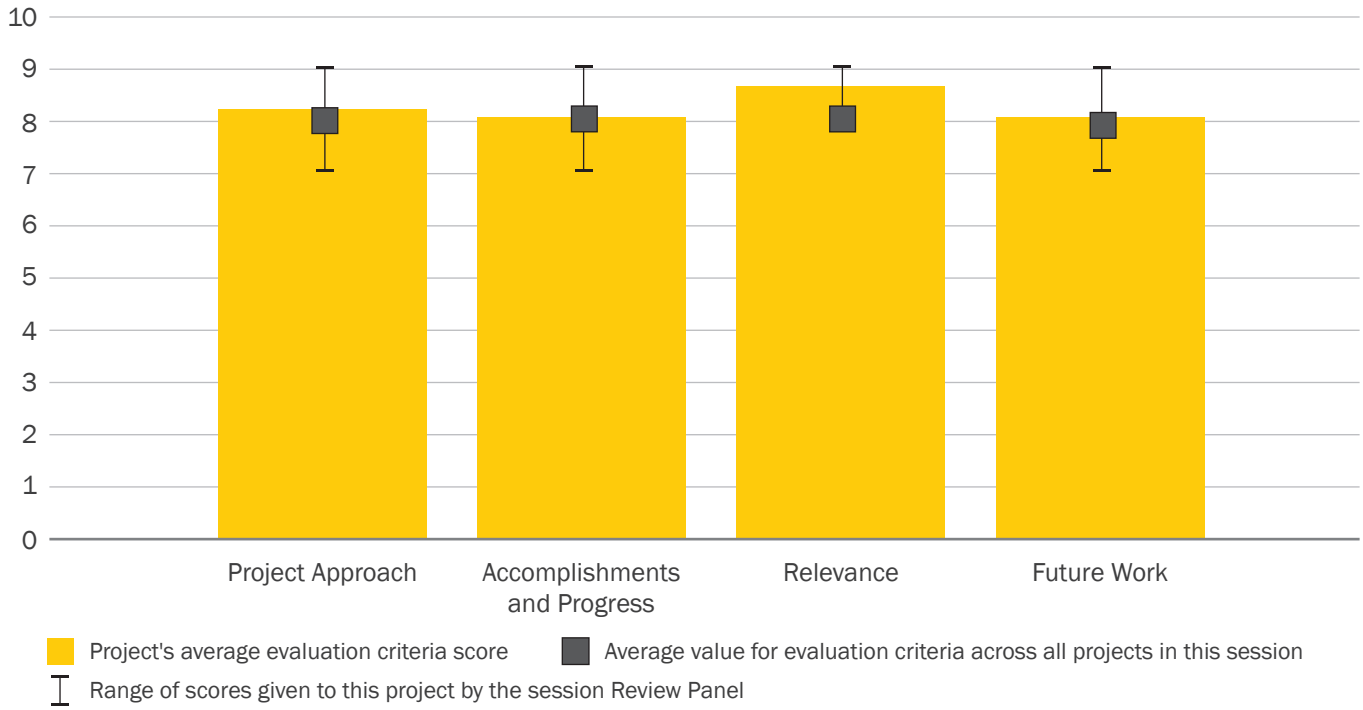


Photo Courtesy of American Process Inc.

are already approved blending components into gasoline. American Process Inc. and Byogy are engineering an alcohol-to-jet demonstration scale facility for 2021 startup.

Overall Impressions

- The presentation clearly underlines the position of the project in building on the technology that American Process Inc. has already developed and is a logical extension. This gives the impression of a technology heading towards commercialization, with a relatively clear path. The use of membrane technology to remove product and allow an intensification of the process looks like a great benefit. Additionally, the shift in acetone production to further alcohol production looks like a worthwhile goal, but the presentation did not go into the consequences of that in terms of the metabolism of the organism and balancing co-factors and energy demands.

  Overall, it is good to see this move forward as part of a full technology package and provide an alternative to other combinations of pretreatments/fermentation of sugars/product recovery schemes in the marketplace.

- This project investigates a novel approach to the mixed feedstock coming out of an acetone–butanol–ethanol fermentation by using an organism that would convert the acetone to isopropanol, giving a feedstock much better suited for biofuel production. However, the project is also facing an acetone-to-isopropanol conversion that is much lower than expected. Further, it is unclear why the economics for this process allow lignin to be burned while the very detailed NREL evaluation requires lignin conversion to a high-value product. This inconsistency will need to be evaluated for the BETO program, as industry will likely gravitate to the least expensive processes.

- This project seeks to develop a novel process design using membrane-assisted fermentation and liquid/liquid extraction to enable lower energy removal of alcohol products. The economics seem good if targets are hit, partly due to the prior work American Process Inc. has done in providing a clean, economical sugar stream. The project is on track so far and is managed by a strict stage-gate program to ensure continued progress. Engineering the strain to shift production from acetone to isopropanol could pose a huge technical barrier, but this was not discussed, so I am not certain if the team has the right expertise to do this.

- This project was very well-presented and is awaiting Phase II approval. Critical success factors, quantitative progress towards metrics, and the value of future opportunities were clearly described. There are remaining challenges with strain engineering and sugar/product recovery, and the project is addressing them. Success would be a commercially competitive n-butanol process, without subsidy.

- Overall, this project has very good management and metrified goals using a stage-gate process to measure progress and direct go/no-go decisions. There are good technical achievements on all ends to allow small-scale demonstration of continuous fermentation and liquid/liquid extraction with novel configuration (separate C6/5 fermentations) and benchmark economics and life-cycle analysis. This is a very good demonstration of how the American

Process Inc. AVAP process sugars, which come from genetically engineered clostridia and good industry-academia collaborations, can be utilized effectively for mixed alcohols.

PI Response to Reviewer Comments

- The AVAP pretreatment provides clear fractionation of hemicellulose, cellulose, and lignin from a variety of biomass sources—about 90% monomeric sugar yield. Additional C5 sugar conditioning is applied for bacterial fermentation, which is less tolerant to inhibitors than yeast.
- Phase I established that AVAP C6 sugars do not require any conditioning, and C5 fermentation targets were reached after certain conditioning steps. The Phase I innovation with concurrent C5 and C6 fermentation productivity of the hydrolysates approached those of dextrose and xylose in a continuous fermentation scheme. The integration of non-toxic extractant to remove solvent from the fermentation permeate allowed recycle of raffinate and resulted in improved yield and productivity due to recycle of sugars, nutrients, and metabolic intermediates.
- Phase II will focus on the economics of the identified conditioning steps. The experimental matrix seeks to eliminate unnecessary steps and then determine the minimum necessary treatment to meet project targets. A complete mass balance of sugars and inhibitors will be constructed to evaluate the techno-economic optimum configuration. Finally, the whole process will be simulated, and process integration principles will be applied to find minimum utility requirement. Should there be excess lignin available, this can supply to the existing lignosulfonate market for additional revenue.
- Isopropanol producing Clostridia exist in the wild. State-of-the-art genetic engineering tools will be used by a subcontractor to rectify the isopropanol production in the robust AVAPClo clostridium. American Process Inc. will perform verification of the long-term viability of the resulting bacteria in the continuous fermentation system. The isopropanol conversion is targeted to be fully complete, but at least 80% is required to meet the current economic projections. We will update the life-cycle analysis and techno-economic model at the conclusion of the extended-duration runs.
- The separation of solvents from fermentation broth using liquid/liquid extraction proved efficient. The organic-to-aqueous ratio and number of solvent stages will be optimized, and scale-up for the commercial plant will be obtained via vendor.
- The DOE target MFSP at below $3/gge by 2020 is achievable from AVAP process generated sugars using pine feedstock, as well as minimizing sugar loss in conditioning and performing successful genetic engineering with value engineering to reduce capital cost. The production of a lignosulfonate byproduct is a very real potential upside, if the process integration proves excess lignin is available.
- struggle to match. Moving forward with all viable options is the best path. Clearly, we did not perform to the level we had hoped. However, we do feel that there are some very positive results coming from the work performed. Given that we were venturing into unknown terrain, there was always a risk to delivering a scale-up ready process.

ENGINEERING CLOSTRIDIA FOR N-BUTANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS AND CO_2

(WBS#: 2.3.2.203)

Recipient:	The Ohio State University
Principal Investigator:	Shang-Tian Yang
Project Dates:	8/1/2015–7/31/2017
Project Category:	Ongoing
Project Type:	FY 2013—Incubator: DE-FOA-0000974
Total DOE Funding:	$1,232,148

Project Description

This collaborative project between the Ohio State University, Green Biologics, and the University of Alabama aims to engineer novel Clostridium strains to produce n-butanol from low-cost lignocellulosic biomass and gases (CO_2 and H_2). Biobutanol is an advanced fuel that can fit the existing fuel infrastructure and directly replace gasoline in auto engines without modification. This project focuses on the metabolic engineering of Clostridium cellulovorans, a cellulosome-producing acidogen, for directly converting cellulose to n-butanol and ethanol, as well as carboxydotrophic acetogens to produce ethanol and butanol from CO_2 and H_2.

The engineered strains will be used in a consolidated bioprocess integrated with *in-situ* butanol separation to alleviate butanol toxicity and reduce energy consumption. The proposed co-fermentation process using both cellulose and CO_2/H_2 for biofuel production can greatly increase product yield from the biomass feedstock while also reducing greenhouse gas emissions by over 50% compared to current processes for butanol production. Metabolic and process engineering will be aided with proteomics and metabolomics analyses. The final optimized process is expected to be able to produce n-butanol from biomass, such as corn stover, at $2.25/gallon ($3.00/gge), which is much lower than the current butanol price (~$6.25/gallon) in the chemical market and would be competitive to use in the fuel market.

Weighted Project Score: 8.2

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

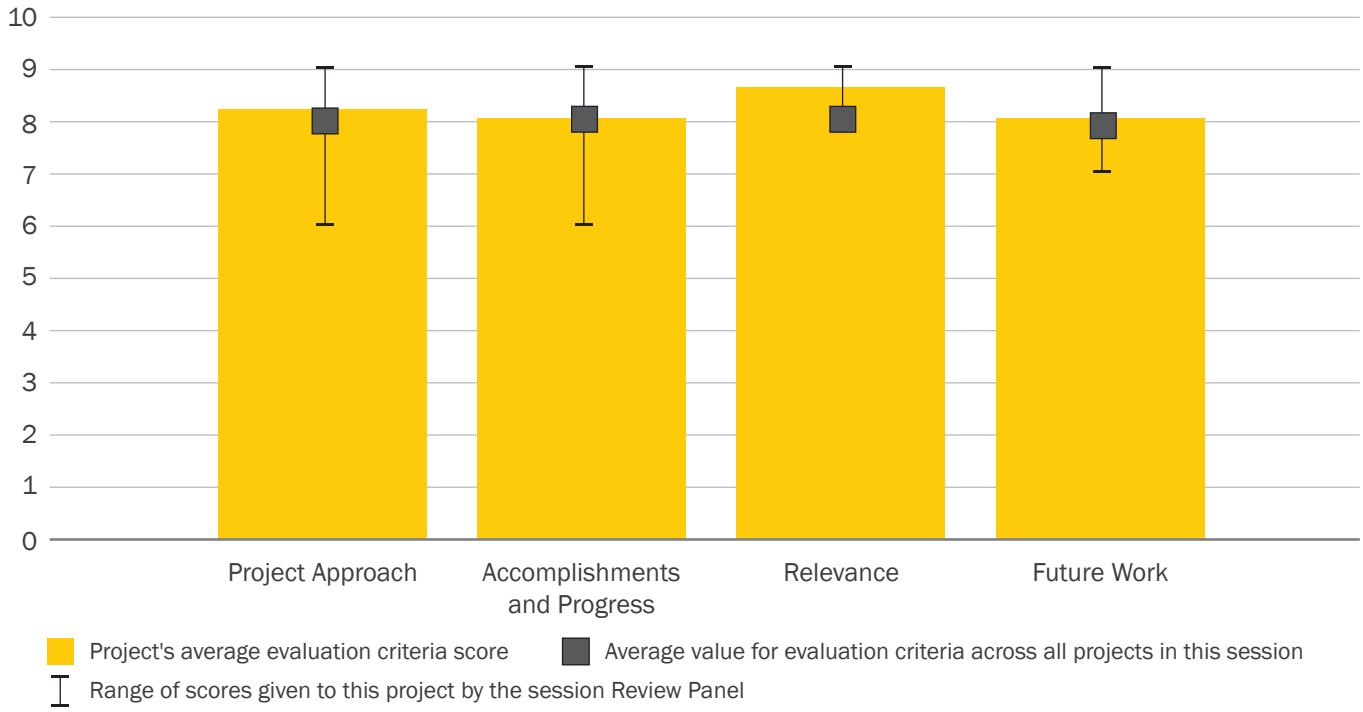


Photo Courtesy of The Ohio State University

Overall Impressions

- The presentation gave a strong impression of a well-structured, well-organized project that has brought together the right set of partners. The team has shown an excellent mix of logical research steps and innovative approaches, with a high degree of understanding of the underlying biology. The use of milestones, work breakdown structure, and go/no-go decision points looks very good.

  In terms of academia/industry partnerships and a project focused on BETO goals, this absolutely looks like a model project and a great use of funding.

- The PIs present a novel approach for direct conversion of biorefinery cellulose to biofuels through genetic engineering. Targeting cellulose as the substrate is a worthwhile goal, and if productivity issues can be developed, this might be a nice alternate approach to mixed alcohols. They further plan to improve carbon utilization by developing organisms that can consume CO_2 generated during fermentation and convert it into butanol. This is an interesting and potentially promising approach, but the team needs to update their preliminary economics in the short term to evaluate whether the overall process has industrial viability.

- This team is taking a novel approach to butanol production using two species of Clostridium: one that can consume cellulose and another than can consume CO_2. Engineering two organisms is a lot of work, so the team should do more thorough evaluation of the economics compared to the cellulosic organism alone (which should still offer benefit compared to the current state of the art due to CBP). Specifically, the team should determine how much benefit is gained from using the excess CO_2, considering that external H_2 would have to be added.

- Furthermore, it may be simpler to just engineer the first organism to uptake hydrogen, which will boost the alcohol yield due to the extra redox (as shown by methyl viologen addition), which would mean less CO_2 production in the first place. Overall, the net stoichiometry and redox balance is the same, whether it happens in one organism or two.

  For the targets set, the team has made great progress, but there is still a long way to commercialization with a lot of challenges, both biological and engineering.

- This is a well-organized project and is making good progress towards converting both biomass and "waste" CO_2 to fuel molecules in a CBP-like process. I personally favor the co-fermentation approach over asking one CBP organism to do everything. With similar strains, there is a reasonable chance of developing a robust single tank co-culture during both growth and production. Scale-up will be exciting!

- This is a very good example of an industry-academia relationship with direct contribution to BETO's mission and goals. The project team came with novel solutions (e.g., co-culture) and overcame many technical gaps in the way with very structured and metrified goal setting and milestones deliverables.

PI Response to Reviewer Comments

We appreciate the positive comments and confirmation from the reviewers on our progress so far. Regarding engineering the cellulolytic strain to uptake hydrogen, this would be very difficult to do, as uptake hydrogenases are complicated and difficult to express in a heterologous host. In contrast, we are taking the approach to engineer the strain with minimal CO_2 and H_2 production, so most substrate carbon will be in the final product, butanol. Any CO_2 and H_2 released from the cellulolytic strain will then be captured and used by the carboxydotrophic strain.

We understand that there is a long way toward eventual process scale-up and commercialization of the technology. Nevertheless, to demonstrate the technology concept and its feasibility and economical and environmental benefits in 2 years would meet the goal of this incubator program. Further development and commercialization decisions will be based on the results of TEA and life-cycle analysis studies toward the end of the project.

SECOND-GENERATION MIXOTROPHY FOR HIGHEST YIELD AND LEAST-EXPENSIVE BIOCHEMICAL PRODUCTION

(WBS#: 2.3.2.205)

Recipient:	White Dog Labs
Principal Investigator:	Shawn Jones
Project Dates:	9/1/2016–9/30/2018
Project Category:	New
Project Type:	FY 2014—Incubator II: DE-FOA-0001320
Total DOE Funding:	$1,539,826

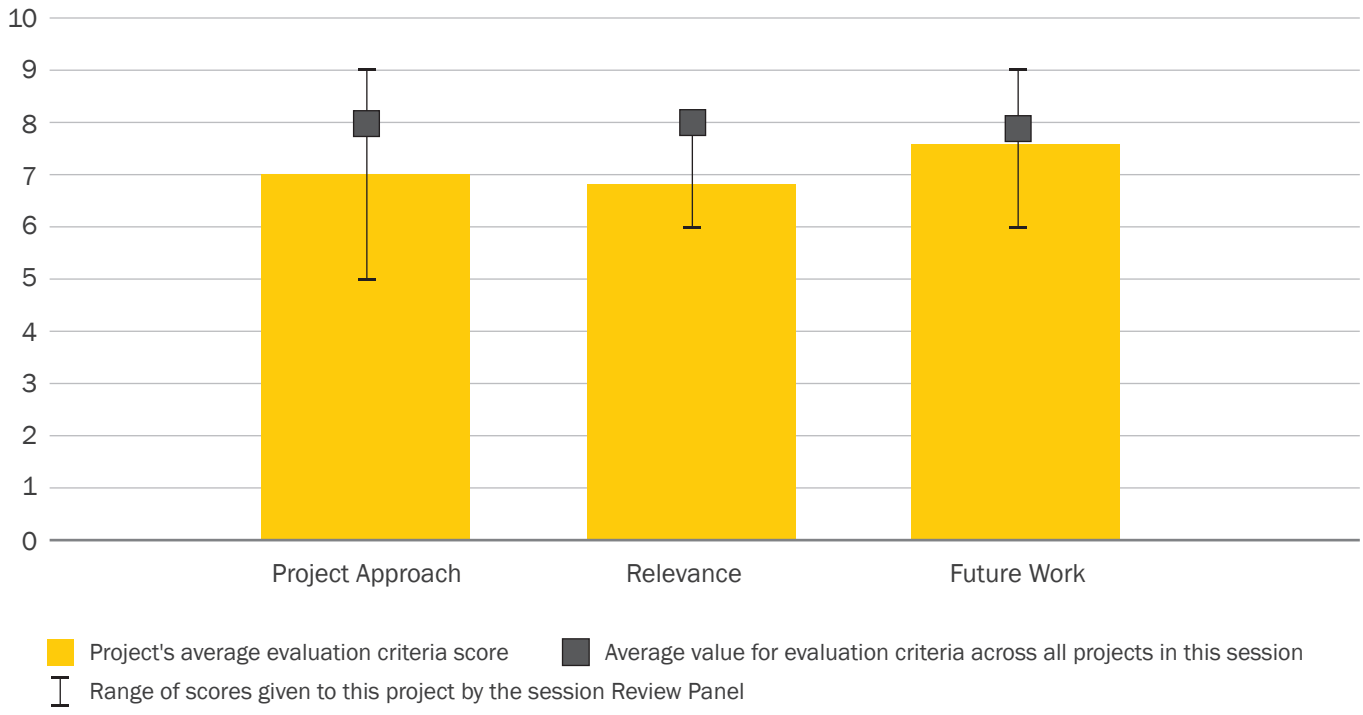
Project Description

The primary economic driver for second-generation biochemical/biofuel processes is feedstock cost and costs associated with feedstock's conversion to fermentable carbohydrates; therefore, maximizing the carbon yield of products is critical. However, with most conventional fermentations, at least one-third of the carbon feedstock is converted into CO_2 to produce the desired reduced products. To overcome this limitation, we have developed a fermentation technology called MixoFerm™ (also known as anaerobic, non-photosynthetic mixotrophy), which uses microorganisms capable of simultaneously consuming both organic (sugars) and inorganic (CO_2) substrates. With this technology, the CO_2 produced during the catabolism of sugar can be fixed back into product and significantly improve the carbon yield.

In this project, we plan to demonstrate the improvements in carbon yield by producing acetone from cellulosic hydrolysates at a mass yield at least 130% the theoretical maximum from conventional fermentation. In addition to the improved yield, we will demonstrate industrially relevant productivities and titer using a continuous, cell-retention fermentation system. This technology is a transformational platform improvement for biochemical/biofuel production as it can dramatically improve cellulosic carbon yields and be applied to nearly any metabolite of interest.

Weighted Project Score: 7.3

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



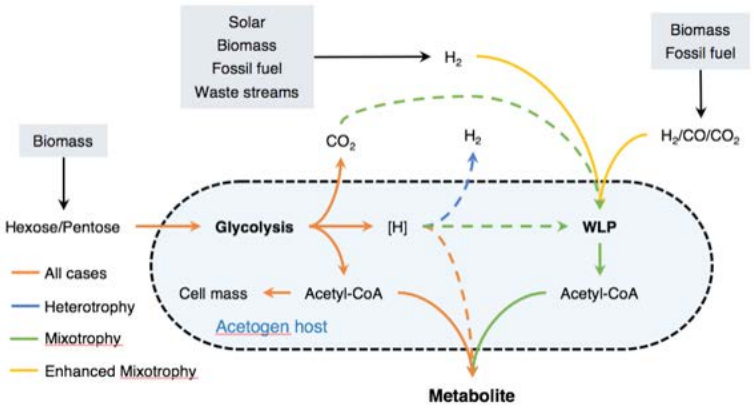


Photo Courtesy of White Dog Labs

Overall Impressions

- The goal of using mixotrophy to bring in carbon through the Wood-Ljungdahl pathway in conjunction with fermentation looks interesting.

  Adaptation of the strain to utilize glucose in addition to fructose through a combination of genetic manipulation and strain evolution was a good approach that appeared to work well. Addition of the Wood-Ljungdahl pathway and demonstration that the H_2 addition created the anticipated boost in yield on glucose looks good (the published work).

  The approach to future work looks oversimplified and overly optimistic. Dealing with lignocellulosic hydrolysates, in terms of performance of the organism and issues with continuous fermentation, is likely to be a significant challenge.

- The PIs are using an interesting concept of CO_2 capture and conversion to improve the carbon yield of a process targeting acetone as a product. However, the lack of any TEA or idea of what the cost of their acetone will be in comparison to commercially produced material is a significant weakness within the context of the BETO program. The PIs need to work closely with their program manager to develop a more compelling description of their project and its justification as a potential industrial process.

- Using a mixotrophic organism maximizes the benefit of carbon from biomass by re-utilizing some of the CO_2 given off. White Dog Labs is developing an organism that uses this process to produce acetone from cellulosic sugars. So far, they have made excellent progress toward their goals and have overcome initial challenges around glucose uptake and operation of a continuous cell-retention membrane. Beyond this project, a huge challenge will be scale-up for this fermenter configuration. In addition to the membrane fouling issue, contamination can be a big problem at industrial-scale continuous bioprocesses.

- In addition, it is not clear how acetone production will be profitable, regardless of technical success, since it is available at very low cost as a byproduct of the petrochemical industry. More market intelligence should be gathered, and then a thorough TEA performed.

- The opportunity to achieve 130% of conventional liquid fermentation yield is tantalizing. The project is making good progress to convert an acetogen to a mixotroph that can utilize biomass sugars. The cost-competitiveness of the process is an open question. The statement was made that the acetone could compete in the high-purity specialties market, but not the bulk market. Are there higher-value opportunities for this interesting technology?

- Overall, this is a good team with vast knowledge in strain engineering, fermentation, and process development, who can deliver, as illustrated in their recent publication, that increased yield of lignocellulosic sugars fermentations can be augmented with syngas fermentation. The business decision of focusing on acetone needs to be revisited, as this commodity chemical will be hard to replace with a biologically derived one. As the nature of this proj-

ect is to use acetone only to exemplify technology feasibility, this can be considered later as the project advances to higher TRL.

PI Response to Reviewer Comments

Most comments have been addressed in the previous comments. The additional comment made here regarding contamination of the process has been considered. We are planning for the entire fermentation process (both fermentation vessel and membrane filter) to be fully steam sterilizable in case of contamination. Additionally, in the plant design, we are building in multiple independent fermentation vessels and filter systems so that if one is down, the entire process does not stop. We are also working with an industrial design firm with deep experience in proper design of fermentation systems to reduce the possibility of contamination. Obviously, we cannot design a system to completely prevent contamination, but we are taking precautions to reduce the possibility and building in strategies to correct for a contamination, should it happen.

FERMENTATION PRODUCTION OF TRICARBOXYLIC ACID CYCLE (TCA)–DERIVED CHEMICALS USING CELLULOSIC SUGARS

(WBS#: 2.3.2.206)

Recipient:	Lygos Inc.
Principal Investigator:	Jeff Dietrich
Project Dates:	10/1/2016–9/30/2018
Project Category:	New
Project Type:	FY 2014—Incubator II: DE-FOA-0001320
Total DOE Funding:	$1,709,466

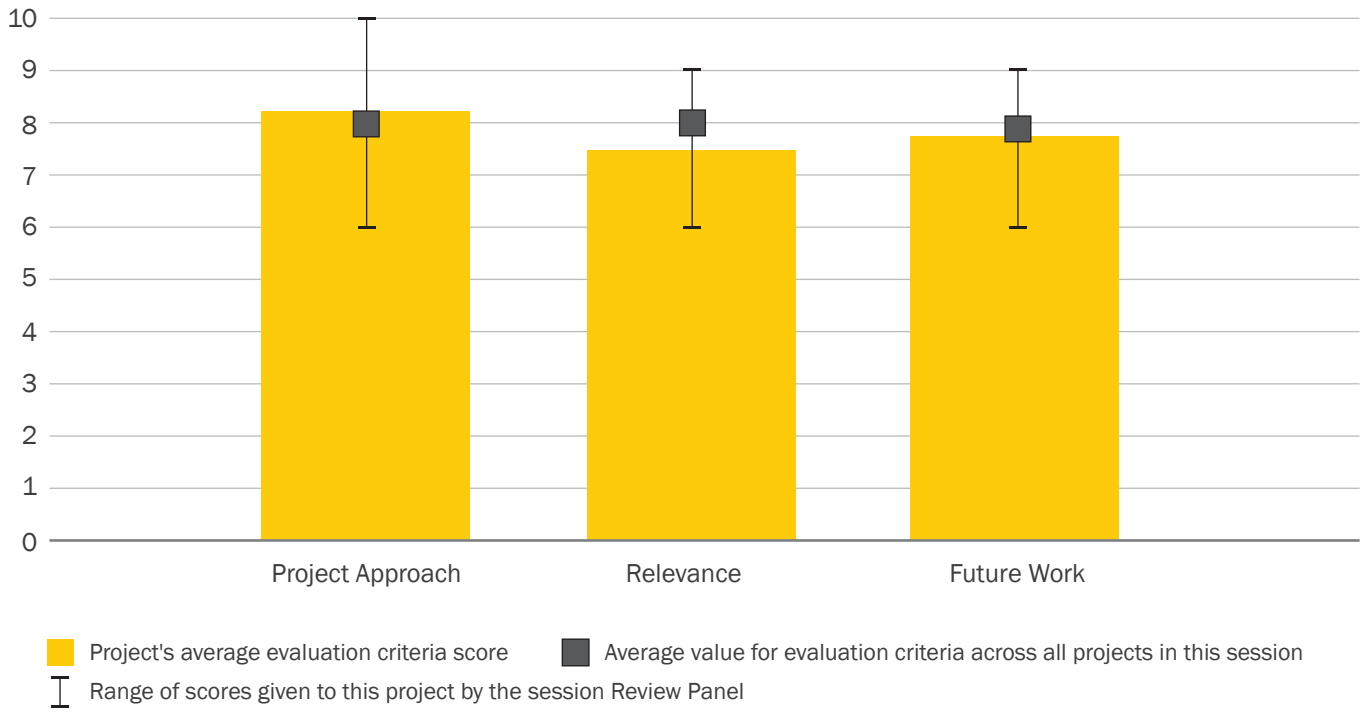
Project Description

Currently, the United States' chemicals industry is almost completely dependent on petroleum and natural gas feedstocks. Lygos is addressing this problem by developing microbial catalysts to convert renewable cellulosic sugars into higher-value commodity and specialty chemicals. Lygos is a part of the overall strategy to replace the whole barrel of oil and specifically targets "bio-advantaged chemicals," compounds that are expensive to make petrochemically and that can be produced biologically for less than the petrochemical raw material cost. These are chemicals where the market size is constrained by production cost, and a lower-cost, biological process can enable market growth.

The goal of this project is to develop an integrated process from cellulosic glucose through fermentative production of a high-value chemical derived from the tricarboxylic acid cycle. Biochemicals produced from the tricarboxylic acid cycle are excellent targets for fermentative production: they can be produced at high efficiencies and rates, driving production costs down. The outcome of successful project completion includes a cost-advantaged process to a high-value biochemical with a net reduction in greenhouse gas emissions relative to the competitive, petrochemical process. Commercialization of the technology as a bolt-on plant in an integrated biorefinery can also improve integrated biorefinery economics, driving biofuel production cost to below $3/gge.

Weighted Project Score: 7.8

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



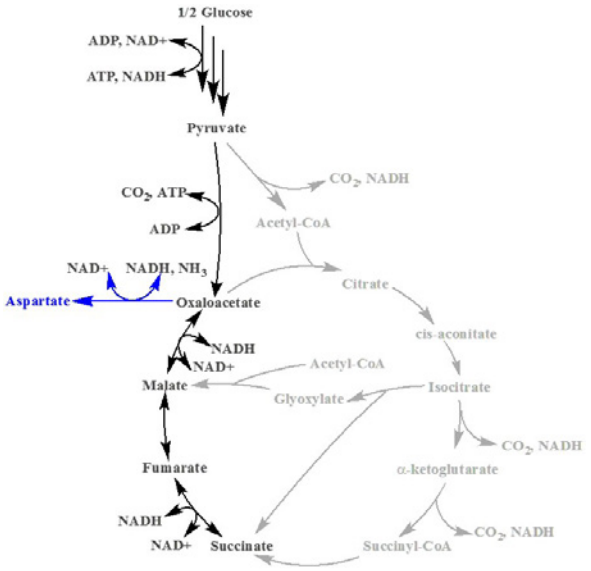


Photo Courtesy of Lygos Inc.

Overall Impressions

- The presentation was very clear, and the Lygos team demonstrated that they have a good grip on the steps they need to take to develop a novel process. The target of producing an organic acid from cellulosic sugar streams seems reasonable (with the caveat that the exact target molecule hasn't been disclosed). This aspect and the synthetic biology requirements for the project build on the technology platform that Lygos has built in the previous BETO-funded project. Overall, this looks like a great extension of that earlier work and again shows a well-thought-out approach.
- This is a very early-stage project and is based on a large amount of proprietary information. The broad strokes of the plan seem reasonable, as it appears to be a conventional organism-identification/engineering process, but a more detailed evaluation will need to wait until more results are obtained.
- Lygos is developing an acid-tolerant yeast strain for production of an undisclosed organic acid two steps away from the reductive tricarboxylic acid cycle. Due to CO_2 incorporation in the reductive tricarboxylic acid cycle, theoretical yields are very high. Technically, the team seems very well-positioned to hit project goals.
- Lygos demonstrated at Stage 1 incubator that they are capable to reduce effectively to practice the design-build-test-learn cycle, even without automation, with a clever biosensor and high-quality data analysis approach. I'm confident that they can deliver on the 2017 milestone and enable another BETO relevant product to enable a more sustainable production of organic acids utilizing waste stream (CO_2).

PI Response to Reviewer Comments:

- To clarify the difference in work plan between years 1 and 2: In year 1, we aim to demonstrate the activity of novel or poorly characterized enzymes in the engineered host; we divided the work plan into three modules, with each focusing on addressing one of three enzyme activities required for product biosynthesis under the anticipated commercial fermentation conditions. The goal at the end of year 1 is to successfully integrate all three modules into a single production host (i.e., providing the prototype strain). Achieving this goal will have addressed the majority of the enzyme and pathway risk. In year 2, the focus is placed on strain and process optimization; mass balances on glucose consumed will be used to assess the pathways that should be up- or down-regulated to direct flux away from biomass, CO_2, and byproduct formation. Additionally, in year 2 we begin both fermentation and downstream process development, two aspects of the technology that are stage-gated until after the prototype strain is successfully constructed.

  The fermentation milestones are all assessed using the same supplier of cellulosic glucose (and same lot number of glucose). This choice was intentional and allows us to perform longer-term studies to assess how strain, fermentation, and downstream processing process modification effect impurity levels at each stage in the integrated process (and enable us to draw more accurate comparisons with data from years earlier). Based on the impurity profile reported by the commercial supplier and our

in-house knowledge of strain tolerance, we do not anticipate impurity buildup to reach toxic levels in the fermentation. However, we will continue to monitor this (potential) problem over time and modify the strain or fermentation process as needed if issues are uncovered.

The reviewer's comment to provide more technical details in future public presentations is noted, and we look forward to providing a more in-depth discussion of the technology shortly as the intellectual property is published.

INTEGRATED PROCESS FOR COMMERCIAL PRODUCTION OF FARNESENE FROM DOMESTIC LIGNOCELLULOSIC FEEDSTOCK

(WBS#: 2.3.2.207)

Recipient:	Amyris
Principal Investigator:	Gale Wichmann
Project Dates:	10/1/2016–12/31/2019
Project Category:	New
Project Type:	FY 2016—MEGA-BIO
Total DOE Funding:	$7,000,000

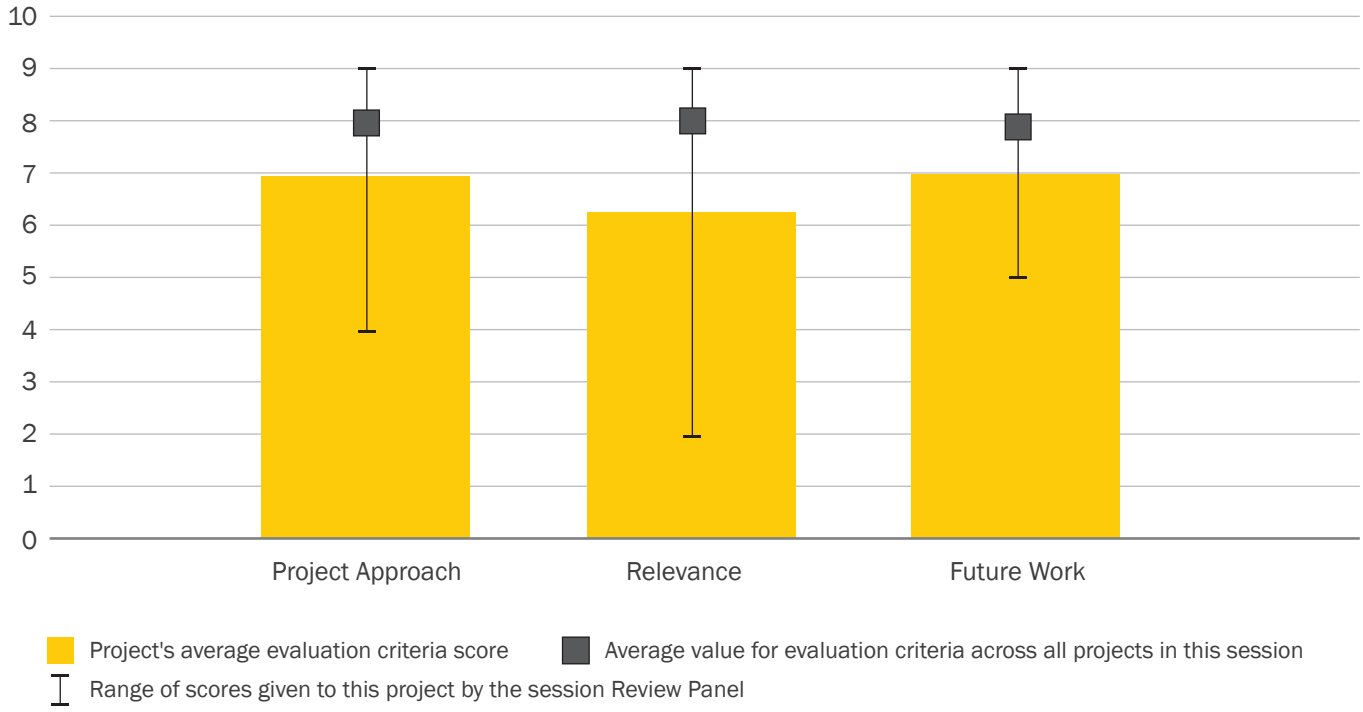
Project Description

This project proposes an integrated process for commercial production of farnesene, a versatile platform chemical, from domestic lignocellulosic feedstock. This project will develop an engineered yeast strain and a scalable, lignocellulosic-based manufacturing process for the production of farnesene for fuel and bioproducts from woody feedstocks. The work will be carried out by Amyris, Renmatix, and Total, three commercial entities with complementary capabilities.

Renmatix will employ its Plantrose® process that uses supercritical water to fractionate hemicellulosic and cellulosic sugars from pine. Amyris will develop a yeast strain and process for cost-effective conversion of Renmatix's cellulosic sugars into farnesene that is of equal quality to that produced today using cane syrup. This will involve engineering a farnesene manufacturing strain to consume the xylose found in biomass-derived sugars that will be resistant to cellular inhibitors present in the cellulosic sugar feedstocks. Total will conduct a thorough engineering study and TEA to provide production cost estimates and develop a rigorous life-cycle analysis to assess the environmental impact in support of the project's go/no-go decision points.

Weighted Project Score: 6.8

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



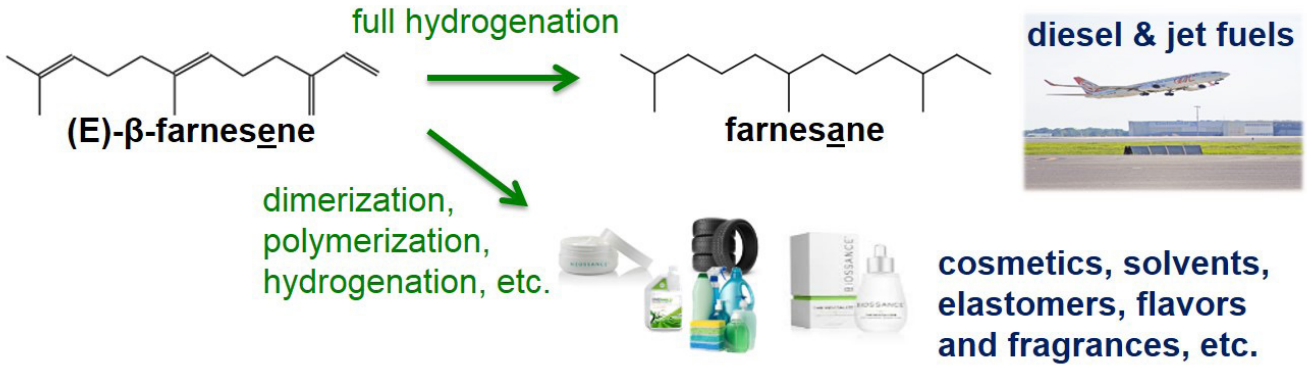


Photo Courtesy of Amyris

The final project goal is to develop a manufacturing-ready process to produce farnesene from cellulosic sugar in the United States at a manufacturing cost of $2.00/L.

Overall Impressions

- The project looks like a very appropriate combination and further extension of existing technologies at the partner companies. This builds on each of their expertise and has a goal that will provide a useful commercial process. It seems to have a good blend of solid technical foundation (e.g., Amyris' current farnesene process) and technical risk (extending to cellulosic sugars). There are technical challenges that can obviously be worked on separately, but a key component for the project will be cross-checks between the strain development effort and the pretreatment process development effort and regular transfers of pretreated material.

  Overall, the project looks to have been set up very well, making good use of the partners' expertise.

- Although the project was presented as an approach to fuels, the discussion seemed to pivot when the costs were examined to become a project targeting products. Close coordination of the Amyris team with BETO management will help to identify their exact interest in carrying out this work and improve its message, as they already make farnesene profitably in Brazil.

- Amyris is using this project to lower the cost of farnesene by the use of cellulosic sugars provided by Renmatix. The process for farnesene production is relatively mature compared to other product targets in the BETO portfolio; thus, this work is incremental in nature rather than groundbreaking. Technology is already available for xylose utilization in yeast, so it is rather straightforward to introduce these into the farnesene production strain. The project is already at a very high TRL, so this would be more appropriately funded by other mechanisms (e.g., loans) than by a BETO grant. In addition, since farnesene is not cost-competitive as a fuel, the product will likely be directed to small niche markets for the foreseeable future.

- This is a strong project with partners that have extensive experience in their respective areas. Farnesene is already profitable in non-fuel markets at $2/L from sugarcane syrup. Extending the feedstock range of the yeast to biomass-derived sugars and incorporating a new C5 metabolic pathway seems like a logical extension. The technical milestones are reasonable based on the SOT in the industry.

PI Response to Reviewer Comments

- We definitely were planning to use evolution strategies as well as rational engineering strategies for overcoming inhibitors.

  Earlier DOE-funded work at Amyris gives us confidence that our xylose utilization goals are feasible. However, the final >95% consumption goal is for the end of the project in 2019, not for the end of this year (2017).

For both the xylose consumption and the tolerance goals, we certainly plan to leverage existing technology where it is available to us. However, for IP and licensing reasons, it is often not possible to work with outside entities, either academic or industrial.

To address the reviewer comment about using a BETO grant versus a loan for this work, it is important to remember that while there are markets in which Amyris can sell farnesene profitably, neither Amyris nor Renmatix are yet profitable companies. Therefore, neither company can afford to fund projects (or would be willing to take a loan) for any project that does not have immediate revenue potential. Funding (in the form of a grant) is necessary to ensure a cellulosic-farnesene project is conducted. While both Renmatix and Amyris see the benefits that could ensue from this project, there is currently no logical justification to fund this work internally.

BIOLOGICAL CONVERSION OF THERMOCHEMICAL AQUEOUS STREAMS

(WBS#: 2.3.2.301)

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Gregg Beckham
Project Dates:	10/1/2013–9/30/2017
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$500,000
DOE Funding FY 2015:	$750,000
DOE Funding FY 2016:	$750,000
DOE Funding FY 2017:	$750,000

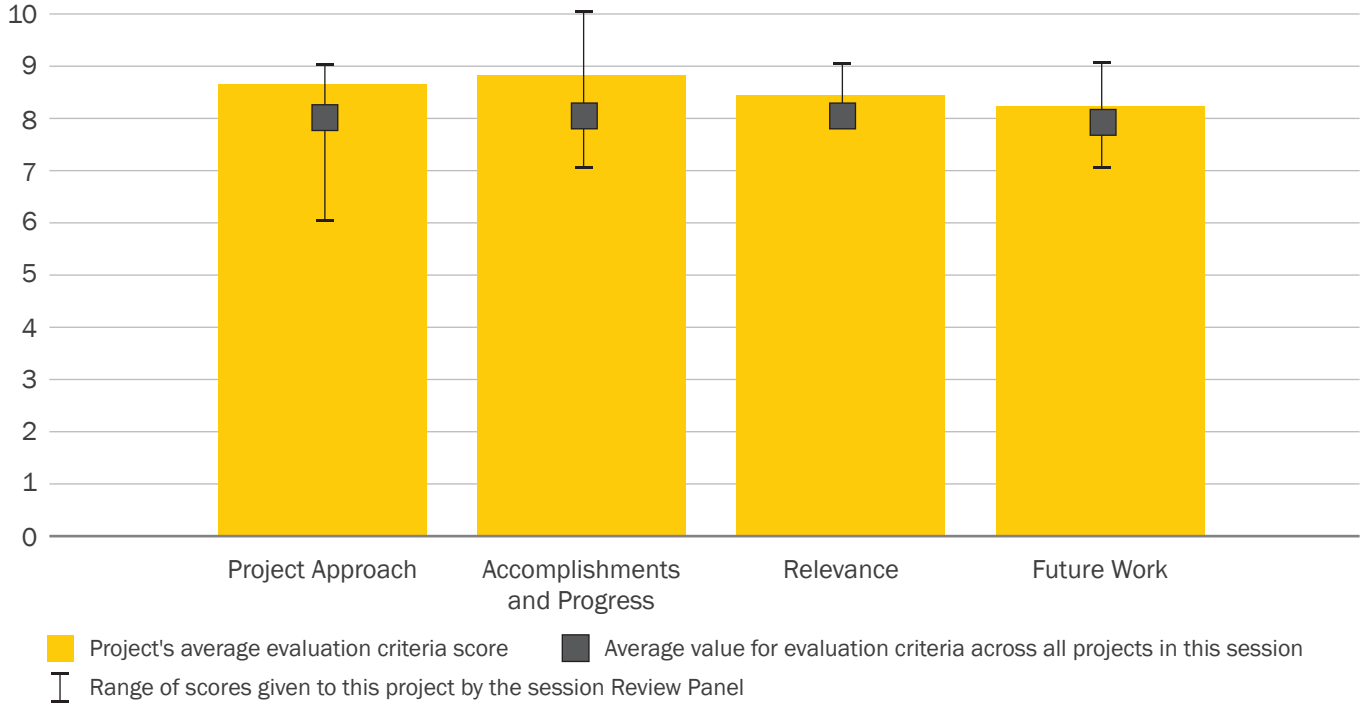
Project Description

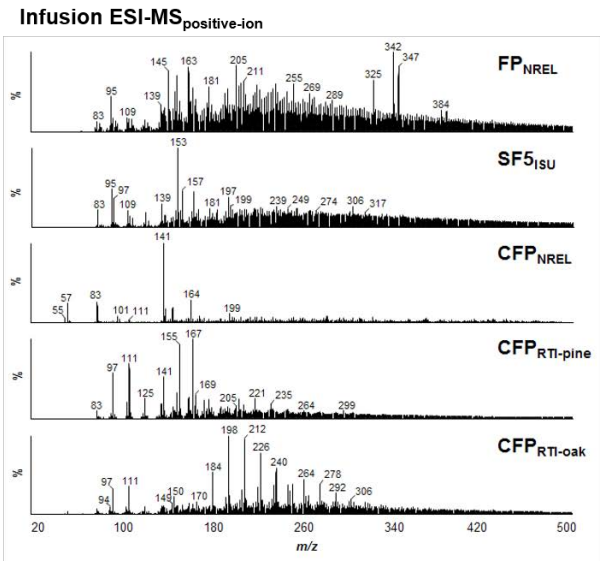
This project aims to develop integrated biological strategies to valorize the "waste" carbon present in thermochemical aqueous streams from pyrolysis processes. Overall, this project has two aims: first, we will develop robust analytical methods to characterize aqueous streams from thermochemical processes (e.g., fast and catalytic fast pyrolysis) with national laboratory, industrial, and academic collaborators; second, we are adapting the biological funneling idea—originally developed for lignin valorization—to convert carbon in thermochemical waste streams to value-added products (PHAs).

The motivation for this project is to transition from a process cost for wastewater to a process credit wherein process economics are improved via a value-added co-product. To date, we have conducted characterization of over 25 thermochemical aqueous streams with near complete mass closure and have identified >200 compounds, in turn enabling a down-selection to an ex-situ catalytic fast pyrolysis stream for focusing future strain development efforts. For the second aim of the project, we are employing a robust aromatic-catabolic microbe, Pseudomonas putida KT2440, to convert a wide range of carbon in aqueous streams, and we have demonstrated that over-expression of the native protein quality control machinery enables a two-order-of-magnitude improvement in the toxicity tolerance—a key scientific challenge for making

Weighted Project Score: 8.5

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





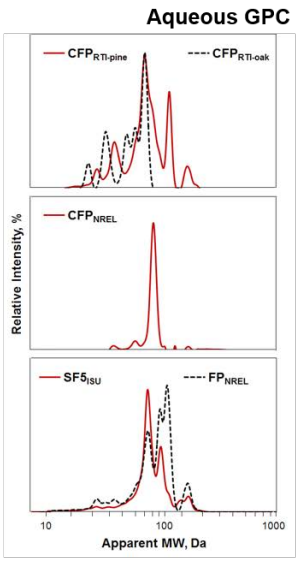


Photo Courtesy of National Renewable Energy Laboratory

this process concept contribute positively to a $3/gge cost target for 2022 via thermochemical conversion.

Overall Impressions

- The project looks well-managed, has a good and economically relevant target, and has made great progress so far. The chemical analysis of the waste streams and reaching the degrees of mass closure seen is in itself a tour de force. The pathway engineering strategy in P. putida looks very sound, with pathways identified to address the molecules seen in the waste streams. Only the biology will tell us what the limits of adding several new pathways to a single host may be, in terms of robustness or performance.

  The routes taken to increasing tolerance to protein damage have produced very good results (a 200x improvement in tolerance is impressive and suggests application to other systems). It would be good to bear in mind that, given that the strains may be able to actively digest the toxins, a fed-batch process may help the strains tolerate much higher levels of toxins than can be handled in batch.

  It would be interesting to think through the economics of applying the system to actively detoxifying other process streams, leaving behind sugars, for example, that can be readily utilized by a yeast system.

  Overall, this a great effort by the team, producing novel technical paths and excellent results.

- The PIs are developing one of the more exciting processes described, and the number of questions are a result of probing the potential of this approach. The ability for a single organism to navigate a huge range of functionalities and structures has the potential to be applicable in a wide range of areas. There are clear challenges, but the PIs recognize what is needed. Although it is a significant challenge to find an organism that can deal with a mixture of hundreds of materials, the PIs have found routes to make the funnel bigger. The presentation tells a nice, positive story regarding a concept that is quite straightforward.

- Aqueous waste streams account for 3%–10% of biomass carbon, currently sent to wastewater treatment. This project aims to create a monoculture that can not only remediate this stream, but turn it into a value-added product. One of the biggest accomplishments is the analytical characterization of these streams. P. putida was engineered to broaden substrate range, and a surprising increase in tolerance was obtained by overexpression of just GroES/EL

(heat shock 10 kilodalton protein 1 (Hsp10)/another protein of 57 kilodaltons).

The tolerance improvement here is likely sufficient. The organism has been engineered for utilization of various substrates, but the biggest challenge ahead is improving the utilization rates so that they will match the waste production rate without needing an enormous bioreactor. Also, if the waste treatment step is to be avoided, the organics have to be removed to an extremely low level. This is often challenging as catabolic pathways shut off at low substrate concentrations.

- Waste valorization is one of the keys to cost-effective biorefineries, both bio- and thermo-conversion processes. The use of bio-funneling is a clever approach to upgrading myriad waste carbon components into a value-added product, and possibly saving catalyst cost by decreasing the severity of the thermochemical conversion (allowing bioconversion to "mop up"). Since the 2015 review, PHA has been identified as an exemplary product, and a TEA is in progress and should lead to quantitative metrics moving forward.
- This is a good example of high-risk/high-gain project. It is a very ambitious project with dynamic moving targets of variability of waste stream composition and multiple toxicities (e.g., membrane fluidity, protein generation and repair, global stress response, acid tolerance, etc.). These challenges make it difficult to judge if this project will make it to the end goal, which is transfer to industry to develop a fermentation process for the complete use of wastewater from thermochemical processes to a biobased product. If successful, it will make a big impact on the economics of the thermochemical processes.

PI Response to Reviewer Comments

We thank the reviewers for the positive feedback and constructive comments. We completely agree that the approaches being developed here could potentially be useful for hydrolysate cleanup.

LIGNIN UTILIZATION

(WBS#: 2.3.4.100)

Project Description

This project aims to develop viable, scalable, and robust processes to produce value-added co-products from lignin, which will contribute $2–$4/gge MFSP credits to the 2022 BETO hydrocarbon fuel cost target of $3/gge. The project was founded upon the economic necessity to produce chemicals alongside fuels in a lignocellulosic biorefinery to ensure renewable hydrocarbon fuel selling prices are competitive with fossil-based fuels.

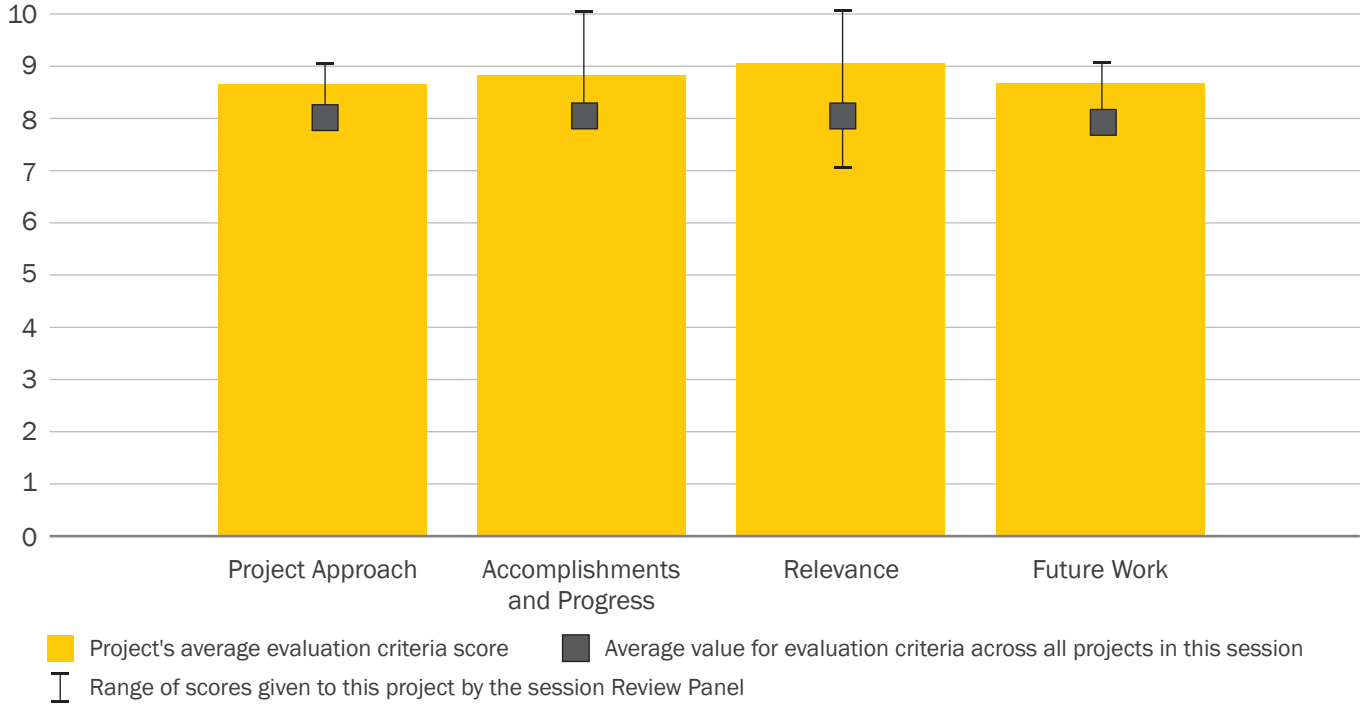
The Lignin Utilization project has two technical goals: first, we aim to isolate and depolymerize lignin-rich streams to high yields of aromatic monomers, and second, we aim to employ the "biological funneling" approach to convert heterogeneous slates of aromatic compounds to single-target chemicals, thus overcoming the heterogeneity challenge in lignin valorization. For the 2022 cost target, we have chosen adipic acid as a target from lignin via a bio-derived muconic acid intermediate.

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Gregg Beckham
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$1,000,000
DOE Funding FY 2015:	$1,000,000
DOE Funding FY 2016:	$1,000,000
DOE Funding FY 2017:	$1,500,000

To date, we have developed two high-pH treatments to recover high yields of lignin from whole biomass and are beginning to develop active, robust catalysts to further depolymerize lignin-rich streams using oxidation at high pH level. For adipic acid production, we have

Weighted Project Score: 8.7

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



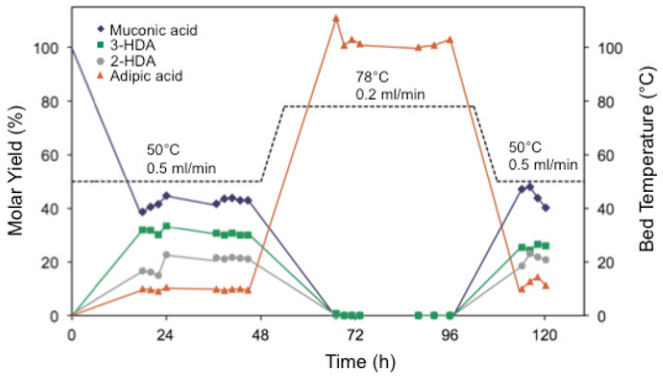


Photo Courtesy of National Renewable Energy Laboratory

demonstrated nearly 100% yield from a few relevant lignin-derived model compounds. The 2022 outcome of this project will be a fully integrated process from lignin to adipic acid at yield targets predicted from TEA.

Overall Impressions

- The project direction looks good. It has the right balance of analysis to understand the chemistry of the depolymerization processes and catalysis and process development. Obviously, it is closely tied to the Targeted Microbial Development project, and those appear to be closely managed. The approach covers a lot of ground in a logical manner and looks to be making good progress.

  The final determination for the success of the project will perhaps not be whether you can break down lignin chemically, or whether products can be used by a microbial strain (although both of those are great to demonstrate and certainly worthwhile to keep developing), but whether the process is economic in its own right; doesn't interfere with the economics of the sugars process (as is being addressed in this project); and is modeled properly in the TEAs covering both parts of a process scheme. Looking at some of the other TEAs, it can start to look like the lignin process is being used as a way to say "and then we reach $3/gge in 2022 with lignin valorization." It would be good to start making sure the numbers being applied to other TEAs from the lignin-derived co-product have some foundation in a modeled-out, full-on lignin stream/biofuel process.

- Lignin deconstruction and conversion is a critical part of biorefinery development and will be a key contributor to meeting BETO's goals. This project is doing an excellent job of addressing this challenge and has come up with interesting potential solutions to (1) converting lignin into a mixture of more-tractable low-molecular-weight compounds for eventual conversion, and (2) demonstrating high-yield conversion of lignin models (coumarate, ferulate) to muconate, an adipic acid precursor. Although the focus has been on NREL's current alkaline pretreatment, the project plans to make catalytic systems that work with lignin from any pretreatment process...however, this seems like a very large challenge, so it might be wise to test depolymerization and catalytic conversion with lignins from a range of known pretreatments.

  While all the pieces are not yet in place, the PI has done an excellent job of breaking the project down into manageable parts and addressing each part in turn. As a result, the project has a good chance for success.

- The Lignin Utilization program is essential to reaching the $3/gge objective, as lignin valorization is a key assumption of all the TEA performed. Nonetheless, this work is extremely challenging, and the team has done an amazing amount of work to de-risk both lignin depolymerization and upgrading. The team has also put together two steps that require very different scientific disciplines and, in addition, the need for complex analytical chemistry. The project has been very well-managed so that these groups all work well together, united toward the same overarching goals.

- It is commonly accepted now that lignin valorization will be the key to the success of near-term biorefineries. This is a well-designed approach to a

difficult problem. The proof of concept for each of the pieces of a process has been demonstrated (lignin extraction, depolymerization, and bioconversion to muconate). The challenge ahead is to integrate these pieces into a relevant process (could be pulp and paper first) with bioconversion of complex lignin aromatics. A number of new unit operations are being proposed, so keep an eye on the TEA and good luck! This project has the possibility to change the biorefinery equation.

- Overall, this is a very good project and a great example of how to connect things from end to end. This should be, in the future broken, down to lignin de-polymerization group (pretreatment, analytic, separation etc.) and upgrading group (i.e., Agile BioFoundry, Targeted Microbial Development, etc.).

PI Response to Reviewer Comments

- We thank the Review Panel for the positive feedback. As noted, we are conducting TEA with detailed models for the lignin valorization process trains. These TEAs are now being incorporated in the BETO MYPP to outline strategies and key process metrics to meet the out-year $3/gge cost goal through lignin utilization. We agree that this will be a key component of lignin valorization process economics.

We agree that the catalytic challenges here are considerable. Namely, the primary challenge going forward is developing catalytic systems that can cleave both C-O bonds and C-C bonds, the latter of which is especially challenging to do selectively at moderate temperatures. Fortunately, many groups are now working on C-C bond cleavage from a mechanistic standpoint, so we can leverage work from the scientific community in this vein to develop improved oxidation catalysts in a rational manner, which is a key aim going forward in the Lignin Utilization project.

We thank the reviewers for the positive comments on the integration and multidisciplinary aspects of the project. In terms of the way that this project is managed, we have tasks focused on analytics, lignin depolymerization (including pretreatment), and conversion to value-added compounds (both catalytically and biologically). In addition, the Lignin Utilization project works closely with other projects to leverage expertise and capabilities, including the Targeted Microbial Development project (for strain development), the Separations Consortium and the Separations Development and Application projects, and the various catalyst projects being funded by BETO at present, including the Computational Chemistry and Physics Consortium and the Advanced Catalyst Synthesis and Characterization Project.

RENEWABLE CARBON FIBER CONSORTIUM

(WBS#: 2.3.4.200-202)

Project Description

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Adam Bratis
Project Dates:	8/1/2015–12/31/2018
Project Category:	Ongoing
Project Type:	FY 2013—Carbon Fiber: DE-FOA-0000996
Total DOE Funding:	$5,325,790

The primary, overarching objective of the Renewable Carbon Fiber Consortium is to demonstrate the production of carbon fiber–based materials from acrylonitrile (ACN) produced from lignocellulosic biomass-derived sugars at a modeled ACN cost of $1/pound. The ultimate deliverable at the end of the project is 50 kg of ACN, which will be converted into a carbon fiber component for performance testing.

To accomplish this overall goal, the Renewable Carbon Fiber Consortium work is split into two phases. During Phase I (20 months), the team has been exploring the technical and economic viability of three biological/catalytic hybrid pathways that use biomass sugars as feedstocks at bench-scale and will demonstrate the production of at least 50 grams of ACN with concomitant polymerization, spinning, and testing of fibers. In Phase II (also 20 months), the team will optimize ACN production efficiencies and address remaining cost drivers for one of the pathways and scale up to 50 kg of ACN production. This material will then be used to produce a carbon fiber composite that will be tested side by side with a conventional carbon fiber composite to ensure equivalent product properties.

Weighted Project Score: 8.9

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

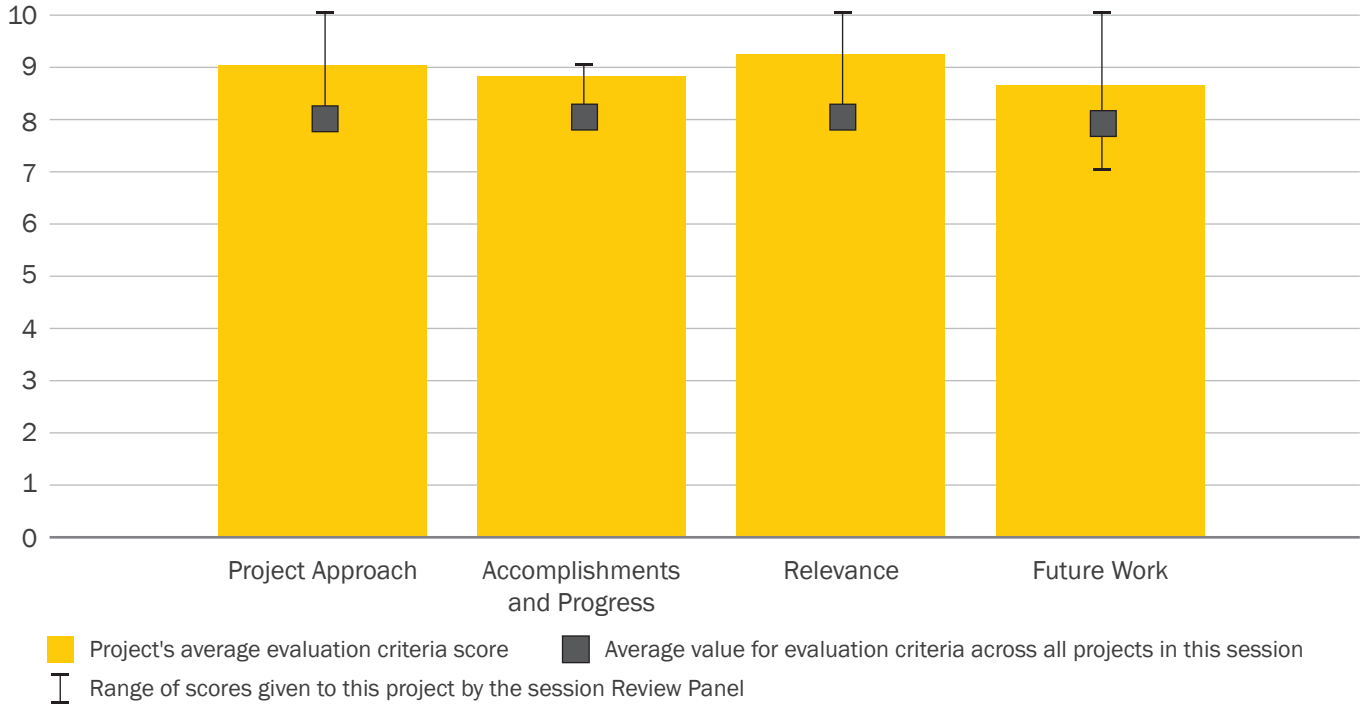


Photo Courtesy of National Renewable Energy Laboratory

To achieve these outcomes, the Renewable Carbon Fiber Consortium has assembled a team of experts across the value chain from the national labs (NREL, Oak Ridge National Laboratory, and Idaho National Laboratory), academia (University of Colorado, Colorado School of Mines, Michigan State University) and industry (Biochemtex, Johnson Matthey, DowAksa, and Ford).

Overall Impressions

- Overall, this is a really impressive project, not only for the technical trajectory, but the way the project has been run: the setup of the consortium, logical evaluation of options, use of TEAs, identification of advantages over other technologies, checking of the performance of the outputs early on, and engagement with industry partners. All these hit the right points. In several ways, this looks like a model project for public/private collaboration.
- The bio-ACN team has accomplished an elegant combination of biochemistry and catalytic conversion to develop a new route to a well-recognized industrial chemical. The effort proves that just because the target is simple, one should not assume that good science cannot be done. This project should be considered as one of BETO's wins for the program. It is a very nice effort and one that has an excellent potential for commercial success. Further, the work has been submitted as a publication to Science, and I believe it will have an excellent chance of acceptance.
- The current ACN production from propylene is a complex and hard-to-control reaction, with a toxic byproduct. This program will provide a renewable route, which will also be cost-effective according to the TEA. The team has achieved all Phase I goals and appears to be on track for achieving the ultimate $1/pound goal. A key decision the team must face is to engineer the 3-hydroxypropionic acid (3HP) organism for use of cellulosic (second-generation) sugars at low pH or use lactic acid organism which is ready, but has less ideal downstream process. The barrier to the 3HP strain using the biomass sugars is unclear. Is it the C5 content or the presence of inhibitors? Either of these could be overcome with some research effort. Before making a decision, do a return on investment assessment of the extra work it will take to accomplish this. Lactic acid may be quicker to market, but with 3HP you can also leverage other outlets besides ACN (such as acrylic acid).
- The presenter made a strong case for ACN and carbon fiber as targets for sugar upgrading. The chemistry and biology are mostly known, so the project is empirically determining the best combination of strains, intermediates, and process. Sufficient ACN has already been made for carbon fiber production and testing (no results presented). The project is also developing alternative new nitrilation chemistry for ACN production. This is a very interesting project.
- Overall, this is a good project and on the right track. Achieving high TRY of low-pH 3HP or lactic acids with a high quality of product in the end (minimal impurities) is going to be a prerequisite for this project. The project team is well aware of the challenge and is partnering with the right teams to overcome it. The team is advised to partner faster with industry to accomplish this or its build own strains, which might take longer than the project and BETO

can afford. If the latter is the case, it is advised to use the Agile BioFoundry and/or advanced strain development and evolution tools to accelerate. The nitrilation and generating relatively pure bio-ACN seem to be feasible.

PI Response to Reviewer Comments

- We thank the Review Panel for the positive comments and constructive feedback.

  We note that we have not met the Phase I targets for the 3HP strain in terms of productivity and yield on biomass hydrolysate, but we have done so for lactic acid. The 3HP strains are quite inhibited by compounds in biomass hydrolysate. Moreover, the overall yield of 3HP in E. coli is lower by at least a factor of two relative to lactic acid, from a theoretical yield perspective.

  While we fully agree with the reviewer that overcoming biomass hydrolysate toxicity and C5 sugar utilization is a problem that can be solved, it will likely be a fairly long undertaking relative to the overall integrated process development timeline for this project. As such, we do not feel that this is a realistic effort to achieve the Phase I targets for TRY for 3HP production. Moreover, the higher inherent yields (approximately double) on a mass basis of lactic acid relative to 3HP, the ability to produce lactic acid at low pH industrially, and the ability to produce lactic acid anaerobically suggest that lactic acid is advantaged from a biological perspective. As the Review Panel notes, the chemistry is somewhat different and requires more steps for lactic acid, and we are currently conducting rigorous TEA and life-cycle assessment to understand the tradeoffs. Preliminary comparative TEA of the 3HP cases and the lactic acid cases suggests that both can achieve the target of <$1/pound, and using the chemical approach we are pursuing on lactic acid, we can achieve nearly 100% yield of acrylic acid as well.

  In terms of the SOT for strain development, we note that we have achieved >100 g/L at >3 g/L/hour and near-theoretical yield of lactic acid from biomass hydrolysate using a natural thermophilic strain. We think that this offers us a rapid way to meet Phase I targets and to scale up the process in Phase II.

ENGINEERING THERMOPHILES TO PRODUCE DROP-IN FUELS FROM SYNGAS

(WBS#: 2.3.4.204)

Recipient:	Kiverdi
Principal Investigator:	Dr. George Rudenko
Project Dates:	10/1/2015–9/30/2017
Project Category:	Ongoing
Project Type:	FY 2013—Incubator: DE-FOA-0000974
Total DOE Funding:	$886,322

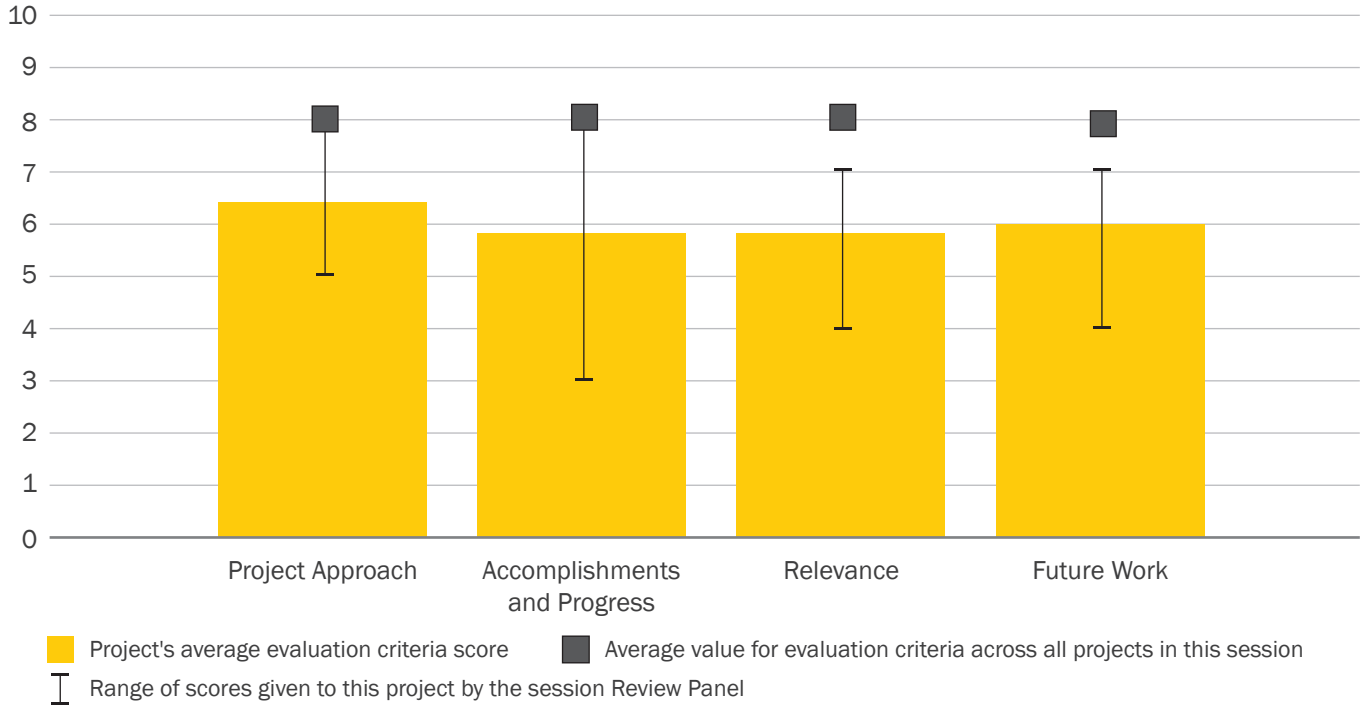
Project Description

Conversion of renewable feedstocks to bioproducts is limited by the heterogeneous and recalcitrant nature of the feedstocks. Multiple-step preparation of biomass for microbial conversion increases cost, complexity, and waste. An alternative is converting biomass to syngas, a more homogenous carbon monoxide (CO) and H_2 output that can be consumed by microbes. The use of syngas as a microbial feedstock represents a new combination of technologies for BETO's Annual and Multi-Year Program Plans, where the use of syngas as a feedstock is currently limited to chemical fuel synthesis. The biological conversion of gasified biomass into fuels is limited by the lack of robust syngas-utilizing microbes amenable to genetic engineering and tolerant of syngas impurities.

To address these issues, Kiverdi and NREL are jointly developing genetic tools and fermentation technologies to produce terpenes in thermophilic syngas-consuming microbes. Foremost, we are establishing basic and essential genetic engineering capabilities for transformation, selection, stable plasmid-based gene expression, and gene knock-outs, building new capabilities from previous advances in these fields. We aim to demonstrate the applicability of a microbial system for fuel synthesis by producing limonene, a next-generation

Weighted Project Score: 6.0

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



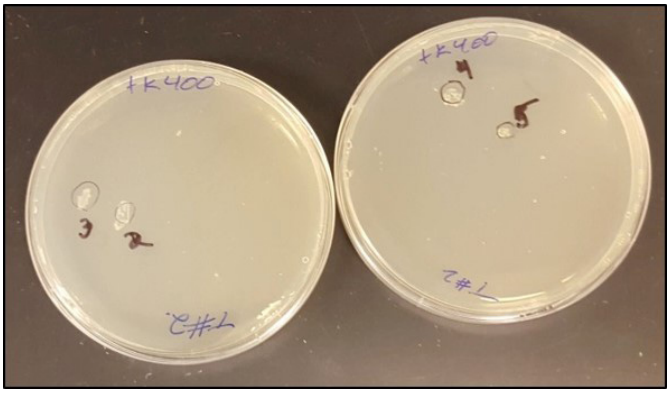


Photo Courtesy of Kiverdi

terpenoid biofuel, through the heterologous expression of thermostable enzymes.

Overall Impressions

- The project was hugely ambitious: selecting a new class of potential fuel molecules; starting from the point of screening for a new thermophilic chassis organism; requiring a plant enzyme to work at relatively high temperatures in a bacterium; needing to develop genetic methods; working in an anaerobic system. For a metabolic engineering project in this timeframe, this represents a massive challenge.

  The question is going to be how far can the project team get in the remaining 6 months, and what can be proven out to support further investment? A good-quality TEA would be crucial to demonstrate the benefit of producing this type of product in a thermophilic host, particularly on the product recovery side. Demonstration of targeted integration of a marker in the genome would be good to show that gene deletions can be made effectively.

  Given so many moving pieces and critical tests to complete, I would highly recommend looking at the project management tools being used. It did not come through strongly in the presentation that this was a central part of the project.

- The PIs have successfully evaluated a number of thermophiles and developed genetic tools that have resulted in a thermophile that incorporates thermotolerant enzymes for the eventual production of terpenes. However, the project also needs to evaluate the necessary syngas specification, determine productivity, and evaluate costs of the terpenes as a function of syngas composition, whether gas shift will be needed and the amount of fuel that can be reasonably produced by this route.

- Various groups are looking at engineering syngas-utilizing organisms and have made some progress. This team is taking a slightly different approach, using thermophilic organisms instead of the standard set of CO/H_2 utilizers used in most research. Although high temperature could offer some advantages, it is not clear that these advantages outweigh the ability to leverage previous work for better-characterized organisms. Similarly, how do the economics of terpene production compare to making alcohols, and does it merit the additional work required? Otherwise, the team has made good progress on goals but is still a long way from commercialization.

- This project aims to convert syngas to monoterpenes in thermophilic anaerobic continuous fermentations. Each of these aspects has challenges, and the project has had numerous false starts in strain selection. Original and revised goals are not defined. It is a nice concept and worthwhile to develop such a strain for the future. There is a long way to go to demonstrate it in a process.

- The project team, despite the many challenges, managed to sort a few pieces (e.g., strain, expression tools, etc.). The project, in my mind could, have started differently as more of an exploratory work to vet some of the underlying assumptions under Kiverdi and/or NREL through laboratory-directed R&D. The many challenges appeared unrealistic to overcome, and BETO should consider analyzing the technical and economic feasibility of such projects more rigorously in the future.

PI Response to Reviewer Comments

- We appreciate that the reviewers recognize the ambitious nature of this project; we are likewise aware of this fact. Given this, we have focused efforts almost entirely on moving the research forward as effectively as possible. This focus was reflected in the Peer Review presentation, which was almost entirely focused on the scientific accomplishments, while the TEA and management plans were sparsely addressed. We appreciate this feedback and have responded to these comments by accelerating efforts to complete the TEA at NREL and hiring a "VP of operations" at Kiverdi to formalize and integrate the use of appropriate management tools.

  We are aware of efforts on other fronts to engineer CO/H_2-utilizing microbes and agree that following on that work could be a reasonable course of action. We originally decided to pursue anaerobic chemoautotrophic thermophiles because long-term continuous fermentation on industrial scales is very susceptible to contamination. Such contaminations significantly impact the economics of any bioprocess, from yogurt and cheese manufacture to pharmaceuticals. We hypothesized that the selective pressures associated with thermophilic and chemoautotrophic growth would permit longer-term fermentation runs and significantly reduce batch losses and production costs, thereby improving the economics relative to mesophilic approaches. An argument can be made that these advantages do not "merit the additional work"; however, this has yet to be formally considered and tested, and therefore, we proposed to initiate the work needed to test these ideas.

  We focused on monoterpenes as a product because they command a higher price (on a per-weight basis) than the short-chain alcohols produced by the more standard CO/H_2 strains. It is thought these higher-value, near-term commercial applications can help finance future development of the strain and process beyond the BETO project, in order to reach the performance levels and scale ultimately required for fuel production. This general strategy of focusing on fuel molecules with higher-value, near-term chemical applications has been adopted widely in the space. Additionally, as a fuel, the monoterpenes have a number of superior characteristics compared to short-chain alcohols. Since they are middle distillate hydrocarbons, without oxygen, they have significantly higher energy density and specific energy than short-chain alcohols. The chemical characteristics of monoterpenes also offer greater potential in jet fuel applications than alcohols. The PI disagrees with the view that this project supports more rigorous project vetting. Understandably, funding agencies want to have an investment portfolio with maximal returns. However, if projects are judged entirely on alignment with previous work, ongoing work, and "assured" commercialization in the term of a 2-year project, much is lost. I encourage BETO and other federal funding agencies to continue to resist the temptation to only fund the most conservative projects and continue to support out-of-the-box thinking and research. Innovation and a diverse research portfolio cross-pollenate thinking and lines of investigation, and it is my view that this kind of diversity fosters creative solutions and true innovation regardless of the current metrics for "success."

  We appreciate the reviewers' time and effort to consider our project and would respond positively if further feedback was requested.

DEVELOPMENT OF A SUSTAINABLE GREEN CHEMISTRY PLATFORM FOR PRODUCTION OF ACETONE AND DOWNSTREAM DROP-IN FUEL AND COMMODITY PRODUCTS DIRECTLY FROM BIOMASS SYNGAS VIA A NOVEL ENERGY-CONSERVING ROUTE IN ENGINEERED ACETOGENIC BACTERIA

(WBS#: 2.3.4.205)

Recipient:	LanzaTech Inc.
Principal Investigator:	Sarah Ye
Project Dates:	10/1/2016–9/30/2018
Project Category:	New
Project Type:	FY 2014—Incubator II: DE-FOA-0001320
Total DOE Funding:	$1,441,115

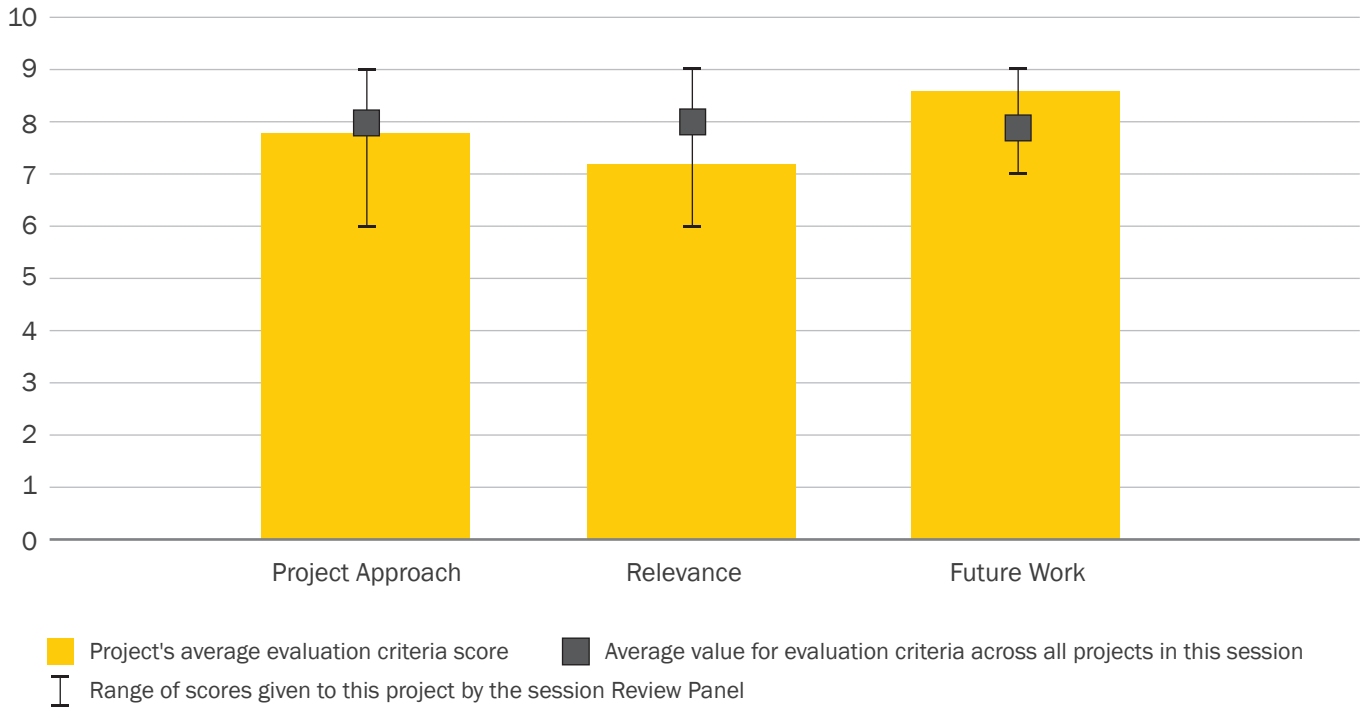
Project Description

LanzaTech and Oak Ridge National Laboratory are developing and scaling up a process to sustainably produce acetone and downstream drop-in fuel and commodity products directly from biomass syngas via a novel energy-conserving route in engineered acetogenic bacteria. This offers a safer and more environmentally friendly production method for acetone than the current phenol-dependent method, and the product will have significantly lower greenhouse gas emissions.

The developed process offers a cost-competitive route to acetone and enables biofuels at or below DOE's $3/gge target. In addition, it also provides an attractive biological alternative to traditional sugar-based acetone-butanol-ethanol fermentation by enabling utilization of non-food biomass resources as fermentation feedstocks. Challenges include the following: (1) Acetate as a byproduct reduces yield and stability. We addressed this by developing a synthetic acetate-independent pathway. (2) A synthetic pathway requires improvements in efficiency. The project is developing a screen to select more-efficient enzyme variants. By month 12, we will

Weighted Project Score: 8.1

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



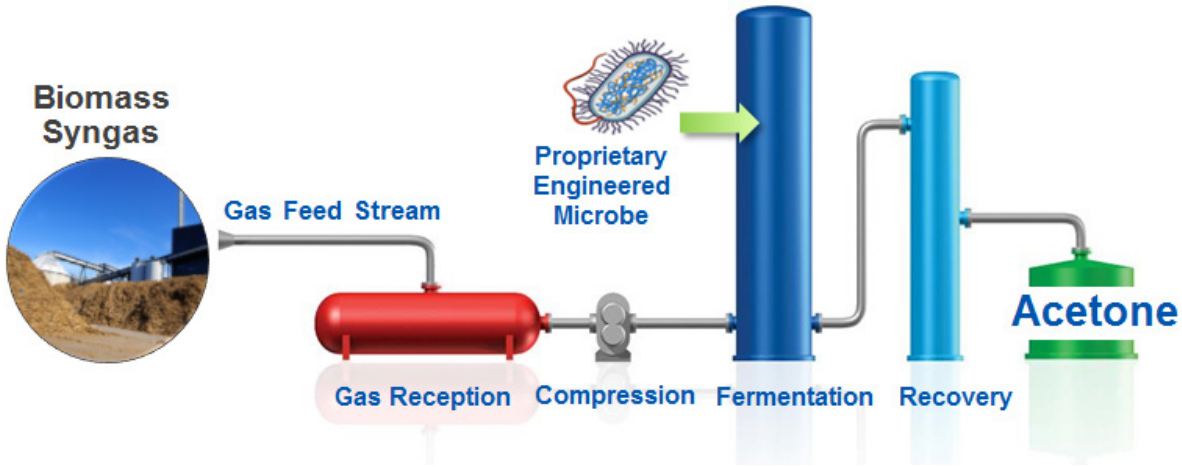


Photo Courtesy of LanzaTech Inc.

demonstrate fermentation stability for acetone production for 7 days at 50% of the commercial rate and 35% of commercial titer. By month 24 (project end), we will demonstrate stable acetone production at a commercially viable rate and titer for 4-day stable production in a scalable reactor.

Overall Impressions

- The project looks to be very well-structured and organized on a project management basis. The tasks look to be well within the technical capability of LanzaTech; they build on LanzaTech's internal technology development efforts and what seems to be a long-term collaboration with Oak Ridge National Laboratory.

  The technical approach looks sound, removing competing pathways for the flow of carbon to acetone and expression of a novel pathway that would favor growth by production of adenosine triphosphate.

  More information on the market potential for acetone would have been useful (explanation for future drop-in phenol production). Otherwise, production of a molecule that has existing routes to fuels, in particular, from a wide range of biomass feedstocks via syngas looks to be in alignment with BETO goals.

- The project has assembled a nice array of tools and methodologies to address the problem, but it would greatly benefit from a much clearer presentation of the potential cost of acetone through this process. It's a commodity and must compete on cost. If this process has a much higher cost, then it will ultimately be noncompetitive in the chemical market.

- LanzaTech is developing a Clostridium strain for conversion of syngas to acetone. They have a lot of experience engineering these strains and developing gas fermentation processes, so the chance of success is high. However, it is not clear how acetone production will be profitable, regardless of technical success, since it is available at very low cost as a byproduct of the petrochemical industry. More market intelligence should be gathered, and then a thorough TEA performed.

- This project has the potential to replace acetone production (which is declining while demand is increasing) from petroleum-derived phenol and significantly reduce greenhouse gas emissions. Two pathways are being developed in parallel, one with much greater yield potential than the other. TEA might help to focus the work if only one path is economically viable.

- The project is well-structured and building upon a good long-term relationship between Oak Ridge National Laboratory and LanzaTech. The novelty of increasing flux to acetone through a unique metabolic pathway will enable, if successful, higher flux and

a more sustainable production process of acetone. The business case for "green" acetone is questionable, and the project team and BETO are advised to rigorously evaluate the merit of this product.

PI Response to Reviewer Comments

- The demand for acetone in the U.S., European, and Asian markets is estimated at 6.4 million tons per year and is valued at $7 billion per annum. Acetone is also a direct precursor of valuable downstream products, such as direct drop-in fuels, fuel additives, polymers, and important chemical building blocks. In addition to its direct use, acetone can serve as a platform intermediate for conversion to a number of downstream products, including propylene ($125 billion), isobutylene ($25 billion), bisphenol A ($10 billion), poly(methyl methacrylate) (a fast-growing $7 billion market), and drop-in fuel isooctane, further diversifying the utility of renewable acetone as a co-product.

  The wide range of uses for acetone, in conjunction with the increasing average market price (the 2016 price was roughly 50% higher than 2015), will help offset the effective cost of the fuel product, thus enabling us to meet the $3/gge target set forth.

  LanzaTech has developed detailed techno-economic models that we use to consistently evaluate the economics of our process. Acetone as co-product can enable ethanol fuel production with a target price of $3/gge. Per this TEA analysis, a 1:1 acetone to ethanol ratio enables meeting this $3/gge fuel target.

BIO-SYNGAS TO FATTY ALCOHOLS AS A PATHWAY TO FUELS

(WBS#: 2.3.4.207)

Recipient:	Dow
Principal Investigator:	Devon Rosenfeld
Project Dates:	10/1/2016–12/31/2018
Project Category:	New
Project Type:	FY 2016—MEGA-BIO
Total DOE Funding:	$1,988,690

Project Description

The Dow Chemical Company is developing a process for the bioconversion of biomass-derived syngas to fatty alcohols as a pathway to biofuels. The fermentation of bio-syngas from lignocellulosic biomass will decouple the biofuel supply chain from the food chain. The production of intermediate fatty alcohols offers a unique opportunity to traverse the "valley of death" for biofuel process and infrastructure development by leveraging the robust chemical markets and high-margin applications of fatty alcohols and their derivatives.

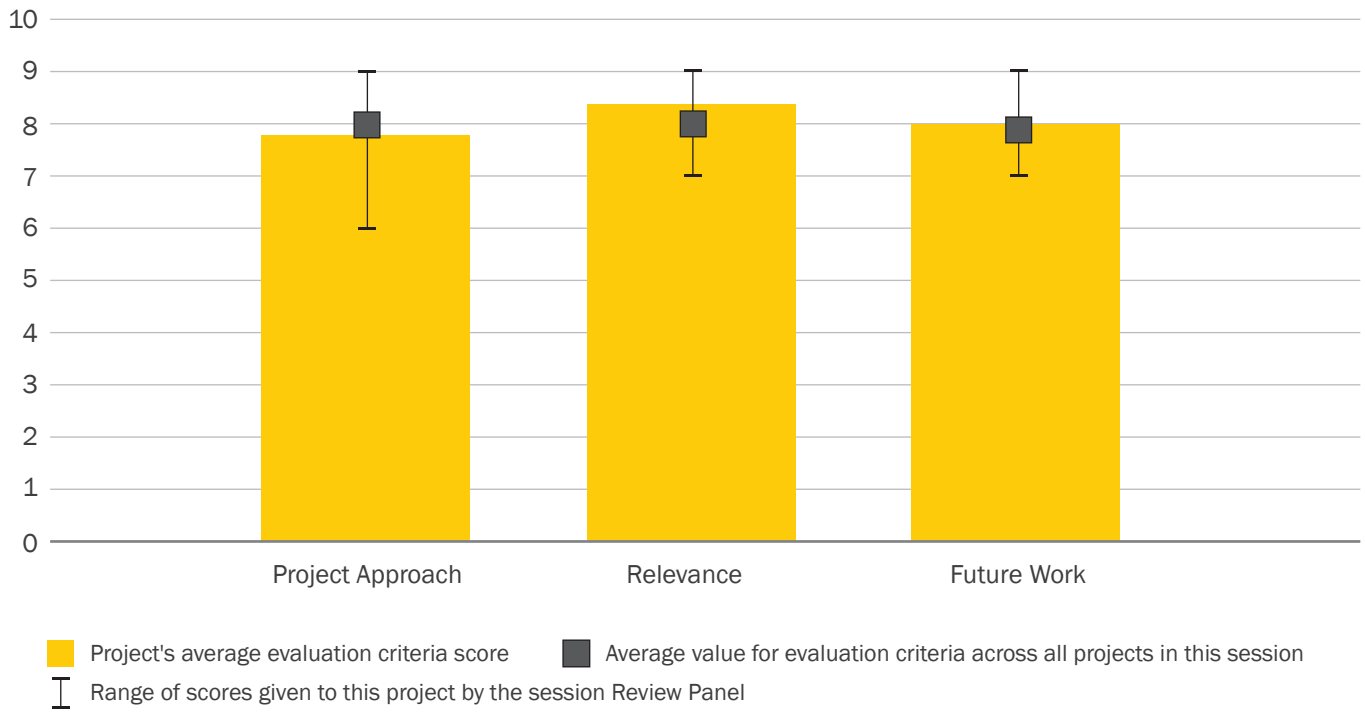
Previous laboratory experiments validated that enzymatic pathways developed within Dow produced fatty alcohols by syngas fermentation within LanzaTech's Clostridium and demonstrated bottlenecks limiting overall alcohol yield. In the proposed research, we'll deploy the syngas fermentation and strain engineering expertise of LanzaTech, computational modeling capabilities of Northwestern University, and process development expertise of Dow to remove bottlenecks discovered in the previously developed metabolic pathway—maximizing the production of C6–C14 alcohols and devising means to purify the products for channeling into the chemical derivative and fuel markets. This process will change the paradigm for biofuels production, enabling the sale of biofuel for <$3/gge while vastly improving sustainability.

Overall Impressions

- Overall, this looks like a worthwhile effort to further improve a syngas–to–fatty alcohols process across

Weighted Project Score: 8.1

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



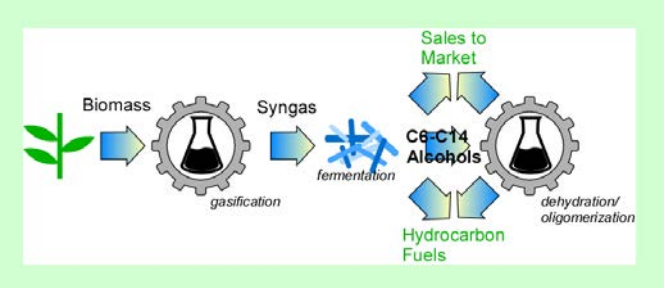


Photo Courtesy of Dow

several technical aspects and drive down costs. Some further explanation of the economics and markets for intermediate fatty alcohols (C6–C14) and a broader view of the technical challenges and risks in the project would have been helpful.

- This is a very early-stage project with no results yet. The overall plan seems reasonable, but the team will need to show the effect of syngas composition and source on the ability to make lipids.
- The project aims to convert syngas to C6–C14 fatty alcohols, which are existing chemical products and can be converted to fuels. If successful, this could be a breakthrough technology that could contribute significantly to the MYPP goals. This is a very challenging strain engineering effort, but if successful, the experience of the two partners positions them well for commercialization. The team may be underestimating the challenge of identifying enzymes that work preferentially on longer-chain-length compounds. They are leveraging Northwestern's computational methods to identify target enzymes, but predicting substrate range and specificity is unreliable. So, many enzymes should be screened, and the need for enzyme engineering should be anticipated.
- This is a challenging metabolic engineering project that has gotten off to a good start due to the unique expertise of each of the partners. This will be a fun project to watch in coming years. Product markets are well-understood and accessible by the lead partner.
- The project is well-aligned with BETO's MEGA-BIO FOA and, if successful, will provide confidence at Dow and LanzaTech to continue harnessing the findings. I advise the team to boost the key performance indicators of the project, as achieving 100 mg/L at 4 mg/L/hour in 2 years between very experienced teams at LanzaTech and Dow is low-balling a milestone. I would encourage the team to offer a higher bar after year one if successful.

PI Response to Reviewer Comments

- The economics of our process are confidential; however, we have process and techno-economic models that we will use to consistently evaluate the economics of our process. Our target molecules, C6–C14 straight-chain, terminal alcohols, have widespread consumer chemicals applications, including use in detergents and soaps, personal care and cosmetics, plasticizers, corrosion inhibitors, and lubricating fluids. Our target alcohols are a significant part of the global fatty alcohols market where 2014 demand surpassed 2,300 kilotons in 2014 ($3.5 billion).

  This market is projected to grow by 5.1% annually to approach $5.5 billion in 2023. The market outlets for our target alcohols will enable our ultimate aim of biofuel production by supporting infrastructure development to traverse the "valley of death." Ethanol made by this process will be converted to jet fuel. The end goal is both to sell the fatty alcohols for high-margin chemicals applications and to channel them into the fuels market as both diesel blendstock and as diesel/jet fuel after hydroprocessing or direct enzyme-mediated conversion. The technical challenges are the strain and enzyme engineering to mitigate bottlenecks and promiscuous pathways.

  The project titer and productivity targets are significantly higher than what we presented during the Project Peer Review meeting. These targets remain confidential and were created using the state of our technology upon submission of our proposal and application of techno-economic models.

BENCH SCALE INTEGRATION

(WBS#: 2.4.1.100)

Project Description

The Bench Scale Integration project develops bench-scale integrated biomass-to-fuel conversion processes for pilot-scale demonstration to meet BETO's $3/gge 2022 fuel cost target. The project focuses on improving YRT from the enzymatic hydrolysis and fermentation process steps. Outcomes include successfully demonstrating a bench-scale process meeting the necessary criteria to reduce the risk of scaling; generating data for NREL's annual SOT reports, which track research progress and cost improvements; and validating new technology when possible. Bench-Scale Integration focused the last 2 years on using fermentation science to improve productivity of several processes.

By managing nutrients and moving to a fed-batch process, we doubled lipid fermentation productivity from 0.32 g/L/hour to 0.68 g/L/hour. For 2-3 butanediol production, optimizing aeration levels in fermentation vessels doubled the titers from 10 g/L in shake flasks to 20 g/L in fermenters. In addition to fermentation research, the project team worked with three commercial enzyme companies to test two of NREL's pretreated feedstocks using an enzymatic hydrolysis assay: deacetylated dilute acid and deacetylated mechanical refined corn stover. One of the enzymes produced 85% glucose yield from deacetylated dilute acid at 10 mg protein loading, reducing the loading in half. Taking the improvements in lipid productivity and enzyme reduction, we showed a $1.8/gge decrease in the MFSP for FY 2016.

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

Weighted Project Score: 8.5

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

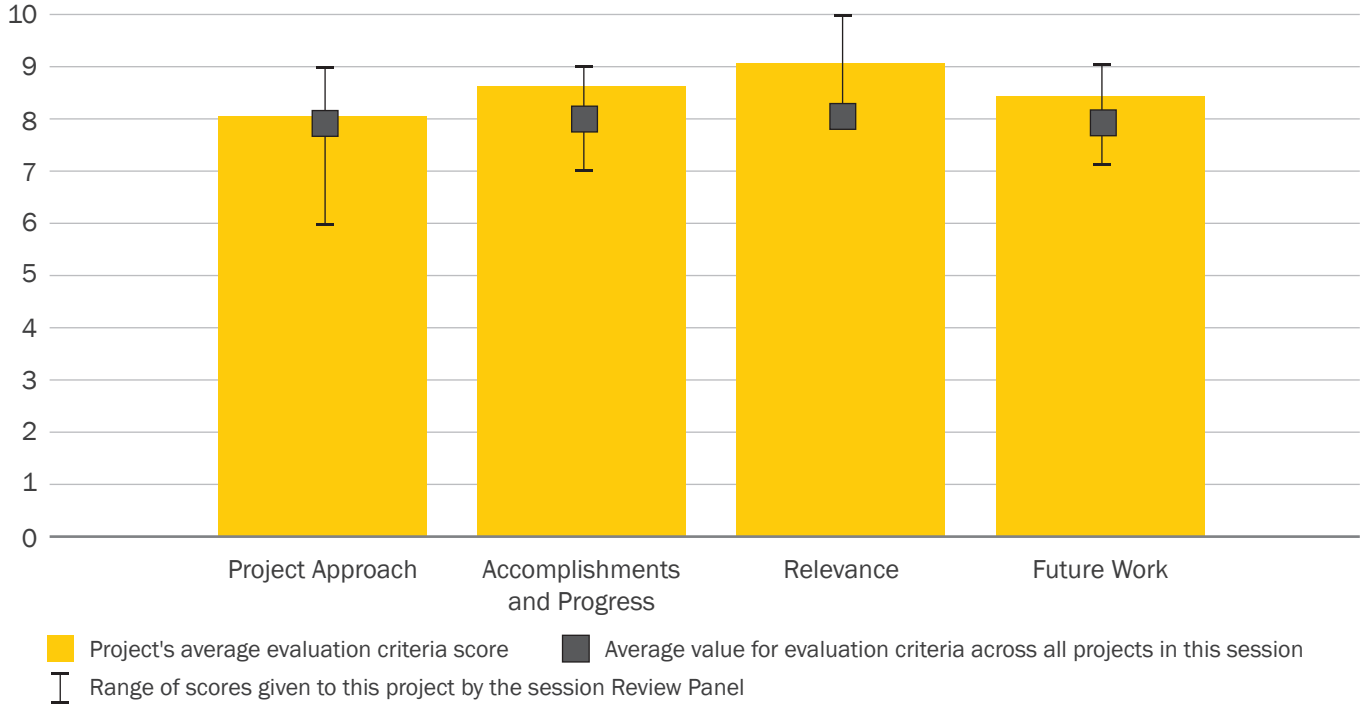


Photo Courtesy of National Renewable Energy Laboratory

Overall Impressions

- The project is a key component for the NREL program and looks to be well-run. There are obviously a lot of moving pieces in bringing together different technologies and processes, but it certainly looks under control. Processes obviously get expensive at larger scale, and fewer tanks will be available, so it is essential to have a platform that allows multiple replicates to be performed across multiple conditions. It would have been good, actually, to get a quick table of how many vessels are available for the team and at what working volume.

  The project has several functions: optimizing process conditions to get the best out of the material being developed by other projects in the NREL program; comparing the materials being generated (e.g., deacetylated dilute acid vs. deacetylated mechanical refined pre-treated material); and helping industry directly by benchmarking material, such as enzyme packages. In this regard, NREL can fulfill an important role—many companies go along a particular technical route, particularly with pre-treatment regimes, so NREL can really help in showing how these compare to each other.

- The bench-scale operations provide a critical element to addressing BETO's goals of getting laboratory-scale technology into the marketplace. By providing an intermediate step between the laboratory and the pilot unit, potential problems in scale-up can be worked out prior to large investments in pilot operations. Since this is a service function of the program, it is likely more difficult to establish milestones against which progress can be measured. Further, its integration and collaboration with the ABPDU and other scale-up operations in the program could be more clearly defined. However, this is an important component of the BETO program that needs to receive continuing support as a service function.

- The Bench-Scale Integration program provides a bridge between shake flask and pilot scale, and it serves as the next step following organism development. The team has demonstrated significant improvement in SOT for succinate, 2,3-butanediol, and lipids, and it has made improvements to the facility (e.g., online mass-spec for off gas analysis) that improve the quality and value of the data produced. In the future, the team should strive to be even more interactive with the strain development team and work with them to develop a small-scale model that can accurately represent aeration conditions in the fermenter. Since the strains are sensitive to aeration, such a tool may help optimize conditions and select the most robust strain prior to fermentation testing.

- This project is an indispensable resource for testing new bioconversion process designs, improving TRY through fermentation optimization, tracking progress, updating SOTs, and providing data for TEAs. This project interacts with many NREL projects and bridges the gap between shake flask results and pilot-scale validation, supporting NREL bioconversion projects and industrial partners.

- This is a good project with a clear outline of the challenges and opportunities and good plan to mitigate them. This project, like other service-based ones, should be evaluated differently—perhaps together with the other service-based projects for a 5-year plan or longer on how they enable the BETO's mission and vision.

PI Response to Reviewer Comments

We thank the reviewers for their positive comments and appreciate their acknowledgement of the importance of Bench-Scale Integration's role in developing biofuel fermentation processes at bench scale to facilitate scale up to the pilot plant. We recognize the importance of working closely with the strain development groups to evaluate strains in process-relevant conditions and providing important feedback on strain performance.

The project is closely aligned with pretreatment, pilot-scale integration, analysis, and separations projects where we share data on pretreated feedstock evaluations, share performance data for techno-economic modeling and SOT reports, provide feedback to the separations project on biomass sugar quality, produce material for downstream processing, and develop robust fermentation processes for pilot plant scale-up.

We also maintain a close association with industry by providing information on biocatalyst performance in a process context, which we hope will aid in scale-up. For future work, we agree with the reviewers that we should develop a small-scale aeration model and plan to work with our strain development and process simulation projects to accomplish that task. It would be prudent for this project to leverage other DOE facilities like the ABPDU in our development work.

SEPARATIONS DEVELOPMENT AND APPLICATION

(WBS#:2.4.1.101)

Project Description

The Separations Development and Application project performs separations R&D to improve the efficiency and economics of producing and recovering biofuels from biomass. It supports BETO's 2022 $3/gge production cost goal and aligns with BETO's MYPP strategic and performance objectives to produce advanced biofuels from biomass sugars (and other carbohydrate and lignin derivatives).

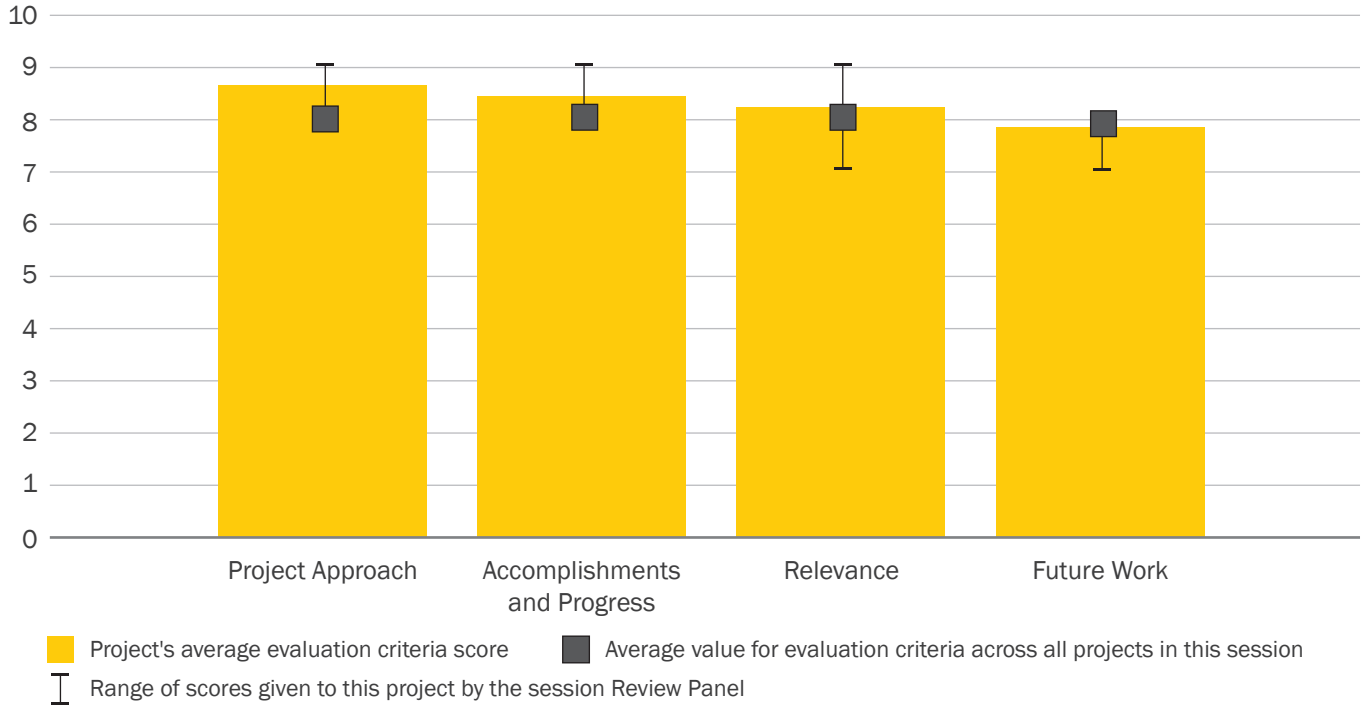
The separations the team is researching, developing, and improving include upstream solid-liquid separations and hydrolysate liquor concentration to prepare biomass sugar streams for biological upgrading, as well as further cleanup of such hydrolysates to prepare them for catalytic upgrading, and also downstream recovery of an intracellular lipid product (fuel precursor) produced from the sugar stream. The project also advances development of continuous enzymatic hydrolysis technology, which offers significant cost-reduction potential over batch processing if it can be robustly demonstrated.

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Jim McMillan
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$750,000
DOE Funding FY 2016:	$750,000
DOE Funding FY 2017:	$750,000

The project's scope and schedule are driven by the need to identify and establish integrated process(es) to be pilot demonstrated at NREL in FY 2022, which requires major process elements (including integral separations) to be defined and ready for piloting by the end of FY 2019. We have been using and will continue to use TEA informed by performance data from this project to guide and refine R&D directions/priorities.

Weighted Project Score: 8.3

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



Overall Impressions

- Overall, the project just looks really well-run and logically organized, while providing both basic studies and innovative approaches. The technical area is one that can have a significant impact on costs and also provides a technical route that can enable other parts of the process to either work better or be more economic. Indeed, it is possible that some of these solutions (product recovery, recycling of enzymes) can take some pressure off areas of biology and reduce the need to reach very high performance targets (e.g., product tolerance or very low enzyme loading levels) that would otherwise be required to make a process economic. The approach on several of the topics is very helpful, in taking a wide view of the possible solutions (e.g., the seven basic methods tested first for the yeast cell lysis task) and then down-selecting to the best for further optimization.

  As a side note, this is probably the best-organized and clearest presentation in the 2017 Project Peer Review.

- This project described success in several different separation processes directly applicable to BETO programs. Further, the work was closely and clearly linked to potential cost reductions important for meeting the 2022 BETO milestone. This is an effort worth continuing, as separations will remain a significant cost contributor within the biorefinery. As the project goes forward, better coordination with the Separations Consortium will be expected.

- This program aims to address two of the most-costly separations processes in biological conversion. The relevance to the MYPP cost targets is clear, as separation conditions directly impact the process cost. The project has clearly established targets and has come most of the way towards reaching them through a combination of novel methods and classical process optimization. The main challenge not addressed here is how well the processes scale from the laboratory to the pilot/commercial scale. In addition, due to the "moving target" nature of the project with uncertainty in upstream composition, further optimization may not be fruitful. Instead, spend the effort on novel technologies that could provide a step change.

- Low-cost, efficient separations are one key to bringing down the cost of biofuels and bioproducts, and this team is addressing critical issues. Some false starts are inevitable when processes and unit operations are developing in parallel (e.g., incompatibility of flocculation with lignin upgrading). This project is working with algal R&D to take advantage of cell lysis advances and adapt separations accordingly. Continuous enzymatic hydrolysis is a nice demonstration, and I'll be watching as insoluble solids increase (>10% insoluble solids and alternate membrane technologies are tested for enzyme passage. This is nice work and should benefit from the Separations Consortium in the long run, and vice versa.

- The project history and relevance to BETO's mission is clear, and the technical and management approaches are sound. The need for good separation technologies to aid in the cost cutting of hydrocarbon fuels is a must, and the team is well-equipped to contribute to it. The good relationship and cross talk with the separation group and industry knowhow will be mandatory to future success. The team has great initiative and competency to build upon.

PI Response to Reviewer Comments

- The reviewers' constructive comments and recommendations are appreciated. As noted, regardless of which specific fuel pathway is down selected for further development in the biochemical platform, separations will continue to be an important cost factor in integrated biorefining. As the project goes forward, coordination with the nascent Separations Consortium will need to increase, and this is planned.

Similarly, we need to maintain/grow our interactions and collaborations with industry, and we plan to do this in close collaboration with the Biochemical Conversion Platform's Pilot-Scale Integration project, which has responsibility for demonstrating pilot scale-up/integration of separations technology solutions developed by the Separations Development and Applications project.

In all cases, this project focuses on developing separations solutions that will be both cost-effective and scalable. Nonetheless, there are on ongoing challenges to identifying and acquiring suitability flexible bench- and pilot-scale systems for all separation processes being researched, and this further demonstrates why the Separations Development and Applications project must continue to coordinate its equipment selection activities closely with the Pilot-Scale Integration project.

These challenges notwithstanding, we agree that there is a "moving target" issue in developing separations processes to recover intermediates or products for which the production processes themselves are still under development, and that, as a result, further optimization of identified separations methods (like those to recover intracellular lipids) will not be that informative until further production technology down-select has occurred. Consequently, going forward, this project will strive to avoid further separations process optimization in favor of focusing on development of novel technologies, such as continuous enzymatic hydrolysis, that have the potential to lead to a step change in overall production cost.

BIOCHEMICAL PROCESS PILOT-SCALE INTEGRATION

(WBS#: 2.4.1.102)

Project Description

The Biochemical Process Pilot-Scale Integration project's high-level goal is to take technology developed at the bench scale and demonstrate its performance at pilot scale, producing data for TEA meeting BETO's 2022 biofuel cost target. To facilitate the work, we maintain the functionality and operational readiness of the biochemical pilot plant located at NREL, and we evolve its capability to perform process-relevant integration work for BETO and industrial clients. We also solve critical scale-up issues that usually only manifest at pilot scale prior to technology deployment. However, processing biomass feedstocks remains a challenge at pilot scale, particularly handling a variety of raw biomass materials.

In the past 2 years, we acquired a rotary drum filter and disk stack centrifuge for process development efforts.

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Dan Schell
Project Dates:	10/1/2003–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$2,000,000
DOE Funding FY 2015:	$2,000,000
DOE Funding FY 2016:	$2,000,000
DOE Funding FY 2017:	$2,000,000

We measured the residence time distribution in a continuous pretreatment reactor and showed that pretreatment severity has little effect on the residence time distribution. We found that separating liquor from alkaline-pretreated material is difficult. We also assessed the ability to predict pretreatment results in large-scale reactors from small-reactor system data. Finally, we generated more accurate performance and cost information for processes requiring aeration using either stirred tank or bubble column reactors. The pilot plant continues to be used by industrial clients, and six new industry-based projects began in FY 2015/2016.

Weighted Project Score: 7.9

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

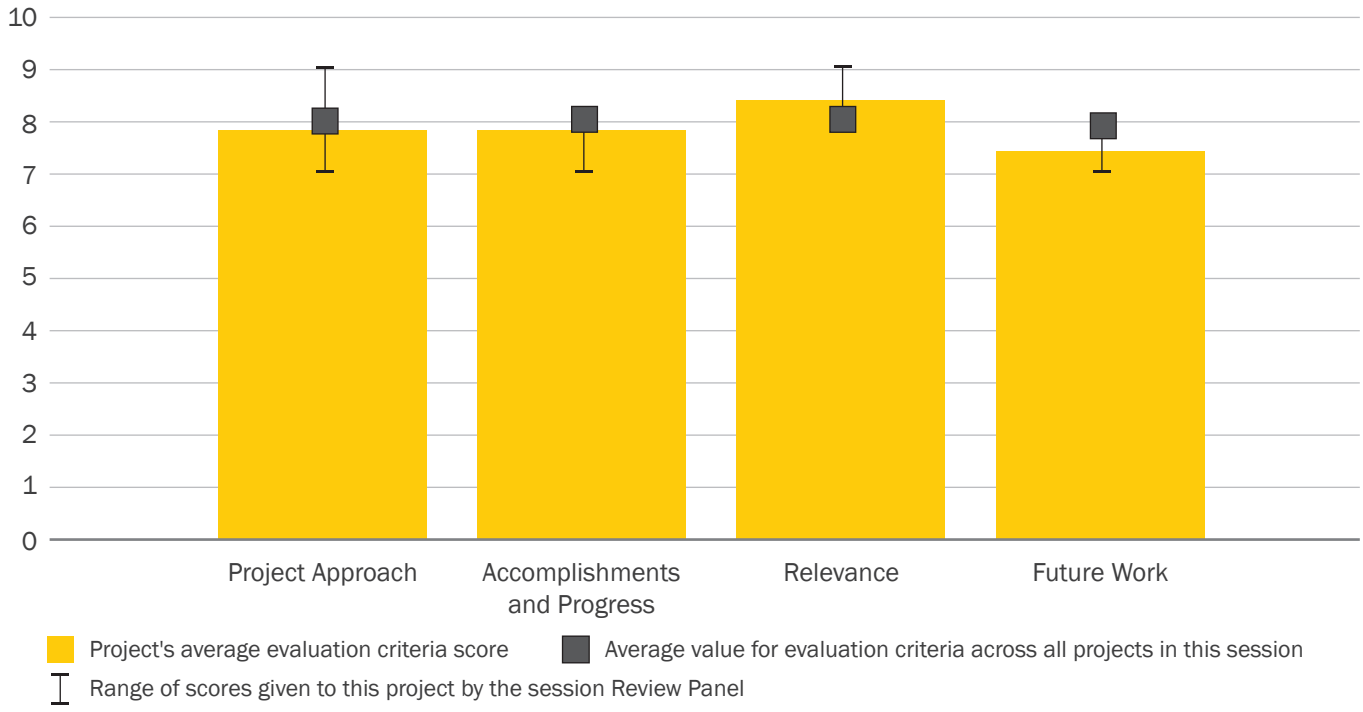


Photo Courtesy of National Renewable Energy Laboratory

Overall Impressions

- Overall, the pilot plant operations look to be fulfilling an essential function for both the internal needs of the NREL projects and external stakeholders. This scale is obviously an essential step for companies to go through to validate technologies and identify potential roadblocks, but it is an expensive proposition. Having a single facility that can cover a range of unit operations, processes, and configurations that may have a significant effect on the performance of biological systems under development at companies makes a lot of sense economically. This is a perfect example of how a national laboratory can fulfill an important role in lowering development costs and risks for industry and allow industry to cast a wider net than they would be able to on their own.
- The process development unit is an important component and capability to have for a program that is dedicated to eventual industrial use. It is hard to measure progress against goals, as this is another "responsive" activity, like the bench-scale program. However, NREL has designed a facility with equipment relevant for industrial testing, and something that appears to be busy for most of the time.

  The presentation and discussion suggested that the process development unit operates in a bit of a vacuum with regard to other larger-scale capabilities within the program (bench, ABPDU). Going forward, better communication and coordination between these operations would streamline management and would improve industry's ability to identify the best location for scale-up of their processes.
- The pilot plant is a core capability to BETO that is essential to achieving the ultimate $3/gge milestone. The team has achieved one of the primary ongoing objectives, which is to keep the plant operational and with the most relevant technology. They have also provided scale-up services to some projects to give valuable guidance on recognizing scale-up problems. It is not clear how the projects are chosen and prioritized. This has to be a programmatic decision, rather than just based on who comes to them with a project. I think this program would benefit from even more funding to ensure that such a critical resource remains high priority. Regardless of the organism used and the products chosen, pilot validation is necessary.
- This facility has been supporting the bioethanol industry for many years. The pilot plant is evolving with the industry, is a valuable resource supporting AOP objectives at NREL, and is also a great resource for external parties. Pilot-scale reactor facilities are fairly rare, and process integration at this scale is necessary to de-risk commercial scale.
- The pilot-scale facility is a must-have step in any scale-up and, as such, is a very important part of BETO's mission to validate near-demo-scale-ready technologies. The team is specialized and knows their work and used good practices of mixture designs and scale-up/down models to address scale-up issues. The challenge of managing publicly available data is understood. The project has good cross talk with the bench-scale integration and analytics team and works closely with the Separation Consortium. Overall, there is good management and a good technical approach. It is recommended, though, to

integrate a community of pilot plant facility and know-how (APBDU, NREL, SCADA at PNNL, bench-scale validation, and the Separations Consortium and analytics projects) somehow, as they all are continued, needed support functions.

PI Response to Reviewer Comments

- We appreciate the reviewers' comments and their efforts reviewing this project. We will continue to evaluate pilot-scale processing needs and acquire capabilities with BETO's support to make the biochemical pilot plant a relevant facility for industry and BETO to develop and test new hydrocarbon biofuel production technologies. As technology development continues and process options for pilot-scale verification are identified, we will continue to increase our collaborations with other BETO projects, in particular, the Bench-Scale Integration project. An even closer collaboration is planned between the Bench-Scale Integration project and this project beginning in FY 2020, and indirectly with industrial and academic stakeholders. A capabilities workshop with all BETO facilities performing pilot-scale work, which would include industry representation, might be useful for soliciting recommendations for new equipment and how best to use these various facilities.

TARGETED MICROBIAL DEVELOPMENT

(WBS#: 2.4.3.102)

Project Description

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Mike Himmel
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$1,900,000
DOE Funding FY 2016:	$1,900,000
DOE Funding FY 2017:	$1,900,000

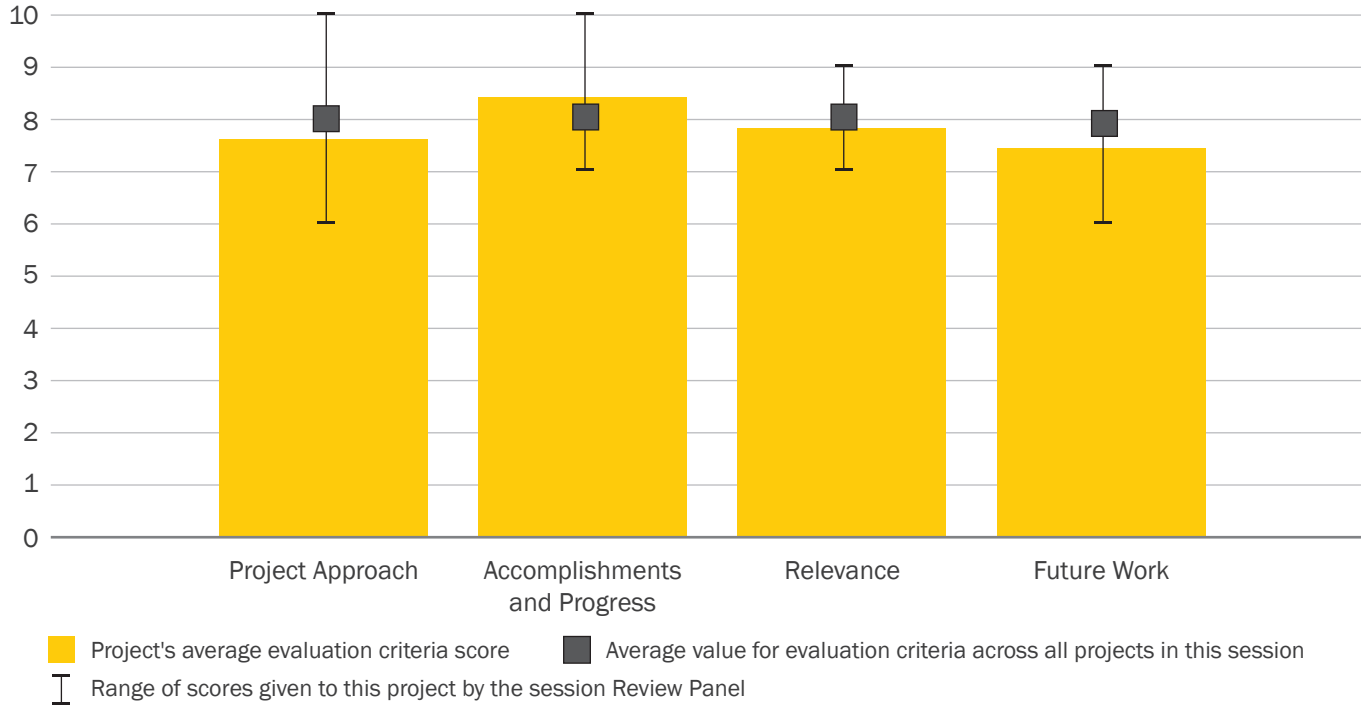
The Targeted Microbial Development project will investigate and recommend promising pathways for advanced biological upgrading of biomass sugars and lignin to hydrocarbons and co-products, supporting the DOE-BETO 2022 goal of enabling advanced hydrocarbon fuels at $3/gge. By applying metabolic engineering and synthetic biology tools, we are working to engineer microorganisms for efficiently upgrading sugars to hydrocarbon intermediates and valorizing lignin for chemicals production.

Task 1 focuses on engineering Zymomonas mobilis to produce a mixed ethanol and 2,3-butanediol (BDO) from sugars, which provides product flexibility for upgrading the BDO intermediate to hydrocarbon fuels or chemicals (e.g., butadiene). Task 2 works on metabolic engineering of Pseudomonas putida to produce muconate from lignin monomers. Task 3 investigates production of long-chain fatty alcohols (as secreted products) as biofuel precursor molecules by engineering oleaginous yeasts. We are also investigating novel CBP concepts that can reduce the cost of producing hydrocarbons.

Overall, Targeted Microbial Development provides leading technologies for producing reduced-cost fuels and high-carbon-efficiency intermediates amenable to separations and catalytic upgrading to hydrocarbon fuels

Weighted Project Score: 7.8

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



and identifies future sugar upgrading technologies. We also seek to develop a critical knowledge base, enabling BETO and the bioenergy industry to deploy production of third-generation hydrocarbon biofuels from biomass.

Overall Impressions

- The project has identified what seem to be relevant target pathways for co-products in order to improve overall economics. The metabolic engineering strategies selected are generally reasonable and reflect the expertise present at NREL in metabolic engineering. However, in the context of the current SOT with regard to synthetic biology in both industry and academia, the scope of the metabolic engineering strategies laid out here seem very limited. It is hard to think that progress towards both the TRY for each task and the understanding gained would not be faster and a more efficient use of resources with high-throughput strain generation approaches.

  Given the complexity of cellulase and hemicellulase mixtures that are known to be required for effective deconstruction of pretreated lignocellulosic material, it is hard to see the study of expression of cellulases in otherwise non-producing hosts as an effective use of resources, given the remaining challenges in pathway engineering for the three metabolic routes.

- One of the NREL strengths is developing a clear understanding of how biochemical systems work and then linking that understanding to processes that meet BETO's strategic direction. This knowledge-based approach is proving itself through the identification of systems that support BETO's goals, for example, the discovery of organisms that can attain nearly theoretical yields of muconate from lignin derivatives. This capability will be critical for current projects and ongoing efforts to engineer organisms that can selectively and rapidly convert both lignin and carbohydrates to high-value products.

- The Targeted Microbial Development program addresses three separate strain engineering projects. All three use different organisms and represent different product/co-product opportunities. The use of P. putida to convert lignin monomers to muconic acid is especially exciting, since relatively little work has been published to address this challenge. Progress has been very impressive on all efforts. The use of metabolic models in conjunction with experiments was shown to be essential to this work. However, the challenges that lie ahead may be even greater, and the team should make sure to use all resources available to guide strain and process development. This includes omics analysis, diagnostic experiments to identify bottlenecks, and adaptive evolution to overcome tolerance challenges.

- Three pathways to upgrading or co-products are being pursued in this project. The mixed ethanol/diol product is the most advanced, and BDO titers have dramatically improved with metabolic engineering and fermentation optimization. This is a nice, original, co-product proof of concept. The team has done a good job of recognizing the complexity of the separation and fermentation scale-up, and they are considering their options. Lignin upgrading to fatty alcohols and muconate are successfully demonstrated concepts. They are further from their target titers. Two of the tasks are considering scope changes due to their understanding of the economics and/or robustness of scalability. This project is doing a good job of using TEA to make decisions.

- This is a good team with ambitious targets, which are relevant in aiding and expediting metabolic engineering of various hosts and are aligned with the MYPP goals. The overall TEA of fatty alcohols should be evaluated as this might be too challenging of a target for the oleaginous yeast program. However, thorough benchmarking of progress toward the TEA goal will likely keep things in spec, and the team, together with Hal Alper's collaboration, has the skill set to tackle the technical challenges.

PI Response to Reviewer Comments

- We thank the Review Panel for the supportive comments and helpful feedback. In terms of high-throughput synthetic biology–based strain engineering, we are attempting to actively deploy a rapid genome-scale editing tool in P. putida KT2440 currently in collaboration with a world-leading synthetic biology group. In addition, we are leveraging modern systems biology tools (proteomics, transcriptomics, and metabolomics) to identify bottlenecks and adaptive laboratory evolution to improve both exogenous and endogenous pathways. If these approaches are successful, we will be able to very rapidly modify and improve flux through aromatic-catabolic pathways in this robust host.

  We are also attempting to deploy in Zymomonas a rapid genome-scale editing tool, such as CRISPR, which will enable us to be more efficient in high-throughput strain generation. We are also leveraging modern systems biology tools (proteomics, transcriptomics, and metabolomics) to identify bottlenecks. As explained in the presentation, the CBP work is our lowest TRL effort and is thus treated as exploratory and low priority. Regarding expression of Cel7A cellulases, these proteins are known to be difficult to fold in yeast. We, and others, assume that enzymes from GH families 5, 10, and 11 will be less of a problem. With that said, we agree that there are remaining challenges in pathway engineering for the three metabolic routes.

  Thank you for your comments. It is nice to hear that we are doing a good job of using TEA to guide our research. For example, we are working to develop anaerobic pathways, which will scale more effectively than microaerophilic or aerobic fermentations. With guidance from NREL's TEA analysis, we consider 2,3-BDO as precursor to butadiene as an alternative strategy, possibly for products valorization of a fuels production process. Our primary focus is maximizing fuel yields in our base case. In fact, we considered a route that takes both ethanol and BDO to hydrocarbon fuels.

PROCESS INTENSIFICATION FOR THE REDUCED COMMERCIAL CAPITAL EXPENDITURE OF BIOFUELS PRODUCTION USING DYNAMIC METABOLIC CONTROL

(WBS#: 2.4.3.200)

Recipient:	Duke University
Principal Investigator:	Michael Lynch
Project Dates:	10/1/2016–9/30/2018
Project Category:	New
Project Type:	FY 2014—Incubator II: DE-FOA-0001320
Total DOE Funding:	$1,691,595

Project Description

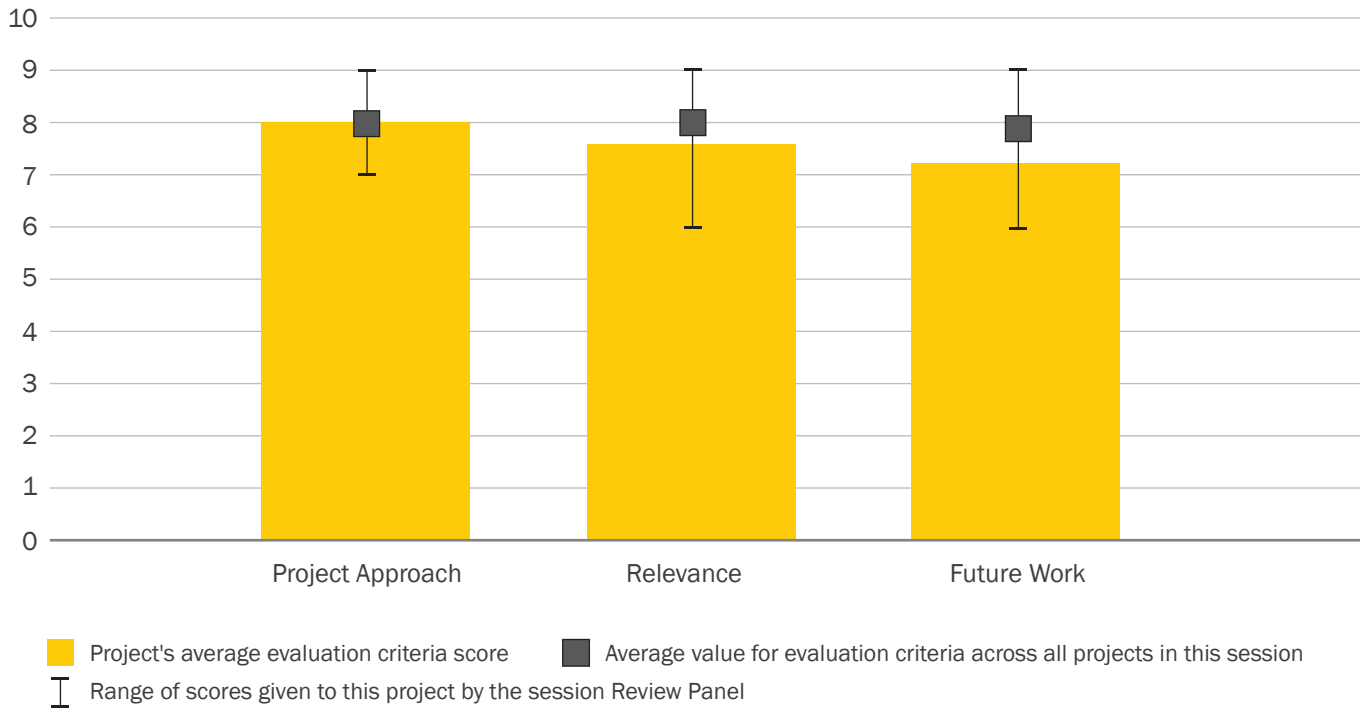
This program is aimed at greatly reducing the capital costs for commercial-scale biobased processes by developing semi-continuous fermentations that can achieve unprecedented volumetric fuel production rates. Currently, we estimate these processes can reduce capital requirements with a 5- to 10-fold increase. Our approach is to utilize advanced two-stage technology, leveraging synthetic metabolic valves in the semi-continuous fermentation of farnesene and related terpenes. Synthetic metabolic valves convert growing cells into active, non-growing, stationary-phase biocatalysts, which can be concentrated and recycled, improving volumetric rates. The key performance metrics to be demonstrated include production rates greater than 25 g/L/hour. and final product titers >500 g/L.

Overall Impressions

- The project contains an interesting central idea—genetic control of metabolic pathways that are required for growth but not product formation and, therefore, can be "turned off" after a growth phase. This adds another dimension to standard metabolic engineering strategies, which, to date, largely rely

Weighted Project Score: 7.5

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



on "fixed" mutations that, for example, seek to permanently eliminate side pathways and are therefore limited to those not essential for growth. The route also has the potential to provide an alternative to fermentation process controls that rely on limitation of a key nutrient to control growth (e.g., phosphate or nitrogen).

What is not clear currently is how the concept will play out when using real-world lignocellulosic sugar streams as the carbon source rather than clean sugar mixtures. Going a long way down the path of genetically optimizing a host strain in relatively benign conditions on clean sugars may create a technical risk, given that the cell physiology required to tolerate toxins in most lignocellulosic hydrolysate streams could be quite different.

The commercial benefits seemed to be tied to intensification of the process—generating a high volumetric productivity by recycling cells and maintaining high biomass concentration during fermentation. This is likely to be a much harder task on lignocellulosic sugar streams than on clean sugars in the laboratory or even sucrose or starch-based sugar streams at scale. It may require separate saccharification of high solids concentrations and then very stringent removal of residual solids. The capital expenditure benefits from the high biomass concentration and continuous operation in this scheme may be offset by more unit operations needed upstream and downstream.

- The PIs are pursuing an interesting biochemical approach for the biorefinery and have established a reasonable initial plan. More detailed evaluation will need to wait until more results are in.
- The goal of this program is to develop a semi-continuous process that can reduce capital requirements significantly. The project leverages technology to switch metabolism from growth to production and maintain production phase indefinitely as the product is removed. The latter may be specific to a product that can be separated easily so as not to build up to toxic levels and/or stop the reaction due to reaching thermodynamic equilibrium. However, the ability to tune gene expression dynamically can be leveraged for any fermentation configuration. The team at Duke and DMC Limited (Dynamic Metabolic Control) has pioneered this technology and seems to be well-suited to reach the objectives. The main challenges will be around scale-up, which are not specifically addressed in this work: behavior of the metabolic switching in large heterogeneous environments, engineering of the semi-continuous process, and possible contamination during long fermentation runs.
- This is an ambitious project. It is being demonstrated on farnesene production by E. coli, but success in this process could lead to similar process intensification approaches for other bioprocesses. The robustness of fermentations utilizing this metabolic control switch will be critical.
- Overall, this is a very ambitious project with a well-structured team that has already demonstrated its ability to rapidly develop strains with high flux to products using its dynamic metabolic control models. This high-risk/high-gain project, if successful, will allow a pipeline of such approaches toward other hydrocarbon fuel molecules from lignocellulosic sugars. The risk factors to take into account are the cost of sugars and the ability to overcome sugar toxicity, as well as the engineering of feeding system to allow the high feed of sugars, dealing with the generated water and product separation. The plan to tackle these risk factors is reasonable, and I'm wishing the project team the best of luck in accomplishing these.

PI Response to Reviewer Comments

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

BIOCHEMICAL PROCESS MODELING AND SIMULATION

(WBS#: 2.5.1.100)

Project Description

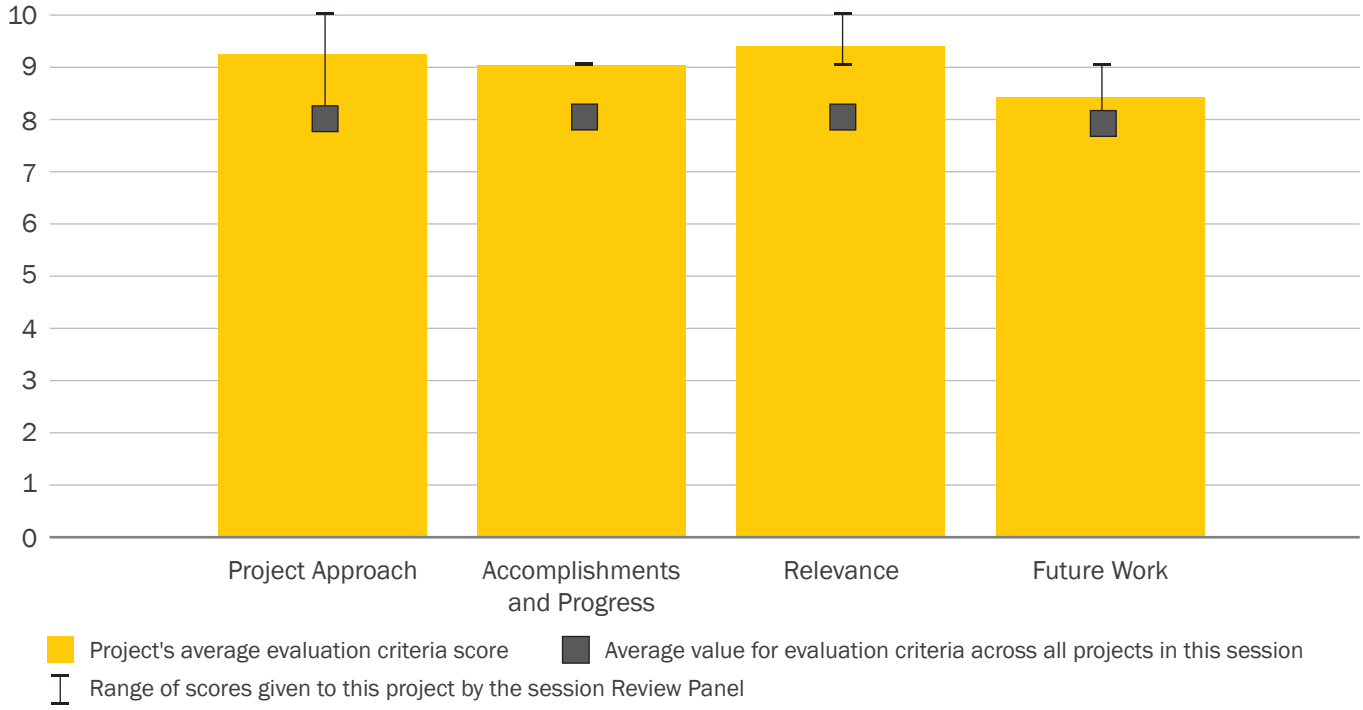
The Biochemical Process Modeling and Simulation project aims to reduce the cost and time of research by applying theory, modeling, and simulation to the most relevant bottlenecks in the biochemical process. We use molecular modeling, quantum mechanics, metabolic modeling, fluid dynamics, and reaction-diffusion methods in close collaboration with pretreatment, hydrolysis, upgrading, and TEA. The project's outcomes are increased yields and efficiency of the biochemical process, added value to products, and reduced price of fuels by specifically targeting catalytic efficiency, reactor design, enzyme efficiency, and microbial design.

We work closely with experimental projects to identify problems and iterate with experiments to find and refine solutions. By working with experimentalists, we decide on problems that can be solved with simulation that could otherwise not be solved or would take too long with experiment alone to reach BETO's targets. We have produced solutions that have resulted in a 10x increase in muconate yield, an aerobic reactor model that predicts cost changes with size and design, and catalyst enhancements that increase muconate upgrading from 10% to 90% utilization, and we have also designed enzyme mutations for enhanced hydrolysis and metabolic tuning. We find methods to overcome specific barriers

Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Mike Crowley
Project Dates:	10/1/2004–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$1,575,000
DOE Funding FY 2015:	$1,500,000
DOE Funding FY 2016:	$1,500,000
DOE Funding FY 2017:	$1,500,000

Weighted Project Score: 9.0

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



and continue to develop those methods. This project is essential in the process of selecting the final processes for 2022 biofuel production targets.

Overall Impressions

- Overall, the combined efforts in the three task areas look excellent. The goal of using computational modeling to reduce the scope of experimental testing and validation is very valid, and this is certainly an active area in enzyme development, synthetic biology, and process development elsewhere. The NREL team can certainly add to that trend and make significant contributions by putting tools and results into the public domain.

  It would be good to see how the metabolic modeling effort will interact with the Agile BioFoundry effort. From the examples shown, it wasn't clear how many suggested mutations were generated for the precise metabolic engineering efforts. It may be useful to consider explicitly the involvement of the metabolic model technology in the "learn part of the design-build-test-learn for the high-throughput strain generation effort in the Agile BioFoundry.

- Computational modeling was brought into the program several years ago as a means to reduce uncertainty in experimental design and to help provide insight regarding reactions and processes. This goal has been nicely met, and the presentation described a number of success stories about the interaction of computation and research. Overall, the potential for modeling work to be applicable to industry is clear. This is an important component of the NREL work and a useful tool for experimentalists. This is a nice program and a nice presentation that provides an overview of an area of huge potential importance for BETO.

- The Biochemical Process Modeling and Simulation program uses theory and simulation to predict performance at ranges of conditions not studied experimentally. The goal is to reduce the experimental effort by using these results to set tighter boundaries on the range of conditions/strains/pathways tested experimentally. So far, this effort appears to be extremely successful in making advances that contribute to reaching goals of multiple other programs.

- Biochemical Process Modeling and Simulation contributes valuable, actionable insights across bio- and thermo-conversion projects. From micro- to macro-scale, this project provides targets for protein engineering, carbon flux manipulation, and reactor design, to name a few. They work closely with the projects, and predictions are tested empirically to both improve productivity and performance and to advance the model. Numerous successful examples were described. Biochemical Process Modeling and Simulation also contributes to TEAs. Modeling insights can save time, explore larger design space, and suggest novel changes for experimental validation. The organization and collaboration of this team seems exemplary.

- Progress has been made on many aspects of modeling and simulation. The diverse topic landscape and the need to specialize in each discipline to enable expediting the cross talk between modeling and wet laboratories may require more focus in the future. However, so far, this appears to be a working model with a highly accomplished team. This is a very relevant support function and very much in line with enhancing the discovery and decision making for hydrocarbon fuels and chemicals, biologically and chemically. I expect to see more of this computational approach in the future to guide decision making, as knowledge in this field is growing through academia and industry. Great job, and keep the momentum going.

PI Response to Reviewer Comments

- We value the Review Panel's work in evaluating this project and are grateful for the efforts and insights given. We plan to continue to increase our impact

and collaboration in experimental efforts, increase the productive dialogue, and apply our efforts and expertise to the most relevant problems, as determined by TEA analysis of highest-impact topics, applicability of methods, and ability to deliver helpful solutions in a useful timeframe. The connections to TEA are very high on our priority list, and we have made significant progress in developing the collaborative mechanisms and dialogues; there is still a lot of room for progress and improvement. There are two aspects to this collaborative work: information flow in each direction.

Biochemical Process Modeling and Simulation depends heavily on TEA to identify the most important places to work and which projects will have the most impact on achieving the BETO goals. We also aim to improve the precision and, hopefully, accuracy of the TEA, where possible, through more physics-based models and parameterization. This is a non-trivial undertaking, largely due to differences in scale and computational complexity and simplicity in the two endeavors. However, we plan to continue to make the connections and contributions stronger and more impactful. One specific example of current collaboration with the TEA team is the calculation of maximum theoretical yield in platform microorganisms that are more accurate than what was used by the TEA team in the past. Previously, these theoretical yields came from approximation, extrapolation, or literature searches.

We are already working closely with Agile BioFoundry in P. putida research and will continue to increase our collaboration as we find applicable. We are aware that the number of projects can exceed our capability, and we will continue to down-select so our impact is the greatest.

ANALYTICAL METHODS DEVELOPMENT AND SUPPORT

(WBS#: 2.5.1.101)

Project Description

The goals of the Analytical Development and Support project are to enable biofuel and bioproducts R&D at NREL by ensuring high-quality analytical data and to advance the tools available to the wider community through method development and globally adopted procedures. Our project is divided into two tasks: one task to improve existing analytical methods and to develop and implement new methods, and one task to maintain existing analytical facilities at NREL and to provide outreach to external stakeholders. We actively cultivate partnerships with industry, academia, and other government laboratories, based largely on our reputation for excellence in analytical chemistry.

The Analytical Development and Support Project is best known for our publicly available LAPs, which provide detailed methods for the summative analysis of biomass materials. Our work is relevant to the overall goals of the program because robust, accurate, precise analytical methods that can be easily and widely implemented help decrease the costs associated with analytical measurements; this is a critical enabling activity both for other NREL researchers and for the larger biofuels research community.

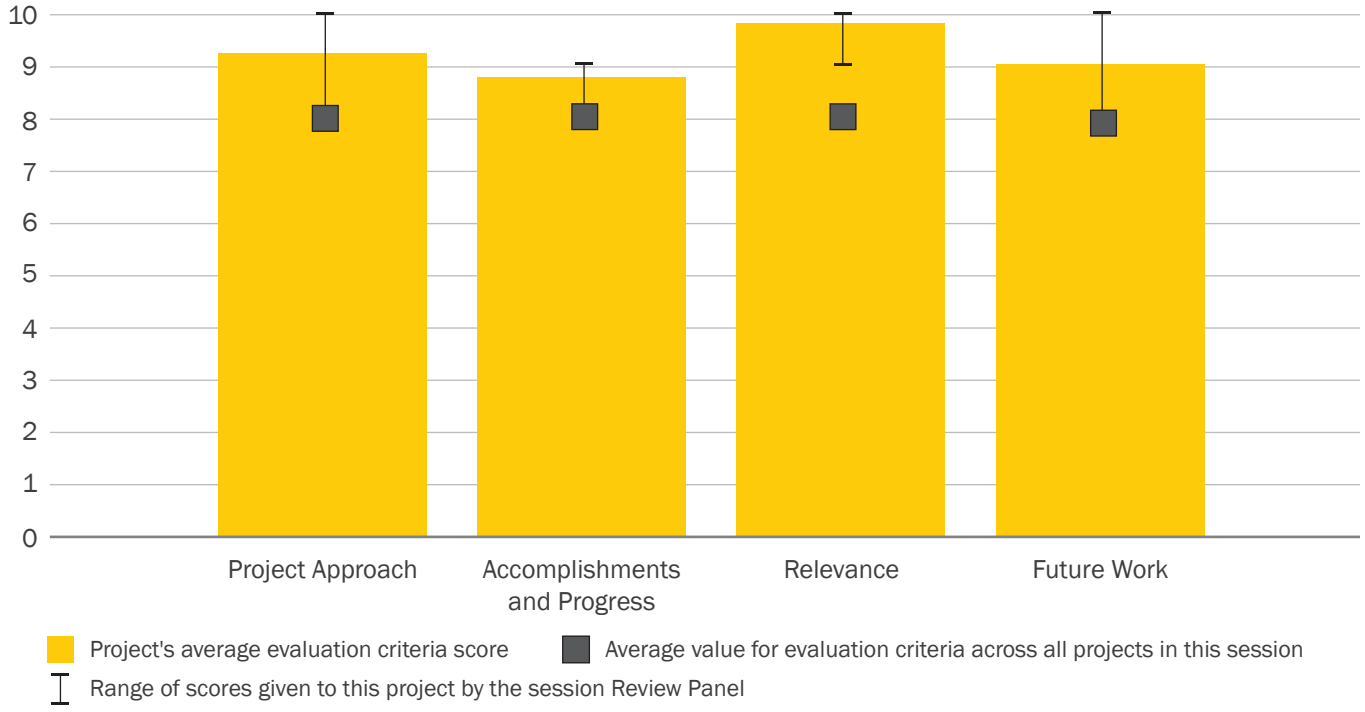
Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Ed Wolfrum
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$1,200,000
DOE Funding FY 2016:	$1,200,000
DOE Funding FY 2017:	$1,200,000

Overall Impressions

- Overall, the project team looks to be doing really well in combining the delivery of quality data to

Weighted Project Score: 9.2

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



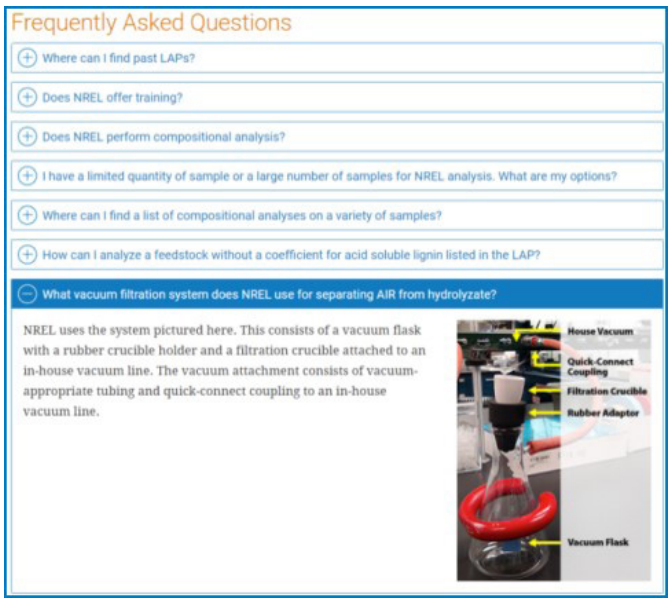


Photo Courtesy of National Renewable Energy Laboratory

researchers working on internal NREL projects and outside partners with the development of new methods. The project highlights the role that a national laboratory can play in doing work that is central to a range of processes and releasing data into the public domain, thereby saving industry a significant amount of money. The laboratory also appears to perform an important role in standardizing analytical methods across the industry. Although, in the end, industrial projects are deemed successful or not by the economics of production, there are many steps along the way where accurate benchmarking can help them find out if they are on the right path and reduce the risk that their own analytical methods are misleading. The outreach effort performed by the group is phenomenal.

- What's not to like? This is one of the most important parts of the BETO program. Biomass analysis is difficult, fussy, and can give wildly different results if carried out incorrectly, or even if carried out in different laboratories. By supporting an effort to standardize methodology and distribute this methodology to the larger biorefining community, more credible data can be obtained to help direct internal research and TEA, as well as providing information to support industry's investigation of biomass as an alternative feedstock. Although the team might consider incorporating nuclear magnetic resonance and data mining into their effort, I would echo previous reviews on this superb activity: keep doing what you're doing.
- The purpose of this program is to develop analytical tools (Task 1) and supply high-quality data (Task 2) to the entire Biochemical Conversion Technology Area. It is one of the invisible machines that keeps everything running smoothly. They have done a commendable job running samples in a timely manner for multiple projects and delivering high-quality data (accurate, precise, consistent, and relevant). In addition, the team supports their instruments and those in other laboratories. All of these activities are extremely important to keeping the projects running without delay. The team also supports industry and other national laboratories with standards and methods.
- This project/team is well-known for developing and sharing new analytical methods that enable the industry. Protocols are published and become industry standards, with tens of thousands of page views and thousands of downloads. They continue to identify the most relevant needs. This is exactly the team that should be working with the U.S. Environmental Protection Agency to define the methods for D3 Renewable Identification Number credits.
- The high-quality data is maintained through the good skills and expertise built in the group and good maintenance of the equipment. The challenges of keeping up with moving-target needs from project teams and quickly developing sufficient, robust methods are well-addressed. Having to specialize on many analytical methods for sugars, enzymes fermentations, pretreatment, etc., and maintaining four laboratories with a large number of analytical instruments is well-handled by the project leader. Challenges with pilot-scale analytics are also

well-addressed. I recommended having a better data repository system to enable data sharing, faster analysis, and, later, perhaps data mining and modeling. On a more organizational level, BETO is advised to consider spacing the review cycle of such an important supporting function. This is not a project in my mind, but mandatory to BETO's success competency.

PI Response to Reviewer Comments

- We thank the reviewers for their detailed comments on this project. Our main focus is on analytical data quality, and we believe that our support of four different laboratories and multiple instruments is an extremely valuable (if somewhat "invisible"') contribution to the NREL Biochemical Technology Area as a whole.

  We believe our other internal-facing activities to coordinate analytical chemistry and maintain analytical laboratories and analytical instruments ensure consistent and high-quality analytical data in the most cost-effective manner possible. We agree that a more robust Laboratory Information Management System for data tracking and delivery would be helpful. We will examine our current efforts on scientific data management and look for cost-effective ways to improve this. We will also continue our method work to develop robust methods for constituent mass balances for pilot-scale alkaline pretreatment experiments.

  We believe that our external-facing activities, principally the maintenance and periodic updating of our website containing LAPs, along with our involvement with ASTM, help to provide a set of common analytical methods with established precision and accuracy. This serves the broader research community and saves many different research groups significant time, effort, and expense trying to "reinvent the wheel." We believe our work to develop, maintain and then license near-infrared calibration data sets for rapid biomass compositional analysis is a powerful and cost-effective method to enable the larger biofuel/bioproducts industry by decreasing the cost of primary analytical measurements. Our ongoing work helping to develop robust, standard methods to characterize corn ethanol plant process intermediate streams for proper fuel Renewable Identification Number valuation should help bring clarity to an issue of concern to many stakeholders. In the future, we will continue our external collaborations, including with our external colleagues addressing Gen1.5 ethanol issues.

  higher than hydrophobic membranes, or if they are made from much less expensive materials, then hydrophilic membranes may be the most cost-effective option for streams containing more water than organics. Alternatively, if the aqueous stream contains less water than organics, then hydrophilic membranes may be the best option, although this is not the case for most aqueous product streams.

  Traditional separation methods for acetic acid from water include energy-intensive distillation processes. Membrane separations have the potential to improve the energy efficiency of the process. In future work, we will conduct more separation tests to provide better data to improve the techno-economic analysis in order to justify membrane performance and economics.

  Regarding dewatering, we agree with the reviewer. Too many downstream upgrading and separation steps will negatively impact economics. Sending the concentrated organics to hydrotreating for fuel production does not require additional equipment, but impact on fuel selling price may not be significant. Thus, converting organics to higher-value chemicals is necessary, but additional costs will depend on conversion performance/selectivity, separation technology, and product selling price.

ADVANCED SUPERVISORY CONTROL AND DATA ACQUISITION (SCADA) FOR BIOCHEMICAL PROCESS INTEGRATION (WITH BEND)

(WBS#: 2.5.1.102)

Recipient:	Pacific Northwest National Laboratory
Principal Investigator:	Jim Collett
Project Dates:	10/1/2016–9/30/2019
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$300,000
DOE Funding FY 2016:	$300,000
DOE Funding FY 2017:	$300,000

Project Description

Commercial viability of advanced biofuel biorefineries will depend on their ability to process lignocellulosic feedstocks that may vary significantly with seasonal conditions and by regional source. Moreover, BETO has identified that maximizing the incorporation of lignin solids into the final fuel is a critical factor for cutting production costs of advanced hydrocarbon biofuels to $3/gge. Industry representatives at the 2014 BETO Process Integration and Carbon Efficiency Workshop specifically endorsed attaining complete bioconversion of high-solids feedstocks into value-added fuels or products. As such, we are developing process analytical technologies (PAT) to optimize bioconversion of biomass feedstocks with variable compositions and high levels of suspended lignin.

The objectives of this project include the following: (1) to enable real-time tracking of critical process parameters in bioconversions of variable, high-solids feedstocks within bioreactors via the novel application of dielectric spectroscopy and near infrared spectroscopy tools that comply with industrial PAT standards, and (2) to reduce bioconversion scale-up risks by using PAT to optimize bioreactor process control systems in the laboratory under actual industrial conditions and also by

Weighted Project Score: 8.6

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

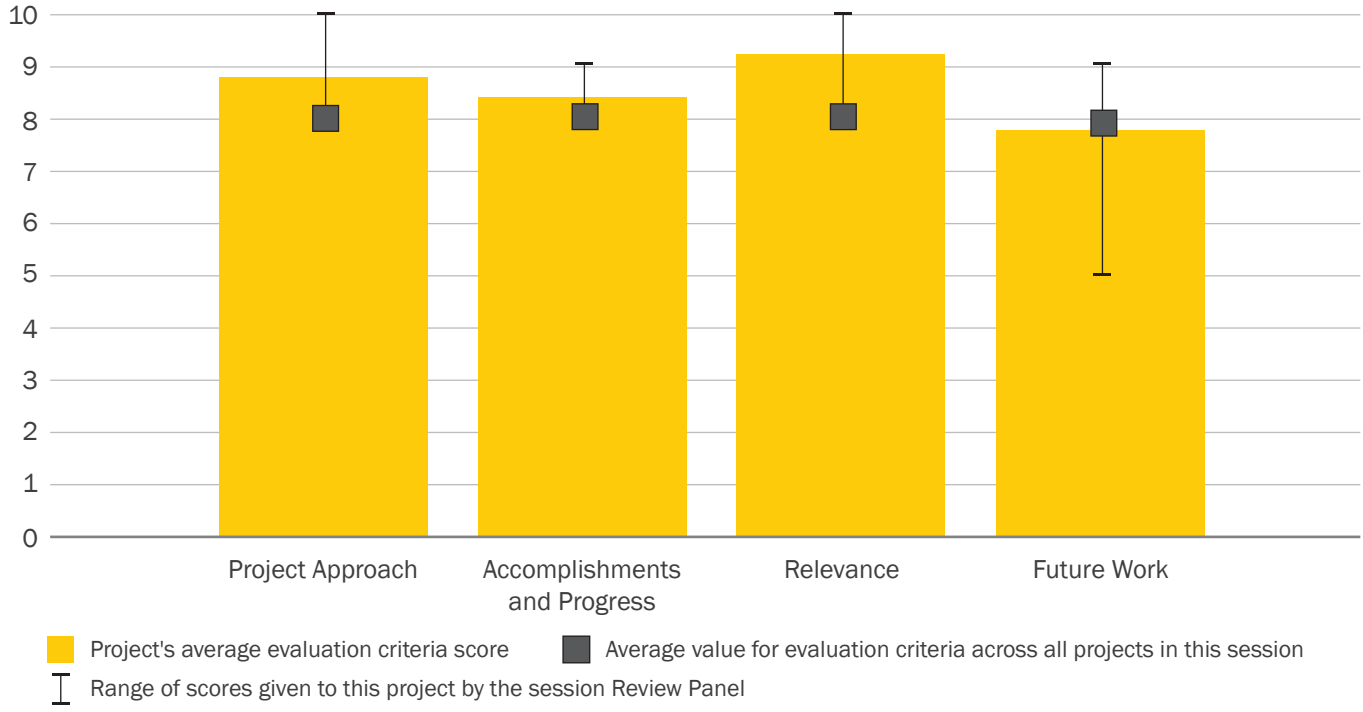


Photo Courtesy of Pacific Northwest National Laboratory

ensuring that these same PAT tools and control systems will scale up and directly integrate into the SCADA networks of commercial biorefineries.

Overall Impressions

- The project has addressed an important area that has attracted some attention in industry but is certainly a growing field and currently under-exploited. The team can provide great value to the biorefinery industry (and also further afield) by testing out the validity of the three technologies under study when applied to the complex mixtures seen in cellulosic bioprocess streams.

  A further benefit is the software side of this project, testing out tools that can be integrated to bring together the data from online and offline monitoring, process conditions and set points, and starting material. Demonstrating a system and advising companies (particularly small companies) on configurations they can build would be a key output of the project.

  Looking at the other presentations at the review, there are some obvious collaborations within the national laboratory system that could be beneficial here, particularly with the NREL analytical effort (developing a broader chemometric model for near-infrared spectroscopy on feedstocks for instance), and the computational modeling effort also at NREL.

  Overall, this is a really great idea producing very promising results.

- As bioprocesses move from the laboratory to industrial use, successful scale-up is critical. However, understanding and monitoring these larger-scale operations is equally important. This work is developing some nice tools for following processes in real time and providing rapid feedback regarding the operation of a process. The integration of these techniques with laboratory management software and their utility in helping process automation is a real strength. As the project goes forward, better integration with the existing large-scale facilities in the BETO portfolio is recommended.

- The objective of this program is to develop complex control systems for fermentations and other unit operations in bioconversion. This is important for second-generation biofuels in particular because of variability in feedstocks and intermediate streams that affect downstream processes. The team has taken an innovative approach, implementing off-the-shelf instrumentation for a particular program (oleaginous yeast fermentation) with the eventual goal of generalizing to any unit operation. Due to support from instrument vendors, this program was operated with relatively low DOE funding. So, the return on investment for BETO was high. The overall system will likely not be ready to contribute to

2022 goals, but it may be essential for commercial viability of biorefineries. The use of machine learning to incorporate analytical data and metadata is especially promising. Development will take a long time (in particular, collecting enough data to build the model), so now is the right time to start.

- Introducing commercially proven technology to improve monitoring and automated control has significant potential impact on performance and cost. Reducing the need for manual intervention, sampling, and technical expertise may improve efficiency and reproducibility. There are many variables in these bioenergy processes, and reducing a few could have big impact!
- This is a great and much-needed approach. The team has realistic and achievable goals: reducing manual sampling, improving process control, minimizing loss runs, and enhancing operation excellence. This is very much desired and can drive many BETO's sponsored laboratories to accelerate their R&D time and improve de-risking strategies. I would heartily recommend integrating this effort across other pilot facilities and, where possible, small-scale and bench-scale facilities as well.

PI Response to Reviewer Comments

- We thank the reviewers for their constructive feedback and encouragement.

  We appreciate the reviewers' multiple recommendations that we share details of our methods for integrating online spectroscopy into our bioreactor operations with the NREL Process Development Unit and the ABPDU, and we hope to assist them in deploying advanced PAT within their pilot-scale operations as well. We have already reached out to these teams and look forward to working with them more closely during the next 3-year cycle of this project.

  We will coordinate our further development of chemometric predictive models for biorefinery operations with the NREL Biomass Compositional Unit to take advantage of their well-established LAPs for rapid analysis of biomass feedstocks. Our collaboration could assist future biorefineries with a consistent, plant-wide approach for chemometric analysis and PAT integration. Moreover, we will share with them our progress in deploying the open-source LabKey Laboratory Information Management System database so that they may consider using it for managing their data and analytical workflows as well.

  We are also grateful for the verbal recommendation made by one of the reviewers at the Denver meeting that we consider using the OSI PI (process intelligence) system for integrating online spectroscopy data with other sensor, event, and metadata to support chemometric model development. PI is an enterprise-scale database software suite that is already in use at PNNL to support DOE-funded research on electrical-grid SCADA systems. We are now examining how to link the LabKey Laboratory Information Management System with the PI Data Archive for automated integration of offline sample data with online spectroscopy data, other sensor data (such as pH and temperature), and event frames (such as feed pump activation). The integrated time series data may then be matched with process metadata (such as microbial strain information and batch recipe) within the PI Asset Framework to provide more meaningful context to observed process trends. PI analysis tools, such as BatchView and ProcessBook, may then be used for the more rapid assembly of training data sets with a richer collection of class variables, which facilitate identification of latent variables that currently confound robust application of our near-infrared spectroscopy chemometric models across multiple bioreactor runs with varying feedstock lots and operating conditions.

In response to a reviewer's request for further details on how our PAT tools will enable advanced control systems: In a fed-batch operation to produce hydrocarbons from oleaginous yeast, near-infrared spectroscopy appears promising for controlling hydrolysate feeding rates throughout the full course of the process. During logarithmic growth, dielectric spectroscopy may be used for tracking viable cell mass to enable automated shut-off of nitrogen feeding at a targeted cell density to induce the yeast cell lipid synthesis at a point in the batch trajectory that maximizes overall hydrocarbon production. Raman spectroscopy appears promising for tracking the rate of intracellular lipid accumulation, which can enable optimization of hydrolysate and oxygen feeding algorithms that maximize hydrocarbon productivity while potentially minimizing metabolic overflows into organic acids and other unintended side products. Although the relatively small budget for this project limits the scope of our activities, we see many other immediate opportunities to apply advanced PAT tools throughout the biorefinery; the use of dielectric spectroscopy for real-time tracking of enzymatic hydrolysis has already been demonstrated, and there appear to be many potential uses for the extremely rugged BallProbe (manufactured exclusively by project partner MarqMetrix) for Raman spectroscopy–based process control in upstream and downstream thermochemical operations at high temperatures (>350°C), and high pressures (>6,000 psi). We look forward to working with our industry partners, our counterparts at the other national laboratories, and BETO to pursue such opportunities as time and budget allow.

AGILE BIOMANUFACTURING FOUNDRY

(WBS#: 2.5.3.104-112)

Project Description

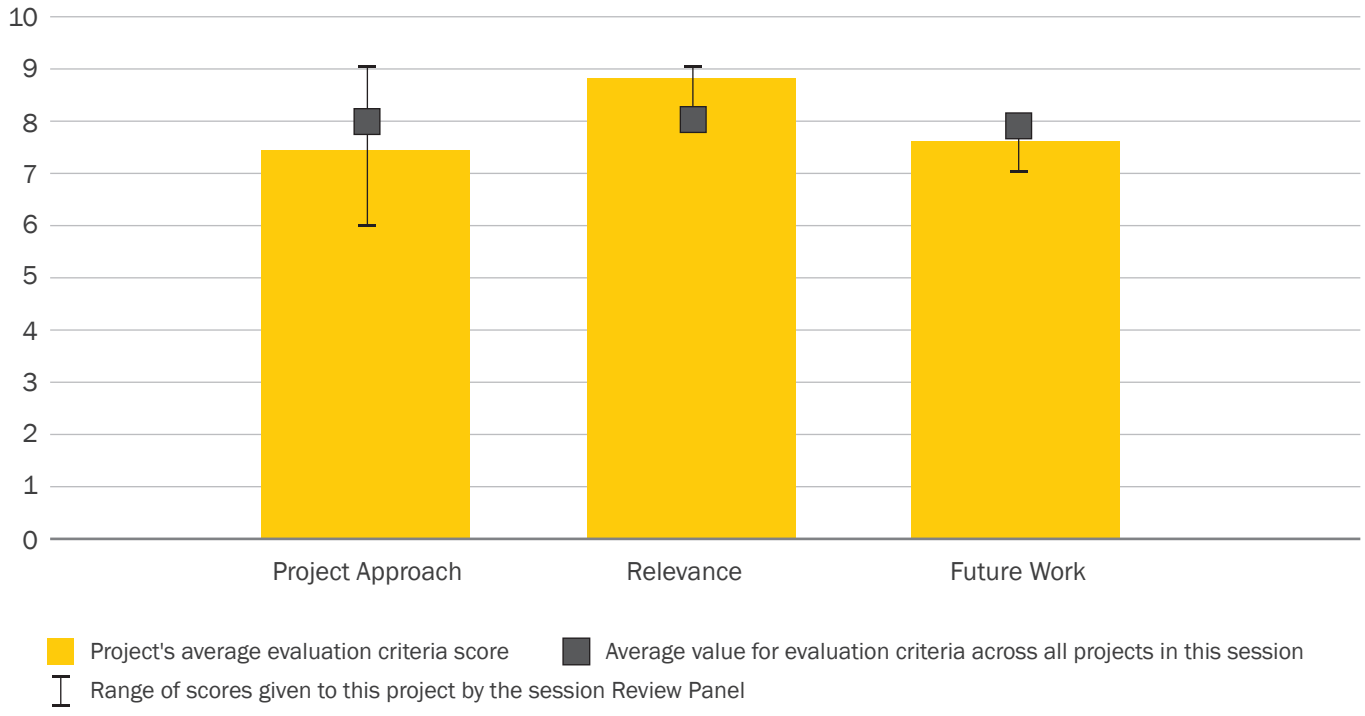
The overall goal of this project is to enable a biorefinery to achieve a positive return on investment through a 50% reduction in the time to scale-up of fuel and chemical production, compared with the current average of ~10 years. This will be accomplished by establishing a distributed Agile Biomanufacturing Foundry (Agile BioFoundry), consisting of a consortium of nine national laboratories that will productionize synthetic biology for industrially relevant, optimized chassis organisms. The Agile BioFoundry will constitute a public infrastructure investment that increases U.S. industrial competitiveness and enables new opportunities for private-sector growth and jobs.

Key challenges to be addressed include the efficient catalytic upgrading of sugars/aromatics and gaseous and bio-oil intermediates to fuels and chemicals; costs of production; and data availability across the supply

Recipient:	Lawrence Berkeley National Laboratory, National Renewable Energy Laboratory, Pacific Northwest National Laboratory, Sandia National Laboratories, Los Alamos National Laboratory, Oak Ridge National Laboratory, Argonne National Laboratory, Idaho National Laboratory, Ames Laboratory
Principal Investigator:	Nathan Hillson
Project Dates:	10/1/2015–9/30/2019
Project Category:	New
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$2,400,000
DOE Funding FY 2016:	$1,200,000
DOE Funding FY 2017:	$16,200,000

Weighted Project Score: 7.9

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



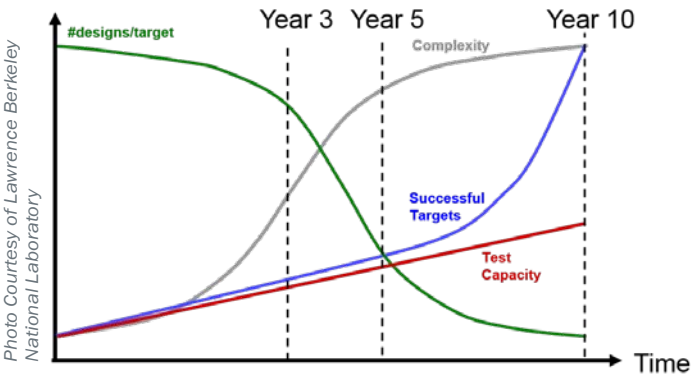


Photo Courtesy of Lawrence Berkeley National Laboratory

chain. The outcomes of this project will include a 10-fold improvement in design-build-test-learn engineering biology cycle efficiency and new intellectual property and manufacturing technologies effectively translated to U.S. industry, ensuring market transformation. The Agile BioFoundry directly supports BETO's mission and goals and contributes to addressing BETO's Conversion R&D FY 2020 and FY 2021 milestones.

Overall Impressions

- The consortium has a lot going for it. As described in the presentation, there are companies offering synthetic biology platforms to clients, but the technology improvements and learnings will most likely remain closely guarded secrets and be developed separately without cross-interaction. There is a real benefit to having a publicly funded effort developing core tools, engaging with a range of internal and external partners, and being relatively open-source about the tools that are developed. This should provide a system for companies to either use directly as a facility or learn from in developing their own systems. The focus should perhaps be on saving small companies money by not having to all do the same thing in developing tools, providing access to expensive automation equipment and perhaps software tools that would require coding skills that are not present in every company.
- Strengths include the following:
  - The consortium represents a very timely effort that could allow companies to reduce strain development costs, increase speed to market, and de-risk adoption of new technology.
  - It connects people and other resources in the national laboratories to reduce redundancy in technology development and improve cooperation.
  - It offers the opportunity to "push the envelope" and develop platform strains that have been adapted for use in more challenging industrial processes, such as the use of cellulosic sugars.
- Weaknesses include the following:
  - Geographic separation of resources may create problems in communication, sample transfers, and disconnects in technology adaptation (e.g., organism onboarding).
- The team has presented an interesting approach to a key problem in getting products to market. However, they do not yet have a compelling argument as to why and how their approach will be better than other potential approaches to the problem. In addition, the rationale for their choice of product targets needs to be strengthened, as it isn't clear that reducing the cycle time to, say, adipic acid, would be generally applicable to other materials, even those in their current portfolio or specific targets that might be suggested by their industrial advisory board.
- The Agile BioFoundry was created to develop capabilities in synthetic biology that will reduce the commercialization time for a bioprocess. BETO recognizes the need for technology-enabling platforms, and thus, the program specifically addresses one of the missions stated in the MYPP. The program was well-conceived, taking input from the DOE and industry experts, and has a solid management structure and a full-time program manager to ensure accountability. The 3-year targets are very ambi-

tious, but if they are achieved, this would represent a significant advancement. In my opinion, the capabilities, host organisms, and knowledge base that this consortium produces, which can reduce timelines across the industry, is more valuable than hitting milestones on the particular molecules they have chosen.

- In addition to the technical challenges that will arise for each specific product/host combination, there is a logistical challenge with having different tasks spread out among multiple locations. This will have to be managed very closely to ensure there are no unreasonable delays in transferring data, strains, samples, etc. Also, there is no substitute for real-time communication to solve technical problems. In analyzing and interpreting the results of fermentation, for example, it is important to include the fermentation engineer, the scientist who created the strain, and a data scientist all in the conversation. Finally, more emphasis should be placed on the performance gap between small-scale culturing and bench-scale fermentation, which is a well-known problem in the field.
- The presentation was well-organized and delivered. The introduction of new host strains with validated robustness will be welcome and should lead to new bioproducts. The project objective is to level the playing field and enable more participants to introduce new products more quickly, which has high relevance for BETO. The metrics are aggressive (reducing cycle time with new organisms), and the team should be willing to drop combinations that aren't working and have a process for making such decisions. The starting point is known hosts with PIs who have experience with those hosts, which de-risks getting some early successes. Managing the activities of a nine-laboratory consortium will be a challenge, and the organization has thus been carefully considered.
- This is a good initiative and very much needed across the national laboratories and industry. It will be useful for companies to tap into this knowledge in the future and use the infrastructure instead of forming their own. The PI is encouraged to look deeply into high-throughput fermentation techniques mastered by enzymes and biobased chemicals and fuels companies (e.g., Codexis, Verenium, Genomatica, and Amyris). This will foster the team's ability to better conduct the "predictive high-throughput fermentation" concept using a reliable, robust, consistent, and reproducible scale-down model. I will also encourage the PI to form a strong liaison between fermentation and the high-throughput team, as this will likely become a bottleneck to the candidates' nomination for testing process.

PI Response to Reviewer Comments

- We appreciate the detailed input from our Review Panel and will seek to incorporate the feedback as our consortium moves forward. As a distributed effort, we clearly may face some operational challenges, although these are offset by the Agile BioFoundry's ability to leverage physical and human resources across distributed national laboratories. The Agile BioFoundry's full-time program manager, together with regular communications across the consortium (via teleconferences, webinars, informatics servers, SharePoint, annual in-person meetings), will help mitigate the communications risks. Sample transfer risks (i.e., sample stability, sample loss) will be assessed through local/proximal compared with remote sample analysis, and the incidence of lost samples will need to be assessed on an ongoing basis. Disconnects in technology adoption, unfortunately, can be a very significant challenge even within a single location/institution, although it may be further exasperated across geographic locations (especially if there are overlapping methods/infrastructure alternatives). It will be an operational

imperative to standardize workflows and data-exchange formats wherever possible.

What sets the Agile BioFoundry apart from other foundries is that we seek to develop and distribute publicly available tools, methods, and strains aimed at broadly benefiting the biofuels and bioproducts industry. Whereas private foundries are incentivized to develop proprietary tools and organisms, the Agile BioFoundry is a publicly funded effort aimed at delivering technology that will enable industry to either leverage our resources through partnership or adopt our methodologies for developing bioproducts. In comparison to the publicly funded Defense Advanced Research Projects Agency Living Foundries program, there are distinct programmatic and technical differences between the aims of the two efforts. Where the Living Foundries program is primarily focused on developing biological pathways to materials that cannot be achieved through transformations of petroleum feedstocks, the Agile BioFoundry is focused developing biological pathways for producing advanced biofuels and renewable, high-volume chemicals.

The Agile BioFoundry is pursuing multiple target/hosts to demonstrate that the methods, software, and technologies can be productively applied across product classes. The process and rationale for selecting the three target/hosts pairs for FY 2017 (and the 15 pairs for FY 2017–FY 2019) was described during the review, and the details have been provided to BETO. In addition, we will consult the Agile BioFoundry Industry Advisory Board during future evaluation and selection phases to ensure that our prioritized targets and hosts remain aligned with industry's needs.

Regarding scaling processes from high-throughput, small-scale experiments to pilot-scale process demonstration at NREL and the ABPDU, we recognize there are new challenges associated with each increase. The Agile BioFoundry workflows will leverage design of experiments and small-scale culture to select strains to grow in bench-scale bioreactors. Bench-scale fermentation provides critical data for the "learn" component of design-build-test-learn, both to inform future designs and to develop predictive models that may be applied to small-scale experiments. Transfer from bench-scale bioreactors to pilot-scale fermentation will be reserved for mature strains and processes when there is need and value.

As we progress, the Agile BioFoundry's milestones are written to explicitly drop underperforming target/host pairs. This requires operational discipline, which will be enforced not only by Agile BioFoundry leadership, but also by BETO. The process is largely guided by TRY metrics and go/no-go decision points, although other metrics (e.g., diminishing learn/strategic value) will also play a role in certain circumstances. We note the Review Panel's comments related to developing broader, operational milestones aimed at demonstrating a successful platform, as opposed to those that emphasize TRY. We thank the reviewers for their valuable input, which will be considered in guiding the direction of this program.

MAXIMIZING MULTI-ENZYME SYNERGY IN BIOMASS DEGRADATION IN YEAST

(WBS#: 2.5.3.200)

Recipient:	J. Craig Venter Institute
Principal Investigator:	Yo Suzuki
Project Dates:	3/15/2013–10/31/2016
Project Category:	Sun-setting
Project Type:	FY 2012—Synthetic Biology: DE-FOA-0000719
Total DOE Funding:	$1,266,224

Project Description

Carbohydrate-active enzymes from various organisms are known to act synergistically in biomass degradation in nature. We aimed to develop synthetic biology technologies to facilitate incorporating some of this synergy into the saccharification process to make the process efficient and cost-effective. Our approach builds on pioneering work by others.

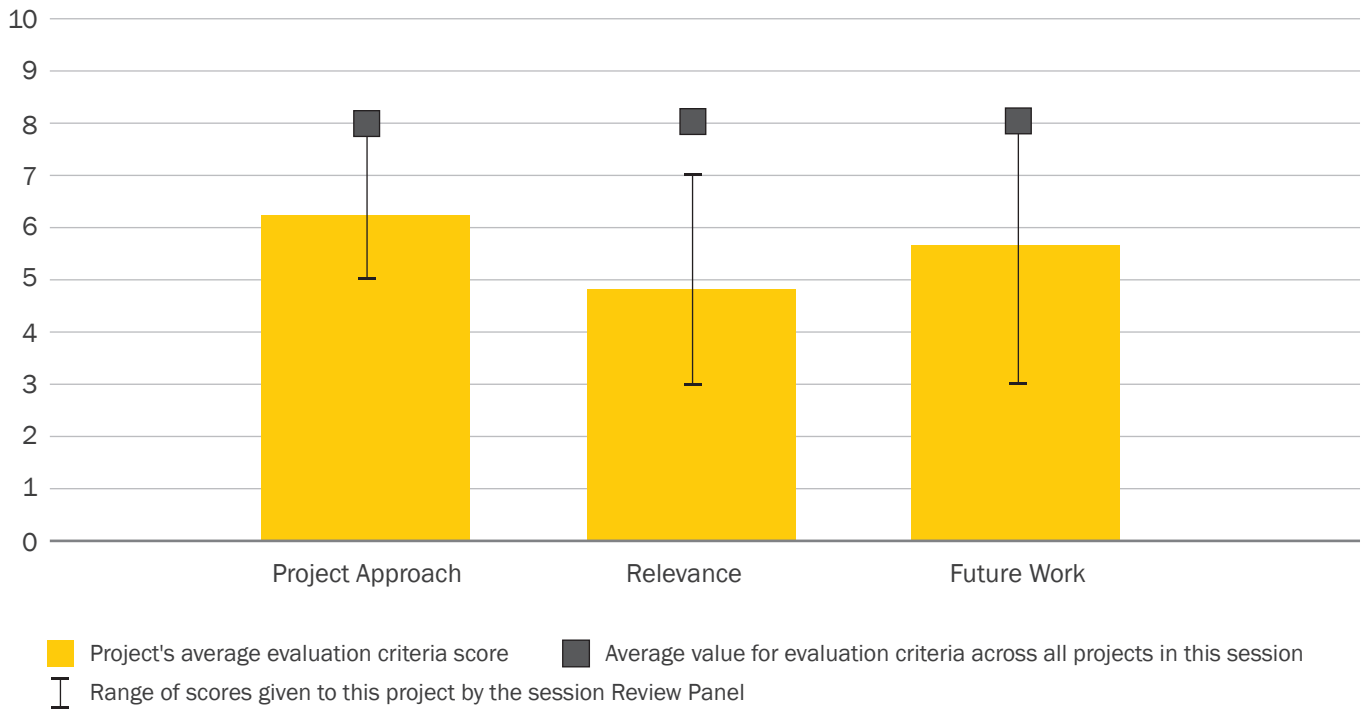
Using a pipeline for gene synthesis, strain construction, and strain testing, we achieved functional expression of 45 enzymes in Saccharomyces cerevisiae, representing 11 glycoside hydrolase families. We generated a scaffold with nine sites for enzyme loading. Using CRISPR-mediated engineering, we generated 10 strains, each containing nine enzyme constructs across the genome.

Our project's unique goals were shuffling of enzyme constructs and identification of optimal enzyme combinations. We mated the multi-enzyme strains and examined the haploid progeny from one pair extensively.

Using a growth-based assay with pretreated corn stover, we screened 626 progeny and selected three strains to be used for validation. We showed that 20%–30% more ethanol is produced with these strains in a selected condition over the baseline, with a comparable strain not making any cellulase. Our synthetic biology approach can be expanded to additional enzymes and feedstocks. The identified enzymes can be prepared as recombinant proteins and tested for suitability for inclusion in the next generation of enzyme cocktails.

Weighted Project Score: 5.4

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



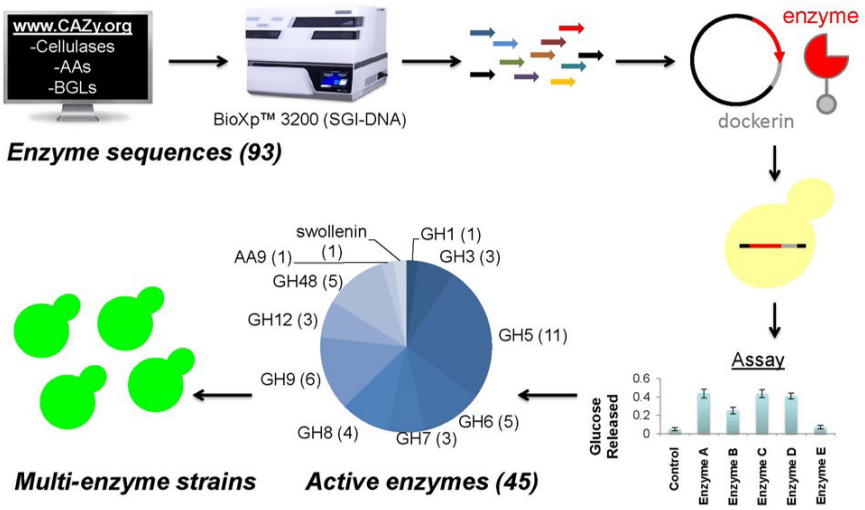


Photo Courtesy of J. Craig Venter Institute

Overall Impressions

- The project contained an interesting idea: introducing expression cassettes for heterologous enzymes into separate locations in the yeast genome to allow shuffling of combinations. The main benefit of using yeast, therefore, was in generating large numbers of combinations of enzymes. It was very unclear from the presentation how many combinations of the enzymes were generated and screened. The testing phase for the final strains appeared to be rather cursory. If there were benefits of expression of cellulases in the yeast strain in reducing exogenous enzyme dosing, this would have been good to examine better using dosing studies. No mention was made of novel synergies detected in the system.
- Overall, what we heard was a story describing a lot of things that didn't work and a lot of problems. They had some successes, and the general concept was interesting, but the approach simply didn't pan out.
- The team developed an efficient workflow for screening multiple cellulases in combination, using automated gene synthesis. The concept of leveraging possible synergy is a good one, though there were no proposed mechanisms of how synergistic interactions would occur. Once the platform was developed, the amount of actual screening performed was small. Given the high-throughput workflow and the resources available at J. Craig Venter Institute, which is likely why this project was selected for award, I would have expected that a larger number of genes and combinations would have been screened.
- Considering the stated advantages of an existing infrastructure for rapid synthetic biology pipeline, new state-of-the-art facilities, the first BioXP machine, Novozymes as a partner, and generally being at the forefront of synthetic biology, this project was poorly executed. The change from the CBP approach after the 2015 Peer Review seems to have thrown them off of their game.
- Overall, this was a novel way capitalizing on J. Craig Venter Institute's synthetic biology strength. The project didn't deliver the expected outcome and not unexpectedly. Many groups, with and without acceleration through synthetic biology tools, failed in this route, and not unexpectedly, this one did too.

PI Response to Reviewer Comments

- We thank the reviewers for the positive comments, the constructive criticisms, and the good understanding of our project. We realize that our presentation did not emphasize where we started for producing the results we presented. This may have resulted

in some of the negative comments. When we started, synthetic biology studies usually involved model cellulose substrates. We believe that we developed technologies to help link synthetic biology with real biomass substrates. This is an important step forward. At least two technologies we developed are already used at NREL to accelerate BETO-funded projects. We agree with reviewers on the importance of tasks they mentioned, but some of the tasks are apparent only now because advances were made in our research. We would like to think of them as valuable ideas for our future studies.

Regarding the lack of clarity mentioned by the first reviewer on how many enzyme combinations were screened, we estimate to have screened 70% of 512 combinations of enzymes from a cross of two multi-enzyme strains. Mechanistic studies of synergy were out of scope for the project, but we are excited to conduct these studies.

Regarding comments by the second reviewer, we agree that we have had successes and disagree that the approach did not pan out. We can agree that the project has not yet produced exciting results, but the contribution we made in the field is impressive, as mentioned above in our first paragraph. We expanded on almost every aspect of previous synthetic biology work and incorporated a real feedstock. A decision was made not to pursue CBP and to focus on developing a discovery tool for enzyme synergy after the 2015 Project Peer Review meeting, so the goal set at the beginning of the project for CBP applications was not met. A story of a lot of things that did not work is expected for a pioneering (low-TRL) work, which our project was supposed to be based on the FOA.

Regarding related comments by the third reviewer, we disagree with the poor execution, because low-TRL projects are expected to have a lot of uncertainties. We conducted productive research by dynamically applying our excellent skills to solving problems.

Regarding the comments by the fourth reviewer, we agree that the scale of screening was small. We wished to screen more, but much of the time had to be devoted to developing technologies that formed the basis of screening. However, we can continue screening, and we are writing a paper to disseminate our technologies for others to use.

Regarding the comments by the fifth reviewer on CBP, the reviewer knows this, but we would like the public to also know that the focus of our project shifted after the 2015 Project Peer Review meeting, as described above in our third paragraph. We had successes with the revised goals. The decision not to pursue building CBP organisms based on yeast within the timeframe of our project was excellent, but advances in synthetic biology in yeast are rapid and are making the boundaries between yeast and other organisms blurry. Rewriting the complete genome will soon be possible in yeast. It will then be feasible to incorporate desirable characters from various organisms into yeast.

We would like to benefit from excellent advice from the reviewers and develop future projects around their ideas. Thank you for the opportunity.

SYNTEC—SYNTHETIC BIOLOGY FOR TAILORED ENZYME COCKTAILS

(WBS#: 2.5.3.201)

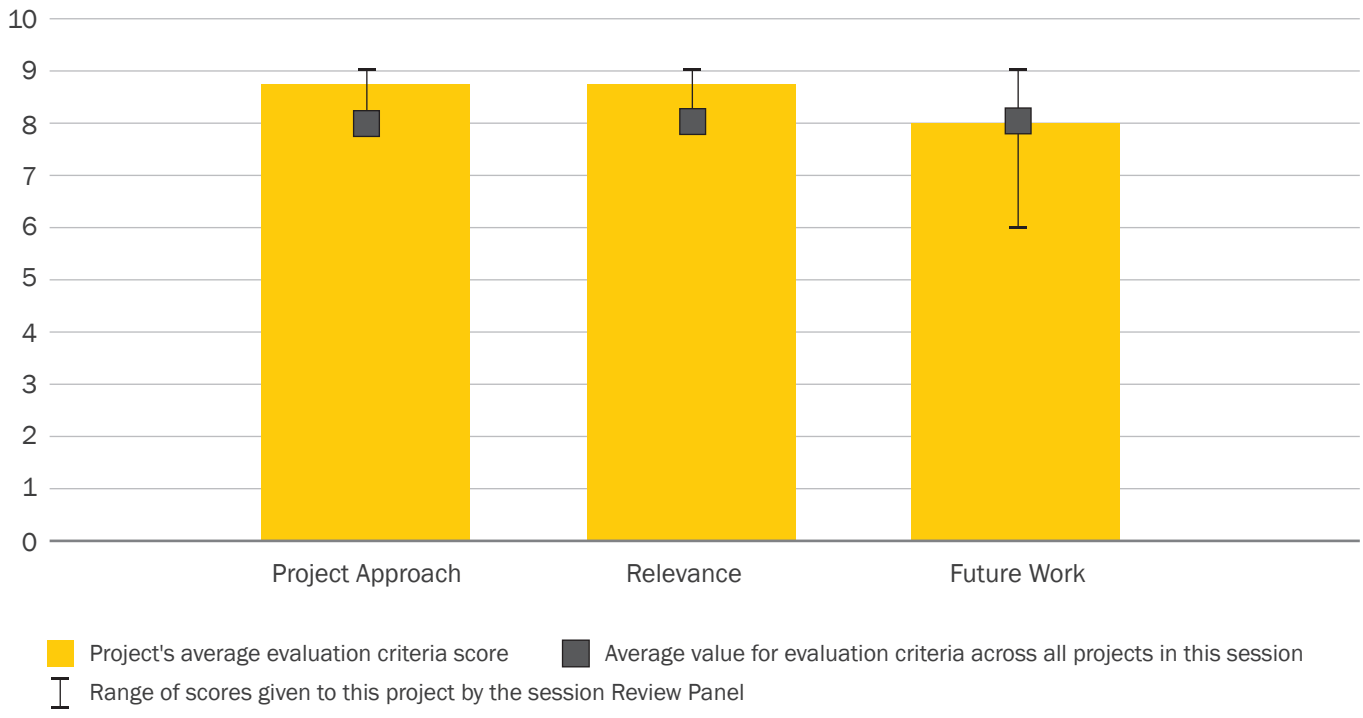
Project Description

Using a novel enzyme screening method inspired by synthetic biology, Novozymes developed new technology that allows for more rapid tailoring of enzyme cocktails. The methodology can be applied to specific feedstocks and/or coupled to address a specific hydrolytic conversion process context. Using combinatorial high-throughput screening of libraries of enzyme domains, we can quickly assess which combination of catalytic modules delivers the best performance for a specific condition. To demonstrate the effectiveness of the screening process, we measured performance of the output catalytic cocktail compared to CTec3/HTec3 (Cellic CTec3 is a commercial cellulase and hemicellulase complex from Novozymes, and Cellic HTec3 is a commercial enzyme for hemicellulose hydrolysis from Novozymes). The test substrate was ammonia fiber expansion pretreated corn stover. CTec3/HTec3 was assayed at the optimal pH and temperature and in the absence of any pH adjustment. The new enzyme cocktail discovered under SynTec was assayed in the absence of pH adjustment and at the optimal temperature. Conversion is delivered by SynTec enzyme at a significant dose reduction relative to CTec3/HTec3 at the controlled pH optimum, and without titrant required to maintain pH, which delivers additional cost savings relative to the current state-of-the-art process. Using a techno-eco-

Recipient:	Novozymes Inc.
Principal Investigator:	Sarah Teter
Project Dates:	7/1/2013–12/31/2015
Project Category:	Sun-setting
Project Type:	FY 2012—Synthetic Biology: DE-FOA-0000719
Total DOE Funding:	$2,500,000
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$500,000
DOE Funding FY 2017:	$500,000

Weighted Project Score: 8.6

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



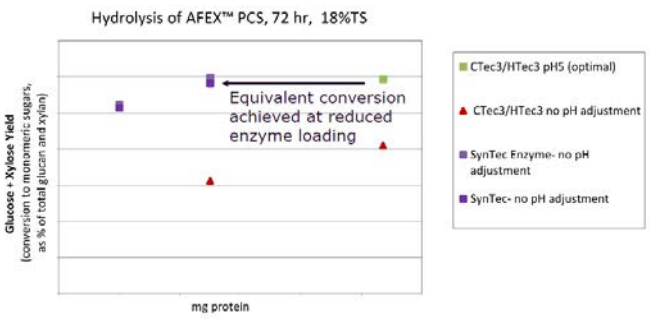


Photo Courtesy of Novozymes Inc.

nomic model developed by MBI, this improved enzyme cocktail led to reduced biomass sugar production costs that were 1.4x lower than for the base case (CTec3 used under its optimal conditions).

Overall Impressions

- The project investigated an interesting hypothesis and developed some useful tools for further work in the area. Their most interesting discovery was that supporting multiple enzymes on a scaffold is no more effective than using a cocktail of individual enzymes. Can this result be used to streamline future formulations of cellulase systems?
- In this project, Novozymes developed a rapid cellulase screening protocol leveraging protein scaffolds to incorporate the potential benefit of enzyme co-localization and synergy. Although the targets were not quite met, impressive improvement was achieved. Once the platform was established, this occurred very quickly, demonstrating good potential for future application. I have no doubt that significant progress can be made if this work were to be continued. The project also contributed to general knowledge base on cellulases. No co-localization benefit was found. Some new synergy was elucidated, but nothing remarkable.
- A clever combinatorial high-throughput screening methodology was developed and demonstrated to identify a significantly improved enzyme composition. The methodology can be used to identify tailored enzyme combinations for different substrates, operating conditions, etc. A composition of 11 enzymes added to CTec3 seems like a complex undertaking for commercialization. It could be a starting point for simplification and removal of minor contributing components. Modifying the composition to eliminate the need for pH control is useful and cost saving. Translation of results to a relevant filamentous fungal host could verify the usefulness of the tool in accelerating commercial development.
- The project goals and technical challenges are well-aligned with BETO's mission to reduce enzyme hydrolysis costs and were largely achieved by a novel screening method to expedite the search through natural diversity and mutagenesis of combinatorial libraries of enzymes, debottlenecking the major challenges—enzyme purification and quantifications. This is very nice development and will allow Novozymes to offer a better package with a lower enzyme load and a broader pH operation spectrum. Kudos for that.

PI Response to Reviewer Comments

- Regarding the potential for engineering a production strain for enzymes discovered in this project, we agree that simplification of the cocktail might be attractive. Generally, we embark on production of "tailored" cocktails by generating a production strain as needed when we have a specific customer in mind. Thus far, we have delivered commercial cocktails for five customers who are operating commercial-scale biorefining facilities. Tailoring a commercial product for ammonia fiber expansion pretreated corn stover (or similar), and absence of pH control, would be warranted if there were a specific customer intending to utilize this type of process at commercial scale.

DESIGN AND OPTIMIZATION OF BIOCHEMICAL / BIOFUEL PRODUCTION WITH BIOSENSOR-GUIDED SYNTHETIC EVOLUTION

(WBS#: 2.5.3.203)

Recipient:	Lygos Inc.
Principal Investigator:	Eric Steen
Project Dates:	3/1/2013–10/31/2016
Project Category:	Sun-setting
Project Type:	FY 2012—Synthetic Biology: DE-FOA-0000719
Total DOE Funding:	$1,783,072

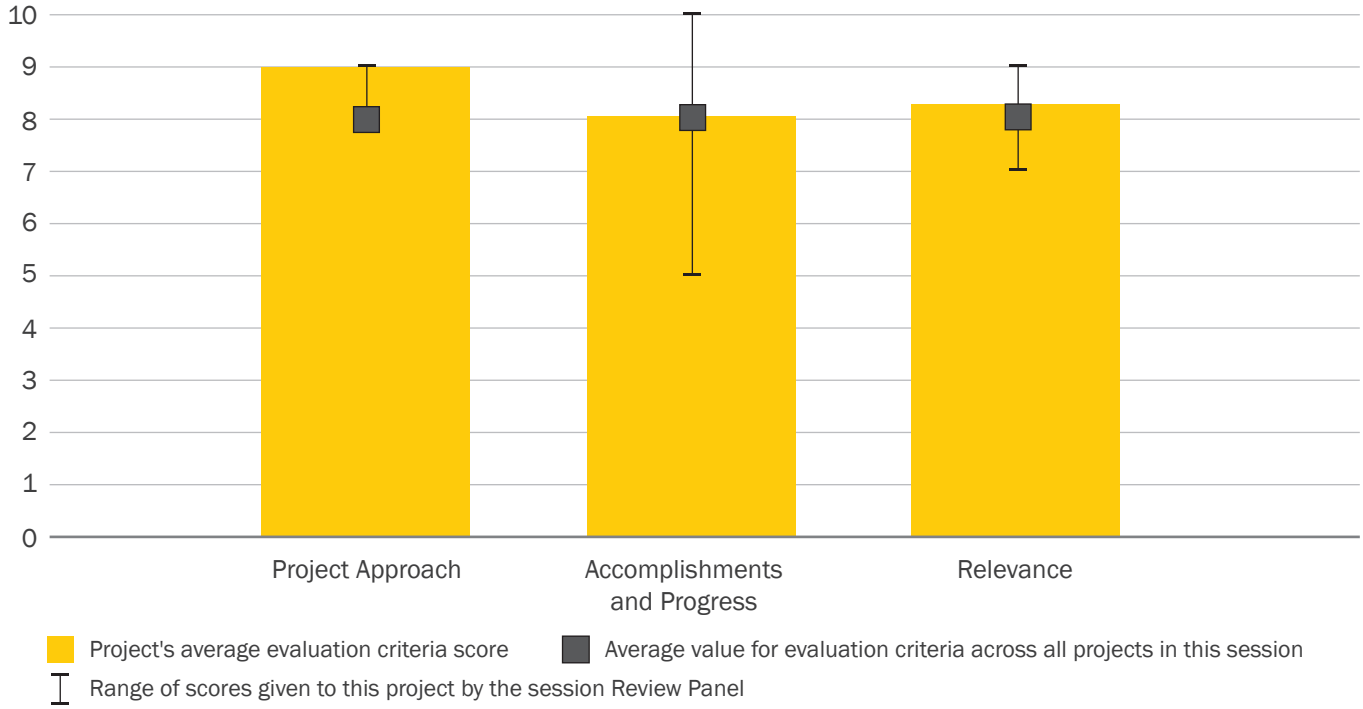
Project Description

Using novel strategies derived from synthetic biology, this research effort supported Lygos' development of a biocatalyst for conversion of sugar feedstocks into fine and commodity chemicals. Specifically, Lygos targeted compounds that are currently derived from petroleum using inefficient, ecologically hazardous chemical processes. To date, market expansion for select compounds has also been inhibited by high production costs and low process yields. Lygos developed high-yielding microbial catalysts that will be cost-advantaged relative to existing chemical routes. The goal of this project was to develop rapid, inexpensive methods to generate biocatalysts that employ non-food biomass feedstocks to make the valuable chemicals and decrease our reliance on petroleum.

In this project, Lygos scientists and engineers built and employed a process for engineering biocatalysts, encompassing software design tools, physical DNA editing tools, high-throughput screening strategies using biosensor-guided evolution, and a software management system for statistical analysis. While the approach was deployed for production of a single, specialty biochemical, malonic acid, it could be broadly applicable to other products and biocatalyst engineering efforts. Finally, our strain engineering efforts were validated in a fermentation process to 50 liters, using cellulosic sugar.

Weighted Project Score: 8.3

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



Overall Impressions

- The presentation gave a strong impression of a well-run project that has achieved its goals. The slides showed a clear logical progression and comprehensive approach, with good use of project management methodologies. Overall, the project shows development of a solid, functional, iterative strain construction and metabolic engineering platform that could be applied to a range of target products.

  The first target chosen is a relevant commercial chemical, and the metabolic pathway suggests a benefit in incorporating CO_2 into the product to raise the yield on sugar above 1. This individual presentation does not disclose TRY for malonic acid achieved so far, but the selection of target and approach to strain and process development look sound.

  The project is a great demonstration of how public funding can allow private companies to take a risk and develop technology that has the potential to generate much larger economic benefits down the road, and should provide a great return on investment.

- Lygos carried out a well-organized program in genetic engineering that displayed a nice combination of computational work with organism development. The transition to malonic acid production added utility to the process by providing access to a known chemical product. However, the costs are currently quite high, and the plan to target boutique users is not sustainable in the long run.

- High-throughput methods for optimizing biocatalyst performance are needed to speed up the pace of all metabolic engineering projects, which would benefit BETO and the entire industrial biotechnology industry. One of the goals of this program was to develop a biosensor, but unfortunately this was not sufficient for a reliable, high-throughput screen. Hopefully more work will be done on this since tying a biosensor to growth could enable very high-throughput selection with application to many projects. The particular choice of malonic acid as the target is questionable. Their preliminary TEA determined a potential production cost, but neither the current price nor the market need were discussed.

- This is a good demonstration of implementation to practice using a novel strain and design-build-test-learn model to allow faster development of a biobased chemical—which, eventually, if it is economically viable, will allow a more sustainable production of this and other molecules down the road. Now that Lygos has a working workflow and developed the skills and expertise to accelerate strain and process development timelines, it will evidently become a faster process for them for any new molecule. Great work and good luck on partnering with industry on scaling this up.

PI Response to Reviewer Comments

- Lygos greatly appreciates the DOE support and reviewers' positive feedback on our successfully organized and executed project, including a combination of computational software tools, genetic engineering tools, and high-throughput biosensor screening with a focus on producing bio-malonic™ acid. This support and project was critical for Lygos in developing our malonic acid technology.

  Lygos is pleased that, overall, the reviewers' comments recognize the value of our approach in building a platform for rapid engineering of microbes and the specific success the project achieved in demonstrating commercial readiness for our flagship product, bio-malonic™ acid. Now that the platform for engineering is in place, continued improvement of the bio-malonic acid process and future target products are expected to advance even more efficiently.

  We appreciate the reviewers' feedback that high-throughput methods are required for optimizing biocatalyst performance and advancing the field,

which was one of the goals of developing a biosensor. We indeed developed a reliable, high-throughput screen using the biosensor, where we attained a high correlation of the biosensor to high-performance liquid chromatograph readings of bio-malonic acid concentration (R2 0.9). We believe this strategy could be broadly impactful to the industry. The screen we developed enabled Lygos to conduct numerous design, build, test, and learn cycles and identify genetic modifications that improved process performance, attaining more than 2,100 strains built and screened per scientist. In the future, Lygos may implement the biosensor screen on a robotics screening platform to further decrease labor requirements and improve screening capacity beyond 2,100 strains per scientist.

We appreciate the reviewers' support for Lygos' product selection of malonic acid. As one described, "The project team seem to have clearly thought about the choice of target molecule (A top 30 molecule from the DOE analysis,[32] the size of the market, sequestration of carbon, and reduction in production of a toxic byproduct by displacement of the petrochemical process."

In more detail and as described in the project, bio-malonic acid is an ideal target for bio-production and was identified previously by DOE as one of the top 30 molecules that could be produced from lignocellulosic sugars. First, it represents a common node of metabolism from which numerous other biological products are derived. Second, it is a toxic, expensive, cyanide-derived product today, and production is restricted to China. Third, because of Lygos' bio-malonic acid pathway's high theoretical yield, it can be produced at lower cost and enable major market expansion. Specific market and pricing discussions are confidential and were beyond the scope of the public forum. However, Lygos' proprietary technology to produce bio-malonic acid promises to enable better quality and expanded supply compared to the current cyanide-based product. We plan to compete on all of these elements as we continue to commercialize bio-malonic acid.

We look forward to continuing to advance and commercialize bio-malonic acid.

[32] T. Werpy and G. Petersen, eds., T*op Value Added Chemicals from Biomass: Volume I—Results of Screening for Potential Candidates from Sugars and Synthesis Gas* (Golden, CO: National Renewable Energy Laboratory, August 2004), https://www.nrel.gov/docs/fy04osti/35523.pdf.

SYNTHETIC MICROORGANISMS TO ENABLE LIGNIN-TO-FUEL CONVERSION

(WBS#: 2.5.3.205)

Project Description

The proposed research aims to address one of the most challenging issues in lignocellulosic biorefinery: utilizing lignin for fungible products. The research integrates synthetic biology design of microorganisms, fractionation of lignin, and fermentation optimization.

The lignin-to-lipid platform will achieve more complete usage of carbon in biomass, increase the net energy gain and carbon balance, enhance the economics and self-sufficiency of biorefineries, and alleviate the shortage of lipid for biodiesel production.

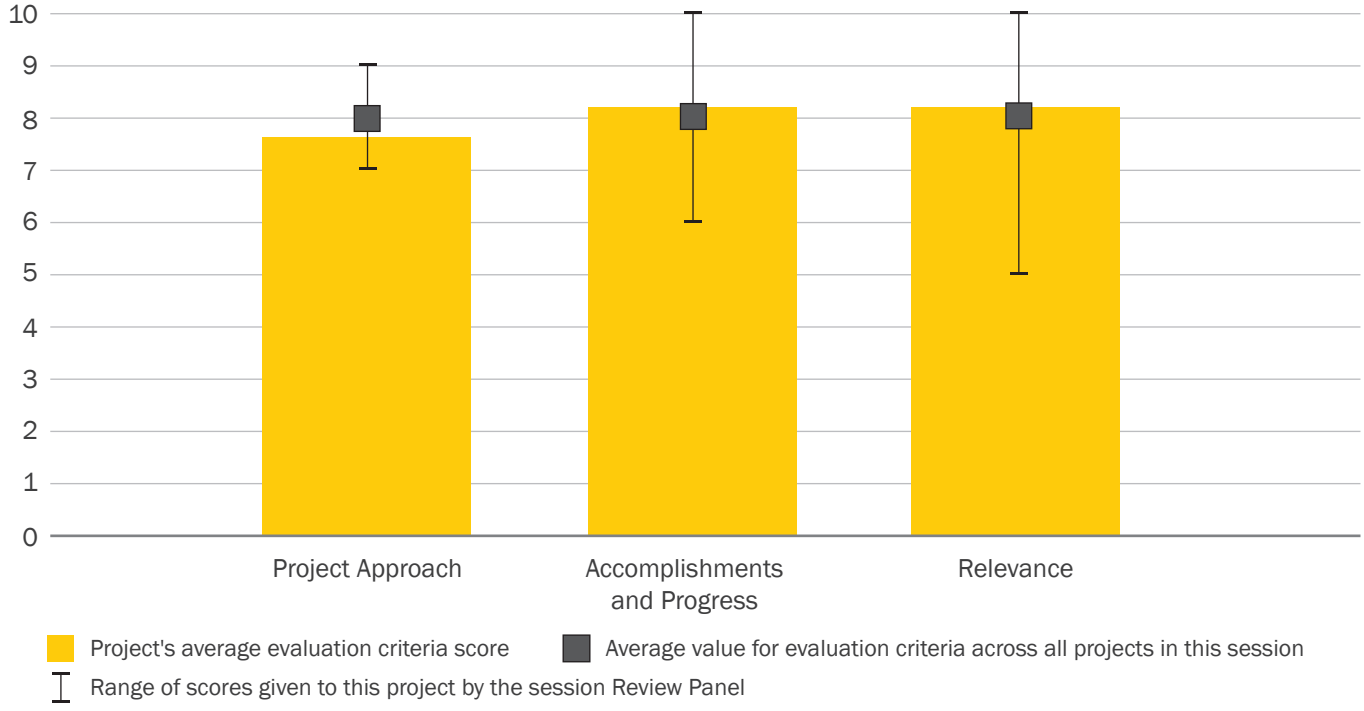
Recipient:	Texas AgriLife Research
Principal Investigator:	Joshua Yuan
Project Dates:	7/1/2013–6/30/2016
Project Category:	Sun-setting
Project Type:	FY 2012—Synthetic Biology: DE-FOA-0000719
Total DOE Funding:	$2,399,977

The research will address three major technical barriers: lignin depolymerization, carbon flux to higher lipid yield, and optimized fermentation for scaling up.

We have achieved most of the major technical milestones. First and foremost, we have achieved 11 g/L of lipid titer for biorefinery waste conversion. Second, we have achieved over 120 colony-forming units/milliliter laccase production and 13.6 g/L protein secretion from Rhodococcus opacus.

Weighted Project Score: 8.1

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



Overall Impressions

- The project has some elements that look promising: laccase production in a Gram-positive bacterium at 13 g/L and lipid production from lignin material. However, the presentation of the data and progress towards goals was fragmented and difficult to follow. It was challenging to see from the presentation exactly where the project currently is in relation to an economically viable process. Obviously, there are almost 4 years of experimentation underlying the presentation, but it really would have benefited from more selective choice of data and clearer explanations, particularly on the metabolic engineering for lipid production and the different forms of lignin streams being used.
- The PI has developed an interesting process for converting lignin into higher-value lipids. The increase in value will be smaller with biodiesel as the product, but converting the lignin to a more tractable fuel is a useful contribution to the larger BETO program.
- This project has developed a single organism for enzymatic lignin fractionation and subsequent conversion of monomers to lipids. Both laccase expression and lipid content of this strain are quite impressive. The team should evaluate whether there is any advantage in using two separate strains for laccase expression and monomer conversion. The project is on track to hit the milestones, though this is a challenging effort and still a long way from commercialization. There is potential to consolidate this work with other lignin valorization projects within BETO.
- This project is moving into the final stage, a scale-up to 2 liters with concomitant TEA. Nice progress was made in laccase secretion for depolymerization and metabolic engineering to increase lignin-to-lipid production by Rhodococcus. I'm surprised that Rhodococcus can apparently secrete laccase for depolymerization and still maintain TRY for lipid production. Many papers, and two patent applications, were written related to this process, and it has apparently been licensed.
- This is a good and energetic team. They are doing a lot of good work in the right direction in close alignment with industrial partners (Archer Daniels Midland and ICM Inc.), which will likely, if technical and economic viability can be realized, end up in a commercialization track. The PI is advised to have a routine benchmarking against critical TEA analysis as a way to articulate progress toward economic goals. Great job!

PI Response to Reviewer Comments

- We appreciate the reviewers recognizing the progresses and impact as impressive.

  First, regarding to the choice of data and clarity of presentation, we apologize that we had to summarize the data from more than 10 publications, the efforts of utilizing different substrates, and the research ranging from microbial engineering to process optimization, all within 15 minutes. The project itself has evolved during the past 4 years. At the time of initial selection, there was very little research on lignin bioconversion in the field. The project, thus, was focusing on synthetic biology engineering of microorganisms to convert lignin. During the past 4 years, the project evolved to focus on bioconversion of various biorefinery streams, where the substrates, scope, and technologies all expanded significantly. The substrate itself expanded from kraft lignin to several biorefinery waste streams from acid and ammonia fiber expansion pretreatments, and produced by ADM, Michigan Biotechnology Institute, and ICM, Inc. The initial focus of metabolic engineering was also expanded to integrate fractionation technology development and fermentation optimization with strain engineering to improve bioconversion. The evolving proj-

ect scope, the multiple publications generated, the multi-scale deliverables, and the overall complexity of the project might have contributed to some confusion of the presentation.

Second, the reviewer has raised a very good question regarding the balance of laccase secretion and lipid production. When the enzyme is produced at very high titer (e.g., >5 g/L), the lipid production can be significantly compromised. In order to mitigate the challenge, we developed two alternative strategies for bioconversion: (1) consolidated lignin processing and (2) fractionation (enzymatic or chemical) followed by fermentation. We can use the R. opacus strain to produce laccase enzyme and subsequently use the enzyme to fractionate lignin for fermentation, or we can use consolidated processing, where the same R. opacus strain produces enzyme and lipid. We have developed a series of strains producing laccase at different levels for different applications.

Third, regarding the benchmarking against critical TEA, we agreed with the reviewer. The project has an initial TEA, and we have evaluated how technical progresses contributed to economic feasibility. More comprehensive analysis is being carried out using the ASPEN model, with lipid production platform bolt on at a cellulosic ethanol plant.

Fourth, regarding the consolidation of this work with other lignin valorization work, we have already discussed synergy and collaboration with the relevant PIs. Importantly, based on our discussion and project review, the efforts among different waste utilization projects are very complementary to one another, and there is no significant redundancy. These multiple projects enable the development a complementary and complete portfolio of technologies for multi-stream integrated biorefinery. It will also help to de-risk the technology development and improve the productivity and accountability.

ENZYME ENGINEERING AND OPTIMIZATION (TARGETED CONVERSION RESEARCH - RATIONAL DESIGN)

(WBS#: 2.5.4.100)

Project Description

The Enzyme Engineering and Optimization project targets technologies that ensure the DOE 2022 target of 10 mg cellulase/g cellulose is met. Our approach is to engineer improved glycoside hydrolase family 7 (Cel7A); however, the minimal existing genetic tools are a major barrier. We have determined the following: (1) some natural variants have higher activity than the T. reesei (industrial) enzyme, and better enzymes still reside in nature; (2) some of these enzymes may have expression problems in industrial hosts, but this can be overcome; and (3) we can describe on a structural level the biochemical function of cellulases.

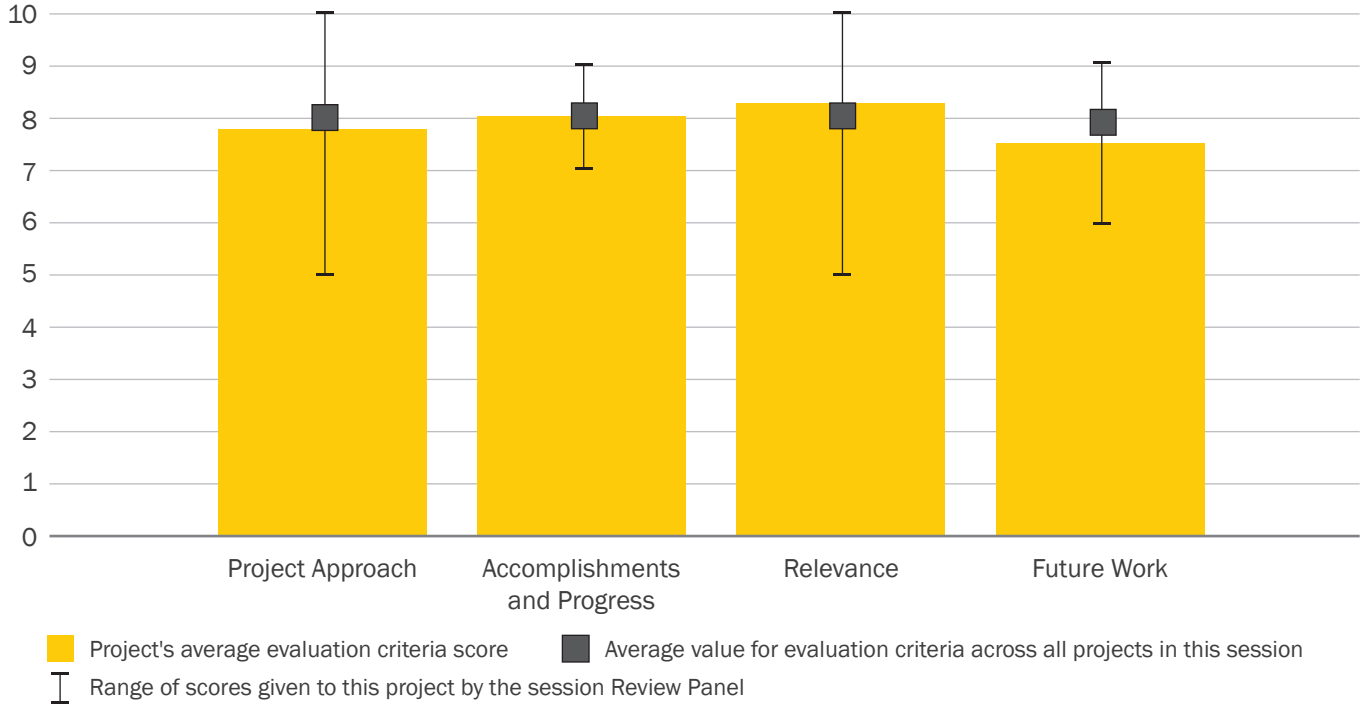
Recipient:	National Renewable Energy Laboratory
Principal Investigator:	Mike Himmel
Project Dates:	10/1/2015–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$1,600,000
DOE Funding FY 2016:	$1,600,000
DOE Funding FY 2017:	$1,600,000

Limited understanding of Cel7A processing in T. reesei restricts rational study of this enzyme. To address this challenge, we model cellulase action, design and build modifications, and measure performance. We are applying computational algorithms to a natural diversity library to parse out Cel7-specific codon usage to enable better heterologous expression.

Collaboration with PNNL's Environmental Molecular Sciences Laboratory is revealing how Cel7A is processed and trafficked in T. reesei, which should enable

Weighted Project Score: 7.9

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



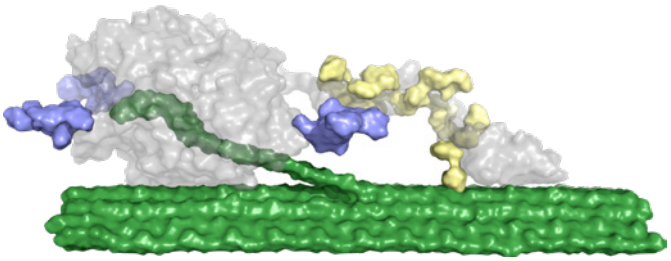


Photo Courtesy of National Renewable Energy Laboratory

strain improvements. We also use our expertise to enhance metabolic pathways of advanced biofuels and products. We collaborate closely with Targeted Microbial Development to screen variants of pathway genes and hand off winners to Targeted Microbial Development for in vivo testing for increased product TRY. Our ability to generate enzyme activities across a range of levels means Targeted Microbial Development can balance enzyme rates to optimize metabolic flux.

Overall Impressions

- The project is built on the idea that detailed study of individual enzymes through basic research on structural biology will lead to improvements in industrial enzyme mixtures. The project has generated some scientifically interesting results (making the performance of the Tr Cel7A like that of the Pf Cel7A by swapping sub-domains, investigating the role of glycosylation sites in Cel7A activity and stability), but there is a weakness is in closing the loop and demonstrating the performance of single enzymes and improved mixtures in deconstruction of pretreated biomass and in measuring expression of native and mutated variants in a relevant host.

  There are significant opportunities for the project team to provide practical benefits to biorefinery and industrial enzyme companies: release of platform T. reesei strains for single enzyme production and advantaged cocktails; a comprehensive study on the benefits of codon usage optimization for synthetic genes in T. reesei; a broad survey of cellobiohydrolase 1 biodiversity and performance; and extension of the study into enzyme compositions across the wide range of pretreated material to which NREL has access.

  My main feedback would be to encourage the project team to consider in full the technical risks and benefits of a particular route early on (e.g., the risks of generating data that do not repeat in a production host versus the benefit of using an easier host, such as yeast). The benefits of throughput may not outweigh the risks of artifacts. Also, I think it is worth stepping back and considering the practical benefits that can be supplied to companies in the industrial biotechnology field through enzyme selection and production, versus what may be scientifically interesting results.

- The PIs have developed a highly interesting program to improve the generation of reactive cellulases and development of new cellulase systems for the production of sugars in the biorefinery. The work has clear industrial potential, and the team would benefit from including additional descriptions of this work's links to commercial application. The dots are all there, and just a little more time spent connecting them will really prove the utility of this work.

- The primary focus of the Enzyme Engineering and Optimization program is to develop better cellulase activity. Since this enzyme represents up to 25% of biorefinery cost, this is a very important task. One might think that after 10 years of active research, there is not room for significant improvement in cellulase activities. However, their results indicate that this is not the case and that there are several exciting avenues to more active and robust enzymes. In fact, given the relevance, it may be worth putting more resources on this work. Future targets are not well-defined. How much improvement are they targeting, both in the near term and long term?

A secondary objective is to aid metabolic engineering projects by improving enzymes that are critical to their success. There are also no quantitative targets set for these enzymes. This work must be closely managed to make sure it doesn't distract from the cellulase work. In the future, perhaps this should be two separate programs.

- The project history and relevance is of most importance to drive cost reduction of bioconversion technologies. The overall technical and management approach is sound and logical. Emphasizing the need for a publicly available chassis fungal strain is well-understood as a mitigation strategy and democratization of knowledge. Commercial viability appeared to be sound based on the TEA and industrial partners' testimonies. An opportunity to expedite even further is to focus a critical mass of resources on holistic development of this category (enzyme engineering, production host, separation, etc.). Another opportunity would be to do the metabolic pathway enzymes discovery at the individual teams or partnered with an academic laboratory for prospecting. The PI is already hinting for such in the event of a budget cut.

PI Response to Reviewer Comments

- We thank the Review Panel for the supportive comments and helpful feedback.

This is exactly our overall goal, and we would welcome any opportunity to work with producers to exploit these results and advances. Note that the yeast screening work was approached in exactly this way (i.e., a quick screen based on the new finding from Mascoma). We continue to strive to be introspective regarding our expenditure of resources and time when driving toward goals important to DOE and industry.

The Review Panel made a very good point regarding cellulase metrics, and we will clarify in future AOP planning. The challenge for us is that only the enzyme companies know what it costs to produce commercial cellulase formulations. We thus necessarily take the approach that improving the key component enzyme performance will always result in a reduction in cellulase cost, regardless of the exact production and formulation path taken by a particular company. The enzyme companies appear to agree that this assumption is valid. The observation that progress in Task 2 (metabolic enzymes) is not as strong as that in Task 1 (enzymatic hydrolysis) is due in part to the disparate respective funding levels. A second cause, explained in the oral presentation, is that the Task 2 work was recently initiated. In planning the FY 2016–FY 2017 AOP, we perceived a more urgent need to make progress in the cellulase arena; this decision can be reassessed for 2018.

LOW-ENERGY MAGNETIC FIELD SEPARATION USING MAGNETIC NANOPARTICLE SOLID ADSORBENTS

(WBS#: 2.5.5.100)

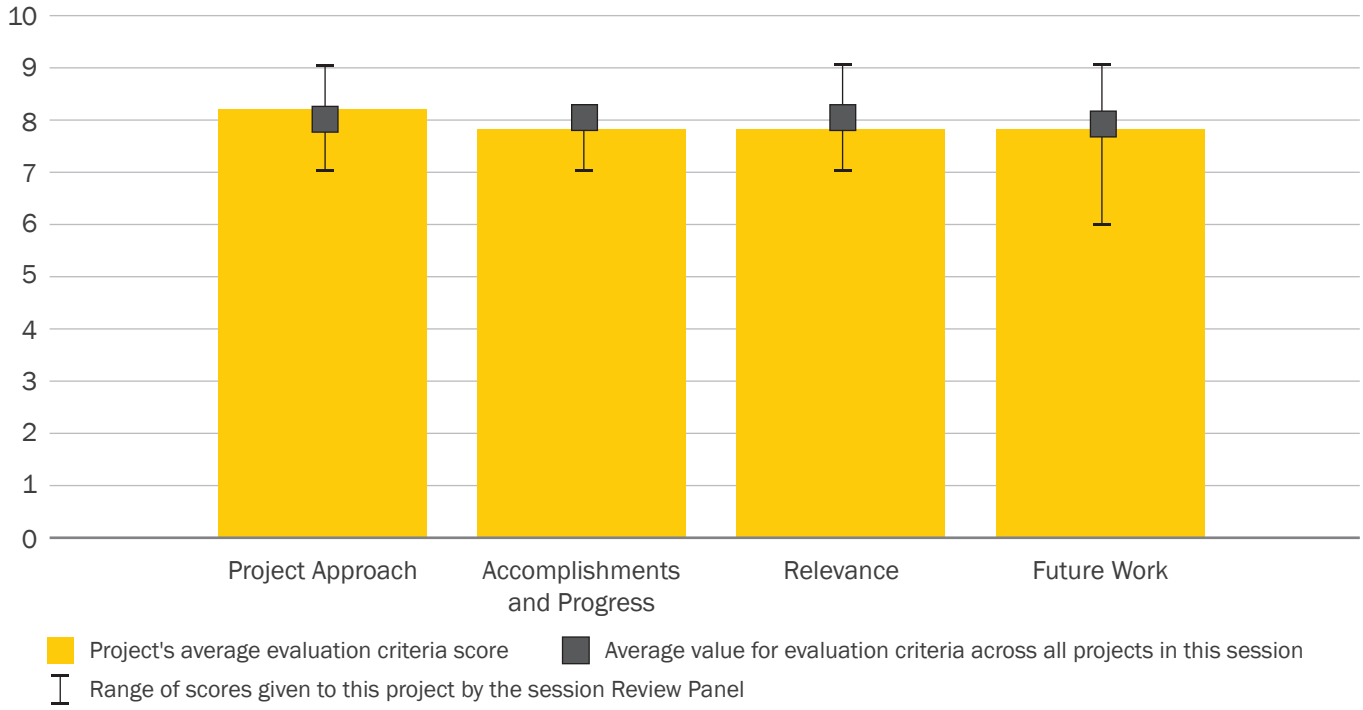
Recipient:	Argonne National Laboratory
Principal Investigator:	Phil Laible
Project Dates:	7/25/2011–9/30/2018
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$300,000
DOE Funding FY 2015:	$300,000
DOE Funding FY 2016:	$300,000
DOE Funding FY 2017:	$300,000

Project Description

We are exploring energy-efficient technology to improve process economics for separations of fuels and products. Nanostructured adsorbents (NA) produced using heterogeneous vapor-phase polymerization successfully adsorb target hydrocarbons. Tailored NA surface treatments enable adsorption of long-chain isoprenols with high affinity and specificity. The NA's capacity for hydrocarbon far exceeds their weight. The materials swell upon binding and have conditional properties that allow for facile removal from bioreactors. Magnetic and/or low-pressure, mechanical compression routinely releases >95% of the hydrocarbons adsorbed during a cycle. On the 10-L scale, NA has proven stable and reusable for tens of cycles and has allowed economics to be compared at the 100-cycle level. Little, if any, materials are lost during each cycle. Current efforts focus on scaled syntheses using commercial production methods. Future NA advancements will focus upon expanding adsorption specificity to include a range of other products produced via biochemical conversion routes. As compared to current, commonly practiced solvent extraction and distillation methods, the costs associated with this novel approach can be much lower, especially as multi-cycle operations are proven. Applications for this process-in-

Weighted Project Score: 7.9

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



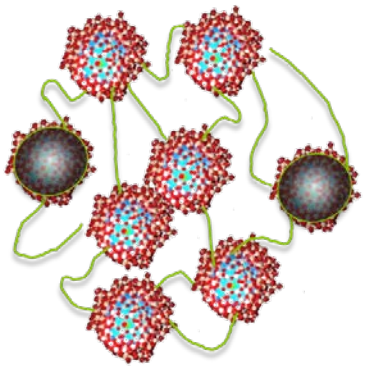


Photo Courtesy of Argonne National Laboratory

tensified approach are greatest for fermentation practices where products are exported into the culture medium and product inhibition is exhibited at low titer.

Overall Impressions

The project has put forward what looks to be a novel solution to the recovery of chemical or fuel products, with the possibility of also removing inhibitors.

The basic generation of material and down-selection to more economic versions looks good, with demonstration of high recovery rates over multiple rounds. The only concern I have is how the performance looks when applied to full-on process configurations (residual solids, complex mixtures of product, cells and pretreatment side products). It would be good for the team to select some of these from the BETO portfolio and see how the material plugs in to existing processes and process streams.

- The PIs have developed an interesting approach for separating hydrocarbon biofuel from a fermentation system. The economics are promising, and the process has been demonstrated at large scale. The project will benefit from testing the process on mixed hydrocarbons. The biggest challenge may be convincing industry that this method is preferable to more conventional separation methods currently in use.
- This program developed nonabsorbent particles for biofuel separation from the fermentation broth. This would enable removal at low concentrations in situ, preventing buildup of the fuel to toxic levels. The team generated a lot of results and met milestones. The method has the potential for large cost savings, but the particles themselves are currently too expensive. Efforts on new production methods were investigated, but they were trimmed so that effort could be focused on demonstrating separation efficacy.
- Separations projects like this have the potential to significantly reduce process costs and be game changers. This need is the basis for forming the Separations Consortium, which this project will join. Specific product removal, reduced inhibition, and continuous fermentation are very worthy goals and can be enabling across bioprocesses. This project did a nice job of leveraging previous work and down-selecting when the original particle was found to be too expensive. Economics and process integration of a new unit operation will be critical.
- This is a very good demonstration of skills and expertise deployed on the right target with a great potential for separation cost savings and upside potential for other projects, like protein separation.

PI Response to Reviewer Comments

- We thank the reviewers for their kind words. We have only one comment/presentation regret: We can see where the down-selection slide on magnetic nanoparticles did not clearly explain that, although these starting materials were abandoned, that we went ahead successfully with lower-cost, non-magnetic replacements that showed conditional properties (e.g., float or sink when binding a threshold amount of biofuel/bioproduct) that were just as good for bioreactor integration/processing as

magnetic-based operations. Thus, we have magnetic-type functionality of the adsorbents without the costs associated in their synthesis. We wish this portion of the talk could have been expanded (as it was in 2015). We just needed to justify the title of the project and how we moved away from magnetic starting materials (in 2017). It seems that four of the five reviewers understood this point, but it is now apparent that we could have been clearer in our description of the technology as it moves forward with lower costs.

SEPARATIONS CONSORTIUM

(WBS#: 2.5.5.501-508)

Project Description

The Separations Consortium aims to move cost-effective, high-performing separations technologies to market faster through coordinated research at the national laboratories that targets challenges relevant to industry and BETO's priority conversion pathways. It addresses BETO stakeholder feedback that separations technologies merit near-term R&D that will reduce biofuel and bioproduct production costs. After a study of separations challenges in BETO priority conversion pathways, the eight-laboratory consortium formed into five teams in the following areas: preserving biochemical catalysts and biochemical, thermochemical, algal, ionic liquid process-based separations.

A TEA and life-cycle analysis team provides technical teams insight into the economic and environmental effects of their work. A steering committee and an industrial advisory board provide guidance. The consortium's future work includes conducting research to enable cost-effective separations relevant to lignin

Recipient:	Argonne National Laboratory, Idaho National Laboratory, Los Alamos National Laboratory, Lawrence Berkeley National Laboratory, Oak Ridge National Laboratory, National Renewable Energy Laboratory, Pacific Northwest National Laboratory, Sandia National Laboratories
Principal Investigator:	Jennifer Dunn
Project Dates:	5/1/2016–9/30/2019
Project Category:	New
Project Type:	AOP
DOE Funding FY 2014:	$0
DOE Funding FY 2015:	$0
DOE Funding FY 2016:	$500,000
DOE Funding FY 2017:	$3,000,000

Weighted Project Score: 8.1

Weighting: Approach–25%; Relevance–25%; Future Work–50%.

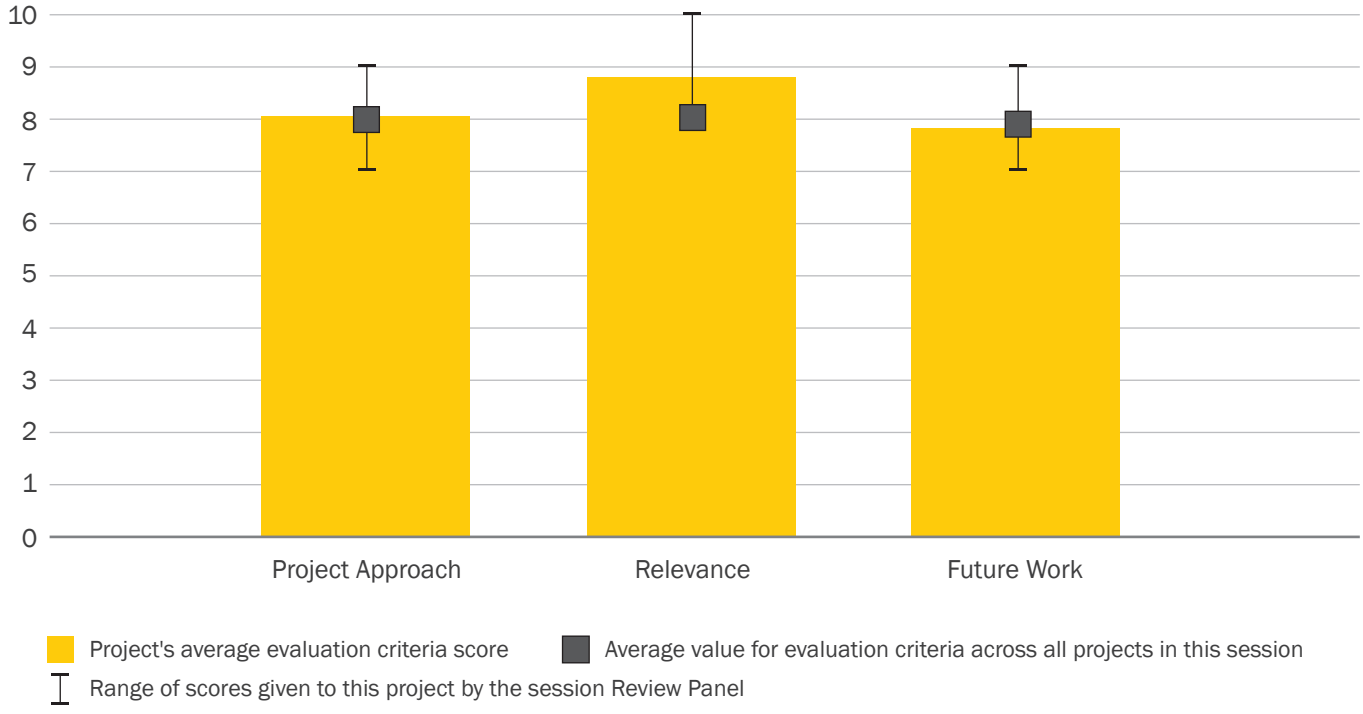


Photo Courtesy of Argonne National Laboratory

valorization and fermentation product target recovery, increasing thermochemical catalyst lifetimes through removing contaminants, using ultrasonic processing and membranes to improve efficiency of dewatering in algal processes, and using tailor-made materials to selectively adsorb toxins in fermentation. The consortium, currently in its second quarter, has met with its industrial advisory board and gathered feedback regarding current projects and future direction.

Overall Impressions

- The core concept for the consortium is very solid. This seems like exactly the type of work that represents a good use of public funding—addressing central problems faced by industry for which solutions can enable further cost-effective development work. The structure allows the different national laboratories to contribute to each other's sub-projects and share ideas and knowledge, without being so codependent that the work is slowed down.

  The consortium looks to be well-organized and appropriately structured and has taken an excellent approach in using the expertise already present in the national laboratories in identifying key process problems relevant to producing biofuels and renewable chemicals from non-food feedstocks and potential solutions to be evaluated. There are real prospects here for the group to put forward and develop process solutions that can make the biology or catalytic chemistry being developed more economically viable.

  An important aspect will be interaction with the outside world. It is certainly useful to have an industrial advisory board, and there will undoubtedly be additional outside companies that can either give useful information on alternative technologies and materials or provide new requests for technology development opportunities, so it will be good to make sure the consortium maintains an outreach effort to essentially advertise its efforts. Providing a well-publicized website with information and a route to interacting with the group is a great idea. It will be useful to monitor the effectiveness of the website in disseminating information and encouraging engagement and to get feedback whenever possible.

- The consortium has the potential to be a valuable addition to the BETO portfolio, but it would be greatly strengthened by a stronger justification for the specific separation technologies chosen for investigation. Leveraging the strengths of the partner laboratories is important, but the choice must be coupled with a rationale describing how and why some technology will be better than other, less-expensive or exotic approaches.

- Separations research has historically not been a major part of biotechnology research, though it is a critical component of a cost-effective biorefinery. The Separations Consortium brings together experts from various locations and focuses research on the most high-priority separation challenges that

will provide the most cost benefit. As separations accounts for 25%–50% of bioprocessing cost, this work is essential to meeting BETO's cost targets. The team took a systematic approach to identifying relevant projects by finding the key challenges in various conversion pathways. They engaged industry experts (and will continue to do so) and coordinated with other BETO programs to ensure the continued relevance of their work. Managing such a large, diverse portfolio of activities may be a challenge. However, the team came up with a great idea to have "stream stewards" to make sure there is consistency in the feedstock or product streams provided to each group for the lifetime of the project.

The team has made good progress in the short time they have been active, and they have a well-defined roadmap for the future. In their approach, the team is looking at novel technologies that will provide more than just an incremental improvement in performance and cost reduction. However, some of these approaches are high-risk, and no contingency plans are given.

- The presentation was well-organized and delivered. The planned work of this consortium is aligned with MYPP goals and has the potential to make real improvements to BETO-funded bioprocesses and enable industry cost reduction through improved efficiency. The target selection approach seems very well-considered. SMART goals are recommended for all separations projects, with multiple opportunities for success. This is a key work stream, and I look forward to following the progress of this consortium!
- This is a great initiative, and I am looking forward to seeing how this important topic is implemented and embedded in every project. It is essential and, in my experience, if taken seriously, can either make or break projects, which should be the case before launching any project in the future.

PI Response to Reviewer Comments

- We thank the reviewers for their many positive comments regarding the relevance and importance of this effort to BETO and to industry and regarding the organization and approach of the consortium. We are looking forward to technical progress within the consortium and continued and expanded interactions with industrial stakeholders.

  In overall comments, reviewers highlighted the need for exterior engagement. The project team is extremely committed to working with industry, as well as other researchers. Collecting input from these stakeholders was a key driver of the industry listening day scheduled in May 2017, as well as ongoing interactions with our advisory board, which provides feedback on the consortium's current portfolio, research progress, relevance to industry, and plans for future work.

  Reviewers noted the need to justify technology choices, especially the rationale behind working with a less-common technology rather than conventional technology. To address this comment, the consortium will continue to use TEA and quantitative metrics to evaluate the technology progress and to underpin the Separations Consortium's project portfolio.

  Finally, reviewers noted the importance of key project management elements, including SMART milestones and contingency plans. The team is very committed to robust project management, with BETO's support. Risk and associated mitigations, along with SMART milestones, are included in the AOPs of every participating laboratory. We appreciate the feedback to highlight these success criteria more broadly. In addition, each team has go/no-go decision points. The team will pursue timely and robust decision making and prioritization of efforts based on laboratory-based performance data and TEA. If a process or technology does not appear to address critical process economic gaps in an efficient manner, the team is structured to re-focus on other efforts.

ADVANCED BIOFUELS PROCESS DEMONSTRATION UNIT (ABPDU)

(WBS#: 2.6.1.101)

Project Description

The ABPDU was established to provide scale-up and commercialization services to the biofuels and bioproducts community, including industry, academia, and the national laboratories. This AOP project covers expenses related to facility readiness, process benchmarking, and business development. While, generally, ABPDU partners and customers pay for the incremental costs associated with the work they do at this facility, this base budget is required to operate the facility in a not-for-profit, work-for-others model. The partnerships enabled by this BETO-supported model allow advancement of key technologies from early stages to deployment in industry, bringing value to the entire biofuels and bioproducts community and providing high-visibility examples relevant to the BETO mission.

Recipient:	Lawrence Berkeley National Laboratory
Principal Investigator:	Todd Pray
Project Dates:	7/13/2010–9/30/2017
Project Category:	Ongoing
Project Type:	AOP
DOE Funding FY 2014:	$2,750,000
DOE Funding FY 2015:	$2,740,000
DOE Funding FY 2016:	$2,750,000
DOE Funding FY 2017:	$2,500,000

In order to provide cutting-edge technical services and process development expertise, the ABPDU will repeatedly baseline its processes to ensure team training and robust performance across all unit operations, from deconstruction through fermentation, separations, purification and analytics. The ABPDU will also maintain and upgrade its physical plant to offer access to technologies, processes, and analytics in demand by its clients, whether using small-scale process optimization capabilities or scaling up to the 300-L fermentation suite and/or the facility's 100-L biomass deconstruction and chemical catalysis capacity.

Weighted Project Score: 8.4

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

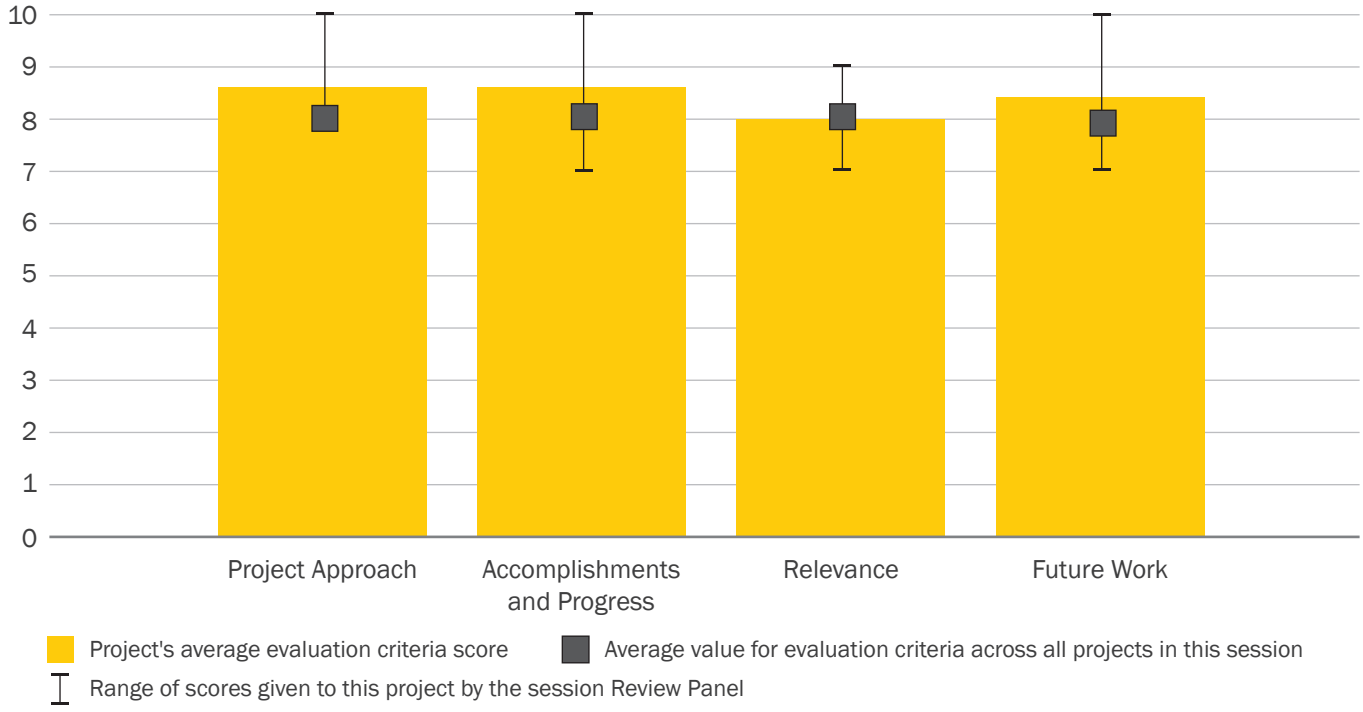


Photo Courtesy of Lawrence Berkeley National Laboratory

Overall Impressions

- I have to say, I'm really glad something like the ABPDU exists. It seems to fulfill an important role in helping small innovative companies test out ideas and materials while minimizing the amount of investment they may need to make in equipment. Just looking at the client list shows the impact that the unit has. It is important to think more broadly about the growing bioeconomy than biofuels alone, and ABPDU can help move forward some of those more innovative ideas about uses for sugars and ways to reduce greenhouse gas emissions.

  Although there is some overlap in scale and perhaps some technologies with NREL, the ABPDU seems to fill a different niche. Certainly, companies should be able to decide which facility they want to work with, and the location in the California Bay Area opens up a different set of collaborations than a location in Colorado, given that process development is a fairly hands-on operation. It does make sense, though, for the ABPDU to be connected to the Agile BioFoundry and Separations Consortium, to make sure the ABPDU can provide another option for the output of those groups, in terms of either platform strains or new processes.

  Personally, I'm not convinced that the ABPDU should aim to reduce its funding from BETO to zero right away. The overall return on investment looks pretty good in helping small companies move forward, often at a critical time in their life cycle when technology needs to be proved out, but capital for investment in expensive fermentation and processing equipment may be limited. Within BETO's portfolio, this looks like a very good use of funding.

- The existence of larger-scale facilities within the BETO system is important, as they provide a centralized outlet for industry to come in and validate their operations. The ABPDU has had success generating business for their facility and also in reducing costs to BETO. However, their interaction with other larger-scale facilities within the program (NREL's Process Development Unit and NREL bench scale) seems limited or nonexistent, to the point that it appears more competitive than collaborative. It would streamline operations if these valuable facilities were more coordinated and had a single point of contact to help guide potential customers to the most appropriate facility for their needs.

- The ABPDU provides scale-up and commercialization support for a variety of projects relevant to the bioeconomy. Their services include all aspects of bioconversion, from biomass to pure product. Over the last few years, it has grown into a state-of-the-art facility that provides high-quality work and supports commercialization of industrial processes. The center has managed its projects well using an agile management system for resource allocation, enabling an impressive number of projects to be com-

pleted with a modest size team. A possible activity for the future is to work with the NREL Integrated Biorefinery Research Facility to determine which is most suitable for which type of project, enabling the best use of resources. Potentially, completed projects at the ABPDU can then move to the NREL facility for larger scale.

- This group has been quite successful enabling industry partners to optimize processes and scale and move bioprocesses towards commercialization. BETO has several mechanisms for funding small business ventures, and the ABPDU is a valuable incubator resource from process demonstration through technology transfer. ABPDU facilities and staff are versatile, having worked on bioprocesses to make chemicals, materials, biomass, and protein products. Initiating a system of project coordination with other pilot facilities (e.g., NREL) in the national laboratory system is highly encouraged.
- This is a nice effort to establish another pilot facility (closer to where it is needed) to enable small businesses and national laboratories with quicker and cost-effective turnaround on their small-scale fermentations. This is a very nice accomplishment in onboarding new projects and methods to enhance the customer and project team needs (e.g., auto sampler, high-throughput analytics, etc.). Also, there are great testimonies from customers, which I hope triggered the backfilling of projects to have this facility operate as a cash-neutral or minimally subsidized entity. The top challenges are keeping abreast with the technology and having skilled experts to operate, which the PI seemed to nicely establish. Cross-talk with the other pilot plants, bench-scale validation, and analytics seem to be gaps where the PI and BETO are encouraged to facilitate better in the future with good handover of protocols, techniques, etc. Overall, this project has made good progress and is on the right track to fulfill BETO's goals.

PI Response to Reviewer Comments

- The ABPDU team appreciates the positive feedback from the reviewers on our process development, scaling, and piloting work with industry and national laboratory partners. We've been very focused on process flexibility, training, sound project management, and overall organization and leadership development for the team, and this has allowed us to successfully execute efforts with 30 companies over the past 3 years or so and play a role in developing commercial processes that have been successfully launched. We've also been able to participate, drive, and contribute meaningfully to several BETO consortia projects, such as the Co-Optimization of Fuels and Engines, the Separations Consortium, the Agile BioFoundry, and the Feedstock-Conversion Interface Consortium.

  A very helpful suggestion from several reviewers is that the ABPDU should coordinate more closely with specific facilities and groups, such as the Pilot-Scale Integration team at NREL's Integrated Biorefinery Research Facility, as well as the Bench-Scale Integration and Analytical Development and Support project groups at NREL. We're very committed to this and see a lot of value in doing so. We've been very proactive and focused on project and business development with industry partners; a number of them have already gone on to work with the Integrated Biorefinery Research Facility at NREL and the pilot-scale algae cultivation facilities at Sandia National Laboratories in Livermore, for example, using shared project plans under the Office of Energy Efficiency and Renewable Energy's Small Business Vouchers program. It is one of our key goals to help companies transition to larger-scale facilities, and we actively help them in transferring technology following development of robust processes at the ABPDU.

Working with BETO and our partners at other national laboratory scale-up capital research assets, we are planning to develop a request for information to solicit feedback and input on existing and new capabilities that would be useful for industry stakeholders as they scale up and commercialize their technologies. The collective funding for all these shared resources at the national laboratory allows each team to complement the others while also providing a unique value proposition to their academic and industry partners.

To conclude, the national laboratories and BETO have an opportunity to more clearly articulate and better communicate the resources available at sites such as the ABPDU at Lawrence Berkeley National Laboratory and the Integrated Biorefinery Research Facility at NREL. This will allow companies and other potential users to quickly find the most suitable team and facility to meet their needs in terms of scale, technical capabilities, location, and process expertise. The ABPDU will do what we can to ensure that the collaboration between national laboratories remains strong, to best enable BETO's goals and the technology developers in industry that are making great progress in growing the bioeconomy for biofuels, biobased chemicals, proteins, and materials that will spur our domestic manufacturing base, create jobs, and leverage the abundant natural resources inherent in biomass.

IMPROVING TOLERANCE OF YEAST TO LIGNOCELLULOSE-DERIVED FEEDSTOCKS AND PRODUCTS

(WBS#: EE0007531)

Recipient:	Massachusetts Institute of Technology
Principal Investigator:	Dr. Greg Stephanopoulos
Project Dates:	10/1/2016–9/30/2019
Project Category:	New
Project Type:	FY 2015—BRDI
Total DOE Funding:	$1,500,000

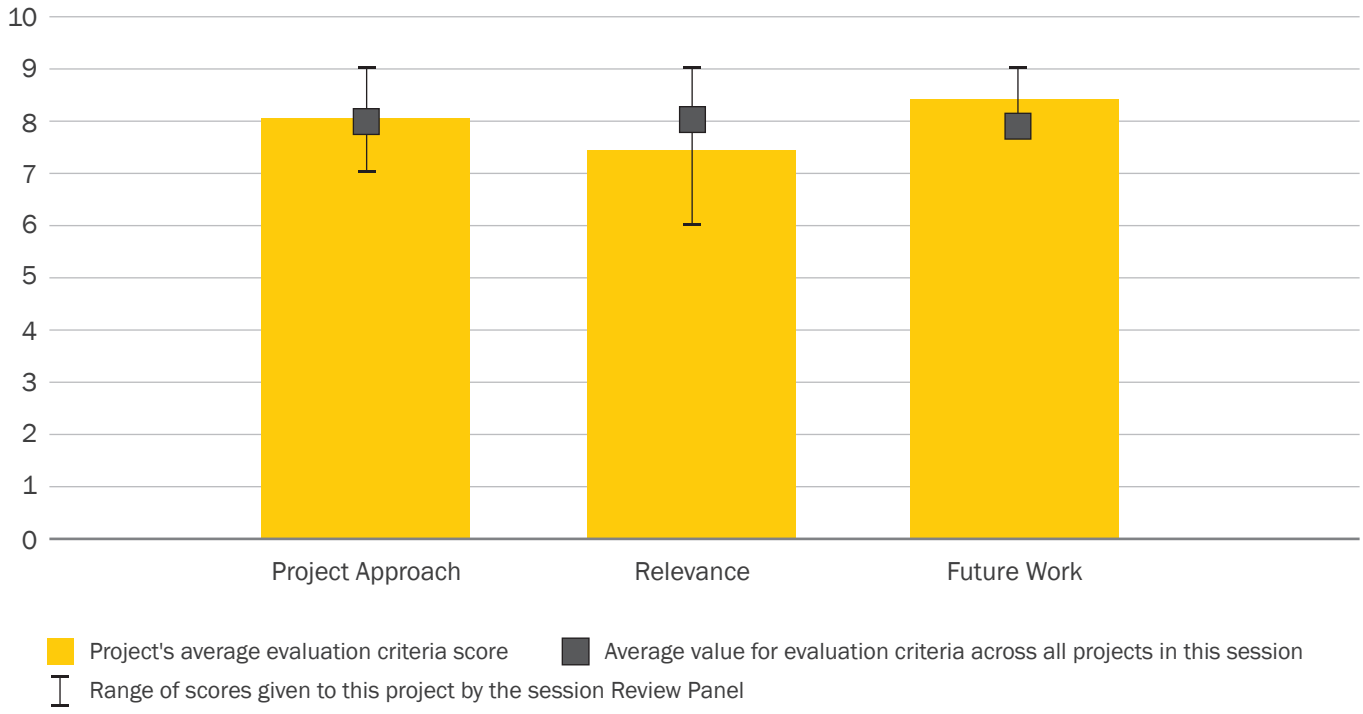
Project Description

Combined substrate-product toxicity in microbes is one of the major constraints hampering the scale-up and economic competitiveness of bioprocesses based on lignocellulosic feedstocks. Hydrolytic pretreatments of biomass release numerous compounds impinging on cell viability; the three most significant to yeast (the industry dominant biocatalyst) and common to all plant sources are furfural, hydroxymethylfurfural, and acetic acid. Furthermore, the desired end product, such as ethanol, typically attacks a multitude of host cellular functions via mechanisms yet to be fully understood.

We propose to enhance lignocellulosic fermentations in yeast using nutrient adjustments proven previously to boost alcohol tolerance, combined with genetic modifications aimed at alleviating hydrolysate toxicity. First, we will systematically characterize the component toxicities in biomass hydrolysates and quantify their relative impacts on ethanol production. Subsequently, we will engineer tolerance to these inhibitory compounds using strategies combining enzymatic detoxification with specific chemical modification of the fermentation medium. Finally, we will assess the extent to which these tolerance methods are transferrable beyond ethanol production, specifically, to yeast processes synthesizing the antifreeze precursor monoethylene glycol.

Weighted Project Score: 8.1

Weighting: Approach–25%; Relevance–25%; Future Work–50%.



Overall Impressions

- The project addresses a serious difficulty, and often a technical weakness, that limits many production systems using lignocellulosic hydrolysate streams. Use of these sugar streams with the attendant inhibitors is a lot different than using clean sugar streams, such as sucrose or glucose from starch, and places a major physiological burden on the cell. It would be very good for the Massachusetts Institute of Technology group to fully address the nature of the damage that the inhibitors cause (individually and in combination) and to identify mitigation strategies that allow higher concentrations of sugar streams to be used, or more severe pretreatments, and reduce the metabolic burden on the cell (hence improving yields).

  The project team seems to have a very good understanding of what they want to do and have presented a fairly clear, well laid out plan.

- This is an early-stage project with little information on which to base an evaluation. However, the approach is based on strong publications from the PI. More detailed evaluation will need to wait for initial results to come in.

- This project leverages experience and knowledge base at Massachusetts Institute of Technology for engineering microbial tolerance to develop S. cerevisiae strains with improved tolerance to inhibitors present in biomass hydrolysate. The relevance is clear, but the team should have more guidance from industry to make sure they are addressing the right factors. The approach is straightforward, and the team's history inspires confidence in achieving success. Ethylene glycol production target is achievable, but since there is no proof of concept so far, it could present unknown challenges.

- The project is testing a membrane-based general tolerance mechanism for ethanol in a strain that will also be able to convert toxic compounds in biomass hydrolysate to alcohols. This detoxifying and ethanol-tolerant strain has the potential to reduce bioconversion process cost with respect to detoxification. It is not clear to me how many in the industry are still practicing detoxification, nor whether the membrane-based tolerance mechanism is combinable with previous progress made in yeast ethanol tolerance.

- Overall, it is an ambitious goal to capitalize on novel molecular tools to enable higher tolerance of yeast strain to inhibitors present at lignocellulosic sugars for producing ethanol and monoethylene glycol. These products are commodities, and as such, the economic feasibility of the project should be evaluated thoroughly in the near future. Nonetheless, as technical advancement in understanding of yeast tolerance enables the academic and the national laboratories, a community with more knowledge in this field could be an advantage. If, in the end, Biochemtex or any other partner will license this to be used in the lignocellulosic ethanol domain as an economically superior strain, then BETO has accomplished its mission. If monoethylene glycol can be produced economically later from C5 sugars as biobased chemicals to enable hydrocarbon biofuels in an integrated biorefinery, that is even better.

PI Response to Reviewer Comments

- Most of the comments have been addressed in the previous sections. Overall, despite some differences in the approach with industry, we concur that more interaction can only help with keeping our goals grounded and relevant. We look forward to cultivating stronger connections as this project progresses.