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INTRODUCTION

The Lignin Utilization Technology Area is one of 14 related technology areas that were reviewed during the 2019 Bioenergy Technologies Office (BETO) Project Peer Review, which took place on March 4–7, 2019, at the Hilton Denver City Center in Denver, Colorado. A total of 11 projects were reviewed in the Lignin Utilization session by five external experts from industry, academia, and other government agencies.

This review addressed a total U.S. Department of Energy (DOE) investment value of approximately $20,312,772, which represents approximately 2.4% of the BETO portfolio reviewed during the 2019 Peer Review. During the Project Peer Review, the principal investigator (PI) for each project was given 30 minutes to deliver a presentation and respond to questions from the review panel.

Projects were evaluated and scored for their 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 Lignin Utilization Technology Area, full scoring results and analysis, the Review Panel Summary Report, and the Technology Area Programmatic Response are also included in this section.

BETO designated Dr. Jay Fitzgerald as the Lignin Utilization Technology Area Review Lead, with contractor support from Ms. Jessica Phillips (Allegheny Science & Technology). In this capacity, Dr. Fitzgerald was responsible for all aspects of review planning and implementation.

LIGNIN UTILIZATION OVERVIEW

Lignin constitutes 15%–40% of biomass carbon, but it is currently considered a waste stream in biorefineries, generally burned for heat and power. Techno-economic modeling suggests that for current biochemical routes to hydrocarbon fuels to be economically viable, lignin must be transformed into a higher-value coproduct stream that is able to reduce effective fuel cost by >$2 per gasoline gallon equivalent (GGE). Given BETO’s focus on large commodity markets for coproducts, analysis suggests that >40% of the carbon in a lignin-rich stream will need to be converted into value-added products in a biochemical conversion process to achieve this reduction in fuel cost.1

The Lignin Utilization Technology Area focuses on research and development (R&D) of biological, thermochemical, and hybrid approaches to lignin valorization in support of cost reduction for biochemical conversion pathways. Approaches to valorize lignin fall into two main categories:

1. Breaking lignin down into small molecules and upgrading those into value-added products

2. Utilizing the native structure of polymeric lignin for materials applications.

Projects in the Lignin Utilization Technology Area work closely with projects in the Biochemical Conversion Technology Area and the Bioprocessing Separations Consortium to utilize real lignin streams and produce purified products.

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LIGNIN UTILIZATION REVIEW PANEL

The following external experts served as reviewers for the Lignin Utilization Technology Area during the 2019 Project Peer Review.

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<tr>
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<tr>
<td>Emma Master*</td>
<td>University of Toronto</td>
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<td>Joseph Bozell</td>
<td>University of Tennessee</td>
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<td>Matthew Tobin</td>
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<td>Jeff Scheibel</td>
<td>J. J. Scheibel Consulting LLC</td>
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<td>W. Michael Sanford</td>
<td>Prisma Renewable Composites</td>
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*Lead Reviewer
TECHNOLOGY AREA SCORE RESULTS

Average Weighted Scores by Project

- NREL (2.3.4.100): Lignin Utilization - 8.6
- NREL (2.2.3.106): Lignin First Biorefinery Development - 8.0
- NREL (2.3.2.100): Biological Lignin Valorization - 8.0
- NREL (2.3.2.301): Biological Conversion of Thermochemical Aqueous Streams - 7.9
- NREL (2.3.1.501): Gas Phase Selective Partial Oxidation of Lignin for Coproducts from Biomass Conversion - 7.5
- ORNL (2.3.2.104): Metabolic Engineering for Lignin Conversion - 7.0
- PNNL (2.3.4.101): Oxidative Valorization of Lignin - 7.0
- Ohio University (2.3.1.205): Biomass Electrochemical Reactor for Upgrading Biorefinery Waste to Industrial Chemicals and Hydrogen - 6.8
- NREL (2.2.3.500): Electrocatalytic Oxidation of Lignin Oligomers - 6.8
- Texas A & M (2.3.1.206): Upgrading Lignin-Containing Biorefinery Residues for Bioplastics - 6.8
- SNL (2.3.2.101): Biological Lignin Valorization - 6.7

Sunsetting, Ongoing, New
A broad range of conversion strategies and product targets are represented by BETO’s Lignin Utilization Technology Area projects. Both physicochemical and biological pathways are represented, including electrochemical and solvolysis approaches, improved and low-cost oxidation catalysts, and engineered microorganisms with ability to funnel useable lignin monomers to specific chemicals. Moreover, multiple projects directly integrate upstream lignin processing technologies with downstream bioconversions to specific products. A good example of this is the Coprocessing of Lignin and Residual Sugar (CLARS) process, which demonstrates improved overall product formation through low-severity pretreatment of the starting biomass. Likewise, the Lignin First Biorefinery Development project improves upon thermochemical approaches with its reductive catalytic fractionation (RCF) method to stabilize reaction products from lignin, ultimately producing aromatic monomers and oligomers of value or that can be funneled to specific end products through microbial processes.

Lignin breakdown strategies mainly concentrate on carbon-carbon (C-C) bond cleavage, but also include carbon-hydroxyl group (C-OH) conversion to the more reactive carbonyl group (C=O) functionality. Synthesis of lignin model compounds, application of robotic systems for sample handling, and robust application of statistical methods have together advanced the development of technologies that convert industrially relevant lignin streams into targeted end products.

The main products targeted to date include diacids (e.g., muconic acid, adipic acid, terephthalic acid, and itaconic acid), medium-chain alcohols, polyhydroxyalkanoates (PHAs), aromatics, hydrogen, and low molecular weight lignins for resin applications. The multiple end products considered reflect the complexity of the starting material and its potential value in many applications.

Each project demonstrated good relevance to BETO’s overall effort to reduce biofuel costs through coproducts from underused biomass fractions. As next steps, it will be important to consider challenges relating to techno-economic analysis (TEA), technology scale-up, and product recovery.

Project presentations included reasonable descriptions of the project management process and approach, including resources, skills, critical success factors, goals and objectives, and meetings/interactions. However, some reviewers noted that risk analysis (identification, weighting, and mitigation) was underrepresented in most projects. More rigor here would be a welcome upgrade and would help to prioritize project tasks and de-risk project activities.

IMPACT

Lignin represents a significant challenge to the biorefinery, as its heterogeneous structure (as either native or technical lignin) has historically seemed to defy efforts to accomplish its selective conversion to high-value products. The projects chosen by BETO hold the potential for overcoming this challenge.

Biochemical work being supported by BETO targets a 40% yield of usable monomers from isolated, technical lignin, which is an ambitious goal in itself. The program further aims to use genetically modified *Pseudomonas putida* to convert those monomers to key industrial chemicals, including various diacids, medium-chain alcohols, and PHA. Despite a potentially growing market, the choice of PHAs as a target product may be a difficult one, as there is a history of commercialization difficulties. Licensing activity around some of the developed PHA technology signals industrial interest; however, the focus on PHA would be strengthened by performing a TEA to consolidate assumptions, and clarification of whether this effort generates PHAs that are significantly different than those generated through previous work.
**LIGNIN UTILIZATION**

*P. putida* strains were developed that transform syringol and compounds from syringyl lignin. In most cases, however, engineered *P. putida* strains were tested for ability to convert ferulate or *p*-coumarate to targeted diacids and medium-chain alcohols, with typically 30% conversion efficiency. DOE’s Agile BioFoundry Consortium, along with DOE’s large-scale sequencing and synthetic biology platforms, are expertly leveraged by the lignin bioconversion projects. By consequence, these projects have already demonstrated the potential to biologically funnel defined lignin-derived compounds to specific chemicals, while uncovering fundamental insights into regulation circuits and tolerance mechanisms that influence lignin bioconversion efficiency. The eventual impact of this program will depend on ongoing efforts to directly funnel complex and changing aqueous streams resulting from lignin pyrolysis or other pretreatments. Moreover, as there are few industrially useful (and manipulatable) organisms available, a licensing or other customer-friendly approach to getting new *P. putida* strains into industry hands could further advance BETO objectives.

Parallel to bioconversion pathways, BETO complements their long-term thermochemical efforts to convert lignin via pyrolysis with electrochemical options for controlled depolymerization to aromatics and hydrogen gas, as well as improved oxidation catalysts for C-C bond breakage. Bond cleavage between lignin monomers and oligomers has been highlighted previously as a critical node in achieving favorable economics via lignin valorization. To convincingly demonstrate the impact of corresponding advances, however, it will be critical to prove economic conversion of complex mixtures to specific final products. The program would further benefit from a clear description of how the newly developed catalysts and approaches offer advantages over similar, earlier efforts that evaluated a wide range of conditions and catalysts.

As a whole, the impact of the lignin effort benefits greatly from the National Renewable Energy Laboratory's (NREL’s) analytical capacity for lignin analysis. Two of the current lignin projects expand upon this core capacity by synthesizing new lignin compounds that support the analytical characterization of processed lignin samples or are used in microbial and enzyme screens. Compounds that support biological screens provide a differentiating resource, because we can only find what can be screened for, and many compounds relevant to lignin conversion cannot be bought commercially. So far, compounds developed for screening reflect native lignin structures. This is valuable for some projects—for example, the Lignin First Biorefinery Development project. As a next step, however, compound synthesis for biological screens ought to leverage the information now available through other projects, which clarify main lignin structures present in pretreated lignin streams that remain difficult to biologically convert to target chemicals.

Relating to this aspect, the impact of both bioconversion and physicochemical conversion efforts suffer slightly by the use of lignin model compounds or representative native lignins, which likely have little connection to the actual structure of the technical lignins available for conversion to high-value products. To this end, each project would be strengthened by defining exactly the sources of lignin intended for use.

**INNOVATION**

Scientifically, the panel was presented with a solid and innovative program. The scientific strength of the program was illustrated by the effort to frequently publish research results, and in top journals. Moreover, the program contains efforts to understand the molecular-level processes that control lignin conversion. For example, the Pacific Northwest National Laboratory (PNNL) and NREL efforts to understand mechanisms in lignin pyrolysis may lead to greater process control and address the very long-standing issue of product complexity in lignin thermochemical conversion. More broadly, incorporation of these types of fundamental efforts need to remain at the core of biomass research efforts, as it couples basic scientific knowledge to successful biorefinery development and bridges fundamentals with applications. The willingness of BETO to include such efforts—in contrast to efforts supported by earlier incarnations of BETO—is a real strength of the program.

The program has identified adipic acid as a key industrial target from their lignin conversion work, and a successful development of an industrially viable production of bio-based adipic acid from lignin would be truly innovative. Nonetheless, the program’s focus on ferulate and coumarate as adipic precursors would be
strengthened with a clearer justification of why (yield, conversion, availability) these are good models of what the biorefinery will actually have to work with. The same question would apply to the choice of base-catalyzed depolymerization (BCD) lignin as an initial feedstock. If (1) BCD lignin fails to be recognized as a viable industrial starting material and (2) the amount of ferulate/coumarate is insufficient to make an economic case, do these processes have a chance to be adopted at a commercial scale? The program needs to be clear that it is not backing into a pretreatment simply because it gives the largest amount of the substrates (ferulate/coumarate) needed for bioconversion. If industry determines that other pretreatments are better, this work will remain scientifically interesting, but will fail to meet larger BETO goals.

SYNERGIES

Presentations in the lignin session did a good job in describing existing and possible synergies. A particular strength was seen in the NREL effort where multiple presentations by different PIs referred to one another and how their work was informing a colleague’s effort. The NREL electrochemistry project is attempting to increase the utility of lignin oligomers generated during BCD deconstruction. NREL’s biochemical work is trying to convert the small amount of lignin generated during thermochemical treatments. Electrochemical transformation of lignin incorporates work from the electrochemical CO₂ reduction project. The panel saw again and again that problems arising in one project were addressed in parallel projects. The program’s multipronged approach to lignin conversion and utilization frequently described applicability to parallel efforts in pyrolysis, biological funneling, and oxidative or reductive conversion.

Whereas projects led by national labs appear to be tightly integrated, the competitive projects appeared less well connected. For example, both Oak Ridge National Laboratory (ORNL) and Texas A&M University described work on PHAs in their projects. A clearer description of the level of integration and coordination between the labs continuing to examine PHAs would be helpful. Notably, the presentation from Texas A&M University was rather long, and yet it was difficult to discern specific advances since the last review and rationale for changes in direction (e.g., apparent discontinuation of laccases). Emphasizing connections between PHA projects across BETO is strongly recommended.

Similar aims and approaches were also noted between the Ohio University and NREL electrocatalysis projects. Despite this, the only point of connection appeared to be the provision of lignin by NREL to Ohio University. Clarification on how these efforts differ from one another (other than specific product targets) is important.

Mutual benefits are expected through closer collaboration of national lab and competitive projects, given the availability of complementary analytical tools, data analysis methods, and TEA expertise.

FOCUS

First and foremost, BETO’s willingness to support multiple projects and approaches to lignin conversion is a real strength and must remain a driver in future work—the technology for lignin conversion still requires a broad-based and flexible effort to define the best approaches, even if it takes longer to reach an answer.

The project reviews, however, would have benefitted from a more detailed description of the base case biorefinery. The background information presented by BETO (e.g., block diagrams in the Multi-Year Program Plan) are clear at a high level, but the specifics of the feedstock, separation processes, and targets being used as benchmarks for GGE analysis would provide a better understanding of the value and feasibility of each project. For example, additional context would clarify if the base case is using a pretreatment that is scalable and likely to be adopted by industry. This is particularly relevant for electrochemical approaches, where important advantages (e.g., selective lignolysis, hydrogen cogeneration) must be shown in light of an overall process to justify the apparent complexity of this option. Additional context would also clarify the merit of generating monomers from lignin that are also generated from carbohydrates (e.g., adipate, muconate). Similarly, deeper context would help to underscore key differences between the hydrogenolysis work through the Lignin First Biorefinery Development project and the similar work being actively pursued through the BioEnergy Engineering for Products Synthesis program at Spero Energy.
Bioconversion research teams have made an impressive and focused effort to develop *P. putida* as a platform host for lignin conversion to valuable chemicals. Many fundamental insights have emerged from that work, which also have applied relevance. Moreover, molecular tools and methods established along the way provide an important resource for the broader research community. Whereas the concerted effort to engineer *P. putida* for lignin conversion presents a strong point of focus that links several research teams, it will be important to further justify this strategy in light of the potential advantages afforded by microbial communities, especially when attempting to transform particular toxic process streams.

Several projects presented a well-organized TEA template developed at NREL. However, this methodology was not employed uniformly. There is an opportunity to use the TEA more effectively as a research tool, to focus efforts, and illustrate the most feasible technology development pipelines. For example, a TEA for technologies at initial, mid, and final stages of the project could help teams (1) report progress in terms of the percentage of the way to reaching an economically feasible solution and (2) point to specific constraints to economic feasibility, uncovered by the TEA, which then informed their research direction.

At the same time, there is an opportunity to develop a “TEA-light” evaluation so that a promising program would not be missed because sufficient data were not yet available to confirm feasibility. This type of evaluation would be a “best-case” scenario to prioritize low-TRL projects where the cost of an effort could be evaluated assuming each step reached the maximum possible yield (i.e., a simple demonstration of broad economic possibility), but, more importantly, an initial demonstration of where the greatest impact of research would be on the cost of a process.

**TECHNOLOGY DEVELOPMENT PIPELINE**

Each lignin project demonstrated high scientific quality; however, in several cases, the path to commercialization was less clear. This may not be a weakness at this stage—the concept of selective lignin conversion is still poorly understood, and the efforts to carry out more fundamental efforts is worthwhile. It should be given sufficient time to reach fruition without being fully driven by efforts to reach commercialization.

However, even at a preliminary stage, a project’s discussion of potential industrial utility needs to make sure that their rationale is well laid out. As an example, the NREL project in gas phase lignin oxidation of lignin to phenols describes a coproduct value of about $0.90/lb. If this is the value used in the TEA, it appears at odds with the price of pure phenol that only commands a value of $0.40–$0.50/lb. As another example, in a conservative industry such as wood products, it is difficult to envision ready acceptance of electrochemical processes, despite the large amount of interesting transformations that it can enable. In this case, rather than production of phenolic resins where many options already exist, it will be important to demonstrate a pipeline to products that leverage unique transformations from electrochemical processes.

For several projects, the corresponding TEA shows that research has led to a projected reduction in cost. However, it was not clear that they could achieve the numbers necessary to make the best use of lignin in the portfolio. It would be helpful to clarify/justify the metrics used when reporting product targets. For example, reporting gram of product/gram of lignin would help compare technologies and their progress. Relating to this, corresponding metrics should be in terms of the initial lignin source, rather than only isolated individual phenolic streams.

Lastly, scale-up required to achieve commercial relevance and plans for product recovery will be important to carefully consider as a next step. In most cases, such challenges were not addressed in the project presentations. A hard look at scalability risks and product recovery options is needed to demonstrate a convincing technology development pipeline.

**RECOMMENDATIONS**

The review panel sees the following as key recommendations for the program going forward:
• Continue the multifaceted approach by supporting multiple lignin conversion efforts. This strategy will be critical to understanding the technology space needed for selective and high-value use of lignin. At the same time, strengthen each project by supporting efforts to carefully consider anticipated challenges relating to scale-up and product recovery.

• The integration of science and molecular-level understanding with industrial applications should continue, as this will form the best foundation for process development and control. As part of that effort, support research activities that utilize industrially relevant lignin sources, and synthesize the most challenging lignin-derived compounds for bioconversion processes.

• There are opportunities to improve the focus of the program, but it must not be done at the expense of promising but unproven approaches to lignin conversion. TEA approaches can be used as a tool to help uncover the most important research questions. For example, the technology development pipeline for each project could be extracted from a TEA performed at initial, mid, and final stages of the project. As part of that effort, teams could highlight examples where a TEA informed the research direction, and clarify metrics as well as key assumptions (e.g., product yield based on relevant lignin source, anticipated market size for targeted products, anticipated environmental benefits).

• Where numerous lignin monomers are obtained, teams should look for ways to group together these monomers or dimers, maximizing total yield for alternative high-value uses. For instance, one suggestion is to sulfonate the mixture to provide water-soluble chelant mixtures, which may be applicable for industrial use.

LIGNIN UTILIZATION PROGRAMMATIC RESPONSE

OVERVIEW
The program would like to thank the reviewers for their time and thoughtful comments throughout the review process. The program responses to reviewer recommendations are found in the following section.

Recommendation 1: Portfolio diversity.
The panel recommended continuing to support multiple approaches to lignin valorization to ensure industrial relevance while strengthening approaches to scale-up. The program will continue to invest in a variety of lignin valorization technologies while ensuring that at least one technology will be able to be benchmarked for a state of technology. We are also actively pursuing scale-up challenges through multiple competitive awards involving industry as well as the BETO Bioprocessing Separations Consortium.

Recommendation 2: Fundamental understanding and lignin sources.
The panel recommended continuing the integration of molecular understanding with industrial applications as well as utilization of industrially relevant lignin sources and synthesis of model compounds. We will continue to pursue molecular understanding of lignin along with industrial applications as suggested by the panel. Diversified sources of lignin will be used in the portfolio, including deacetylation and mechanical refining-enzymatic hydrolysis (DMR-EH) lignin from a variety of sources, acid/base pretreated corn stover, as well as poplar and miscanthus. The Lignin Utilization Technology Area has synthesized >20 model compounds, many of them containing recalcitrant carbon-carbon linkages, and will be testing and sharing those compounds in Fiscal Year (FY) 2020.
Recommendation 3: TEA.
The panel noted that highlighting examples of where TEA has been used in the past to guide research as well as key assumptions during the peer review would be useful. We agree and will implement steps to make sure that these are included more thoroughly in the template for national laboratory projects. For competitive projects, the program utilizes independent experts who conduct a verification process, including vetting TEA assumptions as each project is negotiated. This verification process will be highlighted in the next peer review in order for the panel to have a better sense for the process.

Recommendation 4: Grouping of streams.
The panel recommended that searching for practical ways to group deconstructed lignin streams to maximize yield would be very useful. We agree and are currently investigating two main strategies to group monomer/dimer streams. First, we are investigating ways to funnel monomers/dimers into single products either through metabolism or through gas phase catalysis through projects at NREL, ORNL, and Sandia National Laboratories (SNL). Second, we are exploring ways to functionalize monomers to make use-based groupings in projects at NREL, Spero Energy, and Clemson University.
LIGNIN-FIRST BIOREFINERY DEVELOPMENT

National Renewable Energy Laboratory

PROJECT DESCRIPTION

The Lignin-First Biorefinery Development (LFBD) project aims to develop an industrially relevant, lignin-first biorefining concept to fractionate lignin and polysaccharides through RCF. RCF is an active stabilization method wherein lignin is first solubilized from whole biomass through a polar protic solvent, such as methanol, and the lignin is contacted with a hydrogenation catalyst in a reducing environment to cleave aryl-ether bonds and stabilize the reaction products. RCF has been shown to be able to achieve high yields of a narrow slate of monomers—essentially acting as a catalytic funneling process—from hardwood feedstocks at high extents of delignification. The LFBD project is a close collaboration with the Massachusetts Institute of Technology and aims overall to replace standard pretreatment methods that are carbohydrate-centric (e.g., acid pretreatment) with a fractionation method that produces highly digestible polysaccharides and a lignin stream that can be separately valorized, thus equally weighting the lignin and polysaccharides in biomass. Moreover, most hardwood feedstocks are not compatible with the standard biochemical conversion paradigms of pretreatment and enzymatic hydrolysis, which the RCF process could potentially enable. The LFBD project is conducted in close collaboration with the Lignin Utilization project (for lignin analytics and model compound synthesis), the Bioprocessing Separations Consortium (for advanced separations technologies applied to the RCF process), the Performance Advantaged Bioproducts (PABP) via

Weighted Project Score: 8.0

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%
Selective Biological and Chemical Conversion project (for valorization of lignin products), and the Direct Catalytic Conversion of Cellulosics (DC3) and Enzyme Engineering Optimization (EEO) projects for carbohydrate valorization.

The LFBD project comprises two primary tasks: the first focuses on the RCF solvolysis and catalysis steps. The emphasis of this task is to develop an industrially relevant, semicontinuous, flow-through reactor system and associated robust catalyst that can efficiently solubilize lignin from whole biomass and cleave aryl-ether linkages to produce high yields of monomers, respectively. Accomplishments thus far include the construction and commissioning of a new flow system that is highly versatile and has been demonstrated to achieve greater than 35% monomer yield from hardwood feedstocks. This effort also includes lignin solvolysis modeling efforts, which leverage expertise developed in the Consortium for Computational Physics and Chemistry and will ultimately inform reactor optimization for solvolysis efforts.

The second task in the LFBD project encompasses the overall lignin-first biorefining process, with an emphasis on unit operations development outside the solvolysis and catalysis steps, namely separations of the lignin oil, recovery and characterization of water-soluble products, and solvent recovery and recycle. Accomplishments to date include the development of a rigorous techno-economic model in close collaboration with the Biochemical Platform Analysis task that highlights process challenges and opportunities for the RCF concept and preliminary evaluations of basic separations technologies for RCF oil (e.g., liquid-liquid extraction and distillation).

Ongoing work includes the examination of reduced solvent usage, improved catalyst stability, and reduced process severity, all of which can impact the techno-economics of the lignin-first process concept. Evaluations with the DC3 and EEO projects are ongoing for polysaccharide conversion. Additionally, future work includes collaboration with the PABP efforts to valorize the monomers and oligomers resulting from RCF processing towards the goal of 60% lignin conversion to value-added products by the end of the three-year project.

OVERALL IMPRESSIONS

- This project is establishing a process to simultaneously fractionate and valorize lignin and polysaccharides. The process concentrates on producing stable lignin products that are usable by
microorganisms, while minimally impacting sugar recovery and use. The team designed and built a flexible reactor system for their process, which is operating very nicely; as a next step, the scalability of their design will be important to demonstrate.

- Revisiting existing biomass pretreatments that were developed for sugar usage in order to valorize more process streams is an appropriate, fundamental approach to improving economics of biorefineries. This project improves upon thermochemical approaches with its RCF method to stabilize reaction products from lignin, ultimately producing aromatic monomers and oligomers of value. Capital expenditures of the approach need to be considered due to methanol and hydrogen, as well as separation operations to recover multiple products.

- A lignin-first approach is a great idea. Great progress to date on results coming from thoughtful reactor design and processing approaches. Evaluation of commercial catalysts may advance the work faster, especially if they purchase a pelletized catalyst for flow through the reactor systems. Access to a variety of these were recommended to the team to access through a catalyst recycling company. The team modeled a recirculating reactor, but is this really a loop reactor-type design? Catalytic is the way forward for lower temperatures and pressures. There seems to be a resurgence in work on supercritical water approaches as of late, albeit extreme temperatures and pressures require unique, smaller, multiple reactor designs. Has the team considered that perhaps their lowest-cost solvent could be a mixture of their more volatile lignin products? This could perhaps lower the pressure requirements substantially and create a holistic approach versus using external purchased solvents. Perhaps only a portion of the stream could be used as a solvent while the rest goes to other products.

- Overall this is a well-conceived project, which has made some significant progress. Changing the biorefinery paradigm to lignin first versus sugar first could have significant value if this approach enables significantly higher lignin value capture with minimal impact on the fuels derived from the carbohydrates. The team is taking a holistic, integrated approach to the problem and should be commended for that. A quantitative analysis through TEA on how an RCF compares in economic performance versus alternative-process technologies would be a useful added study. This would help assess the value of continuing to follow this approach versus trying to improve and optimize the sugar-first biorefinery.

- The PIs present their take on catalytic biomass hydrogenolysis. This is an old process, but one that shows promise in that it can be used on whole biomass and affords an overall yield of 20%–30% propylphenols from lignin conversion. The potential for using this process to eliminate a biorefinery pretreatment step is important. The project would benefit by including more work on improving the hydrogenolysis itself, and by giving a better sense of its coordination with similar efforts in industry.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewer for the positive feedback. We completely agree that scalability will be critical to demonstrate, and we are working actively on new reaction engineering concepts that would enable fully continuous RCF chemistry to occur, which could significantly reduce solvent demand.

- We agree that the capital expenditures must be carefully considered. we have developed the first publicly available TEA for the RCF process, which will be published soon. This TEA work has helped identify key cost drivers and areas for research.

- The bench-scale work is not currently a closed-loop reactor design, but we are retrofitting existing equipment to be able to do this. Preliminary experiments have already shown that this process concept is feasible. We also completely agree and think that it is a great idea to use some of the lignin components as a solvent. We are planning to investigate this process concept in FY 2019 and FY 2020.
• We have conducted TEA. More details on this will be forthcoming.

• We agree that the hydrogenolysis step must be improved, and we are working towards that in collaboration with multiple academic groups. We are in close contact and directly collaborate with the only company to our knowledge scaling up RCF chemistry at the moment, thus we are keeping close contact with industrial efforts in this space as we can.
ELECTROCATALYTIC OXIDATION OF LIGNIN OLIGOMERS

National Renewable Energy Laboratory

PROJECT DESCRIPTION

Deconstruction of lignin by “lignin-first” approaches like RCF produces functionalized monomers, but a significant portion of soluble lignin oligomers still remain. These oligomers are generally linked through recalcitrant C-C bonds. Thus, selective cleavage of C-C linkages in RCF-derived oligomers into functionalized monomers is critical to the success of lignin valorization. To address this challenge, this project will (1) demonstrate proof of concept for electrocatalytic oxidative cleavage of the C-C linkages in RCF-derived soluble oligomeric lignin to selectively produce functionalized monomers for further upgrading and (2) advance the technology by targeting yield improvements.

Weighted Project Score: 6.8

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%

One standard deviation of reviewers’ scores
OVERALL IMPRESSIONS

- The PIs present an interesting approach for further upgrading of recalcitrant lignin oligomers generated during lignin hydrogenolysis. The science behind the process is quite compelling, as is the project's link to solar photovoltaics. The project would be strengthened by a better understanding of the expected phenolic oxidation mechanism. It also remains to be seen whether electrocatalytic approaches can be demonstrated as useful in an industrial context or not.

- Valorization of soluble lignin oligomers resulting from RCF is targeted by development of electrocatalysts and electrolyte/solvent systems to cleave C-C bonds. Good progress has been made in the initial development. A large increase in usable monomers is possible assuming the project can achieve its yield and selectivity targets. More discussion on the scalability of the technology would be helpful to ascertain likelihood of commercial use and success.

- The very low concentration on model compounds is key concern for this approach. Also concerning is the high concentration of lithium perchlorate (LiClO₄) and the acetonitrile solvent. Conversion of models to date also are fairly low, making this a very challenging program to meet the BETO goal for this project. The next steps on the RCF stream will define whether there is any right to succeed. Platinum electrodes used in the process raises a question: what is the cost of electrodes at a future potential commercial scale?

- This project has already led to three nanoparticle formulations for controlled potential electrolysis studies. Advantages of the electrocatalytic approach include low temperature, low pressure, and potential catalytic selectively. As for the next steps, the team is advised to address questions concerning scale-up and use TEAs as a research tool to evaluate the impact of electricity costs on the economic feasibility of the technology.

- This appears to be a relatively early-stage project with ambitious goals to try a new approach to improve lignin monomer yields. The team has made good initial progress with the proof of concept, although several challenges remain.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their constructive feedback and guidance, especially regarding our future directions. As noted, this project is an early-stage proof-of-concept effort in which we have leveraged a systematic approach to evaluate the process and gain foundational insight into the reaction chemistry. We appreciate the reviewer's support of our initial progress and acknowledge that there are many challenges and key questions that need to be addressed for this process. These challenges and key questions relate to both the scale-up and commercial application of the technology as well as the fundamental reaction chemistry. During the remainder of this project, we will work to address these challenges and key questions, especially in regard to (1) assessing economic viability and identifying key cost drivers and (2) providing a deeper understanding of the fundamental chemistry and reaction mechanisms so that this technology can be advanced.
BIOMASS ELECTROCHEMICAL REACTOR FOR UPGRADING
BIOREFINERY WASTE TO INDUSTRIAL CHEMICALS AND HYDROGEN
Ohio University

PROJECT DESCRIPTION
The goal of this project is to develop electrochemical processes to convert biorefinery waste lignin to industrial chemicals. The additional revenue stream resulting from these lignin-derived chemicals will enhance biorefinery economics and ultimately lead to a reduction in the cost of biofuels. Researchers at Ohio University have partnered with the Biorefining Research Institute at Lakehead University and an industrial partner to evaluate the electrochemical conversion product streams in resin formulations and to determine the potential economic impact of this electrochemical process on the biorefinery. Key to this project is electrochemical conversion product analysis, which is used to determine metrics like the extent of lignin conversion and predominant product compound class (i.e., functionalized aromatic compounds resulting from electrochemical depolymerization of the lignin biopolymer). We are applying robust statistical analysis to understand with confidence how the electrochemical process modifies the biorefinery lignin. The electrochemical depolymerization of biorefinery lignin results in lower molecular weight products that are more reactive than the lignin biopolymer, both of which could render the product stream suitable for incorporation into resin formulations. This is being evaluated by our industrial partner. By the end of this project, we will have gone through two iterations of a continuous-flow electrochemical reactor, increasing the reactor size and the rate of lignin conversion over the project benchmark and the current state of the art. We will have evaluated

Weighted Project Score: 6.8
Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%

One standard deviation of reviewers’ scores
electrochemical reactor product streams in resin formulations, and will have optimized reactor operation to generate streams most suitable for incorporation into resin formulations. We will also have completed a TEA describing how the electrochemical reactor may affect biorefinery economics.

OVERALL IMPRESSIONS

• This is an innovative approach using electrochemistry for controlled depolymerization of lignin to produce high value aromatics. Excellent progress has been made on technical targets such as conversion, purity, and selectivity. Power consumption of the process and cost of electricity are key areas of concern, although the latter may be helped by hydrogen cogeneration during the process.

• This project is well thought out and has solid technical insights. The achievements to date on conversion are impressive, assuming sound analysis results. This project could have used more input from the BETO analysis team or Ohio University resources to confirm preliminary techno-economic input considering the amount of DOE money also involved in this program. This is a very high-potential project but very challenging to reach industrial-scale success because very few large-scale complex oxidative electrochemical processes exist today, if any, as complex as this one with such a variable feedstock. The early progress seems to indicate good potential to improve further towards viable commercial processes. One question: is there a reason why they have not already evaluated multiple sources of lignin that could have been supplied by BETO teams, or was this done in a previous year? Also, I am surprised there is no result on resin formulation work, even if preliminary, considering how long this project has been in progress. Is it not feasible to do a preliminary batch on a small scale to test the properties?

• This project harnesses the ability of electrochemical processes to achieve three significant outcomes: (1) tighter control over the lignin depolymerization process, (2) more reactive lignin species, and (3) a hydrogen coproduct. The emphasis on statistical consideration of large data sets is commendable. The team includes a close industrial partnership, which will help ensure the translation of the research in practice. To accelerate that process, the team can further integrate data from industry partners into the TEA of the product pathway.

• Conceptually, this appears to be a good project and the team has made good progress on the catalyst and process development. This program apparently started at a pretty low technology readiness level relative to other projects, but has come a long way. The inclusion of an industrial partner to assess the products of their process for use in producing a phenolic resin is commendable. In the event that the initial outcome of the phenolic resin evaluation is not positive, the team should consider what their options are, either to modify the process to achieve an acceptable result or to consider other potential outlets for the product mix. The TEA should be used as a tool to assess the degree of flexibility the team has to adjust the process and/or catalyst to make acceptable products. They have all the tools in place as part of the team to do this.

• The project will use electrochemical approaches for the conversion of lignin into smaller fragments targeted at the phenolic resin industry. The partnership offers an interface with a large and well-established industry. Further, the research team is well integrated across the various steps involved in moving from lignin to final products, and the PI brings important expertise in the design of larger-scale reactors that will eventually be needed. There are gaps in the process to date, especially with regard to understanding of the structures being generated during electrolysis and the mechanism of the electrochemical process. Inclusion of nuclear magnetic resonance as an analytical tool will address many of these issues. In addition, the project will be strengthened by a clear description of how this approach to phenolic resins offers improvements over the many other literature efforts to carry out similar activities.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

• The recipients choose not to respond to the reviewers’ overall impressions of their project.
UPGRADING LIGNIN-CONTAINING BIOREFINERY RESIDUES FOR BIOPLASTICS

Texas A&M University

PROJECT DESCRIPTION

The proposed research will integrate the latest advances from a multidisciplinary academic-industrial coalition to address one of the most challenging issues in lignocellulosic biofuel production: the use of lignin-containing biorefinery residues for cost-effective bioproduct production. The success of a modern biorefinery heavily depends on the diverse product streams and the utilization of all fractions of input material. Even though extensive progress has been made to process cellulose and hemicellulose into advanced biofuels, the utilization of lignin for fungible biofuels and bioproducts has not been achieved. Lignin is much more under-researched as compared to cellulose, which has become a setback for the efficiency, cost effectiveness, and sustainability of a lignocellulosic biorefinery. Essentially all current bioconversion platforms lead to a lignin-containing waste stream that needs to be further processed into valuable products. Although a certain amount of lignin (~30%–40%) is needed for the thermal requirements of biofuel production, a modern cellululosic processing plant will have ~60% excess lignin. The utilization of this excess lignin-containing residue as feedstock for renewable fuels and chemicals offers a significant opportunity to enhance the operational efficiency, lower the overall biofuel cost, reduce the net carbon footprint, and improve the replacement of petroleum. The proposed research will translate the latest technology breakthroughs into a transformative lignin-to-PHA route.

Weighted Project Score: 6.8

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%
This project uniquely addresses the mission and goals of BETO with 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; and (3) process integration and optimization by biorefinery on-site scale-up, TEA, and life cycle assessment for the lignin-to-PHA upgrading process. During the past two and a half years, we have made significant progresses in all three objectives, including systems biology-guided engineering of *P. putida* to efficiently convert lignin into PHA, the development of new combinatorial pretreatment and coprocessing of lignin and residual saccharides (CLARS) processes to enhance both lignin and carbohydrate processibility, and the integration of TEA to evaluate the impact of the process for biorefineries and the bioeconomy. The technical advances have translated into 30-times increase of PHA titer from biorefinery waste, more than 20-times increase of conversion efficiency for biorefinery waste-to-PHA conversion, and 50-times decrease of PHA production cost from biorefinery waste, all of which leapfrogged the technology to address an important challenge in modern biorefinery development.

The proposed research will address an imminent challenge of biorefineries with a transformational solution. Scientifically, the bioconversion of lignin at a high efficiency has rarely been achieved. The integration of multimodule design enabled the highly innovative lignin-to-PHA route. Besides the significant scientific merits and innovation, successful development of this single-unit process will allow the conversion of a significant amount of renewable biomass to bioplastics to meet DOE’s near- and long-term targets for production of low-cost biofuels from lignocellulosics. The process will also maximize replacement of petroleum products, as bioplastics have a rapidly increasing market and represent a sustainable way to replace petroleum-based plastics. This novel platform complements the current technologies supported by BETO to enable the utilization of all structural components of cell wall for better cost effectiveness, overall carbon efficiency, and sustainability for biofuels.

**OVERALL IMPRESSIONS**

- The project addresses pretreatment fractionation, strain engineering, and fermentation development to improve overall process streams and enable optimal microbial valorization of lignin to PHAs. Significant progress has been made under lab- and bench-scale conditions. Additional development, scale-up, and refined TEA validation are required to determine whether the process is commercially viable.

- This was challenging work but the team did have some very nice results. Our main concern is they are likely not on scale to meet BETO goals, even if successful at one liter to reach the $5/kg goal. This is based on the fact that they are two times away from the goal. Many bio projects fail not at the one liter stage but at the pilot- or full-scale stage, making most organism approaches higher risk for commercial-scale success rather than chemical approaches. If PHA remains a niche material, then introduction of increased volumes into market could cause collapse. The only viability I see is if they can go way under PHA current price to drive market acceptance and grow the PHA business/acceptance dramatically.

- By investigating both pretreatment and bioconversion processes, the research team developed the CLARS process. The CLARS process harnesses the value of low sugar levels in lignin-enriched streams to biologically transform the lignin components into bioplastics (i.e., PHA). While the team reports high PHA yield from lignin, corresponding measures are mainly based on depletion of ferulic acid. As a next step, it will be important to determine yields from the total lignin supplied to the engineered *P. putida* strains that accumulate PHA. Notably, the team also demonstrated benefits of using a laccase/mediator system to increase lignin utilization by *P. putida*; however, the robustness of this approach should be confirmed.

- The PIs present a scientifically interesting project that also incorporates the novel concept of biochemical lignin conversion. By optimizing the pretreatment leading to monomers, the PI has been able to improve the yield of *p*-hydroxyxycinnamates needed for eventual conversion to PHAs. The project would benefit from a clearer description of the economics, a better quantification of the active substrates in the lignin extract, and an improved justification for the choice of PHAs as the target material.
This appears to be a very broad and ambitious program that has attacked the problem of lignin valorization from a fairly holistic approach to the biorefinery process. The team should be commended for the range of their accomplishments and overall progress. The team needs to be more clear as to how much of the PHA produced is actually from the lignin and how much from residual sugars, and how much of the PHA produced from lignin can be attributed to the improvements achieved in this project.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewer's comment that "significant progress has been made under lab- and bench-scale conditions." Indeed, the project achieved >30-times increase on PHA titer, >20-times increase on lignin-to-PHA conversion efficiency, and >50-times decrease on minimal selling price of PHA. We agreed with the reviewer that "additional development, scale-up, and refined TEA validation are required," which are exactly the goals of budget period two of the project. Now that the budget period one validation has just passed, the project is moving to budget period two. In addition, we are working with Altex Inc. and Danimer Scientific on the commercialization and scale-up of the technology.

- We appreciate the reviewer's acknowledgement of "very nice results." The team has two general approaches in addressing the market risks. First, in recognition the market risks, the team has already worked with ICM Inc., Altex Inc., and Danimer Scientific on the scale-up of the technologies. In particular, considering the recent ban on trash import from China, there is a significant increase in demand for biodegradable plastics. Danimer Scientific and other companies are working on significantly increasing the PHA production in the United States to address this challenge. The team is working with these companies to broaden the PHA market. Second, and importantly, the team has also developed multistream biorefinery strategies, where different bioproducts can be developed to avoid the market saturation. These lignin-based products include carbon fiber, asphalt binder modifier, lipid, bioplastics, and other bulk products. The future solution could be that different biorefineries could adapt various bioproducts based on market demands to avoid saturation.

- We appreciate the reviewer's acknowledgement of the CLARS process to harness the low-value sugars. We have evaluated lignin conversion efficiency with both ferulic acid and lignin-based methods. We agreed with the reviewer that even though the ferulic acid-based evaluation is very accurate, the lignin-based evaluation is complicated by the complex substrate. We are identifying a solution for this challenge in budget period two. For laccase-mediator-based fractionation, the recent technology development demonstrates that an efficient fractionation can be achieved without laccase. We aim to avoid the laccase-based method due to the potential high cost of laccase.

- We appreciate the reviewer's acknowledgement for improved yield of monomers and eventually PHA conversion. We did include TEA analysis in the "relevance" section, and more thorough TEA analysis of different scenarios are being carried out in budget period two. Both lignin and residual sugars are active substrates in the lignin extracts, along with some organic acids, which complicates the exact contribution of each component to the eventual PHA yield. However, such complication does not impact the economics, as none of these components, including the residual sugars at low concentration, have other economic values. As aforementioned and shown in the economic analysis, PHA is a perfect target for biorefinery products due to: (1) higher value, (2) large market potential due to the recent waste-management crisis, (3) serving as one of the many products for lignin utilization, and (4) funnelling diverse aromatics into single products to reduce the separation challenge.

- We appreciate the reviewer acknowledging the holistic approach. We actually have estimation of how much PHA is from lignin and sugar, respectively, though we do agree that such estimation needs to be better defined during budget period two. However, as aforementioned, the detailed evaluation of how each component of biorefinery waste contributes to the PHA yield will not impact the overall economics, as none of these components can be isolated for producing high-value products.
GAS-PHASE SELECTIVE PARTIAL OXIDATION OF LIGNIN FOR COPRODUCTS FROM BIOMASS CONVERSION

National Renewable Energy Laboratory

PROJECT DESCRIPTION

This project seeks to develop a catalyst and process that can convert low-value lignin streams from biorefineries into valuable phenolic coproducts by the gas-phase catalytic partial oxidation of lignin pyrolysis vapors. The technology will enable a diversified revenue stream for an integrated biorefinery in which the cellulose and hemicellulose biomass fractions are primarily used to produce a suite of fuels and coproducts, creating valuable phenolic compounds from lignin. The focus on producing simple phenols (phenols that do not contain methoxy functional groups) is due to their high value and market sizes (~$1,100 per tonne, 11,400 kT/y phenol; $3,800 per tonne p-cresol, 3,400 kT/y p-cresol; $6,000 per tonne, 45 kT/y catechol).

The approach for catalyst development focuses on design of catalysts by varying two parameters known to be important in selective oxidation catalysis: (1) metal-oxygen bond strength and (2) active site density. The activities that were performed and will be discussed include: (1) establishing benchmarks on yield composition for various types of lignin pyrolysis vapors, (2) establishing a benchmark with a commercial catalyst for

Weighted Project Score: 7.5

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%
phenol yield from partial oxidation of lignin pyrolysis vapors, (3) computational guidance on materials selection, (4) synthesis and characterization of catalyst materials, (5) reaction testing of catalysts using both model compounds and the whole vapor stream from lignin pyrolysis, and (6) integration of TEA with experiment results. Recent catalyst and process development accomplishments are highlighted by improvements in the carbon yield to the desired phenolic compounds and the resulting estimated impact on the reduction in modeled minimum fuel selling price will be presented.

OVERALL IMPRESSIONS

- The project is on track to develop catalysts and conditions for improved yield of simple oxygenated phenols from lignin pyrolysis vapors. Improvements in yield, catalyst regeneration, and scale-up to demonstrate scalability are on deck. Significant interest from upstream and downstream entities bodes well for commercial interest in a successful project.

- This is a difficult project with many challenges. The project is well managed and well run with clear partnerships and a well-thought-out technical approach. There are two concerns to address in the future, one being the choice of the vanadium catalyst, because vanadium has seen large price increases in recent years due to potential demand in the battery market as well as a reduced supply from a key region in China. Second, the key challenge is the requirement to meet a BETO goal of 25% yield of simple phenolics, which represents a future improvement of 66% yield versus current work results. I believe this could be the most difficult challenge to achieve with the current plan. Still, I commend the team for their excellent collaboration across multiple areas with internal and external partners and their progress to date.

- This appears to be a well-organized program that has made very impressive progress to date. They have made good use of computational modeling to streamline catalyst design and selection. Their future work
plans are targeted at appropriate critical issues for cost reduction and scale-up risk reduction. The team should leverage the interaction with their industry partners to validate the market value of their intended product stream (mixed phenols).

- The PIs have made reasonable progress in their understanding of this pyrolysis process and of the materials that are generated as a result. The project needs more clarity as to the potential markets for mixed phenolics, how a value higher than pure phenol is used in the TEA, and how this project represents a clear improvement over the many literature reports of converting lignin or biomass into phenols.

- This project includes excellent representation of industry partners across the value chain and benefits from a high level of partner engagement. Already, the team exceeded their goal to demonstrate ≥10% yield of phenolics at the lab scale, and developed a catalyst with two-times higher performance than a commercial benchmark. The scalability of the process will be important to demonstrate as a next step.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their detailed responses. In order to address concerns of the catalyst cost, we are exploring multiple types of catalysts, including non-vanadium catalysts (e.g., perovskites and industrially supplied catalysts from partners) and will use a BETO-developed tool (CatCost™) to estimate the scaled-up cost of our catalyst to see if the catalyst cost is a driver and look for opportunities to reduce this cost. The end-of-project goal is to demonstrate a 10% yield to simple phenols on the bench scale, and while this has been demonstrated on the lab scale, improving the overall economics will require additional improvements in the yield to simple phenols, which is why we will continue process/catalyst development throughout the project.

- We are actively working on improving our TEA model to reflect the experimental data and understand the separations costs/requirements. There are existing markets and values for phenol ($1.10/kg), o-cresol ($2.40/kg), m-cresol ($3.90/kg), p-cresol ($4.00/kg), and mixed m-/p-cresol ($2.80/kg). The lower cost of the mixed cresols has the separations costs "built in." There is ongoing work at NREL within BETO projects focused on separation methods and has shown the ability to produce >97% purity fractions of phenol, o-cresol, and m-/p-cresol from the condensed catalytic fast pyrolysis broth from whole biomass. The price of phenol (and all commodities) fluctuates, so we have used a phenol price band in our TEA analyses that is representative of both (1) fluctuations in market prices and (2) fluctuations in the product distribution (i.e., lower-value phenol versus higher-value cresols). Our condensed simple phenols consist of 40% cresols, 30% phenol, and 30% alkyl phenols. We are still determining the appropriate markets/prices for alkyl phenols, but using a weighted average of phenol and mixed cresol values gives us estimated market prices of $2.10/kg, which is in line with what we used in the analysis ($1.90/kg).
BIOLOGICAL LIGNIN VALORIZATION – NREL
National Renewable Energy Laboratory

PROJECT DESCRIPTION

This project aims to develop robust biological processes to produce novel coproducts from lignin-derived aromatic compounds. In collaboration with the Lignin Utilization project (focused on chemical lignin depolymerization and lignin analytics), this project will directly contribute to the minimum fuel selling price targets of $2.50/GGE in 2022. The project focuses on the production of three compounds—muconic acid and two other aromatic-catabolic intermediates—that are relevant to large-market-size, direct-replacement chemicals (such as adipic acid and terephthalic acid) to be able to realistically aid the bioenergy industry. The final project milestone for this three-year project cycle is to produce these compounds at 50 g/L and 90% yield. Moreover, this project is among the three collaborative efforts in the BETO lignin portfolio developing the "biological funnelling" concept, which aims to convert a heterogeneous slate of lignin-derived compounds to a single product—a relatively new concept for the biorefinery. Thus, beyond direct contributions to meeting cost targets through effective lignin valorization, this project is providing foundational scientific knowledge for the burgeoning concept of microbial lignin conversion, which is likely applicable to many types of lignin streams and offers a new approach for the much-needed problem of lignin valorization in an integrated biorefinery.

Weighted Project Score: 8.0
Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%

One standard deviation of reviewers’ scores
The project is distributed across three tasks: the first task focuses on metabolic engineering of a robust aromatic-catabolic chassis microbe, *Pseudomonas putida* KT2440, to convert aromatic monomers found in lignin streams to targeted intermediates, with muconic acid as the first exemplary product. The second task focuses on closely associated bioprocess development efforts using bioreactor cultivation to control pH, feeding rate, and mode and other associated process variables. The two tasks work closely to optimize key bioprocess performance metrics, including titer, rate, yield, and substrate toxicity tolerance. Recent accomplishments from this effort of the project include the development of robust *P. putida* strains and associated bioprocesses that are able to convert hydroxycinnamic acids, which are highly prevalent in lignin streams from agricultural residues and grasses, to muconic acid at high titers, rates, and yields. Key activities include overcoming bottlenecks in upper pathways by overexpression of rate-limiting enzymes, cofactor engineering, expression of heterologous genes in place of native genes, deregulation of carbon catabolism, and associated optimization of the bioprocess parameters such as feeding control. Progress has additionally been made in collaboration with the Denmark Technical University on evolving strains for higher toxicity tolerance to both substrates and products. Lastly, the project has engineered both syringate and syringol turnover in *P. putida*, which enables syringyl-type lignin (which is highly prevalent in hardwoods and grasses) to be converted into value-added compounds. All of these learnings are being applied towards other BETO projects, such as a close collaboration with the Metabolic Engineering for Lignin Conversion project at ORNL and the Agile BioFoundry, to deploy bioprocesses to aromatic-catabolic strains engineered in other projects.

The third task in the project focuses, in collaboration with the Biological Lignin Valorization project at SNL, on the cleavage of dimers and oligomers that are not able to be catabolized by native strains. Our project mostly focuses on understanding the native capacity of *P. putida* to cleave dimers and oligomers, whereas the Biological Lignin Valorization project at SNL aims to discover new catabolic capacities for inclusion into a chassis strain. To date, our project has discovered that *P. putida* likely packages enzymes into outer membrane vesicles for extracellular lignin catabolism.

**OVERALL IMPRESSIONS**

- This program describes an important strategy to build an organism to valorize lignin-derived compounds biologically. The work is in a good position to benefit from progress in the Lignin Utilization program and appears to be doing so, for example by utilizing model substrates and potentially enabling the building of mock substrate mixtures to focus on limiting compounds as it moves toward authentic.
substrate compositions. The Agile BioFoundry is mentioned briefly in the project description but it is not clear to what extent information is flowing and in what direction. Discussion with the Agile BioFoundry regarding \textit{P. putida} development is a clear win-win, as the Agile BioFoundry is working on building a \textit{P. putida} chassis. One could easily see implementation of a Design-Build-Test-Learn strategy (or at least a mini-Design-Build-Test-Learn in the early stages of discovery) for gene dosing (copy number) and stacking of tolerance factors and "on-pathway" substrate utilization/product genes as they emerge, and are likely to add value to move the program quickly. The discovery of a secreted enzyme system is interesting and may provide additional strategies to exploit this for extracellular degradation of potentially cytotoxic compounds such as soluble lignin-derived oligomers. The team is encouraged to further explore the secretome of the organism to determine its potential and limitations, and optimize key enzymes.

- The team has done an outstanding job making progress across many challenges that arose during the project for such stretching goals. This team worked well together, showing solid management of program and good collaboration across the various other teams inputting information and product streams for evaluation. Again, from experience, biological conversion on such complex materials is highly challenging and the team is commended for taking on this challenge and making great progress. This program has a chance to contribute substantially to BETO goals by funneling many products into single key materials when lignin processing produces so many compounds. I would hope this project could find a way to continue beyond the September end date.

- The PIs continue a scientifically excellent project for the biochemical transformation of lignin into muconate, and ultimately adipic acid. High-yield success in this project through biological funneling will be a nice addition to biorefinery development. Demonstration of this approach on a wider range of lignins and a clear TEA will further improve the project's impact.

- This project has made excellent progress towards identifying new enzymes able to cleave the five most relevant linkages in lignin, and then transferring that capability to \textit{P. putida} to increase lignin bioconversion into valued biochemicals. In addition to using lignin model compounds, the team developed \textit{P. putida} strains that can utilize syringyl lignin, transform syringol, and produce muconic acid from base-catalyzed depolymerized lignin. As a next step, strain tolerance to fluctuations in feedstock composition and slight changes in pretreatment conditions will be important to quantify.

- This project fits nicely in the lignin valorization portfolio and is well integrated with other relevant programs. The team appears to have made very substantial progress on titer, rate, and yield performance in model systems, which presumably is carrying over to the performance in "real" lignin streams. It appears that currently the levels of rate and titer they can achieve in these real streams is limited by the upstream processes’ abilities to produce a high-enough concentration of addressable carbon.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We appreciate the positive comments and feedback. Indeed, we will on-board some of the tools developed in the Agile BioFoundry (e.g., biosensors and transformation tools), and much of the work originating in this project has informed engineering in the Agile BioFoundry as well, so synergies will exist in both directions. We are certainly investigating the secretome in more detail as bandwidth permits and also leveraging fundamental discoveries made in the DOE Office of Science-funded and academic efforts.

- We completely agree that this approach needs to be demonstrated on a wide range of lignins. We are actively engineering a \textit{Pseudomonas putida} chassis that is able to convert syringyl (S), guaiacyl (G), and hydroxyphenyl (H)-type lignin monomers, and hence will ideally be useful for lignin samples from multiple upstream catalytic treatments and biomass sources.
• We agree that upstream changes will modify the organism needs and balance. At present, we are attempting to establish a baseline strain that is able to catabolize H-, G-, and S-type lignin monomers that will be produced from a wide variety of biomass samples.

• The comment regarding the upstream process abilities dictating the titers, rates, and yields is absolutely correct: namely, the upstream lignin depolymerization process dictates the amount of "bioavailable" aromatic carbon for the microbe to convert. We are working in close collaboration with the Lignin Utilization project to enable higher amounts of aromatic carbon that is biologically accessible now.
BIOLOGICAL LIGNIN VALORIZATION – SNL
Sandia National Laboratories

PROJECT DESCRIPTION
Depolymerization of lignin and subsequent upgrading of lignin-derived intermediates to fuels and chemicals is key to meeting BETO’s 2022 goal of $3/GGE; however, utilization of lignin has not been successfully demonstrated in an industrially relevant manner. This is in part due to our limited understanding of both the compounds biological systems produce when they depolymerize either native lignin or lignin coming from a biomass pretreatment process and which of the produced compounds microorganisms metabolize. This project is focused on developing fundamental understandings of microbial and enzymatic depolymerization of lignin and answering several key questions: To what extent can lignin can be enzymatically depolymerized? What intermediates are produced? Which of these intermediates are consumed by microbes? What are the enzymes involved in lignin degradation and metabolism? Answers to these questions will provide insights into the requirements for biological upgrading of lignin, which is critical to the work being done in a companion project at NREL developing microbial strains and bioprocesses to convert lignin-derived monomers and oligomers to products.

Realizing the difficulties of top-down approaches in which lignin depolymerization is studied using either native lignin or lignin from biomass pretreatment processes, in this project we are taking a bottom-up approach

Weighted Project Score: 6.7
Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%
in which initial studies are being performed using model dimer compounds containing the canonical C-O and C-C linkages in lignin. This allows us to develop a fundamental understanding of how lignin-metabolizing organisms modify lignin-derived intermediates, to examine how defined bond types are enzymatically cleaved and to identify the enzymes involved in these processes. The knowledge derived from these studies will be used to guide analysis of studies on mixed bond-type lignin model polymers and lignin coming from process-relevant approaches, such as the BETO-funded Deacetylation, Mechanical Refining, and Enzymatic Hydrolysis project.

We have studied a set of 16 microbes reported to be capable of degrading lignin on a set of commercially available model dimer compounds and a set of newly synthesized model dimers containing a fluorescent reporter group. We synthesized two phenolic and two non-phenolic ether compounds linked to the highly fluorescent 4-methylumbelliferone compound. A versatile peroxidase from the *Bjerkandera adusta* cleaved the C-O bond in the absence of hydrogen peroxide (H$_2$O$_2$) and at low concentrations of exogenously added manganese(II) chloride (MnCl$_2$), suggesting manganese peroxidase activity dominates and the Mn binding site is fully occupied. These compounds have also been used as substrates in the presence of the 16 identified microbes, and several produce enzymes capable of catalyzing C-O bond cleavage in both phenolic and non-phenolic ether compounds.

We also investigated dimer degradation and modification by 16 microorganisms using a panel of C-O (SGE, VGE, GGE) and C-C (DDVA, pinoresinol) bonded dimers. A subset of these microorganisms, including *Exophiala alcalophila*, *Sphingobium sp*. SYK-6, *Delftia acidovorans*, *Rodococcus jostii*, *Pseudomonas putida* mt-2, and *Streptomyces viridosporus*, modified all five compounds, establishing they either secrete ligninolytic enzymes or are capable of incorporating and metabolizing these compounds. Additional studies of the kinetics of GGE and pinoresinol modification by *Sphingobium sp*. SYK-6 were conducted and showed the presence of multiple intermediates expected to be produced based on its published aromatic metabolism pathways. Similar experiments examining the modification of dimer compounds by combinations of the subset of microorganisms that modified the five dimers are ongoing and future experiments will include studies using a commercially available synthetic lignin polymer with defined structure and mixed bond types (two C-C bonds and one C-C bond).

**OVERALL IMPRESSIONS**

- The project is the bottom-up counterpart to other biological lignin valorization projects, seeking to discover enzymes from a set of organisms showing an ability to work on a set of synthesized lignin dimers. This is an important complement to those programs and may provide valuable leads for heterologous genes and their encoded enzymes that can be leveraged into potential chassis hosts such as *P. putida*, or perhaps as hosts in their own right (although this seems less likely given resources and stage of development).

- The PIs present a potentially interesting expansion of their *P. putida* work by investigating the reactivity of a broader family of lignin-converting organisms. If successful, the project could identify systems tailored to key structural units in lignin and alternative biochemical approaches to lignin-derived chemical products.

- An important and distinguishing capability of this project is its focus on the synthesize lignin model substrates that facilitate enzyme and microbial screening. The focus on compound synthesis to support enzyme and microbial screens addresses a central limitation to enzyme discovery. To build from the project's success, the team is advised to now focus on the synthesis of lignin model compounds that retain structures that are difficult to transform by known enzymes and microbes (e.g., condensed C-C linkages).

- The team took a sound technical work approach. I do like the approach to look at mixtures of enzymes/organisms that may have synergistic impact. I am concerned that no realistic samples of lignin
or lignin products have been tried and progressed organisms only on model compounds. Testing of realistic samples early is needed to define how far away they are from future goals.

- Overall, this is a meaningful project which fits well in the BETO-funded portfolio of lignin valorization programs. The team has made good progress in developing model compounds that fluoresce to indicate bond cleavage as a means of tracking lignin degradation activity. However, it appears that most of the effort to date has focused on the more tractable C-O linkages, which may not be particularly relevant in technical lignins, which would be the substrate in the context of a biorefinery process. Hopefully in the balance of the project they will make progress on the C-C linkages comparable to the strong progress they have made on addressing the C-O linkages.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We are taking a "bottom-up" approach, in as much as we are as this project was conceived as an alternative approach to lignin depolymerization by exploring and exploiting the enzymatic diversity present in select microorganisms with reported capabilities for degradation of aromatic compounds.

- We agree and are currently synthesizing a C-C linked model compound. Synthesizing a C-C bonded compound to include a compound that fluoresces upon bond breaking proved difficult, so we are now synthesizing a compound suitable for a fluorescence resonance energy transfer-based assay.

- Our plan is to continue to move toward more complex lignin samples. Our current set of model dimers are very useful for identifying new lignin-utilizing organisms and evaluating potential metabolic pathways and associated enzymes. Using more complex lignin substrates will allow us to identify organism that must be secreting enzymes that break down polymeric lignin to fragments. We are working to understand how microbes break down lignin and utilize the lignin fragments produced. We are trying to fill the gap in understanding of the lignin fragments produced during biological depolymerization and of these fragments that are consumed and utilized. This will inform synthetic biology approaches to lignin valorization, but as the commenter suggests does require investigating more complex lignin compounds and polymers.

- Continuing the comments above, our approach is to first tackle C-C bond cleavage by generating a C-C linked model compound(s) and then move on to investigating more complex lignin polymers containing mixed bond types. While we agree with the comments suggesting we focus future work on C-C bond cleavage and are indeed working on this, we also think β-O-4-containing compounds are relevant. A primary goal of this work is to address gaps in knowledge around lignin products produced from biological depolymerization of lignin and of these products that can be used by microbes to sustain growth and are thus target fragments for use as substrates by microbial hosts engineered to metabolize them into valuable products. It is still difficult to predict if fragments produced from technical lignins lacking β-O-4 bonds will provide fragments amenable for microbial conversion. Control of processes to produce desired targeted intermediates amenable to upgrading may require pretreatment processes that leave β-O-4 bonds intact. Our results have shown that compounds containing β-O-4 bonds (e.g., GGE, SGE, VGE) are metabolized by microbes that have evolved to utilize β-O-4 bond-containing dimers; thus, from a fundamental biology/biochemistry perspective it remains important to understand the enzymatic routes and enzymes involved in β-O-4 bond cleavage. One important component of this work is to determine the effects of the size and structural complexity of different target substrates with different bond types on the degree of bond breakage by biological systems, as well as identifying depolymerization products capable of sustaining microbial growth. This information is key to developing biomass deconstruction processes that optimize valorization of both sugars and lignin, and may conclude that processes producing lignin streams in which β-O-4 bonds are completely cleaved also produce lignin intermediates less amenable to upgrading via synthetic biology and biomass and, therefore, pretreatment processes may need to avoid β-O-4 bond cleavage. For example, biomass pretreatment processes such as ionic liquids solubilize lignin but do not result in β-O-4 or C-C bond cleavage.
METABOLIC ENGINEERING FOR LIGNIN CONVERSION

Oak Ridge National Laboratory

PROJECT DESCRIPTION

Lignin valorization will be critical for the economic production of biofuels in a biorefinery. Therefore, technologies are needed to convert lignin into more valuable products. However, chemical markets are small compared to fuel markets, so the ability to produce a range of products from lignin would increase the flexibility of biorefineries to meet market demands. One attractive approach for lignin valorization is to engineer microorganisms for the bioconversion of lignin-derived aromatic compounds into products of interest. 

*Pseudomonas putida* KT2440 is a highly robust bacterium capable of efficiently utilizing a variety of carbon sources, including aromatic compounds derived from lignin, and it has recently been engineered to convert lignin streams from plant biomass into muconic and adipic acids. In this project, our goal is to engineer *P. putida* to convert lignin into products derived from other parts of cellular metabolism, thus demonstrating that we can diversify the portfolio of products that can be made from lignin. One example is medium-chain-length polyhydroxyalkanoates (mcl-PHAs), which are natural carbon storage compounds produced by *P. putida* that can also be used as bioplastics. Through a combination of gene deletions and heterologous expression, we have engineered *P. putida* to increase the yield of mcl-PHAs from both model aromatic compounds and depolymerized lignin. This work is being leveraged to also produce medium-chain-length alcohols (mcl-alcohols) from aromatic compounds, because both biosynthetic pathways

Weighted Project Score: 7.0

Weighting for Ongoing Projects: Approach-25%; Accomplishments and Progress-25%; Relevance-25%; Future Work-25%

Average Score

One standard deviation of reviewers’ scores
are offshoots of fatty acid biosynthesis. To further increase the compounds able to be made from lignin, we are also exploring production of molecules derived from the tricarboxylic acid (TCA) cycle such as itaconic acid, and current progress will be discussed. Together, this work is a critical step forward in demonstrating microbial approaches for lignin valorization to enable sustainable biorefineries.

OVERALL IMPRESSIONS

- This project adds to the suite of *P. putida* projects seeking to valorize lignin. The goal of this project is specifically oriented to produce a target molecule from aromatics to a specified titer and yield by on-pathway manipulation, including regulation, deletion, and heterologous gene expression. Initial performance targets are being achieved on both model and lignin-derived substrates; the inclusion of such (more) authentic lignin substrate is greatly appreciated to put the progress into context with conditions more reflective of real-world conditions.

- The PIs are pursuing a relevant project for lignin conversion to itaconic acid (and other chemicals) using novel biochemical approaches in contrast to chemical catalysis. Initial results have shown that discrete chemicals can be derived from lignin, albeit at low concentrations. The future direction of the project should demonstrate the breadth of the conversion with regard to different lignin sources and identify new markets for itaconic that might arise if the cost drops as a result of this research.

- This project applies a carefully designed metabolic engineering approach to increase product yields from lignin substrates, while uncovering general strategies that can be employed to control the performance of engineered organisms. As a result, there is an excellent opportunity to further leverage the outcomes of this work in other BETO projects. In an effort to further utilize TEAs as a research tool, the team could evaluate the impact that improved product yields and costs can have on the market size of the targeted biochemicals.

- The team has progressed the project well and done an impressive job developing this organism for multiple targets. My only concern is that at this stage various BETO sources of lignin from upstream project isolation have not been tested to define the robustness of the engineered organism. Also I suggest, if not already doing so, taking a closer look at past history of LS9, Inc. and current Renewable Energy Group (REG) work in this area, if accessible, to make fatty acids from sugar. This is great project on fatty alcohols and a U.S.-sourced supply could make for an interesting market, if it can be made at current or lower fatty alcohol prices than alcohol made from palm kernel oil.

- This appears to be a strong, well-organized program making good progress, and making good use of TEA to drive metrics and performance assessment. It is encouraging to see what appears to be a good collaborative effort with the NREL team. In addition to the TEA to assess economic impact, it would be helpful to undertake (possibly outside the scope of this project) an assessment of the challenges and key issues to bring a molecule such as itaconic acid in as part of a material substitution versus well-established materials. I suspect that these challenges are underestimated, and they need to be well understood so projects moving forward can be designed with these challenges in mind so they are addressed early.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- Thank you for the positive comments.

- We agree that exploring diverse lignins and market analysis are important directions for the future, so we will continue to collaborate closely with researchers at NREL for both the bioconversion of real lignin streams and for TEA and exploration of market opportunities.

- We agree and plan to continue to work closely with the NREL TEA team to further explore the intersections between performance (e.g., titer, rate, yield), cost, and potential market size.
• Substantial work has already been performed on the robustness of *Pseudomonas putida* in different lignin streams by our collaborators at NREL (and by others), and it will be interesting to explore how our engineered strains perform on these diverse lignin streams. We will also explore any publicly available work on fatty acid production by LS9, Inc./REG to look for lessons learned and potential paths to improvement in our system.

• While the reviewer is correct that exploring the challenges of bringing a molecule to new markets/applications is beyond the scope of this project, we anticipate that we will reach out to potential commercial partners as strain performance (e.g., titer, rate, yield) improves, and that these potential partners could explore new applications. We can similarly reach out to other BETO projects related to downstream processing.
BIOLOGICAL CONVERSION OF THERMOCHEMICAL AQUEOUS STREAMS

National Renewable Energy Laboratory

PROJECT DESCRIPTION

The project aims to biologically upgrade compounds in aqueous streams from thermochemical (TC) processing to value-added coproducts. This work will support the BETO mission towards cost-effective TC conversion by producing coproducts (with the exemplary products, mcl-PHAs or muconic acid) that will result in a switch from a $0.25/GGE cost for wastewater treatment to a revenue stream of a similar magnitude toward lowering the minimum fuel selling price. Utilization of such “waste” carbon is key to the responsive TC biorefinery to produce fuels and chemicals. We predominantly utilize a soil bacterium, *Pseudomonas putida* KT2440, as the organism for this work, as it can catabolize substrates including some furans, lignin-derived species, and organic acids, and it is tolerant to high concentrations of organic substrates.

This project comprises two tasks: the first is focused on comprehensive analytical method development to fully characterize TC waste streams using mass spectrometry coupled to gas chromatography or liquid chromatography, nuclear magnetic resonance spectroscopy, and molecular weight analyses. The second task

Weighted Project Score: 7.9

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%
focuses on the engineering of \textit{P. putida} for improved toxicity tolerance and expanded substrate specificity. The work will ultimately culminate in a bioprocess development effort at the end of the current three-year cycle that will demonstrate a cultivation strategy for biologically converting a waste stream from \textit{ex situ} catalytic fast pyrolysis to value-added products.

To date, the first task has developed a robust protocol for characterizing TC waste streams from multiple technologies, including fast pyrolysis, \textit{ex situ} and \textit{in situ} catalytic fast pyrolysis, and fast pyrolysis with fractionation approaches. These results have been provided to the analysis projects in the BETO portfolio for rigorous TEAs. Technical accomplishments to date from the second task include engineering of a \textit{P. putida} strain combining chaperone overexpression, dehydrogenase engineering, and membrane modifications that are significantly more toxicity tolerant than the wild-type strain. Additionally, we have engineered the strain to be able to convert over 90\% of the carbon found in pilot-scale \textit{ex situ} catalytic fast pyrolysis streams to value-added compounds. Future work includes continued development of \textit{P. putida} towards much higher toxicity tolerance thresholds, additional characterization of \textit{ex situ} catalytic fast pyrolysis pilot-scale derived aqueous streams (including streams from the newly developed platinum [Pt] titanium dioxide [TiO\textsubscript{2}] catalysts), and bioprocess development towards the end-of-project milestone.

It is also noteworthy that this project has larger implications to the BETO portfolio and the synthetic biology and lignin valorization communities more generally; namely, developments in this project will make strides in improving toxicity tolerance of strains to harsh waste streams, and this project will also develop new aromatic-catabolic pathways that can also be harnessed in the context of lignin (as has already been done with discoveries in this project that have translated to the Biological Lignin Valorization at NREL and PET Upcycling projects).

\textit{Photo courtesy of National Renewable Energy Laboratory}
OVERALL IMPRESSIONS

- This is another *P. putida* platform development program that focuses on aqueous thermochemical process waste streams that may have up to 10% waste carbon. Valorizing this level of waste to a useful coproduct, as well as cleaning up the wastewater stream itself, appears to be a worthy target, although it depends on the concentration (a well-developed TEA will bear out the cost-benefit). Strain tolerance is a key issue, and the discovery of a misalignment of substrate composition from TC processing at different scales is key, and presents a challenge. New strategies, strain engineering methods, and potential PABPs have all resulted from this project so far, which is good progress.

- The PIs have presented a scientifically compelling approach to conversion of carbon that would otherwise be lost. The products are reasonable and recognized in the chemical industry. While the specific application of this work to waste streams faces considerable challenges, the broader applicability to lignin conversion is promising, as it is trying to address lignin's structural heterogeneity.

- This project tackles the difficult task to biologically transform low levels of carbon-containing compounds that can be recovered from thermochemical effluents into valuable biochemicals. Although difficult, this objective can reduce wastewater treatment costs incurred by biorefineries, while generating valuable coproducts. This project has made excellent progress. For example, the team has uncovered important differences in aqueous stream compositions as a function of pilot versus bench-scale TC processes. They also made significant improvements to strain tolerance. Codigestion of thermochemical effluents with other industrial process streams represents an important opportunity for future investigation.

- The team has done well coordinating across two very different work programs from analytics to bioengineering and extending the funnel of carbon utilization. Also commended for their ability to work well across different disciplines from process, catalyst team, analytics, and bio team all coordinating well, which speaks to good team management. The project is a success overall, though there is a question as to whether the value return to fuel GGE is sufficient to justify further work, albeit this can contribute learning to lignin bioconversion. Also, there is an interesting development for methyl branched mucconates, which perhaps can be reapplied to lignin programs. Excellent utilization of the waste carbon.

- Overall this is a strong project that has made a number of good technical achievements. I would like to see more explicit statements of the translation of the accomplishments in the basic science, which seem to be numerous, into practical results. For example, what level of carbon utilization has been achieved in bioprocessing of either mock or real catalytic fast pyrolysis wastewater streams, and what is the yield of useful products? How does the performance in these types of tests compare with the necessary targets for commercial viability, and what are the key remaining challenges to be overcome? Maybe all of that is part of future work. This team has done some very solid work to bring the underlying technology along quite far, so it would be good to see their vision as to how that gets translated to a viable, ready-to-deploy technology.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We appreciate the positive and constructive feedback from the review team overall.

- We have achieved 89% carbon utilization of a pyrolysis wastewater stream. The product yield to muconates is approximately >50% of the carbon. The product yield to PHAs, inherently, will be approximately 30%–40% of the carbon. In terms of developing a bioprocess towards a TEA model, we have just developed a rigorous and comprehensive TEA model, and we are baselining strain performance and bioprocess performance against these targets now.
LIGNIN UTILIZATION

National Renewable Energy Laboratory

PROJECT DESCRIPTION

The project aims to develop industrially relevant processes for lignin valorization with a particular focus on (1) chemical catalysis for lignin depolymerization, (2) chemical catalysis for lignin upgrading in collaboration with the Biological Lignin Valorization at NREL project, (3) lignin analytics applications and development, and (4) lignin model compound synthesis. Through rigorous TEA conducted at NREL by the Biochemical Platform Analysis project, lignin valorization has been shown to be essential for meeting the 2022 BETO minimum fuel selling price cost target of $2.50/GGE, and this project aims to contribute directly to that effort. The project comprises three tasks: depolymerization, upgrading, and analytics synthesis, and works closely with other BETO projects across the entire conversion portfolio to ensure that the solutions developed in Lignin Utilization are applicable across the spectrum of lignin isolation and biomass-conversion technologies. The end-of-project goal for FY 2019 is to demonstrate a 40% yield of usable monomers from lignin, and to work with the Biological Lignin Valorization at NREL project to show that the monomers can be assimilated in an engineered aromatic-catabolic microbe.

Catalysis strategies for lignin depolymerization focus on aromatic monomer yield and aim to develop methods that are lignin feedstock agnostic. Accordingly, oxidation is a major thrust area aiming at both C-O and C-C

Weighted Project Score: 8.6

Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%
bond cleavage. The ability to develop catalysts that are able to cleave both C-O and C-C bonds would increase the theoretical lignin monomer yield from many industrially relevant lignin streams, including from acid pretreatment, the pulp-and-paper industry, and the deacetylation and mechanical refining paradigm currently being employed in the BETO conversion portfolio. Accomplishments in this space include the evaluation of alkaline oxidation catalysis across a wide range of conditions and cocatalysts and the development of heterogeneous vanadium-based catalysts. The oxidation catalysis work is culminating with a new catalyst formulation able to cleave both C-O and C-C bonds, which is the major emphasis of the project to complete the current three-year project cycle. This catalyst has been demonstrated to cleave dimers, trimers, and oligomers from reductive catalytic fractionation oil, which is known to contain very few C-O bonds. Deployment of this catalyst system to other lignins is ongoing.

For lignin upgrading, efforts in the current three-year cycle include catalysis and associated process developments to produce the direct replacement, commodity monomers terephthalic acid and adipic acid, both of which are used in very large-market applications. Both of these monomers can be produced from cis,cis-muconic acid, which is being made biologically in the Biological Lignin Valorization at NREL project from lignin monomers produced in the Lignin Utilization effort. To date, we have demonstrated greater than 99% yield of adipic acid from muconic acid at high substrate flow rates and with industrially relevant catalysts (in collaboration with the Chemical Catalysis for Bioenergy Consortium project for development of robust catalysts). In addition, we are developing industrially relevant strategies to convert cis,cis-muconic acid to terephthalic acid via isomerization, Diels-Alder coupling with ethylene, and dehydrogenation. To date, we have demonstrated isolated steps for each chemistry, achieving 70% terephthalic acid process yield.

Lastly, the project developed a comprehensive library of analytical methods to characterize lignin in the solid form to examine lignin chemistry in the cell wall and in residual lignin solids, as well as methods to characterize depolymerized lignin using advanced mass spectrometry methods. In addition to analytics, the project also produces lignin model compounds for catalysis and biology efforts in support of other BETO projects.
OVERALL IMPRESSIONS

• The Lignin Utilization project is tasked with a wide breadth of work that is actually three projects (described as tasks: characterization, deconstruction, and upgrading). Ultimately, the goal is to recover
the highest possible yield of usable monomers from lignin, to be upgraded either chemically or biologically to value-added compounds (e.g., muconic acid, adipic acid, terephthalic acid), all to be done from an economically attractive process. Each of these tasks performed to date have demonstrated progress on track with expectations. A new catalyst for C-C bond cleavage has been identified, a more recoverable base to replace sodium hydroxide (NaOH), better understanding of structure, analytical development, and synthesis of model compounds, and pathways to adipic acid and terephthalic acid have been demonstrated. There is still a long way to go in terms of yields, such as additional catalyst identification and development, process integration (chemical and biological), and, importantly, proving out the economics. The project appears very well managed and integrated with other projects such as Biological Lignin Valorization, which will continue to be essential to keep on track.

- This is a well-thought-out and organized team effort with a solid technical approach. Model compound synthesis upfront to evaluate and define the approach is a great first principle approach for the project. There are excellent project results to date, and the team is on track to assist the fuel dollars-per-GGE longer term and help other teams by excellent characterization of products formed for use by the bioupgrading team. Excellent progress on the recovery of monomers from multiple feed materials with the easier-to-recover oxidative bases versus sodium hydroxide. There are minor questions around processes to convert muconate to dimethyl terephthalate (DMT), including iodine, which can be a challenge to remove from wastestreams and any traces could carry through causing issues with processes downstream. Has the team considered other isomerization approaches that might be easier to separate, such as solid isomerization catalysts for fixed-bed operations? Also is there a reason why nickel, which is cheaper than palladium (Pd), is not used in dehydrogenation to DMT? Or is it just for initial proof of concept?

- This project tackles a key challenge to higher biological utilization of lignin-enriched streams, namely presence of condensed C-C linkages. The approach builds on NaOH-catalyzed lignin depolymerization, now using bases that can be readily recovered and recycled. To facilitate the analysis of lignin products, the team created a library of model lignin compounds, which represents a unique resource that has been made available to other BETO projects. To further demonstrate the applied significance of a base-catalyzed lignin depolymerization process, it will be helpful to report yields of products from the downstream bioconversion step in terms of amount of product per amount of total lignin consumed. The stability of lignin-derived compounds produced by the base-catalyzed process will also be important to verify.

- This appears to have been a broad, effective program that has made significant progress toward its goals while enabling other projects through expansion of the lignin analysis toolbox. The application of analytics and development of a range of model compounds should be leveraged across multiple BETO lignin-related projects.

- The PIs present an important project with direct relevance to the biorefinery. Their effort to generate a reasonable yield of monomers from lignin depolymerization processes that can be used for further biological processing is valuable and addresses a crosscutting challenge to biorefinery development. Improving the clarity regarding certain process steps and the current status of the model versus real lignin work would be helpful.

**RECIPIENT RESPONSE TO REVIEWER COMMENTS**
- We thank the reviewers for the positive and constructive feedback.
- The question about use of iodine is a good one. Iodine catalysis is used industrially today, but we are working towards other options for the muconate isomerization in parallel as well, including heterogeneous catalysis strategies, as the reviewer suggests. In terms of the dehydrogenation chemistry
to produce DMT, Pd is an initial starting point for this reaction to demonstrate proof of concept, as the
reviewer notes.

• We agree on how to report yields. This is indeed a challenge in many lignin conversion processes, but as
discussed at the peer review during the questions, we are attempting to achieve comprehensive mass
closures to be able to provide these yield data to the TEA teams in a rigorous fashion.
OXIDATIVE VALORIZATION OF LIGNIN
Pacific Northwest National Laboratory

PROJECT DESCRIPTION
Depolymerization of the lignin macromolecule to its monomeric constituents provides an opportunity to generate marketable chemicals or value-added intermediates. However, due to its heterogeneity and compact structure integrity, developing an efficient and cost-effective lignin depolymerization method that also produces selective products has been a well-recognized challenge.

During the last decade, PNNL and Washington State University have developed an oxidative valorization of lignin (OVL) pathway to produce selective monomers, such as monomeric phenolic compounds (MPC) and dicarboxylic acids (DCA), which have promising applications. We have tested OVL on more than half a dozen biorefinery lignin samples, and demonstrated that OVL is an efficient lignin depolymerization technology capable of producing MPC in high yield (i.e., 47% based on initial lignin) under mild reaction conditions (i.e., 1 atm, 60°C).

The specific target of this annual operating plan project is to significantly reduce the cost of catalyst and oxidant (both by 50%) utilized during lignin oxidation while maintaining the product yields and conversion process efficiency.

Weighted Project Score: 7.0
Weighting for Ongoing Projects: Approach - 25%; Accomplishments and Progress - 25%; Relevance - 25%; Future Work - 25%
The presentation will report progress made in this project to date as well as the future work and potential impact of this project on sustainable biorefinery development.

OVERALL IMPRESSIONS

- The PIs present good progress on their efforts to convert corn stover lignin into mixed phenols at a specified yield. The primary weakness is that they do not clearly delineate their efforts from the many similar efforts in the literature with regards to improvements and the ability to get mixed phenolics into the chemical industry. A much clearer description of the assumptions used in their TEA would improve the project.

- The OVL project has been successful in demonstrating useful monomer productions in milestone-relevant yields. The process may ultimately be challenged by oxidant and catalyst cost, pending significant further progress on loading or less expensive alternatives to be identified.

- The team has made good progress and has a solid strategy to define the best catalyst systems through high-throughput experiments. The work is at an extremely small scale. I would have liked to see some testing of the current best result at a scale of 100 g or so. Also, there is a big concern over such oxidative processes on a commercial scale, and before scaling the team needs to be clear on potential risks for runaway oxidation reactions unless the lignin itself is a self control for such runaway reactions. The biggest challenge is to reduce cost of catalyst and peracidic levels. One suggestion is to explore self-regenerating peracid or other surface active peracids or aromatic peracids with unique oxidation catalysts that have a high turnover of conversion of acid to peracid regeneration. These may or may not be compatible with the other catalysts but worth exploring from a proof of concept to see if the amount of oxidant loading can be used. See patents by Scheibel et. al. from the Procter & Gamble Company on these iminium ion compounds for use in laundry applications. I cannot say if these materials will be stable to reaction conditions and other cocatalysts. The soils on clothing and dishes are often similar in
challenge to remove, and often aromatic with multiple rings and functionality from the human body, soot from pollution in some countries, polymerized fats/oils, and other complex structures from soil and grass.

- This appears to be a strong project. The team has clearly identified the key challenges and criteria for success and shown very good progress against program milestones. Some high level of assessment of the commercial uses of the OVL products and what a route to commercialization would look like would be additive in defining the key next set of challenges for follow-on work.

- An important development from this project includes a platform to simultaneously screen multiple catalyst formulations. A key aim of this project is to also identify lignin intermediates, which promoted the development of analytical methods for lignin characterization. From a product standpoint, the goal is to increase the phenolic content of lignin to approximately 50%. To reach this goal, it will be important to test selected catalysts on different lignin sources, and evaluate requirements for downstream separation technologies.

RECIPIENT RESPONSE TO REVIEWER COMMENTS

- We greatly appreciate the reviewers’ comments and suggestion for this project. We strongly agree with reviewers that the oxidant and catalyst are the key cost barriers toward the commercialization of this progress. Based on the suggestions, we plan to include two specific tasks in future work: investigate the regeneration of the peracid and test lignin depolymerization at a larger scale (e.g., 100-g level). We will also conduct a more rigorous TEA analysis in the final year of the project. We also plan to expand the research to test the OVL on other lignin sources and determine the market application/value of monomeric phenolic compounds as the funding will allow.

- We must admit that the TEA assumptions should have been better described. Many inputs were assessed, and the primary cost drivers were oxidant consumption and product yield. Although the dollars-per-GGE fuel value shown above each vertical bar on the waterfall chart was based on an assumed monomeric phenolic compound product value of $1.00/lb, the value at $0/lb monomeric phenolic compounds credit is merely the value on the y-axis corresponding to the top of the bar charts. The downward trend in the bar chart illustrated cost reductions against the base case bar at the far left.

- Preliminary TEA shows that oxidant cost is a significant cost driver. Hence, we conducted experiments to better understand peracetic acid decomposition and its interaction with the catalyst and substrate in order to identify less-expensive alternatives. We expect that catalyst cost will also be important and thus conducted a combinatorial study to identify active and less-expensive catalysts.

- Current operating temperature and pressure conditions are low enough that runaway oxidation reactions are not a concern. Indeed, we are excited to find an alternative oxidant and catalyst, as well as applications to selectively produce oxidant reactive species that will produce our desired product and improve oxidant cost and consumption efficiency.