

# CARBON DIOXIDE UTILIZATION

TECHNOLOGY AREA

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## INTRODUCTION

The Carbon Dioxide (CO<sub>2</sub>) Utilization Technology Area is one of 12 technology areas that were reviewed during the 2021 Bioenergy Technologies Office (BETO) Project Peer Review, which took place virtually March 8–12, March 15–16, and March 22–26, 2021. A total of 12 presentations were reviewed in the CO<sub>2</sub> Utilization session by five external experts from industry, academia, and other government agencies. For information about the structure, strategy, and implementation of the technology area and its relation to BETO's overall mission, please refer to the corresponding program and technology area overview presentation slide decks (<https://www.energy.gov/eere/bioenergy/2021-project-peer-review-co2-utilization>).

This review addressed a total U.S. Department of Energy (DOE) investment value of approximately \$13,253,412, which represents approximately 2% of the BETO portfolio reviewed during the 2021 Peer Review. During the Project Peer Review meeting, the presenter for each project was given 30 minutes to deliver a presentation and respond to questions from the Review Panel.

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

BETO designated Ian Rowe as the CO<sub>2</sub> Utilization Technology Area review lead, with contractor support from Seth Menter (BCS, LLC). In this capacity, Ian Rowe was responsible for all aspects of review planning and implementation.

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## CO<sub>2</sub> UTILIZATION REVIEW PANEL

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## CO<sub>2</sub> UTILIZATION REVIEW PANEL SUMMARY REPORT

*Prepared by the CO<sub>2</sub> Review Panel*

### INTRODUCTION

The CO<sub>2</sub> Utilization program was established by BETO in Fiscal Year 2017 to explore the opportunities and potential for converting waste CO<sub>2</sub> into fuels and chemical feedstocks using a combination of biological and artificial pathways—including electrocatalytic, thermocatalytic, biocatalytic, and hybrid combination approaches—powered by renewable energy. BETO’s recent *Rewiring the Carbon Economy: Engineered Carbon Reduction Report* (<https://doi.org/10.2172/1419624>), serves as the foundation and guideline of this program, and the Review Panel agrees with the evaluation that “the CO<sub>2</sub> reduction and utilization technologies discussed have significant potential to impact carbon management and provide economic, environmental, and electric grid stability benefits.” For context, CO<sub>2</sub> reduction and utilization technologies have experienced intense interest both in the United States and internationally, driven by market demands for reducing emissions as well as policies such as the Section 45Q tax credit. The Review Panel agrees that the CO<sub>2</sub> Utilization program is timely and important given the strategic importance and emerging field of carbon capture, utilization, and storage (CCUS).

The Review Panel reviewed 12 projects in total: 9 were national laboratory projects, and 3 were external projects with industry or academia. The panelists conducted extensive question-and-answer sessions and discussions with the principal investigators (PIs), BETO program managers, and other participants. Overall, the panelists were very impressed with the organization of the program and the progress of the projects, especially given the disruptions during the last year due to the COVID-19 pandemic. The portfolio of projects was diverse and ranged from low to high technology readiness levels (TRLs) across a broad range of approaches. Although the Review Panel is impressed with the outcomes of the program so far and the initial learnings from the first phase, the Review Panel recommends that the program take a more focused approach going forward and build an integrated portfolio of projects that advance the most promising technologies to commercialization. In this report, the Review Panel provides summarized review comments and recommendations on program development.

### STRATEGY

#### Impact

The topic of CO<sub>2</sub> conversion has garnered much attention, spurred by recent announcements of governments and industries pledging net-zero CO<sub>2</sub> emissions targets by 2050. Many technology companies—such as Microsoft, Shopify, and Stripe—have committed to supporting technologies that capture and convert CO<sub>2</sub>. Challenge-style competitions, such as the \$20 MM Carbon XPRIZE, have similarly galvanized the innovator, researcher, and entrepreneur communities around CO<sub>2</sub> capture and conversion. Despite all this added attention and excitement, large-scale commercial successes of CO<sub>2</sub> conversion technology are still nascent, and some of the most promising technologies remain at a low to mid TRL. The BETO CO<sub>2</sub> Utilization program is perfectly placed in both timing and scope to help de-risk critical new disruptive technologies that have a clear market demand.

The entire portfolio of projects was well aligned with BETO’s stated objectives, especially around rewiring the carbon economy. Most teams in this program were from the National Renewable Energy Laboratory (NREL), with only one team from Oak Ridge National Laboratory (ORNL). Three external teams included projects with leads from Montana State University (MSU), LanzaTech, and Johns Hopkins University. Aside from one project that covered broader techno-economic analysis (TEA) and life cycle assessment (LCA), most projects fell into three categories: electro- and/or thermocatalytic CO<sub>2</sub> conversion, biocatalytic pathways focused on one-carbon (C1) intermediate feedstocks, and integration of electro/biocatalytic reactors.



The first project presented on TEA performed by NREL and set the stage for clear strategic direction. The Review Panel felt this was an excellent project, with the goal to guide existing and future research and development (R&D) with a deliverable of roadmaps for strategic R&D and a comprehensive design report. Particularly impressive was the level of collaboration with other major actors in the space, including the Global CO<sub>2</sub> Initiative and Argonne National Laboratory, which shows that this project will have far-reaching impact beyond BETO. The project has produced a few high-impact publications and a plan to make results publicly available. The team delivered on recommendations from the 2019 Peer Review by including more sensitivity analysis and benchmarking evaluation metrics, especially for CO<sub>2</sub> electrocatalysis technologies. Going forward, the Review Panel suggests that the team start providing more in-depth case studies with process simulation and detailed TEA and LCA for the most promising technologies. Although the foundational work done by the team is very helpful in setting baselines and context, it would be helpful to see concrete examples of technologies with process units that are as accurate as possible.

The projects on electrocatalytic conversion of CO<sub>2</sub> focused primarily on electrocatalyst development and benchmarking with an emphasis on materials design. The first project, from NREL, focused on diagnostics and standard operating procedure development to consistently test new CO<sub>2</sub> electrocatalysts with a goal of enabling long-term and stable operation of low-temperature CO<sub>2</sub> electrolyzers. The main focus was on the electrosynthesis of CO for use in syngas, and the team presented progress on diagnostic electrochemical measurements, kinetics, and understanding ion exchange membrane influence on the stability of the reaction. The Review Panel felt that this was a needed baselining project that would be impactful to the community. The Review Panel was particularly interested in the team's approach to approximate lifetime through accelerated testing via higher constant currents or faster cycling. Ultimately, the Review Panel felt that any accelerated testing needs to be validated on experiments that run on the scale of thousands of hours. The second project, from ORNL, focused on the development of carbon nanospire (CNS) catalysts for two-carbon-plus (C<sub>2</sub>+) production from CO<sub>2</sub>. Unfortunately, this particular project was heavily impacted by COVID, the lead scientist moved to another national laboratory, and the project is struggling to find the expertise and personnel to continue. Although the initial results were promising, the Review Panel felt that the current densities provided in the project were quite low and that the stability of the reaction provided was also low. This was a lower TRL materials-based project that did not fit well with BETO's objective of rapidly advancing the technology to commercialization. The Review Panel recommends that this project be sunset.

The projects on biocatalytic pathways were diverse and included upgrading of formate using *Cupriavidus necator*, enhancing acetogens to utilize liquid (formate) or gaseous (e.g., carbon monoxide, CO) feedstocks, and engineering new metabolic pathways to utilize *Escherichia coli* for formate conversion. The Review Panel found all biocatalytic projects impressive but felt that some projects were significantly lower in TRL than others, which made it difficult to compare them. For instance, the project that was utilizing clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) 9 tools to modify *E. coli* for formate utilization was impressive from the perspective of fundamental science because the team was able to engineer glycolate production when the wild-type pathway is not capable of doing so; however, the performance and selectivity of 15% was relatively low and therefore unlikely to be scaled. Although impressive, the Review Panel felt that much work needs to be done before it can be practical. In contrast, the work on acetogens for both gas fermentation of CO and liquid utilization of formate showed relatively high carbon efficiencies and success in the team's planned performance. Unexpected synergistic effects were also discovered when mixing different feedstocks, which is interesting and impactful. The project focused on using *C. necator* was also impressive because the team was able to engineer strains that showed production of a high-performance material (2-hydroxymuconate semialdehyde, 2HMS) whereas evolved isolates showed no improvement against wild type. Also, a project focused on utilizing cell-free enzymatic systems for CO<sub>2</sub> capture showed a 20% energy reduction compared to standard capture solution systems; however, the activity gains degrade rapidly, and only 40% activity is retained after 5 hours. Although this is an interesting proof of concept, the Review Panel thought that the project focused on CO<sub>2</sub> capture (rather than conversion) did not fit within the scope of the CO<sub>2</sub> Utilization program. The Review Panel thought that all of these projects were scientifically impressive

but questioned which approach would be most scalable. Going forward, the Review Panel suggests that the program more clearly define performance targets in terms of product amount, rate, and efficiency so that the projects that have potential to scale are better supported.

The last group of projects focused on the integration of both electrocatalytic and biocatalytic processes whereby CO<sub>2</sub> would be converted to either CO or formate electrocatalytically and then fed into a bioreactor to then be upgraded to either alcohols or other chemical feedstocks. Of these projects, one was led by NREL, and the other three were led by external partners. All projects showed progress in scaling up the electrocatalytic and biocatalytic reactors; however, the Review Panel felt that integration and the gains from rate matching electrocatalytic/biocatalytic processes were not clear. Electrocatalytic processes operate at higher reaction rates than biocatalytic processes, which makes the transfer of electrocatalytic products to a biocatalytic reactor suboptimal without some form of intermediate chemical storage. The Review Panel asked the fundamental question: Is it better to scale up the electrocatalytic and biocatalytic processes separately and then connect them through product transfer, or are there significant optimization gains to be made by integrating the two immediately before scale-up? Although projects showed promise in terms of scale-up and testing of product recyclability, it was not clear what the overall benefit was to fully integrate these two processes.

## Innovation

The CO<sub>2</sub> Utilization program, being relatively new, showed quite impressive research output despite delays from COVID. The Review Panel thought that the majority of the projects presented innovative and novel ideas or addressed much-needed gaps in the science and technology communities. The TEA and baselining projects to both set realistic targets and provide consistent operating procedures for electrocatalyst comparisons were particularly useful and innovative in the field. This builds on NREL's reputation as setting technology targets, standards, and baselines in emerging clean technologies, such as hydrogen and solar cells. The projects regarding electrocatalysis were also promising, but the field has matured to show that the major gaps in technology adoption are within the system, including membranes and electrolyte engineering. The most scientifically impactful work from the perspective of fundamental research was the research done on biocatalysis, particularly on utilizing CRISPR-Cas9 tools to engineer new pathways and change strains' catalytic performance. The integration projects were also quite innovative because they are some of the first projects to take this biocatalytic/electrocatalytic integrated approach. The scale-up and demonstration of some of these projects that showed decent stability over long timelines were also very impressive.

## STRATEGY IMPLEMENTATION AND PROGRESS

### Synergies

The Review Panel felt that although some synergies in the program existed, especially on the scale-up and integration projects, the level of coherence between the electrocatalytic and biocatalytic teams was lacking. The program is ambitious, looking to bring together materials scientists, microbiologists, chemical engineers, and biochemists; however, the Review Panel felt that projects were quite independent, and they remain siloed based on experience and topical area. The only crosscutting area that each project worked hard to incorporate was TEA. Although useful in some cases, especially for generating ballpark estimates, the Review Panel found that many projects were simply too early in terms of their TRL to provide accurate TEA. Despite this, there remains much opportunity for further synergies among the electrocatalytic, biocatalytic, and systems integration teams.

The Review Panel also noted that there were quite a few new startup companies in recent years that have made progress, especially regarding electrochemical CO<sub>2</sub> conversion. The Review Panel suggested increasing the number of funding opportunity announcements (FOAs) to allow for more external collaboration with both startups and academics in the space. There also exists an opportunity to collaborate internationally with other national laboratories that have strong CCUS research expertise, such as in Canada and Norway.

## Focus

The overall strategy of the CO<sub>2</sub> Utilization Technology Area is to enable the development of technology that combines electrochemical CO<sub>2</sub> conversion to intermediate feedstocks that then undergo biological upgrading to value-added fuels and commodity chemicals. This technology area fits well within the broader BETO funding portfolio, and it is unique compared to other DOE programs and technology areas. Moreover, this strategy is well considered for eventual practical and scalable CO<sub>2</sub> conversion.

To achieve this overall strategy, the CO<sub>2</sub> Utilization Technology Area funds projects in three broad topic areas: (1) the nonbiological electrochemical reduction of CO<sub>2</sub> to C1 intermediates, (2) the biological upgrading of CO<sub>2</sub>-derived intermediates to valuable products, and (3) developing TEA to inform research goals, product targets, and scale-up. These three funding areas are appropriate for the desired goals, and the technology area appropriately funds a diverse group of projects in each of these three funding areas.

Regarding the first funding area, electrochemical CO<sub>2</sub> reduction is a comparatively mature field of research; however, the overall goal of coupling an electrochemical process to a downstream biological upgrading process leads to technological challenges unique to the CO<sub>2</sub> Utilization Technology Area. It is recommended to focus the efforts of the electrochemical CO<sub>2</sub> reduction work to (1) scaling electrolyzers for the necessary output rate and product concentration for biological upgrading; (2) managing contaminants from likely input CO<sub>2</sub> streams, both in terms of improving electrolyzer durability and measuring the propagation of contaminants into output streams; and (3) developing technologies to integrate the electrolyzer output streams with biological systems.

Regarding the second funding area, this is an important strategic component of the technology area. Given the required fundamental science still needed in this area, it may be beneficial to refocus efforts in this area first on meeting reasonable performance metrics and deemphasize TEA. TEA is likely not useful for such systems until they can convert C1 inputs into the desired products at fast production rates and high titer.

The third funding area is crucial to the success of the program because it can help distinguish between the viability of emerging technologies and provide necessary performance metrics for economic viability. This area might be expanded to include efforts on developing standard diagnostic tools and operating procedures for the funded projects as informed by the needs for integration and through conversations with external stakeholders.

The funded projects are mostly tied to the strategic direction of the technology area, and most are on the cutting edge of the work in their fields. The implementation of these projects could be improved by improving communication among the projects. For instance, communication between the electrochemical conversion and bioconversion projects regarding the composition and concentration of the electrolyzer output streams and necessary bioreactor inputs may help better define performance targets and facilitate future integration of these technologies. In addition, each project should be strongly encouraged to communicate with the projects focused on feasibility assessment.

## RECOMMENDATIONS

### **Recommendation 1: The Review Panel recommends that the program begin to focus on specific technology pathways and support the scale-up of the most promising technologies.**

The program can do this by using the outcomes of this phase to determine which technologies have the greatest chance for success. It is recommended that projects be supported that have a realistic chance of scaling up either with higher current density or more industry-based reactors. For example, many proof-of-concept projects utilize continuous stirred-tank reactors (CSTRs), which are appropriate for this conceptual project but will not work at scale. The program needs to push researchers to think about how their processes will work at scale. Specifically, there needs to be more work on bioreactor design and/or operating conditions to achieve

high gas utilization and end-product productivity. One possible FOA could be supporting projects that have achieved a proof of concept in a CSTR and then supporting scaling them in an industrially relevant reactor design to a pilot stage.

**Recommendation 2: The Review Panel recommends that the program support a holistic and integrated scale-up approach that more fully combines the electrocatalytic and biocatalytic processes.**

The implementation of these projects could be improved by requiring communication among the projects. For instance, the bioconversion projects should communicate their needs for input streams (rate, purity, concentration) to the electrochemical CO<sub>2</sub> conversion projects to inform their production targets. Likewise, the electrochemical CO<sub>2</sub> conversion projects should inform the bioconversion projects concerning the likely makeup of their product outputs, including contaminants and electrolytes (for aqueous products such as methanol or formate). Finally, all projects should be communicating with those focused on feasibility assessment to help inform their target products and conversion metrics.

**Recommendation 3: The Review Panel recommends that the program support a greater number of competitive FOAs to balance the number of internal and external projects.**

The Review Panel understands that the program has already been doing this, but the most recently awarded FOA projects were just approved and did not have anything to present. This is particularly important because there are an increasingly greater number of startup companies and academic universities that have been showing significant progress on the scale-up of both electrocatalytic and biocatalytic processes.

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## CO<sub>2</sub> UTILIZATION PROGRAMMATIC RESPONSE

### INTRODUCTION

The Conversion R&D Program Area would like to extend our gratitude to the CO<sub>2</sub> Utilization Panel for their attentive participation and helpful discussion related to this emerging research area within BETO. Such input from experts in the field has been essential to developing this portfolio, and it would have never gotten to where it is now without such help. As the above summary points out, the Review Panel found the organization of this new program and the variety of projects within it to be commendable and aligned with BETO goals. The technology balance between biological and catalytic options was seen as well appropriate. Specifically, the Review Panel found that the TEA associated with the feasibility study to be especially important for the strategic development of this program specifically as well as to the broad field of CO<sub>2</sub> utilization.

The reviewers pointed out the synergistic opportunity for combining researchers who work on catalyst development with their counterparts who are engaged in process engineering and biological strain development. Such interdisciplinary collaboration was seen as key to developing robust conversion systems. Similarly, additional collaboration between those at the national laboratories and their university and industry counterparts could be leveraged to overcome major barriers in CO<sub>2</sub> utilization.

The Review Panel pointed toward opportunities in funding projects that integrate hybrid approaches to realize practical CO<sub>2</sub> conversion. This can allow catalysis researchers to specify their output stream for the proper engineering of the relevant bioreactors; however, reviewers stressed that direct integration of these technologies is less important at this stage than ensuring that each can achieve meaningful rates and scale. Other opportunities in addressing mass-transfer limitations, separations, and system integration were mentioned as items BETO should consider. The following section specifically addresses the three major recommendations from the Review Panel.



**Recommendation 1: The program should begin focusing on specific technology pathways and support the scale-up of the most promising technologies.**

The program agrees with the Review Panel on the importance of narrowing the scope of activities to be as effective as possible with the funding dedicated to CO<sub>2</sub> utilization. The portfolio has spent the last few years exploring a variety of different activities related to CO<sub>2</sub> conversion that cover many different aspects of CCUS. Moving forward, the program seeks to narrow its efforts to projects that focus on the core BETO strategy of simultaneously developing CO<sub>2</sub> reduction technologies and intermediate upgrading approaches while conducting the proper analyses needed to understand the important economic and sustainability issues in the field.

**Recommendation 2: The program should support a holistic and integrated scale-up approach that more fully combines electrocatalytic and biocatalytic processes and communicates with feasibility assessment efforts.**

The program agrees with the importance of ensuring that the efforts in this portfolio operate in a synergistic manner, given the importance of each effort in the BETO strategy. Given that both CO<sub>2</sub> reduction and the subsequent intermediate upgrading are closely linked, it is essential that each half speaks to the other regarding things such as rates, titers, and impurities; this helps avoid pitfalls while highlighting certain unexpected synergies that arise as each technology advances. Moving forward, BETO will ensure that projects that focus on complementary CO<sub>2</sub> reduction and intermediate upgrading strategies are actively communicating with one another. This will be established in the upcoming suite of projects.

**Recommendation 3: The program should seek to balance the number of internal and external projects.**

BETO agrees with this assessment and is actively attempting to balance the portfolio. As mentioned by the reviewers, several recently awarded projects did not get a chance to share their research because they have just begun and are not yet ready to present. From a numeric standpoint, BETO has more projects funded at the laboratories; however, they are smaller in scale, and their overall dedicated funding is significantly less than those awarded via competitive FOAs. BETO recently expanded its support of CO<sub>2</sub> utilization in competitively funded work by allowing it as a feedstock in several different topic areas in the FY 2021 FOA for the first time. This increase in external efforts will be matched by new national laboratory projects.

# FEASIBILITY STUDY OF UTILIZING ELECTRICITY TO PRODUCE INTERMEDIATES FROM CO<sub>2</sub> AND BIOMASS

## National Renewable Energy Laboratory

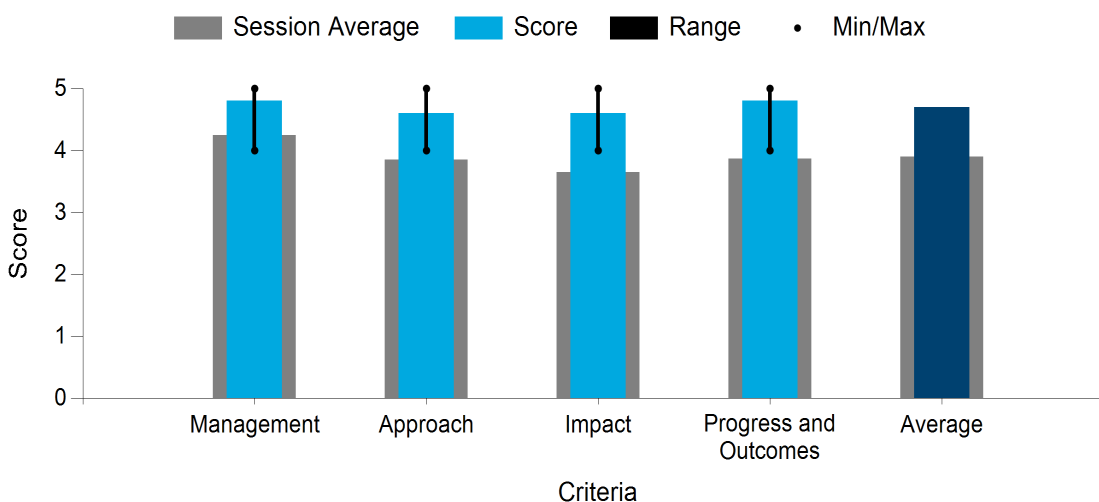
### PROJECT DESCRIPTION

Capturing and converting CO<sub>2</sub> generated from bioethanol facilities into valuable products, leveraging renewable electricity as the primary energy input, could increase overall biorefinery carbon utilization by as much as 40% and provide a means to decarbonize fuels and chemicals production; however, significant uncertainty exists around the costs, carbon intensity, risks, and technical challenges associated with electron-driven CO<sub>2</sub>

reduction. Thus, the overarching objective of this project is to guide existing and future R&D efforts by addressing these knowledge gaps for utilizing renewable electricity and CO<sub>2</sub> to improve biorefinery economics and carbon utilization. By September 2023, this project will develop and publish a comprehensive design report for the integration of CO<sub>2</sub> utilization into two existing conceptual biorefinery designs, which will include conceptual process models, pioneer and n<sup>th</sup>-plant economics, identification and quantification of technological risks, and projections for future cost reductions.

WBS:	2.1.0.304
Presenter(s):	Adam Bratis; Josh Schaidle; Zia Abdullah; Courtney Payne; Jessica Krupa
Project Start Date:	10/01/2020
Planned Project End Date:	09/30/2023
Total DOE Funding:	\$800,000

Average Score by Evaluation Criterion



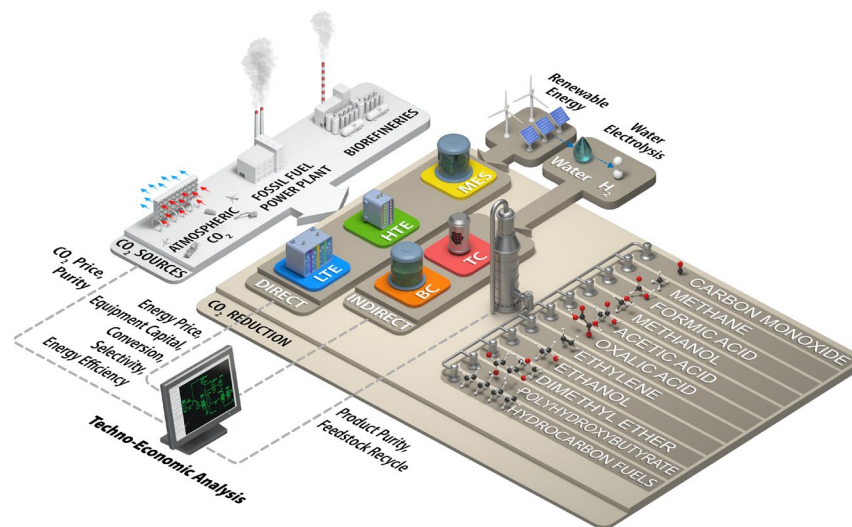


Photo courtesy of NREL

## COMMENTS

- This is a very good project. It is well delivered, and the impact in the field is high. This BETO demonstration project is possible, but it requires a much higher monetary investment. The Review Panel was particularly impressed with the partnership element regarding the Global CO<sub>2</sub> Initiative and Argonne National Laboratory
- The goal of this project is to assess the feasibility of current and emerging CO<sub>2</sub> reduction technologies. The project couples TEA feasibility, and it has an overall approach that focuses on high-level crosscutting evaluations of CO<sub>2</sub> reduction technologies followed by deep dives into specific pathways. A particular strength of the project approach and impact is the partnership with scientific stakeholders to ensure that the team's evaluations are relevant to emerging technologies. The performers have made significant progress, and the focus on providing actionable information to the CO<sub>2</sub> research community makes this an important and impactful project.
- The project is well conceived and effectively managed. The analyses have zeroed in on the critical factors for achieving the overall goal of guiding R&D.

**Strengths:** The project team has made a comprehensive effort to access the relevant information from disparate sources and apply rigorous analysis to achieve their goals. The work has provided a clear approach to understanding the effects of many interrelated parameters on the overall cost and carbon intensity of the technical options the team have selected. Consistent progress toward the project goals has been achieved throughout the effort.

**Weakness:** It would help to have a more direct comparison between the current state of the art for CO<sub>2</sub> production and the metrics needed for economic/carbon intensity viability—in simple terms, what are the target current density, voltage, cost per m<sup>2</sup>, and durability, and how far off are the best available systems? There is uncertainty here, and maybe multiple options are needed, but distilling it to the terms the community is most familiar with would be beneficial.

- The team looks to have a good management structure and is well connected with the larger community. Results are already being shared through publications and reports to BETO. The team's approach of first taking a broad overview of the different CO<sub>2</sub> conversion technologies (completed in the first three-year cycle), followed by narrowing the scope to focus on two conversion technologies is sound and makes sense. From the presentation, it was not clear what criteria will be used to narrow the scope of the design report to two conversion technologies, and a clear justification will be needed. The well-researched, broad overview of the CO<sub>2</sub> conversion technologies can aid the CO<sub>2</sub> Utilization program, as will the design report when completed. These can serve as guides to future research programs. Although narrowing the focus to two technologies makes sense, the team risks lessening the impact of their design work if neither of the two chosen technologies proves feasible or if a new breakthrough in one of the unchosen technologies makes it more feasible. This is a minor risk and one worth taking. The project met the first three-year cycle goal and looks to be on schedule to meet the new project goal.
- This project is important in terms of developing a strategic R&D plan for highly integrated CO<sub>2</sub> utilization processes. The team has made significant progress during the last year, and the data presented were quite insightful. Although the findings were interesting, the current approach is limited to biorefinery cases, and this project can have greater impacts if it considers a wider range of CO<sub>2</sub> feedstocks. Further discussions on the scales of reactor systems (e.g., modular versus large scale) as well as capacity factors would be useful for different processes that are currently being developed.

## PI RESPONSE TO REVIEWER COMMENTS

- We would like to express our appreciation to the reviewers for taking the time to participate in BETO's 2021 Peer Review and for their thoughtful analysis and constructive feedback on this project. Building on our progress to date, we will use the reviewers' feedback to guide our work in the coming years, especially in the areas of (1) considering a wider range of CO<sub>2</sub> feedstocks, (2) evaluating the impact of process scale and deployment configuration (e.g., modular versus centralized), (3) defining technical targets for existing technologies that would improve their commercial viability and communicating this information to the community, (4) balancing our focus on specific technology pathways with the flexibility to adjust as new breakthroughs happen, and (5) maintaining and enhancing our focus on providing actionable information to the CO<sub>2</sub> utilization community.



## ELECTROCATALYTIC CO<sub>2</sub> UTILIZATION

### National Renewable Energy Laboratory

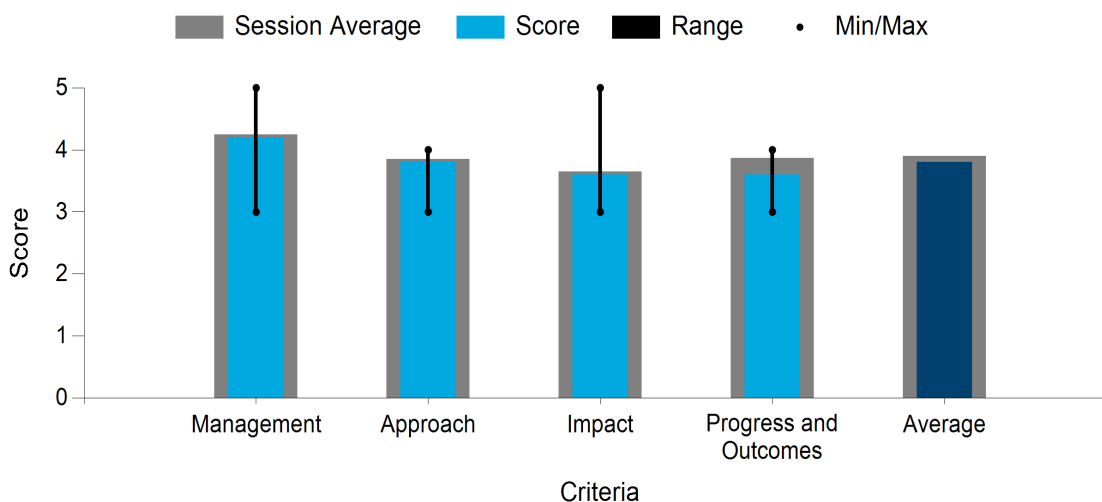
#### PROJECT DESCRIPTION

Existing biomass-conversion processes, such as fermentation to produce ethanol, produce very pure industrial sources of CO<sub>2</sub>. In fact, domestic ethanol biorefineries are the largest single-sector supplier of CO<sub>2</sub> to the merchant gas markets. This provides a unique opportunity for the bioenergy industry: to reduce the cost (minimum fuel selling price) of the primary product (ethanol) by valorization of this large

CO<sub>2</sub> waste stream. In this project, we are focused on studying different types of membrane electrode assemblies (MEAs) for low-temperature CO<sub>2</sub> electrolyzers. Specifically, MEAs using both proton exchange membrane (PEM) and anion exchange membranes (AEMs) are in scope. Although there has been significant recent interest in this field, challenges remain for both types of MEAs before this process can be performed industrially. The goal of this project is ultimately to enable long-term and stable operation at high current densities for low-temperature CO<sub>2</sub> electrolysis. During the past two years, we have developed a diagnostic test for membrane integrity in the assembled MEA, tested a new AEM material, and quantified hydrogen evolution kinetics in PEM MEAs. Moving forward, we plan to focus on the degradation of both catalysts and catalyst supports as well as the development of standardized methods for testing and product quantification. By addressing the persistent issues facing these technologies, we aim to enable to the economic electrochemical conversion of CO<sub>2</sub>.

WBS:	2.3.1.316
Presenter(s):	Adam Bratis; Jack Ferrell; Zia Abdullah
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2022
Total DOE Funding:	\$600,000

Average Score by Evaluation Criterion



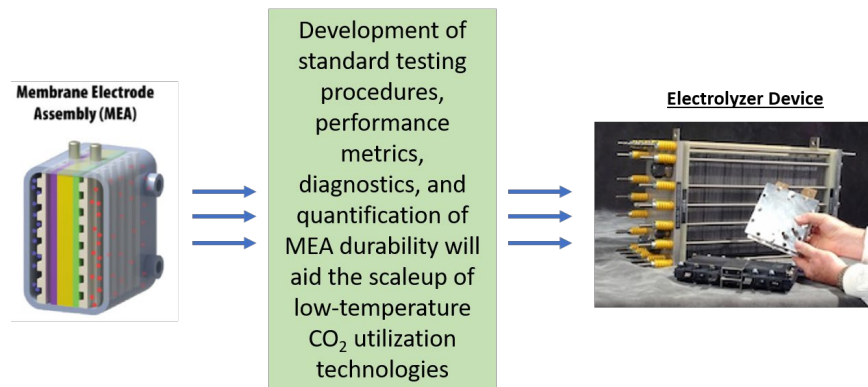


Photo courtesy of NREL

## COMMENTS

- The progress with respect to diagnostics has been impressive. It was useful to show the membrane being developed from other National Energy Technology Laboratory scientists and in-house intellectual property generation. The team has yet to do long-term tests beyond several weeks; currently, they do it at weeks, up to a month. Also, accelerated tests would be higher constant currents or higher cycling, but these may not be the most indicative of industrial conditions.
- It is not clear how this project is directly related to BETO's goals. The project does not need to work on the biologic systems or biocatalysts, but making connections to other BETO efforts would be important. The progress made in the *in situ* study of membrane integrity and Electrochemical Impedance Spectroscopy (EIS) provided valuable insights into CO<sub>2</sub> conversion and the durability of AEMs. It is not clear whether this project belongs to the BETO program.
- The goal of this project is to develop standardized testing protocols for electrochemical CO<sub>2</sub> conversion, with a specific focus on scalable MEA architectures. This project has significant potential to help define performance metrics, identify technical barriers to MEA scaling and implementation, and develop best practices for assessing MEA durability and degradation. All are crucial for practical electrolyzer development. The performers have established an interdisciplinary team with crosscutting expertise that fully leverages the capabilities and knowledge base of the Chemical Catalysis for Bioenergy Consortium (ChemCatBio). The performers have benchmarked the performance of some existing MEA designs to establish baseline performance metrics, and they have identified membrane durability and crossover as major technical challenges. The membrane integrity diagnostic developed by the performers is a very promising rapid screen that has significant potential to aid in membrane durability testing, but the current version of the diagnostic requires a specific MEA design. The impact of the project would be strengthened if this diagnostic could be modified such that it has less sensitivity to MEA design parameters. Moreover, the performers correctly identified accelerated durability testing as a crucial need in evaluating CO<sub>2</sub> MEA performance, and the project would be strengthened by the inclusion of a specific plan to move toward accelerated durability testing.
- The management plan looks strong, and the team is well connected within the ChemCatBio group to leverage expertise. The team has decided to focus on two different electrochemical catalysts and has a good team to further develop them. Although the development of these new catalysts and materials is important, there is not a clear pathway to implement them commercially, in particular to test the catalysts using a real-world feedstock. The new diagnostic tool could have significant impact to the industry, but

again, it is not clear how broadly applicable this tool is or its need in the industry. The team seems on track to meet the go/no-go decision for the project. Future milestones need more specific criteria to measure the project's progress.

- This project is designed to investigate the technical feasibility of CO<sub>2</sub> electrolysis in MEAs by (1) assessing the durability of currently available AEMs; (2) developing methods to evaluate AEMs and detect defects that compromise performance; and (3) comparing cation exchange membrane and AEM MEA cells to determine which is the best design for durability and performance.

**Strengths:** The development of systematic testing protocols to evaluate AEMs under CO<sub>2</sub> electrolysis conditions is a worthy goal because it could bring clarity to a question that is a source of uncertainty in modeling and analysis. Standardized testing could help advance the technology, much like analogous testing at NREL has advanced photovoltaic technology. The emphasis on cells larger than 1 cm<sup>2</sup> is a welcome advance over the vast majority of studies in the field. The EIS methodology is interesting and could be useful for rapidly investigating membrane integrity and/or predicting durability.

**Weaknesses:** The technical objectives of the project are modest relative to the reported state of the art in the field. Published results from Dioxide Materials in 2018 showed >3,000 of stable operation at 200 mA/cm<sup>2</sup> and 3 V for CO<sub>2</sub> to CO electrolysis with sustainion. The go/no-go target for this project defines "long-term" stability as 24 hours with <10-mV/h degradation. This is a very large disparity. The durability of sustainion needs to be confirmed by multiple laboratories and assessed on longer timescales, but this project does not appear to be positioned to accomplish that objective. Given >3,000 hours of stability, it is unclear if the EIS methodology developed in this project will allow for addressing stability over longer durations. It is difficult to assess the degree of technical progress on developing/studying MEA cells for long-term operation given the limited data presented and the disparity noted. The investigation of MEA cells' transporting proton is questionable because these cells will necessarily operate with very large voltages. For mechanistic reasons, the cathode requires a very negative voltage regardless of pH. Operating the anode in acid therefore necessitate a cell that operates at ~4 V or higher for a reasonable current density.

## PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their time and feedback. We agree that standardized testing protocols remain a need to enable CO<sub>2</sub> conversion, and we are excited to be able to participate in this critical research area. As noted in the presentation, initial work in this project has focused on understanding the baseline performance for both alkaline and PEM-based MEAs. We have built advanced MEA testing capabilities, and subsequently we demonstrated reproducible performance for these MEAs, applying multiple analytic techniques to close the carbon balance. Moving forward, this baseline will be used for comparison against new catalysts and/or MEA architectures. Additionally, stable performance of these MEAs has also been demonstrated for up to 100 hours. Additionally, the development of standard procedures for MEA fabrication, testing, and product analysis are also needed to enable this technology. We have developed a method to probe alkaline membrane integrity, and although the current version of this is specific to this MEA, ongoing work will aim to broaden the applicability of this membrane durability test. Also, we recently developed a standard analytic procedure based on 1H nuclear magnetic resonance to detect CO<sub>2</sub> conversion products in liquid solutions; additional standard analytic methods are planned in the coming years. In the coming years, we plan to quantify the rates of major degradation routes at play in these MEAs. This will be used for the development of an accelerated durability test, which remains a critical need for the community. We agree that this type of work is critical for defining performance metrics and identifying barriers to the scale-up and implementation of this technology. To address some specific questions, first, we believe this project is a good fit within BETO's portfolio because BETO recently developed a CO<sub>2</sub> Utilization program, and this project was one of the first efforts here. We agree that there is a large disparity between the long-term MEA testing data produced by Dioxide Materials (>3,000 hours) and the rest of the community, this project included; however,

although the Dioxide Materials long-term data are very promising, they need to be validated, and more detailed data are also desired. We have taken the approach of first understanding the system in detail at shorter times (up to 100 hours), and we have closed the carbon balance on this system, producing complete analysis of both anode and cathode streams. Moving forward, we plan to collect these detailed data in conjunction with longer-term durability tests, where the nature of the catalyst, catalyst support, and membrane will be quantified before and after testing. Notably, the Dioxide Materials long-term data did not provide any characterization of different components of the MEA before and after testing, and these data are needed to determine the most prevalent degradation pathways at play and to quantify degradation rates. So, although the Dioxide Materials long-term data are extremely promising, details on major degradation pathways and rates are needed prior to the development of an accelerated durability test. Finally, MEA testing on real-world feedstocks has not been in scope to date on this project; this work has been a part of another BETO project on CO<sub>2</sub> utilization (PI Michael Resch). We regularly collaborate with this project, and moving forward, we are poised to do impurity testing if needed, and we will compare the performance to that of our established baseline.



## HYBRID ELECTRO- AND THERMO-CATALYTIC UPGRADING OF CO<sub>2</sub> TO FUELS AND C<sub>2</sub>+ CHEMICALS

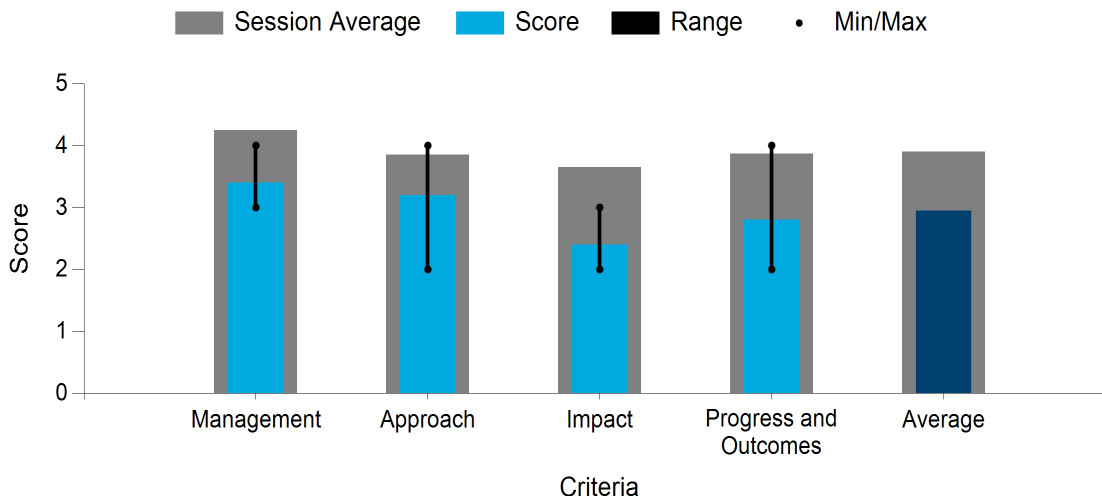
### Oak Ridge National Laboratory

#### PROJECT DESCRIPTION

Electrocatalytic reduction of CO<sub>2</sub> to useful products is a possible pathway toward higher rates of CO<sub>2</sub> recycling and lower rates of fossil carbon utilization. It is also a means of using electrical energy to convert or reduce (in the chemical sense) CO<sub>2</sub> to useful products. The project specifically targets developing an electrocatalytic synthesis approach for the reduction of CO<sub>2</sub> to C<sub>2</sub>+ oxygenates and advancing the state of technology for the electrochemical synthesis of C<sub>2</sub>+ molecules from CO<sub>2</sub> over new CNS-based electrocatalysts. In this approach, bimetallic co-catalysts are developed over CNS catalysts using electrochemical synthesis to tune the CO binding energy to enhance the CO oligomerization rates. Energy in the form of electricity removes oxygen from the carbon and replaces it with hydrogen or other elements, moving the carbon from a low-energy state (CO<sub>2</sub>) to a higher-energy state (alkane, oxygenates). This strategy will become critically important in the future as an alternative to fossil carbon sources and as renewable electricity becomes more available.

WBS:	2.3.1.317
Presenter(s):	Jim Parks; Tim Theiss; Zhenglong Li; Missy Miller
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2020
Total DOE Funding:	\$400,000

Average Score by Evaluation Criterion



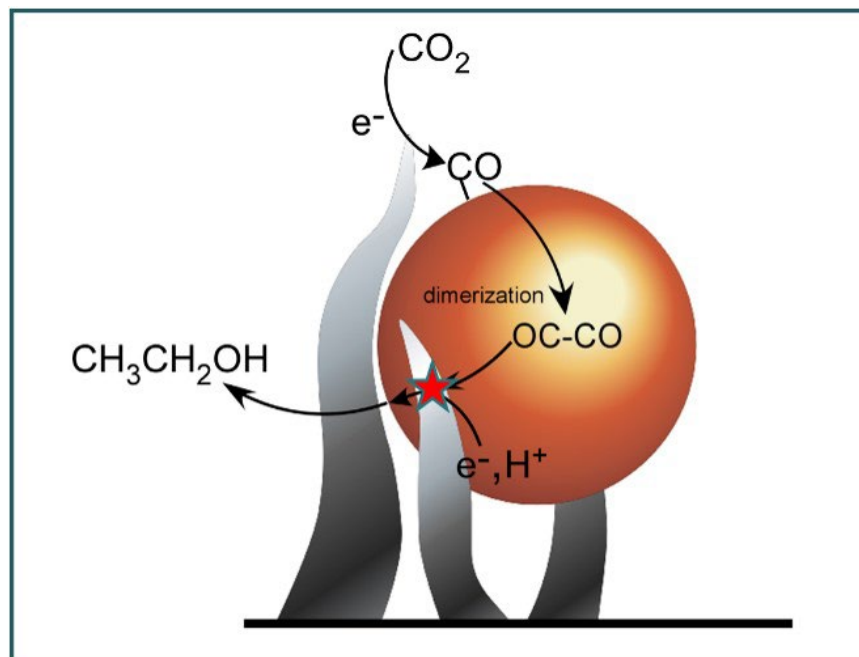


Photo courtesy of ORNL

## COMMENTS

- The project was negatively impacted by COVID, and the main researcher and project lead left the project. The current densities shown and performance seem to be quite low. The catalyst material development work shown has become oversaturated in the literature, and further development of this project is most likely not warranted.
- The goal of this project is to develop a new electrochemical approach for the reduction of  $\text{CO}_2$  to  $\text{C}_2$  products by incorporating copper (Cu)-based monometallic and bimetallic catalysts onto CNS. As part of the project's approach, the performers have developed a new synthesis strategy for generating bimetallic catalysts at the CNS tips. The performers' catalyst/nanospike system shows enhanced activity for  $\text{CO}_2$  reduction compared to the bare CNS, but comparisons to other known catalyst systems were not presented. Moreover, only gaseous products were quantified, but for the most promising systems, up to 80% of the charge may go to producing liquid products. The lack of activity comparisons to known systems and the lack of liquid product quantification make it difficult to assess the impact of the results and whether the project has achieved the project goals. This project would be substantially strengthened by the inclusion of a team member with expertise in liquid product identification and quantification.
- The project seems to be progressing quite well, and the use of the bimetallic catalyst with CNS is interesting; however, it was not clear why bimetallic catalysts would promote the production of  $\text{C}_2$  products. The fundamental basis of selecting these bimetallic catalysts should be explained in depth. Also, the uniqueness of this project was the design of CNS altering the electric fields near the catalysts. It would be great if this project focuses on the mechanistic studies of the catalyst placement in CNS and the role of bimetallic catalysts on  $\text{CO}_2$  reduction producing  $\text{C}_2$  products.
- The team is small, but it is not clear how closely the former PI (Adam Rondinone) interacts with the current PI (Zhenglong Li) and team. Also, connections to the broader community seem to be lacking.

The approach to improve the catalyst system seems to have achieved the goal of creating C<sub>2</sub> molecules, though verification has been delayed because of COVID. There is no clear plan to advance the technology to commercialization, mainly because this is a proof-of-principle project. Although this basic science work is important and can yield industrially relevant technologies (as evidenced by the licensing of the technology to produce C<sub>2</sub> molecules), this project seems out of place given BETO's current portfolio goals. The goal of producing C<sub>2</sub> molecules appears to have been achieved, though final verification has been delayed because of COVID. It is not clear what the next steps of the project are, if any.

- This seed project is investigating electrocatalysts comprising bimetallic particles embedded in CNS for the electrochemical reduction of CO<sub>2</sub> to C<sub>2</sub> products. The target is to improve the Faradaic efficiency (FE) for C<sub>2</sub> products from 46%, which was achieved in a 2016 publication by the ORNL group, to 58%. The strategy is to create bimetallic metal particles embedded in the nanospikes by alloying Cu with palladium (Pd). The project is an early-stage catalyst research project that is not well aligned with the larger BETO CO<sub>2</sub> Utilization program portfolio. The target metrics are not sufficient to move the state of the art in the field, and there has not been significant progress to meet these targets.

Strengths: The nanospike architecture is unusual and relatively unexplored for electrocatalysis.

Weaknesses: The target metric of 58% FE for C<sub>2</sub> products at -1.1 V versus reversible hydrogen electrode (RHE) does not include current density and therefore is of limited value for assessing progress. A meaningful metric that can be used to compare to the state of the art should include current density, voltage, and selectivity (FE). The current densities demonstrated so far for nanospike catalysts are far too low to determine whether they are promising for CO<sub>2</sub> reduction. Current densities in this project have ranged from microamps to a few milliamps, which makes it very difficult to assess the viability of the approach. Recent reports have shown up to 75% FE for C<sub>2</sub> (ethanol and ethylene) at >100 mA/cm<sup>2</sup> at -0.8 V versus RHE (<https://doi.org/10.1038/s41565-021-00974-5>). The results shown for the nanospike catalysts as well as the target metrics for the project are far behind this benchmark. There is no indication that alloying Cu with Pd improves C<sub>2</sub> synthesis.

## PI RESPONSE TO REVIEWER COMMENTS

- We appreciate these great comments. The original motivation of this project is to design bimetallic catalysts to tune the surface \*CO binding so that the coupling products' formation can be promoted, which is the major focus of this small seed project. The laboratory shutdown due to COVID and personnel changes have significantly limited the progress and efforts. When additional resources are available, we hope to further advance this CNS-based electrocatalyst in terms of current density and Faradaic efficiency and benchmark it with other known catalyst systems.

## WASTE CARBON GAS UPGRADING VIA ACETOGENS

### National Renewable Energy Laboratory

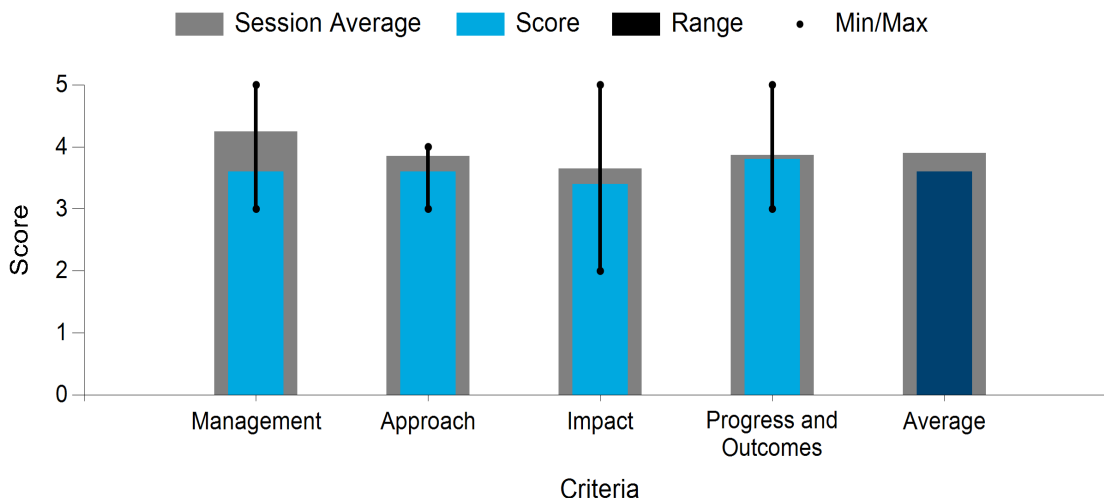
#### PROJECT DESCRIPTION

Waste carbon gas represents a large and diverse set of feedstocks that could be captured and turned into useful products. This includes waste gas emitted from industrial activity, syngas from burned plant biomass or processed municipal waste, and the electrochemical reduction of CO<sub>2</sub>. Currently, carbon gas is being microbially converted to ethanol as a main product; however, ethanol is a lower-value product with a limited market size. Although these microbes can make other higher-value products, there are no commercial processes for generating these other products, leaving a gap in understanding potential implementation for commercialization.

WBS:	2.3.2.106
Presenter(s):	Adam Bratis; Jonathan Lo; Zia Abdullah; Courtney Payne; Jessica Krupa
Project Start Date:	10/01/2017
Planned Project End Date:	09/30/2023
Total DOE Funding:	\$1,950,000

Expanding the products microbially produced from waste carbon gas requires several steps before commercial implementation. We are studying the acetogen *Clostridium ljungdahlii* as a biocatalyst to convert waste carbon gas to the chemical 3-hydroxybutyrate (3HB), a plastic monomer and fuel precursor. For that, we are studying and engineering microbial characteristics for novel 3HB product formation from waste gas streams. This includes metabolic characterization, genetic engineering, gas fermentation scaling, as well as TEA and LCA.

Average Score by Evaluation Criterion





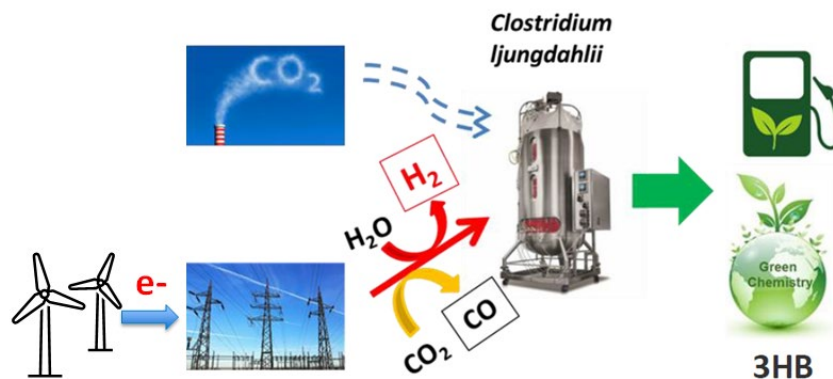


Photo courtesy of NREL

## COMMENTS

- The goal of this project is to develop genetically engineered microbes to convert CO<sub>2</sub> and CO gas streams into C<sub>2</sub> products, specifically 3HB. The performers envision an overall process where CO<sub>2</sub>-rich industrial waste gas is first electrochemically reduced to CO<sub>2</sub>/CO/hydrogen (H<sub>2</sub>) gas mixtures, which are then fed into a bioreactor for conversion to C<sub>2</sub>–C<sub>4</sub> products. Microbe engineering is well underway, and the performers have already developed organisms with enhanced CO<sub>2</sub> conversion efficiency. The performers have also developed a 2-L bioreactor for mixed-gas inputs, and they are on track for scale-up to the target 5-L reactor size. The project approach would be strengthened by increased consideration of the impact of flue gas contaminants on the microbial conversion efficiency. In the performers' envisioned overall process, such contaminants may still be present in the electrolysis output stream, and thus they may be introduced into the bioreactors. A particular strength of this proposal is that the TEA is fully integrated into the research approach, and it has already been used to determine minimum productivity targets for economic viability.
- The team seems capable of performing the project, but it is not clear how frequently the team meets nor how progress is monitored and technical issues are resolved. Although mention was made of Royal Dutch Shell, it is not clear what specific role they play or what interactions with the broader community the team has. Metabolic engineering of acetogens to produce products from syngas is not novel but should accomplish the team's goals. Additionally, no new genetic engineering tools are being developed, though demonstration of the attB integrase in *C. ljungdahlii* could be new. The major weakness is the reliance on metabolic engineering to achieve the project's goals (5 g/L of 3HB in a 5-L system). The fermentation system is discussed, but it does not seem to be a major focus. Few concrete strategies are given on how to progress from 18 mg/L at 100 mL to 5 g/L at 5 L. Also, the current fermentation system discussed is only 2 L, and no mention is made of a 5-L system. The overall impact of this work seems to be nominal. As mentioned, genetic engineering of *C. ljungdahlii* has been accomplished by several groups, and syngas fermentation is a well-known technology. It is not clear what new approach this project brings forward. The proof-of-concept strain has been constructed and scaled to at least 100 mL. Further specific milestone metrics and dates are not given, so it is difficult to assess how well the project will meet the specific outcomes.
- This project aims to make advances in acetogenic gas fermentation to enable the production of products beyond acetate and ethanol. The technical objective of this project is to develop a laboratory-scale acetogenic gas fermentation process that produces 5 g/L of 3HB. The scope of work includes

bioengineering of *C. ljungdahlii*, bioreactor design/optimization, and TEA. As such, the effort is diffuse, and it is unclear how the project will be effectively managed across multiple disparate activities.

**Strengths:** The ability to produce 3HB from acetogenic fermentation could provide a competitive alternative to several other technologies for making 3HB/polyhydroxybutyrate (PHB) that use either sugar or methane feedstocks. It is still quite early in this project, but from the preliminary results with the deletion mutant, there appears to be some traction in engineering the organism to improve 3HB titers. The initial TEA has identified key factors for cost-competitive 3HB production, especially increasing the productivity (g/L/h).

**Weaknesses:** Despite the improvement from 150 mg/L to 345 mg/L, the titer is still more than an order of magnitude off the target. There is not a clear plan as to how this gap will be closed. The TEA is necessarily very speculative at this point. Although there is value in identifying the 3HB productivity target, further TEA at this point seems premature given the very early stage of the 3HB process. Moreover, the CO<sub>2</sub>/H<sub>2</sub> and CO feedstock options are shown to give the same selling price, but this top-line result is not particularly meaningful because there are fundamental issues with each. For CO<sub>2</sub>/H<sub>2</sub>, it is mentioned that the productivity is low. It would help to clarify whether there is any precedent for C<sub>4</sub> productivity at the level used in the TEA. Similarly, it would help to clarify the assumptions around the CO<sub>2</sub> electrolyzer used in the case where CO is the fermentation feedstock.

- This project is very ambitious considering the budget size. There are many parts and collaborations that cover a wide range of topics. Although the data being collected by each subgroup is interesting, the overall project seems to be less focused. The interplay between the TEA and other parts of the project (e.g., strain stability, cocultures) should be further strengthened.
- This project is well managed, and progress toward the goals of the project were clear. The project approach involved developing organisms to develop ethanol instead of acetate using CRISPR-Cas9 engineering, and interesting strains have begun to be disseminated. There remains lots of room to understand the organisms with respect to the response to different syngas mixtures. A gap that needs to be addressed is process engineering.

## PI RESPONSE TO REVIEWER COMMENTS

- Syngas conversion via acetogenic metabolism is beginning to be commercialized; however, these organisms mainly generate lower-value C<sub>2</sub> chemicals, mainly ethanol. Ethanol has a well-understood industrial microbial conversion history, such as separations and product markets. These organisms are normally wild type and have not been scaled; thus, there is a knowledge gap surrounding scaling engineered organisms with regard to performance and strain engineering. Producing nonnative products is likely to have different stresses on the metabolism of these organisms, and there may be selective forces when scaling these microbes that prevent good heterologous product formation. Additionally, many microbial engineering efforts so far have focused on replicating plasmids and have minimal engineering of the native organism itself. Little is known about the performance of engineered strains, especially in scaled systems. Although the TEA is speculative, it is useful for identifying knowledge gaps and areas of research that may have been overlooked or underemphasized. The CO<sub>2</sub> electrolyzer assumptions are used from the NREL team, which has both a state of technology and a “future assumption” metric where they hope CO<sub>2</sub> electrolyzer technology to be in the future. We do have collaborations with those performing CO<sub>2</sub> electrolysis (Royal Dutch Shell and Princeton are part of a collaboration with us doing the electrochemistry) and microbial conversion beyond our model syngas mixtures, so we can test real feedstock streams from electrochemical sources. We have already combined engineering the native pathways of the organisms to reduce C<sub>2</sub> products and increase the 3HB as a product. Genetic engineering tool development in our project has multiple aims beyond heterologous product formation, including testing strain stability in larger systems as well as dealing with syngas mixtures and understanding product profiles as a result of the convergence of biological and industrial

engineering efforts. We are planning on scaling engineered strains to our 2-L system first. The 5-L system is still in development because the gas fermentation scaling system is an NREL center initiative shared across biological conversion platforms. We are simultaneously engineering our strains at a smaller scale to identify biological engineering targets while working at larger scales to see if process improvements can help with some experimental limitations when working with smaller volumes.

## IMPROVING FORMATE UPGRADING BY CUPRIAVIDUS NECATOR

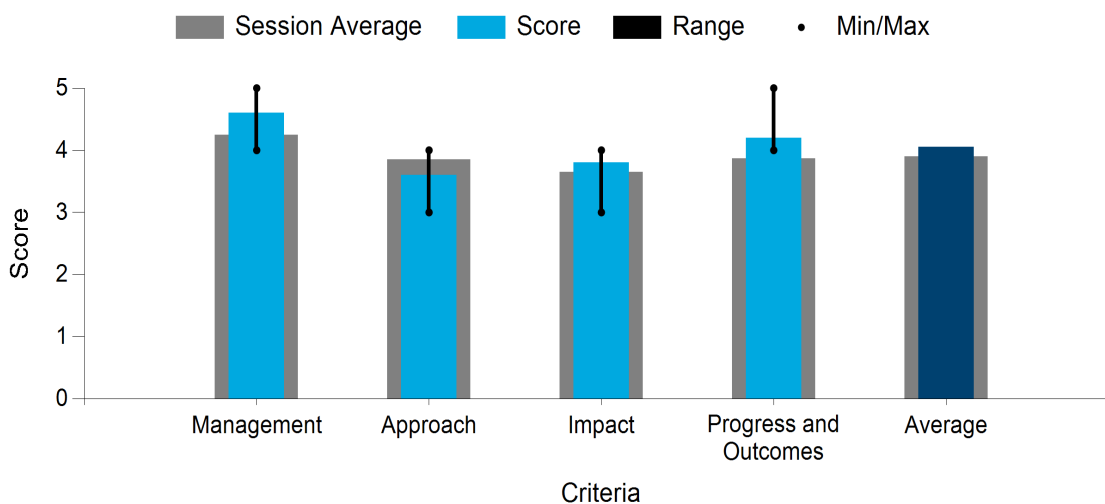
### National Renewable Energy Laboratory

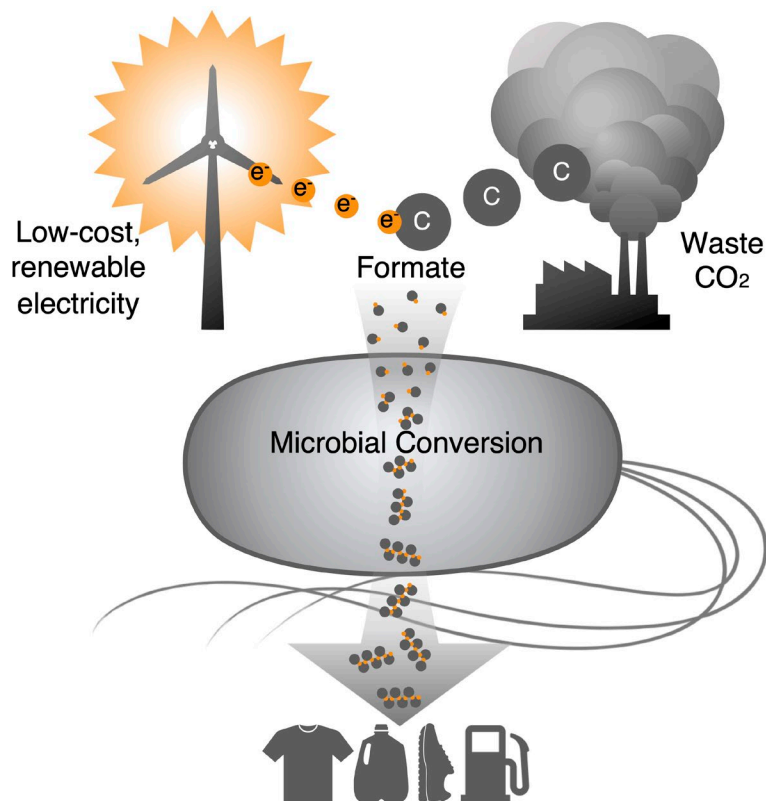
#### PROJECT DESCRIPTION

Formate can be generated by electrocatalytic reduction of CO<sub>2</sub> and has been proposed as a soluble intermediate for the storage of carbon and energy. Biological systems capable of assimilating formate could enable the conversion of formate generated from low-cost renewable energy and waste CO<sub>2</sub> to myriad fuels and chemicals. To that end, the goal of this project is to develop the natural formatotroph *C. necator* as a robust microbial chassis for the efficient conversion of formate to value-added products. Using a combination of laboratory evolution and rational engineering, we have identified mutations and genetic deletions that improve its growth rate and biomass yield on formate by 1.2 times. Using ribonucleic acid-sequencing (RNA-seq) transcriptomics, we are elucidating the mechanisms that underly these improvements, and we anticipate that this will contribute to our understanding of formate conversion. We have also engineered *C. necator* to convert formate to the potential polymer precursor 2HMS by introducing an exogenous pathway and deleting competing pathways, and we have demonstrated production and robust growth using a pH-stat bioreactor. Final TEA and LCA on the biological production of 2HMS from formate will enable DOE and other stakeholders to evaluate the economic and environmental impacts of this project and related technologies.

WBS:	2.3.2.111
Presenter(s):	Adam Bratis; Christopher Johnson; Michelle Nolker; Zia Abdullah; Courtney Payne
Project Start Date:	10/01/2019
Planned Project End Date:	09/30/2021
Total DOE Funding:	\$900,000

#### Average Score by Evaluation Criterion





*Photo courtesy of NREL*

## COMMENTS

- Formate is an interesting alternative to CO as a feedstock for the fermentative production of chemicals and fuels. This project is largely a strain engineering effort to improve formate assimilation and demonstrate a proof-of-concept titer (2 g/L) for the production of 2HMS.

**Strengths:** The project is well managed. There was a clear plan to evaluate the ability to evolve/engineer improvements in both the native pathway and a synthetic pathway, and the team succeeded in achieving the desired improvement (1.2 times) with the native pathway strategy. The combination of genomic analysis and rational engineering has yielded insights into the metabolic reasons for the improved growth and biomass yield, providing valuable knowledge for the field.

**Weaknesses:** The choice of 2HMS as a target was not adequately justified. Given that there is no existing market for 2HMS, it would help to have some description of the target applications or the products that 2HMS could substitute for to evaluate the importance of engineering toward this target. The engineered strains so far have yielded 76 mg/L, which is quite far from the target 2 g/L, and there are only six months left in the project.

- The management plan is clear, as is coordination with other C1 utilization groups. Starting with a natural formate-utilizing microbe is smart, as was the decision to drop the synthetic pathway approach. The selection of the 2HMS product may not be the most appropriate product, but it is sufficient for this proof-of-principle project. The improvement in formate utilization is impressive, and the knowledge gained from the genome sequencing could yield important scientific breakthroughs. Implementation of

the technology is dependent on an economic approach to generate formate from CO<sub>2</sub> (though this is purposefully beyond the scope of the project). The team looks to be on track to meet all project outcomes.

- This project focuses on engineering microbes for upgrading formate to 2HMS, a polymer precursor. The project approach originally focused on engineering microbes for formate conversion along two biological pathways: a native route via the Calvin-Benson-Bassham cycle and a synthetic route via the formolase pathway. An early no-go decision was triggered for the synthetic route when integration of the formolase pathway into the microbe resulted in no enhancement in formate consumption, and this led to a redirection of effort toward improving the native route. This redirection of effort had a beneficial effect on the project's progress, and the performers demonstrated increased activity for formate conversion along the native route for evolved and engineered microbes. The performers demonstrated a 0.5-L bioreactor for small-scale 2HMS production, but they did not indicate an approach to scale this to achieve their milestone of 2 g/L.
- This project is developing biotechnologies to upgrade the C1-liquid intermediate product formate into a more value-added high-performance product. Despite initial setbacks with evolved isolates, engineered strains showed an improvement of 1.2 times; however, the size of the reactor was relatively small, and this initial project shows that there is room for improvement. A question remains whether a formate feedstock is appropriate or would have the highest impact because of its relatively small market.
- This project showed that 2HMS can be produced from formate, and it provided insights into how the formation conversion can be improved. Although the study was thorough and insightful, it lacks the investigation of potential complexities of such biological systems. The effects of impurities and feedstocks with different C1 compounds would be a good addition for future studies.

## PI RESPONSE TO REVIEWER COMMENTS

We thank the reviewers for their careful review of this project and thoughtful comments and suggestions. The overall goal of this project is to develop the natural formatotroph *C. necator* as a robust microbial host for conversion of formate to value-added products, using 2HMS as an exemplary product. We believe the greatest contribution this project can make is in developing an improved base strain for the conversion of formate, so that has been our primary focus. As noted in our presentation, however, 2HMS has functional groups that lend it utility as a polymer precursor. Its production pathway is also closely related to other promising polymer precursors, including muconic acid and beta-ketoadipic acid, both of which can be used to form bio-based nylon and could be pivoted to relatively easily with additional strain engineering. As the reviewers noted, titers need to be improved considerably to meet our goal of producing 2-g/L 2HMS from formate. We should note, however, that the bioreactor cultivation included in our presentation was stopped while the strain was still growing and producing 2HMS, so we expect that additional bioprocess development will enable higher titers. Further, that demonstration was performed using our "first-generation" strain engineered to produce 2HMS. Additional engineering to delete competing pathways has since resulted in a >350% improvement in 2HMS titers in small-scale plate reader experiments, which we expect will translate to higher titers in future bioreactor cultivations. With respect to addressing the complexity of biological systems, as noted in our presentation, we have performed transcriptomics to understand the mechanisms by which formate conversion was improved in our evolved strains. We are still analyzing the results, and we anticipate that an in-depth examination of specific targets identified in these experiments could be the focus of future studies aimed at better understanding formate conversion. We agree that future studies should also be aimed at enabling the conversion of actual CO<sub>2</sub>-derived formate, which is likely to contain impurities.



## ENHANCING ACETOGEN FORMATE UTILIZATION TO VALUE-ADDED PRODUCTS

### National Renewable Energy Laboratory

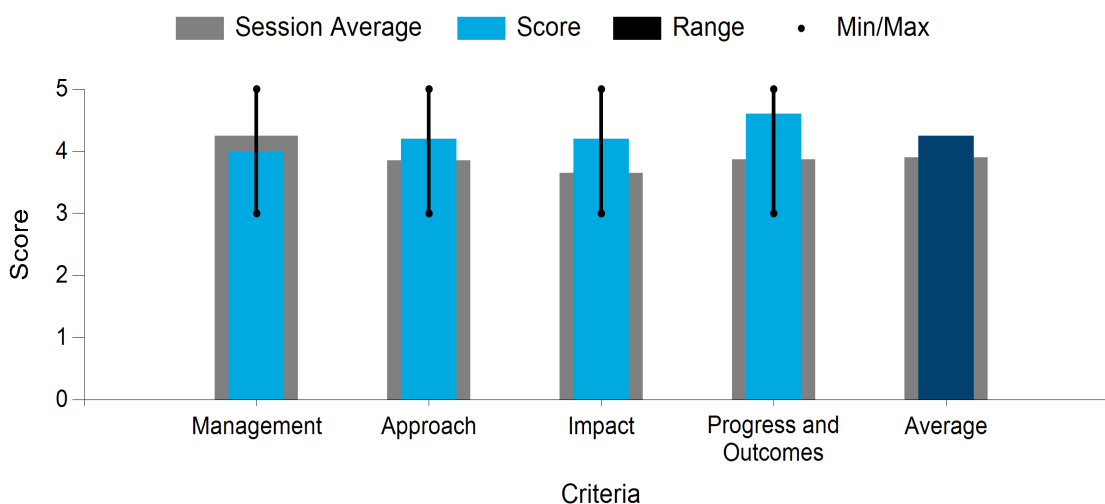
#### PROJECT DESCRIPTION

Electricity from diverse sources is increasingly utilized due to its low cost. Low-cost electricity can be used to chemically convert CO<sub>2</sub> to the liquid C1 compounds formate and methanol. Methanol can also be generated from biogas, or it can be thermochemically produced from H<sub>2</sub> and CO<sub>2</sub>. These feedstocks can then be microbially upgraded to useful chemicals. Formate and methanol are feedstocks for a variety of bacteria and have several advantages to gaseous electrochemical products, including ease of storage and miscibility in liquid. We decided to focus on the acetogen *Butyribacterium methylotrophicum* because of several advantages: it can already utilize liquid C1s; it can natively make the C4 compounds butanol/butyrate; and it has the Wood-Ljungdahl pathway, the most efficient natural carbon fixation pathway.

WBS:	2.3.2.112
Presenter(s):	Adam Bratis; Jonathan Lo; Zia Abdullah; Courtney Payne; Jessica Krupa
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	\$900,000

For this project, as a proof of concept, we are focusing on microbially converting liquid C1s to the C4 products butanol/butyrate. This task primarily relies on two parts: characterizing/improving C1 utilization and improving C4 production. First, we characterized the conditions of C1 fermentation. Then we looked at strategies for improving C4 production formation. In the process, we also developed genetic tools to manipulate the metabolism of these organisms. We have begun consolidating the data generated into a TEA and LCA to understand how an overall process might work.

Average Score by Evaluation Criterion



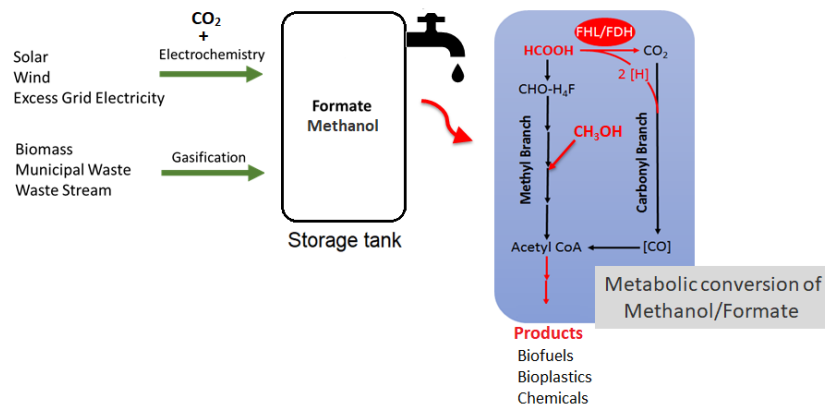


Photo courtesy of NREL

## COMMENTS

- Considering both formate and methanol as C1 compounds is great for the flexibility of the developed technology. The study was systematic and insightful, providing a proof of concept. It is not clear whether the TEA of the process at this stage of the development is meaningful, but the team is considering all the potential challenges.
- Strengths: The project has exceeded the target 2-g/L production of a C4 by reaching 3.6-g/L butyrate with 50% carbon efficiency. The project has also established some basic tools for engineering *B. methylotrophicum*. The engagement with industry is helpful for scoping the best paths forward with liquid C1 feedstocks.

Weaknesses: The assumptions for CO<sub>2</sub> reduction to formate used for TEA are not justified. The cell voltage required to obtain even 100 mA/cm<sup>2</sup> is >3 V, and most cells need >4 V. There are major stability issues. No CO<sub>2</sub> electrolyzer has ever come close to the 300 mA/cm<sup>2</sup> at 2 V used in the TEA. It would help to see more details about the key milestone result (3.6-g/L butyrate, 50% carbon efficiency). It is difficult to reconcile the carbon efficiency with the data on slides 15 and 16, which show rather low selectivity for butyrate. It would also help to see the productivity (g/L/h). The metabolic engineering efforts seemed to yield relatively modest improvement over wild type (slide 16).

Suggestion: It would be interesting to look at a tandem fermentation process with formate to C2 (acetate) with one organism combined with acetate to C4/C6 with another. This is similar to what Siemens/Covestro are doing with the Reticus project.

- The project management structure is not clear, including how often the team meets, how progress is checked, and how issues are resolved. The use of a liquid C1 substrate to overcome mass-transfer limitations with gas makes good sense, as does the selection of a native formate and methanol utilizing microbe. It was unclear how the genetic tools will be used beyond introducing an alcohol dehydrogenase gene. Further development and understanding of the microbe host (*B. methylotrophicum*) could assist the broader community. Implementation of the technology is dependent on an economic approach to generate formate or methanol from CO<sub>2</sub> (though this is purposefully beyond the scope of the project). It seems the main project goal of making 2 g/L of C4 compound from C1 feedstocks has been achieved, but beyond this, no further metrics or progress are given. A more detailed accounting of the projects accomplishments is needed.

- This project developed acetogens as a platform for liquid C1 conversion and was able to demonstrate 2 g/L of a C4 compound. The project found that there is unique synergistic conversion when both formate and methanol mixed. This project demonstrated unique conversion utilizing C1 liquid intermediates, which could be easier to handle and require less separation than gas fermentation. My overall assessment of this project is very positive.
- This project focuses on engineering microbes for upgrading formate to C4 products. The performers made substantial progress and demonstrated a test-tube-scale reactor for conversion of formate to butanol/butyrate at 3.6 g/L titer, exceeding their end-of-project milestone of 2 g/L titer. Their approach incorporates a TEA that suggests methanol as an alternative, more cost-effective feedstock, and the performers tested mixed formate/methanol feedstocks for conversion to C4 products. The future work plans of scaling TEA/LCA to larger reactor sizes and using C1 feedstocks from CO<sub>2</sub> electrolysis will further increase the projects impact.

## PI RESPONSE TO REVIEWER COMMENTS

- Liquid C1 utilization by native formatotrophs is a novel, developing concept that is really in the nascent phases. Although acetogenic conversion of syngas is reaching industrial scale, little is known about how a C1 liquid acetogenic commercial process would function. Demonstrating a proof of concept is the first step to realizing commercial potential, and capturing production metrics are important for identifying further areas of important research. By the end of the project, we are hoping to demonstrate C1 liquid conversion in a fermenter with productivity metrics, which will then feed into analysis done by NREL's team to generate TEA and LCA. Electrochemistry integration, TEA, and LCA are moving targets. As we develop the biological process, research is continuing for the CO<sub>2</sub> electrolyzer, prices are changing, and the increasing value of CO<sub>2</sub> capture and utilization needs to be measured/estimated. The metrics used for the CO<sub>2</sub> electrolysis is a best guess of where the technology will end up, so these estimations are to be taken with a grain of salt because they are somewhat future predictive. Also, we are hopefully going to have an impact on the research directives of those developing CO<sub>2</sub> electrolysis. As we have shown, a mix of liquid C1 compounds is actually advantageous for our microbial platform, which was an unexpected outcome of our research. Many electrolysis systems are designed to pursue a single product from CO<sub>2</sub> reduction, but acetogenic systems can utilize various reduced C1s simultaneously; thus, single C1 products from electrolysis become less important. Integration with CO<sub>2</sub> electrolysis systems may become more important in the future to understand how biology and electrochemistry can best work together. Because these are new organisms, understanding these organisms and developing genetic tools is important, and there are many potential implementations of these organisms. As cited in the comments, a tandem/combined fermentation process has been explored in C1 syngas conversion to C4/C6, which could be a potential implementation of these liquid C1 conversion systems. A key endeavor of this project is to disseminate information about the potential of these organisms and the promise they hold. Syngas conversion has already reached the commercial stage, but it will probably always be hampered by the fundamental gas/liquid mass-transfer problems. This research can alleviate those fundamental problems, either by co-consumption of gases and liquids or by replacement by C1 liquids only.

## SYNTHETIC C1 CONDENSATION CYCLE FOR FORMATE-MEDIATED ELECTROSYNTHESIS

### National Renewable Energy Laboratory

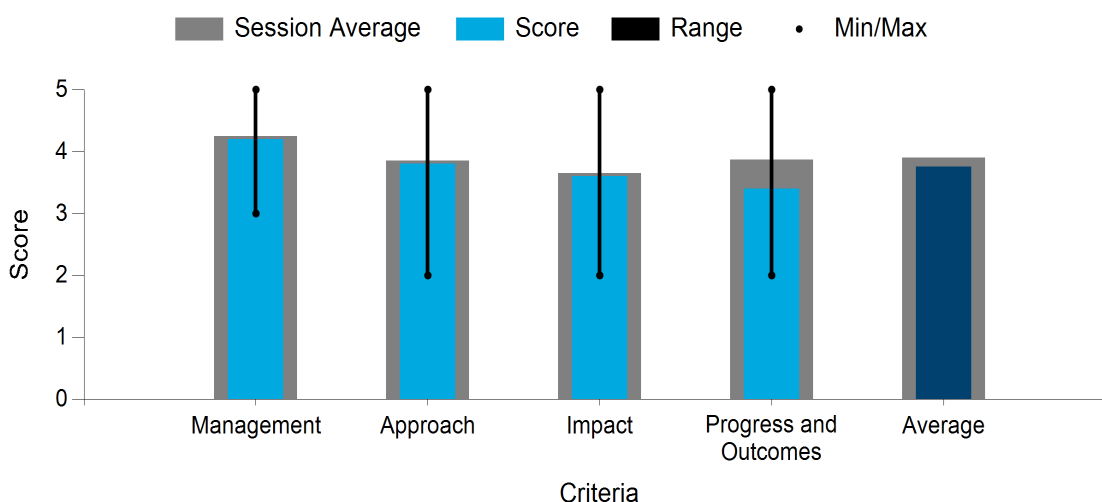
#### PROJECT DESCRIPTION

Formate-mediated electrosynthesis aims to create a novel pathway for CO<sub>2</sub> conversion and biosynthesis. It exploits the best of electrochemistry and biology via an ideal metabolic intermediate—formate, which can be electrochemically produced from CO<sub>2</sub> and biologically upgraded to high-value products. In this project, the NREL team, in collaboration with Lawrence Berkeley National Laboratory, designed and engineered a synthetic formate-utilization

pathway in *E. coli* by utilizing systems-level and high-throughput synthetic biology approaches. *In silico* pathway analysis and experimental synthetic biology work were accomplished comprehensively. Computational analysis indicated thermodynamic feasibility and production potential of the synthetic C1 Condensation Cycle as well as quantitative enzyme requirement in catalytic kinetics. The TEA further identified potential cost drivers in this technology. The synthetic pathway was successfully engineered in *E. coli*, with competing pathways knocked out and supporting genes expressed. The best strains grow mixotrophically on formate and other renewable feedstocks with endowed ability to produce glycolate. The titer of glycolate from mixotrophic *E. coli* cells reached ~4 g/L in shaking flasks. Based on <sup>13</sup>C-tracer analysis, ~15% of glycolate is synthesized from formate and/or CO<sub>2</sub>. The NREL team is performing deeper genome engineering, laboratory evolution, and bioreactor optimization to further improve formate-utilization efficiency and glycolate productivity.

WBS:	2.3.2.113
Presenter(s):	Adam Bratis; Wei Xiong; Zia Abdullah; Courtney Payne; Jessica Krupa
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	\$900,000

Average Score by Evaluation Criterion



## COMMENTS

- The team management plan is clear. The interactions, if any, with the other C1 utilization groups were not clear. The engineering of a novel formate-utilization pathway into *E. coli* is scientifically interesting, but the benefit is not clear, beyond being novel. There are many native formate-utilizing microbes that already use different pathways, and it is not clear that the same amount of effort could not be used to make one of these more genetically tractable. Even if a formate-utilizing strain is constructed, the team will then need to optimize a production pathway, similar to what would be needed in a native utilizer. Although the science is very interesting, this project seems out of place in the BETO portfolio. It is unclear what the major impact of the development of a novel formate-utilization pathway will be because, as presented, the pathway does not have any advantages over existing known pathways. Scientifically, demonstration would be interesting, but I do not see this significantly impacting the industry. Progress has been made, and partial utilization of formate has been achieved; however, it is not clear the team will achieve the 1-g/L glycolate production from formate by the end of the project.
- This is an ambitious project to engineer *E. coli* to convert formate into glycolate, which requires importing an entire pathway and deleting a competing pathway. The project has achieved some success, although the prospects for advancing this technology further toward application are still unclear.

Strengths: Formate to glycolate is a value-added transformation that would enable substantial CO<sub>2</sub> valorization if coupled with electrochemical formate synthesis. The team has succeeded in engineering an organism that produces 4 g/L of glycolate from formate and xylose, which is derived from inedible biomass. The project has generated foundational learnings about formatotroph engineering that will be valuable for further development of this approach to CO<sub>2</sub> conversion.

Weaknesses: The amount of glycolate produced from formate is still low and below the modest end-of-project target of 1 g/L/d. The glycolate productivity from xylose and formate is interesting, but this is a substantially different application that needs further context and analysis to understand if it is worth pursuing. There was no discussion of the theoretical limits to energy conversion with this strategy—specifically the energy content of glycolate at maximum attainable yield divided by the energy content of formate.

- This is an interesting study that aims to produce glycolate from formate. The approach is unique, and the findings seem promising. The modeling effort is a good addition, but additional discussion is needed on how computational validation can aid in the better design of formation conversion pathways. Based on what has been presented, it is not clear what are the critical bottlenecks of this approach to achieve the target yield, titer, and productivity of glycolate (i.e., better controlled reacting environment? This needs to be specific).
- This project focuses on engineering microbes for upgrading formate to glycolate. The performers successfully demonstrated that *E. coli* can be engineered to consume formate and produce glycolate with ~4.1 g/L titer at flask level. This result is of scientific importance because *E. coli* does not naturally consume formate, so the researchers engineered a new formate conversion pathway into these organisms. The performers did not indicate an approach to scale their process to achieve the project milestones.
- This project sought to engineer *E. coli* to convert CO<sub>2</sub> or formate via engineering a novel metabolic pathway. This was a very ambitious and creative project that was high risk and high reward at a low TRL. The project was successful in producing glycolate from the engineered pathway, but formatrophic growth has not been achieved. Although this is a very interesting fundamental science project, the ability for commercialization and scale-up to the industrial level remains to be seen.

## PI RESPONSE TO REVIEWER COMMENTS

- Response to Comment 1: We are grateful for the comment. In terms of how computational validation can aid in the better design of formation conversion pathways, we conducted computational thermodynamic analysis and protein cost analysis. One ongoing effort is to quantitatively profile the proteome of engineered strains. The results will be comparable with the optimal modeling outcomes. The difference will indicate potential pathway targets that need to be further engineered, thus guiding the synthetic biology work. In addition, a better-controlled reacting environment, as the reviewer suggested, will affect glycolate yield and productivity. We are performing process optimization in both shaking flasks and a bench-top bioreactor. Variables that are being investigated include aeration, pH, nutrient compositions, isopropyl  $\beta$ -d-1-thiogalactopyranoside level, and others.

Response to Comment 2: Many thanks for the comment. The ability for scale-up is being investigated by bioreactor experiments.

Response to Comment 3: Thanks for the comment, especially the weakness the reviewer mentioned. We are coupling the metabolic engineering and laboratory evolution strategy for formatotroph purposes. As to the xylose-plus-formate strategy, the theoretical yield of this approach was confirmed by genome-scale flux balance analysis, which shows that the approach can produce glycolate without carbon loss as CO<sub>2</sub>. This could be developed as a practical strategy for bioproduction with carbon neutralization. In addition, preparing a sugar-plus-formate cofermenting culture first and then adapting the culture to formate only proved to be a valid strategy for evolving formatotroph; thus, this work will also help us achieve the goal on absolute formatotroph.

Response to Comment 4: Thanks. The ability for commercialization and scale-up to the industrial level are being investigated by, for example, bioreactor experiments and TEA.

Response to Comment 5: The reviewer's comment is appreciated. We have bimonthly meetings and share progress often with other formate projects. From the perspective of strategies, this project focuses more on synthetic formate conversion; the other two leverage native formate organisms/pathways. We posit that both types of strategies have their own sets of advantages and disadvantages, and all projects are synergetic to each other toward the goal of formate upgrading. Note that even for native formatotrophic organisms, intrinsic limitations still exist in the pathways because formate utilization is evolved for minimal survival purposes, not for high-yield bioproduction. To solve them, we need to go further than only turning native formatotrophic organisms genetically tractable. In this regard, engineering a model microbe (e.g., *E. coli*) could be advantageous because of well-studied metabolic background, simplicity in genetic manipulation, robustness and fitness as industrial chassis, etc. It provides an effective approach to learn biosystems' traits in formate conversion and therefore will shed light on the industrialization way.

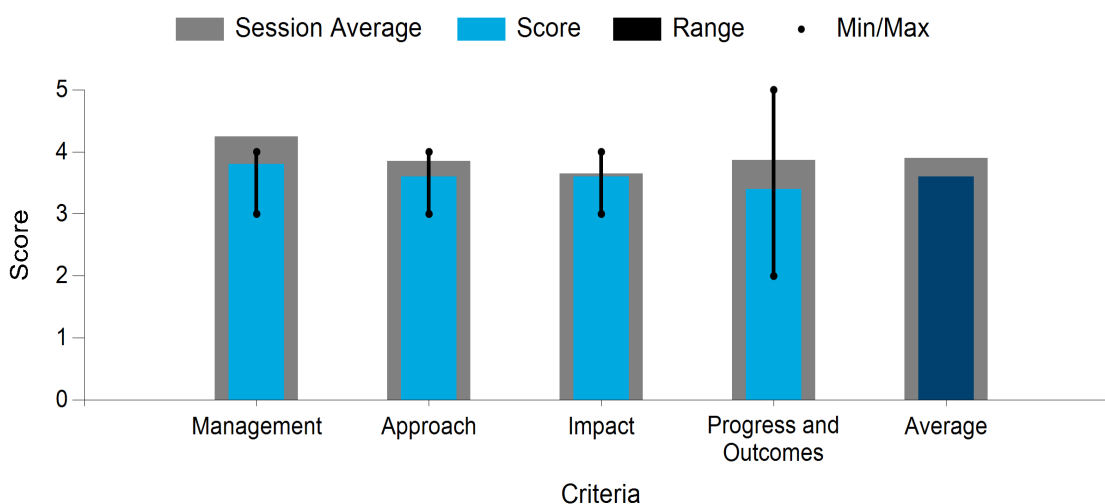


# DEVELOPMENT OF A SCALABLE, ROBUST ELECTROCATALYTIC TECHNOLOGY FOR CONVERSION OF CO<sub>2</sub> TO FORMIC ACID VIA MICROSTRUCTURED MATERIALS

Montana State University

WBS:	2.3.4.600
Presenter(s):	Lee Spangler
Project Start Date:	10/01/2018
Planned Project End Date:	03/31/2022
Total DOE Funding:	\$1,483,983

Average Score by Evaluation Criterion



## COMMENTS

- The approach taken by this project is meaningful in terms of providing insights of scaling up these reactor systems. This project lacks the study at the interface between electrochemical and biological reactors. Two systems seem to be studied independent from each other. It is not clear how water recycle can be achieved and what would be the pH tolerance throughout the system. The overall process integration should be revisited.
- The overall goal of this project is to develop an electrochemical reactor for conversion of CO<sub>2</sub> to formate and then to use this formate as a feedstock for biological upgrading to more highly reduced products. The project is divided into two overall themes: one focused on scaling an electrochemical reactor for formate production and the other focused on organism engineering for formate conversion to ethylene glycol. In the first theme, the performers are using an iterative scaling approach that has allowed them to identify and address technical challenges at multiple scales. This approach has led to optimized cell designs, catalyst coating methods, and graded porosity gas distributors that help overcome challenges identified during the scaling research. In the second theme, the performers developed microorganisms for formate

conversion and tested the conversion efficiency using electrochemical process fluids. The performers have made significant progress in both themes, and the findings have high potential impact in their respective fields; however, based on the presentation, it seems that the two themes are being studied independently, and the strategy for eventual integration of the themes was unclear. The project approach and impact could be strengthened by increased emphasis on the technical challenges associated with reactor integration.

- The project management plan was clear. Although examples of technical risks are provided, possible mitigation strategies are not. The overall technical approach is good. The team is able to focus on the electrolyzer, microbial upgrading, and the integration of the systems. A key challenge is integrating the output of the electrolyzer with the microbial host, and the team has made good progress on this front. The reasoning behind selecting *E. coli* as the microbial host is not clear. A novel first-of-its-kind pathway has been identified, and the team has made good progress in implementing a new pathway for C1 upgrading, but it is not clear this has a significant advantage over a native formate-consuming microbe. The team must implement both the new pathway and the ethylene glycol pathway rather than only an ethylene glycol pathway. By focusing on the entire process and integration, the team is maximizing the possibility of success. Although the novel C1 upgrading pathway is interesting, it is not clear that is a distinct advantage over a native formate-utilizing microbe. The team has made good progress on all fronts and looks on track to meet the milestones. It is not clear when the microbe needs to begin consuming formate, which it has not done yet.
- This project combines formate reactor scale-up with earlier-stage research to develop a formate-to-ethylene glycol pathway in *E. coli*. Both tasks are challenging for different reasons, and they are at very different TRLs. Consequently, there is little that can be accomplished by combining them as opposed to operating these projects separately.

**Strengths:** The generation of graded porosity gas distributors is an interesting technology, and the project has succeeded in scaling up production to accommodate large electrolyzers. Formate to ethylene glycol is an ambitious target but would be very attractive if an optimizable biosynthesis were developed.

**Weaknesses:** The performance of the larger (100-cm<sup>2</sup>) electrolyzers is significantly worse than the smaller (10-cm<sup>2</sup>) electrolyzers, and there is a large gap between the current results and the end-of-project goal. The plan to bridge this gap is not clearly delineated. There is not yet any demonstration of microbial ethylene glycol synthesis from a formate feedstock. The use of formaldehyde is not a reasonable substitute for formate.

- This project focused on scaling up an electrochemical reactor for the conversion of CO<sub>2</sub> to formate, followed by the biological upconversion of formate to ethylene glycol. The project team included partners from academia as well as a startup looking to scale up CO<sub>2</sub> electrolyzers. Although the biological upconversion holds promise, the performance targets for CO<sub>2</sub> to formate (FE ~70%, >4 V, etc.) are far from the state of the art. The TEA was also performed by the startup company and raises questions about its accuracy or reliability. Overall, I was underwhelmed with the electrochemical performance of the scaled-up reactor.

## PI RESPONSE TO REVIEWER COMMENTS

- This project is consistent with the DOE FOA and has the generation of the “intermediate” chemical (potassium formate salt) and its consumption by the engineered bacteria to make the “final” product (ethylene glycol) take place in two separate process steps. The intermediate generation step is an electrochemical process that generates the formate salt using CO<sub>2</sub> and water. In the second step, in a separate bioreactor, the formate salt is fed to the bacteria to produce ethylene glycol. Although the two steps cannot be integrated in the same reactor, both processes—the electrochemical process chemistry and the biological bacteria ability for uptake of formate—are being modified so that the biological

process can be directly fed with the formate product solution from the electrochemical reactor, without need for separations, concentration, or pH adjustment. To date, our work on the integration of the electrochemical and biological conversion processes has focused on the impact of key electrochemical salts on the growth of our target microbial hosts. Our finding that *E. coli* is capable of growth in media containing up to 1.25 million potassium chloride (KCl) has provided a basis for the electrochemical work to optimize this system for formate generation considering this factor. The electrochemical and biological teams continue to iterate on this optimization to determine the optimal conditions conducive to maximizing formate generation and biological upgrading directly from electrochemical process media. We have demonstrated growth of our engineered strains in process media containing a secondary carbon source, and we will continue to refine and improve this aspect. This will be conducted in conjunction with our continued engineering and improvement of biological formate upgrading as we move to *in vivo* ethylene glycol production based on our demonstration of the entire conversion (formate to ethylene glycol) *in vitro*. We also note that given the well-known toxicity of formate to microorganisms—including *E. coli* and native formatotrophs, such as *C. necator*—we are envisioning that a fed-batch process will be needed to mitigate this issue. The continued TEA will further guide these efforts to better understand the best approach for formate conversion (e.g., fed-batch) and recycling (e.g., CO<sub>2</sub>, water, salts) considering the required electrochemical parameters and biological constraints (e.g., pH, formate concentration).

- We proposed a budget period two target of an “optimal” 100-cm<sup>2</sup> reactor, which is a 10-times (one-order-of-magnitude) improvement over the current state of the art for CO<sub>2</sub> to electrochemical liquid products because reactor sizes by other researchers are limited to a maximum of 10 cm<sup>2</sup> (3.3 cm x 3.3 cm). This is because mass-transport limitations of CO<sub>2</sub> and flooding across the gas diffusion electrode (GDE) become factors that significantly reduce the electrochemical performance (current density, FE, continuous operation time) when going from 10 cm<sup>2</sup> to larger sizes. For this reason, most studies focus on small reactor sizes to research performance improvements. To our knowledge, the only study looking at larger sizes provided significantly suboptimal performance for formate, with low stable electrochemical performance (<4 hours) and extremely unreasonable chemical consumption (using expensive potassium hydroxide as consumed/reacted anolyte). The reason other researchers limited the reactor size to a small fraction of the commercial size (1-m<sup>2</sup>) reactor is the extremely complex nature of the CO<sub>2</sub>-GDE-electrolyte contact and mass-transport optimization issues that need to be understood and revised for optimal reactor performance. The project team has taken it upon itself to solve this issue when going from 10 cm<sup>2</sup> to 100 cm<sup>2</sup> in budget period two and up to a 300-cm<sup>2</sup> reactor in budget period three, where OCOchem is working to modify the current reactor designs, and MSU is developing customized-pore graded structures to aid the mass transport and avoid hydrodynamics issues in the electrochemical reactor.

The reason to have MSU in this project is to develop the structures to eliminate and optimize the hydrodynamics issues at larger reactor sizes, which were foreseen at the proposal stage. Unfortunately, the approach and progress in these developments could not be discussed in entirety during the call with DOE. Progress is being made, and we are confident that we will meet the project goals.

OCOchem has been working on iteratively optimizing the hydrodynamics by modifying the reactor configuration and testing in-house-developed reactor parts (3D-printed flow distributor plate and flow promoter mesh) to obtain uniform catholyte distribution without liquid head buildup. MSU has been separately developing structures to act as backing to the GDE that preferentially distributes the gas to soothe the difficulty in doing so with the presence of the liquid head on the other side of the GDE. These will be integrated in the coming weeks and tested, and they will be optimized iteratively to help obtain the desired electrochemical performance metrics.

Corresponding to a recent project milestone (March 31, 2021, after the project peer review meeting), we demonstrated the production of ethylene glycol from formate utilizing identified enzymes *in vitro*. Prior

to this, however, with our discovery and use of a first-of-its-kind biological pathway for C1 upgrading to multicarbon products, our approach to developing this pathway for formate to ethylene glycol conversion has focused on concurrently investigating enzymes for the key C1-C1 condensation reaction (2-hydroxacyl-CoA lyase, HACL) along with evaluating upstream (formate-to-formaldehyde/formyl-CoA) and downstream (glycolyl-CoA-to-ethylene glycol) modules. We agree with the reviewer that formaldehyde is not a reasonable substitute for formate in the envisioned process; however, our approach has allowed us to identify and demonstrate efficient enzymes for all required reactions. Because the HACL catalyzed condensation is a new-to-nature reaction and one that is central to pathway operation, we used formaldehyde as a proxy to evaluate and characterize candidate enzymes. With well-performing (e.g., efficient operation at low formaldehyde concentrations) HACL enzymes in place, we have integrated all pathway modules and demonstrated formate-to-ethylene glycol conversion. This establishes the entire pathway for formate upgrading, which we will continue to optimize and improve.

- Generically, project milestones and specific, measurable, attainable, realistic, and time-related and go/no-go decision points were set to correspond to key technical risks, thereby reducing risk to DOE regarding funding of subsequent project budget periods. Specific technical risks listed were (1) the ability to continuously grade porosity structures, (2) the identification of host strains and enzymes, and (3) performance retention in electrochemical reactor scale-up. These were at least partially addressed through the remainder of the presentation while detailing project progress, but the correspondence to the identified risks was not called out, so we understand why the reviewer made this comment.

For Risk 1, two approaches were made to mitigate it. The first was to perform stepwise grading of porosity, a method we had high confidence in achieving (which we did), and could likely generate needed performance, but was less ideal than continuous grading. The second was to test grading with different material types because particle density affects useable particle concentration in the slurry and therefore gradient range in the resulting structure.

For Risk 2, the mitigation strategy was to identify and test a large number of enzyme candidates and host strains, improving the probability of identifying the needed performance. Milestones have been met in this area. As we continue improving the overall operation of our C1 conversion pathway and engineered strains, we will utilize this large pool of enzymes and required genetic manipulations to improve the pathway and strain performance as needed (e.g., reducing byproducts, improving tolerance to specific compounds).

For Risk 3, we explored methods of depositing catalysts and how robust that catalyst distribution is; methods of improving conductance across the GDE, including use of a metal mesh substrate; and gas diffusion against the pressure gradient caused by the hydrostatic head in the reactor, which is the purpose of exploring the development and use of the graded porosity structures. Our selection of *E. coli* as the microbial host is based on both our novel pathway design and the advantages of this proven industrial organism. *E. coli* is an industrial host used to produce diols commercially (e.g., 1,3-pentanediol, 1,4-butanediol), so it is very relevant for this project given the diol product we are targeting (*E. coli* has been shown to produce and tolerate greater than 70-g/L ethylene glycol). Further, our investigation into *E. coli*'s tolerance to key electrochemical process components (i.e., KCl) determined that *E. coli* has better tolerance to KCl, a salt required at high concentrations for the electrochemical CO<sub>2</sub> reduction component of the process, than other organisms tested (e.g., the halophile *Vibrio natriegens*), with growth observed at concentrations up to 1.25 million. These characteristics give *E. coli* a distinct advantage compared to native formatotrophs that are not as well characterized or industrially proven.

From the standpoint of formate utilization, current approaches toward biological product synthesis from C1 substrates based on natural or synthetic pathways—including native formatotrophs, such as *C. necator*—inherently rely on the central metabolism to funnel C1 substrates to precursor metabolites that feed product synthesis routes. As such, these approaches require concurrent engineering of C1

assimilation, central metabolism, and product synthesis pathways to generate multicarbon products. This is a challenge even for genetically tractable organisms, and it becomes a significant barrier when considering organisms lacking genetic tools and/or requiring laborious processes for genetic engineering. Native formatotrophs are in the latter category, and combined with the relative inefficiencies of all native formate-utilization/-conversion pathways, the use of these organisms for formate conversion is significantly limited in our view. Opposed to working within this framework, we developed a synthetic pathway orthogonal to native metabolism that can directly generate multicarbon products from C1 units. This minimizes the interdependence on native metabolism, providing a more direct pathway (i.e., fewer steps) that circumvents many intrinsic regulatory mechanisms and carbon and energy inefficiencies and does not require complex cofactors. Specific to ethylene glycol production, note that our target product is a direct extension of our formate-utilization pathway, and as such, it does not require extensive engineering of an additional product synthesis pathway (which would be required in a formatotroph). This pathway simplicity and orthogonality facilitates its operation in any host organism, including the use of industrially proven *E. coli*. This, in turn, enables more efficient biocatalysts that offer more process flexibility, including the ability to grow on a secondary carbon source without directly influencing C1 conversion. As we continue to optimize our novel pathway for formate-to-ethylene glycol conversion and integrate the electrochemical and biological conversion, these key pathway and organism advantages will become even more critical.

## PRODUCTION OF BIOPRODUCTS FROM ELECTROCHEMICALLY GENERATED C1 INTERMEDIATES

LanzaTech, Inc.

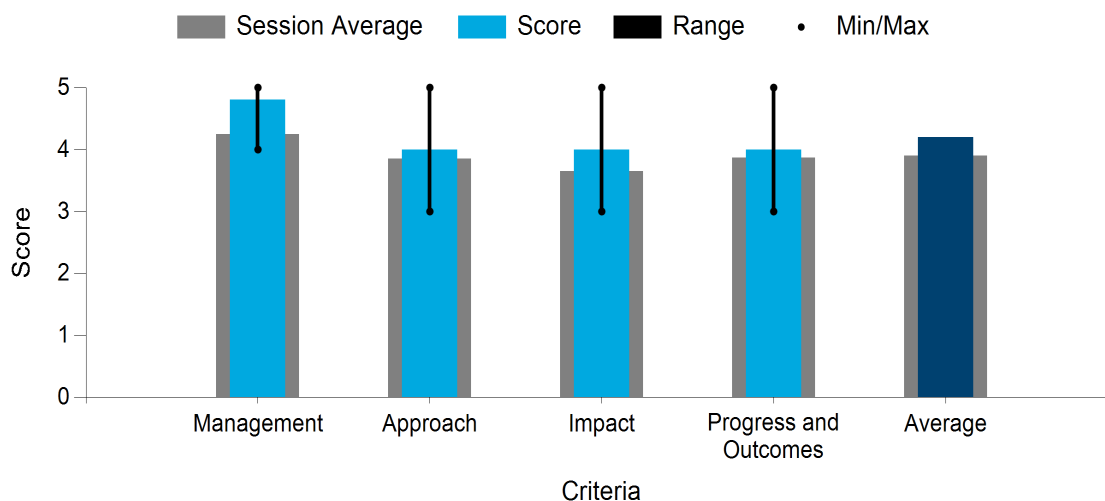
### PROJECT DESCRIPTION

With increasing drivers for renewable chemicals and plummeting sustainable energy costs, there is an urgent need for technologies that convert CO<sub>2</sub> to chemicals with renewable electrons.

The objective of this project is to convert a minimum of 37% of a CO<sub>2</sub> stream to isopropanol. This will be realized with a platform integrating CO<sub>2</sub> electrolyzer technology with gas fermentation. An AEM-based electrolyzer from Dioxide Materials will be integrated with LanzaTech's isopropanol producing gas fermentation strain. CO<sub>2</sub> from the back end of fermentation will be recycled back to the electrolyzer to minimize carbon losses. To aid fermentation yield, the isopropanol strain will be developed to utilize arginine as the sole nitrogen source, which has been shown to provide an adenosine triphosphate (ATP) benefit over ammonia without significantly increased feedstock costs.

WBS:	2.3.4.601
Presenter(s):	Jason Bromley
Project Start Date:	10/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	\$1,350,000

Average Score by Evaluation Criterion





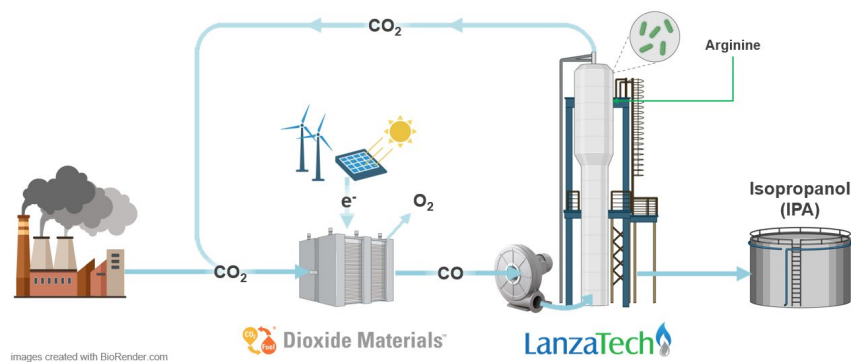


Photo courtesy of LanzaTech, Inc.

## COMMENTS

- The goal of this project is to develop an overall CO<sub>2</sub> electrolysis/gas fermentation system for the conversion of CO<sub>2</sub> to isopropanol. The project approach is focused on optimizing the design of a small-scale CO<sub>2</sub>-to-CO electrolyzer, improving the yield of the fermentation process, and integrating the electrolyzer with the bioreactor. The performers have made significant progress in all aspects of the project approach, they have developed a somewhat modular system for integrating electrolyzers with bioreactors, and they have identified technical challenges associated with the reactor integration; however, the presentation lacked sufficient discussion of the TEA to assess the economic viability of some design choices, such as the use of arginine rather than ammonia as a feedstock, or to assess the scalability of the process.
- The project team has good experience to achieve the project, and the management structure is clear. It is good the team is taking a wholistic view of the process, optimizing the catalyst, fermentation, and integration. The benefit of arginine over ammonia needs more justification. Although the PI says the cost per mol of nitrogen is equivalent, further discussion and justification are needed. The assembly of an all-industry team maximizes the potential successful implementation of this technology at scale (which is one goal of BETO), but it also limits the potential sharing of knowledge because of the proprietary nature of the work; thus, any fundamental benefit of catalyst design, the arginine supplementation, ornithine utilization, or integration will be kept within these two companies, limiting the broad impact of the work. The team has made good progress and met the first go/no-go; however, a full detailing of milestones and end-of-project goals was missing, so it is hard to fully judge the progress.
- The team has great expertise in each part of the overall process. The large-scale challenges investigated by this project are still relevant to the laboratory scale, not the full commercial scale. The project will lead to high-impact results if the future research is more focused on the integration of electrochemical and biological reactors.
- This project seeks to advance the state of the art for electrochemical CO<sub>2</sub>-to-CO conversion and integrate it with acetogenic CO-to-isopropanol conversion. A successful outcome would likely define the state of the art for CO<sub>2</sub>-to-isopropanol conversion (by any system) and provide a basis for a realistic initial assessment of commercial viability.

Strengths: The project is managed well and combines the strengths of the two companies involved. The project has advanced the performance of the electrolyzer in key metrics that will determine the overall

efficiency of the process. The power consumption of the electrolyzer is roughly in line with the end-of-project goal (8 kWh per Nm<sup>3</sup>).

Weaknesses: It is unclear how close the team is to achieving a gas fermentation that meets the target metrics for isopropanol productivity. No results were presented for an integrated system, making it impossible to assess the progress toward the overall CO<sub>2</sub>-to-isopropanol goal or the nature of the integration challenges. The project seems like two projects in parallel at the moment.

- This project was looking to integrate CO<sub>2</sub> electrolyzers from Dioxide Materials with LanzaTech's CO fermentation to produce isopropanol. The project was able to demonstrate an improved and scaled-up CO<sub>2</sub> electrolyzer design with increased energy efficiency and reduced CO<sub>2</sub> crossover. The biocatalysis innovation included co-utilization of arginine and CO with several strains and pathways built. The integrated unit at a reasonable industrial scale has been built, and recycle studies are ongoing. Overall, this is a very impactful and positive project with great technological progress on all parts of the system.

## PI RESPONSE TO REVIEWER COMMENTS

- We appreciate the reviewer for acknowledging the depth of our team's expertise and aptness for our approaches. Although it is true that many of the current integration challenges are associated with laboratory scale—such as accurate flow, composition, and pressure monitoring—the system is set up to address the main risks for commercial scale: electrolyzer robustness and reliability; components in fermentation off-gas that affect electrolyzer performance; full characterization of CO<sub>2</sub> crossover (e.g., at varying feed rates, feed composition, current/feed ratio, current density); and characterization of any effects of the electrolyzer gas on the fermentation (compared with pure synthetic gases of the same composition). Integration of electrochemical and bioreactors is the focus of the project moving forward. We have already identified the major hurdle to an efficient commercial-scale process, which is the CO<sub>2</sub> crossover in the electrolyzer, and we are working on multiple methods for mitigating this.

We thank the reviewer for these positive comments on our progress and assessment of our work. TEA is part of the project, but unfortunately most detail is unable to be shared publicly. The TEA model relies on data generated from laboratory experiments and is updated each billing period.

- We appreciate the reviewer's assessment of our strengths and progress. The isopropanol milestone targets on titer and productivity results are unable to be shared publicly, but we surpassed the budget period one targets in 2019, and we recently surpassed the budget period two project milestones by approximately 20%. The electrolyzer performance data presented were achieved on the integrated system with gas recycle. The isopropanol targets (not presented) were also achieved on the integrated system. Some of the integration work between the arginine and the electrolyzer in budget period two was not presented. On the electrolyzer-CSTR system, co-utilization of CO and arginine was tested and achieved the relevant isopropanol productivity milestone. In addition, the two work streams will come together further in budget period three, where the milestone is to achieve the final isopropanol fermentation milestones on the electrolyzer-CSTR system with arginine as the sole nitrogen source.
- We thank the reviewer for their positive assessment.
- We appreciate the reviewer's assessment of our project team, management structure, and overall approach. Regarding the benefits of arginine over ammonia, the arginine addition provides the nitrogen source with a cost that is similar to that of ammonia at a per-mol-nitrogen basis. In addition, arginine addition supplies extra ATP to support growth, and it can increase energy-intensive product yield based on whole-genome-scale modeling results (as shown in our slide deck and the previous quarterly report).

## INTEGRATING CHEMICAL CATALYSIS AND BIOLOGICAL CONVERSION OF CARBON INTERMEDIATES FOR DERIVING VALUE-ADDED PRODUCTS FROM CO<sub>2</sub>

Johns Hopkins University

### PROJECT DESCRIPTION

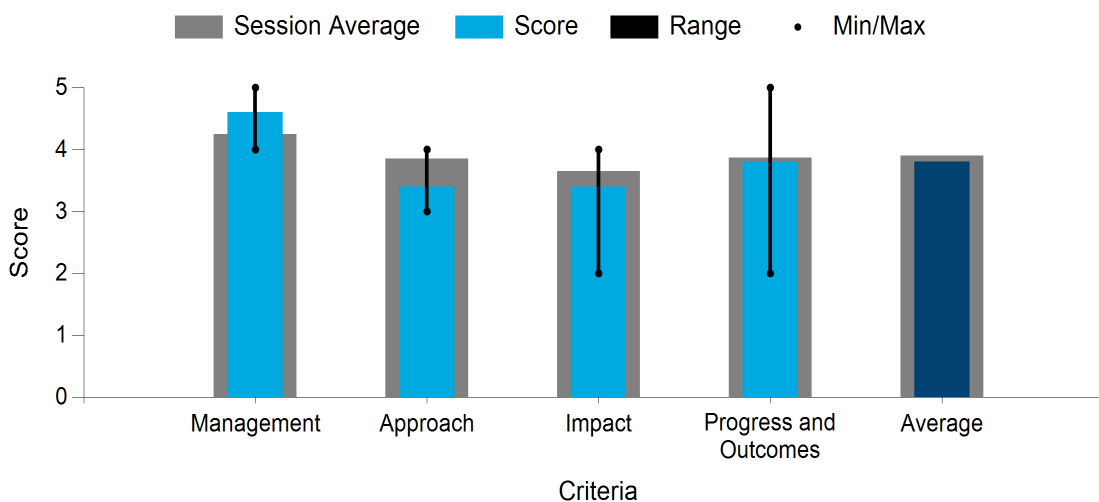
Capturing and upgrading point source CO<sub>2</sub> represents a desirable technological goal to achieve sustainability targets. Electrocatalytic processes excel at reducing CO<sub>2</sub> into simple carbon compounds.

Conversely, biocatalysts excel at upgrading reduced carbon compounds. In this project, we are developing a two-stage integrated platform that leverages the

advantages of both electrocatalytic and biocatalytic systems: CO<sub>2</sub> is converted into methanol, acetate, and formate via electrocatalysis, and these substrates are subsequently valorized using a methanotrophic bacteria. This process has the potential to represent a transformative platform combining electrocatalytic and biological conversion steps into an integrated approach for the bioproduction of commercial products.

WBS:	2.3.4.603
Presenter(s):	Michael Betenbaugh
Project Start Date:	10/01/2018
Planned Project End Date:	11/30/2022
Total DOE Funding:	\$969,429

Average Score by Evaluation Criterion



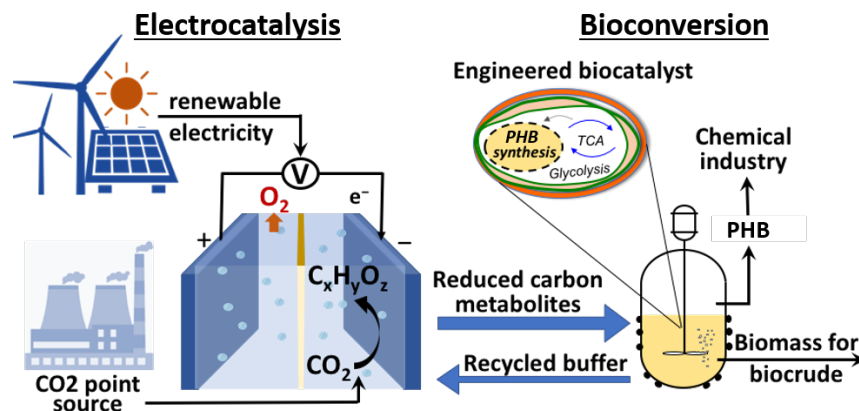


Photo courtesy of Johns Hopkins University

## COMMENTS

- The goal of this project is to develop an overall CO<sub>2</sub> electrolysis/bioconversion system for the conversion of CO<sub>2</sub> to PHB and biomass. The project approach is focused on optimizing electrolyzers for CO<sub>2</sub> conversion to C1 and C2 liquid products, and using these products as feedstocks, engineering microbes for efficient conversion of these C1 and C2 electrolysis products to PHB and integration of the electrolysis and bioconversion systems. A particular strength of the project team is the inclusion of a team member focused on integration, and the research approach includes metrics and analyses for each project theme. The performers have made substantive progress toward meeting their project milestones, but they did not indicate a specific approach for increasing carbon conversion efficiency (CCE) and yield to target milestones. The impact of the project could be strengthened by discussion of how this process will compete with existing technology for PHB production.
- The management structure and risk mitigation measures were very clear. The overall approach is well constructed, with a focus on the entire integrated process. Although theoretically it is good to focus on the entire process, both technologies (the electrolysis and fermentation) are first-of-their-kind technologies, so it may be too ambitious to tackle both in one project. The selection of the specific microbe and metabolic target needs more justification—for example, what are the specific advantages of this strain? The project is focusing on two new technologies (electrolysis and fermentation), so the amount of fundamental knowledge gained is increased. The team has so far made a good effort in sharing what they have learned. The project looks to be on schedule to meet project goals and milestones.
- The team has very good complementary expertise that is needed for this project. They have well identified the risks and their mitigation options. Considering different feedstocks—including formate, methanol, and acetate—for biosynthesis is a great approach. The target FE and CCE seem to be not very ambitious compared to the state-of-the-art values. But the data provided for the integrated systems were insightful.
- This project combines CO<sub>2</sub> electrolysis with metabolic engineering to transform C1 feedstocks into PHB. Both components are at a very early stage. Based on the preliminary analysis presented, it will be very difficult to produce economically competitive PHB even if the technical goals are achieved. There has been some significant progress toward the technical milestones, but there is still a large gap between the current results and the end-of-project goal.

Strengths: Methanol is a challenging product to make electrochemically, and the current density, FE, and CCE reported for methanol synthesis are promising. The idea of supplying a fermentation reactor with a mixed feedstock of liquid C1/C2 products is a nice complement to other more focused strategies, and the production of methanol and acetate at a combined 70% FE and 50% CCE is a good starting point. Proof-of-concept microbial growth on methanol/acetate in the microbial host has been demonstrated.

Weaknesses: There is no indication of the energy efficiency of the CO<sub>2</sub> electrolysis, which makes it difficult to assess the significance of the selectivity results. The reported CCEs for the electrolysis are very high given the multi-electron reduction products (they are higher than optimized systems that produce only 2e- products such as CO), and it would be helpful to see a full account of the carbon balance (CO<sub>2</sub> into cathode, CO<sub>2</sub> out at cathode and anode, C in products). It does not appear that the team has succeeded in producing any PHB to date. The electrolysis and fermentation efforts are at such an early stage that it is difficult to make meaningful progress on integration. TEA is very premature, but if the projections on slide 17 represent best-case scenarios, then it seems that PHB is not a good target. Very little information about the TEA was presented, making it impossible to understand the basis for the projections.

- This project is looking to integrate the electrocatalytic conversion of CO<sub>2</sub> to formate or methanol with bioconversion of these liquid intermediates into PHB. Although electrocatalytically the team was able to meet the stated milestones for formate production, they were not able to produce methanol at >40% FE. This project shows early-stage integration at the laboratory bench scales with relatively low integrated yields that did not meet their milestones. Although the project has academic merit, I do not think it is a practical or scalable approach.

## PI RESPONSE TO REVIEWER COMMENTS

- We would like to point out that there are already industrialized electrolysis technologies. For example, chlor-alkali electrolyzers have been commercially employed to produce caustic soda. Similar systems/devices can be used for the electrochemical reduction of CO<sub>2</sub>. Our TEA is also based on DOE standards for such electrochemical devices/systems.
- In our report presented at the Project Peer Review meeting, we showed that we had met the milestones for CO<sub>2</sub>-to-formate and CO<sub>2</sub>-to-C1/C2 platform chemicals. More recently, we have also shown that CO<sub>2</sub>-to-methanol milestones can also be met by improving the design of electrochemical conversion processes. In the new design, we have ~14% CCE for direct CO<sub>2</sub>-to-methanol conversion and ~80% CCE going to CO, with the rest counted as potential carbon loss due to gas separation (see the following discussion). For subsequent CO-to-methanol conversion, we can achieve >90% CCE because methanol is the only carbonaceous product. Overall, the CCE toward methanol has achieved >80% even when we account for ~5% carbon loss for each electroreduction step.
- We agree with the reviewer that it is challenging to reach >40% FE for direct electroreduction of CO<sub>2</sub> to methanol; however, the major side product is CO, which can be further reduced to CO in a two-step CO<sub>2</sub>-to-CO-to-methanol conversion process. In a recent meeting with the BETO program managers, we have shown that we can achieve ~18% FE for the electroreduction of CO to methanol, which can still be further improved. Plus, with the 33% FE for direct CO<sub>2</sub>-to-methanol conversion, we can now achieve >50% FE toward methanol.
- The energy efficiency can be calculated based on the FE and overpotential from electrocatalysis experiments. For the CO<sub>2</sub>-to-formate conversion, the energy efficiency is near 40%, with a FE of 95% and an overpotential from 0.5–0.6 V. For the CO<sub>2</sub>-to-methanol conversion, the energy efficiency is >30%, with an overall FE of ~50% and an overpotential of ~0.4 V for both CO<sub>2</sub> and CO reduction.

- To improve the CCE in the electrochemical conversion of CO<sub>2</sub>, we proposed to integrate gas separation technologies to recirculate CO<sub>2</sub> and CO. The major task here is to separate H<sub>2</sub>, the only side product of note, from CO<sub>2</sub> and CO when it accumulates to certain extents (e.g., >10%). We would like to point out that pressure swing adsorption (PSA)/temperature swing adsorption (TSA) protocols have been industrialized for such gas separations in natural gas utilization, with <5% carbon loss for each stage (<https://doi.org/10.3303/CET1332310>). In our budget period three research plan, we have a task for the demonstration of such system integration, where we will run laboratory tests of PSA/TSA separations and perform TEA/LCA to evaluate the potential impact on the energy efficiency, CCE, and economics of the whole process.
- Our focus in this research is more on the electrochemical process design and system engineering, less on the fundamental R&D for electrocatalyst development. As discussed, we have made substantial progress on balancing and improving the FE/CCE for the electrochemical conversion of CO<sub>2</sub> to formate or methanol. We hope our work will contribute to the TRL of electrochemistry-based conversions in bioenergy industries. Nevertheless, we have also made technical advancements to boost the durability of cobalt phthalocyanine electrocatalysts for CO<sub>2</sub> reduction. With our innovation, we are now able to run electrocatalysis with this molecular electrocatalyst for several days and produce concentrated liquid products (via electrolyte recirculation), which, to the best of our knowledge, remains a grand challenge in the community for the electrochemical production of methanol from CO<sub>2</sub>. Noticeably, CO<sub>2</sub> reduction to methanol is much more challenging than CO<sub>2</sub>-to-formate or CO<sub>2</sub>-to-CO conversions, whereas our bioconversion studies have shown that methanol is much more valuable than formate or CO for biomass production because of its liquid nature and high energy content.
- We will use a combination of metabolic engineering, carbon flux, and process optimization approaches. Specifically, nearly 30% of consumed methanol can be metabolized into formate and secreted into the medium. We have engineered a 20Z mutant with knocked-out methenyl-cyclohydrolase and formaldehyde-activating enzyme to improve the fixation of methanol into biomass or a target product instead of formate. Second, we demonstrated that growing 20Z in the -Cu growth medium reduced its growth rate but significantly improved CCE from methanol toward cellular biomass and the accumulation of biopolymer storage compounds. Third, we are enhancing the expression of PHB synthesis through applying promoters with higher expression efficiency and codon optimization. Indeed, we have demonstrated earlier that promoter and codon optimization can boost the synthesis of the target bioproduct in 20Z by more than fiftyfold. Finally, we will enhance the CCE through the optimization of bioreactor parameters, including semibatch cultivation dose, intermittent methanol dosing, and the limitation of other key nutrients (e.g., N or P).
- Our latest electrocatalysis strategy allows us to generate methanol as the primary or even the only intermediate metabolite with high electrocatalysis CCE. Moreover, the methanol has the highest CCE among other intermediates (e.g., formate, acetate). We anticipate that our latest progress will result in achieving the total CCE (electrocatalysis CCE x bioconversion CCE) exceeding the final target of 37%.
- The target CCE is for combined (integrated) process from CO<sub>2</sub> to the final product. The high state-of-the-art values are typically for the conversion of CO<sub>2</sub> to formate, which is not a very good substrate for bioconversion with biological CCE <15%. On the other hand, the conversion of methanol into biomass can be achieved at relatively high CCE; however, electrocatalysis of CO<sub>2</sub> to methanol with high CCE is a major challenge.
- The study is focused on *Methylotheobacterium alcaliphilum* 20ZR, a promising bio-platform for the utilization of simple reduced carbon substrates, such as methane and methanol. *M. alcaliphilum* 20ZR is a robust methanol utilizer. The strain can tolerate 10% methanol and show robust growth at 5%. Further, it is one of few cultures capable of growth in the high-molarity carbonate buffers often applied for CO<sub>2</sub> electrocatalysis; thus, it is a potentially superior bio-platform for the utilization of the electrocatalytically



produced C1 and C2 compounds. As part of previous and ongoing DOE-funded research, we established a computational framework encompassing the metabolic network of the culture. A large set of multiomic data—including metabolomics, the RNA-seq data set, and proteomics—for cells grown in liquid cultures with methane or methanol were generated and applied to further improve computation modeling. The flux balance model has been actively applied to inform targeted metabolic engineering. The strain has been improved for the production of succinate, butanediol, muconic acid, and lactate from methane or methanol, all of which are commodity chemicals and precursors for plastic-based polymers. A set of genetic tools for the efficient metabolic engineering of the strains has also been developed or optimized. The current toolbox includes vectors for gene knockout and insertion as well as optimized protocols for gene delivery. In this work, we aim to improve our understanding of the cellular strategies for mixed-substrate fermentation to establish a fully integrated electrocatalysis-bioconversion process for the robust and efficient utilization of CO<sub>2</sub>-derived feedstocks.

- We agree with the reviewer's comments on the importance of mixed substrate utilization fermentation. although methanol can fulfill *M. alcaliphilum* requirements for growth, recent data demonstrate an unsatisfactory CCE of only 61%. A significant portion of the energy is released as heat. These experiments suggest that the central metabolic pathways cannot build enough biomass precursors to keep up with energy production. *In silico* simulations demonstrate that co-utilization of methanol and C1/C2 organic acids can improve CCE dramatically up to 70%. Energy from methanol oxidation is captured to drive acetate consumption and phosphorylation, whereas formate supplies additional carbon and a redox source. Modulation of the carbon flux via acetate auxotrophy can provide an efficient mechanism for directing carbon toward acetyl-coenzyme A (CoA)-based products, such as polyhydroxyalkanoates.
- Strains expressing the PHB-biosynthesis pathway were constructed. The amount of PHB observed is limited; however, electromicrographs suggest the formation of a polymer, and light scattering analysis suggests that the produced polymer is low weight (80 kDa). Thus, additional improvements in both the overall conversion to PHB as well as the modification of PHB to market-attractive polymers, such as copolymers PHB and 3-hydroxypropionate, are desirable. We are currently changing the expression system to increase the PHB production and interlink production with methanol utilization. Once this goal is achieved, we will focus on the integration of the 3-hydroxypropionate biosynthesis pathway.
- Initial integration efforts helped identify key challenges for us to address and focus our team's efforts. Separate advancements in the electrolysis and fermentation steps as a result of our preliminary data leave us confident that we will meet our stated goals for the integrated system.
- Although the final integrated system is not yet fully defined, investigating the myriad factors required to bring the electrolysis and fermentation steps together can still provide useful insights. For instance, the electrolysis cell functions optimally when operating with high carbonate levels, but these carbonate concentrations adversely affect cell growth and carbon conversion efficiency. By identifying and addressing challenges such as these, we are serving to optimize the overall integration of the electrocatalysis and biofermentation steps.
- We are in the process of reviewing alternative PHB production strategies and will include that in subsequent TEA.
- The TEA is informing the development of the technology to determine where investments and advancements should occur as we advance the technology toward scalability and commercialization. Further, we believe our laboratory and pilot-scale studies will be a critical step in demonstrating feasibility for scale-up and commercialization.

- As noted, the TEA is premature and constantly evolving to guide the process development. By incorporating the TEA into our process development, we believe we can identify which steps are challenging and work to address these, such as reducing or eliminating separation costs.
- We appreciate the interest in our work, and we believe we have significantly detailed preliminary TEA. Because of time constraints, we focused on all aspects of the project and did not focus specifically on the TEA. More information on our TEA is available in a recorded conference presentation (<http://applied-energy.org/mitab2020/AEAB%202020%20Bili.htm>). We welcome additional feedback.

## INTEGRATION OF CO<sub>2</sub> ELECTROLYSIS WITH MICROBIAL SYNGAS UPGRADING TO REWIRE THE CARBON ECONOMY

### National Renewable Energy Laboratory

#### PROJECT DESCRIPTION

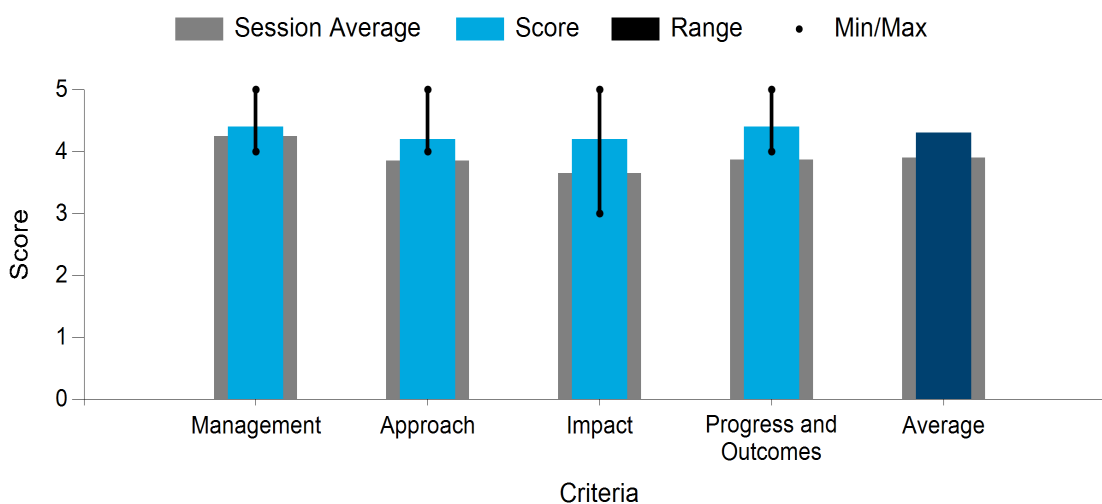
This project has worked toward demonstrating and understanding the conversion of waste CO<sub>2</sub> into value-added fuels and chemicals. If successful, our proposed technology will incentivize CO<sub>2</sub> capture and can add carbon efficiency to biorefineries. We have integrated two conversion technologies to demonstrate a novel approach of combining electrolytic CO<sub>2</sub> reduction with biocatalytic CO upgrading. Industrial partners 3M and Dioxide

Materials have recently demonstrated the electrolytic conversion of CO<sub>2</sub> and water to syngas using inexpensive renewable energy. Concurrently, supporting partner LanzaTech has demonstrated industrial-scale CO fermentation using steel mill waste CO as a substrate.

WBS:	5.1.3.101
Presenter(s):	Adam Bratis; Michael Resch; Zia Abdullah; Courtney Payne; Jessica Krupa
Project Start Date:	09/01/2018
Planned Project End Date:	09/30/2021
Total DOE Funding:	\$1,500,000

This presentation will highlight the investment BETO has made to establish a CO<sub>2</sub> electrolysis test station and CO fermentation at NREL. We will also discuss the process of increasing the size of the electrolyzers to meet fermentation needs, along with the investigation into the effect CO<sub>2</sub> concentration and contaminants have on electrolyzer efficiency, lifetime, and specificity. Additionally, we have begun to examine the downstream impact that electrolyzer off-gas CO-H<sub>2</sub> ratios have on the metabolism of *Clostridium autoethanologen*. Experimental results, along with input from our industry partners, have led to a baseline TEA and LCA. These results enabled the identification of key cost drivers and carbon intensity of the process. We used these metrics to identify technology gaps to iteratively inform the process optimization and potential deployment siting to achieve the economically viable, sustainable conversion of biopower-derived flue gases to fuels and chemical intermediates.

Average Score by Evaluation Criterion



## COMMENTS

- This is a great team with clear goals and approaches. The findings from this project are definitely high-impact and answer key questions on how to integrate electrolytic and biocatalytic reactors.
- The goal of this project is to design an integrated CO<sub>2</sub> conversion process in which CO<sub>2</sub> is first converted to CO in an electrolyzer, and the resulting syngas is used as a feedstock for microbial upgrading to more highly reduced products. The project approach is divided into three tasks focused on electrolyzer scaling, gas fermentation process development, and integration of the electrolyzer and gas fermentation processes. The performers have established a strong team that includes industry partners with expertise in each task. The performers have met with industry stakeholders regarding the possible integration of their design with industrial waste CO<sub>2</sub> feedstocks, and the possibility of such a partnership increases the impact of the project. A particular strength of the project is the research progress studying contaminant tolerance for the CO<sub>2</sub> electrolyzer. Most of the progress thus far has been on designing, optimizing, and scaling the individual electrolyzer and fermentation components; however, there will be significant technical barriers to efficient integration that can only be addressed once integration studies commence. A renewed emphasis on component integration would help identify new technical challenges and strengthen the project approach and impact.
- The project combines CO<sub>2</sub> electrolysis with gas fermentation to provide a route from CO<sub>2</sub> and electricity to C<sub>2</sub> products (ethanol and acetate). The work includes an assessment of the impact of impurities found in CO<sub>2</sub> emission sources on the performance of CO<sub>2</sub> electrolysis, strain engineering to improve gas fermentation productivity, and the integration of a prototype electrolyzer with an L-scale gas fermentation reactor. The project has a generic, nonquantitative goal (slide 4) that makes it difficult to assess progress and impact. Nonetheless, substantial work has been performed, and there are valuable findings from the project to date.

**Strengths:** The project appears to be well managed, and the development of the infrastructure and capabilities for CO fermentation at NREL is impressive. The work on impurities is an important contribution to the field. Understanding the impact of impurities on CO<sub>2</sub> electrolysis is critical for the overall process design and feasibility. The determination that sulfur impurities irreversibly damage the silver cathodes demonstrates that sulfur removal will be essential, both from the original CO<sub>2</sub> source and from recycle loops. Given the progress to date, the team is in a good position to create an integrated electrolysis-fermentation system and provide valuable data on overall performance.

**Weaknesses:** Without a quantitative goal, the project lacks a clear target to focus all the efforts. The base case in the analysis on slide 10 shows 8 g ethanol/L/h. Based on the data on slide 23 (22.3 g/L/d CO consumed, 8.25 g ethanol), it seems that the current productivity is at ~0.3 g/L/h. There is no discussion of the gap or how it will be closed to make the system viable. The hydrogen sulfide (H<sub>2</sub>S) data do not show the timescale—i.e., how long does the electrolyzer have to be exposed to the impurity for the degradation to occur? If the experiments were performed with “pulses” of H<sub>2</sub>S, or the effects were assessed after very short time periods, there could be an underestimate of the sulfur liability.

- The project put together a good, comprehensive team including industry partners. It was not clear how frequently the team met or how progress was tracked and technical issues resolved. The overall approach is well designed, with the team using a real-world gas and focusing both on the electrolyzer and fermentation. This comprehensive approach, along with bringing in industry partners, should increase the likelihood of eventual commercialization. One concern is the focus on improving microbe growth in the absence of a potential product. Although a synthetic pathway can be introduced into the improved

strain later, that strain would most likely need to be optimized again. Additionally, a team would have difficulty with a final TEA because they do not know what product they are making. The comprehensive investigation and inclusion of multiple industry partners increases the likelihood of commercialization; however, without the selection of a final product, it is hard to judge how impactful this work will be. The team seems on track to meet all milestones for the project.

- This project integrates CO<sub>2</sub> electrolysis and microbial syngas upgrading through partnerships with Dioxide Materials and LanzaTech. TEA was performed to determine key cost drivers. Adaptive laboratory evolution was also performed, and initial studies to show the tolerance of electrolyzers to minor contaminants were done. Overall, the initial project outcomes were positive and showed progress. One big gap will be batch matching between electrolysis and biocatalysis processes.

## PI RESPONSE TO REVIEWER COMMENTS

- We thank the reviewers for their constructive and supportive comments. We appreciate the support for the importance of integrating these systems and understanding the effects and possible mitigation of flue gas contaminants. With regard to the current microbial productivity, the TEA metrics reflect those achieved at scale in LanzaTech's proprietary loop reactor. The presented data (~0.3 g/L/h) are merely reflective of current productivity in a batch, bench-scale, CSTR configuration, which has inferior gas mass transfer. Additionally, the biocatalyst employed here is a wild-type bacterium, whereas LanzaTech's industrial process employs a metabolically engineered derivative, which has been optimized for high productivity. Our primary goal is to identify key genetic mediators governing optimal assimilation electrolyzer-derived gas streams, which can then be transferred to production hosts; thus, the project focus is primarily on CO uptake, as opposed to ethanol production, in part to avoid conflict with LanzaTech's intellectual property portfolio and extant funding. CSTR versus loop differential: The latter is optimized for maximal gas-to-liquid mass transfer, which enables enhanced productivity (g/L/h production rate). Additionally, the loop reactor bypasses the necessity for impeller-mediated mixing which, in turn, decreases operating expenditures associated with energy inputs. We agree with the reviewer's assessment that the optimization of strains in the absence of a target product would likely require further downstream optimization; however, as noted, our primary goal is to elucidate the biological mediators of CO/CO<sub>2</sub> assimilation and a systems-level understanding of uptake mechanisms and resultant carbon flux. Thus, we hope to transfer this knowledge into an array of production hosts following the establishment of design principles.
- The contaminants were not introduced to the electrolyzer in discrete pulses, but rather the cathode was continuously exposed to increasing contaminant concentrations, in 12-hour increments, during the 150-hour experiment. For H<sub>2</sub>S, the CO production rate and CO selectivity decreased stepwise with increasing contaminant levels. The goal of these experiments was to investigate the contaminant levels at which cell performance decreased by 10%. Investigating the long-term cell performance on feedstocks with sulfur concentrations commensurate with flue gas is another important step to understanding how sulfur compounds degrade cell performance. Our recent, ongoing studies indicate that exposing the cell to the highest contaminant levels for only 12 hours did not have the same deleterious effect on CO production rate and CO selectivity as the longer-duration experiments presented. This suggests that the total contaminant exposure time in the Q1 experiments did play an important role in the performance loss. We would love to do 500-hour runs; however, those longer time frames are outside the scope of this project period. This is the last year of the three-year annual operating plan cycle, and we have proposed the 500-hour runs for a continuation proposal.

## NOVEL CELL-FREE ENZYMATIC SYSTEMS FOR CO<sub>2</sub> CAPTURE

### National Renewable Energy Laboratory

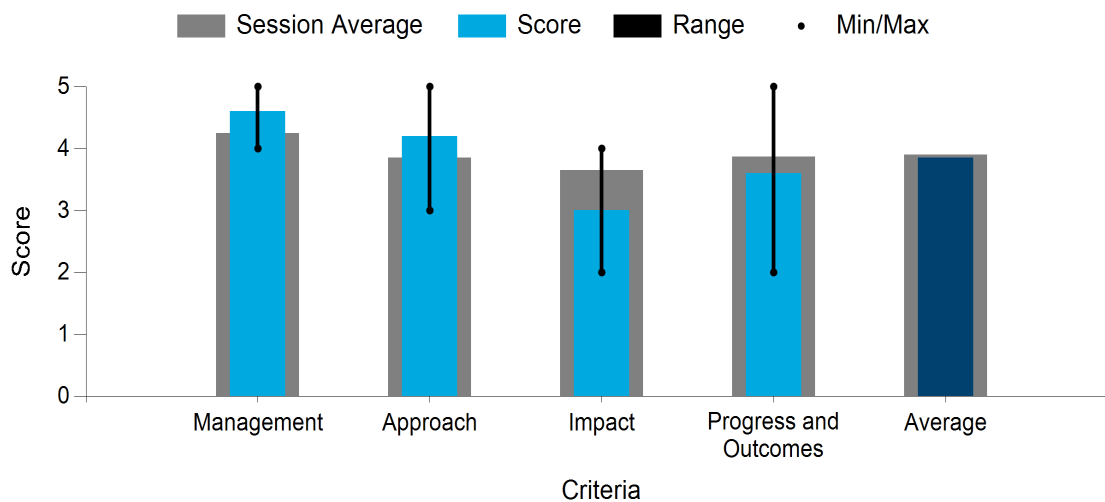
#### PROJECT DESCRIPTION

The goal of the project is to develop a low-energy CO<sub>2</sub> scrubbing technology using immobilized thermotolerant carbonic anhydrase to accelerate CO<sub>2</sub> capture by solvents with low regeneration energy. Fast-reacting monoethanolamine CO<sub>2</sub> absorption solvent requires high regeneration energy due to the high heat of absorption. Alternatively, more benign and sustainable solvents have a lower heat of absorption, but they react slower. Previous studies

with bench-scale and pilot testing have proven the ability of carbonic anhydrase to accelerate CO<sub>2</sub> absorption in alternative solvents in both dissolved-enzyme and immobilized-enzyme forms; however, process improvements are still needed to achieve energy reduction versus benchmark monoethanolamine. We are working on three parallel paths for (1) enzyme engineering to improve the enzyme robustness, (2) enzyme immobilization for process longevity, and (3) solvent evaluation for low-energy regeneration with the goal of improving CO<sub>2</sub> capture efficiency. The ultimate goal of the project is to demonstrate the technology at bench scale and support TEA and LCA enabling 20% energy reduction compared to the monoethanolamine reference case (at 90% CO<sub>2</sub> capture). This technology can be deployed to many industries that generate waste gases to capture CO<sub>2</sub> from biopower, fossil-based power plants, and biogas production and to upgrade natural gas, biogas, and CO<sub>2</sub> for revenues.

WBS:	5.1.3.103
Presenter(s):	Adam Bratis; Min Zhang; Zia Abdullah; Courtney Payne; Jessica Krupa
Project Start Date:	10/01/2018
Planned Project End Date:	05/30/2022
Total DOE Funding:	\$1,500,000

Average Score by Evaluation Criterion





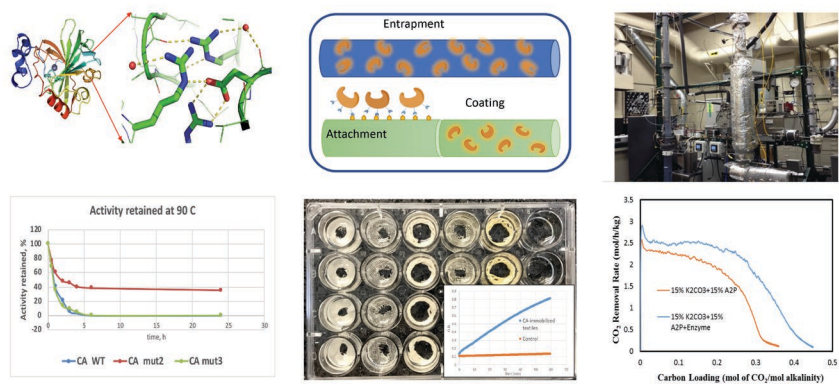


Photo courtesy of NREL

## COMMENTS

- The management plan is clear and involves an industry partner. Roles and responsibilities of each investigator are clear. The multidisciplinary approach and the use of a current industrial enzyme make sense. Although this approach seems to have good potential, the team needs to do a better job benchmarking their process to current CO<sub>2</sub> scrubbing technologies, in particular the economics of the process. The team has made good technical progress and seems on track to meet all project goals; however, it is not clear if they will meet the operating and capital-savings goal because no discussion is given.
- The project is a strong collaboration between two universities and industry that combines the strengths of each organization. Although there has been a delay for unspecified subcontracting reasons, the project is currently on track to meet the end-of-project goal.

**Strengths:** The team has made substantial progress on all three tasks, and the project is being managed effectively. The small-bench integrated carbon capture and sequestration (CCS) system provides critical data on performance under application-relevant conditions. The experiments with this system enable the team to assess whether the laboratory advances on the enzyme or immobilization work are achieving progress toward the overall goal. The initial results with the CCS show promise for the use of immobilized enzymes to improve CO<sub>2</sub> capture.

**Weaknesses:** It is unclear what the thermal stability requirement is for the immobilized enzyme. Given that the CCS testing indicates that an immobilized enzyme is required, it is important to clarify the temperature regime relevant for the immobilized enzyme to guide the engineering efforts. There was no information provided about how the enzyme is being immobilized and what strategies are being pursued to improve the durability and activity of the immobilized enzyme. The CO<sub>2</sub> absorption efficiency measured in the CCS needs to be clarified to indicate how it translates into energy savings on CO<sub>2</sub> capture, which is the project goal.

- This is a unique project in the BETO program focusing on carbon capture. Although it is different from other BETO projects in terms of scope, it fits into the BETO program. The project is based on the successes of previous work by the team, and it was not clear what breakthroughs were made from the past year's study. Even with the improved longevity, it is not clear that it is sufficient (40% activity

retained at 90°C for 24 hours?). Many parts of this study are valuable for the future design of carbon-capture systems, but it would be good to have a clear answer on whether cell-free enzymatic systems are suitable for carbon capture.

- This project focuses on developing an enzymatic carbon-capture system based on immobilized carbonic anhydrase enzymes that operates with reduced energy requirements compared to current carbon-capture technologies. The project approach is divided into three tasks focusing on engineering enzymes for increased robustness, immobilizing enzymes and laboratory-scale testing, and testing solvent compatibility and bench-scale testing. The performers seem on task to meet the project's performance targets. It is difficult to gauge the potential impact of this project because of a lack of TEA or comparisons with other state-of-the-art carbon-capture technologies, and incorporating such analyses and comparisons would substantially increase the project impact.
- This project incorporated cell-free enzymes into liquid sorbent CO<sub>2</sub> capture systems to reduce the energy needed for capture and release by 20%; however, this was a very preliminary study that showed only 40% retained activity >24 hours and a rapid activity loss within 5 hours. Overall stability of the system is an issue. I do not think the project or technology area should continue.

## PI RESPONSE TO REVIEWER COMMENTS

- We thank all the reviewers for their positive feedback by recognizing our strong collaborative efforts, and we appreciate the reviewers' concerns on the suitability/stability of using cell-free enzymatic systems for carbon capture. One major goal of the project is to de-risk the technology by improving the process stability and efficiency via enhancing enzyme robustness for achieving high CO<sub>2</sub> removal efficiency, which includes thermotolerance of the enzyme, solvent compatibility, and in-process longevity. We have made significant progress in improving the enzyme thermostability, we have developed effective immobilization methods, and we have identified relevant benign solvents. The operating temperature range for immobilized enzymes in the absorber column is between 40°C–60°C. Current carbonic anhydrase variants are showing stability up to 90°C, which will provide for minimal thermal degradation of carbonic anhydrase in the operating temperature range from 40°C–60°C. During the preparation and analysis of samples, laboratory evidence showed that after an initial significant activity drop, the leading enzyme candidate is remarkably stable at the 90°C condition. Considering that immobilization will allow the enzyme to be continuously reused, even an initial activity loss will be overcome by longevity in use. We are also in the process of investigating the reasons for the activity drop, and we will explore alternative enzyme production steps to mitigate this. When a solvent can capture more CO<sub>2</sub> (higher capture efficiency), it will take less energy to regenerate the solvent (strip off the CO<sub>2</sub>) on a Joule/per CO<sub>2</sub> mole basis. How much the improvement in capture efficiency versus energy penalty varies for each solvent system is strongly influenced by operating conditions, which is the reason we are proceeding to bench-scale testing in the validated reactor at the University of Kentucky Center for Applied Energy Research to obtain these important metrics. A general estimate is that a 10% improvement in CO<sub>2</sub> capture efficiency will translate to a 10% drop in regeneration energy. A TEA will be conducted as planned in FY 2022 Q2, with comparisons made to current state-of-the-art CO<sub>2</sub> capture systems to assess the impact of the technical improvement.