Rational Optimization of Microbial Processing for High Yield CO_2-to-Isopropanol Conversion

Cooperative Research and Development Final Report

CRADA Number: CRD-20-17114

NREL Technical Contact: Wei Xiong

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Contract No. DE-AC36-08GO28308

Technical Report
NREL/TP-2700-88680
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National Renewable Energy Laboratory
15013 Denver West Parkway
Golden, CO 80401
303-275-3000 • www.nrel.gov

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Cooperative Research and Development Final Report

Report Date: January 24, 2024

In accordance with requirements set forth in the terms of the CRADA agreement, this document is the CRADA final report, including a list of subject inventions, to be forwarded to the DOE Office of Scientific and Technical Information as part of the commitment to the public to demonstrate results of federally funded research.

Parties to the Agreement: Shell International Exploration and Production Inc.

CRADA Number: CRD-20-17114

CRADA Title: Rational Optimization of Microbial Processing for High Yield CO_2-to-Isopropanol Conversion

Responsible Technical Contact at Alliance/National Renewable Energy Laboratory (NREL):

Wei Xiong | wei.xiong@nrel.gov

Name and Email Address of POC at Company:

Jan Klok | Jan.Klok@shell.com (for Nicolas Tsesmetzis | nicolas.tsesmetzis@shell.com)

Sponsoring DOE Program Office(s): Office of Energy Efficiency and Renewable Energy (EERE), Bioenergy Technologies Office (BETO)

Joint Work Statement Funding Table showing DOE commitment:

Estimated Costs	NREL Shared Resources a/k/a Government In-Kind
Year 1 Year 2, Modification #1	$200,000.00 $100,000.00
TOTALS	$300,000.00

Executive Summary of CRADA Work:

This project focuses on the production of the fuel blendstock isopropanol using a CO_2-fixing Clostridium by metabolic engineering and process optimizations. The project will initiate from a baseline isopropanol producer and pursue isopropanol production at high carbon-conversion efficiency. We will lead engineering work by in-depth pathway analyses including thermodynamics optimization, enzyme expense analysis, metabolic robustness analysis, and -omics analysis. The isopropanol production will be optimized via genome editing followed by fermentation optimizations. This project will deliver a novel microbial process that efficiently converts waste CO_2 to isopropanol at ~g/L titer level within 18-months. This project will layout a solid knowledge basis and technology platform for renewable CO_2 valorization to bio-blendstock that help achieve Co-Optima and Shell's goals.

CRADA benefit to DOE, Participant, and US Taxpayer:

The CRADA project described offers several benefits to the Department of Energy (DOE), the Participant (Shell and the National Renewable Energy Laboratory), and U.S. taxpayers.

Benefits to DOE:

- Assists laboratory in achieving programmatic scope: The project aligns with DOE's mission to advance energy technology and promote sustainable energy. By fostering high-yield CO_2-to-isopropanol conversion, the project further propels the DOE's commitment towards sustainable biofuel production.
- Enhances the laboratory's core competencies: The project involved innovative methods of metabolic optimization and genomic engineering, contributing to the lab's expertise in these areas.
- Uses the laboratory's core competencies: The project required the use of advanced bioinformatics tools and expertise in enzyme analysis, proteomics, genetic engineering, and fermentation processes, all areas where the National Renewable Energy Laboratory excels.

Benefits to Participant:

- Advances Shell's long-term objectives: The research has significant potential to shift isopropanol production towards commercial viability, aligning with Shell's focus on developing sustainable energy resources.
- Intellectual property development: The novel microbial process that were developed during this project may hold significant intellectual property value for the participants.
- Enhances core competencies: The project contributed to Shell's and National Renewable Energy Laboratory's expertise in areas such as metabolic optimization, genetic engineering, and fermentation processes.

Benefits to U.S. Taxpayers:

- Enhances U.S. competitiveness: The successful implementation of the project could bolster the U.S.'s standing in the global biofuel market, potentially leading to job creation and economic growth.
- Encourages use of renewable energy: The project's goal of commercializing isopropanol production from waste CO_2 aligns with nationwide efforts to reduce dependence on fossil fuels and transition to renewable energy sources.
- Reduces greenhouse gas emissions: The conversion of waste CO_2 into isopropanol not only helps to recycle greenhouse gases, but it also contributes to efforts to reduce overall greenhouse gas emissions, benefiting the environment and potentially slowing the effects of climate change.
- Cost-saving: Once commercialized, the process could lead to more economical biofuel production, possibly resulting in cost savings for U.S. taxpayers in the long run.

Summary of Research Results:

Task 1: *Systematic pathway analysis in C. ljungdahlii for isopropanol production.*

Task 2: *Systematic genome engineering in C. ljungdahlii for isopropanol overproduction.*

Task 3: *Process optimization to improve isopropanol production under controllable autotrophic conditions.*

Task 4: *Map the neat and blended fuel properties when upgrading isopropanol to additional fuel products for light-duty and heavy-duty applications.*

This summary serves as a comprehensive encapsulation of our 18-month endeavor, entitled "Rational Optimization of Microbial Processing for High Yield CO_2-to-Isopropanol Conversion." In a collaborative effort by Shell and the National Renewable Energy Laboratory, our project was centered around enhancing the sustainable production of isopropanol, a bio-blendstock identified by Co-Optima, from waste CO_2. This was achieved by employing acetogenic bacteria, a special group of microbes capable of fermenting syngas, and leveraging cutting-edge microbe design, metabolic engineering, and process optimization methodologies.

Our technical strategy unfolded in three stages. To begin, we utilized advanced metabolic optimization techniques, including thermodynamic modelling, enzyme cost analysis, metabolic robustness analysis, and high-throughput -omics profiling (**Task 1**). We incorporated our bioinformatics tools for an exhaustive analysis of isopropanol production in acetogen. This furnished valuable insights into thermodynamic feasibility, kinetic optimality, and pathway stability. By coupling these insights with quantitative proteomics analysis, we were successful in identifying potential thermodynamic or kinetic impediments that curtail the conversion of CO_2-to-isopropanol. The representative results of computational modeling please see Figure 1 and Figure 2.

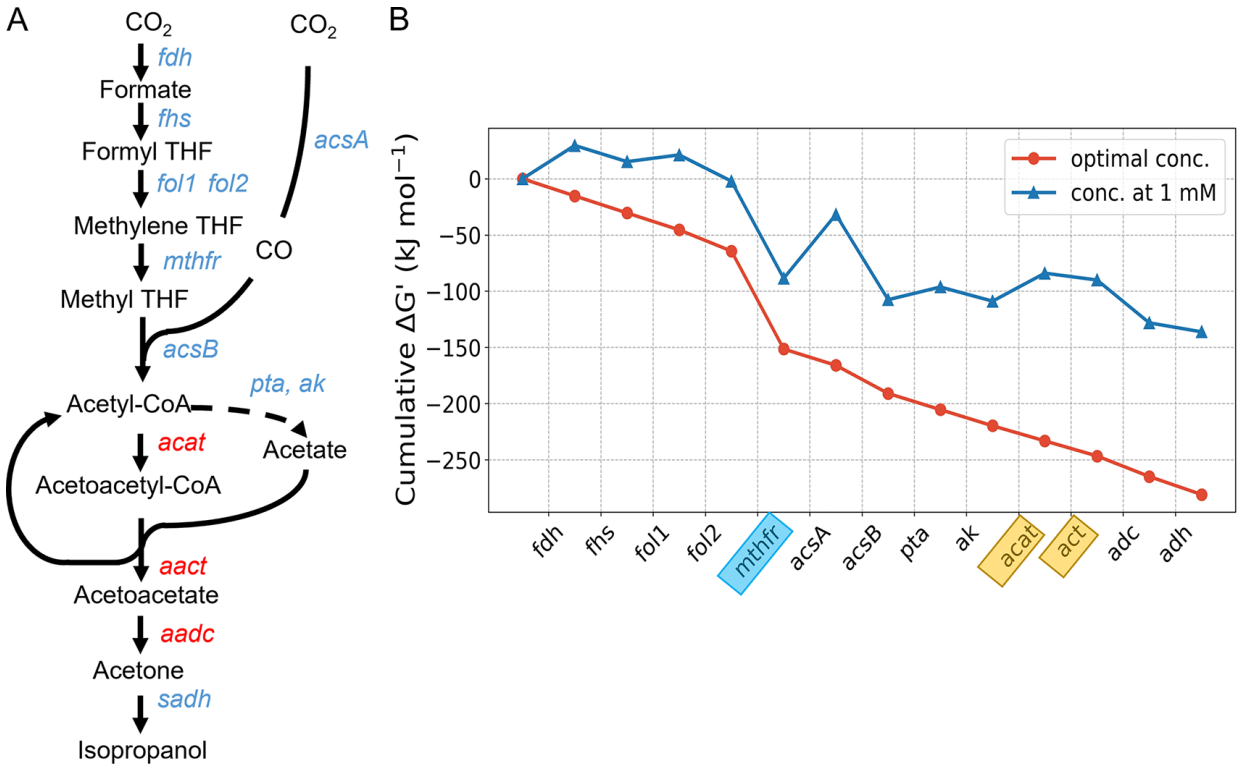

Figure 1 The thermodynamic feasibility of IPA biosynthesis from the acetogenic Wood-Ljungdahl pathway (WLP). (A) The WLP and isopropanol (IPA) pathway. Heterologous enzymes engineered into C. ljungdahlii for IPA production are shown in red. (B) The thermodynamic driving force of the pathway is presented as the cumulative sum of reaction Gibbs energies, ΔG′. The blue line denotes standard Gibbs energies with all metabolite concentrations fixed at 1 mM, and the red line denotes Gibbs energies when the highest reaction ΔG′ is iteratively maximized in the negative direction. The most and least thermodynamically favorable reactions are shown in blue and yellow boxes, respectively.

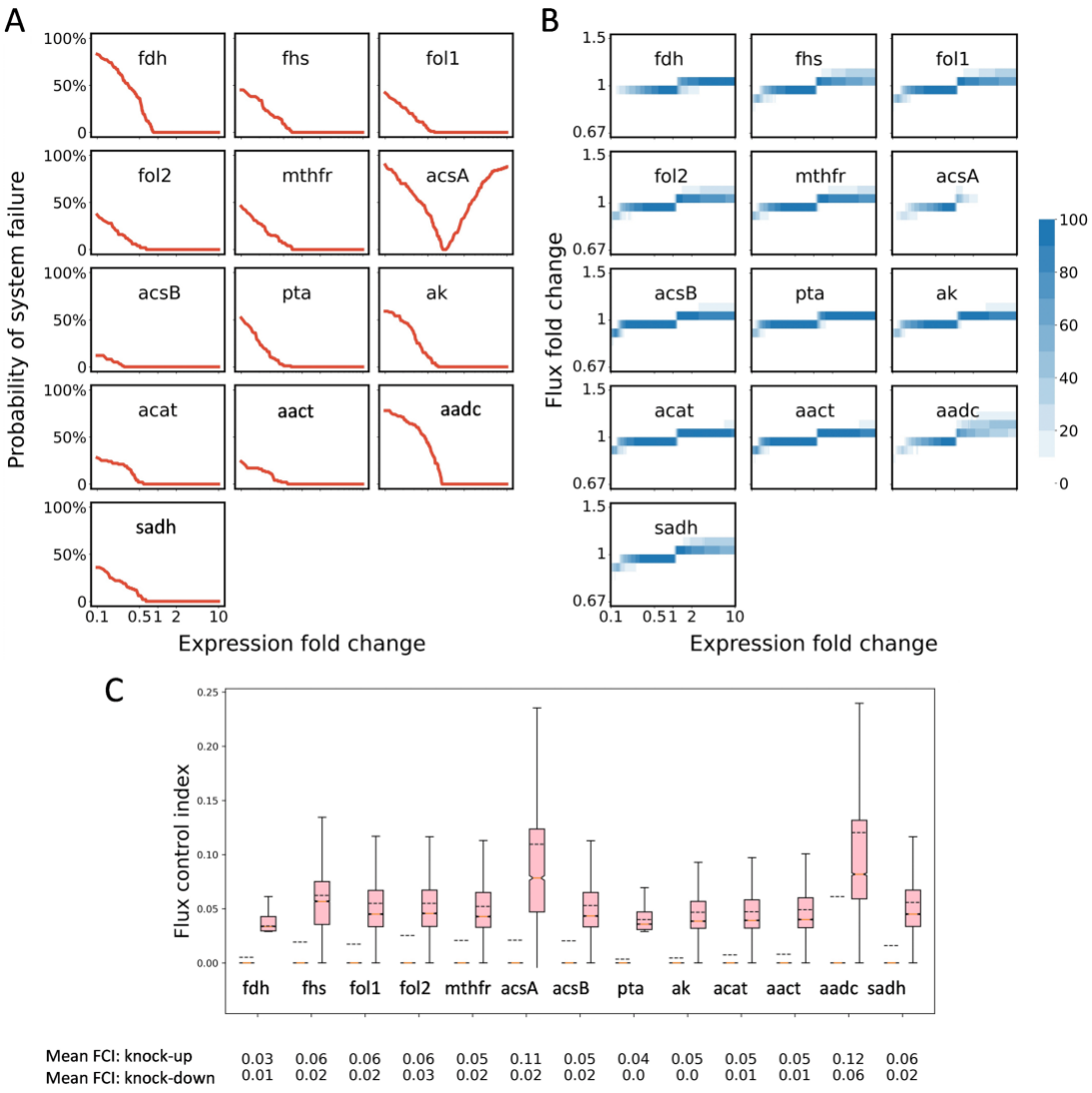

Figure 2 Robustness analysis (A) and flux control indexes (B and C) of the IPA biosynthetic pathway. (A) Pathway robustness is represented by the probability of system failure at various fold changes of enzyme expression levels over the reference state. A pathway is considered to be entering system failure when any intermediate is depleted or overaccumulated, and the probability of system failure is calculated as counts in an ensemble of 100 models which were generated with log-uniformly sampled kinetic parameters from the feasible spaces, meanwhile being subjected to the same flux distribution as the reference state. (B) Flux fold changes by enzyme perturbation were evaluated by an ensemble of 100 models which were generated with log-uniformly sampled kinetic parameters from the feasible spaces, meanwhile being subjected to the same flux distribution as reference state. The results are presented as a set of heatmaps, and the color indicates the number of models of corresponding flux fold change at some enzyme expression level for each enzyme. (C) Based on the visualized results in panel B, flux control indexes (FCIs) were also calculated to quantitatively describe the extent of pathway flux change caused by enzyme perturbations. FCIs for downregulation and upregulation of enzyme expression are plotted in blue and pink boxes, respectively. The mean of the FCI is presented as a dashed line and listed at the bottom (first row denotes upregulation FCI and second row denotes downregulation FCI).

In the second stage (**Task 2**), we focused on enhancing isopropanol production through genome engineering, informed by the targets identified previously. We employed genetic engineering tools to modulate gene expression levels, and evaluated the efficacy of iterative genetic engineering approaches for optimal expression of essential genes involved in CO_2-to-isopropanol conversion. This allowed us to evaluate the effects of gene modification on cell viability and isopropanol production, enabling us to pinpoint the most effective combinations of gene targets for maximizing synthetic isopropanol pathway activity (see Figure 3 for the scheme of a genetic engineering procedures and results).

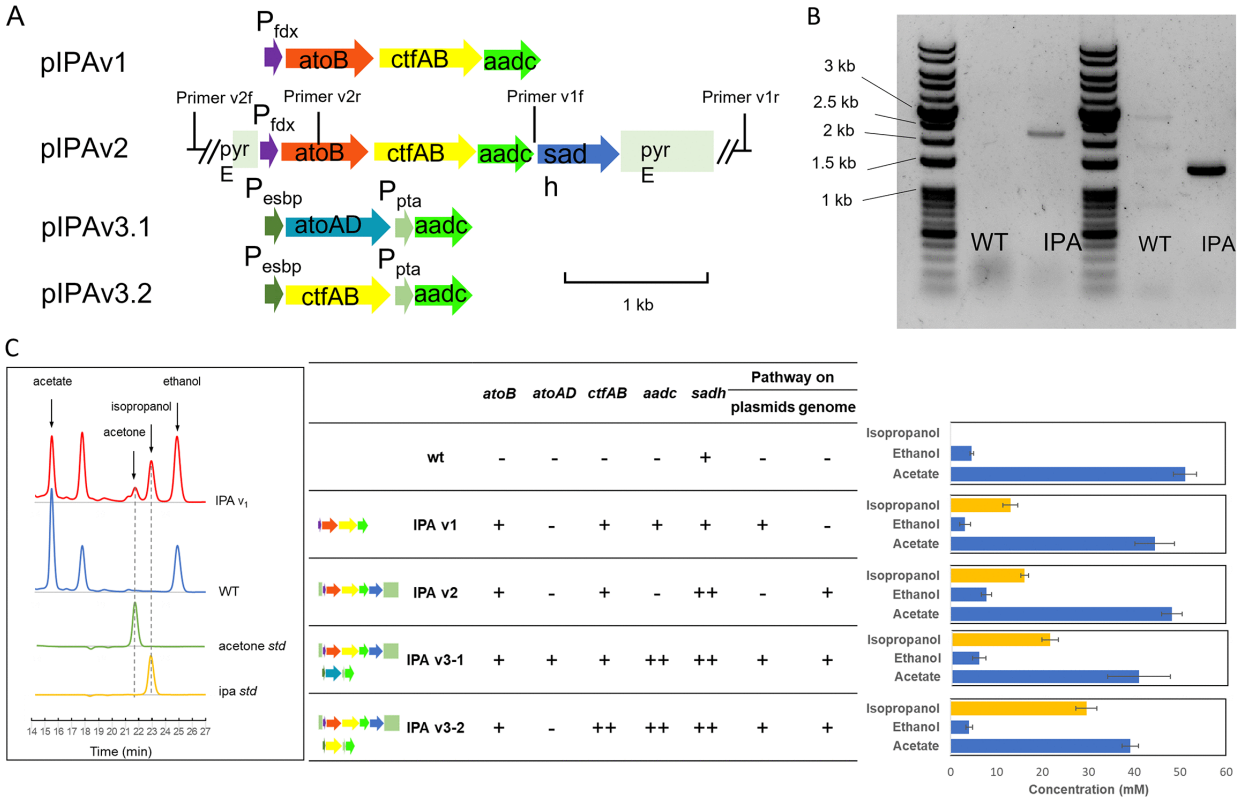

Figure 3 Pathway constructions for IPA biosynthesis. (A) Gene cassettes and expression components on the constructs. (B) PCR results indicating that the IPA pathway on pIPAv2 has been integrated into the genome on the pyrE locus. The PCR templates are genomic DNA from the wild-type C. ljungdahlii (labeled as WT) and IPA v2 strain (labeled as IPA). PCR products using primer v1f- v1r (left two lanes) and primer v2f-v2r pairs (right two lanes) are shown on the gel picture, respectively. The locations the primers are labeled in panel A. (C) IPA-producing strains and their IPA productivities. Shown on the left are the HPLC profiles of the IPA v1 strain and wild-type broths in comparison to the chemical standards. Dashed lines denote the retention time of IPA and acetone. The presence of IPA pathway genes in various IPA strains and the corresponding IPA titer are shown in the middle and right, respectively. "–" indicates that the gene was not present in the strain; "+" indicates one copy of the gene; "++" indicates two copies in the strain.

Lastly, we chose suitably modified host strains and finetuned the fermentation process in bench-top gas bioreactors (**Task 3**). This involved adjusting various parameters, such as gas compositions, gas flow rate, stirring rate, pH, and fed-batch strategies, culminating in a novel microbial process that efficiently converts waste CO_2 to isopropanol with improved titers (see Figure 4).

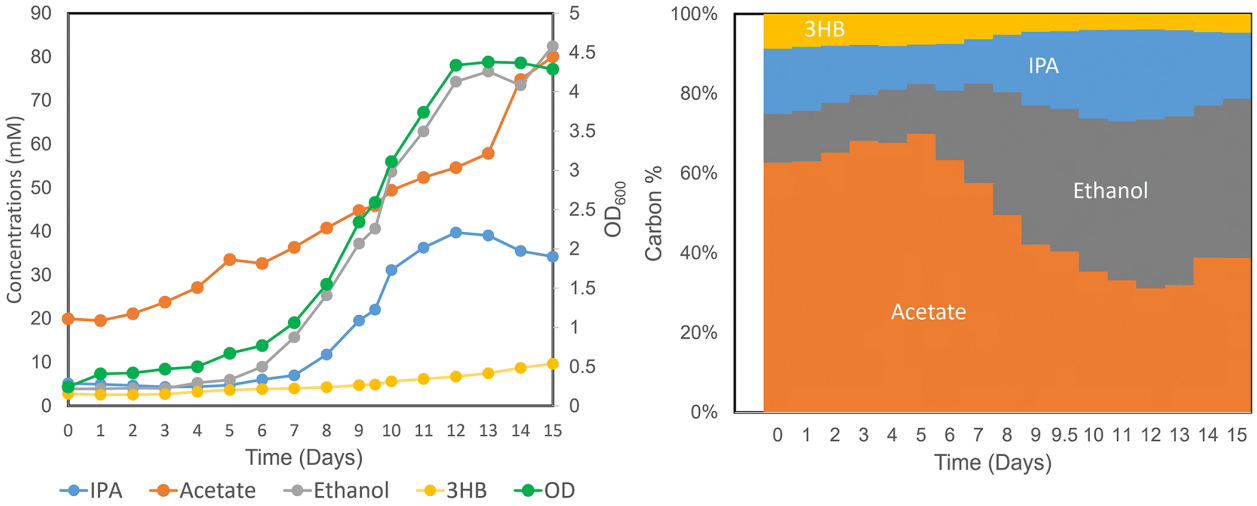

Figure 4 Time course of the IPA v3-2 strain grown autotrophically in a 2-L gas-fermenting bioreactor sparged with syngas (CO/CO2/H2). Shown on the left are the cell OD600 and production of IPA, acetate, ethanol, and 3-hydroxybutarate over time. Shown on the right are the changes of carbon molar yield in the products.

The project has resulted in a significant increase in isopropanol production compared to the initial version, with the engineered strain producing isopropanol in grams per liter under gas-fermenting conditions, using CO, CO_2, and H_2 as substrates. This work has successfully propelled the field of renewable CO_2 valorization to bio-blendstock, nudging isopropanol production towards commercial viability, and contributing to the long-term objectives of both Co-Optima and Shell. Importantly, our project has laid a robust groundwork for future research in this field, illustrating a viable path to recycle greenhouse gas (GHG) CO_2 into isopropanol via CO_2-fixing acetogenic bacteria, aligning with Co-Optima's target of a 60% reduction in greenhouse gases.

In summary, our work firmly establishes the potential of rational design of microbial processing to substantially boost the conversion of waste CO_2 to valuable bio-blendstocks like isopropanol. We are confident that our findings mark a promising step towards a sustainable bioeconomy, bolstering global efforts to curb greenhouse gas emissions and encourage the use of renewable energy sources.

Completing the summation, our work firmly establishes the potential of rational design of microbial processing to substantially boost the conversion of waste CO_2 to valuable bio-blendstocks like isopropanol. (Extensive results can be found in the reference)

Task 4: *Map the neat and blended fuel properties when upgrading isopropanol to additional fuel products for light-duty and heavy-duty applications. (Period: M12-M18)*

This task was initially created to enhance our understanding of fuel properties. However, due to the unavailability of the NREL expert for this particular task, all partners agreed to redirect their efforts primarily towards Tasks 1-3.

References:

Jonathan Lo†, Chao Wu†, Jonathan Humphreys, Shrameeta Shinde, Xin Wang, PinChing Maness, Nicolas Tsesmetzis, Wei Xiong*. Thermodynamic and Kinetic Modeling Directed Pathway Redesign for High-Yields Isopropanol Production in a Gas-Fermenting Bacterium. **mSystems**. DOI: https://doi.org/10.1128/msystems.01274-22

Subject Inventions Listing:

None.

ROI #:

None.