DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

Advanced Biofuels and Bioproducts Process Development Unit (ABPDU) Operations, Lawrence Berkeley National Laboratory (LBNL)

TECHNICAL TASKS 2 and 3

04/05/2023

Conversion Team

Deepti Tanjore and Eric Sundstrom

Lawrence Berkeley National Laboratory

ABPDU

TRANSITION TO TASK 2

Learning from Data for Predictive Scale-up of Biofuel Technologies

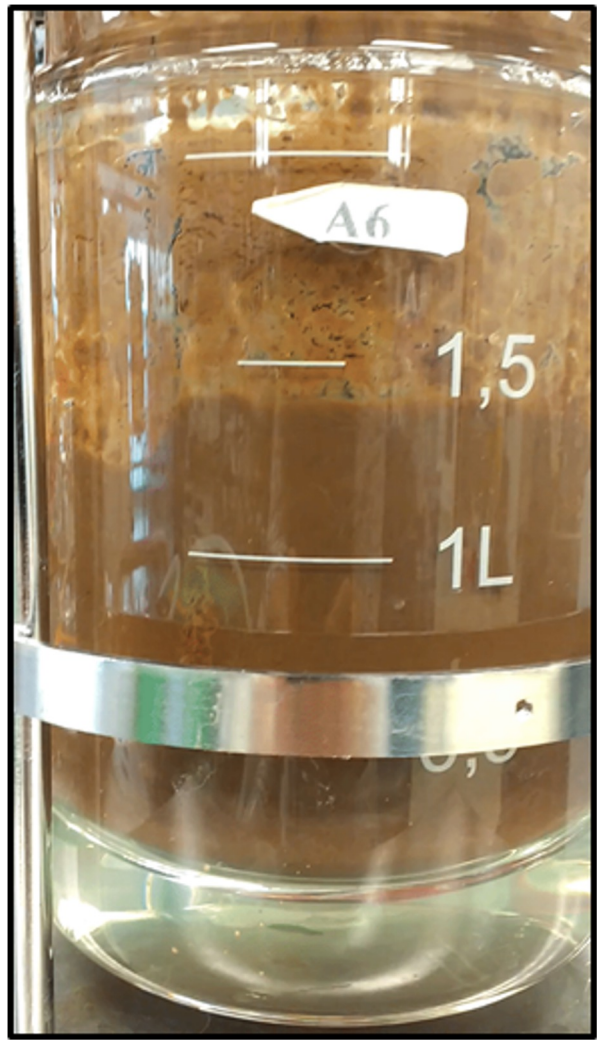
04/05/2023

Conversion Team

Deepti Tanjore

Lawrence Berkeley National Laboratory

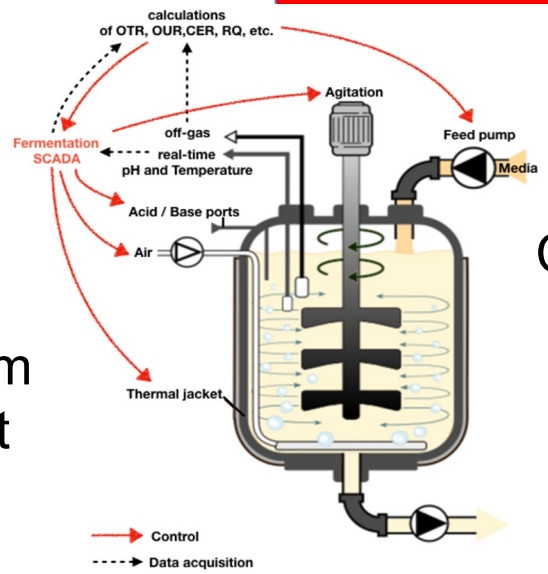
Technical Task Overview
Task 2: Learning from Data for Predictive Scale-up of Biofuel Technologies



Technical Task Overview
Task 2 – Microbial Image Processing

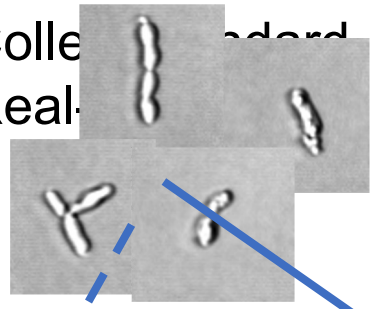
Self-Driving Bioreactor Operation with Biological Changes

Collect standard off-line Data and novel Data, e.g. –omics



Perform Fermentat

Collect standard and novel Real-time Data



Analyze Real-time Data

Control the Bioreactor

Microl ure Optimization Algorithm

Process Engineer

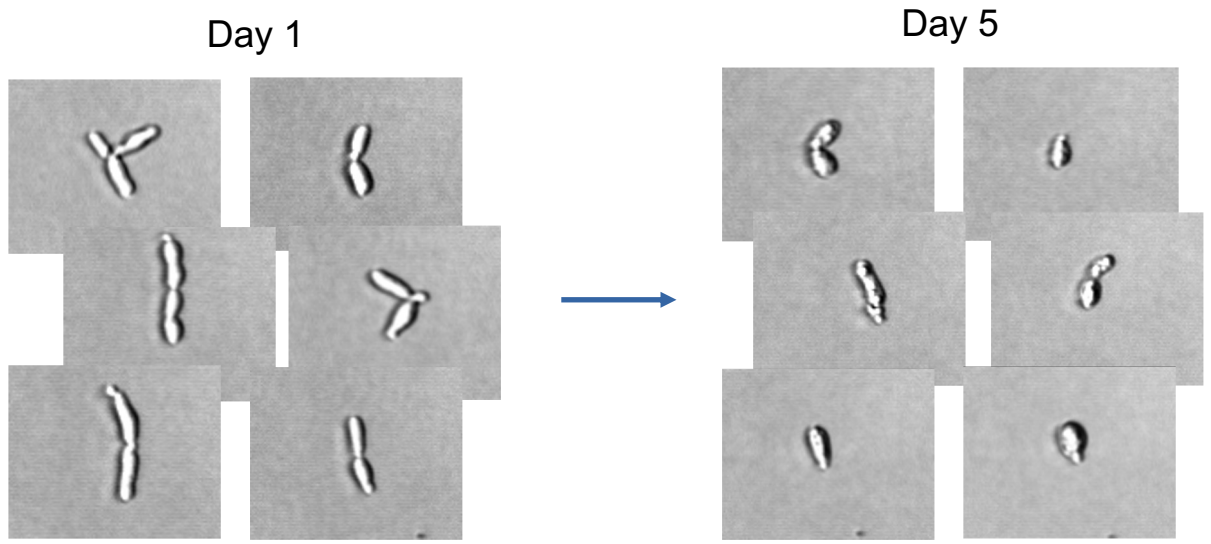
Microbial Experience and Heterogeneity Intuition "Rules"

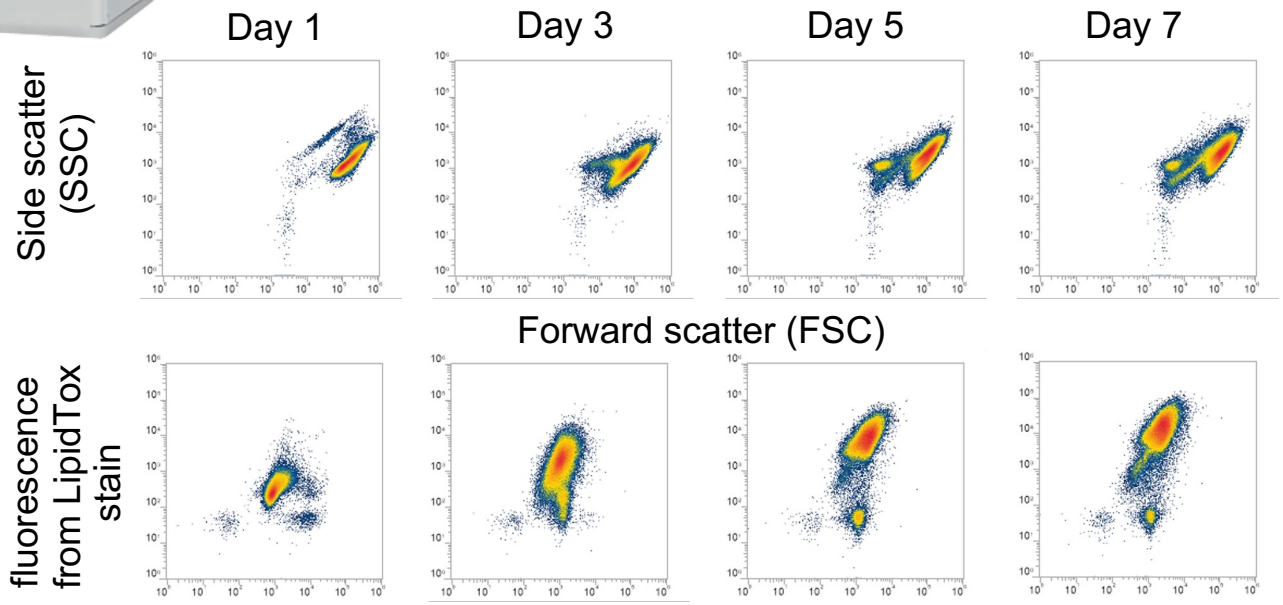
Off-cycle Data Analysis and Documentation

Progress and Outcomes
Task 2 – Microbial Image Processing

Developing Imaging as a Process Analytical Tool

Attune CytPix Flow Cytometer
Thermofisher





Progress and Outcomes
Task 2 – Schedules and Milestones

Milestone Description	Completion Criteria	Date	Milestone Type	Status
Complete necessary hiring to initiate process research activities	Complete postdoc hiring. We are now hiring internal data scientists for 50% time	12/31/2022	Quarterly	Complete
Complete purchase and commissioning of new equipment as suggested by industry collaborators	Purchase and install at least two new equipment capabilities per previous industry requests Flow cytometer has been procured, installed, and commissioned.	09/30/2022	Quarterly	Complete
Apply images and subpopulations' data from flow cytometry to AI models for detection of unanticipated events	Predict at least a single unanticipated event by correlating morphological changes of cells to process conditions in bioreactor	09/30/2023	Annual	Ongoing
Predict anticipated/ unanticipated events by applying rule-based or third wave AI/ ML methods and suggest an intervention to improve fermentation conditions	Predict at least a single anticipated/ unanticipated event.	09/30/2023	Annual	Ongoing
Deliver three conference or academic presentations	Publish at least one paper/ public dataset	09/20/2024	End-of-Project	Ongoing

Impact
Task 2 – Microbial Image Processing

Self-driving bioreactor concept was developed after Deepti Tanjore led a team to participate in DOE I-Corp in 2018. Over 92 companies were interviewed and expressed a strong desire for “high throughput” capacity in bioreactors.

Excited about the self-driving bioreactor as we (IFF) have to go back to drawing board when culturing novel organisms. This technology will be very applicable to microbiome- related work
- Luis Cascão Pereira, Global R&D Ventures Director at IFF during ILD 2023

Analytical capabilities studying live cells are important
- Chong Wing Yung, Associate Director of Global University Relations and External Research (CTO Staff), Agilent during ILD 2023

TRANSITION TO TASK 3

Biomanufacturing Using Gaseous Feedstocks

04/05/2023

Conversion Team

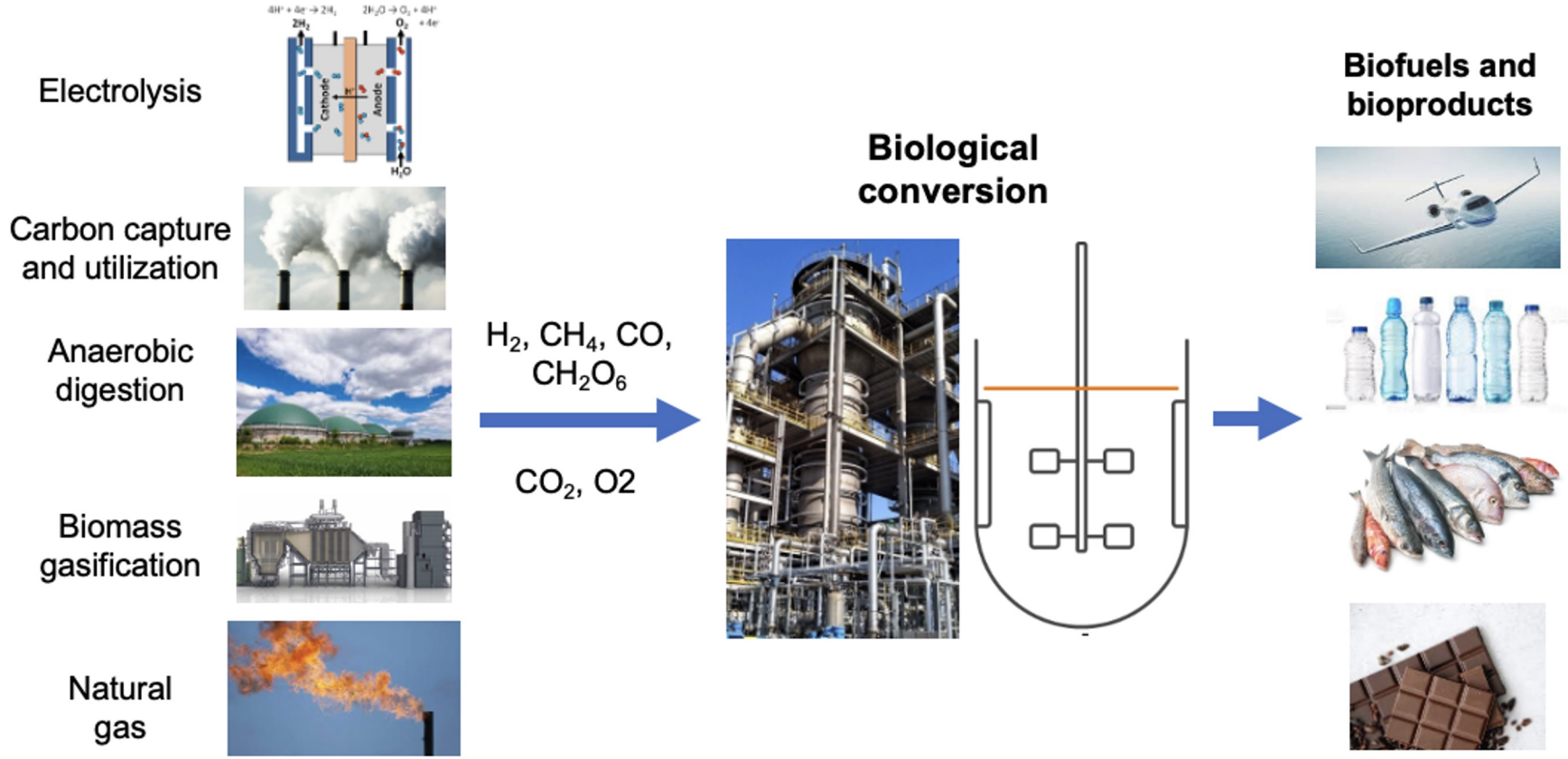
Eric Sundstrom

Lawrence Berkeley National Laboratory

Technical Task Overview
Task 3: Biomanufacturing Using Gaseous Feedstocks

Subtask 3.1: Development of intensified fermentation capabilities for aerobic gas fermentations.

Subtask 3.2: Documentation of best practices for safe and effective scale-up and scale-down.



Bioconversion of gaseous feedstocks aligns with a breadth of BETO and DOE priorities: biomass utilization, CO_2 utilization, and generation of SAF and sustainable materials

Approach: Motivation

Key takeaways from industry outreach

- *Lack of effective scale-down models:*
  - Low gas transfer
  - Variable partial pressures
  - Low gas volumes
  - Limited bioreactor controls
- *Major barriers to safe operation:*
  - Flammability
  - Pressure
  - Gas toxicity
- *Overall lack of standard equipment and best practices:*
  - No off-the-shelf equipment available
  - No established CMO or CRO facilities
  - Best practices for equipment installation, process development, and safety are proprietary and inaccessible

Mission: Develop a state-of-the-art public sector resource for gas fermentation development

Extensive outreach conducted: Multiple stakeholder requests for a national lab gas fermentation capability

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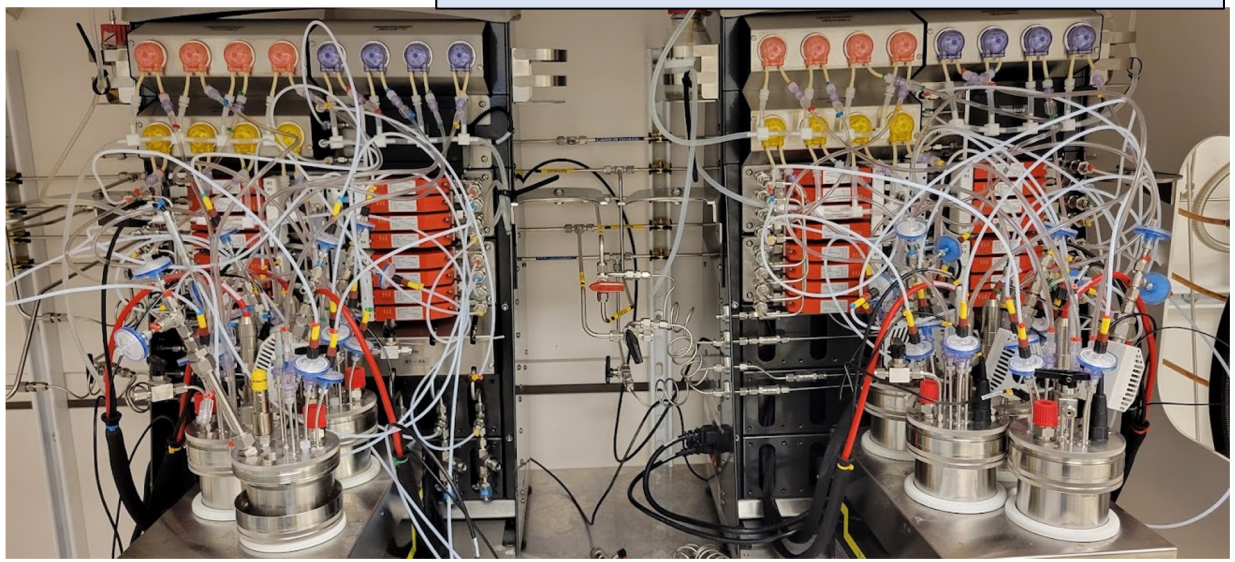
Approach: Schedule and Milestones

Milestone Description	Completion Criteria	Date	Milestone Type	Status
Complete necessary hiring to initiate process research activities	Complete postdoc hiring	12/31/2022	Quarterly	Complete
Complete purchase and commissioning of new equipment as suggested by industry collaborators	Purchase and install at least two new equipment capabilities per previous industry requests	9/30/2023	Quarterly	Ongoing
Complete gas fermenter commissioning and necessary safety approvals	Initiate ethane fermentations for the iMicrobes Agile BioFoundry DFO	9/30/2023	Quarterly	Ongoing
Successfully onboard both aerobic methane and hydrogen conversion in pressurized bioreactors	Demonstrate a >5x improvement in both titer and rate for each process when transitioning from serum bottles to bioreactor.	9/30/2023	Annual	Ongoing
Demonstrate successful operation of intensified aerobic gas conversion processes at above-ambient pressures.	Demonstrate hydrogen and methane conversion in 500mL pressurized fermenters to achieve >10g/L dry cell weight (task 3.1), publish best practices for safe and effective scale-up and scale-down of aerobic gas fermentations (task 3.2)	06/30/2024	Go/No-go	Ongoing

Progress and Outcomes
Hiring and Bioreactor Commissioning

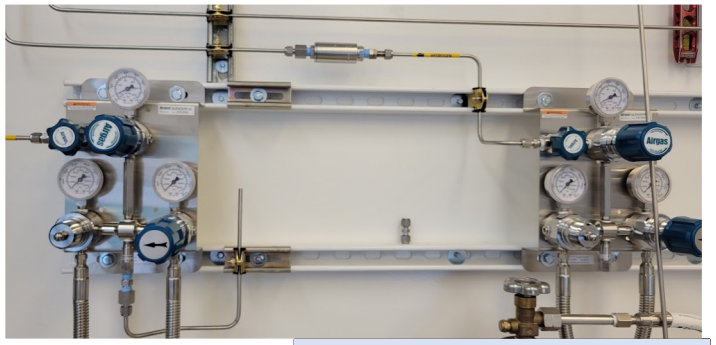
Bioreactor Commissioning:

- **8 x 500 mL and 1 x 20 L bioreactors procured in FY22 with $700k in LBNL funding**
- Stainless vessels, back-pressure control to 5 bar
- Installed in ventilated enclosure with separated spargers, gas monitors
- Multi-gas manifold installed to supply H_2, O_2, CO_2, CO, CH_4, C_2H_6, N_2
- Safety approvals for H_2, CH_4, C_2H_6 in aerobic pressurized fermentation, CO approval ongoing
- Additional requested capabilities procured for installation in FY23: H_2 generator, off-gas GC



20 L vessel

Five-gas manifold



Automated changeover valves

New Personnel

Sara Tejedor Sanz
Senior Process Engineer

Basem Zakaria
Postdoctoral Scholar

AB PDU

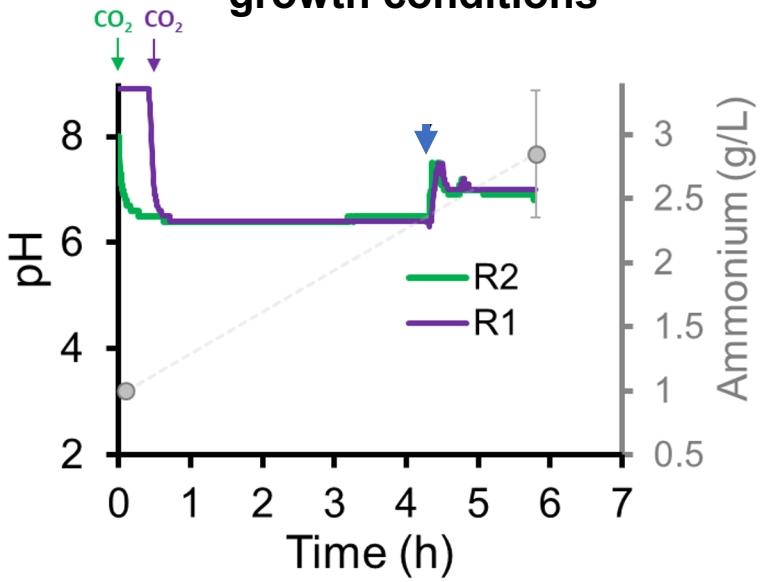
Progress and Outcomes
Process Onboarding for H_2 and CH_4 Conversion

Example processes for methane and hydrogen conversion will be leveraged to develop best practices in the public domain, leveraging engineered strains available from LBNL and BETO programs

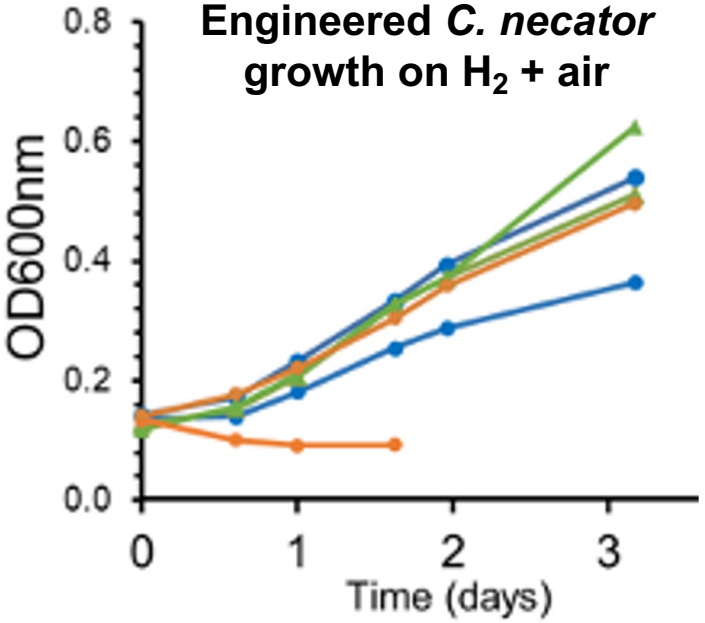
Goal: develop generalizable protocols to achieve >5x increases in both rate and titer vs serum bottle cultivation

- Two processes in active bioreactor testing:
  - Aerobic utilization of hydrogen and atmospheric CO2 with *C. necator*
  - Anaerobic conversion of H_2 and CO_2 to VFAs with microbial consortia
- Two additional processes currently in development:
  - Methane conversion to malonic acid
  - Methane conversion to rhamnolipids
- 5x improvement in growth rates vs serum bottles already documented for *C. necator* fermentation

pH impacts of high CO_2 growth conditions



Engineered *C. necator* growth on H_2 + air



Impact
Accelerating Time to Market for Industrial Fermentations

Two industry collaborations underway – additional projects in development

Checkerspot Pressurized Fermentations

- Simulating manufacturing-scale aerobic fermentation in pressurized miniature bioreactors
- Heterotrophic, pressurized fermentations
- <10% variation from 500mL to 300L across all key metrics

BioMADE

Checkerspot®

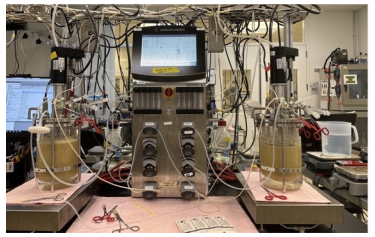
AB PDU

Industrial Microbes ABF DFO

- Predictive scale-up for ethane fermentation to P3HP

Ethanol: Bench scale

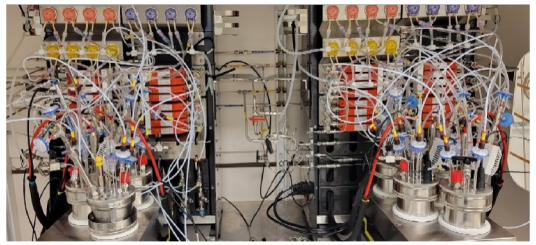
Current SOT – tech transfer



Ethanol : Pilot scale

Recommended strain and process improvements

Ethane: Bench scale

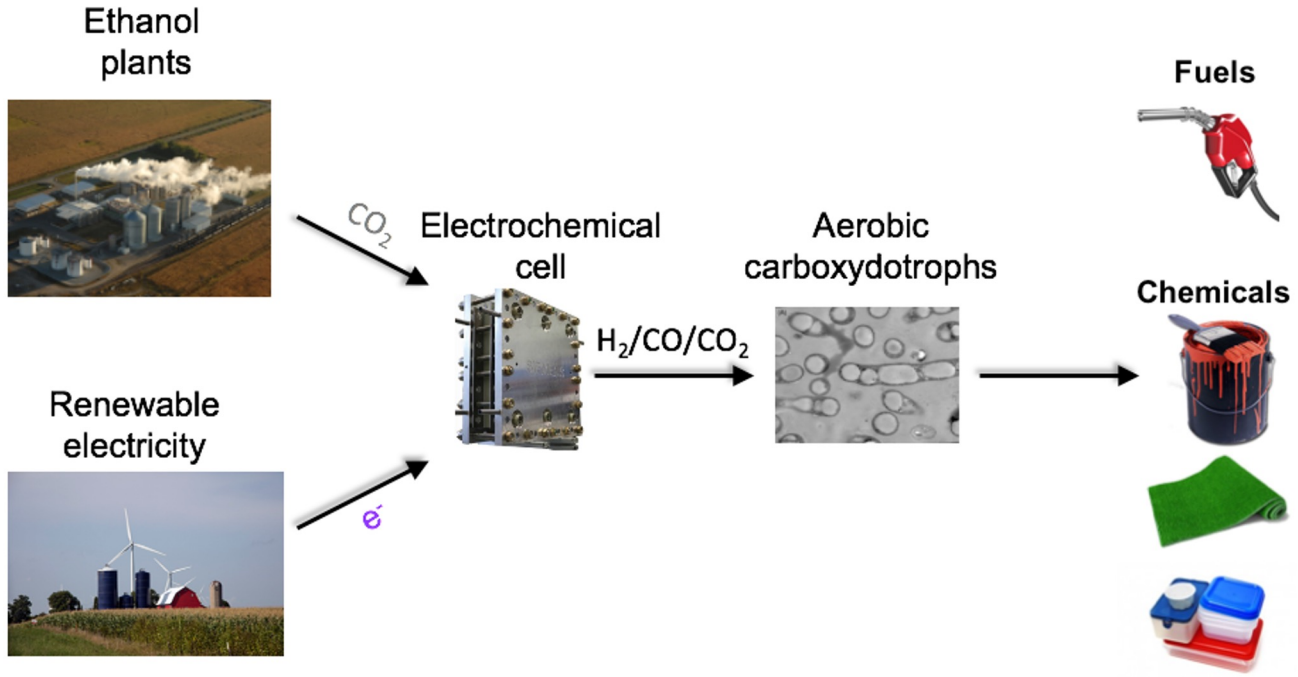


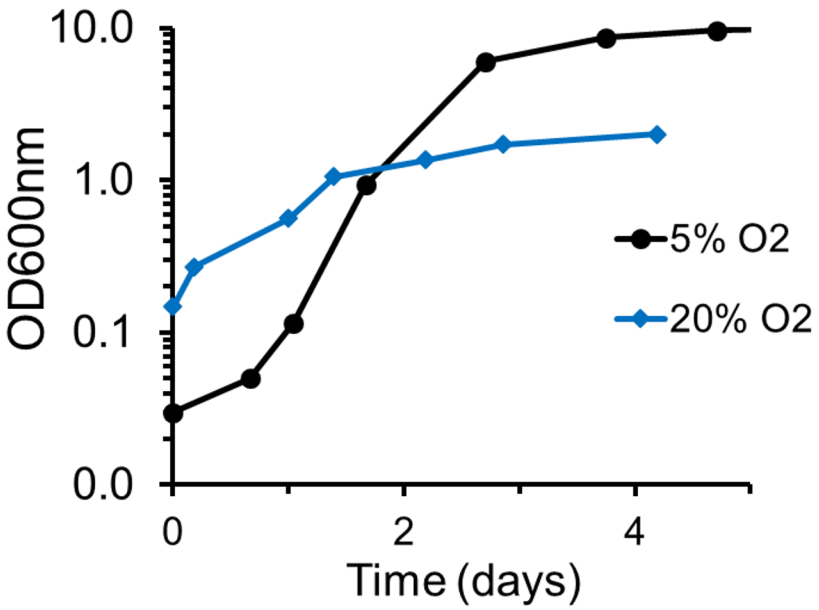
Ethane to 3HP Commercialization

Impact
Providing Differentiated Capabilities to Core BETO Programs

BETO CO_2 Reduction and Upgrading Consortium

- Direct utilization of gas fermentation capabilities developed under ABPDU AOP
- Coupling electrochemical syngas with aerobic bioconversion to sustainable aviation fuels
- *H. Pseudoflava* growth on H_2 / CO_2 / O_2 in pressurized bioreactors currently at 15 g/L DCW – 150x max literature values for bubble columns





Summary

- Significant industry demand identified for a comprehensive public facility addressing gas fermentation process development
- Bioreactor Installation, safety approvals, hiring, and commissioning complete to execute pressurized, aerobic gas fermentations
- Process onboarding complete for two hydrogen fermentation processes, methane fermentation onboarding ongoing
- Capabilities currently leveraged to support two industry collaborations and one BETO consortium – additional collaborations in development
- Continued success will enable rapid de-risking of new gas fermentation technologies with broader dissemination of best practices and lessons learned