Agile BioFoundry

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

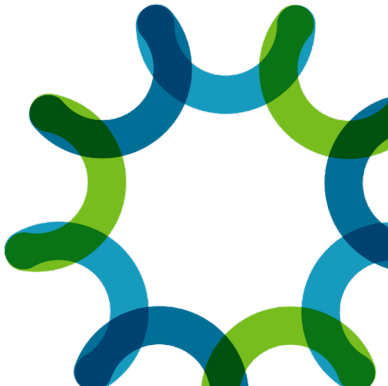
BIOENERGY TECHNOLOGIES OFFICE

DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

Deep Learning for Process Improvement and Predictive Scale-Up of Gas Fermentation

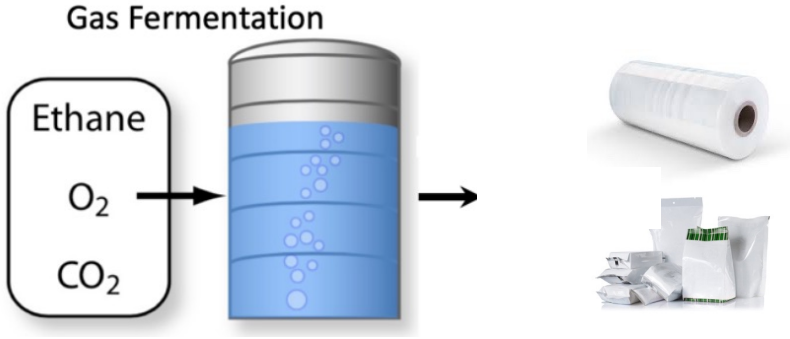
April 5, 2023
Agile BioFoundry Directed Funding Opportunities

Eric Sundstrom
Lawrence Berkeley National Laboratory



Project Overview

Objective: Leverage multi-scale bioprocess development, high-throughput proteomics, and deep learning models to achieve predictive scale-up for aerobic gas fermentation processes



Project team:

Technology platform

Elizabeth Clarke

Amanda Beverly

Bioprocess development

Eric Sundstrom (PI)

Carolina Barcelos

Proteomics

Chris Petzold

Deep learning

Phil Laible

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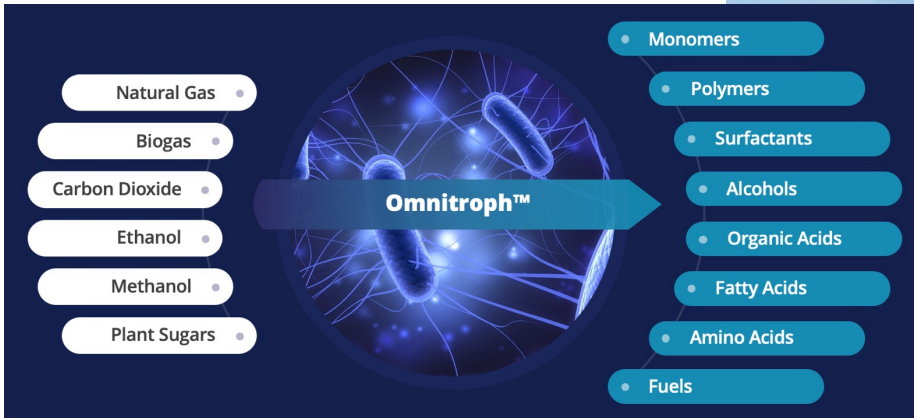
1– Approach: Ethane to P3HP

Industrial Microbes platform: Methane monooxygenase engineered into *E. coli* maximizes both metabolic flexibility and genetic tractability

Initial application: Ethane to polyhydroxypropanate (P3HP)



Ethane conversion

- Available as off-pipeline flared natural gas
- Improved gas transfer and reduced cooling requirement vs methane
- Beachhead for C1 conversion

P3HP

- Biodegradable alternative to polyethylene
- Applications in packaging, films, and agriculture

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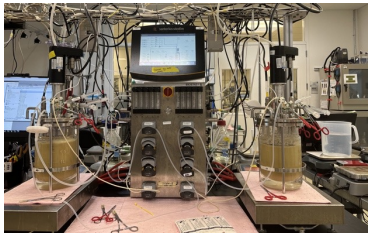
1– Approach: Bioprocess Development

- **IM bioprocess challenges**
  - Process safety
  - No off-the-shelf equipment available
  - No scale-up CMO or CRO facilities available
  - Gas delivery and gas transfer
  - Limited prior art
- **Bioprocess approach**
  - Leverage ABF capabilities: bench scale gas fermentors and 300L pilot capabilities
  - Couple with proteomics and deep learning approaches to predict scale-up performance

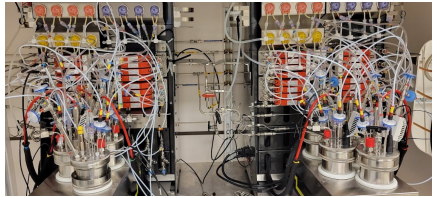
Ethanol: Bench scale
Current SOT – tech transfer



Ethanol : Pilot scale
This project

Recommended strain and process improvements

Ethane: Bench scale
This project



Ethane: Demo and commercial scale

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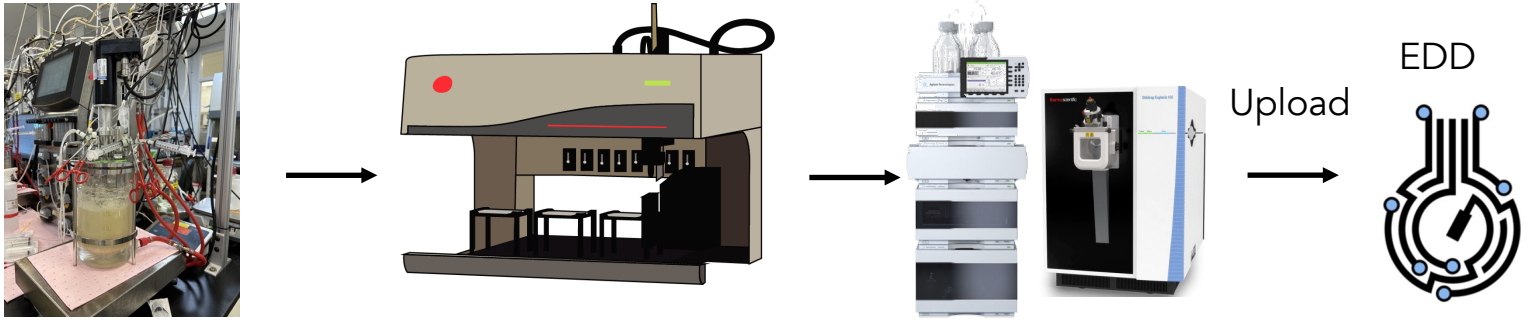
1– Approach: High throughput proteomics



Fermentations and sampling

Automated protein sample preparation

High-throughput proteomic data acquisition

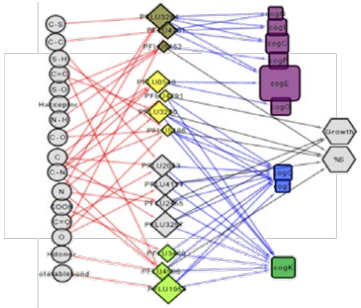
- Sample preparation in 96-well plate format for reproducibility, low variance, and efficiency
- Quantitative proteomic data for over 1200 proteins in each sample
- Analysis of both on and off-pathway expression
- Data uploaded to the Experiment Data Depot (EDD) for the Learn team

1– Approach: Predictive modeling

Varied bioreactor parameters in challenge/recovery experiments (for safety & productivity)

Performance and proteomics datasets

Layered physical/ metabolic/ regulatory models



ML/AI approaches propose additional process challenges

Strain engineering and bioreactor process parameters modified to optimize safe, stable, scaled fermentation

2 – Progress and Outcomes

Six-month goals: Q2 FY23

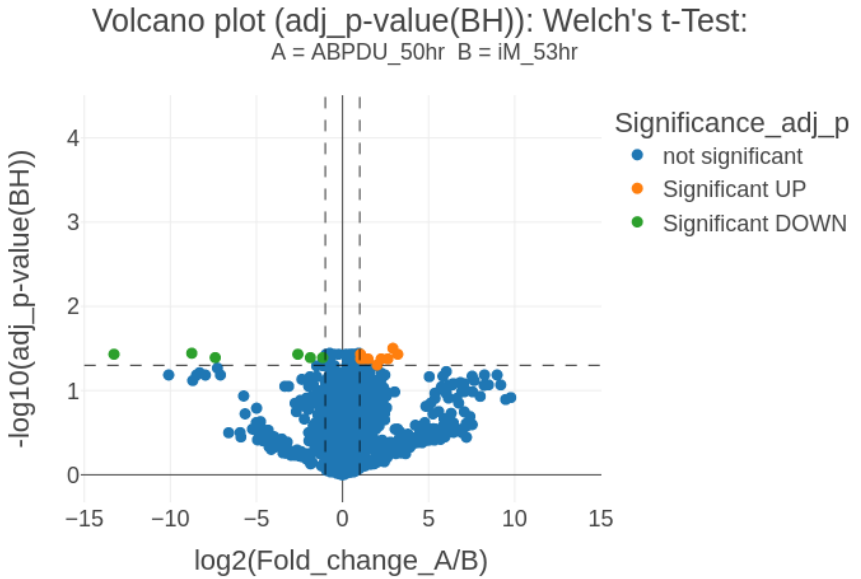
- Evaluate baseline and execute tech transfer
- Harmonize TRY metrics to +/-15% between IM and LBNL

Final project goals: Q4 FY24

- Leverage model predictions to achieve 3x improvement over baseline TRY
- Demonstrate 300L ethanol scale-up within 10% of improved TRY

Current status

- Bench-scale technology transfer ongoing
- Proteomics baseline comparison completed
- Data harmonization and data sharing platforms established
- Initial ML analyses proceeding based on tech transfer training data



High throughput evaluation of protein abundance reveals significant similarity between 2L campaigns at LBNL and IM

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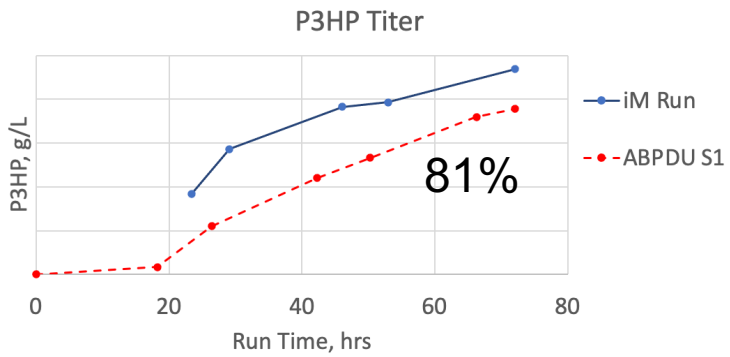
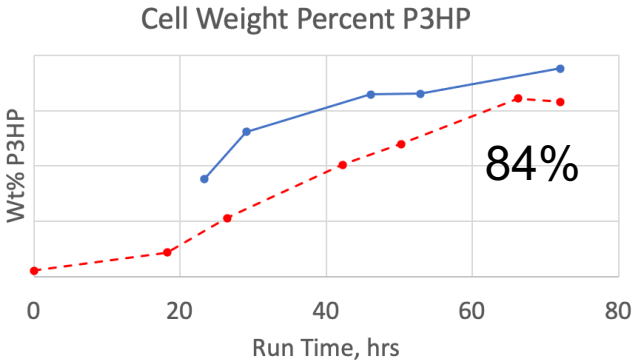
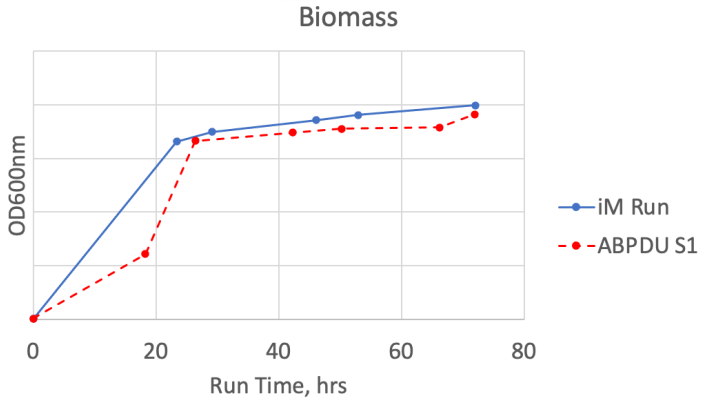
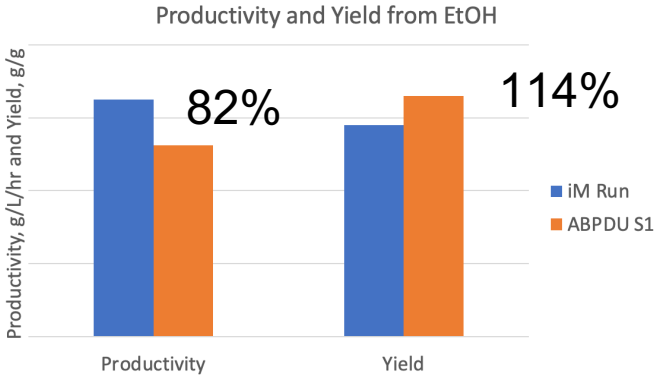
2 – Progress and Outcomes: Technology Transfer









Technology transfer to LBNL currently within 20% of IM baseline across all performance metrics

3 – Impact

- **Mission Relevance:** Accelerates deployment of aerobic gas fermentation technologies while developing technology alternatives to displace recalcitrant plastics with biobased, biodegradable alternatives
- **Industry Impact:** Direct pathway to commercial impact via close collaboration with Industrial Microbes
- **Capability development:** Develops much-needed generalizable protocols and lessons learned for predictive process development and scale-up with aerobic gas fermentations
- **Synergistic Research:** Dissemination of lessons learned and project expertise within ABF, to associated programs – ABPDU, BETO CO2 Consortium, and to the broader biomanufacturing community via presentations and reports

Summary

- **Problem statement:** Can we utilize multi-scale bioprocess development and machine learning to overcome key barriers to successful gas fermentation scale-up?
- **Current status:** Tech transfer ongoing, all metrics within 20% of Industrial Microbes baseline, multi-site proteomics baseline established
- **Final project goals:** Deliver 3x improvement in TRY over baseline and validate at 300L pilot scale
- **Project impact:** Accelerated development and deployment for aerobic gas fermentation processes, displacement of petrochemical polymers
- **Next steps:** Initiate ethane fermentations in Q3 to initiate first gas fermentation DBTL cycle

Quad Chart Overview

Timeline

- *October 1, 2022*
- *September 30, 2024*

	FY22 Costed	Total Award
DOE Funding	*$490k*	*$1M*
Project Cost Share	$220k	$500k

Project Goal:

this collaboration will leverage multi-scale experimentation to create a predictive model for bioconversion of ethane to Poly(3-hydroxy)propionate that identifies productivity improvements, forecasts scale-up performance, and enhances process robustness for gas fermentation.

End of Project Milestone:

Leverage model predictions to improve titer, rate, and yield for P3HP production from ethane by over 3x over initial baseline. Execute 300L ethanol fermentation and achieve TRY metrics within 10% or improved metrics.

Funding Mechanism:

Agile BIoFoundry FY22 Directed Funding Opportunity

Project Partners

- LBNL, Eric Sundstrom (PI), esundstrom@lbl.gov
- Industrial Microbes, Elizabeth Clarke, Liz@imicrobes.com
- ANL, Phil Laible, laible@anl.gov