DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review
WBS 2.4.1.100 Bench Scale Research & Development

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Biochemical Conversion and Lignin Utilization

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This presentation does not contain any proprietary, confidential, or otherwise restricted information

Project Overview

What are we doing? Developing and optimizing fermentation processes to produce bio-based fuels and chemicals for commercial scale-up

- **Near-term focus on producing 2,3-butanediol (BDO) from pretreated corn stover for sustainable aviation fuel (SAF) and low-carbon chemicals**
- Demonstrate fermentation design cases to meet technical and economic goals
    - Provide data for state-of-technology reports to track research progress
- De-risk technology for scaling

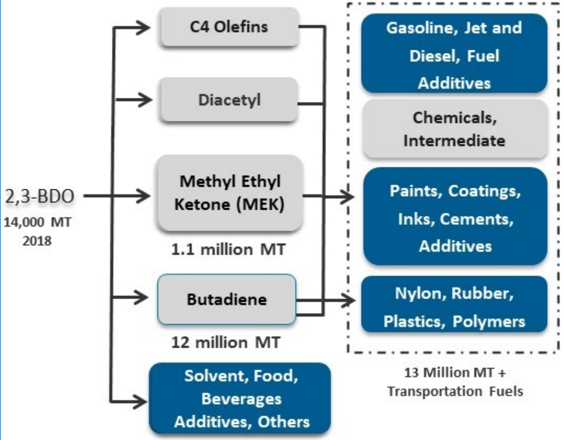
Why is it important? We want robust and industrially relevant fermentations for near-term deployment

Relevance to BETO? Achieving rate, titer, and yields to meet SAF cost target. Advancing tech for near-term commercialization

Why 2,3-Butanediol? Why *Zymomonas*?

1

Highly Upgradeable Chemical



13 Million MT Transportation Fuels + Chemicals Potential

2

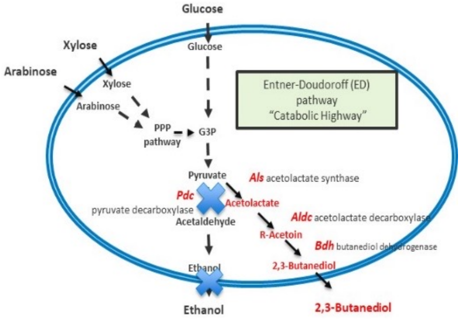
Negative Carbon Chemical

Biobased BDO: Dedicated biorefinery processing corn stover to 2,3-BDO
CI: **- 0.68 kg CO_2e/kg**

Fossil-based BDO: 2-2-butene ethylene to 2,3-epoxybutane to 2,3-BDO
CI: **0.69 kg CO_2e/kg**

3

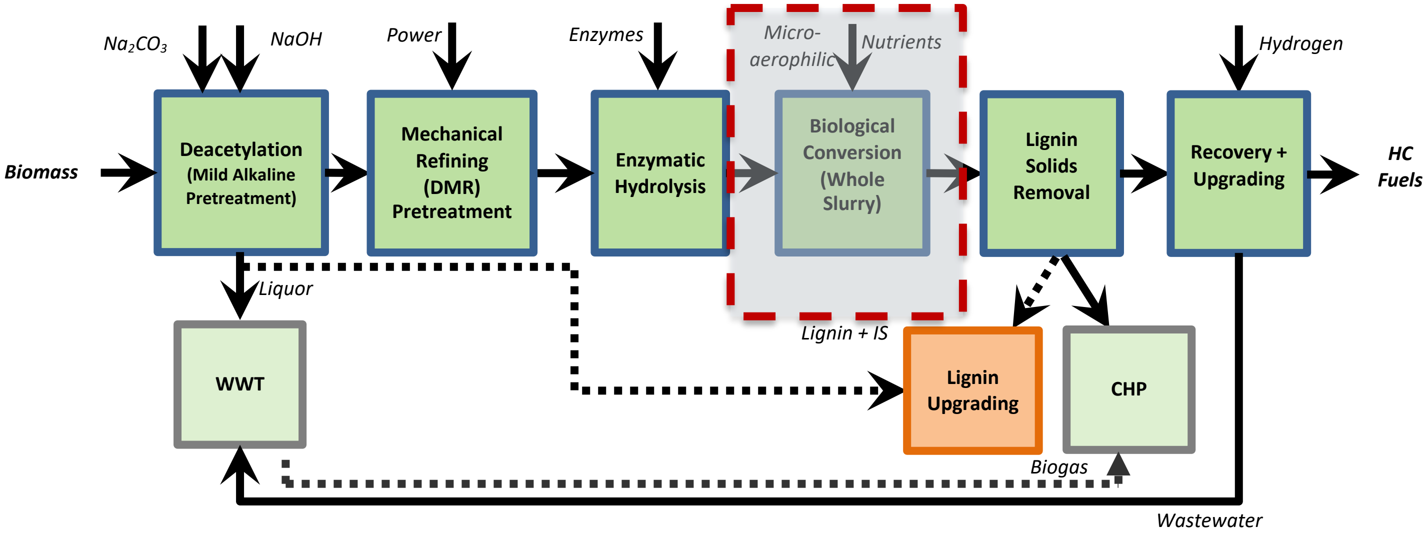
Industrial Strain



- *Z. mobilis* has a high sugar uptake rate (~ 3X faster than yeast)
- Does not make many side products
- Nutrient requirements are minimal
- +20 yrs. working with the strain at volumes up to 9000L
- Ethanologen version used in a commercial cellulosic EtOH process

Design Case

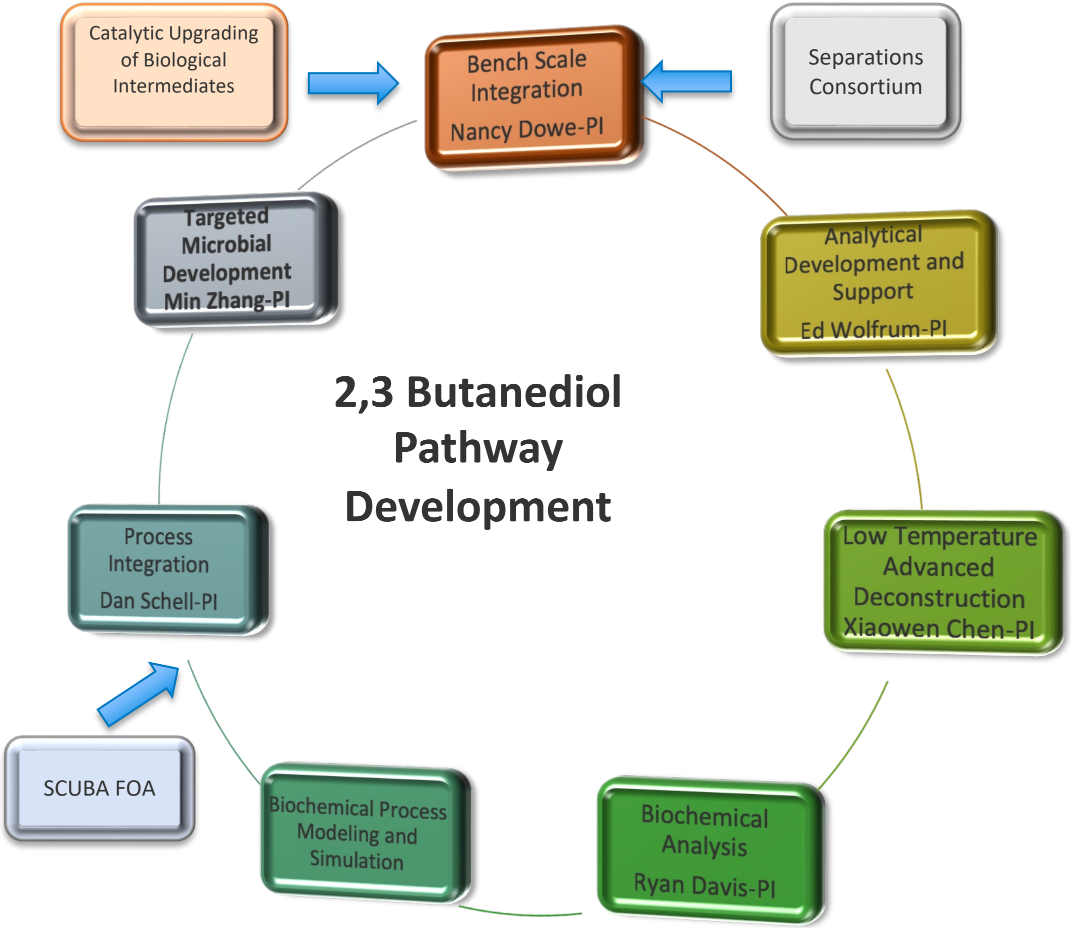
Mixed Alcohols/Diols (2,3-Butanediol) Fermentation Development



<u>Key 2030 TEA Goals</u>

- 90% product yield
- 90% hydrolysis yield
- 100 g/L titer
- 2.6 g/L-hr productivity
- Whole slurry fermentation feedstock

1. Approach – Management & Collaborations

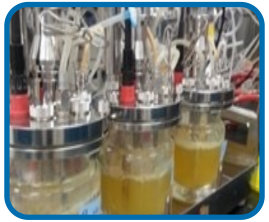


- Project has one task – Fermentation Development
- Project task members (80+ yrs. exp.)
  - Staff Chemical Engineer/Pilot Plant Engineer
  - 3 Staff Microbiologists
  - 2 Staff Analytical Chemists
- Monthly project meetings between project team and strain development PI
- Developed a risk register for project during last peer review cycle.

1. Approach – Project Goals

Goal 1: Demonstrate Technical Targets for Diol (2,3-BDO) to Fuel Pathway Design Case

(Whole Slurry Process)



Goal 2: In Parallel, Develop a High Titer Fermentation from Biomass Liquor to Enable Down Stream Separations and Upgrading

Goal 3: Scaling 2,3-BDO Fermentation for Commercial Applications

1. Approach – Goal 1

Goal 1: Demonstrate BDO to Fuel Pathway Design Case Technical Targets

- **<u>Challenges:</u>** Sugar concentration tolerance of *Zymo* is 160 g/L which is not enough to make 100 g/L BDO (whole slurry titer target).
- **<u>Technical Approach:</u>** Must run a fed-batch using solids or increase sugar tolerance in the strain.Run different process configurations using different forms of whole slurry to achieve titer target.

- **<u>Risk Analysis and Mitigation</u>*:*** Risk register identified fed-batch using solids as medium risk. Rework TEA for a liquor fed-batch process and set new targets for mitigation.

Non-Hydrolyzed DMR CS

High Solids Hydrolyzed DMR CS

DMR CS Conc. Liquor

OR OR

- *Must consider enzymatic hydrolysis vs. fermentation optimal conditions*
- *Rate of cellulose hydrolysis on the strain (glucose is the driving force)*

2. Progress and Outcomes – Goal 1

DMR Conc. Liquor

Similar results to liquor only fed-batch (100 g/L BDO; 90% Yp/s; 95% total sugar utilization); may get some hydrolysis during fermentation; **still need sugar concentration unit operations**

High Solids Hydrolyzed DMR

Must feed conc. solids; 30% TS raised BDO titer from 70 to 80 g/L; 75% sugar yield at 30% TS and 20 mg enz loading; **doesn't meet sugar hydrolysis yield goal or titer**

Non-Hydrolyzed DMR CS

Fed additional 10% TS; 77 g/L BDO, 30 g/L acetoin and 3 g/L glycerol; 84% Yp/s; 64.5% sugar yield at 20 mg enz loading; **doesn't meet sugar hydrolysis yield goal**

Go/No-Go

Down-selected to a liquor sugar feedstock to meet TRY goals.

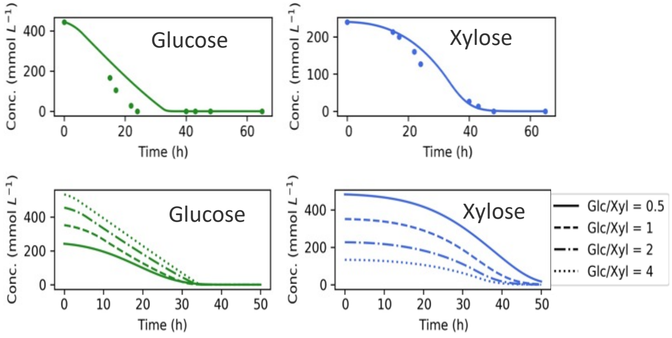
1. Approach – Goal 2

Goal 2: High Titer Fed-batch Fermentation Development from Biomass Liquor

- **<u>Challenges:</u>** Multiple sugars with different uptake rates by *Zymo* makes it difficult to control residual sugar levels which can affect *Zymo's* performance. Sugar ratios are set and can't be changed. Different aeration requirements for different sugars.

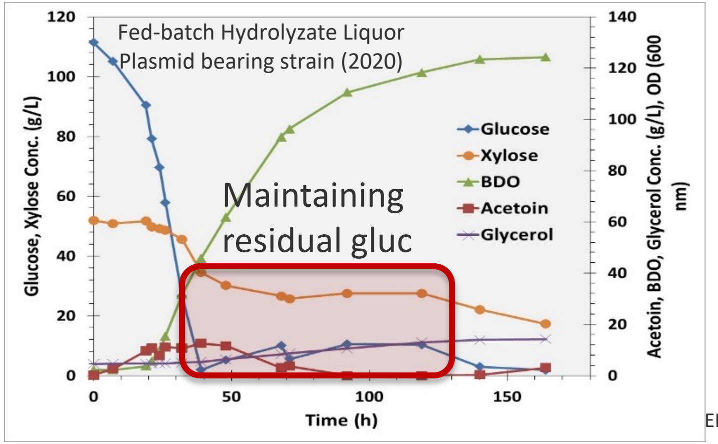
- **<u>Technical Approach:</u>** Maintain 10 g/L residual glucose during feeding; continuous ramp-down of the agitation speed to lower aeration levels when xylose is the primary sugar left.

- **<u>Risk Analysis and Mitigation:</u>** Co-utilizing sugars a risk to meeting productivity goals. Mitigate with strain engineering to improve co-utilization of sugars; Lower titer target.



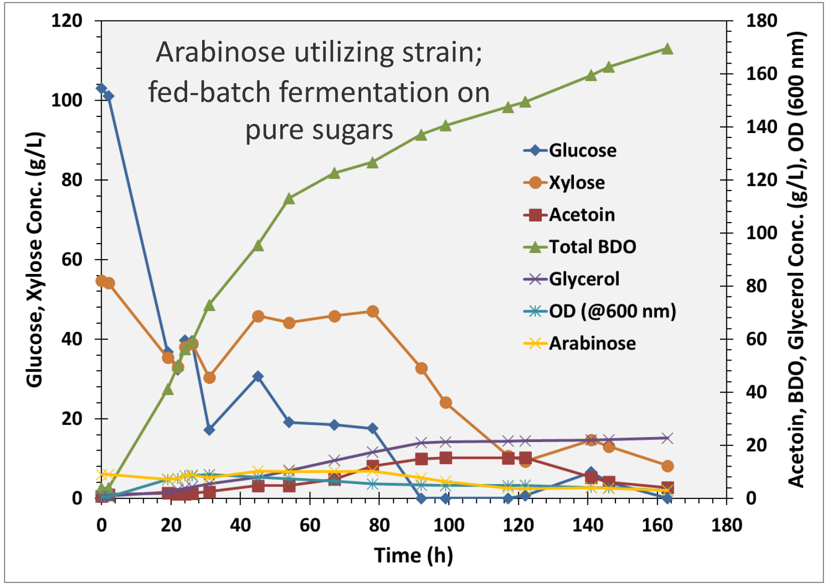
Wu et. al. 2021. Frontiers in Bioeng & Biotech https://doi.org/10.3389/fbioe.2021.707749
2.5.1.100 Biochemical Process Modeling and Simulation

- *20% more ATP during xylose utilization; but*
- *Glucose metabolism is thermodynamically more favorable*



2. Progress and Outcomes – Goal 2

Develop a High Titer Fermentation from Biomass Liquor to Enable Down Stream Separations and Upgrading



Outcome: We Exceeded Titer and Are Approaching Rate and Yield Targets

Parameter	Glucose Fed-Batch
BDO Qp (g/L-hr)	1.04
BDO Yp/s (g/g)	0.43 (86%)
BDO Titer (g/L)	170

New TEA Goals for Liquor Case

- 140 g/L titer
- 90% process yield
- 2.6 g/L-hr

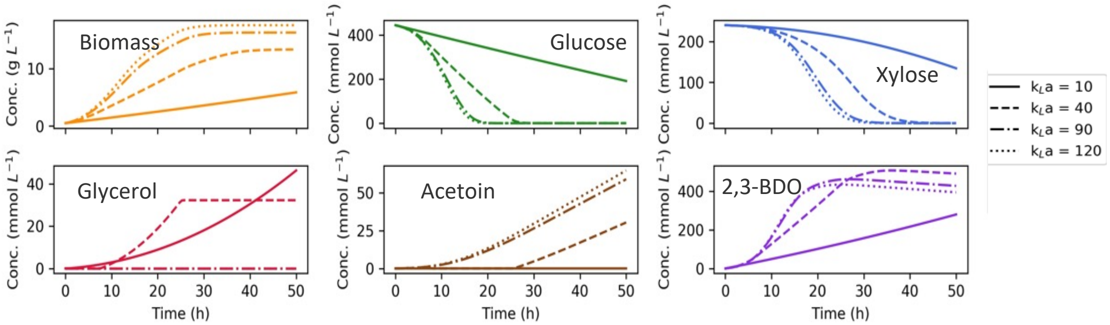
Breakthroughs for High Titer

- New strain **has improved sugar co-utilization** which helps in controlling sugar feed rate
- **Continuous ramp down of agitation** to reduce aeration levels when xylose is the primary fermentation sugar favors BDO production

1. Approach – Goal 3

Goal 3: Scaling for Commercial Applications

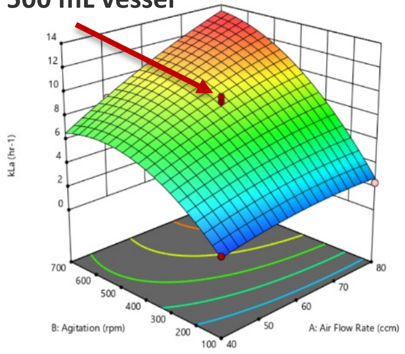
- **<u>Challenges:</u>** Scaling a micro-aerophilic fermentation.
- **<u>Technical Approach:</u>** Develop NIR rapid analysis to monitor sugars and products for process control and map oxygen transfer coefficient and oxygen transfer rate in different types and sizes of bioreactors to develop a method for scaling.
- **<u>Risk Analysis and Mitigation:</u>** Scaling a process is always a risk. Rapid analytical will enable process control to target BDO production. Mapping OTR/kLa in vessels will reduce risk of failed fermentations.



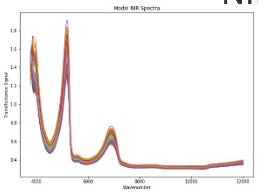
Wu et. al. 2021. Frontiers in Bioeng & Biotech https://doi.org/10.3389/fbioe.2021.707749
2.5.1.100 Biochemical Process Modeling and Simulation

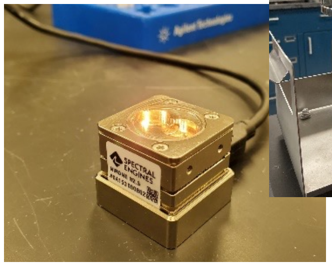
- *Low level of O_2 to balance redox in cell*
- *Faster BDO production at higher k_La*
- *Higher BDO yield at the mid-range k_La*

Optimized OTR/k_La in 500 mL vessel



NIR Rapid Analysis





Scaling 2,3-BDO Fermentation for Commercial Applications
OTR/$k_L a$ Vessel Mapping

500 mL Sartorius Bioreactor

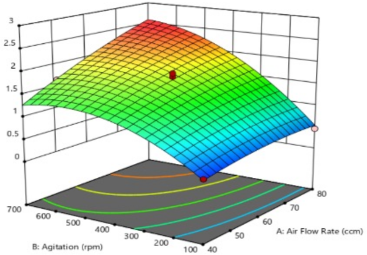
- Overlay air
- Optimized BDO production at 350 – 700 rpm and 60 ccm OTR of 1.57 – 2.02 mmol O_2/L-hr **(Target)**

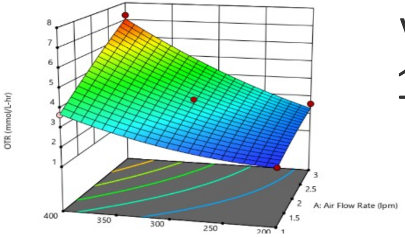
10L BioFlo Bioreactor

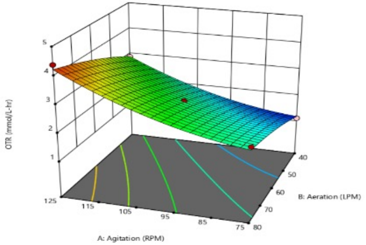
- Overlay air
- For OTR of 1.66 – 2.02 mmol O_2/L-hr model predicts 1 LPM and 200 - 269 rpm

1000L Pilot Plant Bioreactor

- Sparged air
- For OTR of 1.57 – 2.02 mmol/L-hr model predicts 50 LPM and 80 - 98 rpm





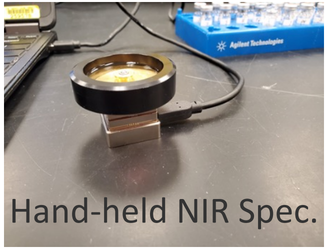


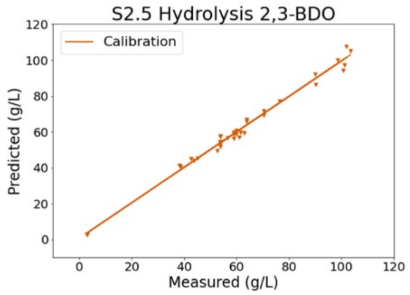
Outcome

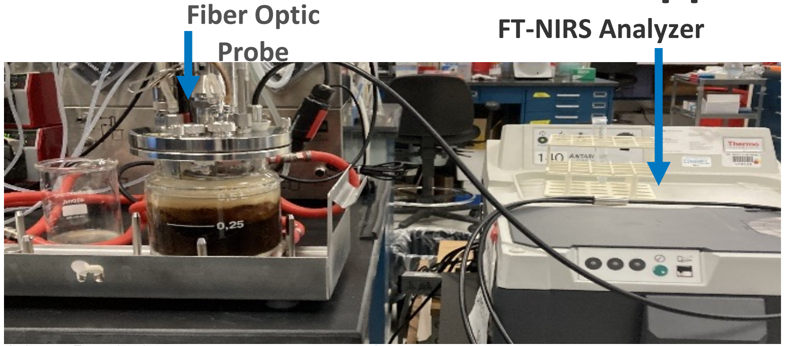
We have an aeration model for several vessels (500 mL, 10L, 100L, and 1000L) and target conditions to test OTR mapping approach for scale-up.

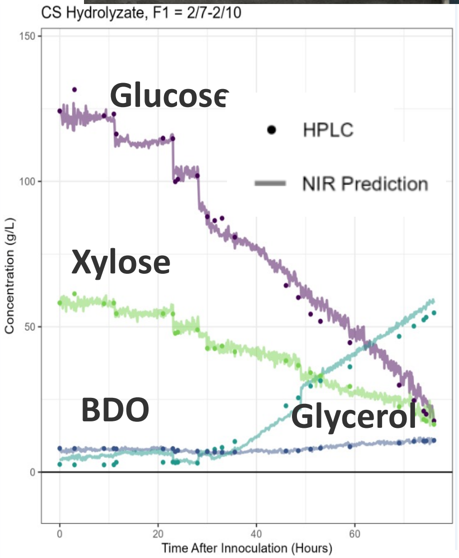
4. Progress and Outcomes – Goal 3

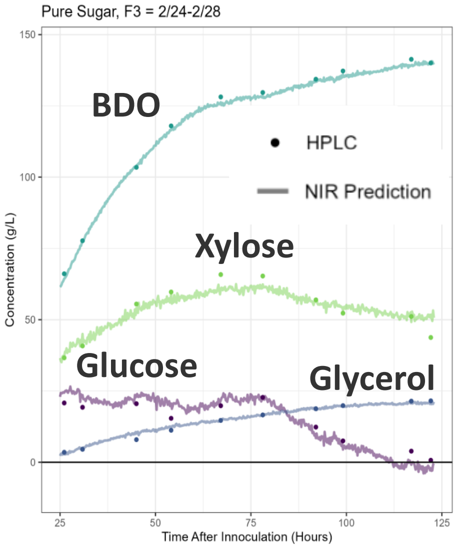
Scaling 2,3-BDO Fermentation for Commercial Applications – Rapid Analysis











Outcomes

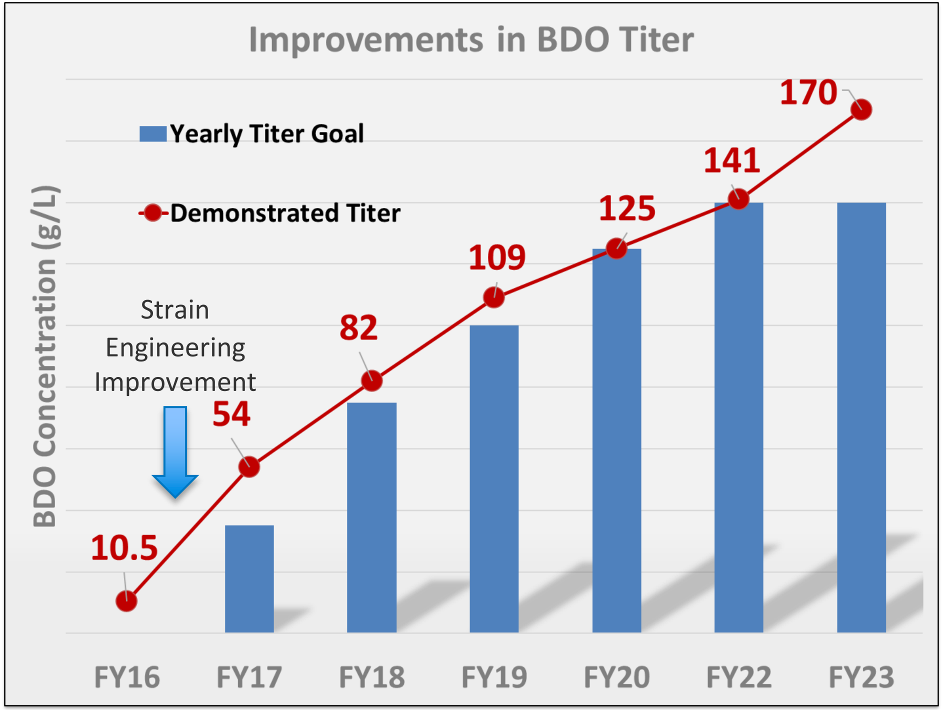
- ❖ Hand-held at-line analysis of Gluc, Xyl, BDO, Acetoin, Glycerol ready to deploy
- ❖ Successful testing of online probe for process control and metabolic R&D

3. Impact

Decrease Conversion Costs and Reduce Commercialization Risks Through Fermentation Process Development and Optimization

Impact on State of Technology and Industry

- **Best in class with titer** on biomass (corn stover) sugar substrates and on mixed pure sugar showing sustained and significant improvements to the fermentation process
- Project identifies and demonstrates operating conditions that **align with envisioned commercial-scale processes**



3. Impact

Decrease Conversion Costs and Reduce Commercialization Risks Through Fermentation Process Development and Optimization

Desseminating Results

- BETO SCale Up of Bench Applications **(SCUBA) FOA project** – BDO to biojet fuel (Georgia Institute of Technology, NREL, ORNL, and ExxonMobil Research and Engineering)
- ROI-22-75 Engineering Zymomonas to utilize fructose and sucrose (4/22/22) which includes fermentation process control
- Copyright to NIR calibration dataset (SWR-22-60) 7/21/22
- Wu, C., Spiller, R., Dowe, N., Bomble, Y. J., & St John, P. C. (2021). Thermodynamic and Kinetic Modeling of Co-utilization of Glucose and Xylose for 2, 3-BDO production by Zymomonas mobilis. Frontiers in Bioengineering and Biotechnology, 9, 653.

Industrial collaboration with fermentation company (BioPrincipia) to scale process creating a 2,3-BDO business case and large supplies for the derivative chemical and SAF markets

Summary

1. **Approach:** Focused on **demonstrating a commercially viable fermentation process** through fermentation process control of aeration and sugar feeding to achieve high titers and yields from lignocellulosic sugars. Developing tools like rapid fermentation broth analysis and k_La/OTR mapping in a variety of vessels to enable scaling.

2. **Impact:** Project **reduces scale-up risks** by moving research from bench to pilot plant. Improvements in fermentation performance have a direct impact on bioconversion costs. Analytical methods developed for **rapid at-line analysis aid in scaling** and can be used by other projects (NIR models copywritten and fermentation process included in recent patent application). **Working with industry through a Technology Commercialization Funded project with BioPrincipia** to scale the fermentation process using industrial waste to create a 2,3-BDO business

3. **Progress & Outcomes:**
   - **Achieved 140 g/L BDO at 84% theoretical process yield and 1 g/L-hr productivity in a fed-batch fermentation** using pure mixed sugars that mimic deacetylated mechanically refined (DMR) corn stover liquor. **BEST IN CLASS**
   - **Deployed a rapid at-line near-infra red analysis** of the BDO broth to aid in fermentation process control
   - **Developed model using Design of Experiments for mapping OTR/kLa** in different sized vessels **for scale-up**
   - Go/No-Go decision to pursue a clarified liquor fed-batch process with new performance targets for cost competitive SAF

4. **Future Work:**
   - **Integrate strain and fermentation development with separations and upgrading projects for successful BDO-to-SAF deployment**
   - Use OTR/kLa mapping and apply to fermentations in different vessels; simulate different zones of aeration expected in large vessels
   - Develop on-line aeration/agitation and sugar feed rate control using NIR probe

Quad Chart Overview

Timeline

- *FY2013 – DEI Plan Not Required*
- *FY2023*

	FY22 Costed	Total Award
DOE Funding	*(10/01/2021 – 9/30/2022)* $750,000	*(negotiated total federal share)* $750,000
Project Cost Share *		

TRL at Project Start: 4
TRL at Project End: 5

Project Goal

Develop scalable bioconversion processes, that produce bio-derived fuels and chemicals with the focus on fermentation R&D to increase titer, rate and yield. The project's near-term focus is on producing 2,3-butanediol from corn stover biomass for SAF production, achieving high titers and yields by controlling aeration levels and sugar feed rates and using rapid NIR analysis during operations for process control.

End of Project Milestone

BDO Scale-up: BDO conversion projects. Meet design target BDO titer (140 – 150 g/L) from DMR corn stover liquor at 1000L or larger scale to demonstrate BDO process design case and work with industrial fermentation stakeholders to develop fermentation protocols for use in future commercial

Funding Mechanism: Lab AOP (3-Yr Merit Review)

Project Partners: NA

Team Members

Ben Wachter
Rob Nelson
Zofia Tillman
Darren Peterson
Holly Rohrer
Ryan Spiller

Thank You

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Xiaowen Chen (PI LTAD – pretreatment)
Ryan Davis (PI Biochemical Analysis)

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Additional Slides

Responses to Previous Reviewers' Comments

Overall Impressions from Reviewers

- "A balanced team approach with good coordination and leveraging with other groups (strain development, engineering, process development, end product uses, pretreatment, etc.). Risks and risk mitigation is addressed in great detail. The approaches used in fermentation development and rapid analysis to meet targets looks very good. The project is within the scope of BETO's goal of enabling cost effective production of biofuels and chemicals. Using near IR to monitor fermentation is useful timely and innovative."
- "This project has the important role in de-risking the scale up of bioreactors for biofuel production. The current focus on 2,3-BDO production with *Zymomonas mobilis* creates a nice integration with other projects in NREL and in the BETO portfolio. The reported progress is impressive. Reaching titers of 125 gBDO/L in fed-batch reactors fed DMR-processed liquor (without solids) and 92 gBDO/L using DMR-processed whole-slurry, both at a 100 L scale, shows that the optimization approach is working."
- "The project's inclusion of a risk register is a strength and a more detailed evaluation of potential barriers than seen for other projects. Process engineering offers an important complement to the program's microbiological work, in that it investigates methodology and infrastructure necessary to transition a biological conversion to pilot and eventual commercial application. As such, it occupies a crucial position between the lab and industrial stakeholder showing stakeholders that laboratory results can also have industrial application."

Responses to Reviewers

- We appreciate the constructive feedback on the project's vision, mission and objectives; to develop industrially relevant fermentation processes for bio-derived products that de-risk scaling, lower carbon intensity, and improve sustainability. We also appreciate the reviewer's acknowledgment of the 100L scale-up that produced kg amounts of product and meeting the 125 g/L titer target. Our results show that our optimization efforts are working and that fermentation engineering solutions can positively affect titer, rate, and yield. The project will continue to use a risk register to address technology development barriers and continue developing analytical methods like NIR and Ramen spectroscopy to aid in fermentation control and enable scaling. Both the risk register and the NIR work were positively received by the reviewers. The Reviewers provided excellent feedback for the project's future work and ways to improve future project presentations. As the Reviewers pointed out, a deeper dive into the economics to present a comparison between starch-based fermentation, biomass-derived fuels and co-products from BDO, and the equivalent fossil-derived fuels and co-products is warranted. We plan to use NREL's pilot scale bioreactors to address scaling micro-aerophilic slurry fermentations to aid in scaling beyond 9000L; developing online and rapid at-line analyses will be important to this effort. Our early work to feed and mix biomass slurries to reach higher solids concentrations for higher BDO titers has been a challenge to produce consistent results at 0.5L scale and we will continue to address this issue.

Publications, Patents, Presentations, Awards, and Commercialization

Publications and Presentations

- Spiller, Ryan, Rohrer, Holly, Chou, Yat-Chen, Davis, Ryan, Zhang, Min, and Dowe, Nancy. Evaluating the Feasibility of Higher Titers and Yields for 2,3-Butanediol Production to Achieve the Biofuel Industry Economic Goal of Under $3.50 GGE. United States: N. p., 2022. Web.

- Wu, C., Spiller, R., Dowe, N., Bomble, Y. J., & St John, P. C. (2021). Thermodynamic and Kinetic Modeling of Co-utilization of Glucose and Xylose for 2, 3-BDO production by Zymomonas mobilis. Frontiers in Bioengineering and Biotechnology, 9, 653.

Commercialization

- 2022 BETO Technology Commercialization Fund Award with BioPrincipia: Commercializing Bio-Derived 2,3 – BDO Production from Industrial Waste $1.7MM

- ROI-22-75 Engineering Zymomonas to utilize fructose and sucrose (4/22/22)

- Copyright to NIR calibration dataset (SWR-22-60) 7/21/22