BETO 2021 Peer Review
WBS 2.4.1.100 Bench Scale Integration

March 9, 2021

Biochemical Conversion and Lignin Utilization

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Project Overview

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

- Innovate and demonstrate fermentation techno-economic design cases to meet technical goals
  - Provide data for state-of-technology reports to track research progress
- De-risk technology for scaling
- Provide industry with operational parameters and procedures for future projects

Why is it important? We want robust and industrially relevant fermentations for bio-derived products with reduced carbon intensity to lower greenhouse gas emissions and improve sustainability

Quad Chart Overview

Timeline

- Project start date: FY2013
- Merit review cycle: FY2021-FY2023
- Project end date: FY2023

	FY19- FY20	Active Project
DOE Funding	$1.5 MM	$6.0 MM

Barriers addressed

Ct-D. Advanced Bioprocess Development: Increasing titer, rates, and yields of bioproducts through metabolic engineering and fermentation processing improvements

ADO-A. Process Integration: Understanding process integration is essential to (1) characterize the interactions between unit operations,

ADO-D. Technology Uncertainty of Integration and Scaling: Determining scaling factors for industry best practice of stepwise scaling.

Project Goal

The project goal is to develop scalable bioconversion processes to produce bio-derived fuels and chemicals with the focus on fermentation R&D to increase titer, rate and yield which are driven by techno-economic analysis.

End of Project Milestone (FY18-FY20)

Demonstrate 125 g/L 2,3-butanediol (BDO) titer and a process yield of 85% theoretical from biomass sugars; a 25% improvement over FY19 end -of-year BDO titer.

Funding Mechanism

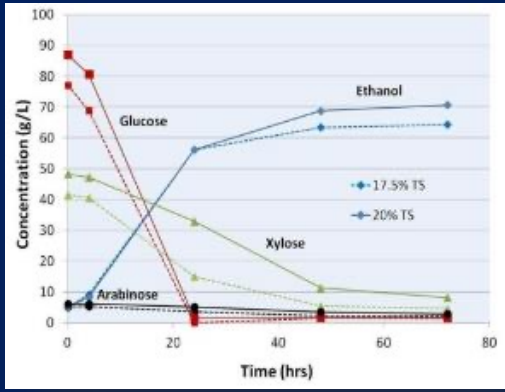
Lab AOP

Project Overview - History

Project has a long history of developing fermentation processes for different biological conversion pathways to produce fuel from biomass

Cellulosic EtOH

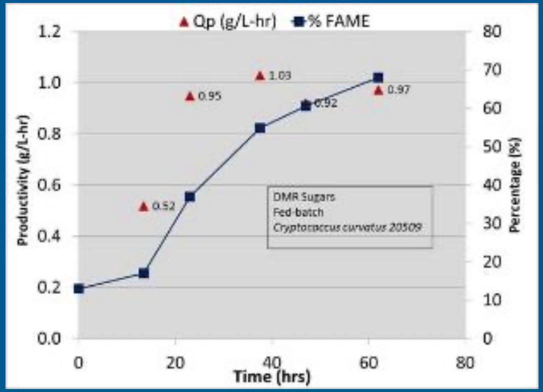
2012 Pilot Demos - $2.15 MESP



Lipid

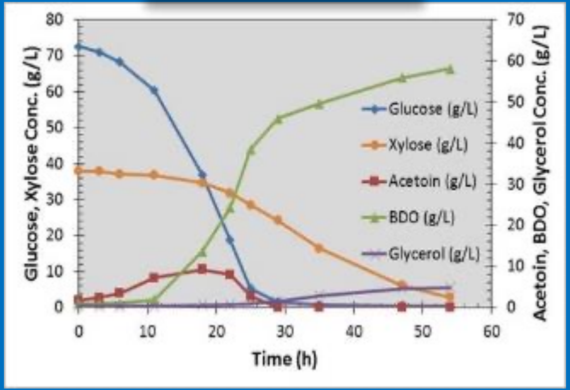
Reduced bioconversion costs by $2.30/GGE (FY15-FY17)

Parameter	FY16 DMR Pretreated Hydrolysates	FY17 DMR Pretreated Hydrolysates
Lipid Qp (g/L-hr)	0.76	**0.97**
Lipid Titer (g/L)	50.0	60.3
Lipid content (wt%)	64%	68%



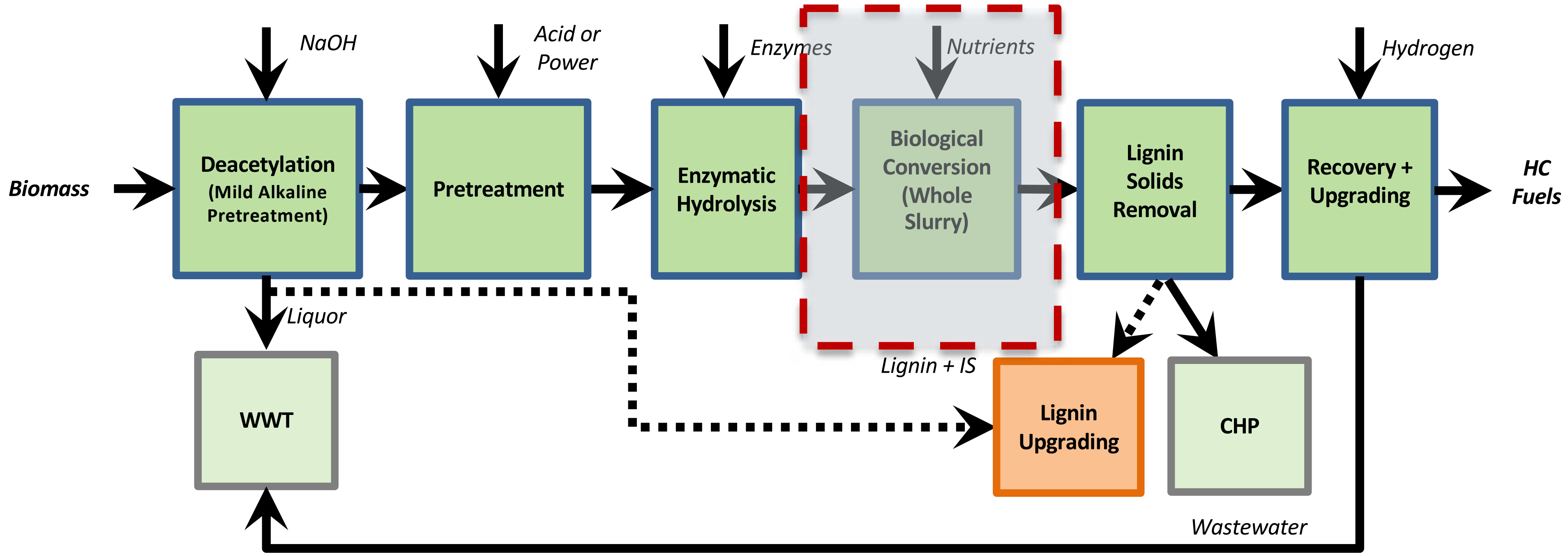
2,3 Butanediol

Increased BDO titer 6X on whole slurry and scaled to 100L (FY17-FY18)



Project Goals

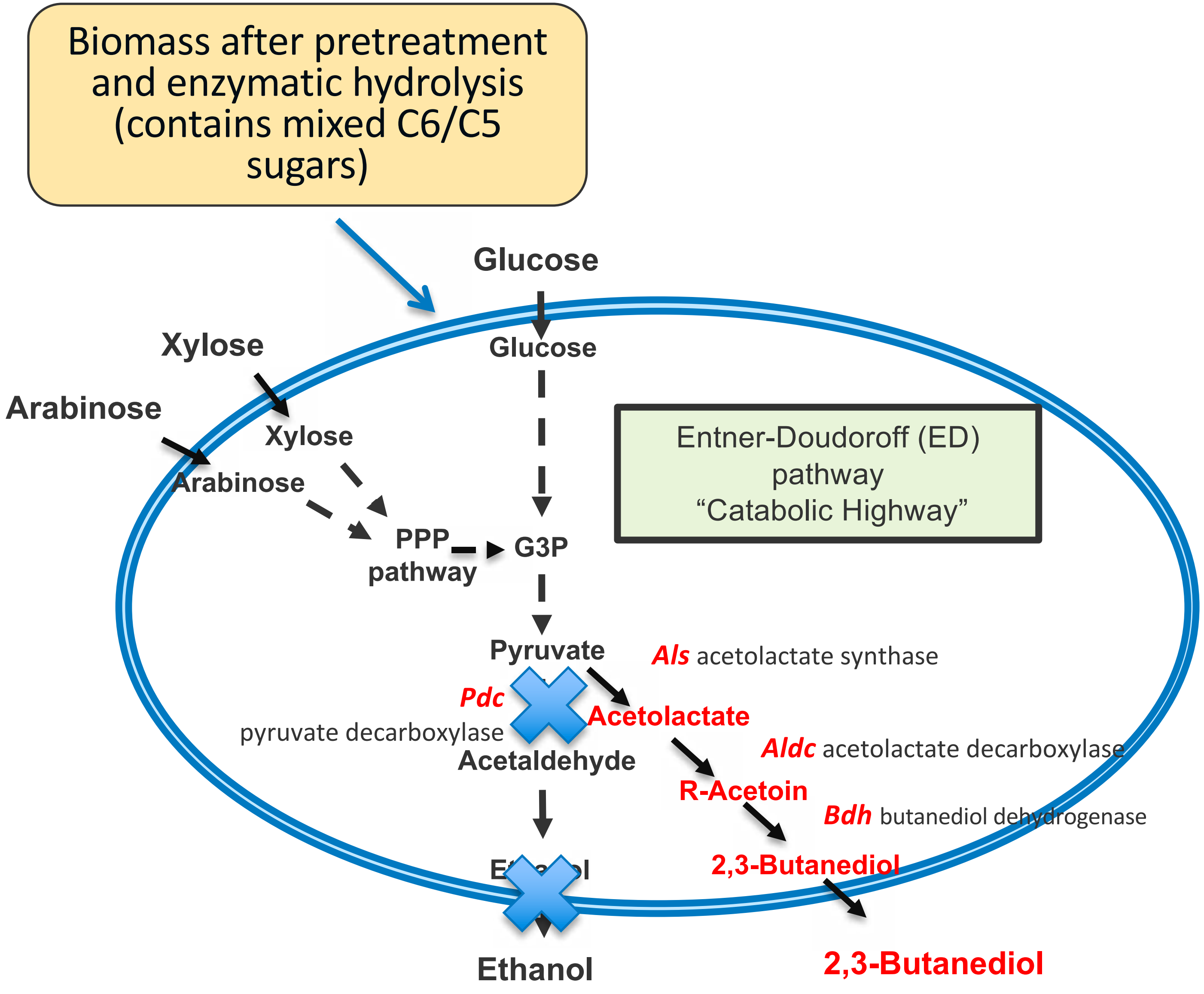
Mixed Alcohols/Diols (2,3-Butanediol) Fermentation Development



<u>Key Goals</u>

- Demonstrate high titers to reduce downstream costs (target 125 g/L)
- Develop a whole-slurry fermentation process to demonstrate BDO design case
  - 2030 TEA technical goals of 25% TS whole slurry; 95% yield from glucose, 90% yield from xylose and 85% yield from arabinose; 2.6 g/L-hr productivity
  - $2.47 MFSP design target cost

BDO Producing Microbe – r*Zymomonas mobilis*



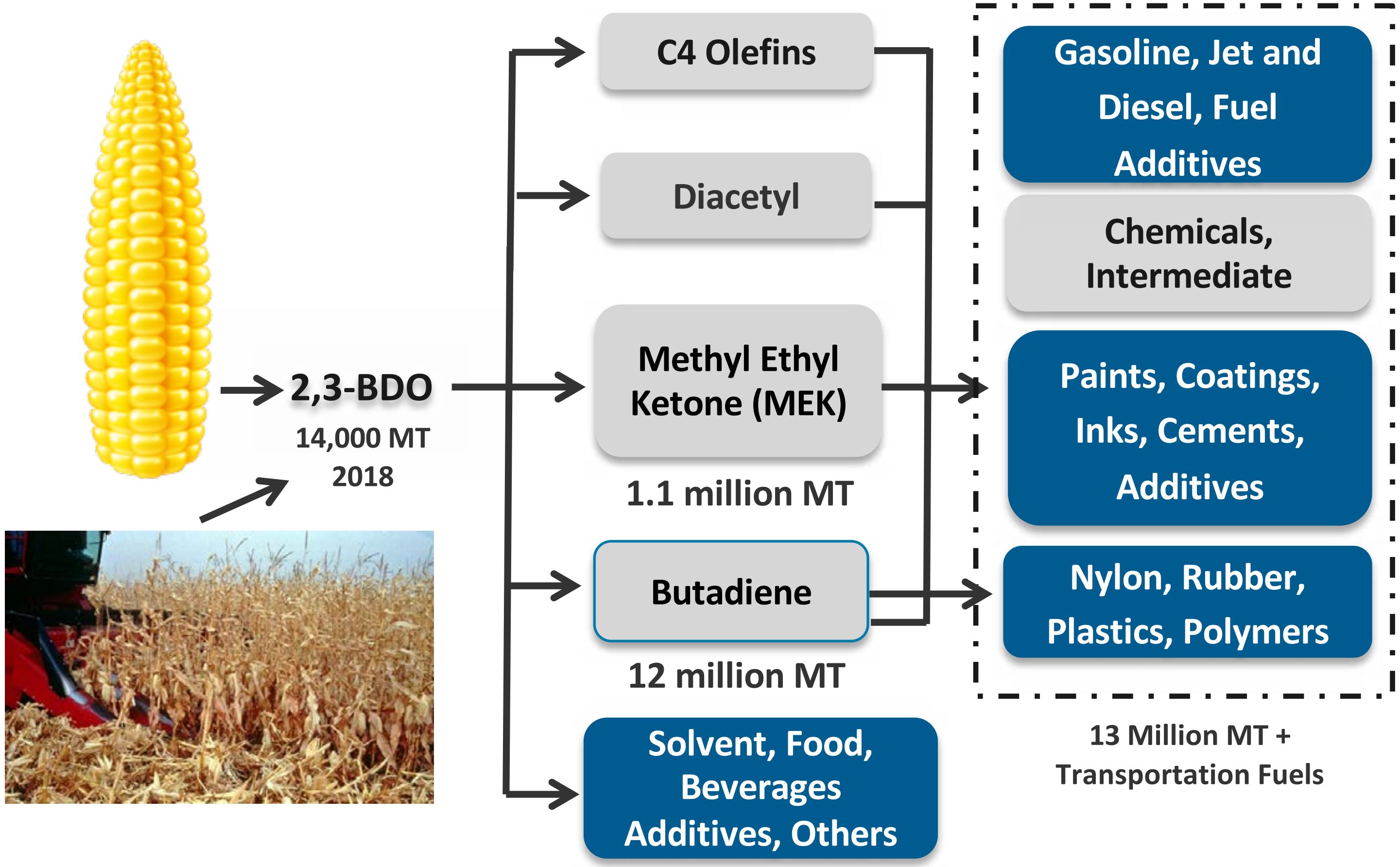
What we like about *Z. mobilis*

- *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

BDO Fermentation

- Potential for high titer, rate and yield
  - ✓ 2,3 BDO is less toxic than EtOH
  - ✓ EtOH pathway is removed
- Need low level of O_2 for oxidizing excess NADH for BDO production

Market Analysis



Key Takeaways: Existing 2,3 BDO market is small, ~14,000 MT per year, with forecasted growth of ~3%. However, BDO derivative markets are a promising opportunity to grow into novel chemicals, polymers, solvents, fuel additives

(Z. Abdullah, L. Tao 2020)

Market Trends

Product	Anticipated decrease in gasoline/ethanol demand; diesel demand steady
Product	Increasing demand for aviation and marine fuel
Product	Demand for higher-performance products
Product	Increasing demand for renewable/recyclable materials
Feedstock	Sustained low oil prices
Feedstock	Decreasing cost of renewable electricity
Feedstock	Sustainable waste management
Feedstock	Expanding availability of green H_2
Feedstock	Closing the carbon cycle
Capital	Risk of greenfield investments
Capital	Challenges and costs of biorefinery start-up
Capital	Availability of depreciated and underutilized capital equipment
Social Responsibility	Carbon intensity reduction
Social Responsibility	Access to clean air and water
Social Responsibility	Environmental equity

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

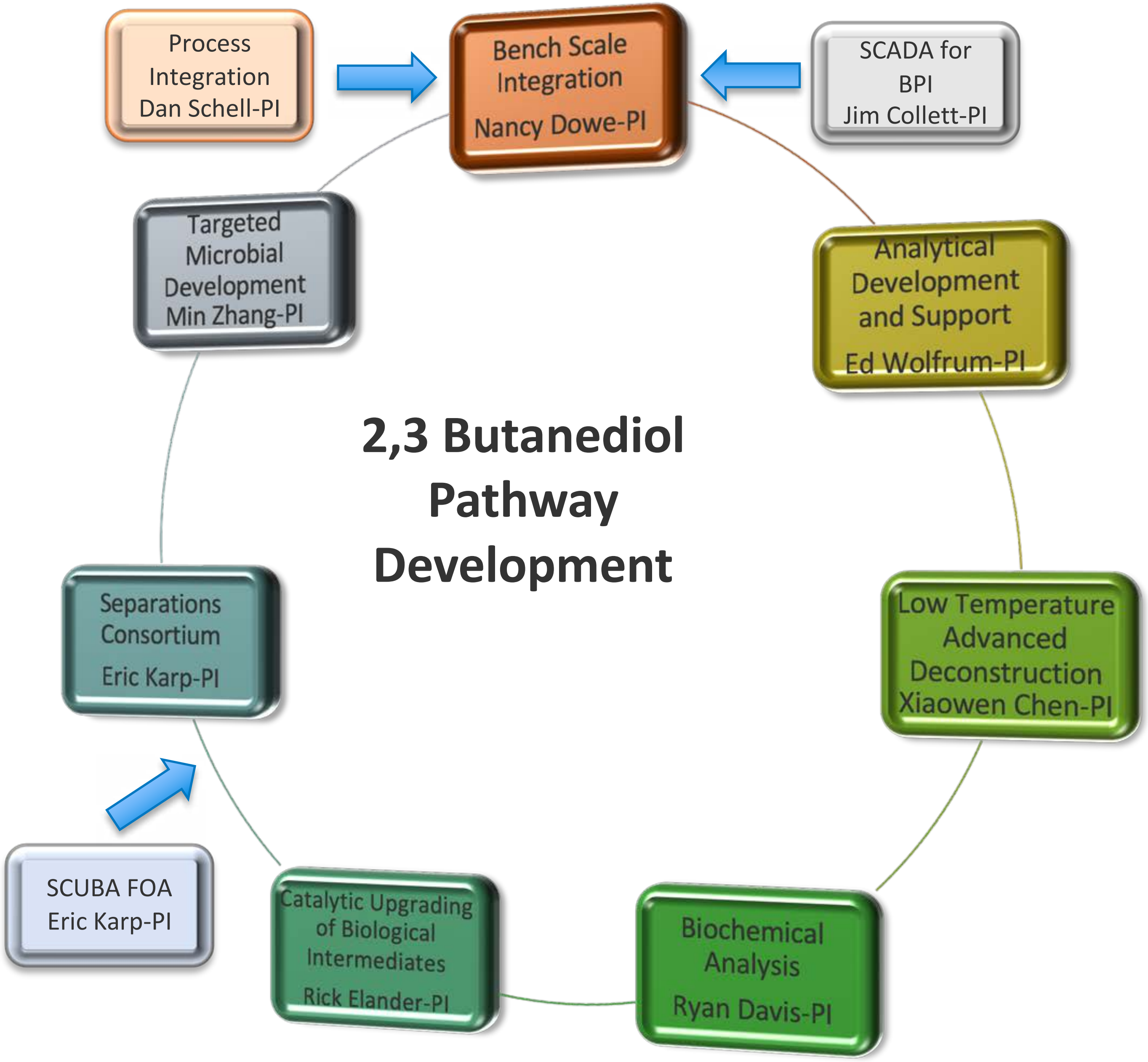
Value Proposition

- 2,3-BDO is a versatile chemical feedstock for producing jet fuels, diesel, solvents, chemicals, and fuel additives
- 2,3-BDO can be produced from cellulosic feedstock; fuels produced could qualify for California's low carbon fuel credits
- Potential drop-in conversion process at corn ethanol plants

Key Differentiators

- Fermentation process is flexible and can be run as a traditional fed-batch on liquid sugars or simultaneous saccharification and fermentation from corn or biomass sources
- Titers of 125 g/L at 84% yield demonstrated at bench; strain is robust; scalable fermentation

1. Management



- Project has one task – Fermentation Development
- Project task members (80+ yrs. exp.)
  - Staff Chemical Engineer
  - Staff Microbiologist
  - Staff Analytical Chemist
  - Staff Pilot Plant Engineer
- Monthly project meetings between project team and strain development PI
- Shared milestones with Biochemical Analysis, Pretreatment (LTAD), TMD
- Provide material for separations and upgrading to develop broth specs
- Engaging PNNL for SCADA and data informatics support and NREL's Process Integration Project for piloting
- Developed a risk register for project

1. Management

Risk register included identifying risks in feedstock, strain development, fermentation process, downstream processing, and economics. Highest risks were identified in downstream processing and economics. This internal risk register helps in building a credible scaling and business plan for external collaborations.

Risk	Probability (H/M/L)	Impact (H/M/L)	Mitigation Plan	Responsible Party	Comments
Fermentation Process					
Scaling a micro-aerophilic fermentation process	L	M	Genomatica has commercially scaled their 1,4 BDO microaerophilic fermentation. Our first attempt at 100L was successful.	BSRD and Modeling projects	Impact on MFSP
The modeled process requires we work with whole slurry which prevents feeding highly concentrated sugar feeds for maximum titer	M	H	Use concentrated liquor (potentially from the continuous enzymatic hydrolysis) for maximum titer and yields. Re-model process.	BSRD and BC Analysis projects	Impact on MFSP
Scaling a whole slurry fed-batch fermentation	M	H	See comment above.	BSRD project	Impact on MFSP
Composition of the broth after fermentation contains contaminants that negatively affect separations and catalytic upgrading.	H	H	Modify growth media and aerate to favor acetoin and BDO production rather than glycerol and BDO (short term). Add more purification steps and look for cost offsets.	BSRD, Separations, and CUBI projects	Impact on MFSP and development schedule

2. Approach

Goal 1: Produce 125 g/L BDO titer from biomass liquor hydrolysate. Higher titers reduce down-stream costs

- **Challenge/Opportunity:** Feeding enough sugar to achieve higher titers will require a fed-batch fermentation. Control over sugar feed rates and aeration levels to balance redox during the fermentation will be necessary to meet this goal.

Goal 2: Transition to a whole slurry fermentation to demonstrate design case technical goals

- **Challenge/Opportunity:** Maintain titers, yields and rates seen with hydrolysate liquor fermentations. Evaluate different ways of feeding biomass solids to increase solids for increased titers.

Goal 3: Develop at-line near-infra red analysis to monitor fermentation products for process control

- **Challenge/Opportunity:** Quick analyses allow for better control over fermentation parameters and aid in scaling. Specifically, changes in aeration levels can affect what products are made (BDO vs. acetoin (pathway intermediate) vs. glycerol (by-product).

Goal 4: Scale to 100L to produce kilograms of BDO for separation and catalytic upgrading projects

- **Challenge/ Opportunity:** Scaling aeration between different vessels sizes, agitation set-ups and geometries is a challenge. Finding correlations between high BDO production and analytics will be key. Feedback on broth specifications will inform fermentation development.

3. Impact

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

Impact to BETO

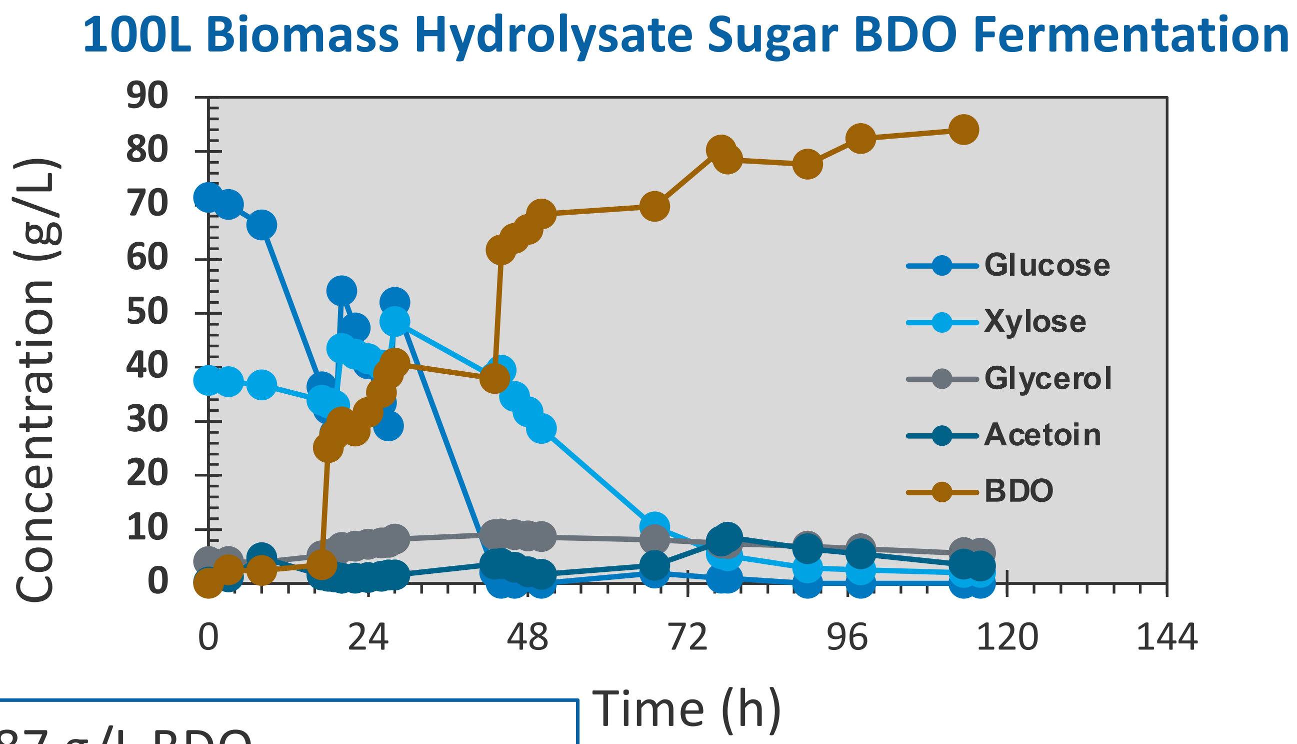
- Project reduces scale-up risk and serves as a validation of conversion technology
- Fermentation improvements have a direct impact on conversion costs
- Developing on-line analytical methods for fermentation process control that can be utilized by other projects
- With industry and consumers shifting to bio-based products, BDO (as a chemical feedstock) has potential to disrupt the fossil-derived chemicals and fuel markets

Impact to Industry

- Project identifies and demonstrates operating conditions that align with envisioned commercial-scale processes
- Working with industry on a BETO SCale Up of Bench Applications (SCUBA) FOA project – BDO to biojet fuel (Georgia Institute of Technology, NREL, ORNL, and ExxonMobil Research and Engineering)
- Working with industry to evaluate other sugar feedstocks for future development

4. Progress and Outcomes

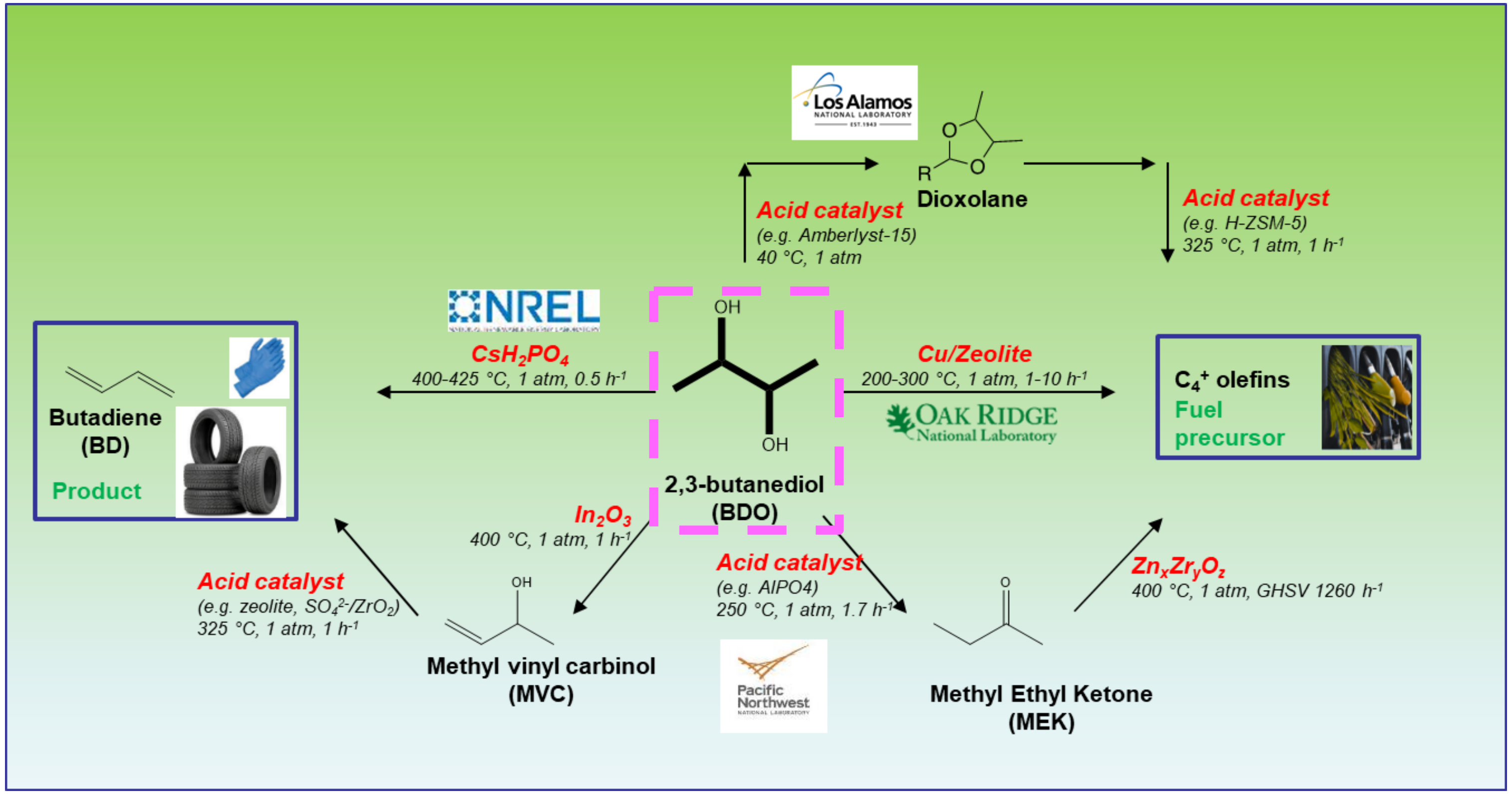
Scale to 100L to produce kilograms of BDO for separation and catalytic upgrading projects



87 g/L BDO
90% Product Yield
0.7 g/L-hr Productivity
7 kg dry BDO produced

4. Progress and Outcomes

Catalytic Upgrading of BDO Fermentation Broth



ChemCatBio
Chemical Catalysis for Bioenergy

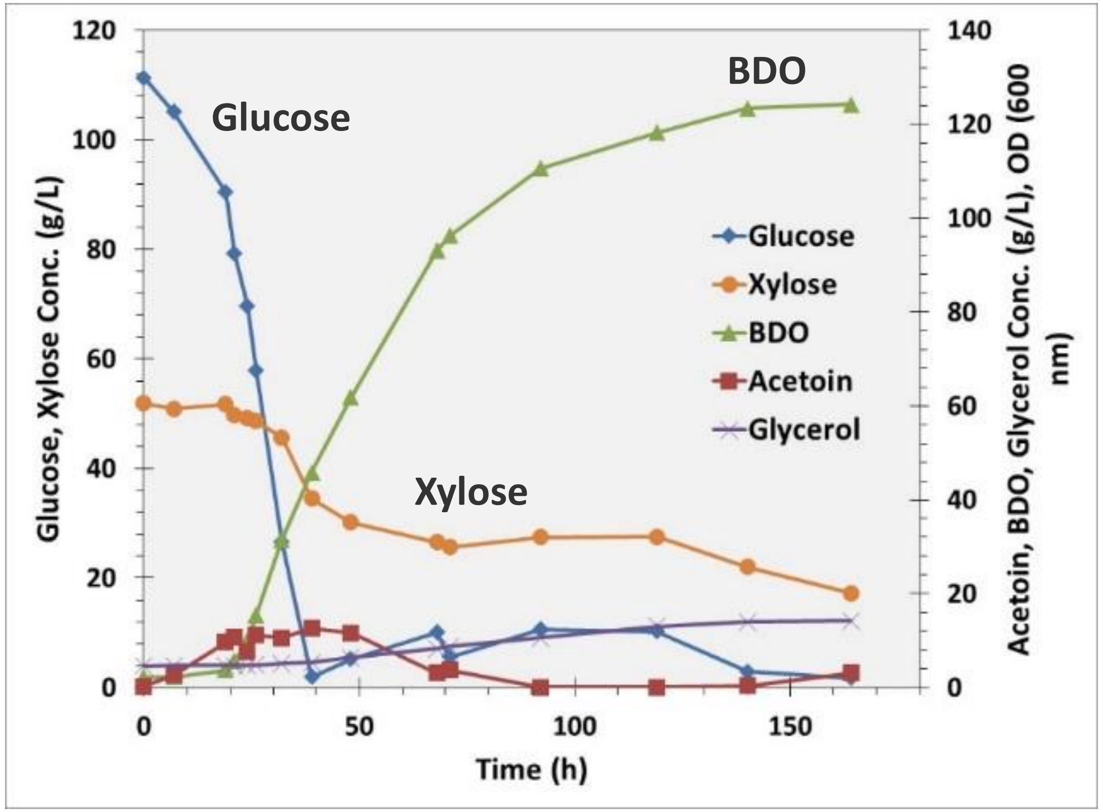
2.3.1.101-104: Catalytic Upgrading of Biochemical Intermediates (ChemCatBio)

NREL | Oak Ridge National Laboratory | Los Alamos National Laboratory | Pacific Northwest National Laboratory

Key Findings

- Presence of water in aqueous fermentation broth does not negatively affect catalyst performance
  - **Lower water content (higher BDO titer) will benefit TEA (reduced energy requirements)**
- Some fermentation by-products are OK, others are not
  - Acetoin: Can be converted to butenes or MEK (for olefins intermediates) at high carbon efficiency
  - **Glycerol: Causes significant coking/ deactivation of upgrading catalysts**

4. Progress and Outcomes



Parameter	FY19 DMR Pretreatment (conc. liquors)	FY20 DMR Pretreatment (conc. liquors)
BDO Qp (g/L-hr)	0.52	0.66
BDO Yp/s (g/g)	0.42 (83%)	0.43 (84%)
BDO Titer (g/L)	107	125

BDO Titer Improvement – FY20 Q4 Milestone Goal

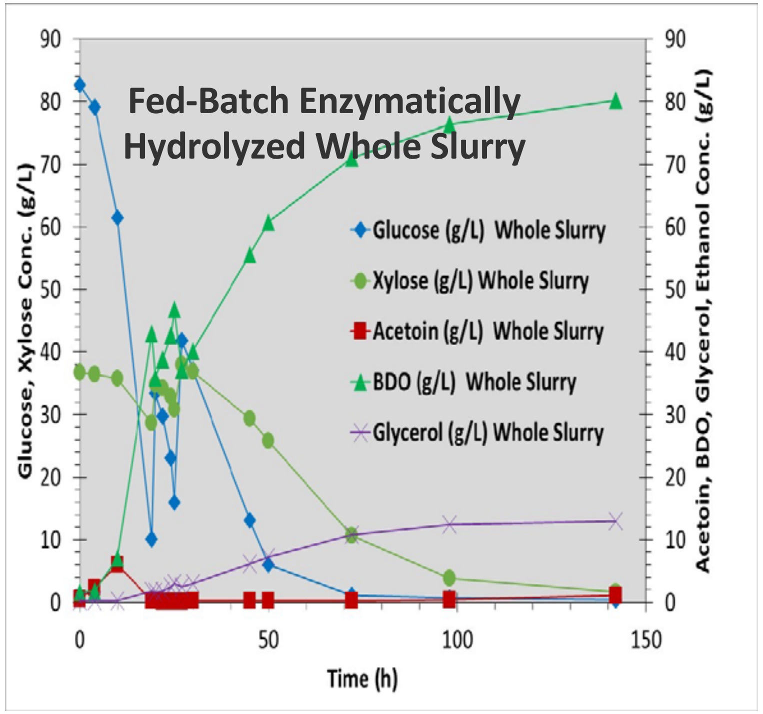
Results: Produced 125 g/L BDO from deacetylated mechanically refined (DMR) corn stover liquor

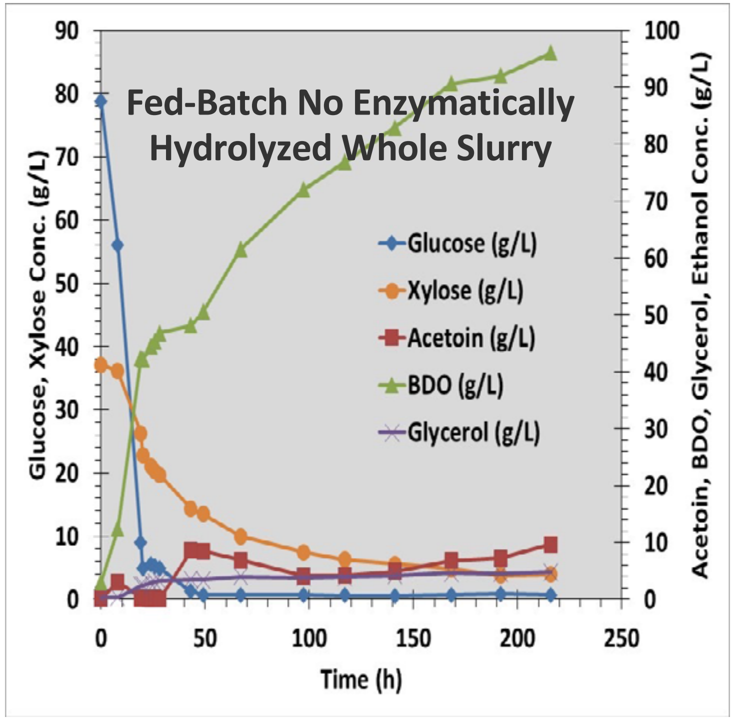
Key Findings: Sugars fed continuously targeting <5 g/L residual glucose by adjusting feed flow rates. Used NIR for near real time analysis for process control. Lowered agitation rates continuously to reduce aeration levels during xylose consumption to maximize BDO production.

Significance: Producing the highest titer on lignocellulosic biomass sugars based on literature and producing comparable titers (150 g/L on glucose) to natural producers (but pathogenic) *Klebsiella* species.

Future Work: Continue developing online control of feeds and aeration (NREL-NIR/PNNL-Ramen Spectroscopy). Utilize off-gas analysis for managing the aeration levels.

4. Progress and Outcomes





Parameter	Batch EH Whole Slurry DMR	Fed-Batch Whole Slurry DMR	2030 Design Case Targets	Fed-Batch Raw DMR Solids
BDO Qp (g/L-hr)	1.4	0.56	2.6	0.4
BDO Yp/s (g/g)	0.45 (90%)	0.43 (84%)	0.47 (92%)	0.48 (94%)
BDO Titer (g/L)	70	80	100	92
Utilization G/X	100%/92%	100%/97%	100%/92%	100%/92%

Whole Slurry Fermentation

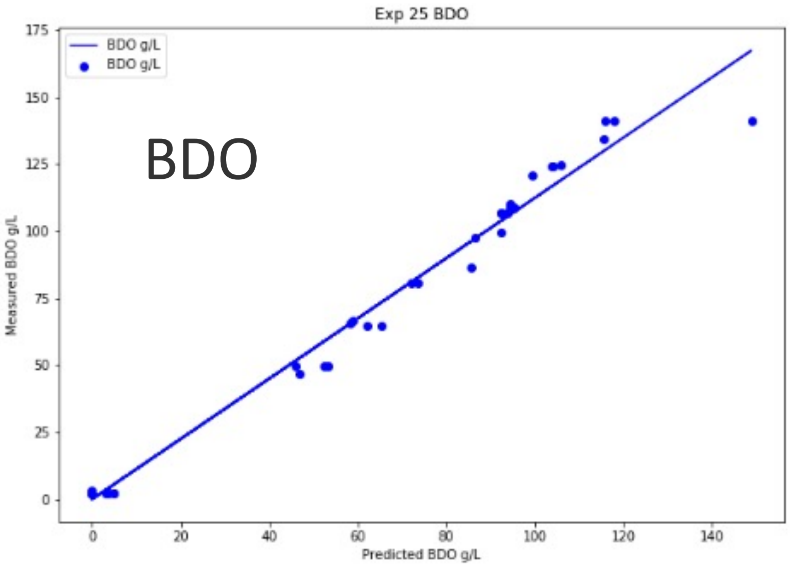
Results: Deacetylated mechanically refined (DMR) corn stover whole slurry fermentations are approaching 2030 performance targets.

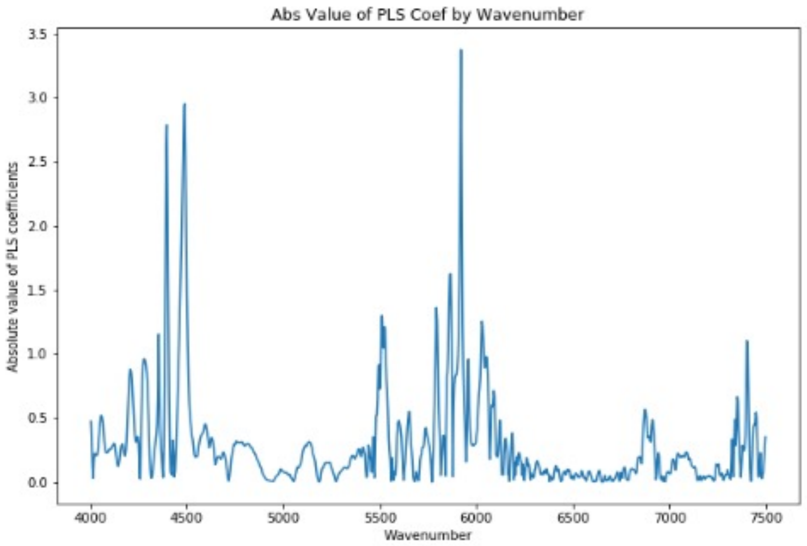
Key Findings: Feeding non-hydrolyzed solids with enzymes increased solids to 28%, titer to 92 g/L and process yield to 94%. Productivity rates were slow (0.4 g/L-hr). Reproducibility was poor.

Significance: $0.85/GGE improvement in MFSP from using whole slurry. Using biomass slurry expands fermentation options for BDO production.

Future Work: Focus on the simultaneous saccharification and fermentation process. Improve reproducibility. Goal of 100 g/L BDO at >1000L by 2023

4. Progress and Outcomes





Near-Infra Red Analysis

Results: A NIR model was successfully developed to predict the concentrations of glucose, xylose, BDO, acetoin, and glycerol. The NIR model had an R2 value of 0.859, and MSE of 55.5 which resulted in and average error of 7.4 g/L. Analysis can be done in 5 minutes.

Key Findings: The quick analysis time enabled better control over the fermentations. Agitation rates and feed rates could be adjusted in near real time.

Significance: Enables tuning the fermentation to meet downstream processing specifications. Aids in scaling.

Future Work: Continue to improve model. Potential to use online NIR probes. Working with PNNL on an alternative analysis (Ramen spectroscopy) and online process control.

Summary

1. **Management:** Cross-functional team working with strain development, feedstock pretreatment, analytical, analysis, two consortiums (separations and catalytic upgrading), and pilot plant operations projects. TEA and risk assessment are used to guide research approach
2. **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 .
3. **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 and on-line analysis aid in scaling and can be used by other projects. Working with industry through a DOE FOA to improve separations and evaluating industrial sugar feedstocks for future development.
4. **Progress & Outcomes:**
    - **Achieved 125 g/L BDO in a fed-batch fermentation** using lignocellulosic sugars from deacetylated mechanically refined (DMR) corn stover liquor.
    - Developing a **DMR whole slurry fermentation** to achieve comparable titers to the liquor that meets the design case technical targets.
    - **Deployed a rapid near-infra red analysis** of the BDO broth to assist with controlling aeration levels through agitation control and feed rates for maximum BDO production.
    - **Produced kilogram quantities of BDO** for the separations and catalytic upgrading consortium research to upgrade BDO to a variety of bio-based fuels and chemicals.

Team Members
Holly Rohrer
Ryan Spiller
Ed Jennings
Darren Peterson

Thank You

www.nrel.gov

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Xiaowen Chen (PI LTAD – pretreatment)

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Additional Slides

Responses to Previous Reviewers' Comments

Overall Impressions from Reviewers

- "Improving process at bench scale and providing predictability for scale up is necessary given BETOs focus on insuring that the technology being developed can be scaled up to industrial scale. The team is focused on developing a fermentation process that can be scaled up so is application driven. Commercial scale up relies in part on technological development. Business decisions are also made based on markets, long term investment and incentives. The team can contribute to improving chances for commercialization as TEA will rely on results from their efforts."
- "The team has had good successes in fermentation development and improvement and scale up to 100L scale. They have done a very good job in attempting to have better fermentation control and analysis and the development of robust fermentation processes with two different microorganisms."
- "This project focused on process development and optimization. The project was initially focused on lipid and BDO production; however, lipid production was dropped due to challenges associated with separations and oxygen transfer. Current work is focused on BDO production. The goal is to develop a process at the ~100L scale. A key accomplishment is the incorporation of off-gas analysis – this will enable them to close the fermentation mass balance. Overall, the work in this project is central to many activities within NREL and BETO. The team is making excellent progress. The milestones are clear. The impact of the project would greatly improve if they explicitly consider product recovery rather than focusing on fermentation titers. In addition, the team should consider how they can generalize these results for new processes."

Responses to Reviewers

- We thank the reviewers for their positive comments and appreciate their acknowledgement of the importance of Bench Scale Research and Development's role in developing biofuel fermentation processes at bench scale to facilitate and de-risk scale up. We recognize the importance of working closely with the strain development groups to evaluate strains in process relevant conditions and providing important feedback on strain performance. The project has historically been aligned with pretreatment, pilot scale integration, and analysis. With the focus of the project on 2,3-butanediol fermentation process development, and as on outcome from the risk register, we have begun to collaborate closely with the Separations and Chem Cat Bio Consortiums to provide material for separations and upgrading research and begin producing fermentation broth that aids the research and development efforts. We continue to maintain a close association with industry by providing information on biocatalyst performance in a process context which we hope will aid in scale-up. For future work, we recognize there are significant challenges in scaling a fermentation process with whole slurry biomass and agree that engaging industrial fermentation experts on vessel design and micro-aeration would greatly help our development and scale-up efforts.

Publications, Patents, Presentations, Awards, and Commercialization

Publications and Presentations

- Solvent-free Spectroscopic Method for High-throughput, Quantitative Screening of Fatty Acids in Yeast Biomass, Eric Knoshaug, Stefanie VanWychen, Holly Rohrer, Nancy Dowe, Min Zhang, Lieve Laurens submitted to Green Chemistry DOI: 10.1039/C8AY02416B (Paper) Anal. Methods, 2019, **11**, 58-69

- Zhang, M., Chou, Y.C., Franden, M.A., Wei, H., Lunin, V., Spiller, R., Rohrer, H., Dowe, N., St John, P., Bomble, Y.J. and Himmel, M., 2019, April. Engineering Zymomonas mobilis as a platform organism for biomass conversion: advances and challenges. In 41st Symposium on Biotechnology for Fuels and Chemicals. SIMB

- Spiller R, Rohrer H, Mohagheghi A, Chou YC, Zhang M, Dowe N. Optimizing operating conditions for production of 2, 3-butanediol using Zymomonas mobilis. In41st Symposium on Biotechnology for Fuels and Chemicals 2019 Apr 29. SIMB

Commercial efforts include evaluating corn starch feedstock from dry mill ethanol plants for potential 2,3 – BDO fermentations