NATIONAL RENEWABLE ENERGY LABORATORY

DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

WBS 2.4.1.100 Bench Scale Research and Development

Conversion

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Goal Statement

Goals: Develop and optimize fermentation processes to produce fuels and chemicals from biomass sugars. Improve the bench scale fermentation laboratory capabilities for internal DOE projects and outside partners.

Outcome:

- An optimized, robust, and industrially relevant fermentation process that reduces cost to meet BETO's biofuel cost target
- Process scales from bench to pilot
- Produce data for State-of-Technology reports to track research progress and validate developed technology
- A flexible and multi-functional bench scale fermentation laboratory

Relevance:

- Show commercial relevance with a developed, integrated, and scalable process
- Provide industry with operational parameters and procedures for future demonstration

Quad Chart Overview

Timeline

- Start: FY2013
- Merit review cycle: FY2017-2019
- 75% complete

	Total Costs Pre FY17** (FY15-FY16)	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$2.2MM	$750K	$986K	$1.5MM

Collaborative Projects

- Targeted Microbial Development
- Pretreatment and Process Hydrolysis
- ChemCatBio Chemical Upgrading of Biomass Intermediates
- Biochemical Platform Analysis
- SCADA for Biochemical Process Integration
- Pilot Integration
- Biochemical Process Modeling and Simulation

Barriers addressed

Ct-D. Advanced Bioprocess Development: **Increasing titer, rates, and yields** of bioproducts **through** metabolic engineering and **fermentation processing improvements** is critical to lowering the costs of fuels and chemicals produced from biomass.

ADO-A. Process Integration: Understanding process integration is essential to (1) characterize the **interactions between unit operations**, (2) identify the **impacts of inhibitors and contaminants** on processing systems

Objective

Develop scalable bioconversion processes to produce fuels and chemicals from biomass sugars ; near term focus on 2,3-butanediol (BDO) production that meets near and long term fuel cost goals of $3 and $2.5/gge. Add to and maintain bench scale fermentation laboratory capabilities

End of Project Goal

Demonstrate an economically viable BDO fermentation process producing **125 g/L BDO at 85% theoretical yield** from cellulosic sugars using minimal aeration that meet $3/GGE (**2.5X increase in titer in three years**)

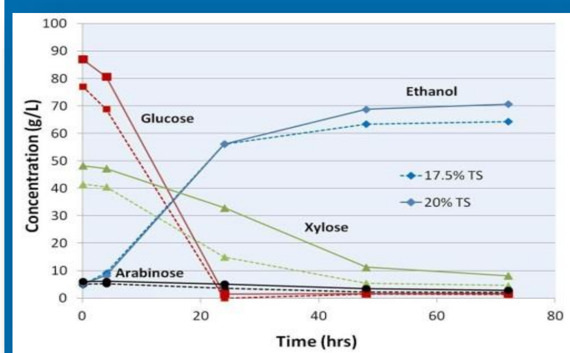
Project Overview – History

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

Cellulosic EtOH

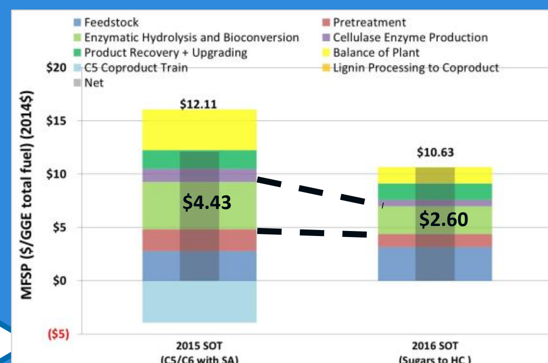
2012 Pilot Demos - $2.15 MESP

	20%	17.5%
EH Glucose Yield	90%	93%
EtOH Process Yield	94%	95%
EtOH Yield from Gluc	97%	97%
EtOH Yield from Xylose	94%	96%
EtOH Yield from Arabinose	51%	63%
Final EtOH titer (g/L)	70	64
Produced EtOH (g/L)	65	59
Xylose Utilization	81%	91%
Arabinose Utilization	53%	67%



Lipid

Reduced bioconversion costs by $1.80/GGE in FY16 over FY15

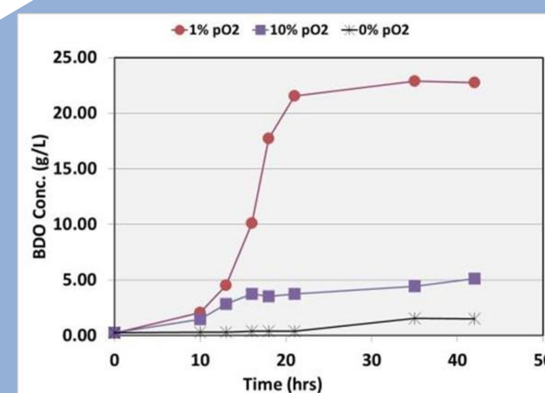


Parameter	FY15 SOT (DDA)	FY16 SOT (DDA)
Lipid Pathway		
Bioconversion volumetric productivity (g/L-hr)	0.34	**0.68**
Lipid content (wt%)	60%	62%
Glucose to product [total glucose utilization]	75% [100%]	78% [100%]
Xylose to product [total xylose utilization]	44% [59%]	77% [100%]

2,3 Butanediol

Early-stage development in 2016 doubled titers

- Focused on titers to enable downstream processing
- Managed oxygen levels in fermentor to favor BDO production
- Targeting redox for optimum pO_2 control



Project Overview – Goals

Lipid Pathway (2017)

- Key goal to **improve productivity** through **fermentation process development**
  - Show 65% lipid content and **0.82 g/L-hr lipid productivity** on biomass sugars; **20% increase** in productivity over 2016
  - Provide data for aeration modeling and quantify non-productive carbon loss to by-products for state-of-technology report

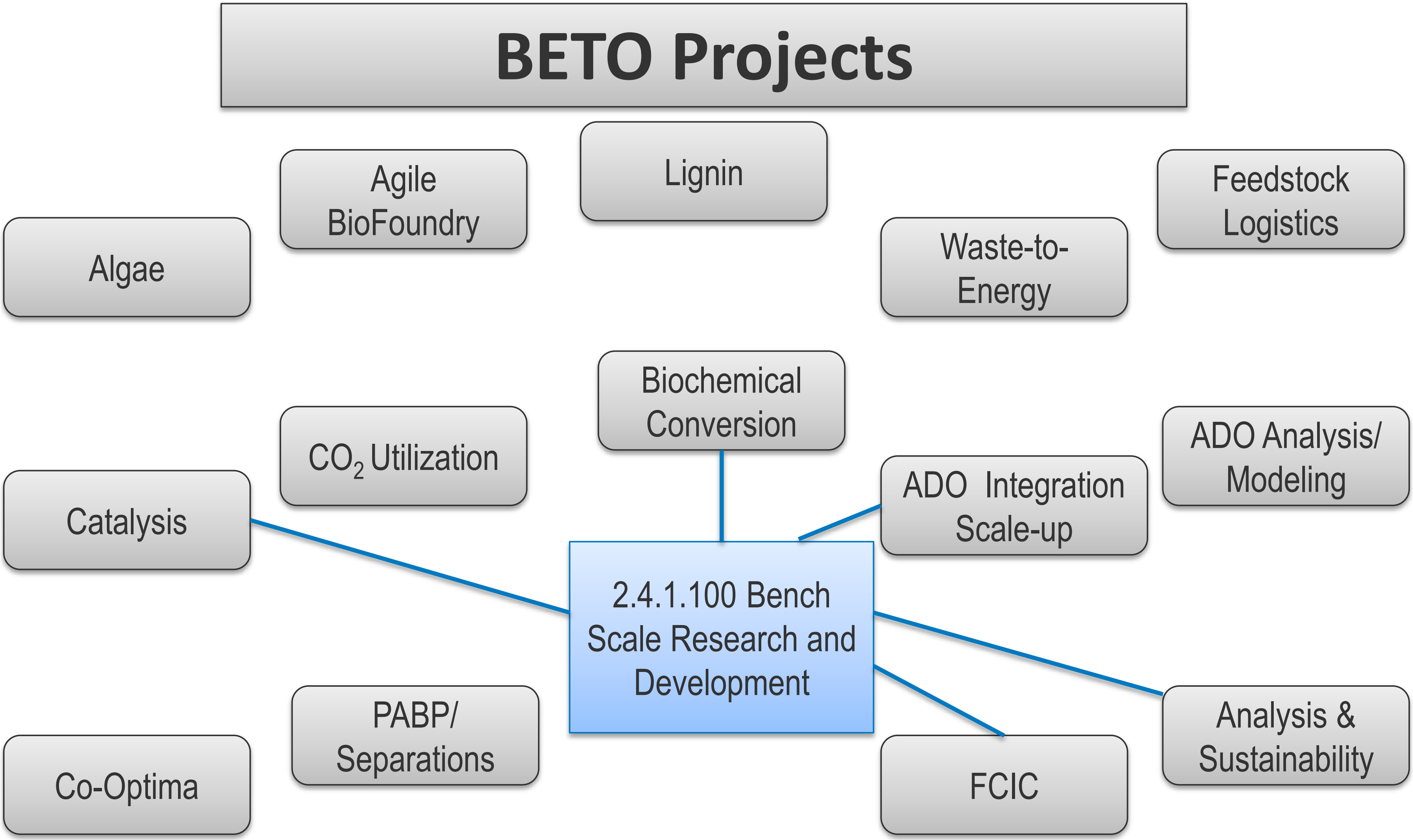
Mixed Alcohols/Diols Pathway (2017-2019)

- Key goal to **increase 2-3 butanediol (BDO) titer** from r*Zymomonas mobilis* through fermentation process development
  - Increase BDO titer from 20 g/L on pure sugar to **75 g/L on biomass sugars by 2018**
  - Determine BDO titer, yields, and productivity on biomass whole slurry for state-of-technology report
  - Produce broth for catalytic upgrading

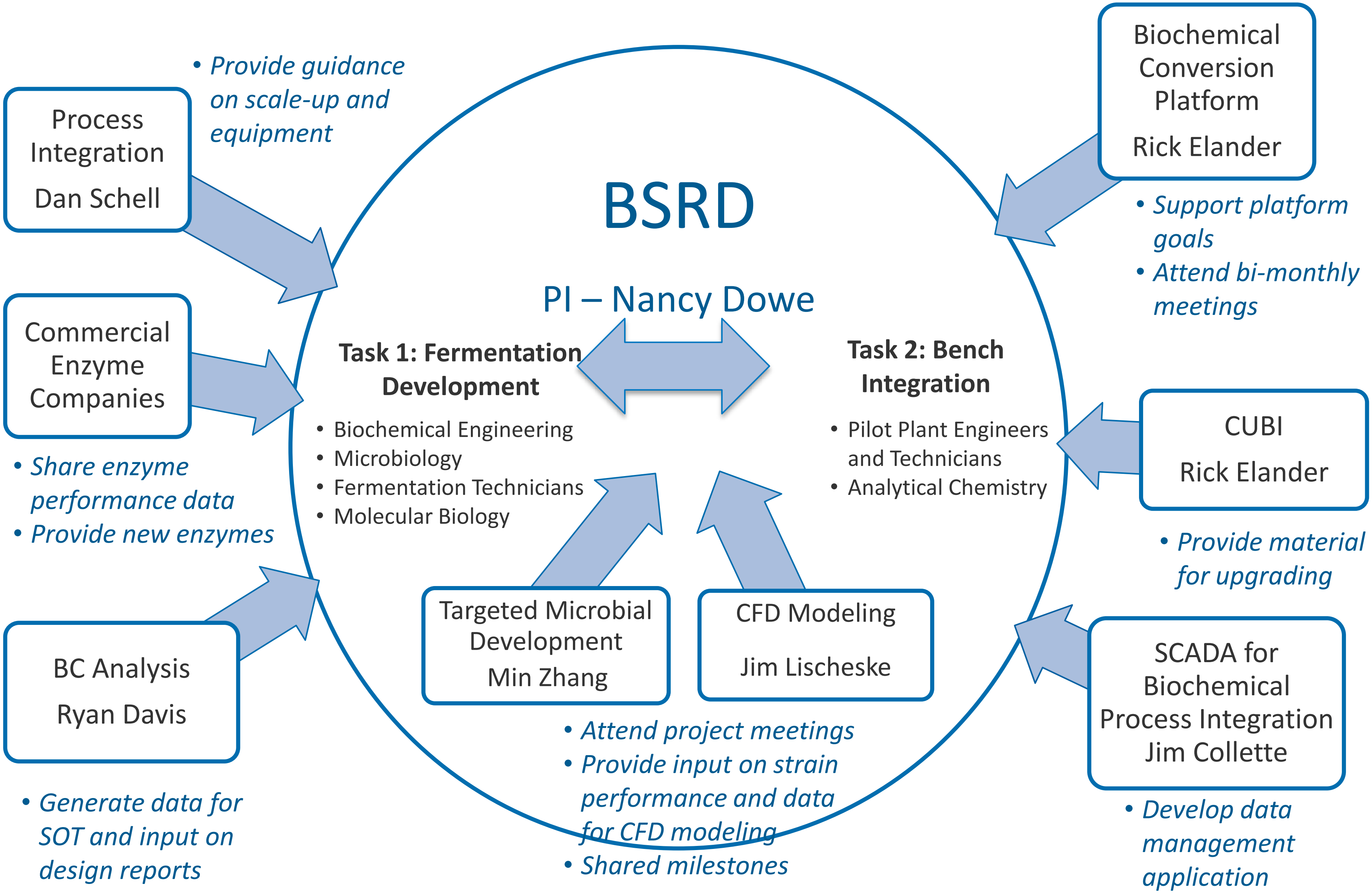
Enzymatic Hydrolysis for Sugar Production (2017-2019)

- Key goal to determine a path forward to supply large quantities of enzyme for pilot runs
- Evaluate commercial enzyme preparations as they become available

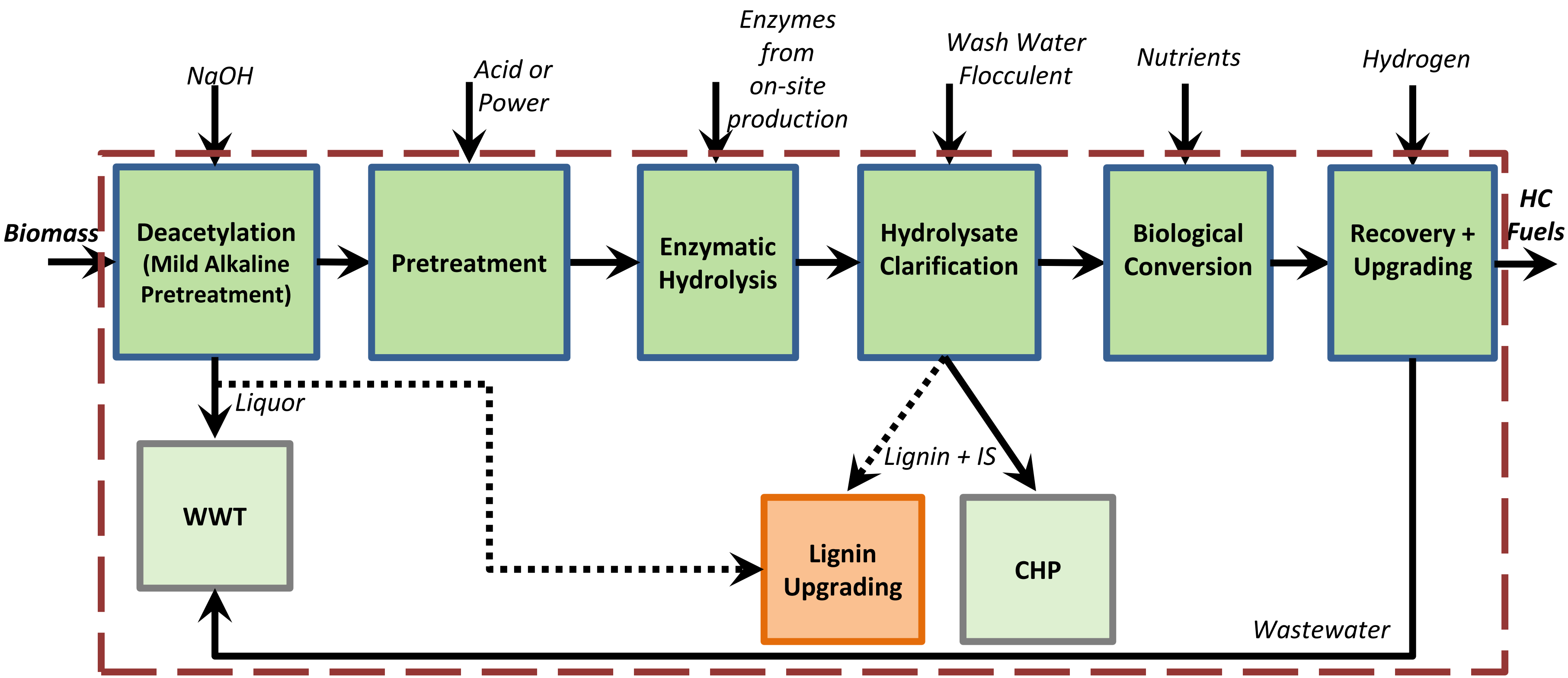
Approach - Management



Approach - Management



Approach - Technical



Four Target Pathways Under Consideration through 2017

- **Intracellular lipids via oleaginous yeast (aerobic)** ✓
- **Secreted long-chain fatty alcohols via yeast (aerobic)** TRL 2-3
- **Short-chain organic acids (anaerobic)** TRL 2-3
- **Mixed short-chain alcohols/diols (anaerobic)** ✓

Approach - Technical

Task 1: Fermentation Development Task

Lipid Pathway

- **Critical Success Factor**
  - Increase lipid content to 65% and productivity to 0.82 g/L-hr
  - Fast productivity reduces fermentor size
- **Challenge**
  - Lipid production is not associated with growth
  - Need optimized conditions for growth and lipid production which is not the same
- **Approach**
  - Develop fed-batch processes for higher titers and productivity
  - Manipulate nutrients for fast growth, high cell density, and high lipid content
  - Use off-gas analysis of CO_2 production and O_2 consumption to understand aeration needs for cell mass accumulation and lipid accumulation

BDO Pathway

- **Critical Success Factors**
  - Demonstrate at least 75 g/L BDO titer using biomass hydrolysate liquor in a fed-batch process
  - Increasing titer reduces down-stream cost
- **Challenge**
  - The recombinant strain has a redox imbalance because of the BDO engineered pathway
  - Micro-aeration will balance the redox – must find the optimized level for maximum BDO production
- **Approach**
  - Develop fed-batch fermentation with micro-aeration control for maximum BDO titer; lower product inhibition from BDO allows for this process consideration
  - Evaluate new strains and adapt fermentation conditions for the specific strain's needs

Approach Technical

Task 2: Bench Integration Task

State-of Technology

- **Critical Success Factors**
  - Produce improved SOT data for annual reports
  - Use new enzymes for enzymatic hydrolysis of feedstock
  - Demonstrate scalability of the BDO fermentation for broth production
- **Challenge**
  - No control over commercial development of new enzymes
  - Micro-aeration can be hard to scale from small bench to pilot plant bioreactors
- **Approach**
  - Use improved fermentation feeding and process parameters for SOT; transition to whole slurry for process cost reductions
  - Monitor by-product formation and off-gas analysis to aid in BDO scale-up
  - Continue to engage enzyme companies when possible

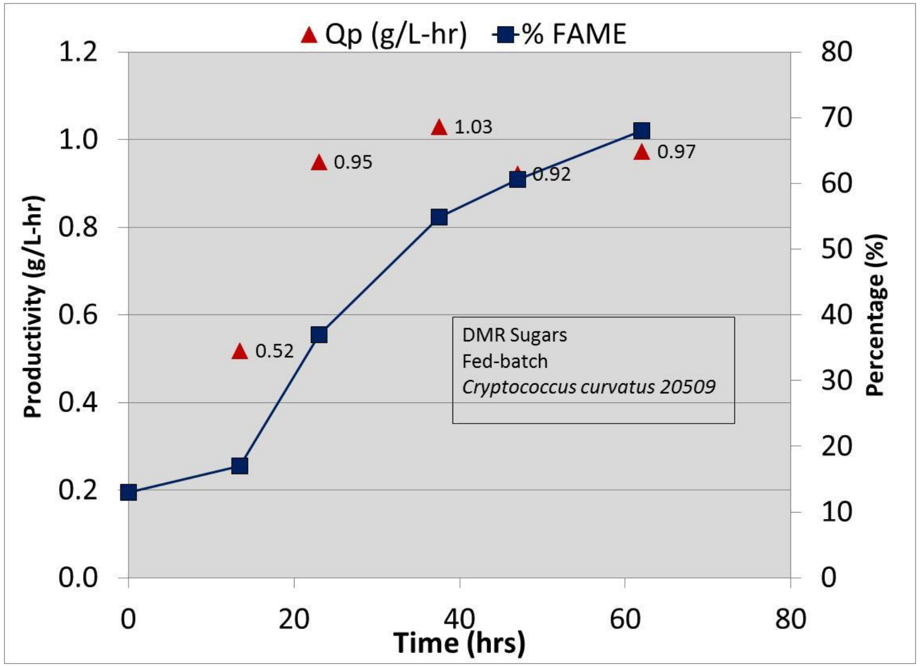
Improve Bench Scale Fermentation Laboratory Capabilities

- **Critical Success Factors**
  - Expand online analysis and data acquisition and control capabilities
- **Challenge**
  - Meeting the research needs of a variety of projects
- **Approach**
  - ○ Add CH_4 and H_2 analyses to mass spec and verify analyses with a test fermentation
  - ○ Design an application to link mass spec data, OUR calculations and feed pump control for lipid production

Accomplishments – Lipid Pathway

Lipid Productivity Improvement

- **Exceeded productivity goal of 0.82 g/L-hr**
- **Modified feeding strategy**
  - Nutrients ratioed to total sugar and added upfront to maximize cell growth
  - Using an organic nitrogen source improved lipid production over inorganic nitrogen



FY17 State-of-Technology

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%

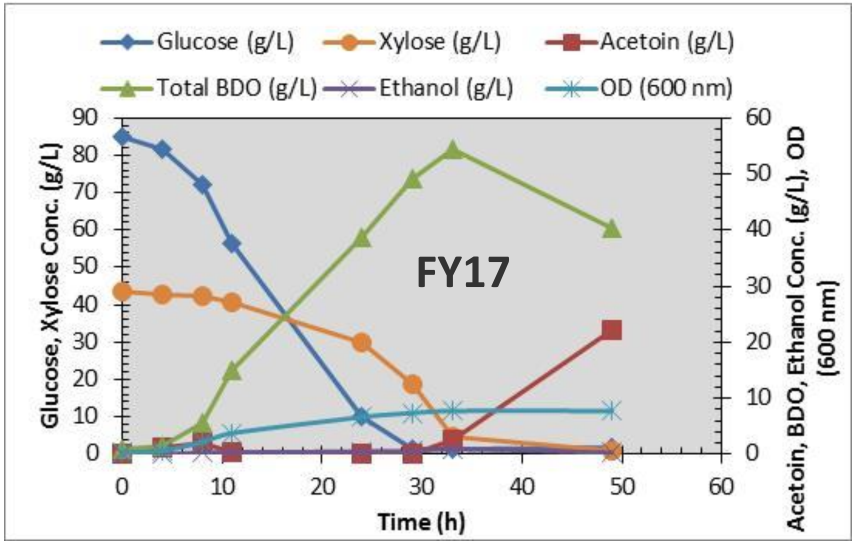
$0.50/GGE MFSP reduction attributed to the lipid fermentation improvements

Mass Balance

	Carbon Closure (%)	Non-Productive Carbon (%)	Lipid Carbon (%)	CO2 Carbon (%)
F1 - *C.curvatus*	**92.7**	**61.9**	**32.1**	**46.4**
F2 - *C.curvatus*	**91.1**	**58.8**	**33.6**	**39.1**

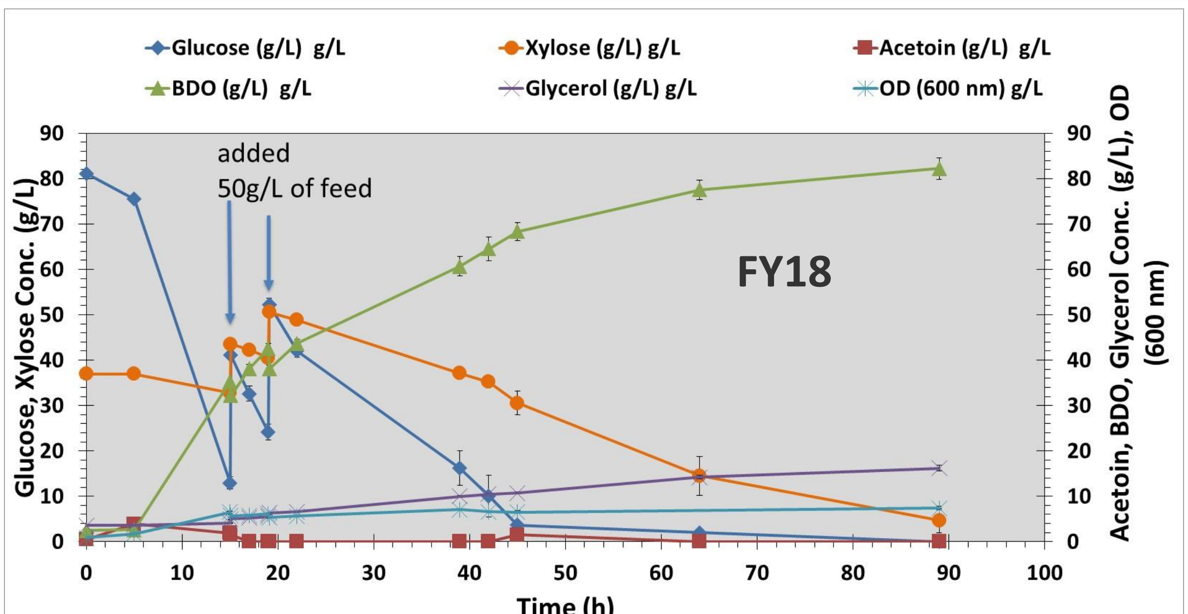
- Developed analysis for exo-polysaccharide (EPS) quantification (non-productive carbon)
- Quantified total CO_2 production using gas mass spectrometer
- Good carbon closure - >90%

Accomplishments – BDO Fermentation Process



Results: Produced **54.5 g/L BDO exceeding 35 g/L goal**, > 95% glucose/xylose consumed, 82% process yield and 1.7 g/L-hr productivity on DMR biomass liquor

Key Findings: Elimination of EtOH production by strain engineering was key to titer increase. Strain is less sensitive to fluctuating micro-aeration levels. Able to run with minimal air overlay

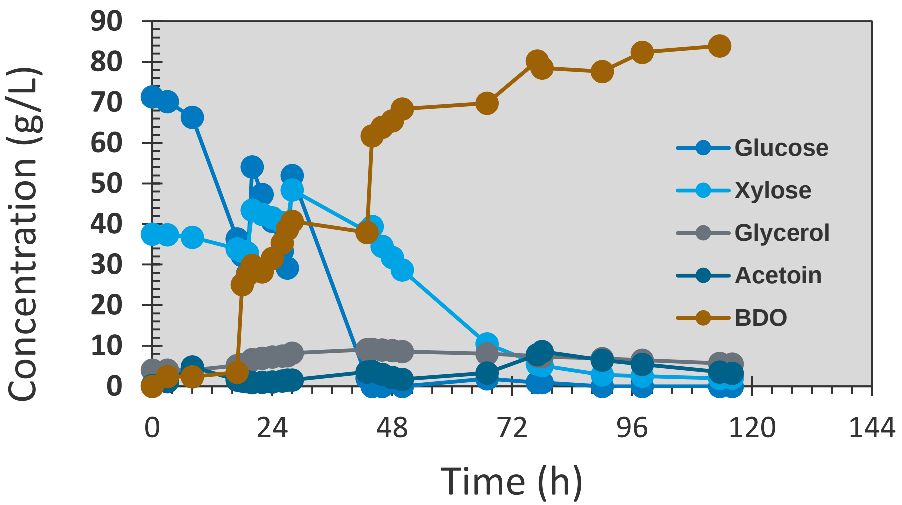


Results: Produced **80 g/L BDO exceeding 75 g/L goal**, > 95% glucose/xylose consumed, 85% process yield and 0.9 g/L-hr productivity on DMR biomass liquor

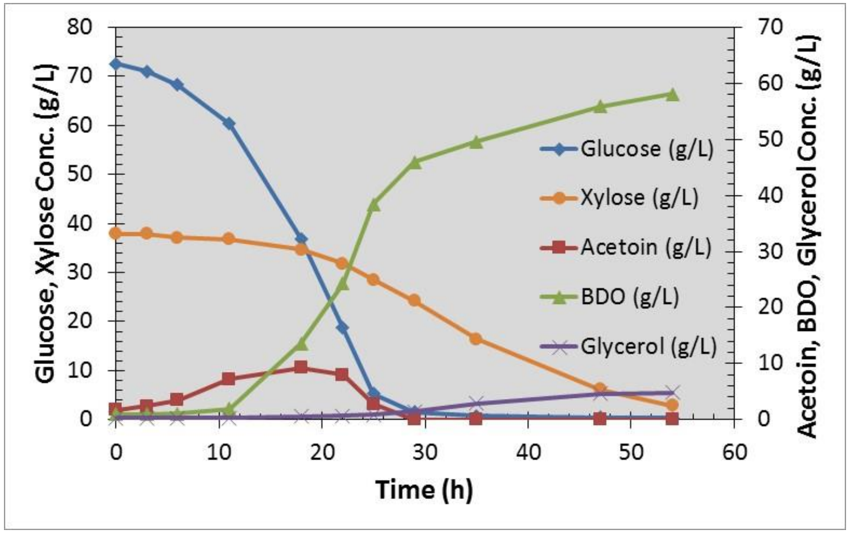
Key Findings: Less aeration needed for xylose conversion to BDO; adjusted agitation rate once glucose was almost consumed to lower aeration levels and maximize BDO production.

Accomplishments – BDO SOT and Scale-up

100L Biomass Hydrolysate Sugar BDO Fermentation



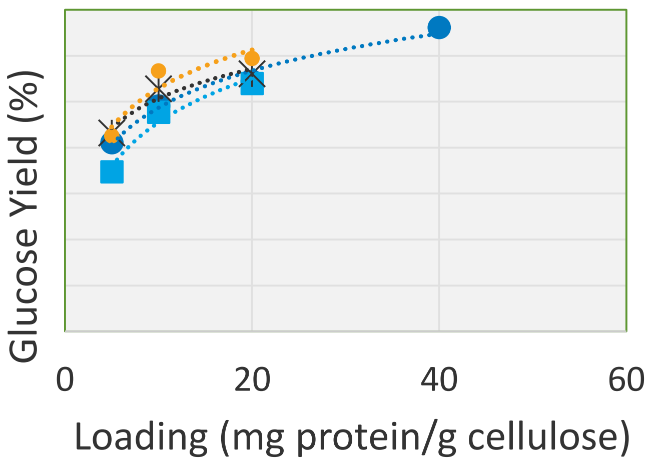
Whole Slurry DMR BDO Fermentation for SOT



Parameter	FY16 DMR Pretreatment (liquor)	FY17 DMR Pretreatment (liquor)	FY18 DMR Pretreatment (whole slurry)
BDO Qp (g/L-hr)	0.23	**1.7**	1.06
BDO Yp/s (g/g)	0.10	0.42	**0.48**
BDO Titer (g/L)	10.5	**54.5**	**58.0**

$0.85/GGE (8%) Improvement in MFSP due to whole slurry

Accomplishments – Enzyme Testing and Fermentation Lab Capabilities

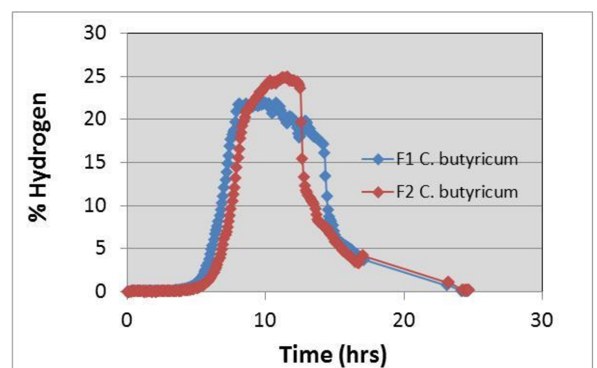


- EC200 enzyme from DSM
  - Received a small sample for initial screening on pretreated corn stover
  - DSM sent us 40L for pilot plant use
- Enzyme operating conditions are favorable for large scale
  - Low operating pH of 4.6
  - Temperature optimum of 63°C

Upgrades to fermentor off-gas analysis

- Able to support up to 24 bioreactors
- Bioreactor size from 0.5 - 9000L
- Flow rate as low as 50 mL/min
- Real-time online analysis of CO_2, O_2, N_2, **CH_4, & H_2**

Automated feeding system



Relevance

Decrease Biomass Conversion Costs Through Fermentation Process Development and Optimization

Industrial Relevance

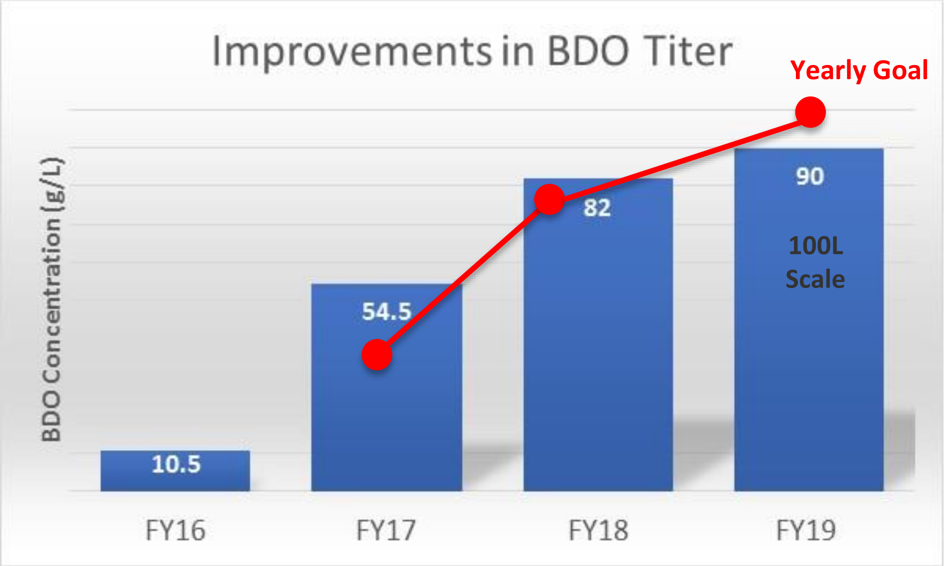
- Demonstrated titers are approaching commercial relevance
- Project identifies and demonstrates operating conditions that align with envisioned commercial-scale processes
- Fermentation laboratory maintained for industrial partners

Project Impact

- BSRD has a direct impact on cost
- Project provides means to test strains, enzymes, and equipment from a variety of organizations in an integrated fashion using biomass sugars

BETO Relevance

- Project focuses on BETO programmatic goals and the 2022 $3/GGE cost target
- Data from project is used in SOT reports to track performance improvements and show yearly cost reductions
- Project reduces scale-up risk and serves as a validation of conversion technology for scale-up



Future Work

Planned R&D through 2020

- Demonstrate titers of at least 125 g/L by optimizing feeds and aeration levels
- Transition to whole slurry to meet cost target goals - R&D on enzymatic hydrolysis
- Screen improved strains from TMD and incorporate into fermentation process
- Develop operational parameters and procedures for scale-up
- Continue producing broth at pilot scale for separations and catalytic upgrading R&D
- Continue to develop LabKey software for data management

Key Milestones

- **100 g/L BDO titer** (FY19 Q4 SMART Milestone)
- **125 g/L BDO titer** (FY20 Q4 SMART Milestone)
- Provide yearly state-of-technology data to track research progress

Go/No-Go

- FY19 develop a risk register for the BDO fermentation process that clearly states identified risks and assess importance to meeting project objectives (performance, cost targets and scalability)

Summary

1. Overview

Develop and optimize fermentation processes to produce fuels and chemicals from biomass sugars. The outcome is an optimized, robust, and industrially relevant fermentation process that reduces cost to meet BETO's biofuel cost target. Project supports improvements to the bench scale fermentation laboratory capabilities for internal DOE projects and outside partners.

2. Approach

Assess the microorganism, product, and TEA to determine fermentation R&D approach. Use fermentation science to develop and optimize conditions like aeration levels, feeding strategies, and nutrient management to reach R&D goals.

3. Progress

Lipid titer and productivity increased from 50 to 60 g/L and 0.76 to 0.97 g/L-hr reducing MFSP by $0.50/GGE. BDO titers from biomass sugars increased nearly 10X (10.6 g/L in FY16 to 90 g/L in FY19) from a combination of strain improvements and micro-aeration optimization. Making **55 g/L BDO from whole slurry helped reduce MFSP by $0.85/GGE.** Upgrades to off-gas analysis increased lab capabilities for a wider range of fermentation projects.

4. Relevance

Project bridges bench-scale development and pilot-scale demonstration. Fermentation development and optimization aligns with envisioned commercial-scale processes. The objectives are aimed at reducing conversion costs and reducing scale-up risks.

5. Future Work

Continue improving BDO titers with a goal of 125 g/L by 2020 to reduce upgrading costs and incorporate whole slurry to reduce solid-liquid separation costs. Develop operational parameters and procedures for scaling fermentation. Produce pilot scale quantities of fermentation broth for separations and upgrading R&D. Screen new strains and incorporate into fermentation process

Thank You

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Response to Reviewers' Comments 2017

Overall Impressions from Reviewers

- "The bench scale integration task bridges the gap between shake flask and the pilot plant to demonstrate production of, e.g. HC fuel from biomass. BSI is an important step in scaling and integrating technology and an opportunity to de-risk. BSI delivers data to track progress towards the $3/GGE HC biofuel target and for TEA. The BSI project has transitioned from EtOH to succinic acid to other products. Maintenance and upgrading the fermentation facilities are part of the task."
- "The program has established an intermediate scale evaluation capability within NREL to bridge lab scale and pilot scale production as a means to work out kinks in the scaleup of biological processes. The goal is to develop conditions at the bench scale that will translate easily into the pilot plant. This is a nice strength of the program."
- "The project crosscuts all aspects of the route from biomass to fuels/products and thus, is a key component for assuring success in the program."
- "This is an exceptionally relevant project for the program. Since BETO's goals are to get technology into the private sector, understanding whether a lab scale operation can be carried out at larger scales is critical."
- "Appears to be good communication between this team and strain development/shake-flask work. However, it would be good to engage strain engineering even earlier in the process, and for the team to consider developing their own scale-down model of fermentation."

Responses to Reviewers

- We agree that we must engage strain development earlier in the process. We are attempting to do that with the butanediol pathway. We are working with the pilot scale integration project, including sharing the development, cost of a scale-down model for fermentation that both projects can use.
- We thank the reviewers for their positive comments and appreciate their acknowledgement of the importance of Bench Scale Integration's role in developing biofuel fermentation processes at bench scale to facilitate scale up to the pilot plant.
- 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.

Publications/Presentations

Eric Knoshaug, Stefanie VanWychen, Holly Rohrer, Nancy Dowe, Min Zhang, Lieve Laurens "Solvent-free Spectroscopic Method for High-throughput, Quantitative Screening of Fatty Acids in Yeast Biomass." Analytical Methods, 2019, **11**, 58-69

Dowe, N. "Collaboration between strain engineering and fermentation scientists is key to developing large scale industrial fermentation processes." Invited talk at 2017 SIMB annual meeting (August 1, 2017 Denver, CO)

Nancy S. Dowe, National Bioenergy Center, National Renewable Energy Laboratory, 1617 Cole Blvd., Golden, CO 80401, Jamie N. Lussier, Life Sciences, YSI Incorporated, 1725 Brannum Lane, Yellow Springs, OH 45387, and Robert M. Gleason, Independent Consultant, 5708 S. Pitchin Rd., Springfield, OH 45502. "Novel Method for the Rapid Simultaneous Measurement of Xylose and Glucose for Bioethanol Production", Poster 2017 SIMB RAFT Conference