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Bench Scale Integration
WBS 2.4.1.100

2017 DOE Bioenergy Technologies Office (BETO)

Project Peer Review

Date: March 7, 2017

Technology Area Review: Biochemical Conversion

Principal Investigator: Nancy Dowe

Organization: National Renewable Energy Laboratory

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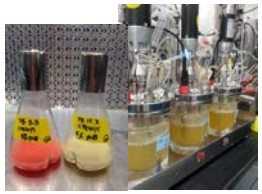
Goal Statement

Project Goal: Develop a bench scale integrated conversion process for scale up that produces HC fuel from biomass at the BETO cost target of $3/GGE by 2022.

Shake Flask



Fermentation Process Development

Pilot Scale

The Bench Scale Integration project is a <u>bridge</u> between Shake Flask and Pilot Scale fermentation

Project Outcome

- Successfully demonstrate an integrated bench scale process for scale up that meets BETO's cost target
- Produce data for State-of-Technology reports to track research progress and validate developed technology
- Optimized and robust fermentation process

Project Relevance

- Important and necessary step in scaling technology
- Incorporate new technology from industry, academia into process when possible
- Generates publicly-available process development data to enable commercial scale-up

Quad Chart Overview

Timeline

- Project Start: October 2014
- Project End: September 2017
- Percent Complete: Approx. 80%

Budget

	Total Costs FY 12–FY14 ($MM)	FY 15 Costs ($MM)	FY 16 Costs ($MM)	Total Planned Funding (FY 17–Project End Date
DOE Funded	$3.7	$1.2	$1.0	$1.0
Project Cost Share (Comp.)*	—	—	—	—

Barriers

Barriers Addressed

- Bt-K Biochemical Conversion Process Integration
- Bt-L Biochemical/Thermochemical Processing Integration
- Bt-G Cellulase Enzyme Loading

MYPP Technical Targets Addressed

- $0.95/GGE Enzymatic Hydrolysis and Conversion by 2022
- $0.41/GGE Cellulase Enzyme Production by 2022

Partners

Collaborators

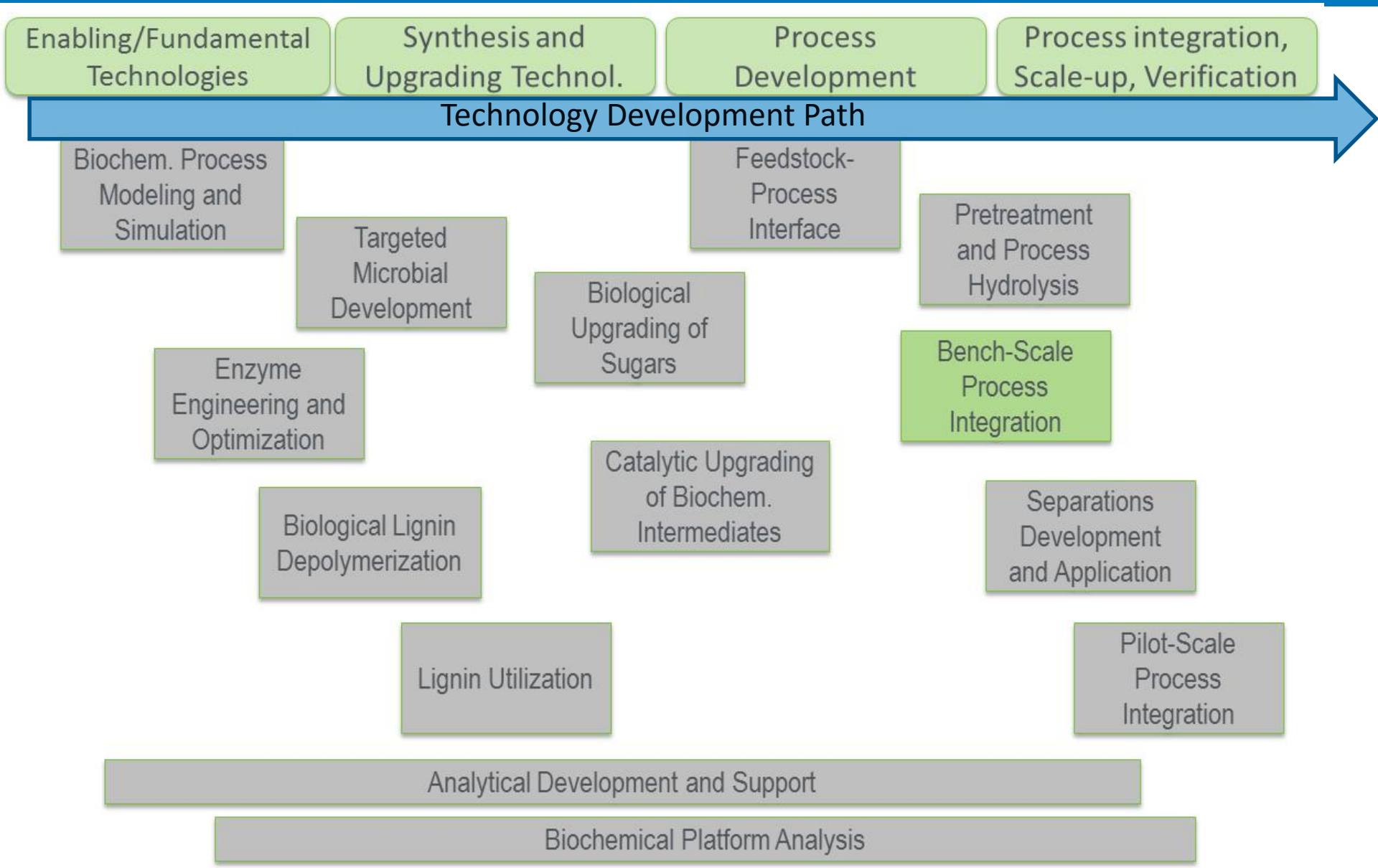
NREL Projects

- Biochemical Platform Analysis
- Analytical Methods Development
- Pretreatment and Process Hydrolysis
- Pilot-Scale Integration
- Separations Development and Applications
- Biological Upgrading of Sugars

Outside NREL

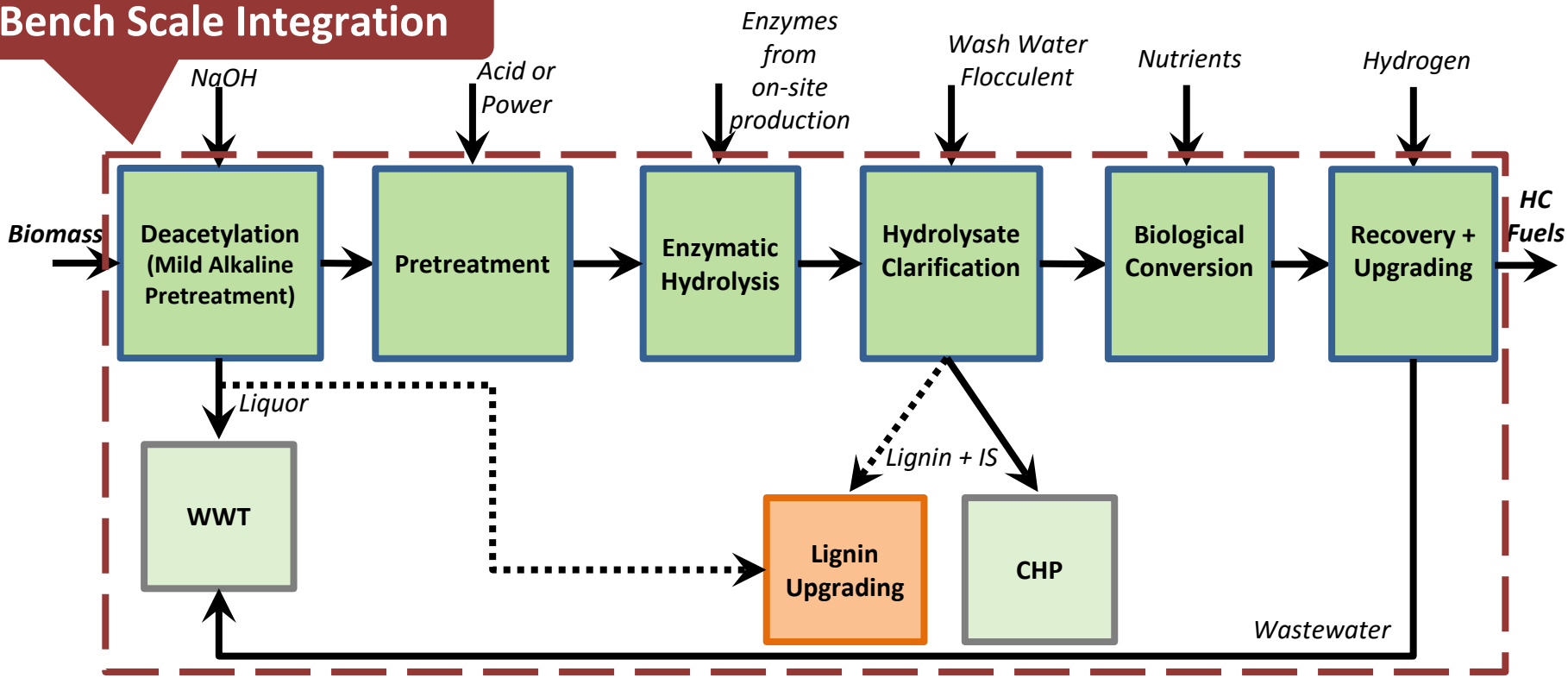
- Novozymes
- DuPont
- University of Pretoria

Project Overview – NREL Biochemical Conversion Projects



Project Overview

Bench Scale Integration



Four Target Pathways Under Consideration

- 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) ✓

Project Overview—Project History

Previous 2017 Process - Demonstrated Technical Targets

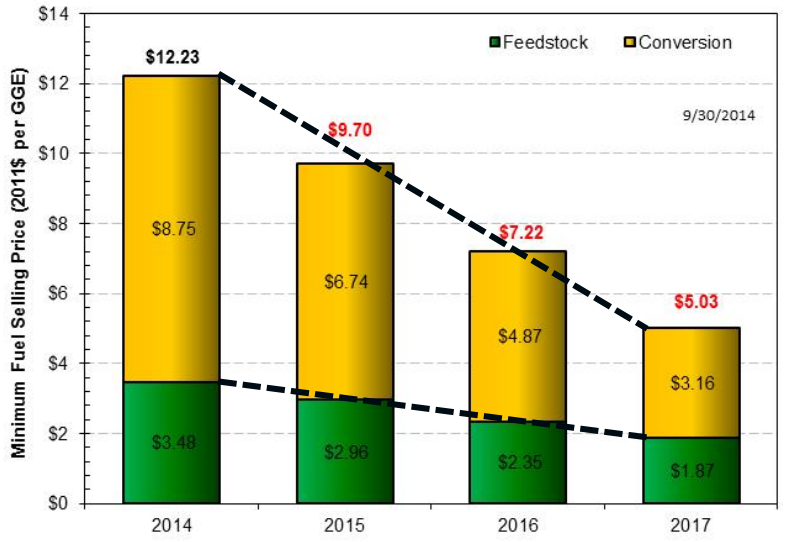
Lipid Technical Targets

Metric	FY14 SOT C6 Biomass Sugars *Lipomyces*	2017 Target C6 Biomass Sugars
Enzymatic Hydrolysis	14 mg/77% Gluc Yield	10 mg/90% Gluc Yield
Glucose Utilization (total)	100%	100%
Xylose utilization (total)	98%	98%
Lipid content	57%	60%
Volumetric productivity (g/L-hr)	0.29 (batch)	0.40 (fed-batch)
Lipid process yield (total sugar-to-product, g/g)	0.26	0.27

Succinic Acid Technical Targets

Metric	FY14 C5 Liquor – Deacetylated PCS	2017 C5 Target
Succinic Acid volumetric productivity (g/L-hr)	0.30 (batch)	2.0 (continuous)
Process yield (total sugar-to-product, g/g)	0.59	0.74
Succinic Acid Concentration (g/L)	43.4	

- Conversion costs need to be reduced
- From TEA, BSI focused on improving productivity and enzyme loading



Project Overview - Goals

Lipid Pathway

- Key goal to improve productivity through **fermentation process development**
  - *2014-2015 (previous 2017 process):* **60% lipid** content and **0.4 g/L-hr lipid productivity** and **2 g/L-hr succinic acid productivity** on biomass sugars
  - *2016 (current 2022 process)*: **65% lipid** content and **0.6 g/L-hr lipid productivity** on biomass sugars; demonstrate 0.82 g/L-hr by end of 2017
- Key goal to show lower enzyme loading and higher glucose yield by testing new commercial enzymes
  - Target is **10 mg** protein/g cellulose and **90% glucose yield**

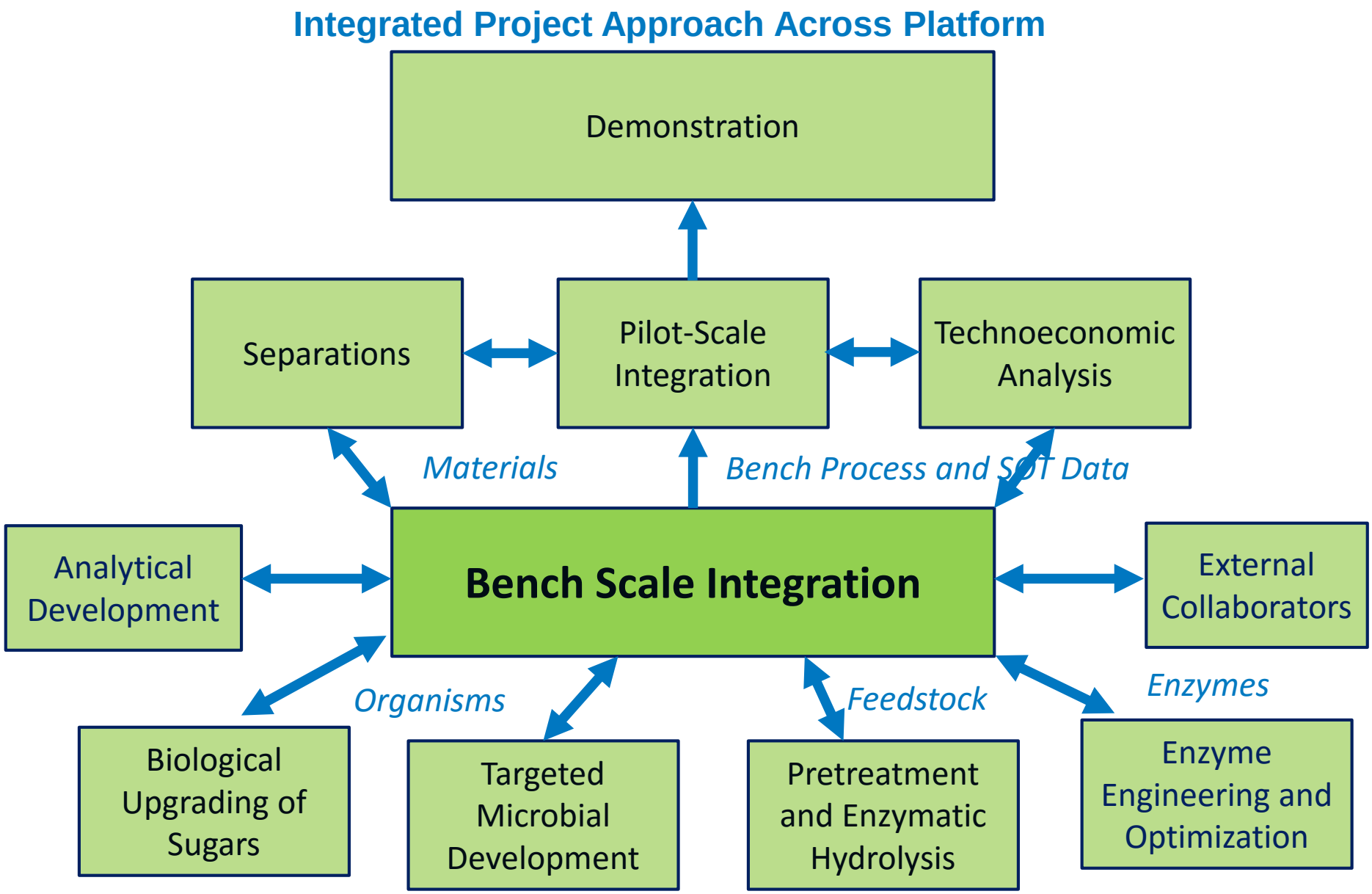
Mixed Alcohols/Diols Pathway

- Key goal to improve 2-3 butanediol (BDO) titer from r*Zymomonas mobilis* through fermentation process development
  - Increase BDO titer from 10 g/L to **20 g/L** on pure sugar; demonstrate 35 g/L by end of 2017

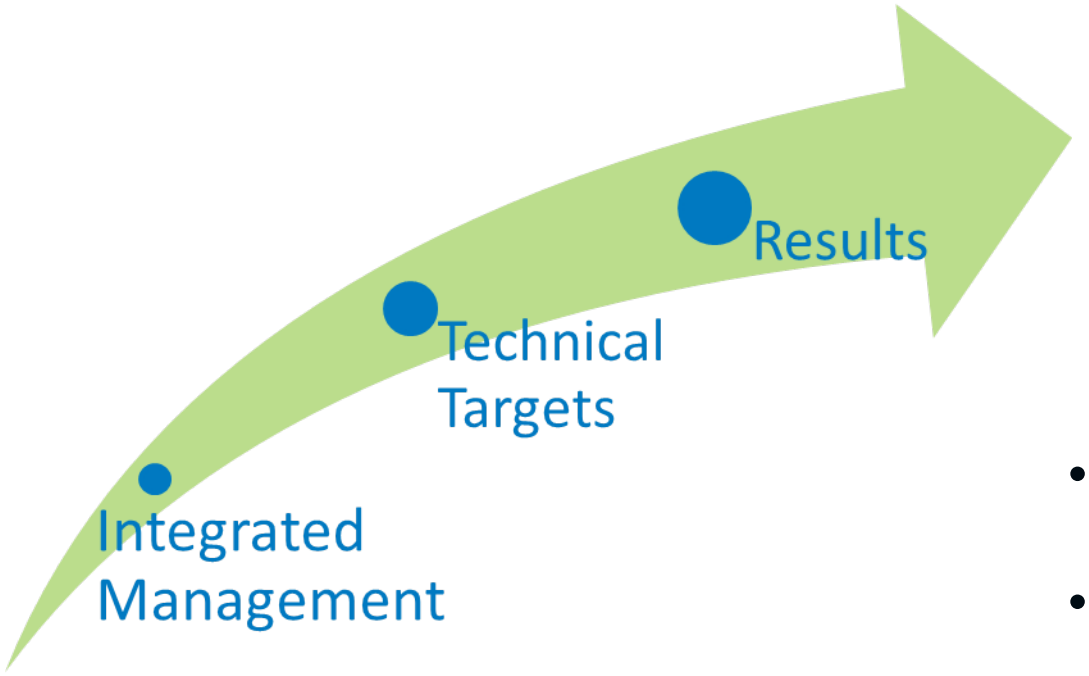
Bench Scale Fermentation Facilities

- Install gas mass spectrometer for off-gas analysis
- Modify existing reactor for larger bench scale enzymatic hydrolysis
- Update fermentor probes

Project Management



Management Approach



Critical Success Factors

Delivery of process suitable for pilot integration and scalable to meet performance and cost targets

BSI Project Structure

Fermentation Process Development ⟷ Bench Integration

Key Challenges

- Process relevant fermentation development
- Integration challenges due to dependency on other core projects to deliver technology ready for integration
- Process complexity and wide range of products to choose for development

Technical Approach—Fermentation Process Development

Lipid Pathway

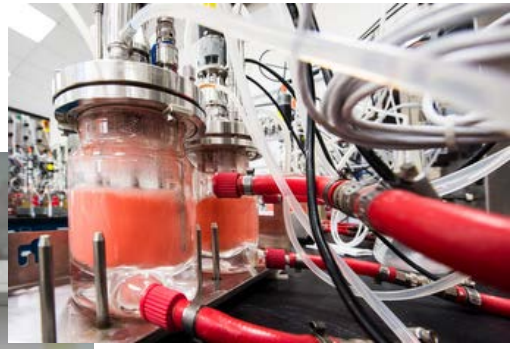
- **Improve fermentation productivity**
  - Increase lipid content to 65% and productivity to 0.82 g/L-hr (end of BSI project goal)
  - Improve succinic acid productivity to 2 g/L-hr (2017 process)

- **Approach**
  - Develop fed-batch and continuous processes for higher productivity
  - Manipulate nutrients for fast growth, high cell density, and high lipid content
  - Understand aeration needs for cell mass accumulation and lipid accumulation
  - Evaluate new developed strains using process-relevant conditions
  - Take advantage of biofilm formation by succinic acid bacteria to increase productivity

BDO Pathway

- **Improve BDO titer**
  - Improve 10 g/L shake flask titer to 20 g/L with further improvement to 35 g/L (end of BSI project goal)

- **Approach**
  - Develop batch fermentation with micro-aeration control for maximum BDO titer
  - Evaluate new developed strains using process relevant conditions



Technical Approach—Bench Integration

Testing New Enzyme Preparations

Demonstrate 85% glucose yield at 10 mg protein/g cellulose loading at 20% total solids

- **Approach**
  - Send pretreated feedstocks to Novozymes and DuPont for testing

Improve Bench Scale Fermentation Facility to Meet Research Targets

Develop off-gas analysis capability, larger bench-scale enzymatic hydrolysis, and update DAC and fermentation control strategies

- **Approach**
  - Re-commission pilot plant gas mass spectrometer
  - Modify Jaygo reactor in pilot plant
  - Use online monitoring and program online measurement to control fermentation parameters (RQ, redox)

Evaluate Pretreated Biomass in an Integrated System

Define best pretreatment process for integration

- Two feedstocks: Deacetylated Dilute Acid (DDA) and Deacetylated Mechanically Refined (DMR)

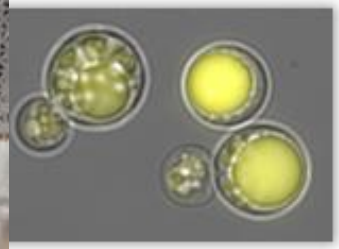
- **Approach**
  - Determine process yields and rates using an aerobic and an anaerobic system
  - Use any new enzymes available

Technical Accomplishments

Technical Accomplishments – Lipid Pathway (Fermentation Process Development Task)

Accomplishment Highlights (Previous 2017 Process)

- **20% improvement** in lipid productivity over SOT—exceeded FY15 target
- **45% improvement** in lipid process yield over SOT
- Nearly **5X improvement** in SA productivity—exceeded FY15 target by 40%
- Met FY15 SA process-yield target



Lipid Production Improvements

Metric	FY14 C6 Biomass Sugars *Lipomyces*	FY15 C6 Biomass Sugars *Rhodospiridium*	2015 Target C6 Biomass Sugars
Glucose utilization (total)	100%	100%	95%
Xylose utilization (total)	100%	59%	98%
Lipid content	56%	60%	60%
Volumetric productivity (g/L-hr)	0.28 (batch culture)	**0.34 (fed-batch)**	0.30 (fed-batch)
Lipid process yield (total sugar-to-product, g/g)	0.17	**0.25**	0.26

Succinic Acid Production Improvements

	FY14 C5 Liquor—Deacetylated PCS	FY15 C5 Liquor—Deacetylated PCS	2015 Target
Succinic Acid volumetric productivity (g/L-hr)	0.30 (batch culture)	**1.4 (continuous culture)**	1.0
Process yield (total sugar-to-product, g/g)	0.59	0.62	0.60
Succinic Acid Concentration (g/L)	43.4	43.3	
Sugar utilization	Xylose—98% Glucose—100% Arabinose—90% Galactose—46%	Xylose—93% Glucose—100% Arabinose—60% Galactose—62%	Xylose—92% Glucose—100% Arabinose—92% Galactose—100%

Technical Accomplishments – Lipid Pathway (Fermentation Process Development Task)

Accomplishment Highlights

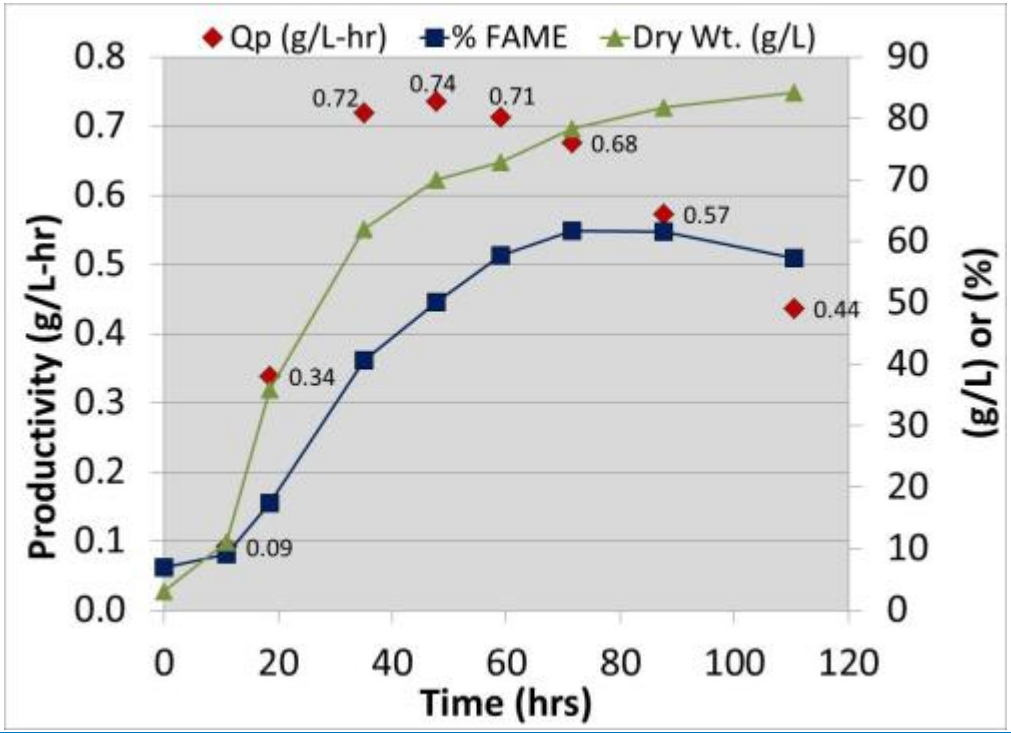
(2022 Process)

- Lipid fermentation **productivity was doubled to 0.68 g/L-hr** from deacetylated dilute acid (DDA) biomass sugars
- FAME remained constant
- Xylose utilization 100%

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%]

Development Highlights

- Managed nutrients differently to grow cells quickly and to higher cell density
  - Increased total sugar fed
  - Determined nutrient requirement as a function of amount of sugar fed for cell mass growth
  - Added all the nutrients up front instead of with the sugar feed
- Switched to *Cryptococcus curvatus* 20509



Technical Accomplishments – BDO Pathway (Fermentation Process Development)

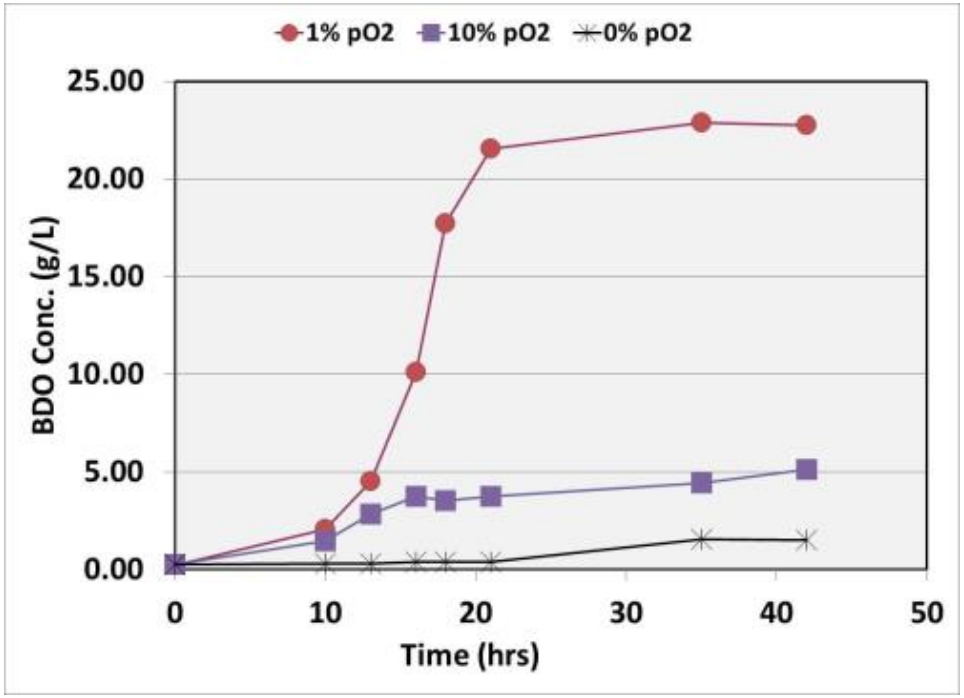
Achievement Highlights

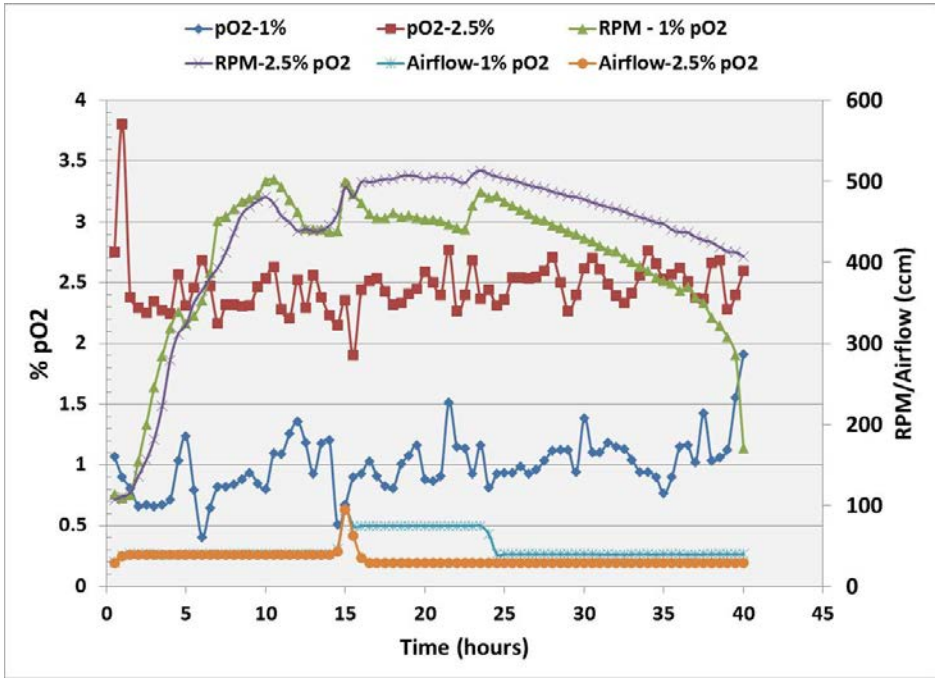
- **Demonstrated 20 g/L BDO**; working towards 35 g/L BDO on glucose
- Determined optimal dissolved oxygen level for maximum BDO production
- Developed a batch fermentation process for strain testing

Development Highlights

- Moved from flasks to fermentors to control pO2 and pH
- Using fermentor control software to adjust agitation and aeration rate to maintain desired dissolved oxygen

100 g/L glucose batch BDO fermentation using r*Zymomonas mobilis*





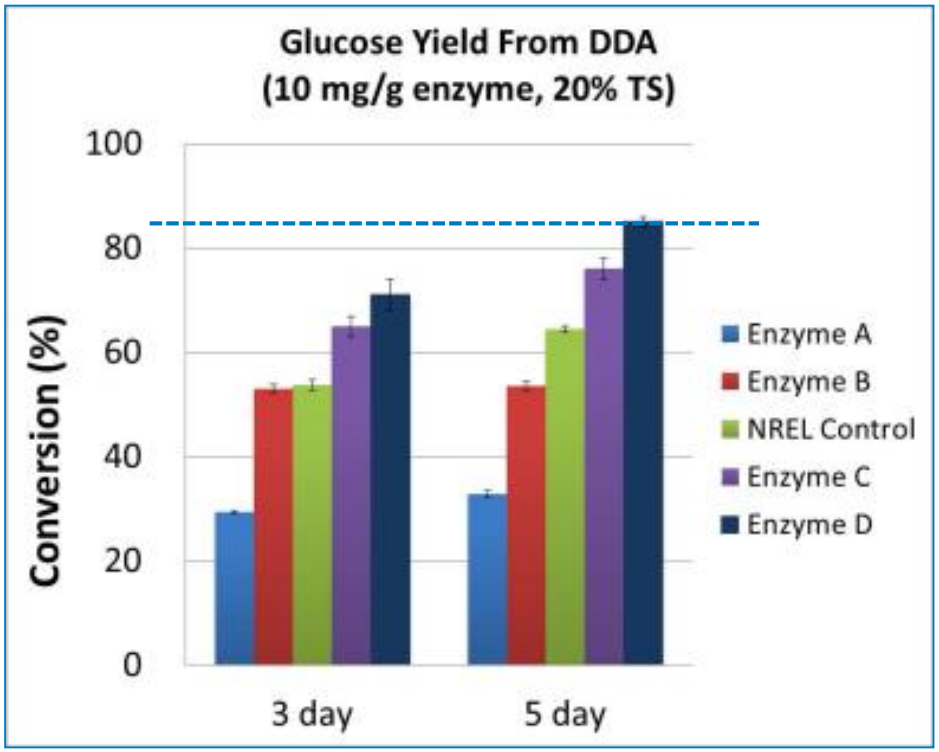
Technical Accomplishments – Enzyme Testing (Bench Scale Integration Task)

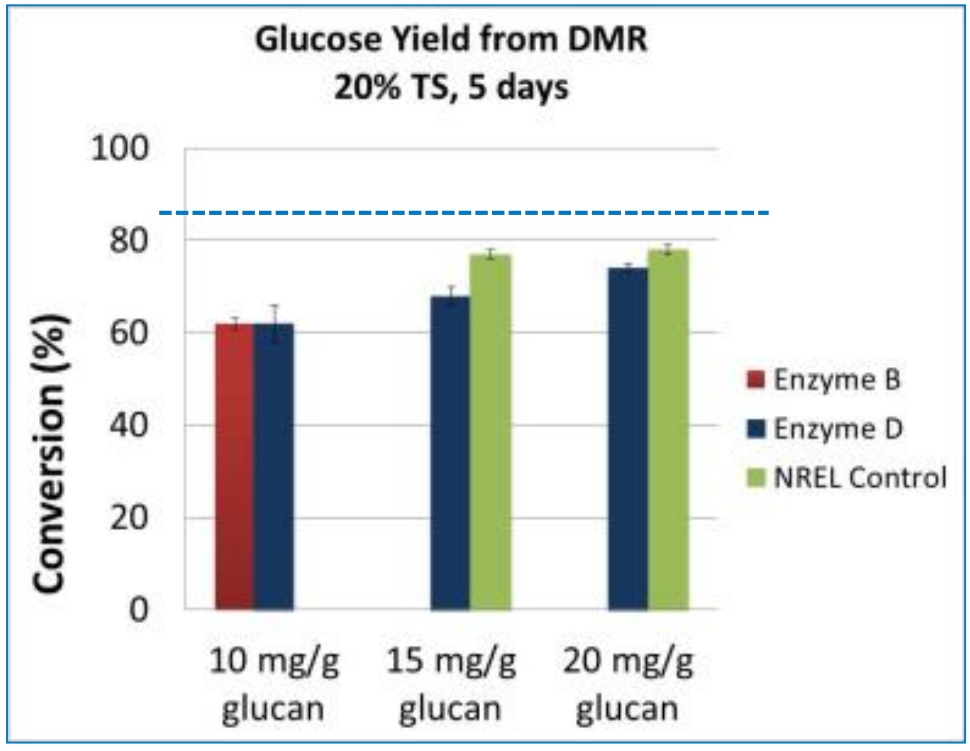
Accomplishment Highlights

- Enzyme **loading cut in half to 10 mg protein/g cellulose loading** (deacetylated dilute acid biomass) from 20 mg FY15 SOT loading
- Glucose increased to 85% over FY15 SOT of 79%

Development Highlights

- Small scale enzymatic hydrolysis assays
- Deacetylated dilute acid (DDA) and deacetylated mechanical (DMR) refined feedstocks sent to Novozymes and DuPont
- In-house testing of Mega Pacific preparation.





Technical Accomplishments – Lipid and BDO Pathway (Bench Scale Integration Task)

Accomplishment Highlights

- Compared deacetylated dilute acid (DDA) and deacetylated mechanical refined (DMR)feedstock in lipid and BDO fermentations
- Better lipid productivity from DMR, otherwise not much difference between the two feedstocks
- Lipid productivity increased **from 0.68 to 0.76 g/L-hr on DMR feedstock**

Development Highlights

- For lipid production, used best nutrient and sugar feeding strategy to achieve high productivity and *Cryptococcus curvatus* 20509
- For BDO, controlled dissolved oxygen at 1% but discovered a lower yield and titer of BDO from mixed biomass sugars when compared to pure glucose

Parameter	DDA Pretreatment	DMR Pretreatment
BDO Qp (g/L-hr)	0.24	0.23
BDO Yp/s (g/g)	0.10	0.10
BDO Titer (g/L)	9.9	10.5
Lipid Qp (g/L-hr)	0.68	0.76
Lipid Titer (g/L)	48.0	50.0
Lipid content (wt%)	62%	64%

Relevance

Bench Scale Integration bridges flasks to pilot demonstration to deliver a process that meets costs and performance

BETO Relevance

- Project focuses on BETO programmatic goals and the 2022 $3/GGE cost target demonstration
- 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

Industrial Relevance

- Project provides means to test strains, enzymes, and equipment from a variety of organizations in an integrated fashion using biomass sugars
- Make information publically available through BETO and peer-reviewed journals
- Fermentation laboratory maintained for industrial partners

Shake Flask

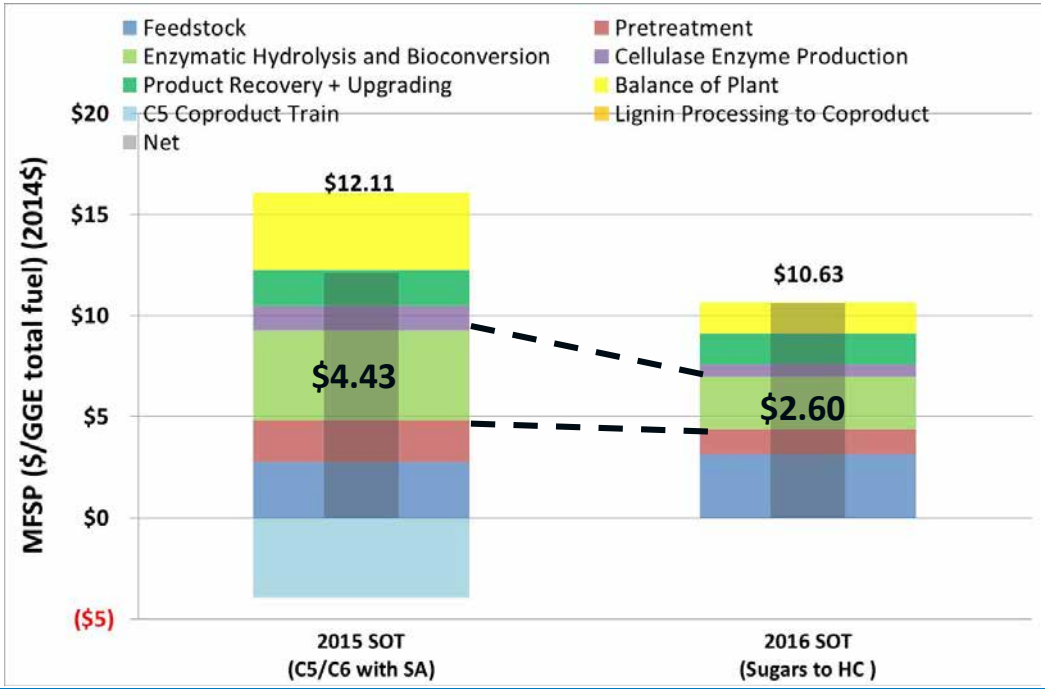
Pilot Scale

Fermentation Process Development

Project Impact

- Bench Scale Integration has a direct impact on cost
- Project identifies and demonstrates operating conditions that align with envisioned commercial-scale processes

BSI reduced the bioconversion costs by $1.80/GGE in FY16 over FY15



Relevance

Develops and maintains a well-equipped bench-scale fermentation laboratory for BETO projects and industrial partner use

- Currently 6 BETO Biochemical Platform projects use the laboratory
- Two Small Business Voucher projects
- Laboratory is designed to handle most all fermentation processes and is available to industry as well as DOE program work

Future Work

Fermentation Process Development Task

- **20% improvement in lipid productivity** over FY16 SOT to 0.82 g/L-hr (FY17 Q4 SMART Milestone)
- **35 g/L BDO titer** (FY17 Q4 SMART Milestone)
- **Quantify aeration needs** for lipid and BDO production (FY17 Q2 Milestone)
- **Identify and quantify fraction of non-productive carbon loss** from lipid-fermentation process (FY17 Q3 Milestone)
- Generate **yearly SOT data**

Bench-Scale Integration Task

- Develop **faster at-line monitoring of fermentation** (i.e., NIR for soluble products, microscope for lipid analysis) (FY17 Q1 Milestone)
- Generate sugar for fermentations
- Continue enzyme evaluations on less-severe pretreated biomass feedstocks

Beyond 2017

- Down select a process for development
  - Must meet cost and performance as determined by TEA
  - Must be integrated and scaleable to pilot plant
  - Fermentation must be robust
- Continue to focus on increasing yield, rate, and titer in the enzymatic hydrolysis and bioconversion steps

Summary

Approach

- Fermentation Process Development Task focused on improving productivity of two fermentation pathways, lipid and BDO, by using different fermentation processes, managing nutrients and sugar feeding, and controlling fermentation parameters such as dissolved oxygen levels
- Bench Scale Integration Task focused on demonstrating better enzymatic hydrolysis through new commercial enzymes, evaluating pretreated feedstocks in aerobic and anaerobic fermentations, and improving the fermentation laboratory to meet fermentation process requirements

Technical Accomplishments

- Produced baseline data on real substrates for FY15 and FY16 SOT which set out-year technical targets.
- Demonstrated reduction in enzyme loading to 10 mg enz/g cellulose on DDA substrate with new enzymes.
- Demonstrated improved lipid productivities with feeding DDA sugars to produce higher productivity (0.62 g/L-hr Qp).
- Demonstrated 20 g/L BDO concentration.
- Lowered the bioconversion costs by $1.80/GGE.

Relevance

- Bridges small scale development to pilot scale demonstration
- Direct impact on demonstrating lower costs

Critical Success Factors and Challenges

- Deliver a scaleable process to pilot scale integration that meets performance and cost targets

Future Work

- Continue improving rate, titer, and yield of the enzymatic hydrolysis and conversion process step
- Down-select the fermentation process with biomass sugars that meet the 2022 performance and cost target.

Technology Transfer

- Collaborate with enzyme companies to develop improved enzymes for new pretreated feedstocks.
- Disseminate process information to DOE and commercial entities.

Acknowledgments

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- Jeff Linger

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Responses to Previous Reviewers' Comments

Overall Impressions from Reviewers

- "This project plays a key role in scaling up and integrating technology developments from different projects. The studies are done in a rigorous and consistent fashion enabling objective assessment of performance improvements."
- "Bench scale integration of the various processing steps is an important stepping stone towards piloting and commercial scale."
- "Good project. 2017/2022 goals will be proven out at this scale before they can go to pilot. Much more efficient way than just going to pilot."

Responses to Reviewers

- We recognize the importance of developing processes in an integrated way because often changes to one part of the process affect multiple areas.
- The project goals are driven by the technoeconomic modeling which keeps the project focused on the R&D necessary to achieve cost targets.
- We maintain a close association with industry by providing information on biocatalyst performance in a process context which we hope will aid in scale-up.
- This project is closely aligned with NREL's strain development, pretreatment, pilot scale integration, analysis, and separations projects. And we are particularly keyed into separations; both from needing biomass sugars for the fermentations and producing material for downstream processing.
- There are multiple shared milestones that keep all the projects working closely together as we develop the technology.

Publications, Patents, Presentations, Awards, and Commercialization

Yang, S., Mohagheghi, A., Franden. M., Chou, Y., Xiaowen, C., Dowe., Himmel, M., Zhang. (2016). Metabolic engingeering of *Zymomonas mobilis* for 2,3-butanediol production from lignocellulosic biomass sugars. *Biotechnology for Biofuels*, 9:189. DOI 10.1186/s13068-016-0606-y

Fei, Q., O'Brien, M., Nelson, R., Chen, X., Lowell, A., & Dowe, N. (2016). Enhanced lipid production by Rhodosporidium toruloides using different fed-batch feeding strategies with lignocellulosic hydrolysate as the sole carbon source. *Biotechnology for Biofuels*, *9*(1), 130. http://doi.org/10.1186/s13068-016-0542-x

Schell, D. J., Dowe, N., Chapeaux, A., Nelson, R. S., & Jennings, E. W. (2016). Accounting for all sugars produced during integrated production of ethanol from lignocellulosic biomass. *Bioresource Technology*, *205*, 153–158. http://doi.org/10.1016/j.biortech.2016.01.024

Salvachúa, D., Smith, H., St. John, P. C., Mohagheghi, A., Peterson, D. J., Black, B. A., … Beckham, G. T. (2016). Succinic acid production from lignocellulosic hydrolysate by Basfia succiniciproducens. *Bioresource Technology*, *214*, 558–566. http://doi.org/10.1016/j.biortech.2016.05.018

Continuous succinic acid production by *Actinobacillus succinogenes* on xylose-enriched hydrolysate. Michael F. A. Bradfield, Ali Mohagheghi, Davinia Salvachúa, Holly Smith, Brenna A. Black, Nancy Dowe, Gregg T. Beckham, and Willie Nicol. 2015. Biotechnology for Biofuels. 8:181

Fermentation Laboratory Capabilities

- Improvements began in 2010 with lab-facility upgrades and first set of Biostat Q+ 500 mL fermentors
- At present we have 36–500 mL (with option to run 3 1L) Q+ fermentors , 2–5L New Brunswick 3000 fermentors, 2–250 mL Applikon fermentors, and gas-to-liquid system with 30 tube reactors for methane work
- Coy anaerobic chamber
- Upgraded to optical dissolved oxygen probes
- Installed gas mass spectrometer for fermentor off-gas analysis
- Jaygo reactor upgrades for enzymatic hydrolysis

- We can run aerobic, anaerobic, microaerophilic, methane feed, with or without solids, batch, fed-batch, and continuous
- 50 kg EH with pH control
- Data acquisition and control
- Currently 6 BETO Biochem Platform projects are using the lab
- Two SBV projects scheduled
- Future improvements
  - Complete piping of all fermentors to MS
  - Improved at-line/online analyses
  - Upgrade control system and add LIMS capability

2016–2017 Project Goals

2022 Biochemical Conversion Process Current SOT and Out-Year Goals

Parameter	FY15 SOT (DDA)	FY18 Projection	FY22 Projection
Enzyme loading (mg/g cellulose)	20	12 (DMR)	10 (DMR)
Hydrolysis glucan-to-glucose	79%	85% (DMR)	90% (DMR)
Hydrolysis residual xylan-to-xylose	26%	85% (DMR)	90% (DMR)
Lipid Pathway			
Enzymatic hydrolysis time (days)	5	3.5	3.5
Bioconversion volumetric productivity (g/L-hr)	0.34	0.82	1.0
Lipid content (wt%)	60%	65%	70%
Glucose to product [total glucose utilization]	75% [100%]	82% [100%]	82% [100%]
Xylose to product [total xylose utilization]	44% [59%]	81% [85%]	81% [85%]
Alcohol/Diol Pathway			
Bioconversion BDO Product Yield		0.43	0.46
Glucose-to-EtOH/BDO [total util.]		35%/60%/[95%]	10%/85%/[95%]
Xylose-to-EtOH/BDO [total util.]		40%/35%/[75%]	15%/75%/[85%]