DOE Bioenergy Technologies Office (BETO) 2015 Project Peer Review

Development of an Integrated Biofuel and Chemical Refinery

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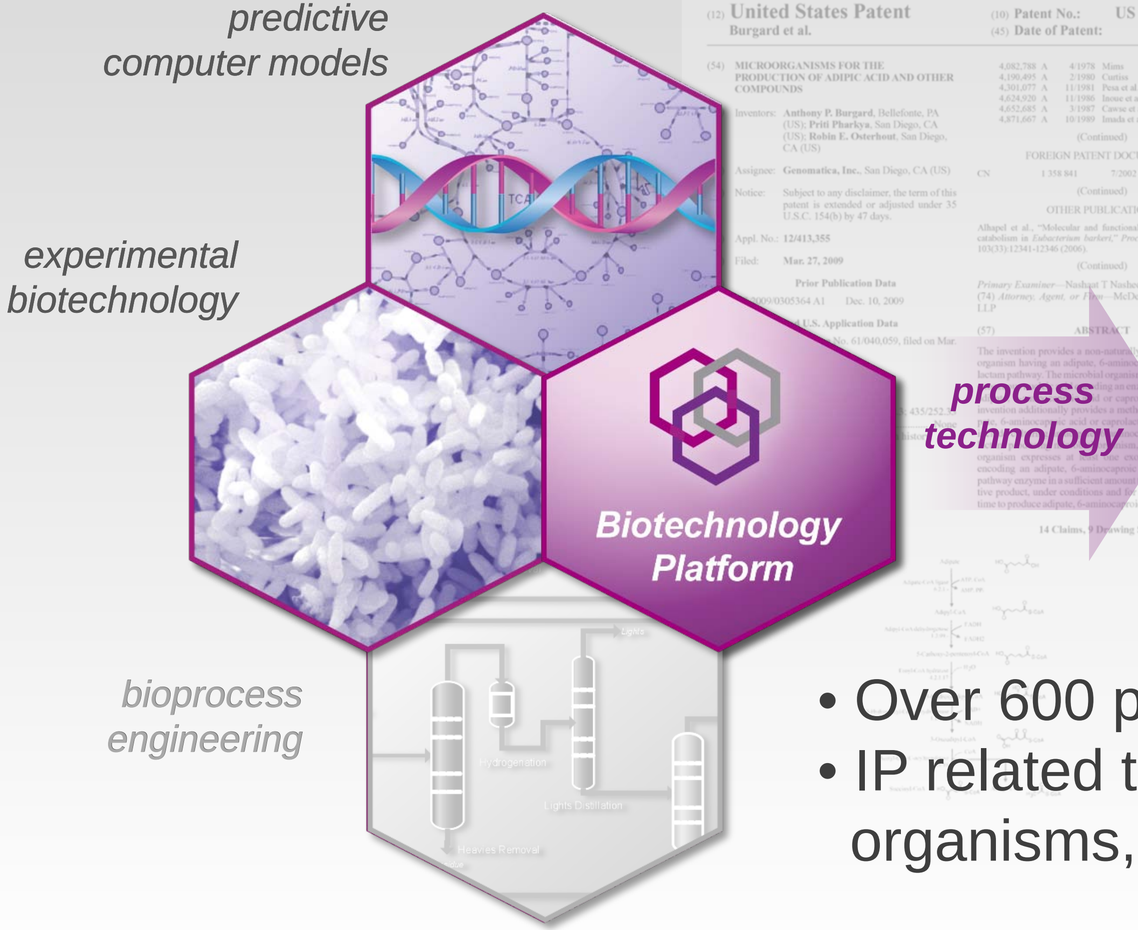
Date: 25 March 2015

Technology Area Review: Biochemical Conversion

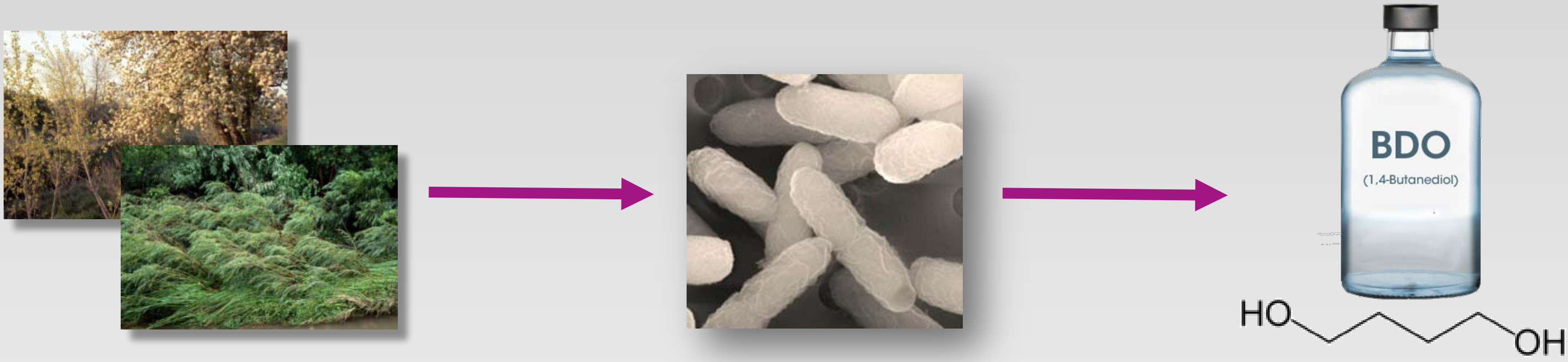
Principal Investigator: Mark Burk
Organization: Genomatica

Integrated biotechnology platform drives innovation, products, solutions



- Over 600 patents and applications
- IP related to 20+ chemicals, organisms, pathways, processes

Goal Statement



Demonstrate the viability and commercial readiness of an integrated biorefinery for low cost production of 1,4-butanediol (BDO), from biomass—deliver the engineered strain and optimized fermentation process to enable the conversion of cellulosic sugars into BDO.

BDO is derived from fossil fuels
Genomatica has developed a glucose to BDO fermentation process

Biomass to BDO could conserve fossil fuels, reduce greenhouse gasses, and provide more flexible feedstock choices

Goal Statement

1: Improving the microbial conversion of cellulosic sugars to BDO.
To deliver commercially acceptable performance and enable scalable integrated biorefineries.

2: Characterizing and improving tolerance to cellulosic hydrolysate.
To deliver commercially acceptable performance and enable scalable integrated biorefineries.

3: Developing and optimizing a scalable fermentation process. *Demonstrate the feasibility and scalability of integrated biorefineries.*

Deliver strains and process for BDO from cellulosic sugars at titer ≥ 70 g/L, and productivity ≥ 2.0 g/L/hr at 30 L scale.

Goal Statement

Results: Surpassed targets for T & R; 90% of yield reached; need for comprehensive approach to biomass-to-BDO; developed improved strains, hydrolysate specification, BDO recovery, and economic models.

Quad Chart Overview

Timeline

- Start: August 2011
- Stage gate: 2013
- End: 1 March 2015 (ext from 9/14)

Barriers

- Barriers addressed
  - Consistency, quality, and concentration of cellulosic sugars in hydrolysates.
  - Glucose – Xylose – Arabinose co-utilization
  - BDO T-R-Y metrics in hydrolysates vs. refined sugar
  - DSP improvements for economics

Budget

	Total Costs FY 10 –FY 12	FY 13 Costs	FY 14 Costs	FY 15 Costs	Total Funding
DOE Funded	853,505	1,259,230	1,938,473	947,908	4,999,116
Project Cost Share (Comp.)	293,400	432,872	442,933	418,329	1,587,534

Partners

- Biochemtex
  - Suppliers of PROESA™ hydrolyates, have worked with Genomatica to reach a specification
  - Biweekly or more frequent consultation with Chemtex staff
- API
  - Supplied API AVAP® hydrolysate
- Other Hz suppliers (OTFF, et al.)
- DOE
  - Worked with DOE officer to manage grant and coordinate changes as needed

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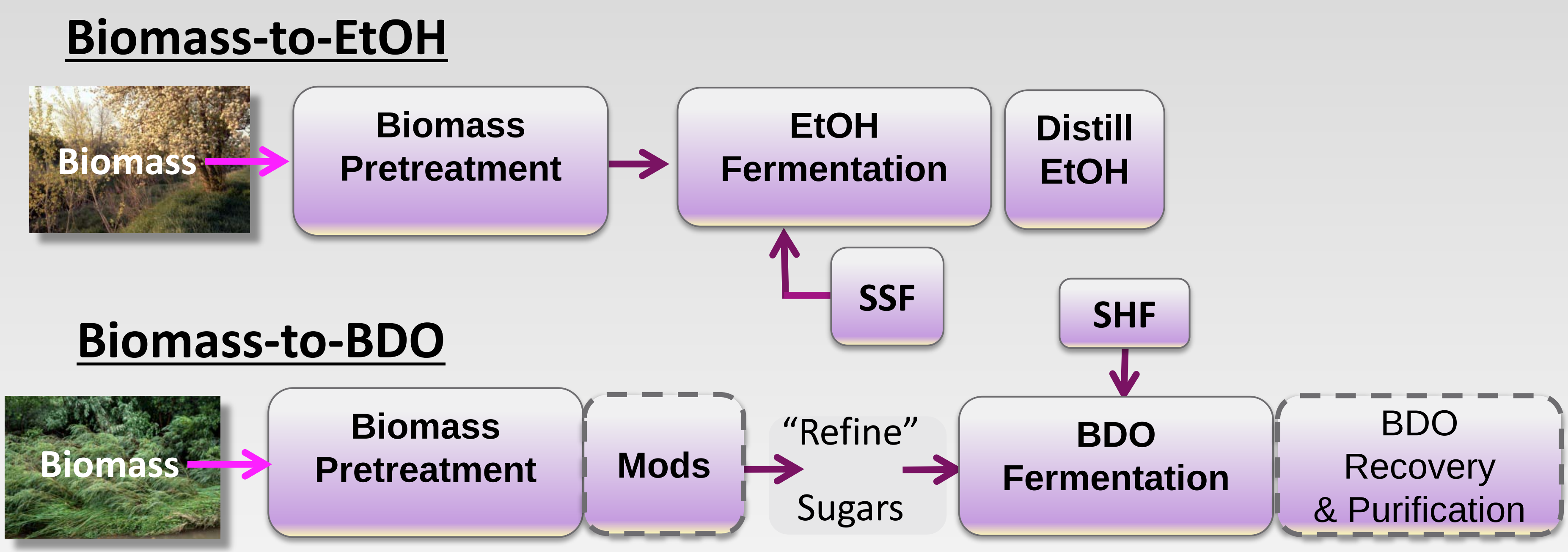
1 - Project Overview: Biomass-to-BDO Process Using Lignocellulosic Sugars



Biomass-to-BDO requires:

1. Pretreatment modifications to produce cleaner, concentrated sugars
2. Process design and economic models for total sugar costs
3. BDO recovery modifications to produce high quality BDO from biomass

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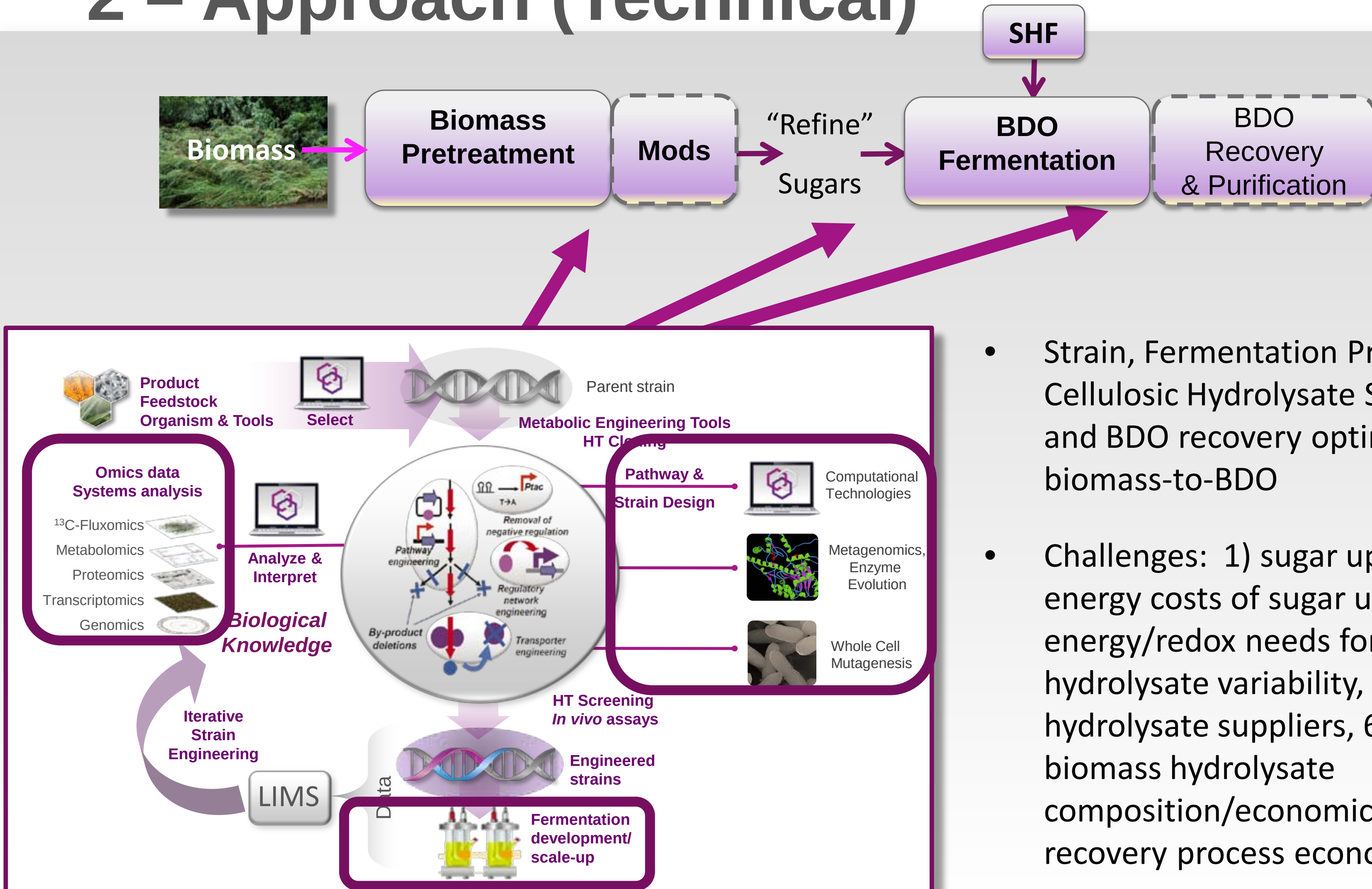
2 – Approach (Technical)



- Strain, Fermentation Process, Cellulosic Hydrolysate Specification, and BDO recovery optimized for biomass-to-BDO

- Challenges: 1) sugar uptake, 2) energy costs of sugar uptake, 3) energy/redox needs for BDO, 4) hydrolysate variability, 5) hydrolysate suppliers, 6) interplay of biomass hydrolysate composition/economics with BDO recovery process economics

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2 – Approach (Management)

- ***T***iter (g BDO/L), ***R***ate (g BDO/L/hr), and ***Y***ield (g BDO/ g Sugars) sufficient for commercialization based on TEA

- C-6 & C-5 sugar co-utilization; complete depletion of fermentable sugars
- T-R-Y in cellulosic hydrolysates
- Composition of hydrolysate ([sugar], other 'impurities', inhibitors, ….)
- BDO *process* for entire process (including recovery)

- Leverage existing knowledge on building BDO producing *E. coli*
- Evolution + directed changes + 'omics to achieve sugar co-utilization and high T-R-Y
- Milestones for each technical metric

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2 – Approach (Management)

Communication: Worked closely with DOE project managers and DOE/NREL Validation Team to refine and direct project.

Project modified; both Genomatica and DOE

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Biomass-to-BDO Approach

Biomass Pretreatment → **BDO Fermentation** → **BDO Recovery**

Focus:

Biomass Pretreatment
1. Remove suspended solids
2. Concentrate sugars
3. Reduce soluble impurities

BDO Fermentation
1. Simultaneous C5/C6 sugar utilization
2. Increase organism hydrolysate tolerance
3. Hit Titer, Rate, and Yield (TRY) targets

BDO Recovery
1. Understand economic impact of increased impurity load on DSP

Adaptive evolution + genomic re-sequencing for sugar co-utilization.
^{13}C flux analysis + metabolomics to ID metabolic constraints limiting performance.

Consider the entire process to develop one that is commercially viable

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Technical overview

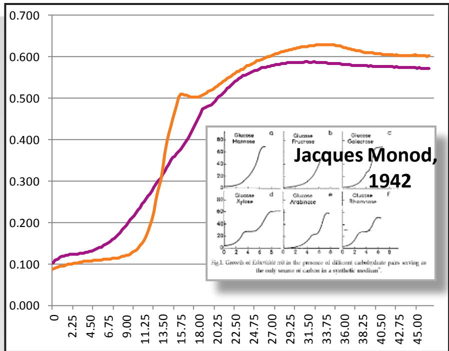
First 2 years, BP-1

- Biochemtex hydrolysate testing and optimization
- Process optimization
- Evolve to C-5 + C-6 sugar co-utilization, ID genes via NGS and introduce into clean background
- Optimize sugar transport efficiency

Final year, BP-2

- Optimize redox, energy, yield, and pathway gene expression for biomass use
- Lignocellulosic hydrolysate specification based on fermentation and DSP
- Optimized strain + improved hydrolysate
- Improved DSP for more economical BDO recovery

Evolved and re-capitulated for glucose + xylose co-utilization



Evolved for xylose – glucose co-utilization; ID'd allele responsible

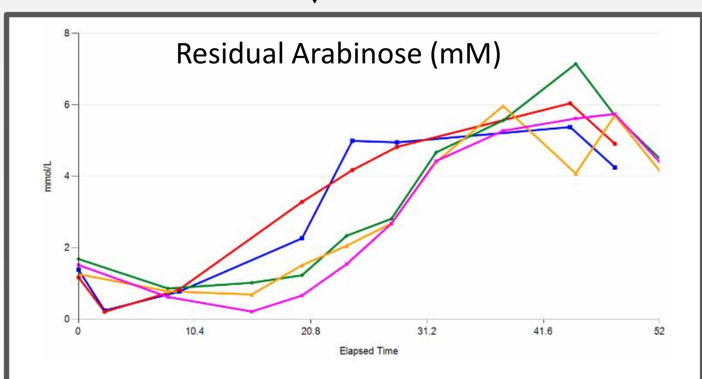
- XUM (xylose utilizing mutant) co-utilizes xylose and glucose
- Then modified for arabinose uptake.

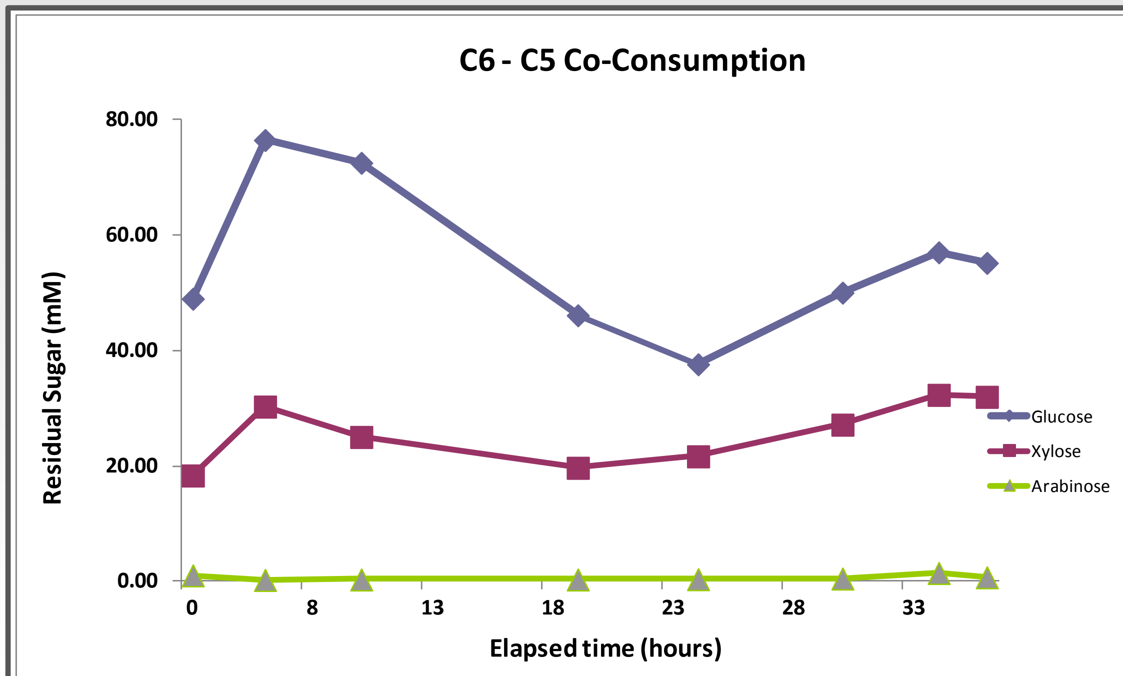
Result: Efficient co-utilization of all 3 sugars during fermentation.

↑

BDO strains have reduced diauxie vs. wt (orange)
Still accumulate other sugars when glucose is present

↓





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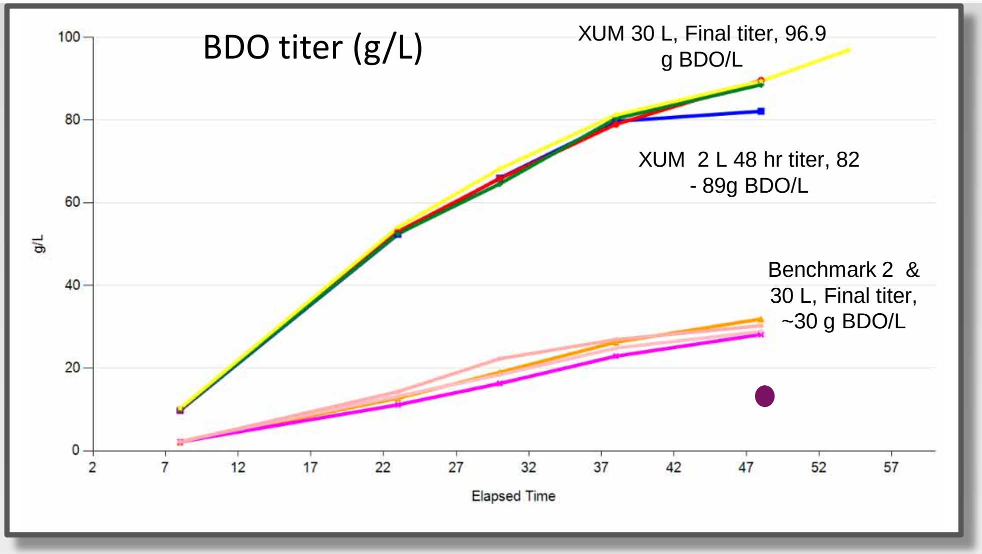
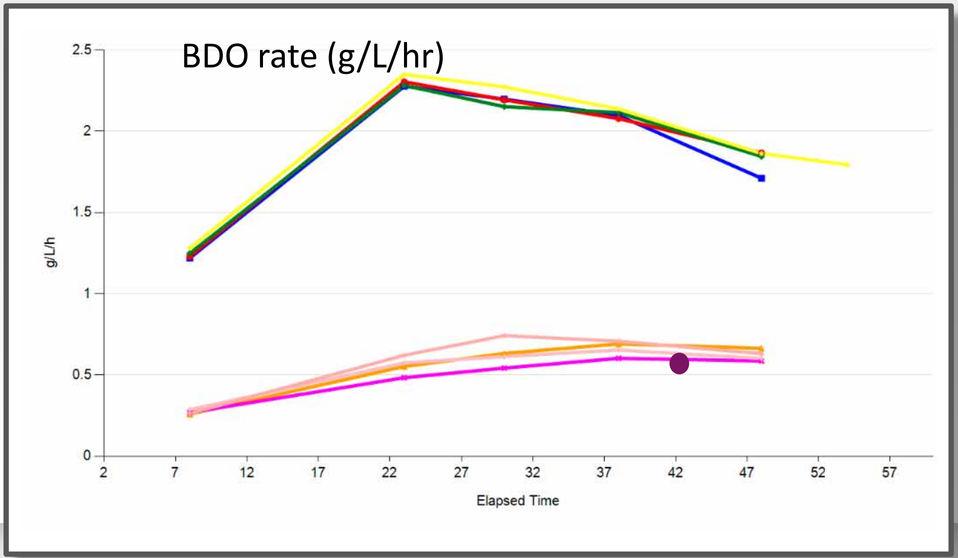
Intermediate (2 yr) DOE/NREL validation visit, XUM biomass strain vs. original benchmark strain, hydrolysate





- Intermediate XUM ara-using biomass strain vs. original benchmark in Chemtex lot 014.2

2 L & 30 L fermentations; **Intermediate XUM, ara-using, same hydrolysate for both 2 & 30 L**

● **Benchmark**

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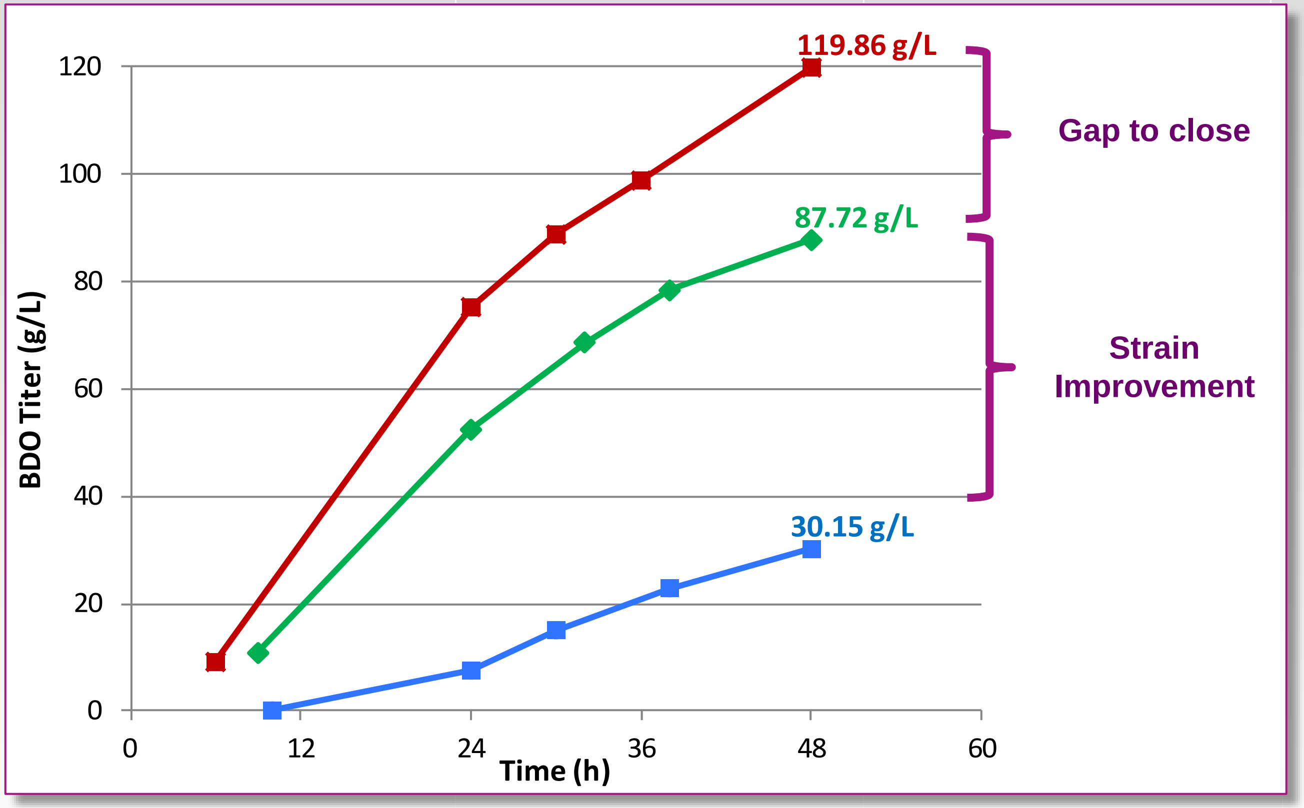
BDO Production using a different feedstock & pretreatment



- Hydrolysate samples via acid pretreatment of wood
- Very clean, high sugar concentration
- Same strain on less clean hydrolysate
- Earlier (original benchmark strain) on less clean hydrolysate

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Different responses to different hydrolysates

Motivation:

- Variation from BDO producing *E. coli* to the many non-sugar components of lignocellulosic hydrolysates
  - *Multiple responses have been observed with varying pretreatments*

- Identify evolution/engineering targets for improving performance in hydrolysate

	Exp 1225	Exp1263	Exp1376	Exp1413
Strain	ECKh7-9	ECKh7-0	ECKh7-9	ECKh7-9, ECKh7-5
Hydrolysate	Supplier A, lot 1	Supplier A, lot 1.2	Synthetic sugar mix + added organic acids feed	Supplier B, lot 1
Feedstock & Pretreatment	Agricultural waste Proprietary	Agricultural waste Proprietary	Reagent grade sugars	Wood Pulp & Paper
Omics	Transcriptomics, metabolomics	Transcriptomics, metabolomics	Transcriptomics	Transcriptomics, metabolomics, proteomics

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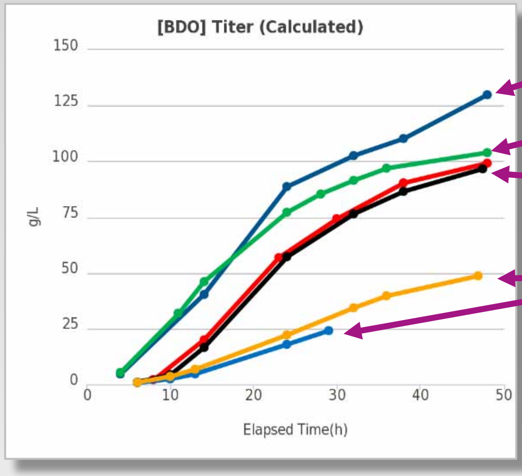
Lignocellulosic hydrolysates from various suppliers (differing pretreatments)

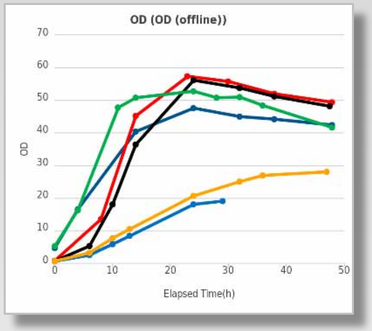


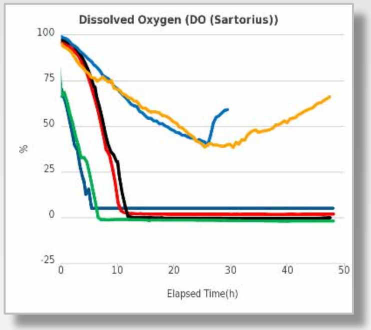
Acid pretreatment, hardwood, XUM strain, earlier

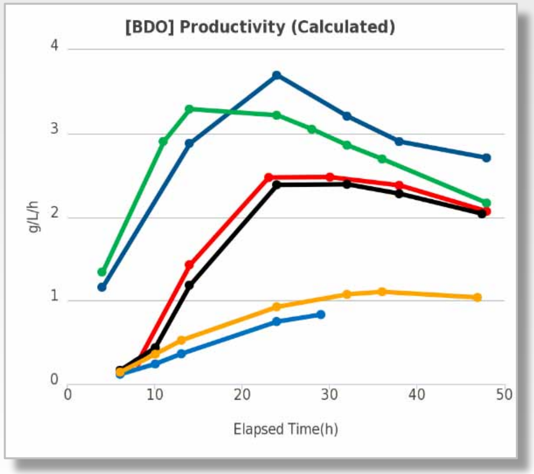
OTFF concentrated 1.3X Latest XUM strain

OTFF unconcentrated Latest XUM strain

Dilute Acid supplier# 1 Latest XUM strain
Dilute Acid supplier# 2 Latest XUM strain







Lignocellulosic Hydrolysates from multiple suppliers

Acid Pretreatment, Hardwood

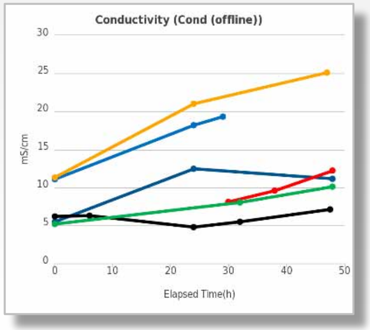
- Very high [sugar], very clean, OLDER strain

OTFF (RSA): Pulp & Paper 'unconcentrated' and 1.3 X by Rotovap

- Performance limited by sugar concentration + furfural/HMF response (omics studies)

2 suppliers using dilute acid pretreatments:

- Performed as expected based on conductivity of the feed



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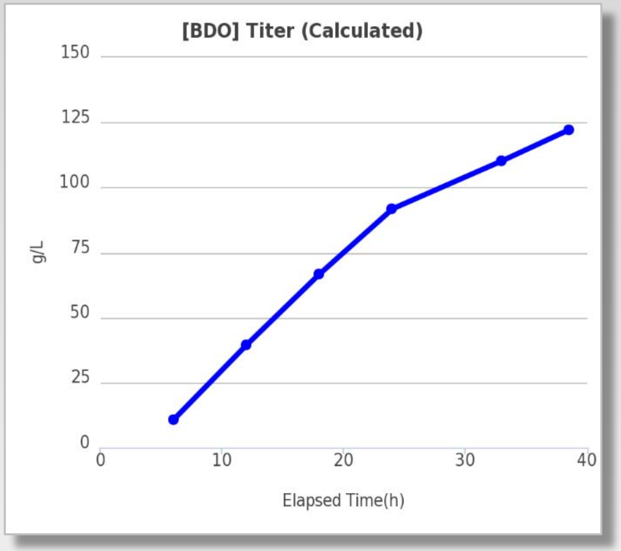
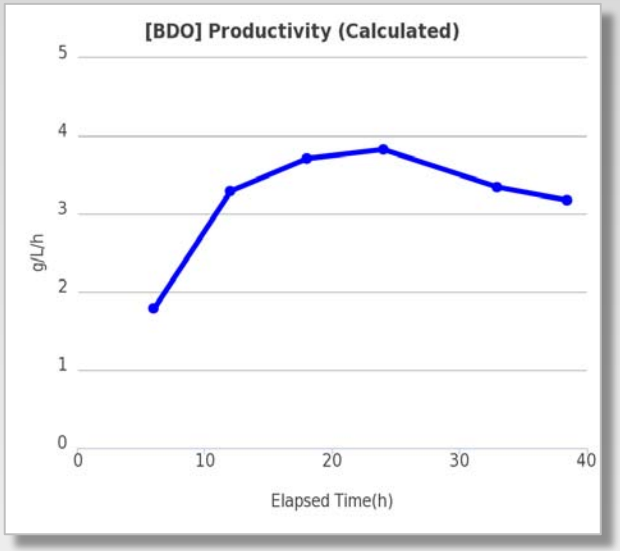
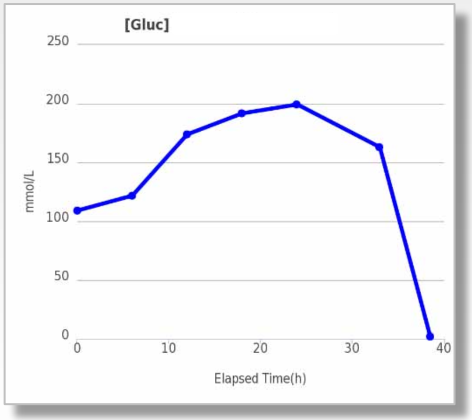
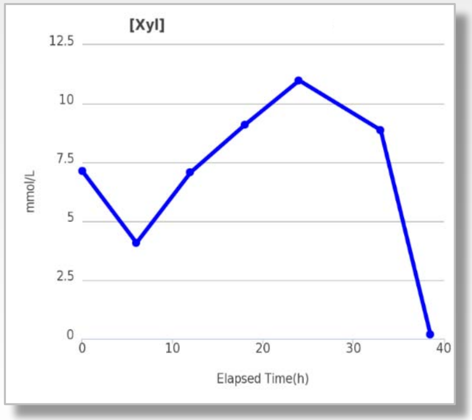
1,4-BDO Production with API-AVAP® Biomass Hydrolysate (agricultural residue)









DOE/NREL final validation

- 30 L scale fermentation w/API AVAP® hydrolysate
- 2 L were run in parallel, similar results
- Biomass-to-BDO strain, Latest XUM strain
- Co-utilized glucose and xylose, both depleted:
  - Glucose >99%
  - Xylose 96%
- Process optimization sped up run, finishing in <40 hrs
- Titer (122 g/L), Rate (3.1 g/L/hr) well above proposed targets (70, 2.5); yield, lower than target.

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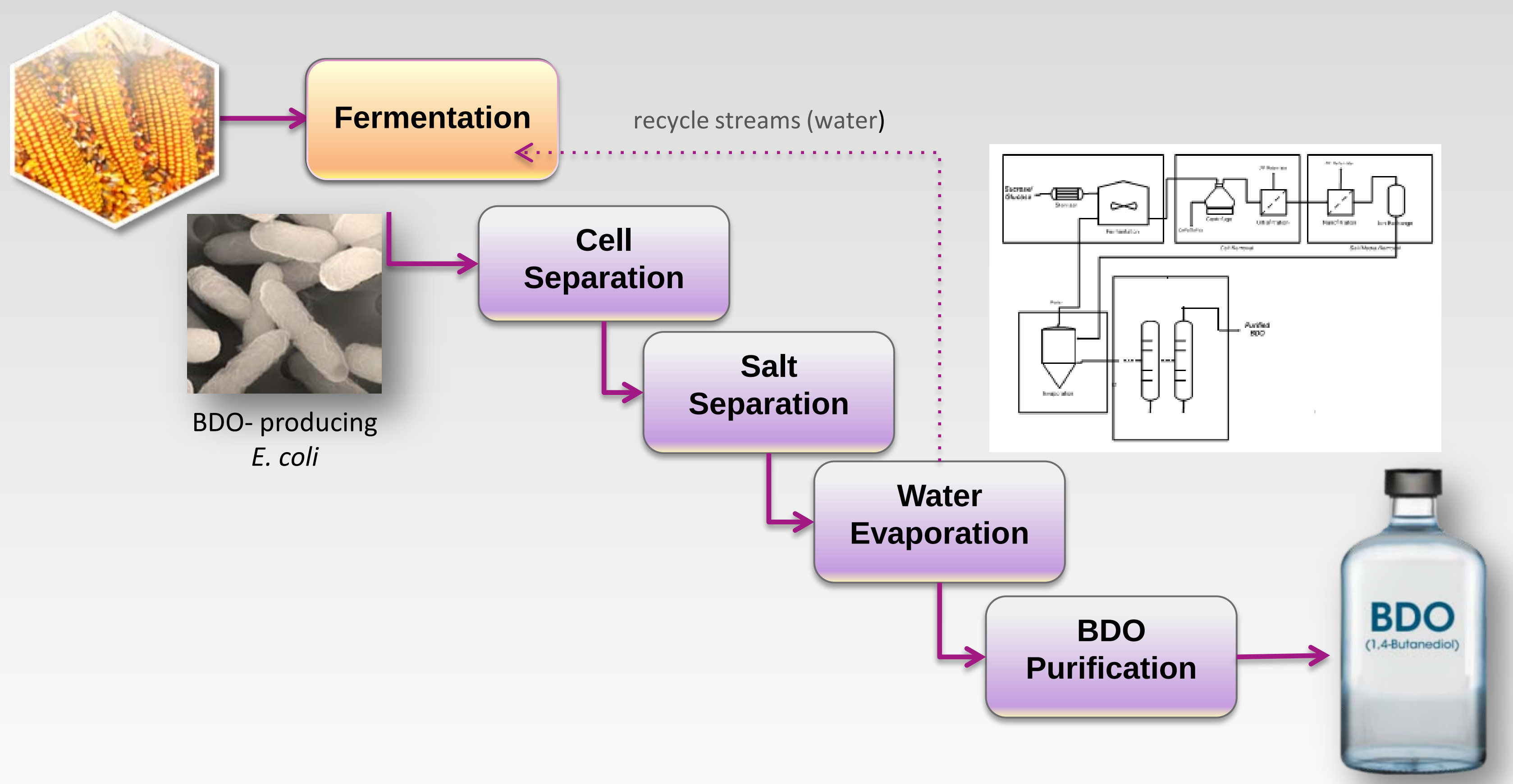
Complete Process Technology for Bio-BDO®



Complete Process Technology for Bio-BDO®

Fermentation

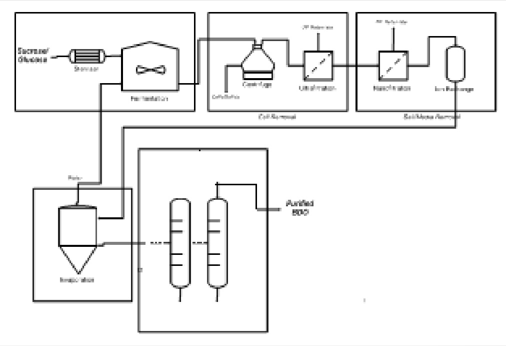
recycle streams (water)

BDO- producing
E. coli

Cell Separation

Salt Separation

Water Evaporation



BDO Purification

Color removal

Therefore, BDO purification involves removing cells, water, salts, etc from the BDO

Relevance and Summary

Relevance

- Increases potential feedstock choices for BDO
- Geographic flexibility
- Lessons on strain design, process design, BDO recovery

Summary

- Range of biomass-to-BDO strains suitable for multiple biomass sources (feedstocks, pretreatments, …)
  - C5/C6 co-utilization
  - Genes to perform better in certain hydrolysates
  - Improved yield genotypes
- Improved understanding of strain genotypes vs. cellulosic hydrolysates
- Evidence that biomass-to-BDO could be a commercial process
- More economical BDO recovery
- **Built economic models to progress potential commercialization**

Acknowledgments

Molecular Biology
Harry Yim
Bob Haselbeck
John Trawick
Wei Niu
Jeff Boldt
Laura Peiffer
Eric Van Name
Chris Wilson
Stephanie Culler
Brandon Chen
Kevin Hoff
Ewa Lis
Fannie Chau
Hongmei He
Shawn Bachan
Jingyi Li
Luis Reyes
Joseph Warner

Microbiology
Catherine Pujol-Baxley
Jazell Estadilla
Jesse Wooton
Jabus Tyerman
Jonathan Moore
Lars Knutstad
Sarah McNees
Jonathan Joaquin
Carla Risso
Ewa Lis

Analytical Sciences
Julia Khandurina
Rosary Stephen
Lucy Zhao
Ahmed Alanjary
Blanca Ruvalcaba
Rainer Wagester
Korki Miller

Enzymology
Brian Steer
Stefan Andrae
Cara Tracewell
Mike Kuchinskas
Wayne Liu
Brian Kinley
Amit Shah
Jacqueline Fritz
Kui Chan

Process Engineering
Joe Kuterbach
Michael Japs
Janardhan Garikipati
Fasil Tadesse
Ben Adelstein
Rachel Pacheco
Daric Simonis
Arvind Kaul
Ishmael Sonico

Computational
Tony Burgard
Priti Pharkya
Robin Osterhout
Harish Nagarajan
Jun Sun
Tae Hoon Yang
Wyming "Lee" Pang
Jungik Choi

Fermentation
Kelsey Yee
Nick Diaz
Sy Teisan
Laurie Romag
Joseph Woodcock
Paul Handke
Gian Oddone
Amruta Bedekar
Rebecca Bratcher
Jason Crater
Akhila Raya
Alex Navarro
Alyssa Doty
Andrew Saarni

Christophe Schilling, CEO
Mark Burk, CTO Bill Baum, CBO
Nelson Barton, VP R&D
Jeff Lievense, EVP, Process Development

U.S. DEPARTMENT OF ENERGY

Award DE-EE0005002 to Genomatica

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our core purpose

Lead the irresistible transition to sustainable materials through our technology and, united with industry leaders, make our world a better place.

our core values

we are real

Results count. Commitments count. Integrity and honesty are absolutes.

we are innovative

We invent, experiment and create—across our entire business. We seek out and embrace differences, to help us think differently.

we are united

We work better together. Shared mission. Shared accountability. Shared learning. Shared success.

we are relentless

We don't give up. We strive for excellence. Our passion flows from our shared vision.

Publications, Presentations, and Commercialization

- Barton, Nelson (VP, R&D, Genomatica) Biomass 2012, 11 – 12 July 2012, Wash., DC
  http://www1.eere.energy.gov/biomass/pdfs/bio2012_final_agenda.pdf
- Trawick, John D. (Research Fellow, Genomatica) 2013 SBFC meeting (2 May 2013)
  http://sim.confex.com/sim/35th/webprogram/Session2437.html
- Trawick, John D. (Research Fellow, Genomatica) 2014 SBFC meeting (29 April 2014)
  http://sim.confex.com/sim/36th/webprogram/Paper26489.html
- Trawick, John D. (Research Fellow, Genomatica) Biomass 2014 (30 July 2014)
  http://www.energy.gov/eere/bioenergy/biomass-2014-growing-future-bioeconomy-agenda

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Responses to Previous Reviewers' Comments

- N/A.

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