2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Development of an Integrated Biofuel and Chemical Refinery

John D. Trawick
Research Fellow, Genomatica

Date: 21 May 2013

Technology Area Review: Biochemical Conversion

Principal Investigator: Mark Burk
Organization: Genomatica

genomatica
sustainable chemicals

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.

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.*

To deliver a scalable fermentation process that employs the engineered microbe to produce BDO from cellulosic sugars at titer ≥ 70 g/L, and productivity ≥ 2.0 g/L/hr at ≥ 100 L scale.

genomatica sustainable chemicals

Quad Chart Overview

Timeline

- August 2011
- July 2014
- ~80% complete

Budget

Funding for FY11(541,971/186,308)
Funding for FY12(852,555/293,074)
Funding for FY13 (253,588/87,173)
2/ $1,000,000.

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

Partners

- Chemtex
  - Suppliers of PROESA^{TM} hydrolyates, have worked with Genomatica to reach a specification
  - Biweekly or more frequent consultation with Chemtex staff

Project Overview

Genomatica has developed recombinant organisms to produce the commodity, 1,4-butanediol (BDO), used in many synthetic polymers.

- BDO producing strains use refined dextrose to make BDO; ties BDO economics to sugar and corn prices.
- Biomass-based sugars are an economical alternative.
- Challenges include:
  - Cheap, consistent feedstock and treatment.
  - Minimizing hydrolysate impurities.
  - Achieving organism performance metrics: titer (T), rate (R), and yield (Y) similar to dextrose-based.
  - Simultaneous utilization of C5 as well as C6 sugars by recombinant *Escherichia coli*.
  - Recovery of BDO is different than ethanol and this impacts design and economics.

genomatica
sustainable chemicals

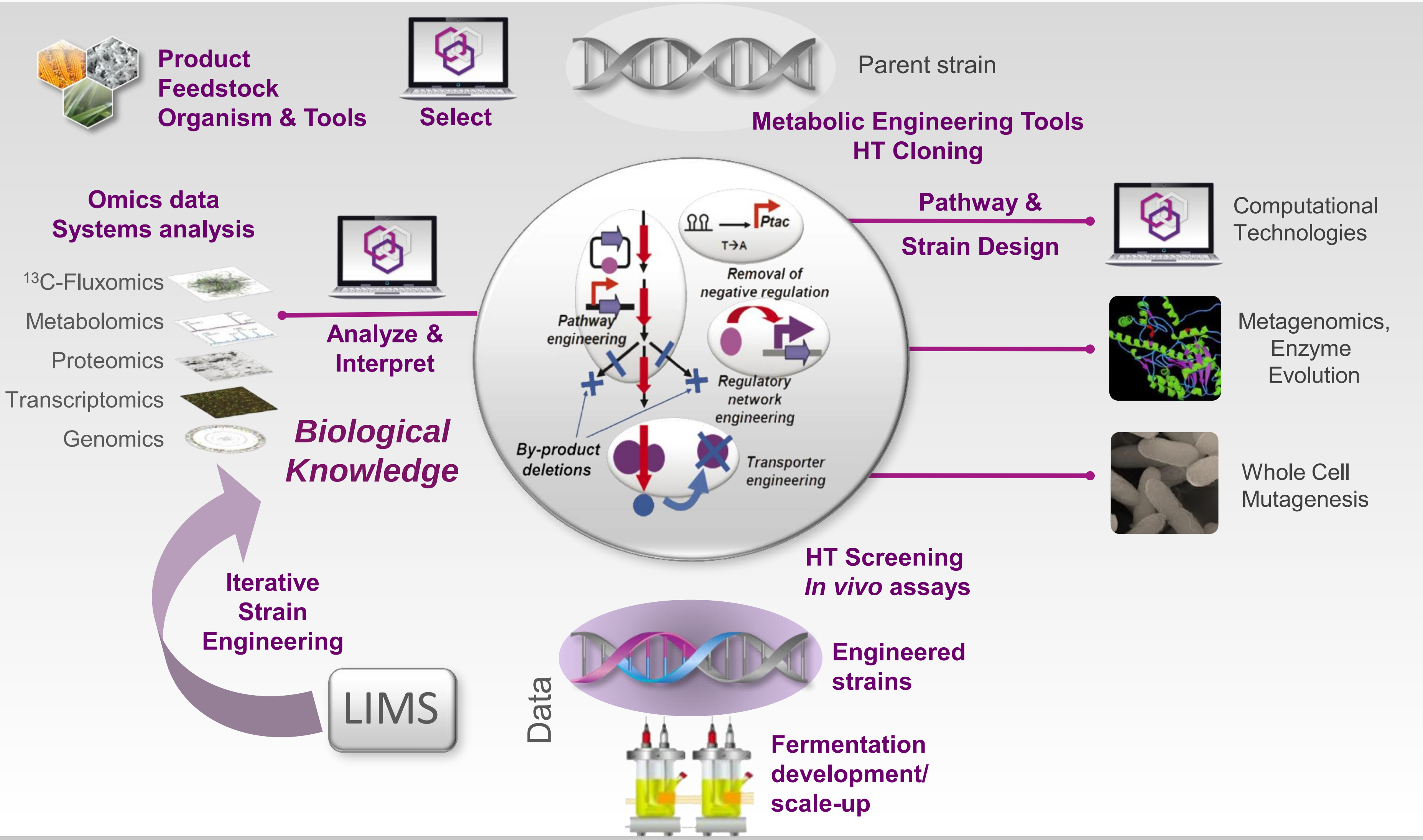
1 - Approach

- *E. coli* to make 1,4-BDO at titer, rate, and yield from lignocellulosic biomass sugars to demonstrate commercial feasibility.
- Critical factors: **1)** hydrolysate quality/composition, **2)** C5-C6 co-utilization, **3)** process impacts on yield and rate.
- **Hydrolysate composition, work with supplier to produce biomass hydrolysates with high and uniform [sugar], low impurities, and minimal toxicity.**
  - Go/no go: achieve 75% T-R-Y of pure glucose at same concentration.
- **Adaptive evolution + genomic re-sequencing for sugar co-utilization.**
  - Go/no go: Efficient C5-C6 co-utilization and move to 'clean' strain
- **^{13}C flux analysis + metabolomics to ID metabolic constraints limiting performance.**
  - Targets to improve energy and reduced cofactors.

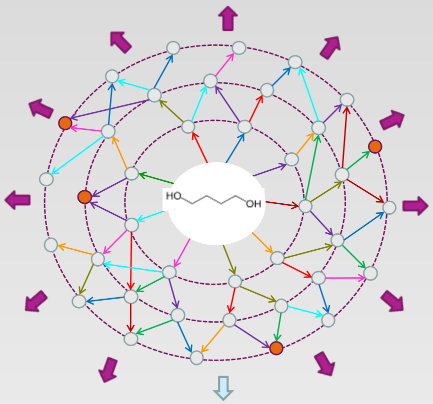
genomatica
sustainable chemicals

Bio-based 1,4-Butanediol

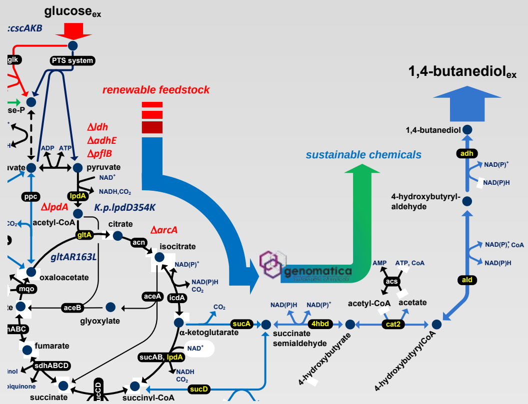
Genomatica's Systems-Based Strain Engineering



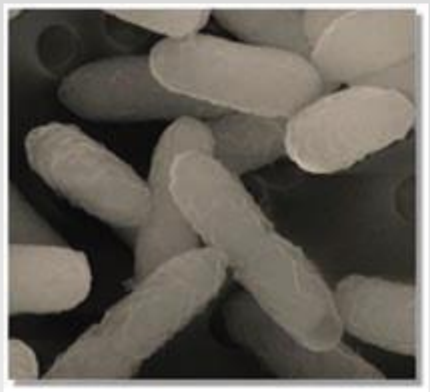
Journey to a BDO Production Strain



Pathway Identification and Engineering



Strain Design and Metabolic Engineering



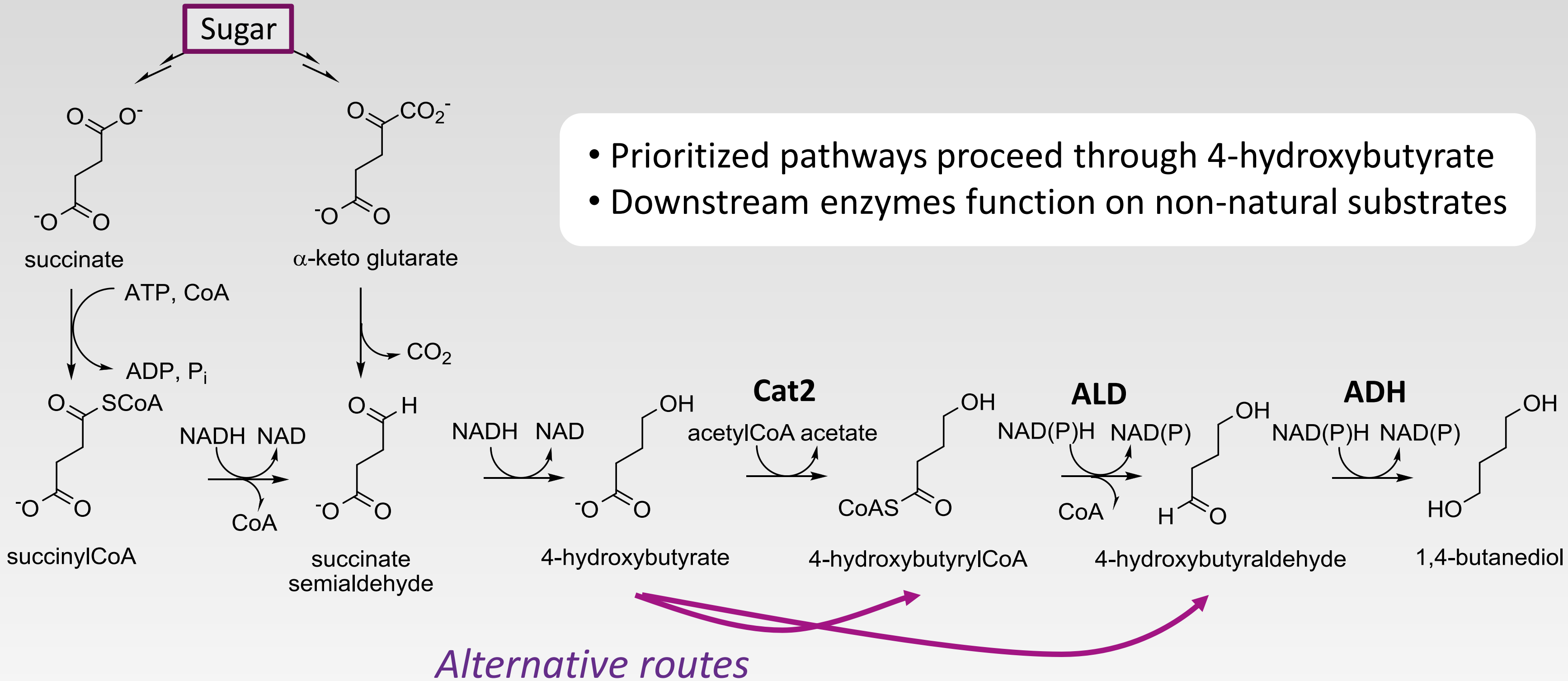
Commercial Strain for BDO Production

Fermentation Metrics → Higher TRY = Lower COGS

- **Titer (g/L) -** Impacts equipment sizing and energy needs
- **Rate (g/L/h) -** Impacts # of fermentors, plant capacity
- **Yield (g/g) -** Impacts feedstock cost contribution

TRY all inter-dependent → reduce by-products, increase rate and yield

BDO Biosynthetic Pathways



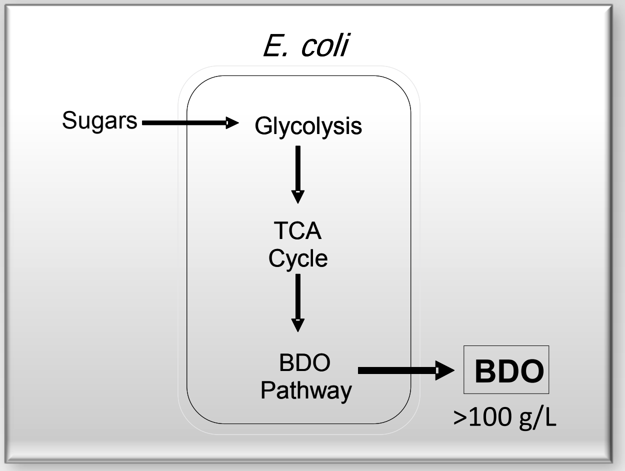
- Prioritized pathways proceed through 4-hydroxybutyrate
- Downstream enzymes function on non-natural substrates

1. Developed enzyme assays and analytical methods for all metabolites
2. Screened libraries of gene candidates for each step – >100 in some cases
3. **Demonstrated seven different functional BDO pathways in *E. coli***

Yim et al., Nature Chem. Biol., 2011

genomatica sustainable chemicals

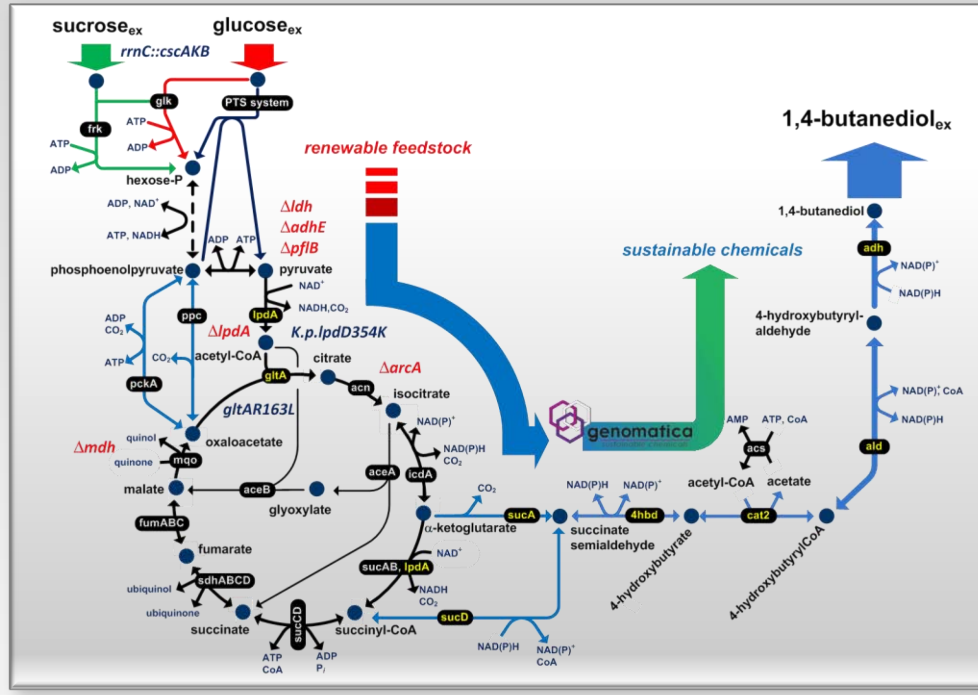
BDO Pathway and Process



$C_6H_{12}O_6 + 0.5\ O_2 \rightarrow C_4H_{10}O_2 + 2\ CO_2 + H_2O$
Max yield = 1 mol/mol (0.50 g/g, 67 C-mol %)

ATP = 0
NAD(P)H = +1

ATP via oxidative phosphorylation

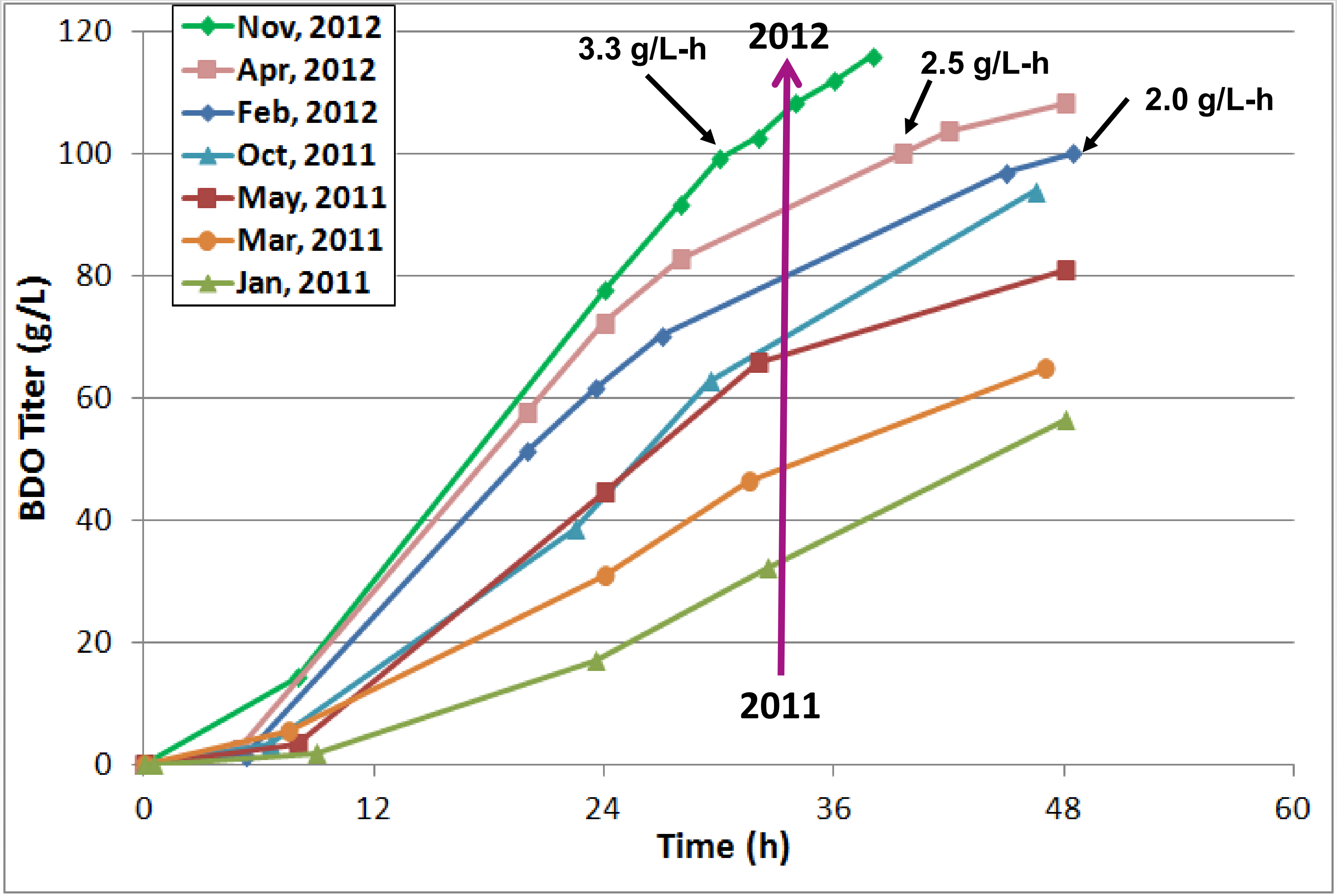


Oxidative TCA cycle flux required for redox needs of BDO pathway

- BDO pathway involves 4 reduction steps – redox intensive
- BDO pathway generates 1 extra NAD(P)H and no excess ATP
- Balance energy, redox and maintain high NAD(P)H/NAD(P)$^+$ ratio
- Microaerobic production (DO ≈ 0) required for optimal performance

genomatica
sustainable chemicals

Technology Platform Drives BDO Strain Performance

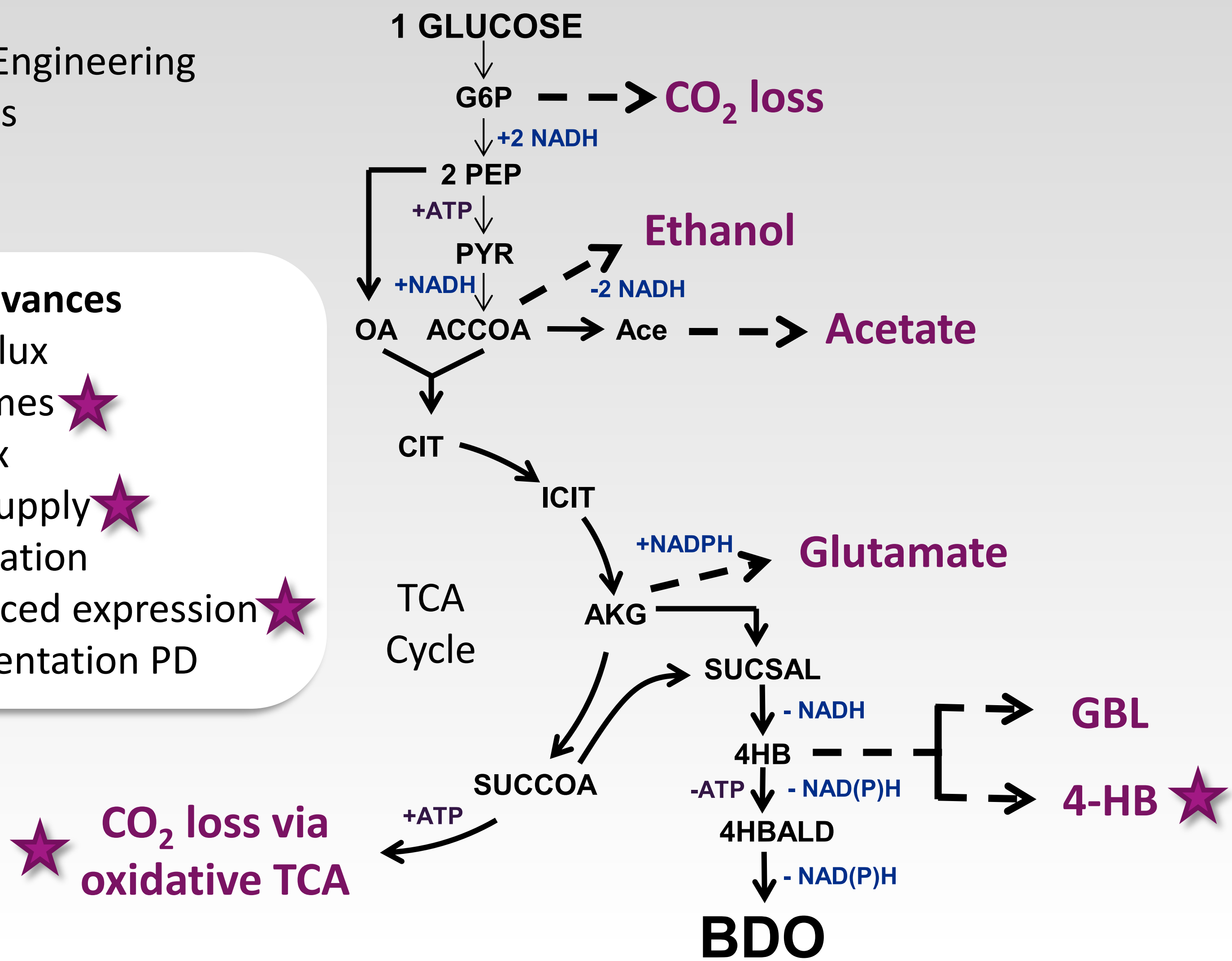


genomatica
sustainable chemicals

Increasing Rate and Lowering By-products

★ Metabolic Engineering Applications

Key Advances
- Backflux
- Enzymes ★
- Redox
- ATP supply ★
- Regulation
- Balanced expression ★
- Fermentation PD



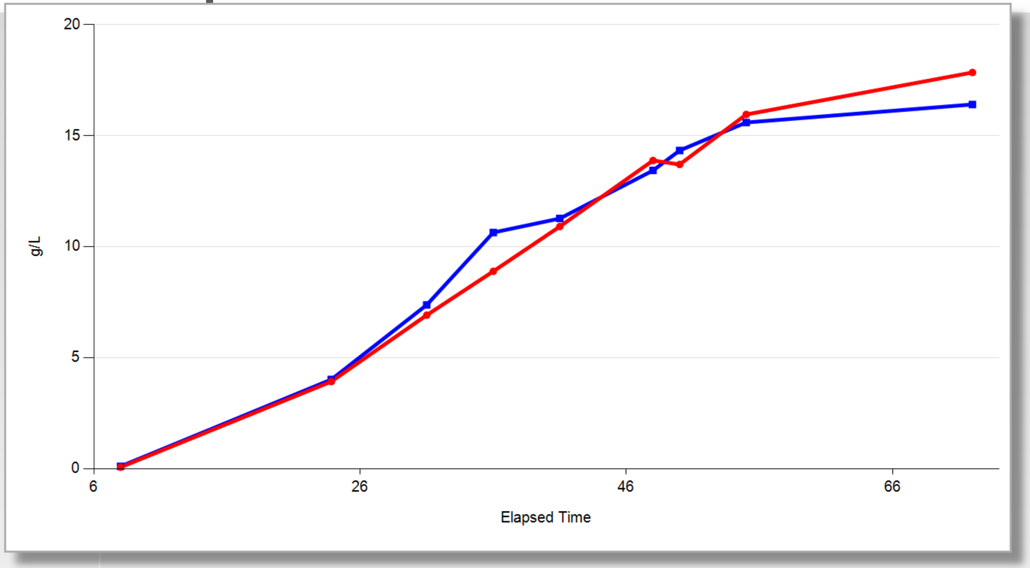
More metabolic steps in a pathway increases avenues for by-products

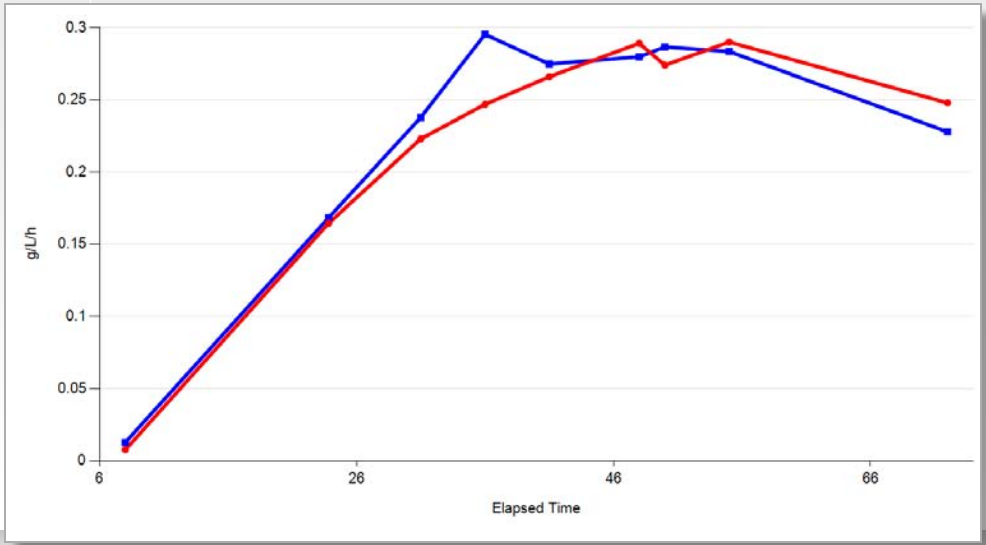
genomatica sustainable chemicals

Approach to Biomass - BDO

- Initial baseline performance (preliminary results in the grant).
- Identification of key constraints on Biomass – BDO and overcoming those constraints
  - Hydrolysate composition, work with supplier to produce biomass hydrolysates with high and uniform [sugar], low [impurities], and minimal toxicity.
    - Hydrolysate improvements with time increased performance.
  - Adaptive evolution + genomic re-sequencing + strain engineering for sugar co-utilization.
    - Acceptable sugar co-utilization selected, designed, and recapitulated.
  - ^{13}C flux analysis + metabolomics to ID metabolic constraints limiting performance.
    - Targets to improve availability of energy and reduced cofactors.

Benchmark performance on biomass, September 2011



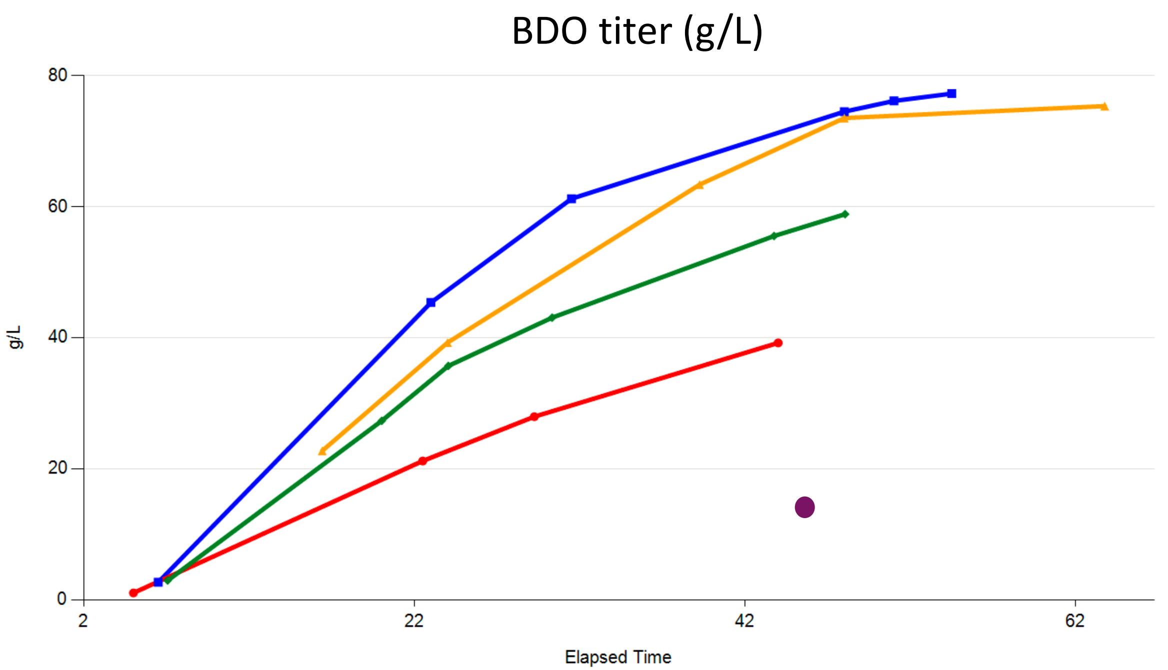


- Benchmark fermentations for DOE/NREL site visit using early lot of hydrolysate and a very early BDO production strain*.
- Titer (16 – 18 g BDO/L) was 24% of grant goal and rate (0.25 g/L/hr) <10% of goal.
- Yield (not shown) >50% of goal.
- A long way to go!

*As used in grant application.

genomatica
sustainable chemicals

Biomass-to-BDO Process Challenges: impurity reduction



35% Glucose
007, Chemtex Hz, low impurities
001, Chemtex Hz, diluted w/sugar
001, Chemtex Hz, untreated
Benchmark, 9/'11, early strain, Hz similar to 001

Strain: BDO Producer
Chemtex Hydrolysate at 35% sugars in feed:

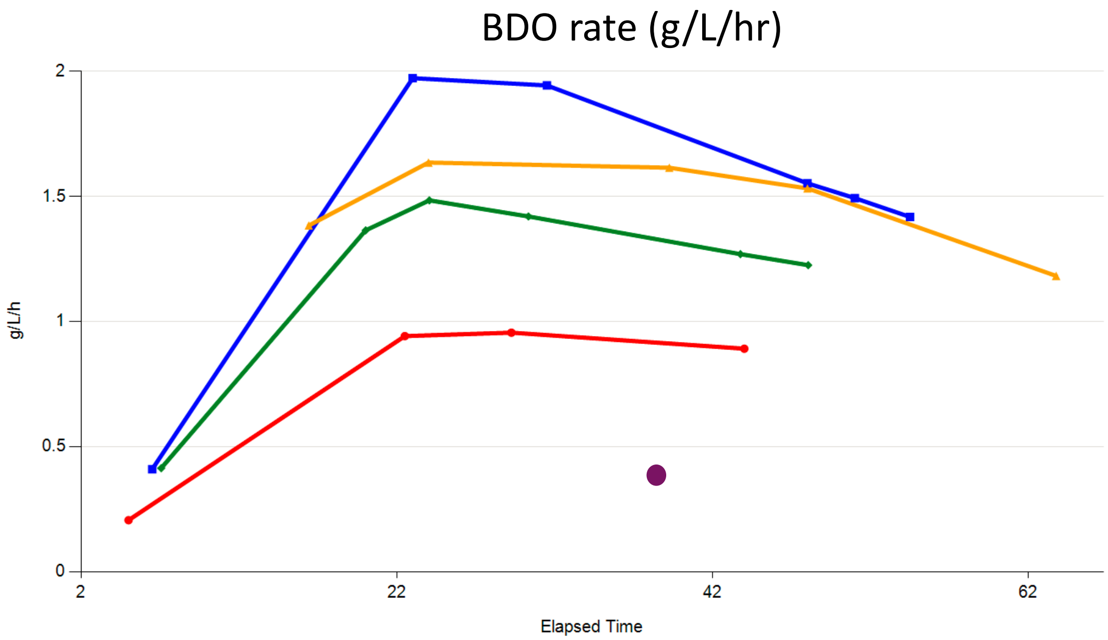
- Lot 001, Untreated Hz
- 001, Untreated Hz Diluted with pure glucose + xylose
- Lot 007: Hz

Chemtex provided hydrolysates to evaluate
- Sugar concentration in a fed-batch process—limits titer and rate
- Lowered impurities; reduced multiple ways

Result: BDO titer approached metric on pure glucose
Strain improvements from original benchmark coupled with Hz improvements.

genomatica
sustainable chemicals

Biomass-to-BDO Process Challenges : Impurity reduction



35% Glucose
Chemtex Hz, low impurities
Chemtex Hz, diluted w/sugar
Chemtex Hz, untreated
Benchmark, 9/'11, early strain, Hz similar to 001

Strain: BDO Producer
ChemtexHydrolysate at 35% sugars in feed:

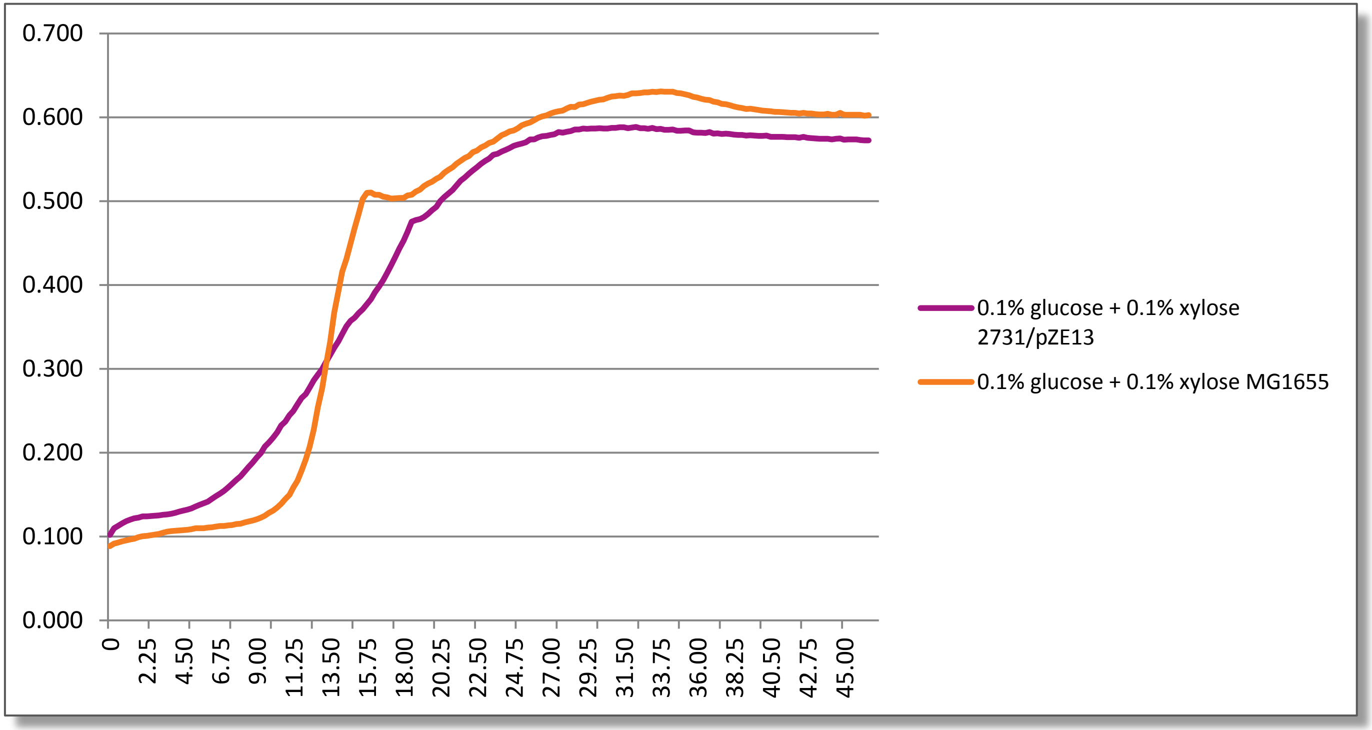
- Untreated
- Diluted with pure glucose + xylose
- Low impurities

Chemtex provided hydrolysates to evaluate
- Sugar concentration in a fed-batch process—limits titer and rate
- Lowered impurities; reduced multiple ways

Result: BDO titer approached metric on pure glucose
Strain improvements from original benchmark coupled with Hz improvements.

genomatica sustainable chemicals

Escherichia coli BDO production strains show diauxic sugar utilization

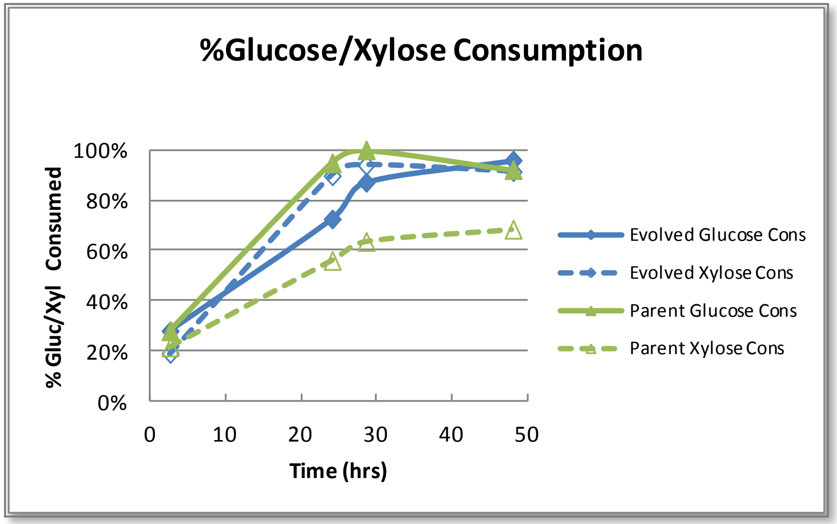


Compared, wt MG1655 vs. a BDO production strain
50/50 glucose xylose, growth curves

Conclusion: BDO production strains have lessened diauxie relative to the wt progenitor.

genomatica
sustainable chemicals

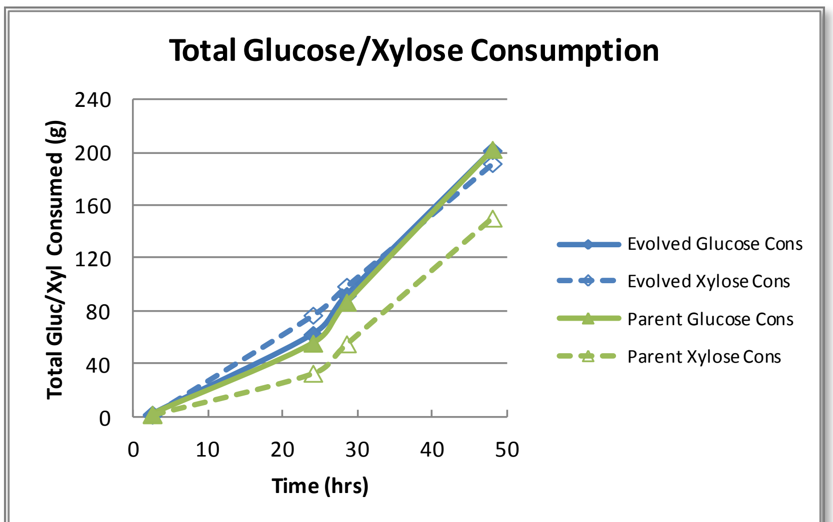
Using Evolution to Improve Xylose Sugar Utilization in Fermentation



Parent unevolved parent BDO producing strain.

Evolved : evolved from Parent for improved xylose utilization.

Fermentation used pure glucose : xylose (1:1), 680 g sugar/L feed

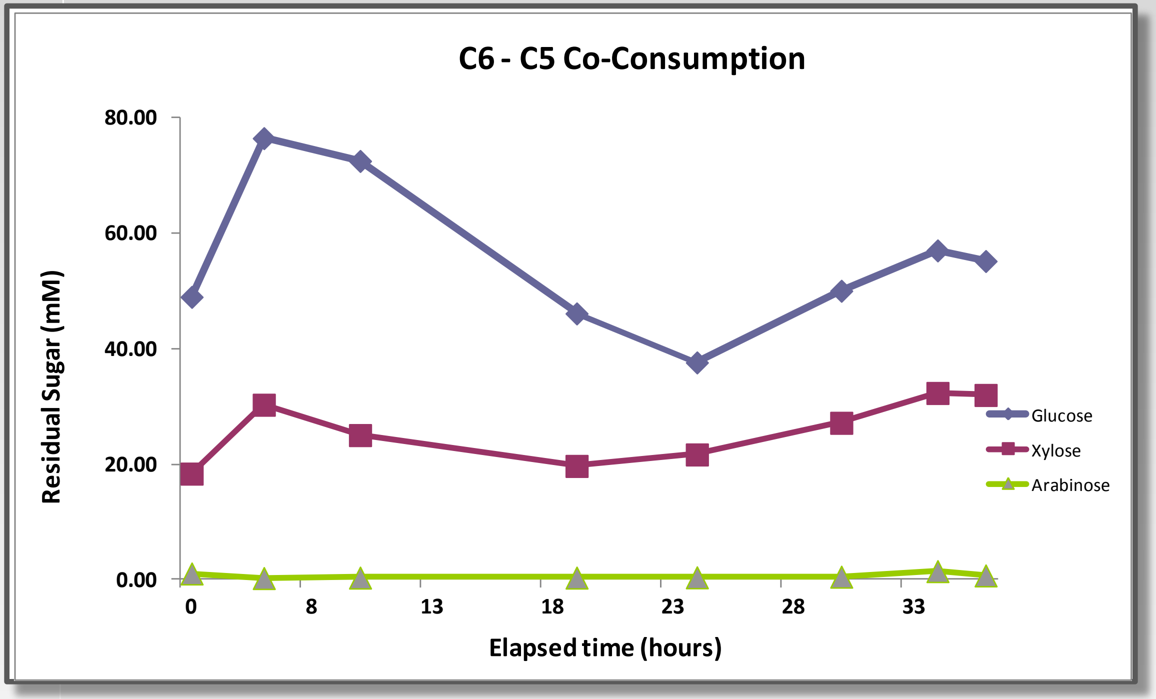


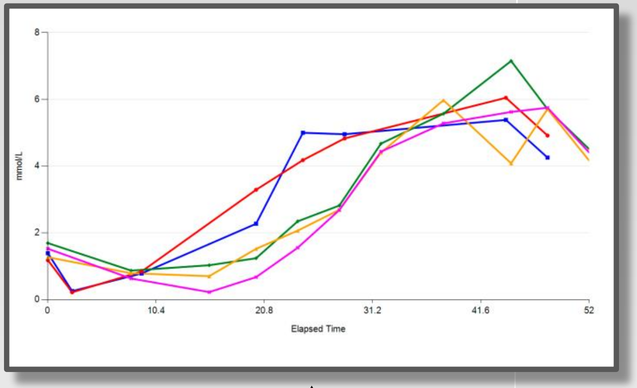
Selection gave a large improvement in xylose consumption in the presence of glucose

- Fermentation in 2 L BR
- Strains made BDO in glucose + xylose;
  - **Evolved** = 70 - 75 g/L;
  - **Parent** = 60 g/L

genomatica
sustainable chemicals

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





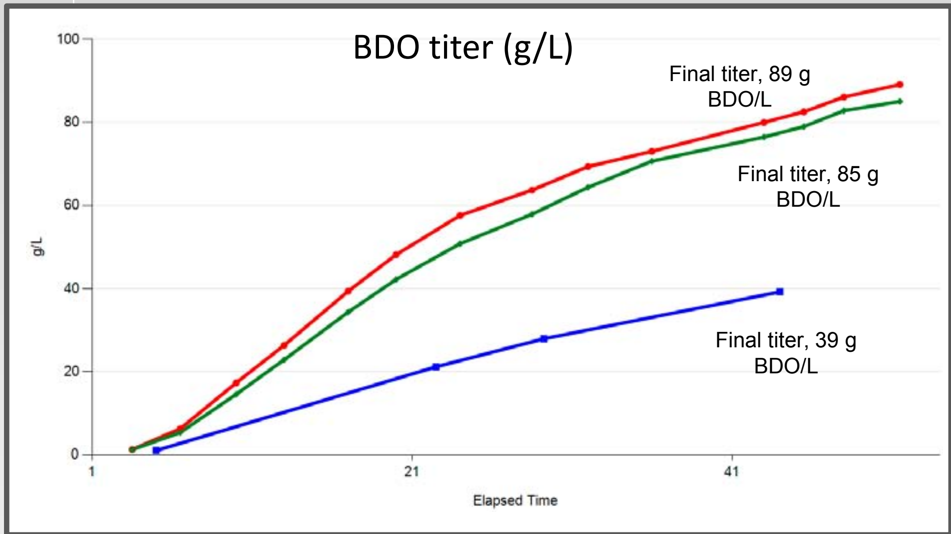
Residual [arabinose] in non-arabinose utilizing strains with similar biomass hydrolysates for comparison.

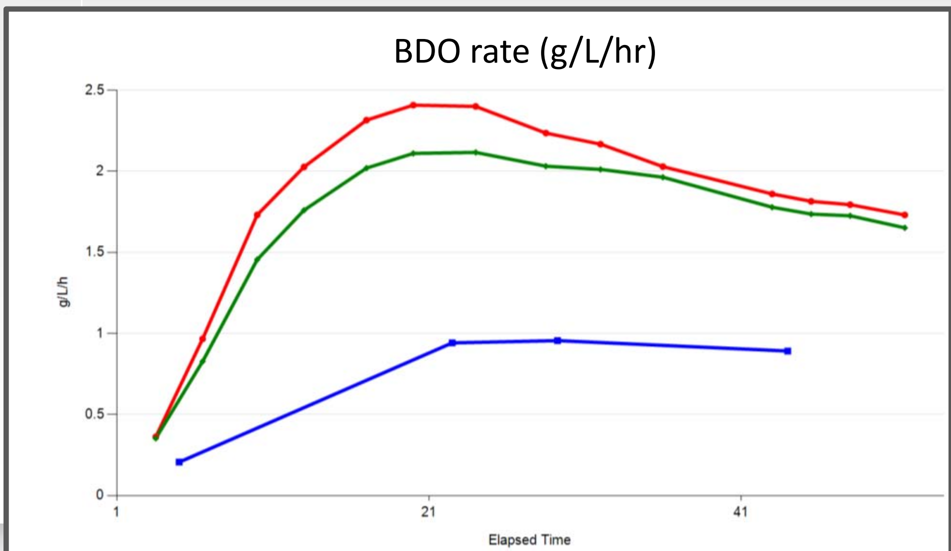
- **XUM (<u>x</u>ylose <u>u</u>tilizing <u>m</u>utant) for xylose co-utilization with glucose was identified and integrated into a clean BDO production host.**
- **A gene for arabinose uptake also added.**

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

genomatica
sustainable chemicals

Improving BDO Performance on lignocellulosic hydrolysates with strain engineering and hydrolysate characteristics



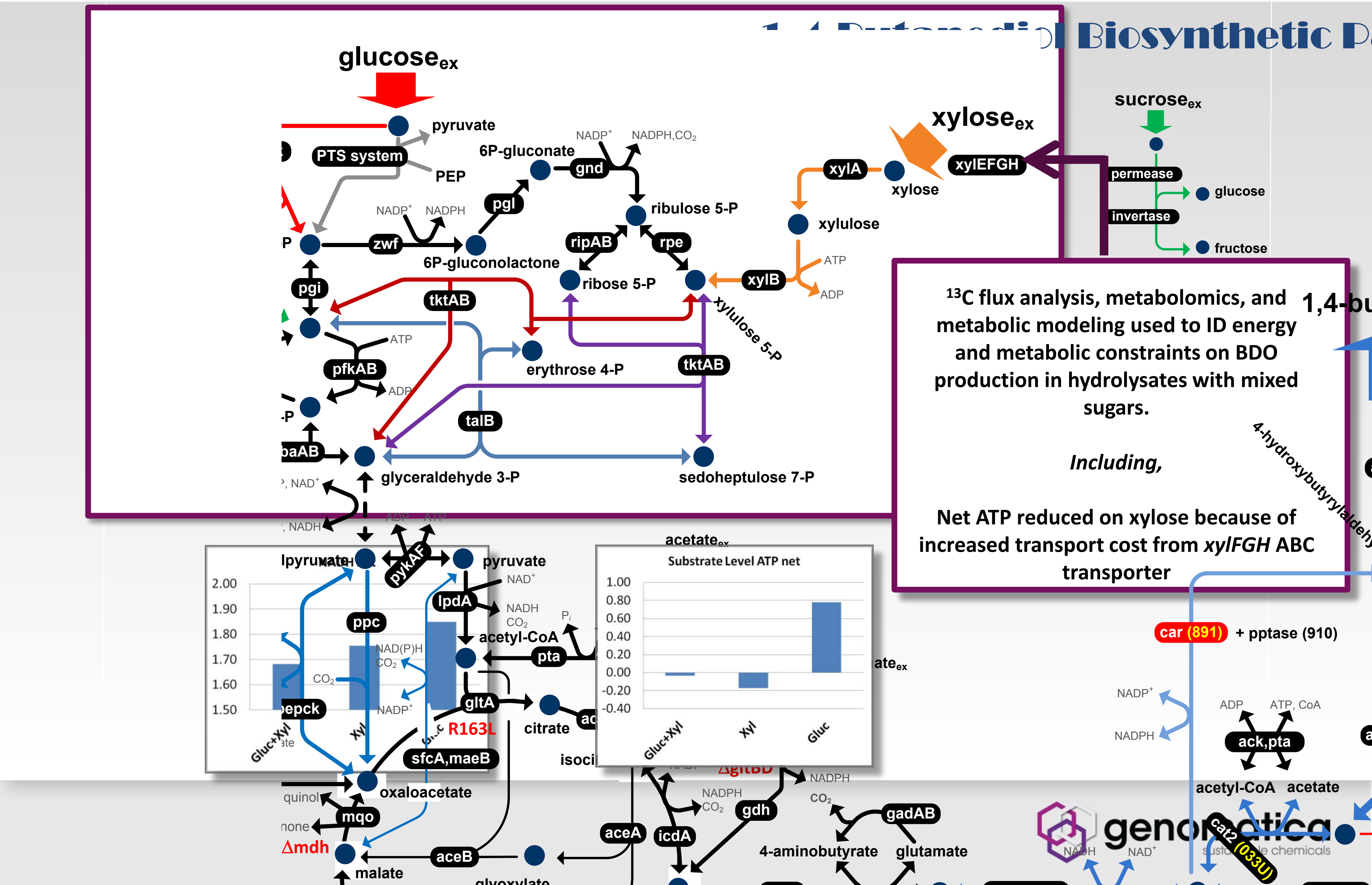


- Better strains and biomass treatment = improved BDO titer and rate
  - Early 2012 diauxic strain, early Hz, Chemtex
  - Late ‘12, Evolved/recapitulated xylose user, Chemtex, low impurities
  - Late ‘12, Evolved/recapitulated xylose user, Chemtex, low impurities
- **>2X in increase in titer, and in peak rate during 2012**
- Challenges remain
  - Consistency/sugar concentration
  - Reducing non-fermentables

Blue	Non-evolved strain	Chemtex Hz-001, early batch
Red	Recapitulated, XUM	Chemtex Hz-012, improved
Green	Recapitulated, XUM	Chemtex Hz-010, improved

genomatica
sustainable chemicals

Cellulosic biomass (xylose) and metabolism



[13]C flux analysis, metabolomics, and metabolic modeling used to ID energy and metabolic constraints on BDO production in hydrolysates with mixed sugars.

Including,

Net ATP reduced on xylose because of increased transport cost from *xylFGH* ABC transporter

Key Accomplishments and challenges

- **Improved hydrolysate composition—addresses impurities, sugar concentrations**
- **Glucose – Xylose – Arabinose co-utilization**
- These and other changes have given at small scale fermentation:
  - Chemtex hydrolysates: **86 – 89 g BDO/L** titer, **1.8 g/L/hr** rate
  - *DOE grant goal (30L scale): 70 g BDO/L titer, 1.5 g/L/hr rate*
- Potential drains on energy and reducing power identified via metabolomics, ^{13}C flux analysis, and metabolic modeling.
- Have multiple proposed changes in process of testing/implementation

genomatica
sustainable chemicals

3 - Relevance

- Develop biomass-to-1,4-butanediol (BDO) increase chemical production from sustainable feedstocks in a cost advantaged process that will be competitive with petrochemicals
  - Achieve performance sufficient for commercial viability (*BDO titer of ≥ 70 g/L, and rate ≥ 2.0 g/L/hr*) **Grant Tasks 1 & 2**
  - Scalable process to integrate into biomass based biorefineries (*at ≥ 100 L scale*) **Grant Task 3**
  - Recovery of BDO along with process that is cost competitive. **Grant Tasks 1, 2, & 3.**

- **Application of the expected outputs:** Bio-BDO from cellulosic biomass, cost advantaged, that can be incorporated into the integrated biorefinery concept.

genomatica
sustainable chemicals

4 - Critical Success Factors

- Success factors defining technical and commercial viability.
  BDO titer of ≥ 70 g/L, and rate ≥ 2.0 g/L/hr at ≥ 100 L scale.
  - Robust organism meeting these targets (technical); >75% of the goal
  - From biomass, BDO purity equal to industry needs (market); can do.
  - Costs comparable or less than pure glucose "Gen 1 BDO" process (business); working towards this with improved strains, hydrolysates, process.

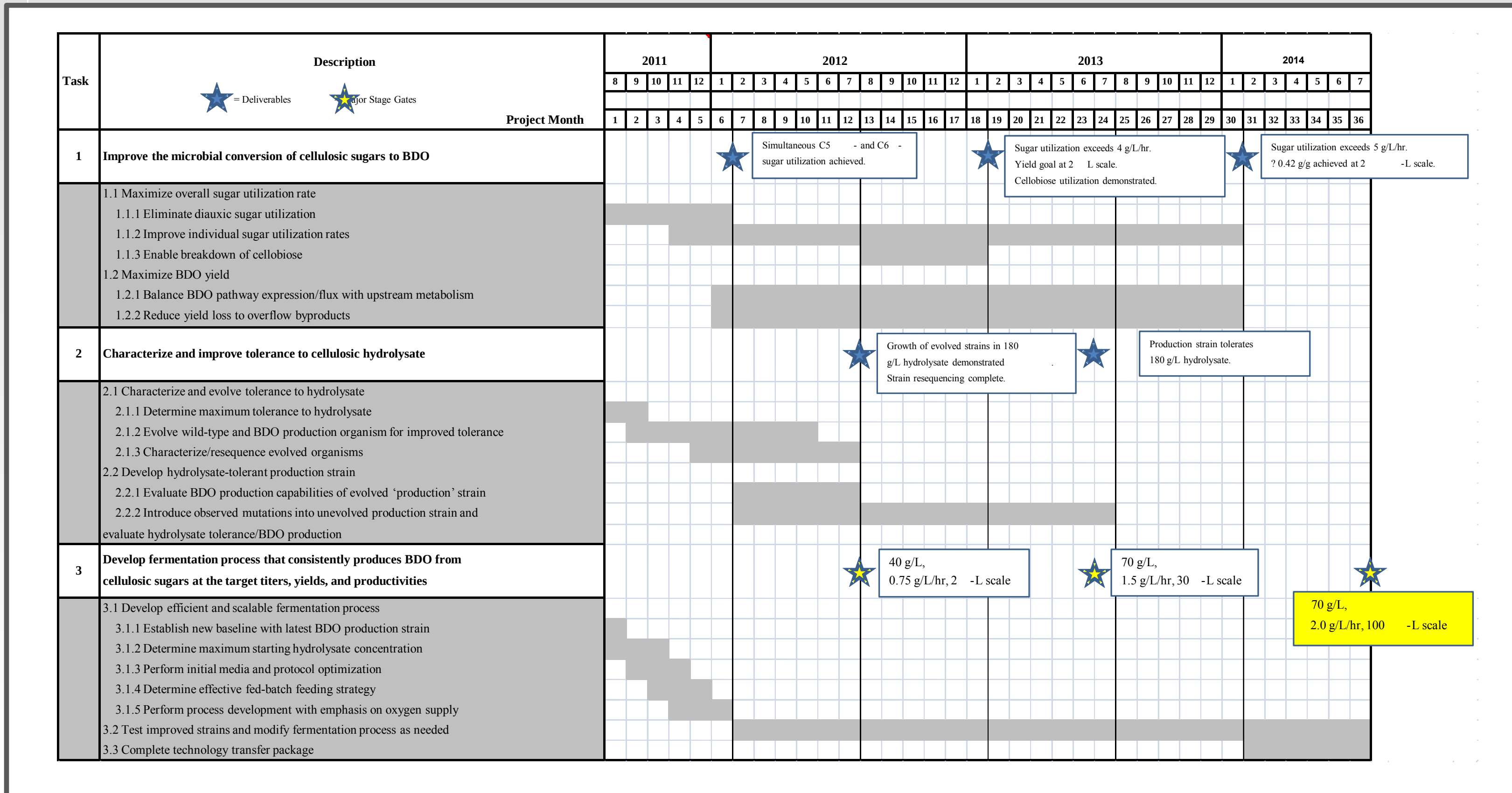
- Potential challenges (technical and non-technical).
  - Cellulosic hydrolysate consistency/specs for optimum fermentation and downstream recovery.
  - Strain performance in hydrolysates, especially energy cost to cell affecting yield and productivity.

- Advance the technology and impact the commercialization of biomass/biofuels.
  - BDO strain/process/recovery are all distinct from ETOH or biofuels; will be a new opportunity.
  - Commercially viable strain and process, biomass-to-BDO, will be very attractive to Genomatica customers.
  - Begin to set specifications for biomass sugars for chemical processes

genomatica
sustainable chemicals

5. Future Work

- Improve yield and incorporate sugar co-utilization into multiple strains.
- Go/no go reaching T-R-Y targets at both 2 L and larger scale
- Test new lignocellulosic hydrolysate feedstocks and treatments.



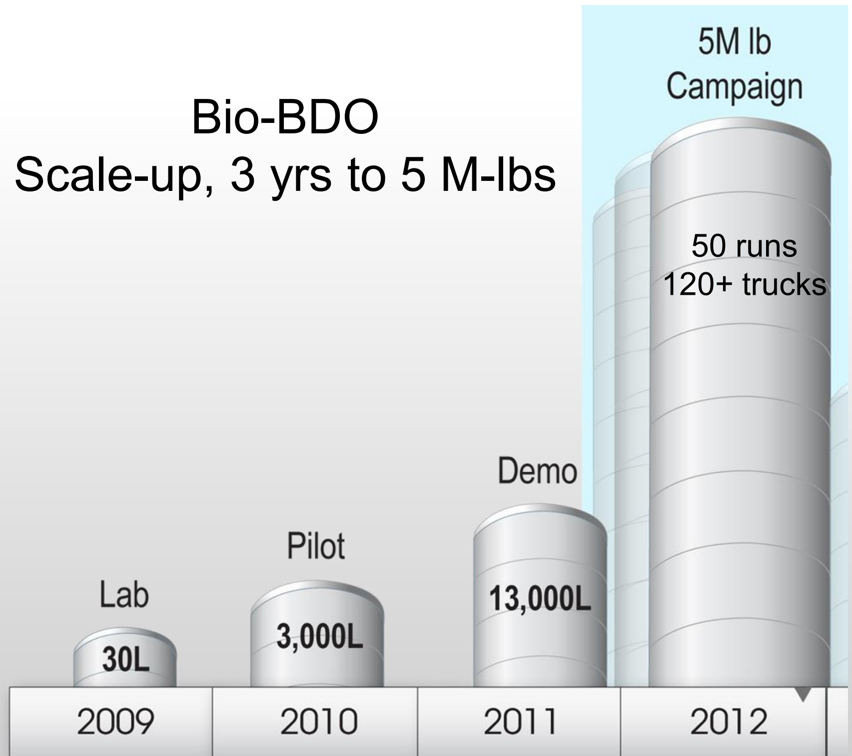
genomatica
sustainable chemicals

Summary

1) Approach
    1) Strain design and engineering coupled with adaptive evolution and ‘omics technologies to ID key constraints on feedstock quality and price.
    2) Hydrolysate evaluation and improvement to reach specification.
2) Accomplishments
    1) **5X improvement in titer; rate and yield are approaching goals.**
    2) **Major C6 and C5 sugars can be co-utilized to max yield and performance**
    3) **Key metabolic constraints ID’d**
    4) Working towards hz specification
3) Relevance
    1) Enable commercial bio-BDO from lignocellulosic biomass sugars
4) Critical Success factors and challenges
    1) Biomass sources meeting needed fermentation and BDO recovery specifications.
5) Future Work
    1) Yield and pathway enhancements to reach or exceed grant metrics.
6) Technology transfer

Bio-BDO commercial progress

Bio-BDO
Scale-up, 3 yrs to 5 M-lbs



BASF
The Chemical Company

BASF, world's leading BDO producer licensed to produce renewable 1,4-BDO using Genomatica's process

Toray Industries, Inc. produced partial bio-PBTs from **bio-BDO** at same specs as petro-PBT

TORAY
Innovation by Chemistry

Genomatica Biomass to BDO Contributors

<u>Molecular Biology</u>
John Trawick
Ewa Lis
Joseph Warner

<u>Chemtex</u>
Alberto Ceria
Piero Ottonello
Francesco Cherchi
Kevin Gray

<u>Microbiology</u>
Carla Risso
Jesse Wooton
Jonathan Joaquin

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

<u>Process Engineering</u>
Michael Japs
Janardhan Garikipati
Fasil Tadesse
Matt White

<u>Computational</u>
Tony Burgard
Priti Pharkya
Robin Osterhout
Jun Sun
Tae Hoon Yang
Jungik Choi
Harish Nagarajan

<u>Fermentation</u>
Dan Beacom
Sy Teisan
Laurie Romag
Joseph Woodcock
Gian Oddone
Amruta Bedekar
Jason Crater
Akhila Raya

U.S. DEPARTMENT OF ENERGY
Award DE-EE0005002 to Genomatica

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

genomatica
sustainable chemicals

Questions?

Thank you

John Trawick

jtrawick@genomatica.com

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) scheduled for the 2013 SBFC meeting (2 May 2013) http://sim.confex.com/sim/35th/webprogram/Session2437.html

Note: This slide is for the use of the Peer Review evaluation only – it is not to be presented as part of your oral presentation, but can be referenced during the Q&A session if appropriate. These additional slides will be included in the copy of your presentation that will be made available to the Reviewers and to the public.

Responses to Previous Reviewers' Comments

- N/A.

Note: This slide is for the use of the Peer Review evaluation only – it is not to be presented as part of your oral presentation, but can be referenced during the Q&A session if appropriate. These additional slides will be included in the copy of your presentation that will be made available to the Reviewers and to the public.