U.S. Department of Energy (DOE)
Bioenergy Technologies Office (BETO)
2017 Project Peer Review
Fermentative Production of Tricarboxylic Acid Cycle-Derived Chemicals Using Cellulosic Glucose

March 9th, 2017
Biochemical Conversion

Principal Investigator: Jeffrey Dietrich
Lygos, Inc.

Introduction to Lygos

Engineering yeast to convert sugar into high-value chemicals

- **Founded 2010**
- **25 Employees**

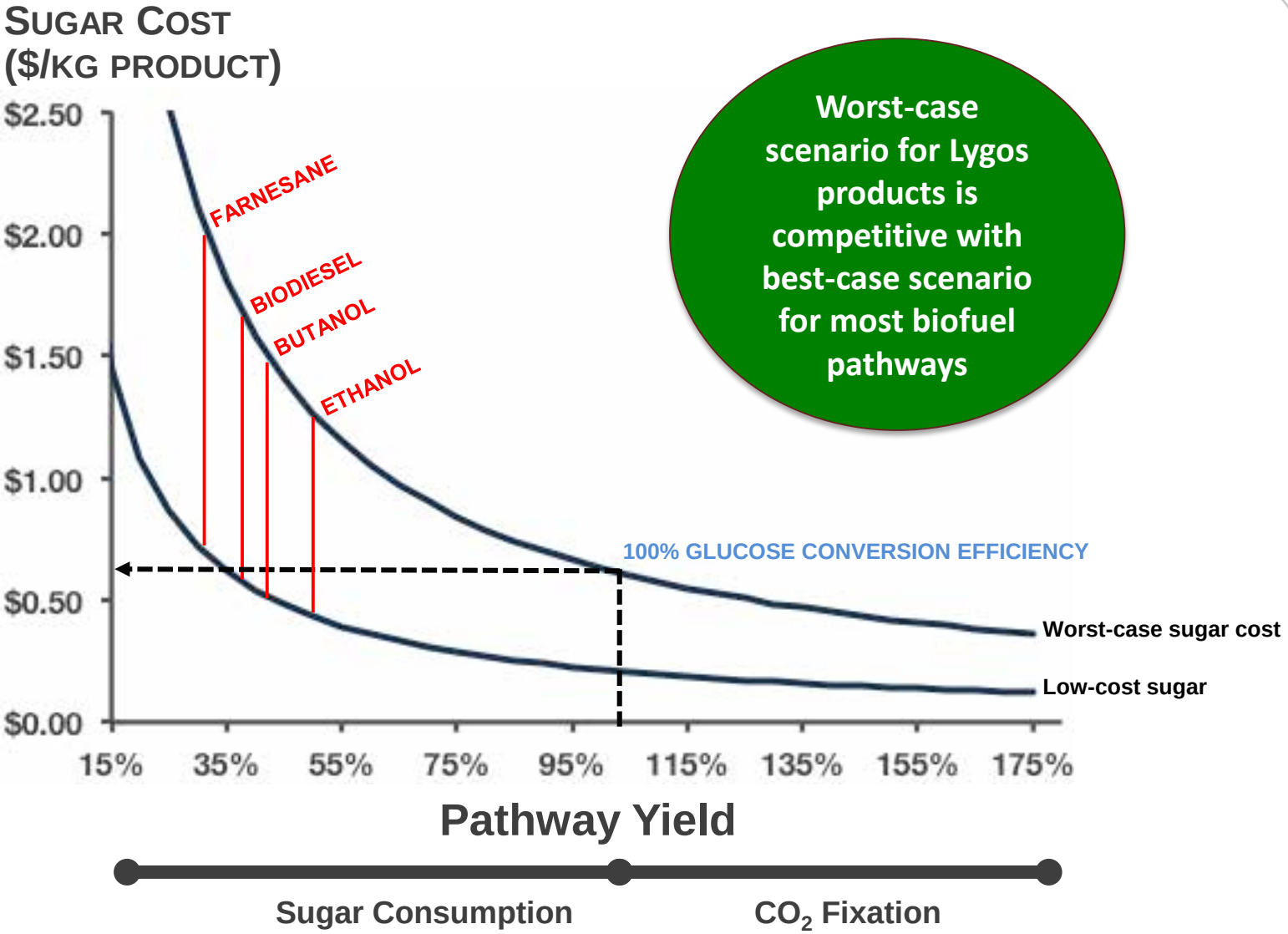
- **Bioenergy Technologies Incubator I:** malonic acid (scaling in 2017)
- **Bioenergy Technologies Incubator II:** new organic acid product

Eric Steen
CEO, PhD UCB Bioengineering

Jeffrey Dietrich
CTO, PhD UCB Bioengineering

Jay Keasling
Investor, Founder; Science Advisor

Lygos produces bio-advantaged chemicals



Worst-case scenario for Lygos products is competitive with best-case scenario for most biofuel pathways

Goal Statement

Anticipated Outcomes

- **Demonstration of an integrated process from cellulosic glucose to a purified organic acid product***
- **Proceeding along a path toward cost-advantaged economics based on improved fermentation yield, titer, and productivity**

Relevance to Department of Energy and BETO goals

- **Reduce dependence on foreign oil and manufacturing**
  - **New technology to existing chemicals that can be cost-competitively produced in the U.S. from domestic biomass**
- **Develop biochemical processes that support and improve integrated biorefinery economics**
  - **Target products where biochemistry can outcompete incumbent petrochemical producers on price and quality**
  - **TCA cycle enables access to chemicals where is CO_2 sequestered, providing a boost to process economics**

*Confidential prior to patent publication

Quad Chart

Timeline

- **Start Date:** October 1st, 2016
- **End Date:** September 30th, 2018
- **% Completion:** 17%

Barriers

- Demonstration of novel, high-yielding metabolic pathway (Ct-H)
- Improving fermentation performance (e.g., yield, titer, productivity; Ct-H)
- Integrated process development, sugar through purified chemical (Ct-J)

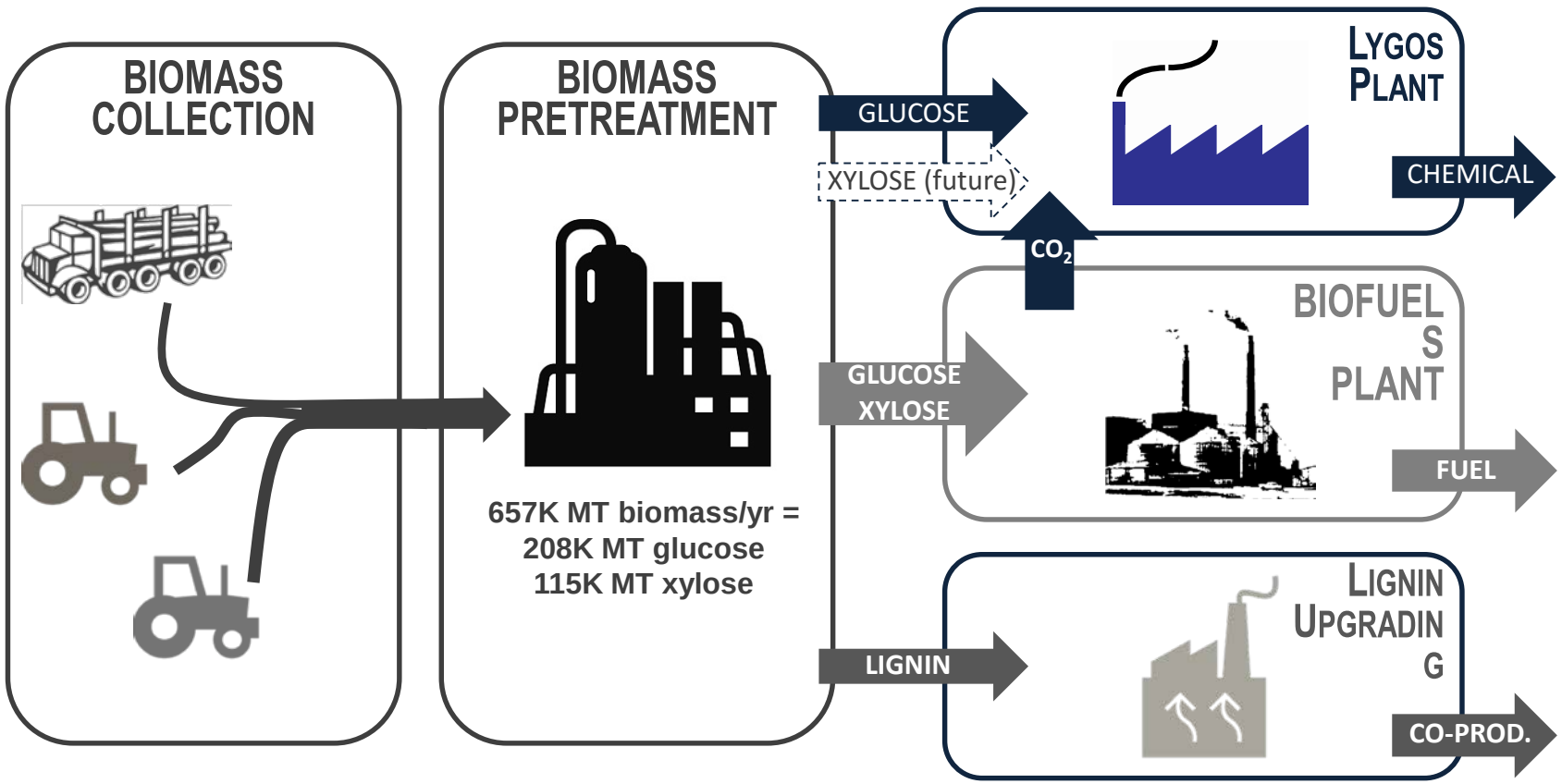
Budget

	Total Costs FY 12 – FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End Date)
DOE Funded	$0	$0	$52,800	$1,314,800
Project Cost Share	$0	$0	$13,200	$328,700

Partners

- Commercial cellulosic sugar provider (confidential; no cost-share)

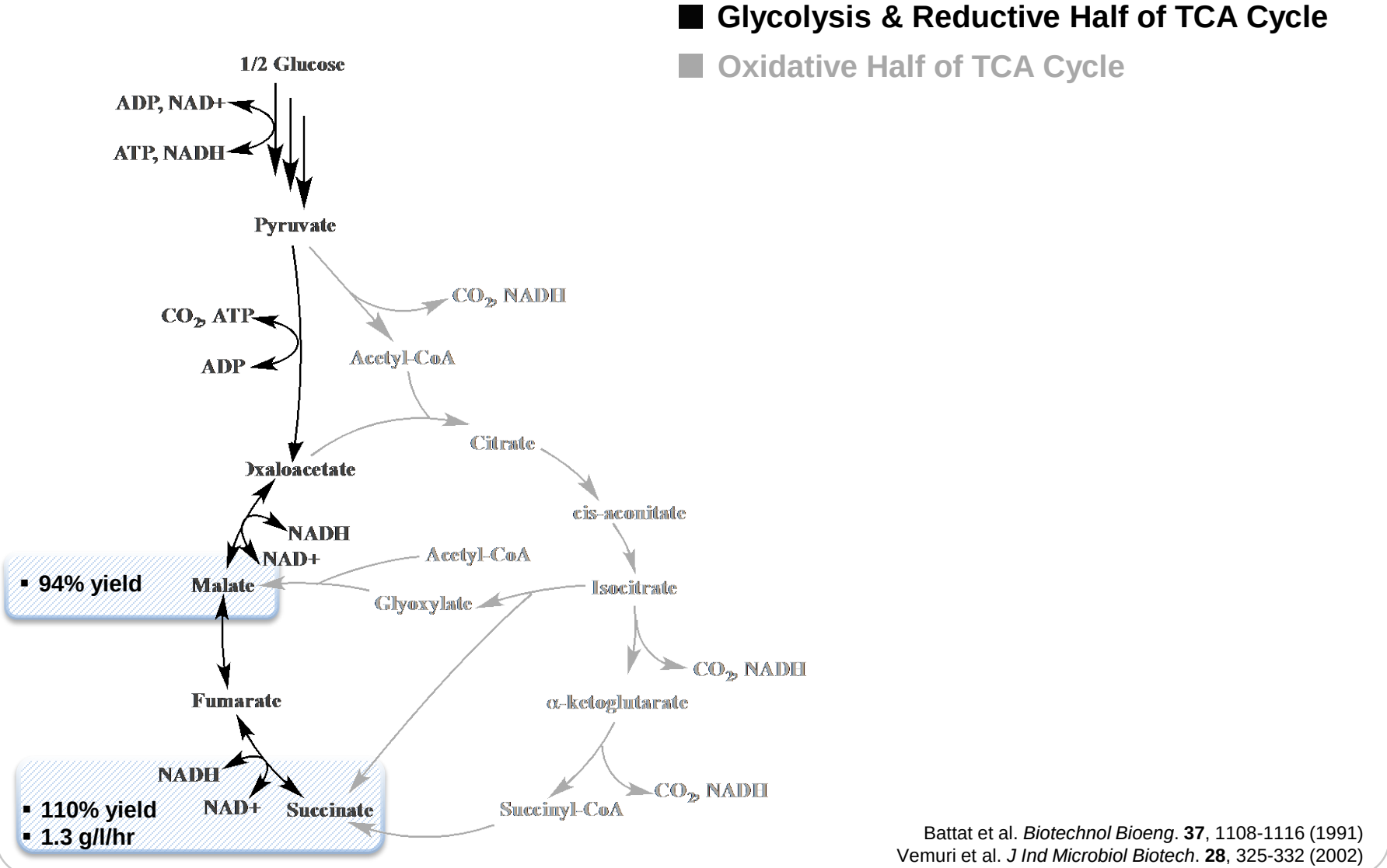
Project Overview: Integrated Biorefinery Vision



Biochemicals can improve integrated biorefinery economics

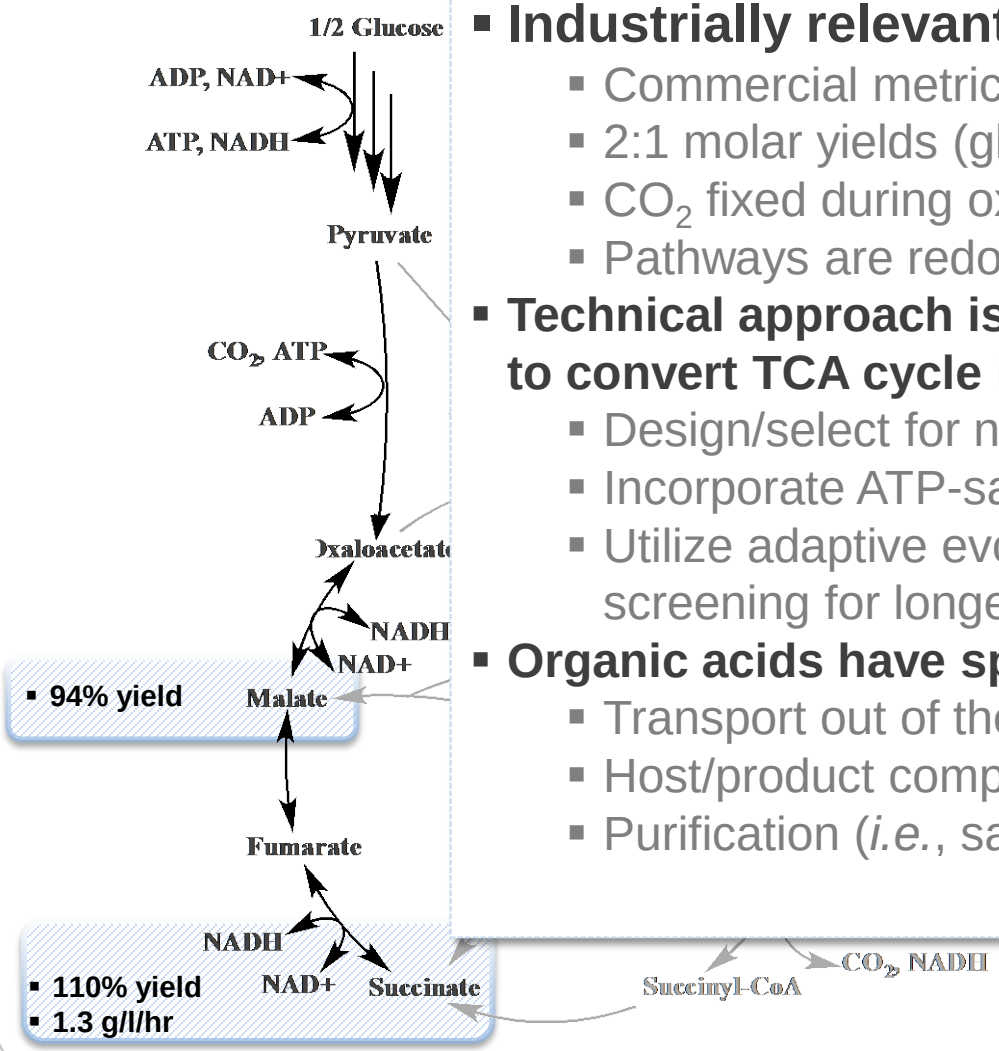
Conversion of C6 sugars to higher-value chemicals (incorporate C5 longer-term)
Use of CO_2 waste stream as additional carbon source (long-term goal)

Project Overview: Targeting TCA Cycle-Derived Chemicals



Battat et al. *Biotechnol Bioeng*. **37**, 1108-1116 (1991)
Vemuri et al. *J Ind Microbiol Biotech*. **28**, 325-332 (2002)

Project Overview: Targeting TCA Cycle-Derived Chemicals



- **Industrially relevant TCA cycle (reductive half)**
  - Commercial metrics reported on intermediates
  - 2:1 molar yields (glucose basis)
  - CO_2 fixed during oxaloacetate biosynthesis
  - Pathways are redox balanced or near balanced
- **Technical approach is to express non-native enzymes to convert TCA cycle intermediates to desired product**
  - Design/select for new enzyme activities
  - Incorporate ATP-saving enzymatic activities
  - Utilize adaptive evolution and high-throughput screening for longer-term strain improvement
- **Organic acids have specific technical considerations**
  - Transport out of the cell
  - Host/product compatibility (*i.e.*, acid tolerance)
  - Purification (*i.e.*, salt reduction)

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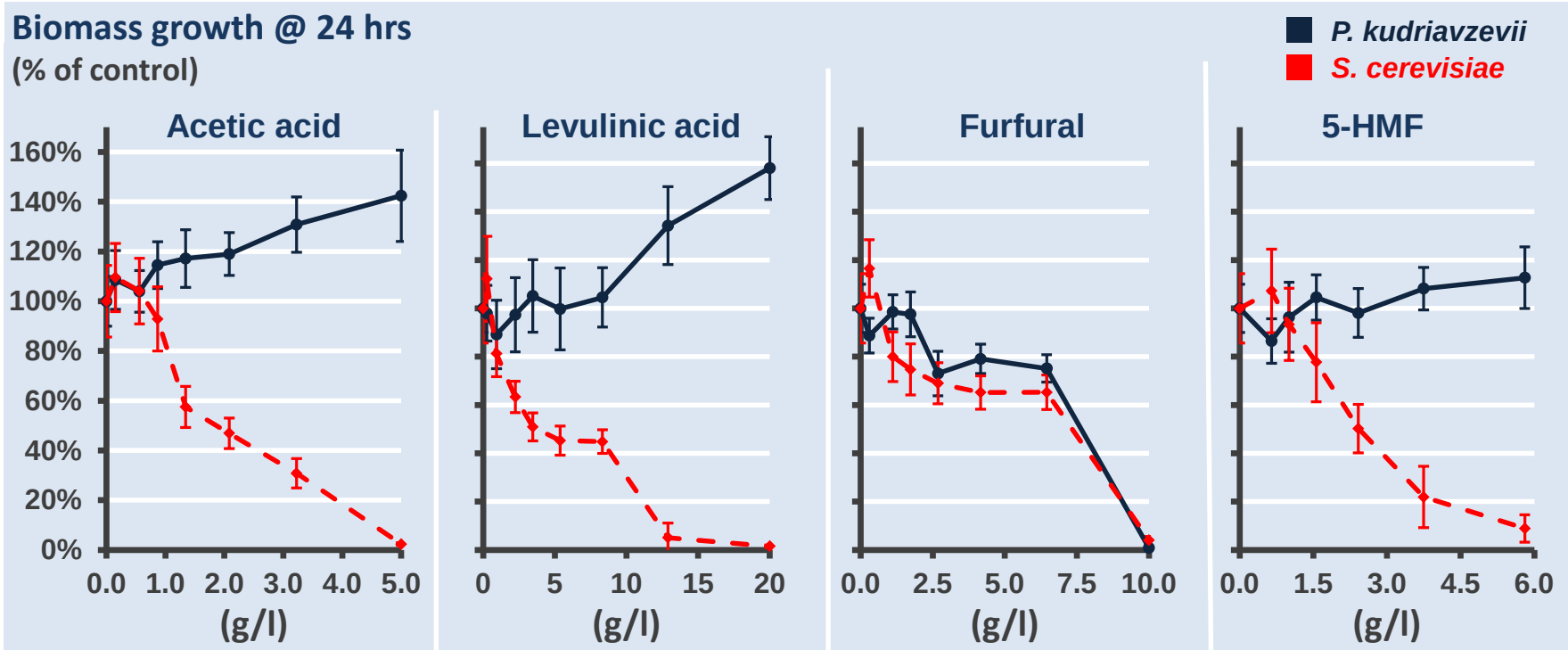
Project Overview: Strain choice

Engineering crabtree-negative yeast, *Pichia kudriavzevii*

- Wild type host is acid, temperature, salt, and solvent tolerant
- Wild type host is resistant to common cellulosic hydrolysate inhibitors
- Strain engineering tools and fermentation methods developed at Lygos
- Lygos strain evolved for a higher glucose consumption rate



Management Approach

- **Management approach and structure**
  - **PI manages over-arching project direction and reporting**
    - Guides Directors on process integration, technical hurdles, and techno-economics
    - Monthly, scheduled technical updates provided to DOE management
    - Quarterly technical and financial reports submitted to DOE
  - **Directors manage Departmental specific aims**
    - Manage day-to-day completion of technical work in the laboratory
    - Participate in contingency planning as unanticipated technical challenges are encountered
- **Quantitative milestones driven by techno-economic analysis and address prioritized technical risks**
- **All strain and fermentation performance data is managed in LIMS database**

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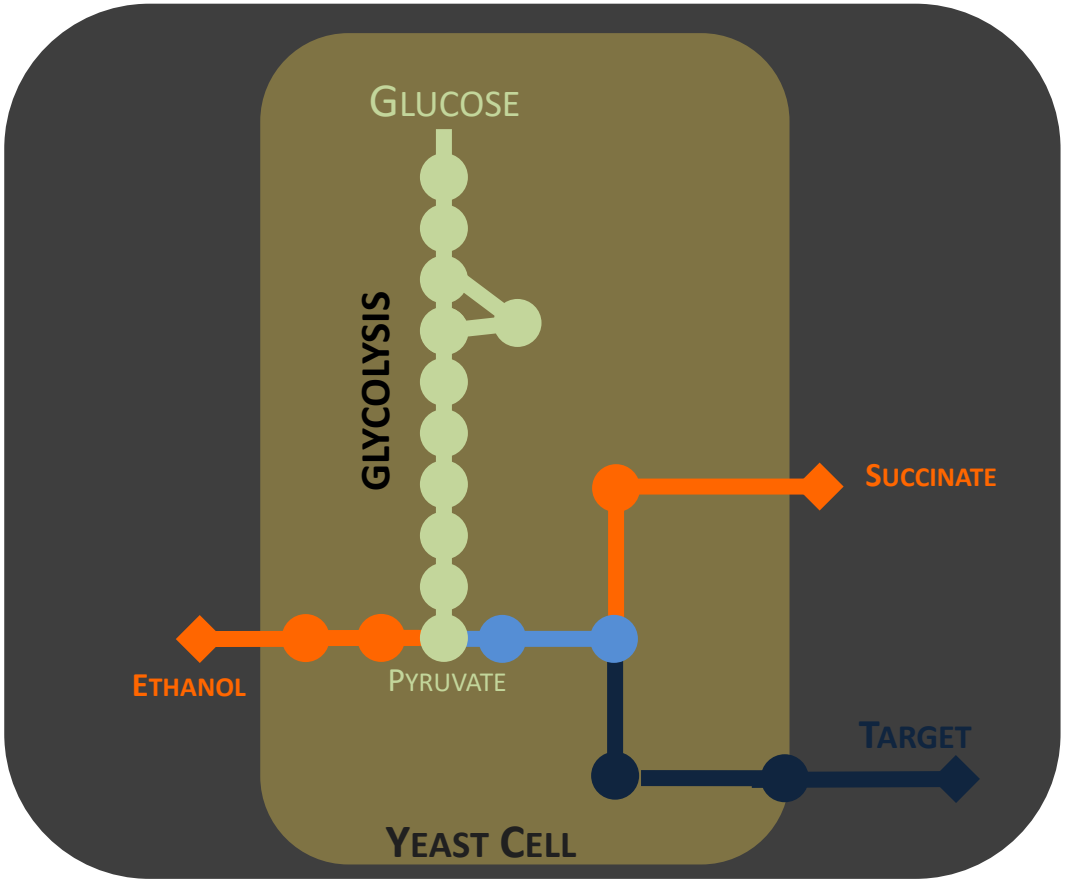
Technical Approach: Year 1 Task Structure



- Native pathways (decrease expression)
- Biosynthetic pathway (overexpress)
- Non-native enzyme screening

- **Decrease native byproduct formation (based on fermentation conditions)**
- **Demonstrate non-native enzyme activities**
- **Proof-of-concept pathway demonstration**

Note: metabolic pathways are outlined to be illustrative only

Technical Accomplishments/Progress/Results

- **Identification of genes associated with *P. kudriavzevii* overflow metabolism under planned commercial fermentation conditions**
  - Sequence homology used to identify candidate genes in *P. kudriavzevii* genome (e.g., pyruvate decarboxylase, dehydrogenase)
  - Demonstrated absence of byproduct formation following gene knockout
- **Identification of native transcription factors responsive to planned commercial fermentation conditions**
  - RT-qPCR to assay specific genes and their cognate promoters
  - RNAseq to identify additional, unpredicted promoters
- **Demonstration of novel pathway enzyme activity**
  - Development of complementation assay for enzyme screening
  - Screened enzyme library, demonstrating desired enzyme activity in multiple hits

4 – Relevance

By the completion of this BETO project, demonstration of process commercial viability is anticipated

Relevance to BETO

- **Demonstration of process from cellulosic glucose to higher-value chemicals, improving integrated biorefinery economics**
- **TCA cycle-derived chemicals sequester CO_2, providing a route to valorize waste CO_2 streams**
- **Integrated process development**
  - **Target products where biochemistry can outcompete incumbent petrochemical producers on price and quality**
  - **TCA cycle enables access to chemicals where is CO_2 sequestered during production**

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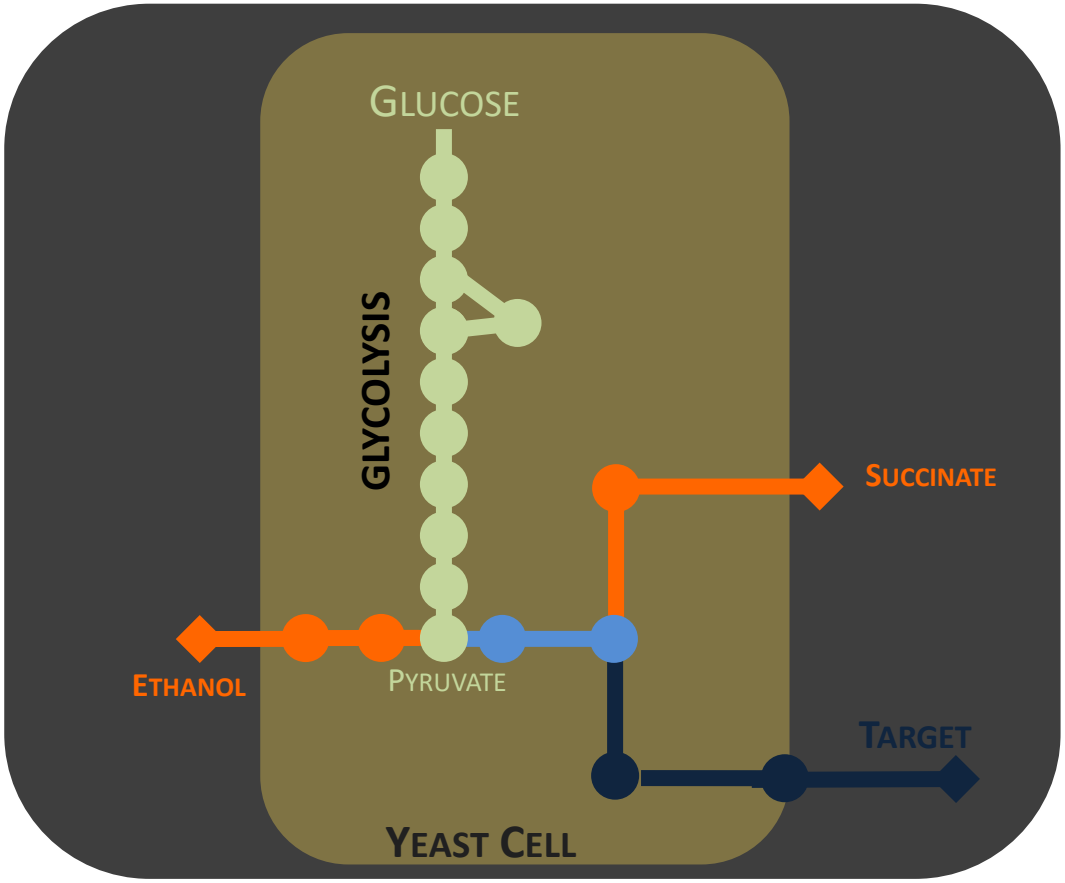
Future Work: Year 1 Tasks and Objectives



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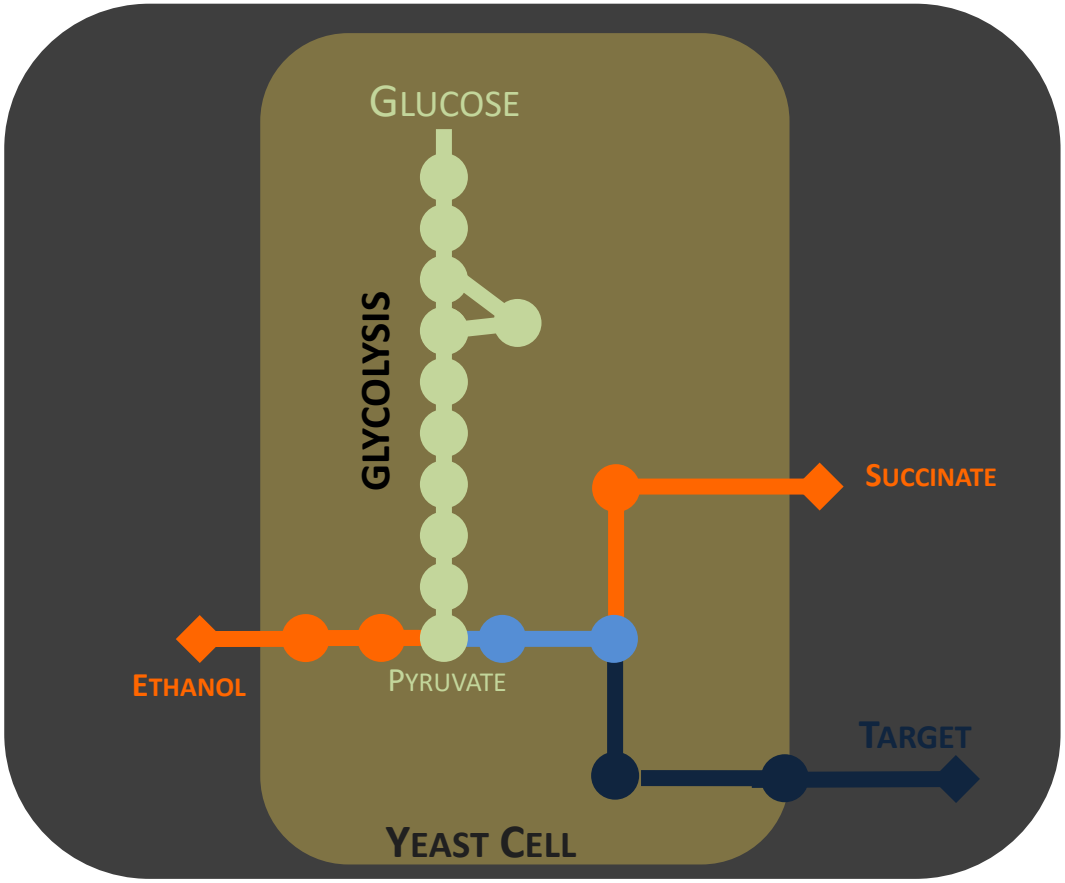
- **Objective #1: Regulatory network/genome engineering to reduce byproduct formation under envisioned, industrial fermentation conditions**
- **Objective #2: Demonstration of non-native pathway enzymes**
- **Objective #3: Product transporter screening and characterization**

Future Work: Year 1 Go/No-Go Decision



- Native pathways (decrease expression)
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Year 1 Go/No-Go Decision: demonstration of prototype strain performance using cellulosic glucose as fermentation feedstock

- All enzymes critical for energy efficient biosynthesis will be incorporated
- Fermentation metrics (e.g., yield and rate) to inform Year 2 optimization

Future Work: Year 2

Year 2: Process optimization, integration, and scaleup

- Cellulosic glucose (one supplier) used throughout the Year 2 workplan
- Progressive fermentation milestones on yield, titer, productivity; goals are driven by techno-economic model
- Purification milestones (yield) using integrated process materials (i.e., cellulosic glucose through purified chemical)
- Final validation based on integrated process scaleup (10X scaleup)

0.5 mL

1000

0.5 Liter Reactors

10x

2-15 Liter Reactors

Future Work: End of Project Goals

- **Strain and metabolic pathway that are de-risked**
  - No “discoveries” remaining
  - Anticipate incremental optimization/improvements will still be required
- **Fermentation process with metrics (yield, titer, and productivity) that warrant start of pilot plant scaleup (tech transfer ready)**
- **Purification process with metrics (e.g., yield and purity) where all unit operations have been technically de-risked**

1000

10x

0.5 mL

0.5 Liter Reactors

2-15 Liter Reactors

Summary

- **Overview:** Engineering acid-tolerant yeast to consume cellulosic glucose and produce a TCA cycle-derived organic acid. Developing an integrated process from feedstock to purified chemical.
- **Approach:** Prototype strain demonstration in Year 1, transitioning to process optimization and integration in Year 2. Final validation based on process scaleup.
- **Technical Accomplishments:**
  - Identified necessary byproduct genes and necessary regulatory components
  - Demonstrated new enzyme activity needed for product biosynthesis
- **Relevance**
  - Ct-H: efficient catalytic upgrading of sugars to chemicals
  - Ct-J: process integration
- **Future Work**
  - Construction of prototype, engineered strain
  - Milestones driven by estimated, scaled cost of goods sold (COGS)

Additional Slides

Not applicable – new project

Publications, Patents, Presentations, Awards, and Commercialization

- **Patents:** International patent application PCT/US16/6158 was filed during the project performance period and contains work performed under this award.