U.S. Department of Energy (DOE) Bioenergy Technologies Office (BETO) 2017 Project Peer Review

White Dog Labs Incubator II

Second-Generation Mixotrophy for Highest Yield and Least Expensive Biochemical Production

Yielding synthetic biology's promise

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Biochemical Conversion

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Goal Statement

- The goal for this project is to develop and demonstrate a fermentation process to produce acetone from a cellulosic feedstock at a mass yield at least 130% the previous theoretical maximum.
- The primary outcome will be a continuous acetone fermentation process achieving industrially-relevant metrics at the 3 L-scale.
- This is a **platform technology** that can be adapted to almost any biochemical or biofuel of interest.
- Improving carbon yields from expensive cellulosic sugars can help lower the operating costs of cellulosic-based processes and lead to greater adoption of the technology.

Quad Chart Overview

Timeline

- September 1, 2016
- September 30, 2018
- 15% complete

Budget

	Total Costs FY 12 – FY 14	FY 15 Costs	FY 16 Costs	Total Planned Funding (FY 17-Project End)
DOE Funded	-	-	-	$1,539,826
Project Cost Share (WDL)	-	-	-	$390,000

Barriers

- Improving carbon yields of biochemicals and biofuels
- Adapting microorganisms and fermentation system to use hydrolysate feedstocks
- Achieving stable, continuous production metrics

Partners

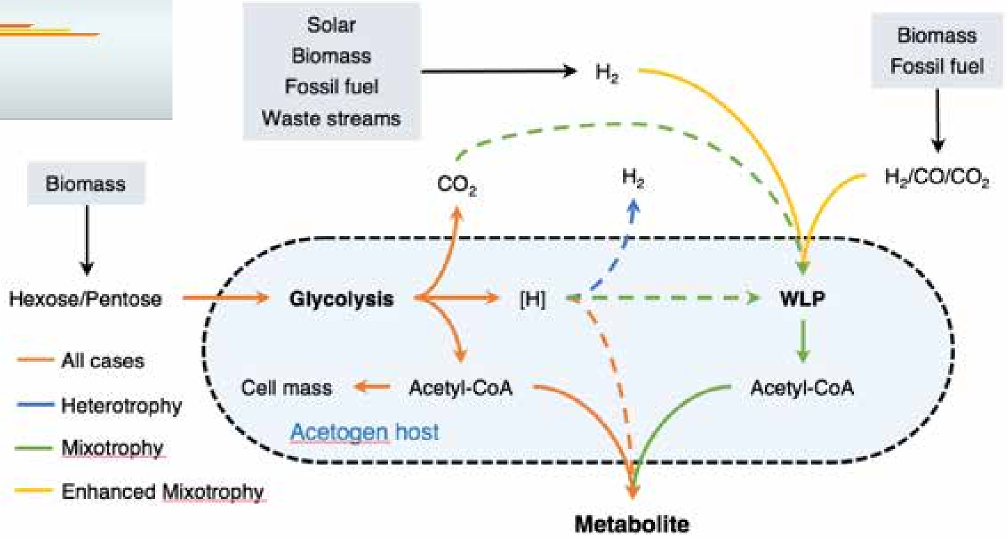
- White Dog Labs (WDL) is responsible for all activities
- We are interacting with several cellulosic feedstock providers to use real cellulosic hydrolysates

Project Overview

nature COMMUNICATIONS

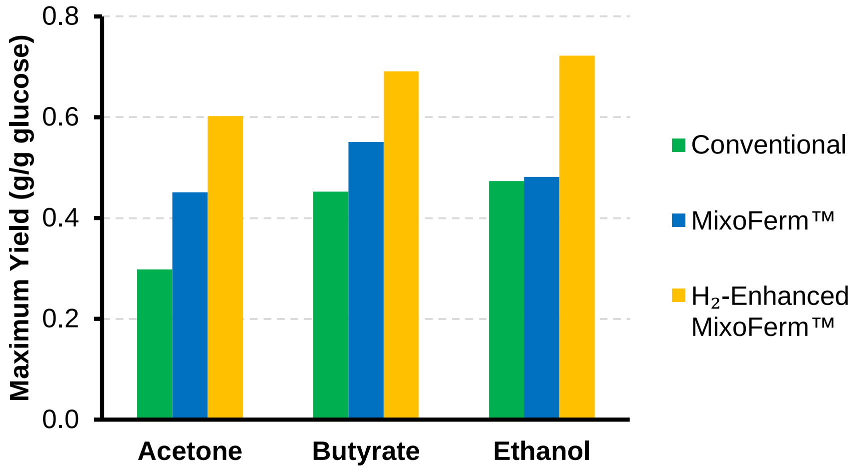
Article | OPEN

CO_2 fixation by anaerobic non-photosynthetic mixotrophy for improved carbon conversion



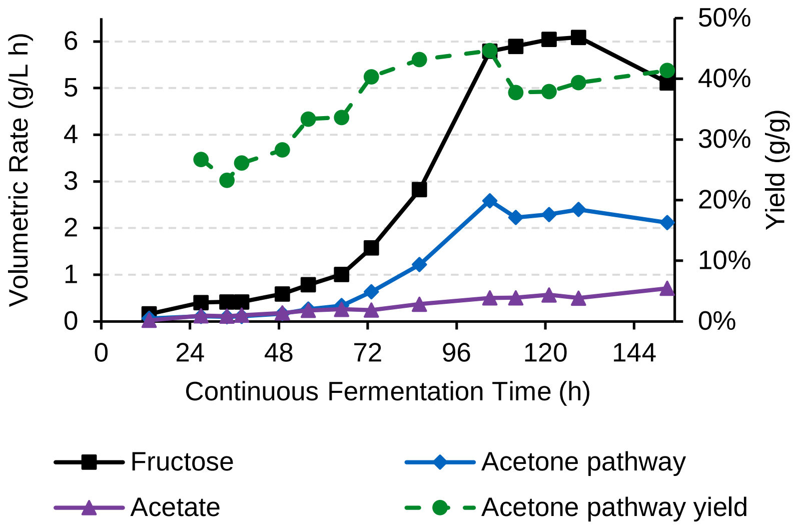
<u>MixoFerm™</u> combines conventional fermentation and gas fermentation. Microbes are able to fix CO_2 produced during catabolism of sugar to produce more product and increase *<u>Carbon Yields</u>*.

Project Overview



NAD(P)H/ Acetyl-CoA	Yield increase	Example molecules
0	51%	Acetone
1	22%	Butyric acid
2	2%	Ethanol

Constructed a MixoFerm™ acetone strain and process



- Achieved **>90%** MixoFerm™ maximum yield
- Preferred C6 sugar is *fructose*

Need to adapt strain and process to use cellulosic hydrolysate

Approach (Management)

- All project activities take place at WDL facilities by WDL employees
- Work locations:
  - Microbiology laboratory – activities overseen by PI
  - Fermentation facility – activities overseen by CEO
- Short update meetings very Mon, Wed, and Fri with all participating scientists to coordinate tasks and discuss results
- Project review meetings take place once a month (led by PI) to update on progress and discuss any critical issues

Approach (Technical)

Strain Development

Generate glucose-utilizing strain

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Impart acetone pathway

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Achieve Yield KPI on C6/C5 mix — **Go/No-Go**

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Adapt to hydrolysate feedstock → Optimize system to achieve target KPIs

Fermentation Development

Adapt system to hydrolysate feedstock

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Optimize system to achieve target KPIs

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Demonstrate KPIs in continuous 3L process

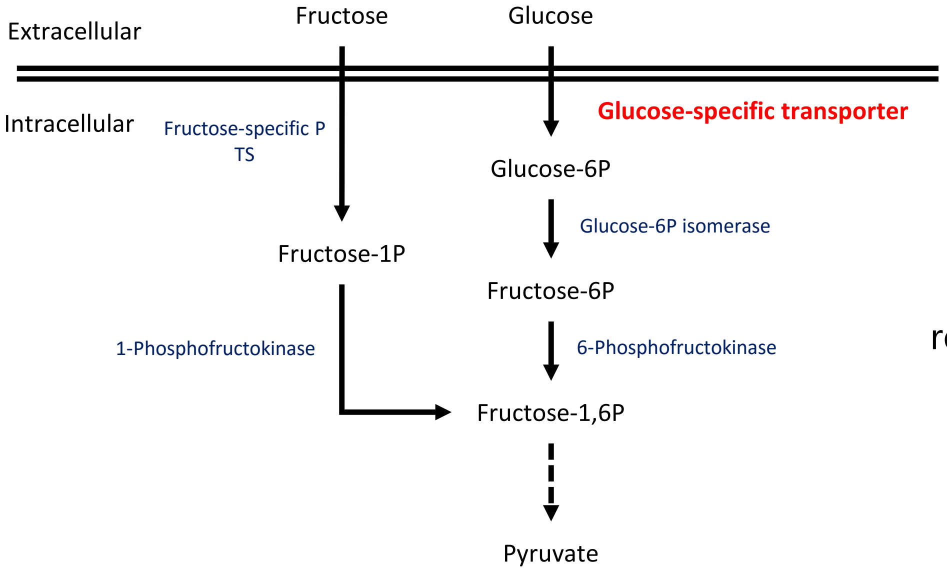
Major Challenges

1. Adapting fermentation system to use hydrolysate feedstocks which contain higher solids content. Solids could clog cell-retention system → Modify Cleaning In Place (CIP) procedures or perform more frequently
2. Adapting strain to grow on hydrolysates. Hydrolysates can contain inhibitory molecules → Adaptively evolve strain to overcome inhibition

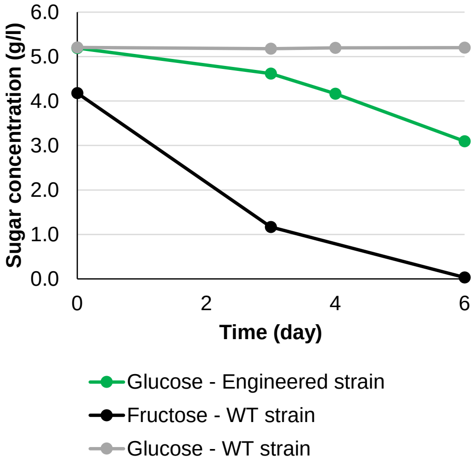
Project outcome: Scale-up ready fermentation process

Technical Accomplishments

Generate glucose-utilizing strain



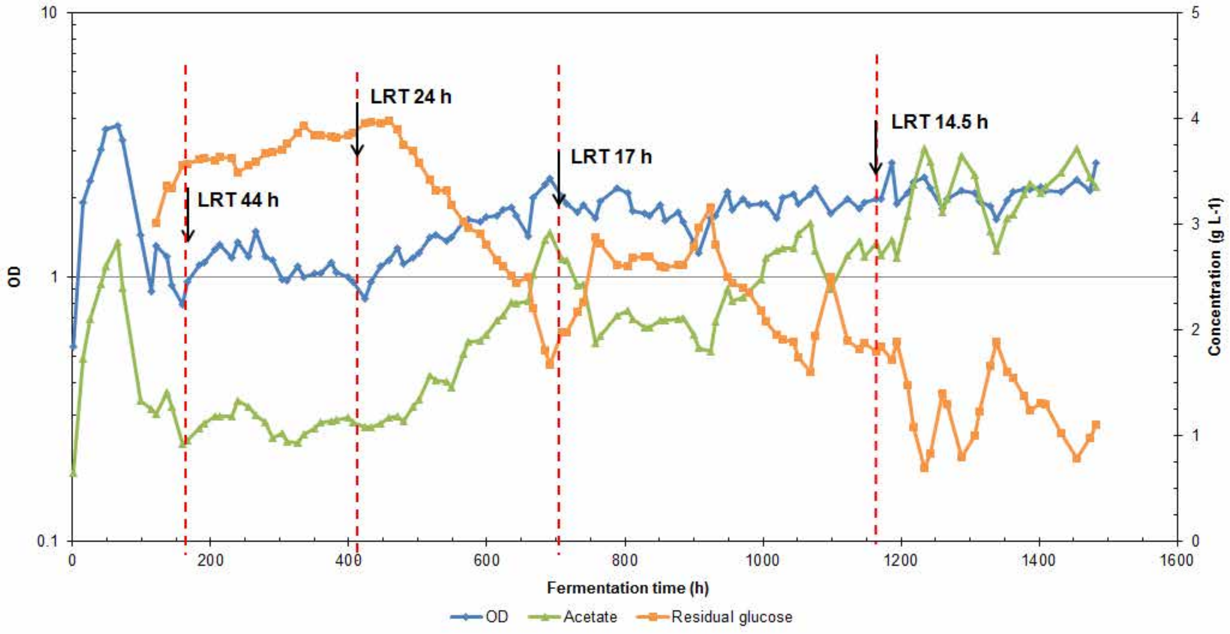
Introduced recombinant glucose-specific PTS genes



Permanently integrated the best PTS gene into the chromosome

Technical Accomplishments

Evolved strain in a chemostat fed with glucose to achieve a faster utilization rate



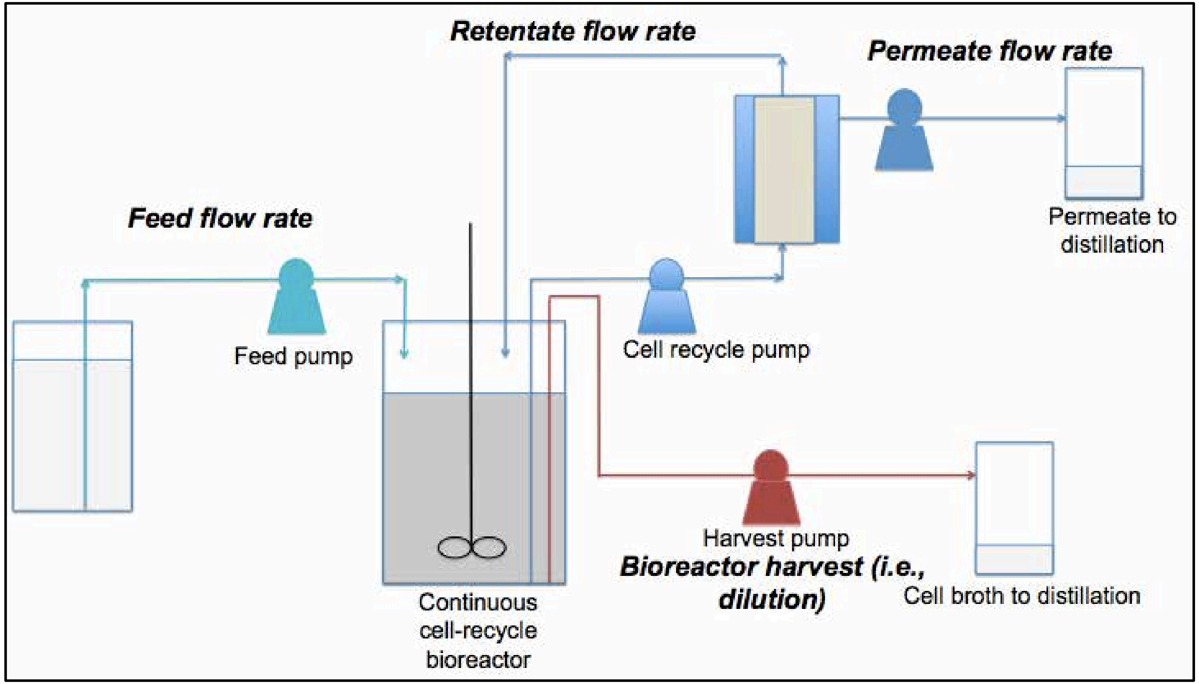
Dilution rate continually increased, selecting for faster growing cells.

Achieved a glucose utilization rate equal to the fructose rate.

Next step is to introduce the acetone pathway into this strain

Technical Accomplishments

Adapt fermentation system to use a hydrolysate feedstock



Testing different:

- Cell retention membranes
- Cleaning procedures

Working to achieve continuous operation with hydrolysates

Relevance

Lower operating costs of cellulosic-based processes by **improving carbon yields** of biochemicals and biofuels

- Addresses a critical issue identified by BETO:
  "Develop strategies for **conserving carbon** and hydrogen in conversion and upgrading processes"
- Improving carbon yields can directly lower operating costs and thus costs of final fuels by producing more unit product per unit feedstock.
- This is a ***platform technology*** to produce a wide array of biofuels and biochemicals using a common chassis microorganism and process. Success will demonstrate a proof-of-concept process to be replicated and adapted to other specific fuel molecules.
- Goal is to demonstrate industrially-relevant production metrics (3 g/L/hr productivity and 40 g/L titer) at the 3 L fermentation-scale.

Increase efficiency of fermentation processes

Future Work

Strain Development

Generate glucose-utilizing strain ✓ Completed

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Impart acetone pathway →

Tasks — **Currently underway**

1. Construct optimized expression cassette for acetone production
2. Integrate expression cassette into the chromosome
3. Confirm acetone KPI yield (≥43wt%) on glucose/xylose mix

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Achieve Yield KPI on C6/C5 mix ← **Go/No-Go**

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Adapt to hydrolysate feedstock →

Tasks — **Currently underway**

1. Test growth on different hydrolysates and determine inhibitory effects
2. Adapt acetone-producing C6/C5 strain to hydrolysates

→ **Strain goes to fermentation**

Strain development tasks are on track to be completed on time

Future Work

Fermentation Development

Adapt system to hydrolysate feedstock

Currently underway

Determine operating parameters to maintain continuous operation using hydrolysate feedstocks

Goal: >200 uninterrupted hours of operation

Optimize system to achieve target KPIs

Tasks

Currently underway using pure sugar feedstock

1. Determine operating parameters to achieve all KPIs
2. Develop production medium

Goal: >336 hours of acetone production

Demonstrate KPIs in continuous 3L process

Project goal is 6 weeks of continuous acetone production at target KPIs (yield, titer & productivity)

Summary

- This project is targeting acetone production from a cellulosic feedstock with a *mass yield at least 130%* the previous theoretical maximum as a proof-of-concept **platform technology**.
- The goal is to help lower operating costs of cellulosic-based fermentations by improving the carbon yield of products.
- Strain and fermentation development tasks are on-going:
  - Have successfully engineered a glucose-utilizing strain
  - Tasks so far are on schedule (project completion Sept. 2018)

Additional Slides

Responses to Previous Reviewers' Comments

This is the first time this project is being reviewed, and no Go/No-Go Review meetings have taken place yet.

Publications, Patents, Presentations, Awards, and Commercialization

No publications, patents, presentations, or awards have resulted from this work yet.

Currently, there are no commercialization efforts for this work.