2013 DOE Bioenergy Technologies Office (BETO) Project Peer Review

Bioenergy Demonstration Project: Value-Added Products from Renewable Fuels

May 23, 2013
Technology Area Review: Biochemical Conversion

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University of Nebraska

Goal Statement

- Third generation cellulosic ethanol is an emerging solution that will make strong contributions to American domestic energy needs. Technology underlying this process will replace commodity enzymes with engineered microbes to convert biomass derived lignocellulose feedstocks into biofuels and value added chemicals. The approach used here is based on consolidated bioprocessing. Thermoacidophilic microbes belonging to the Domain Archaea, will be employed for the deconvolution and saccharification of lignocellulose to maximize biofuel yields. Biomass pretreatment (hot acid) will be combined with fermentation using an extremely thermoacidophilic microbial platform. The identity and fate of released sugars will be controlled using metabolic blocks combined with added biochemical traits where suitable.

- LC/MS analysis supported through the newly established Nebraska Bioenergy Facility will provide general support for bioenergy researchers at the University of Nebraska

Quad Chart Overview

Timeline

- **Project start date:7-01-2008**
- **Project end date 6-29-2013/14**
- **Percent complete: 90%**

Budget

- Funding for FY11 $50K/20%
- Funding for FY12 $50K/20%
- Funding for FY13 $50K/20%
- 5 years / $380K per year av.

Barriers

- Barriers addressed
  - Bt-J. Fuels Organism Development
  - Bt-F. Cellulase Enzyme Production Cost
  - Bt-H. Enzyme Biochemistry

Partners

- Algae Researchers UN-L
- Sandia Ntl. Lab contractors
- DOE-JGI

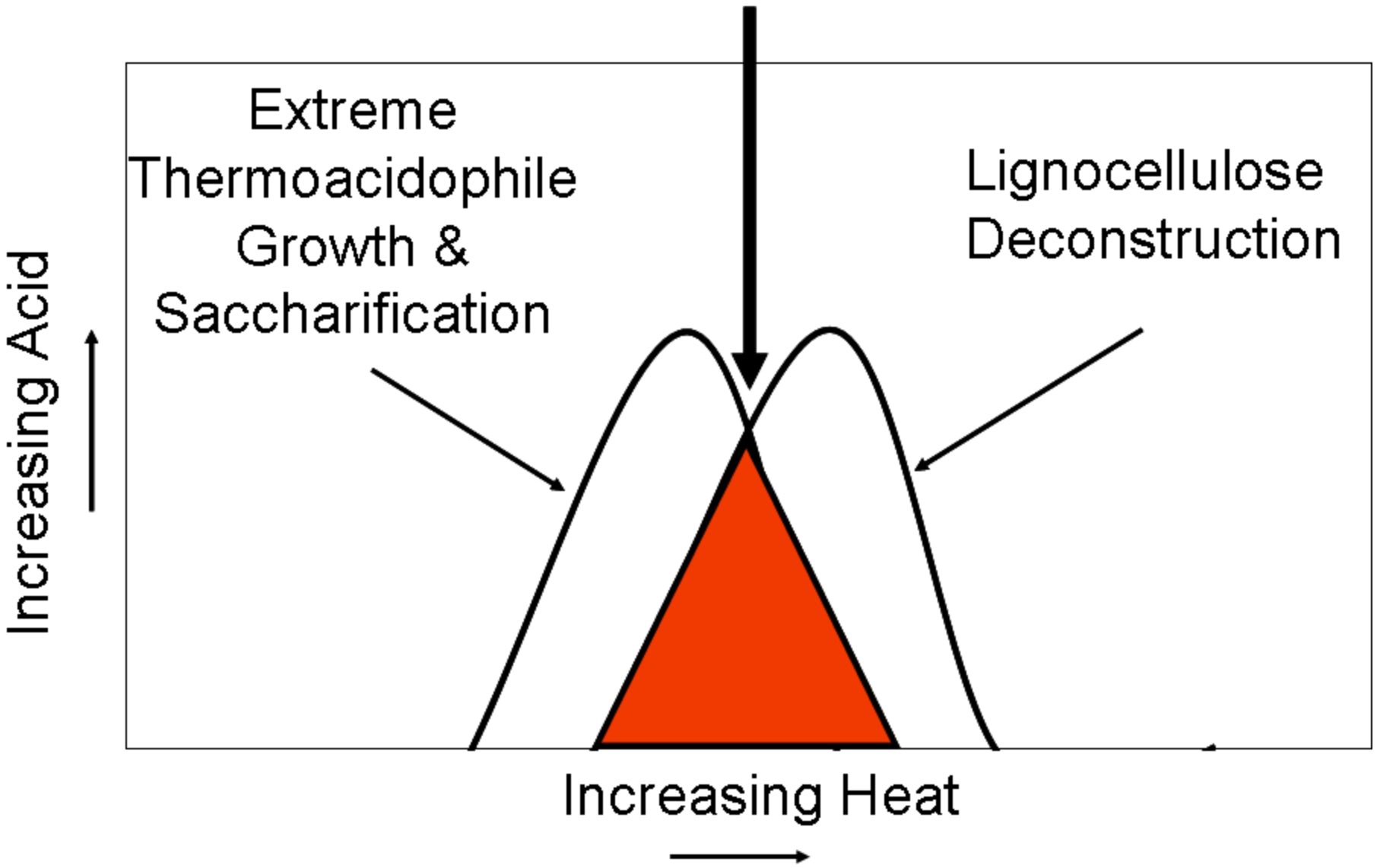
Project Description:

- This project will examine the utility of diverse thermoacidophilic taxa for biomass processing through a combination of genetic, biochemical and chemical methodologies to achieve a reduction in pretreatment costs and process time. A core facility will be established to further regional efforts on development of bioenergy crops.

Goals and Objectives:

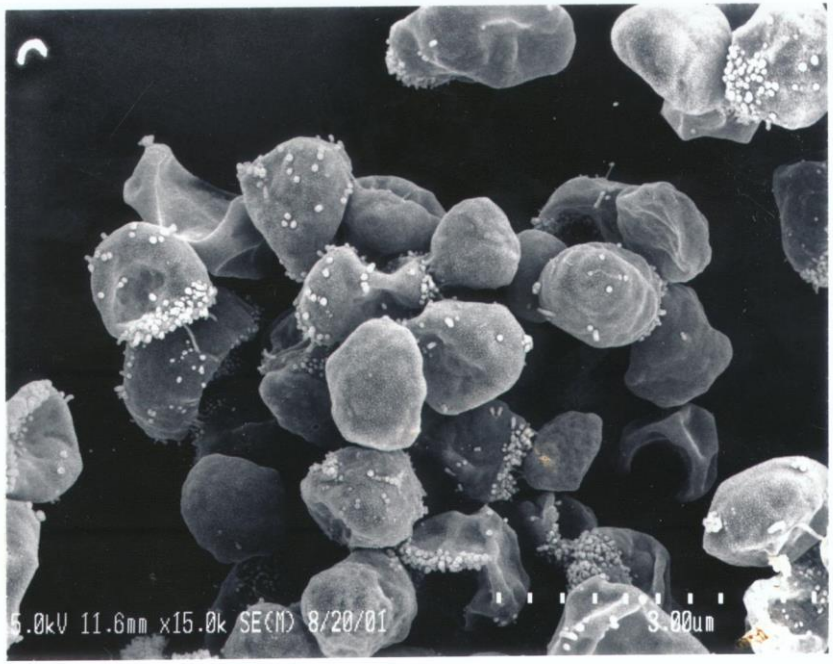
- This is a consolidated bioprocessing project. Using methods pioneered by the Blum lab, the utility of extremely thermoacidophilic microbes will be established for the deconvolution and saccharification of lignocellulose to maximize biofuel yields. Biomass pretreatment (hot acid) will be combined with fermentation using an extremely thermoacidophilic microbial platform. The identity and fate of released sugars will be controlled using metabolic blocks combined with added biochemical traits where suitable. Acquired equipment will also be used to create a Bioenergy Core Facility (BCF) that will provide general support for bioenergy researchers at the University of Nebraska.

Consolidated Biomass Processing Using Saccharifying Thermoacidophiles



Extremely Thermoacidophilic Archaea

- Acidophilic hyperthermophiles that grow at pH 3-5, 65-95°C.
- Crenarchaeota phylum.
- Chemoheterophic and obligate aerobes (with introduced fermentation traits).
- Used globally for scale-up oxidative biotransformations.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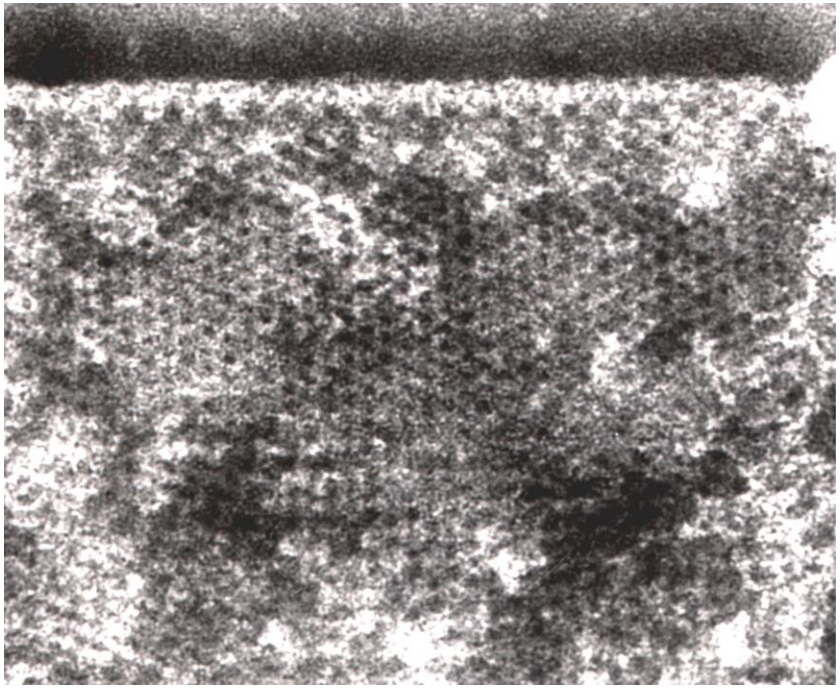


1- Approach

A. “Screening thermoacidophilic taxa” for the ability to deconvolute lignocellulose and depolymerize associated carbohydrates

B. Evaluate and respond to formation of “inhibitors” that arise during incubation of lignocellulose under heated acidic conditions

C. Identify and engineer “sugar flux channeling and catabolic blocks” that control and where necessary redirect metabolic pathways to maximize sugar concentrations

D. Expand the hydrolytic capacity of extremely thermoacidophilic microbes through the “addition of deconvolution traits”.

E. Establish the Nebraska Bioenergy Facility (NBF) at the University of Nebraska-Lincoln.

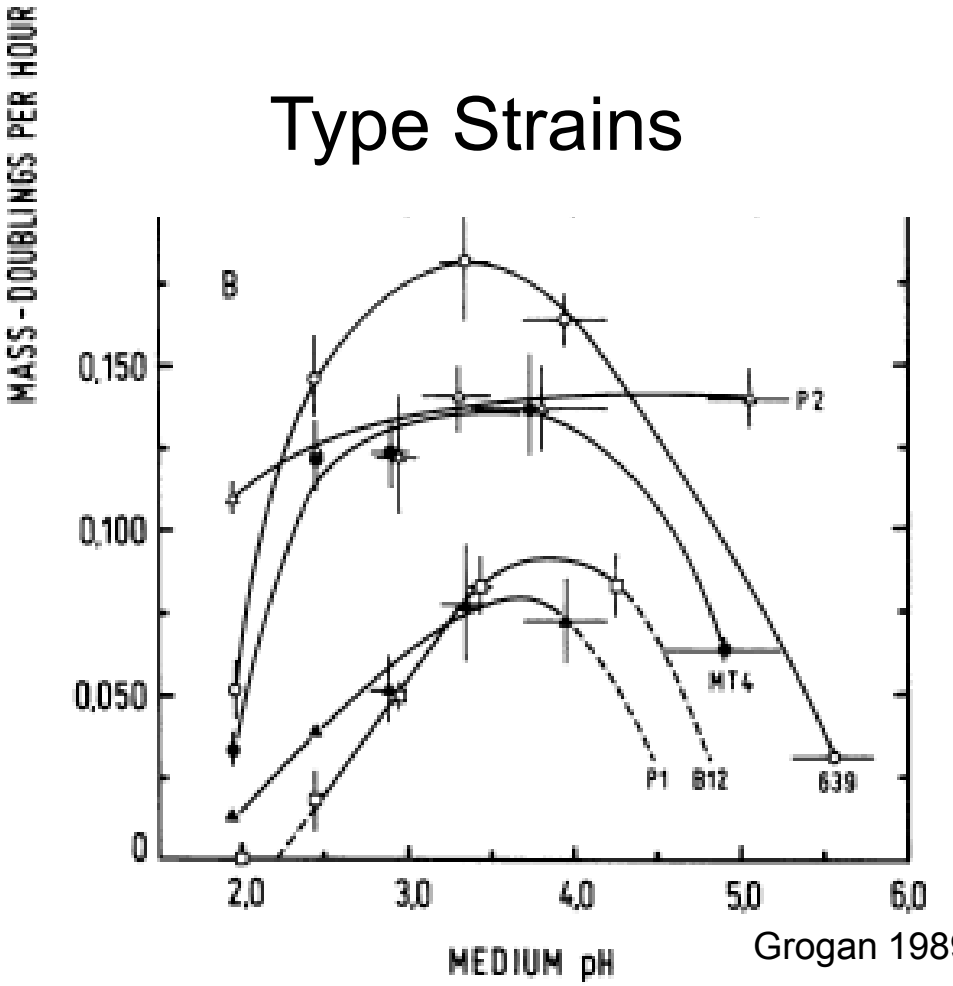
2 - Technical Accomplishments/ Progress/Results

Summary of Work to Date - Accomplishments (FY13-current):

- SARC (super acid resistant crenarchaeota) cell lines established new record in thermoacidophily approaching pretreatment compatibility.
- Mechansims underlying resistance to fermentation inhibitors including furfural and HMF have been identified and engineered into cell lines.
- Type species cell ines have been engineered that are catabolically blocked for glycolysis.
- Hot acid-stable endoglucanases have been characterized in vivo and in vitro.
- A platform system has been discovered that confers hot acid resistance on enzymes based on protein glycosylation.
- The UNL Bioenergy core facility founded with DOE funding continues to conduct algal and extremophile bioreactor fermentations (in addition to LC-MS analysis of lipids) for university researchers.

Thermoacidophilic crenarchaeota: pH optima 3.5 – 4.0 Temp optima 75-80°C

Type Strains

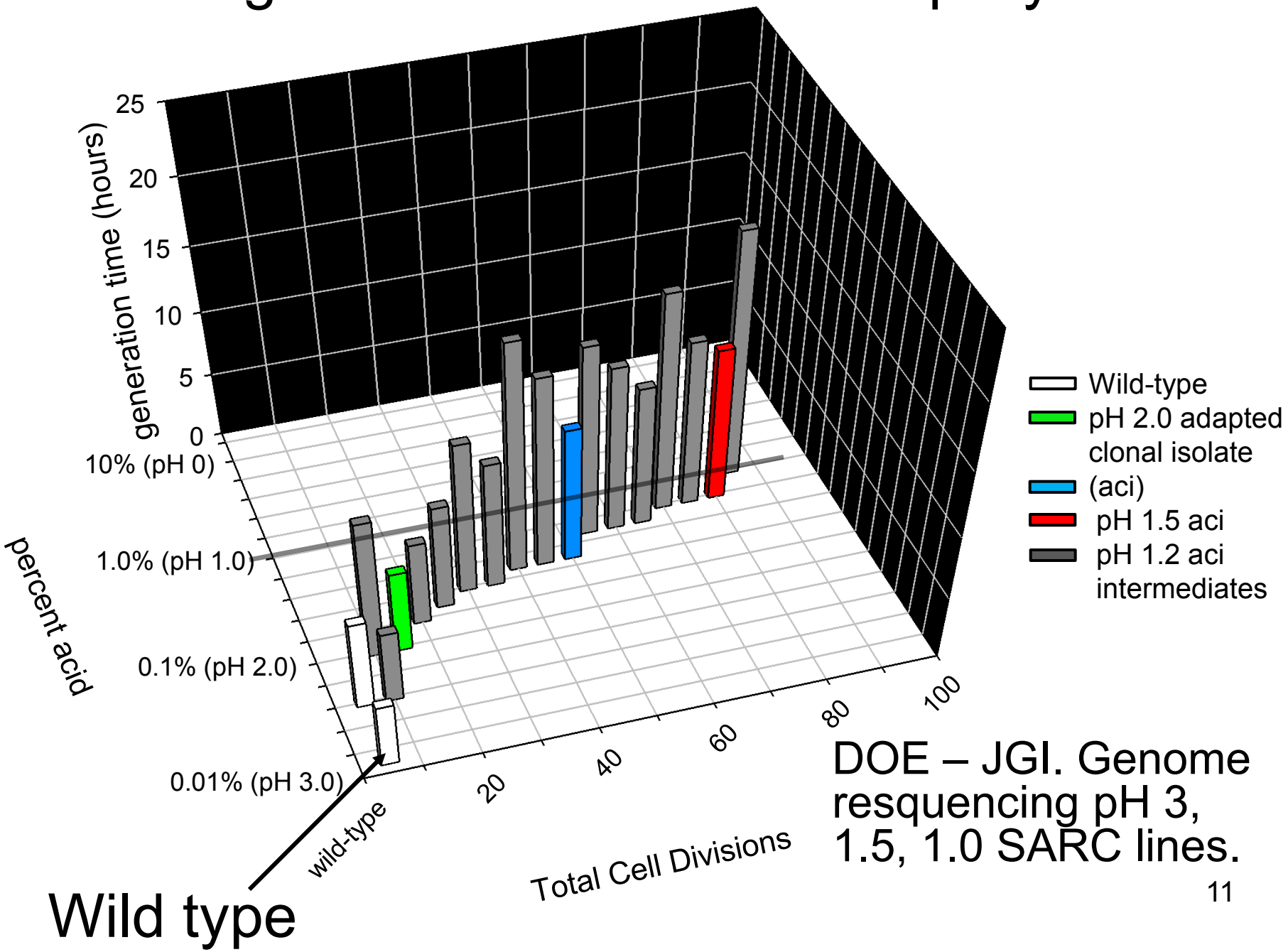


Grogan 1989

New Isolates

- **Analysis of new environmental isolates revealed trade off between more extreme acidophily and heterotrophy**
- **Retention of heterotrophy seems coupled to higher pH optima**

Evolving Increased Thermoacidophily: SARC

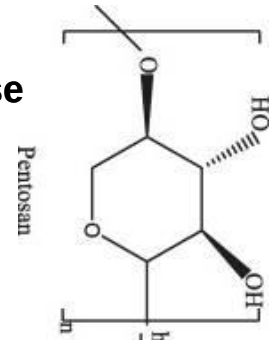


DOE – JGI. Genome resequencing pH 3, 1.5, 1.0 SARC lines.

Wild type

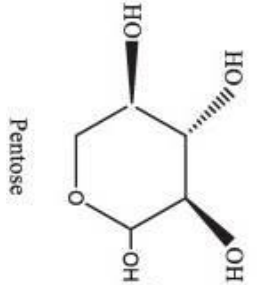
Fermentation Inhibitors: ADH-10 Inactivation Results in Resistance to Furfural & HMF

Hemicellulose



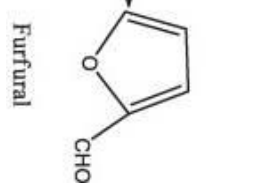
hydrolysis

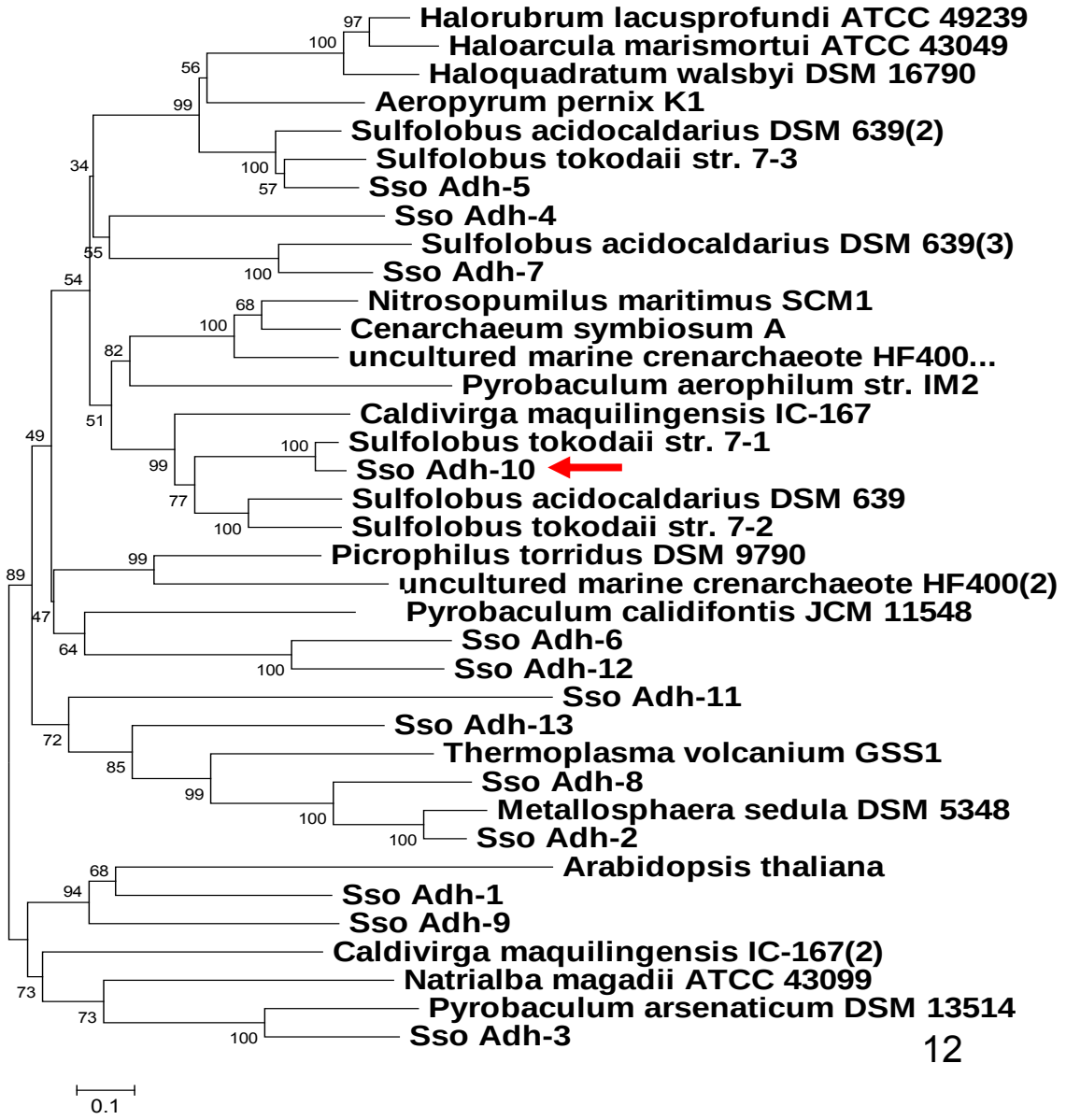
Pentose



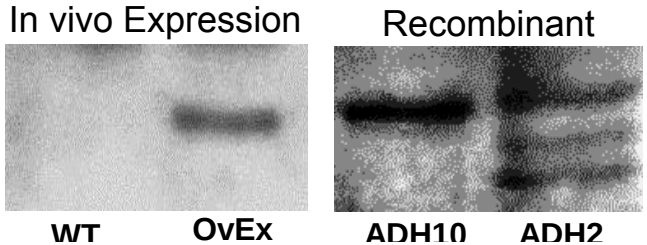
dehydration
cyclization

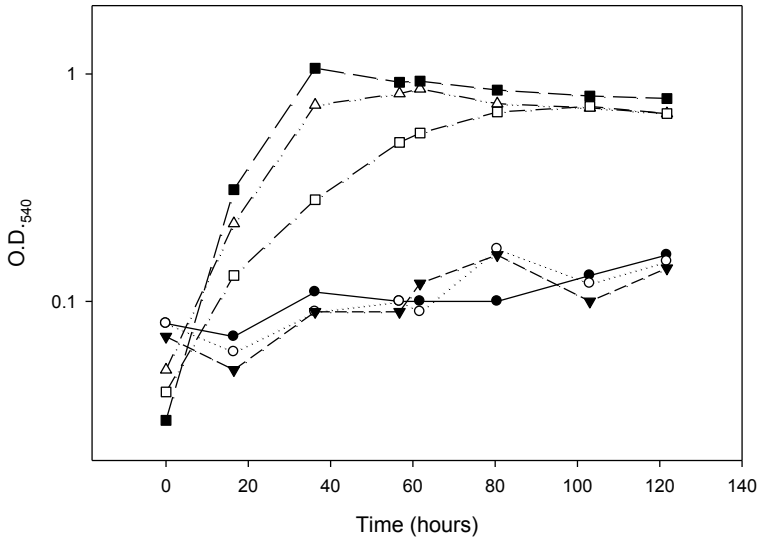
Furfural

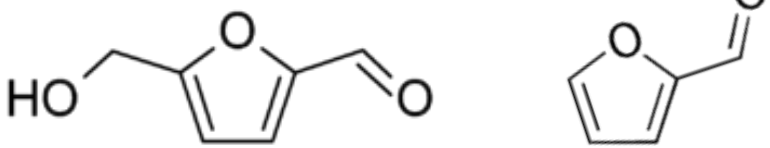


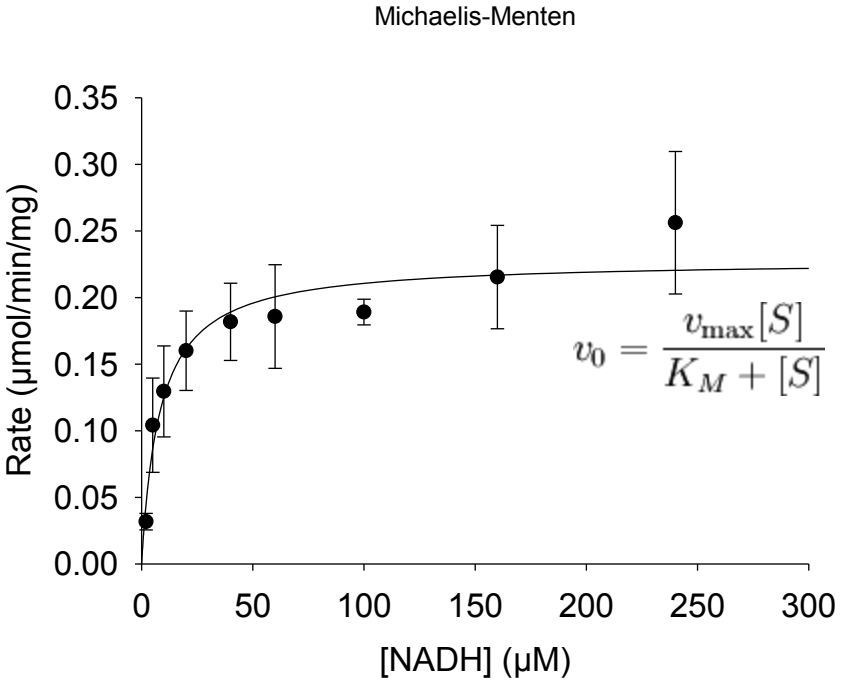


Fermentation Inhibitors: ADH-10 Inactivation Results in Resistance to Furfural & HMF





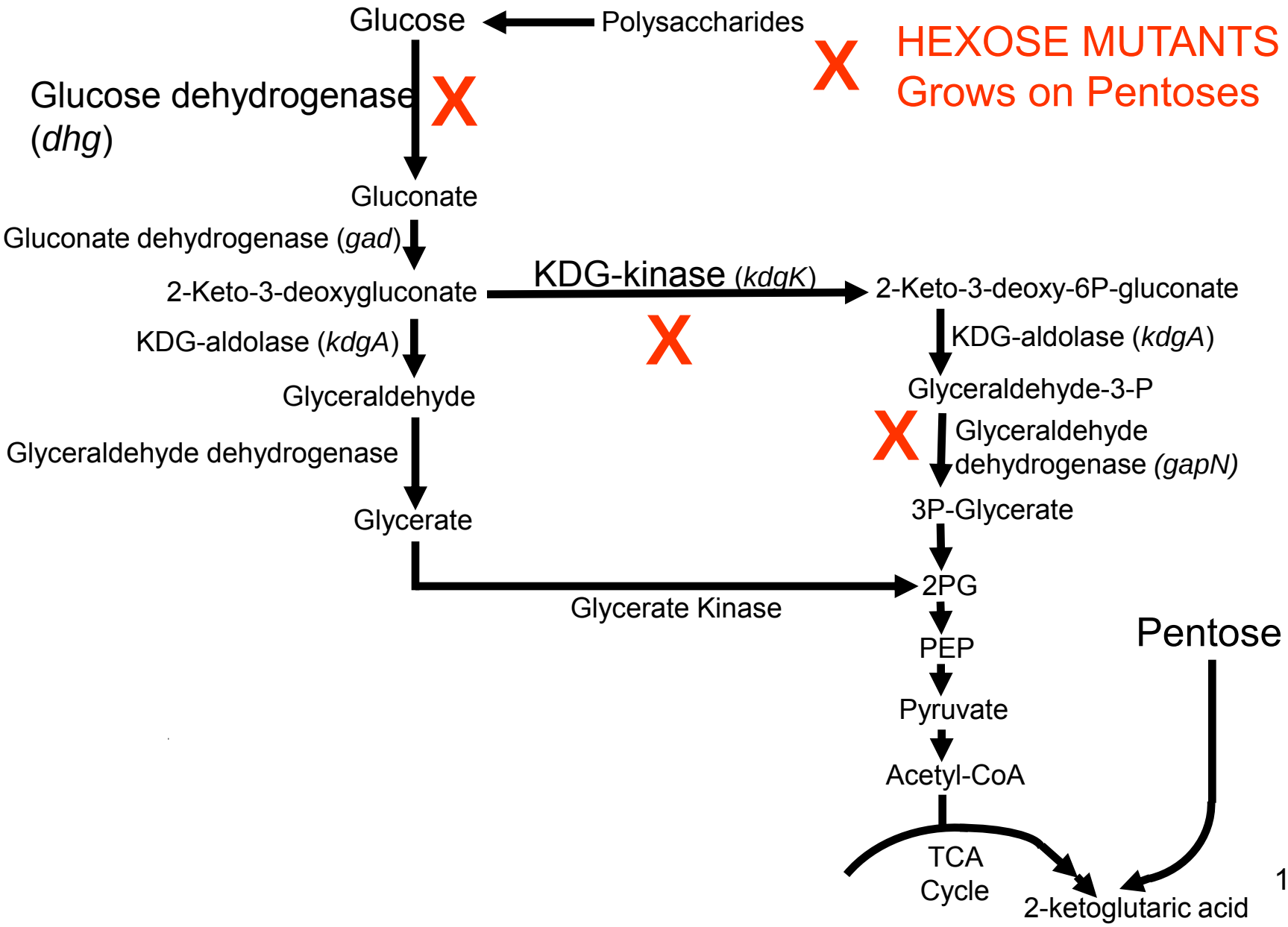




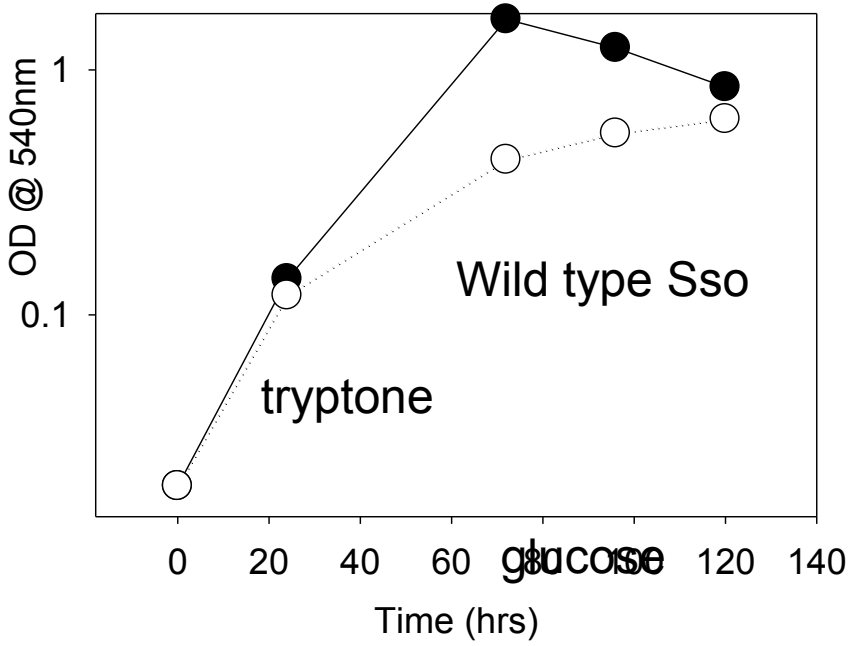
V_{max} = 0.23 ± 0.02 U/mg
K_m = 8.2 ± 3.0 µM
R^2 = 0.9547

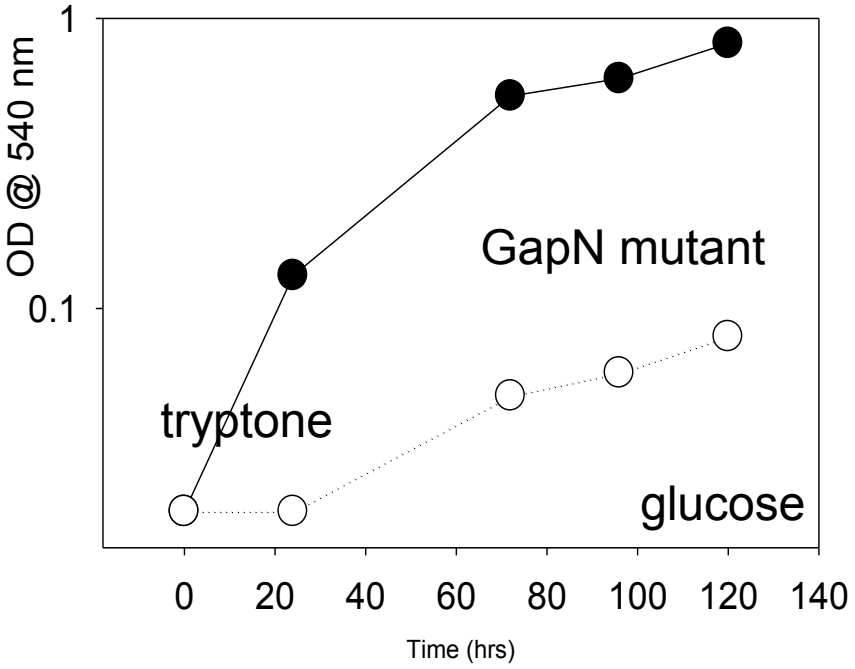
[furfural] = 625 µM (~40K_m)

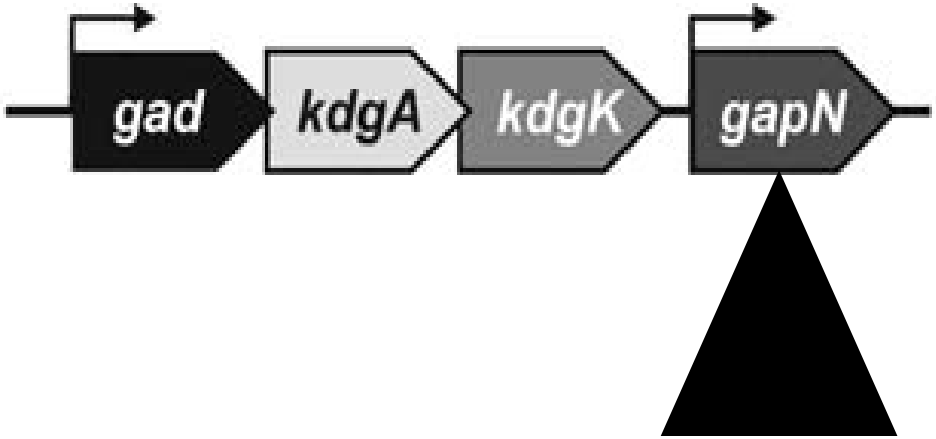
Metabolic Blocks



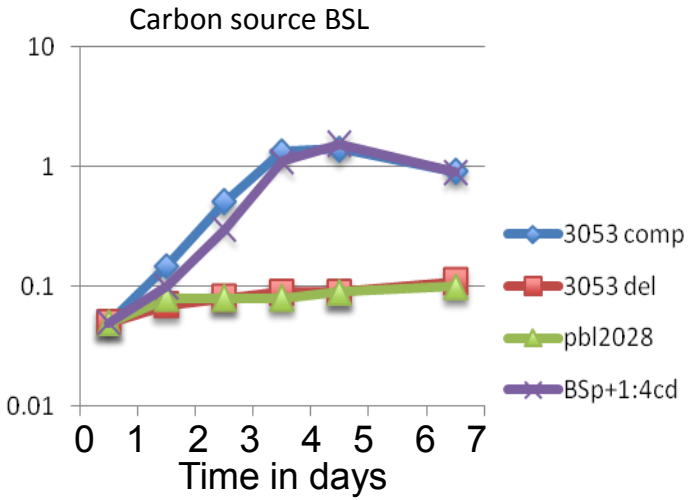
Metbolic Block by Glycolytic Pathway Inactivation



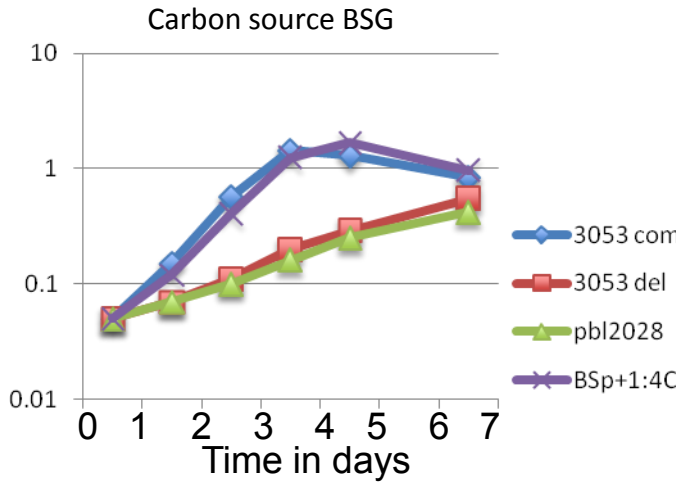


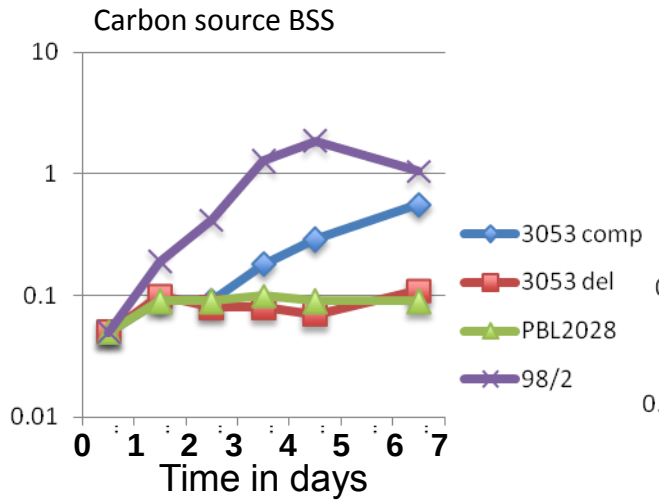


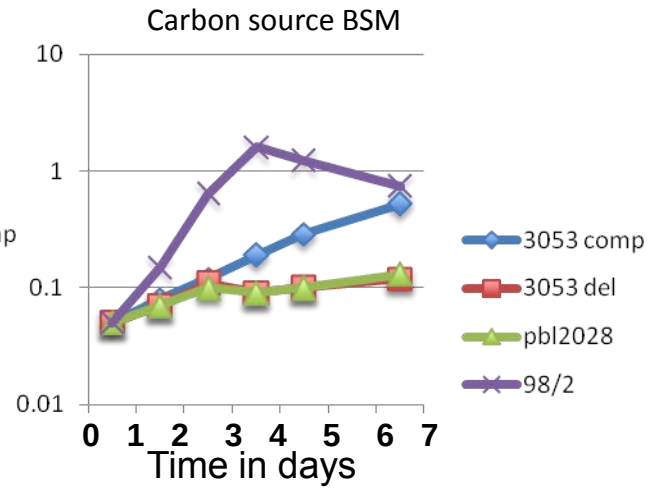
Metabolic Blocks:Transporter Inactivation

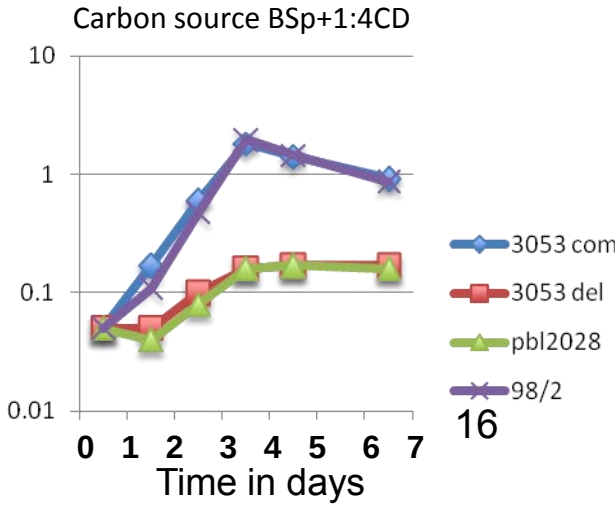


Y-axis: OD @ 540nm
BSL-Lactose
BSG-Glucose
BSS-Starch
BSM-Maltose
CD – cellodextrins mix



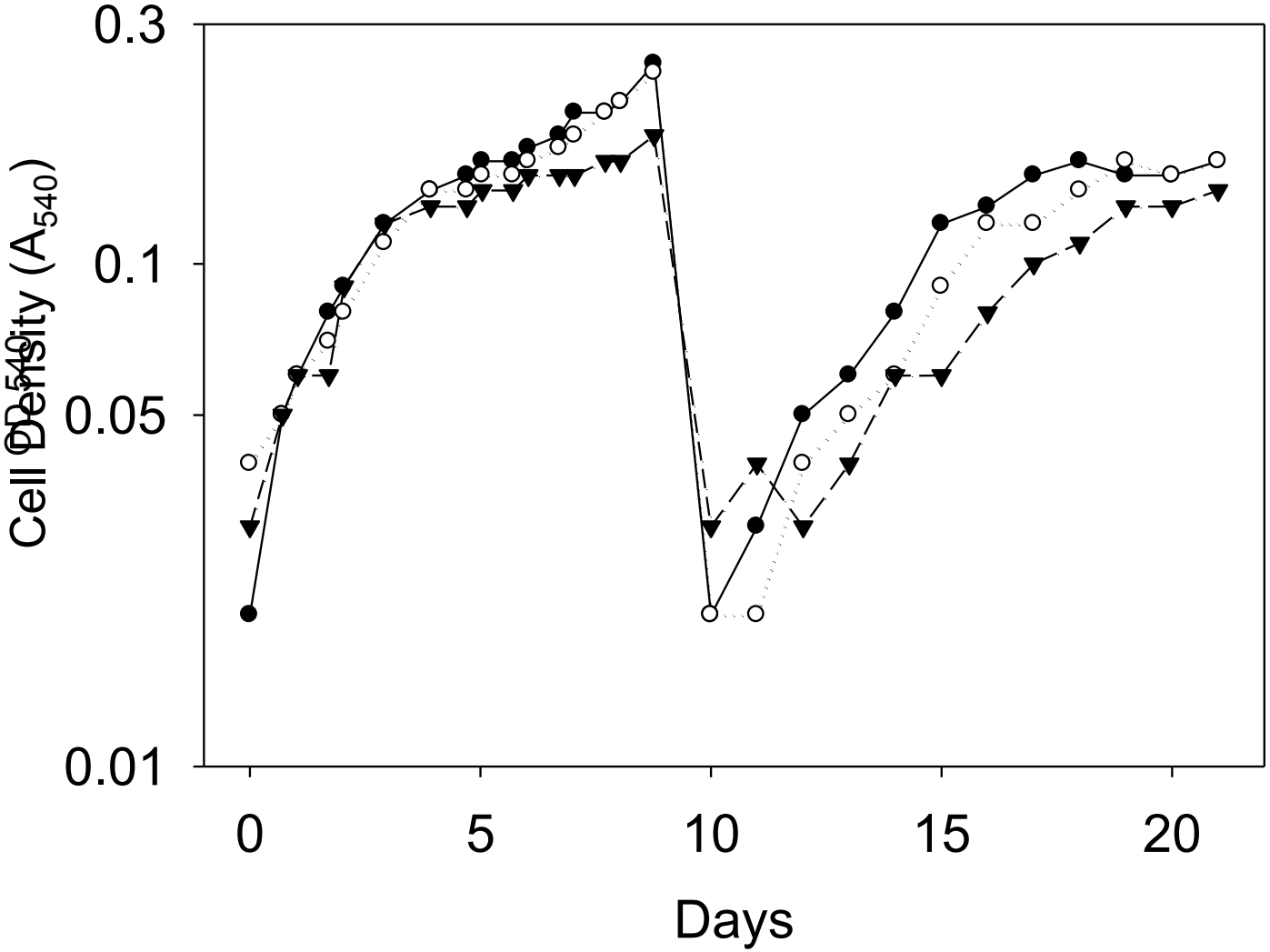




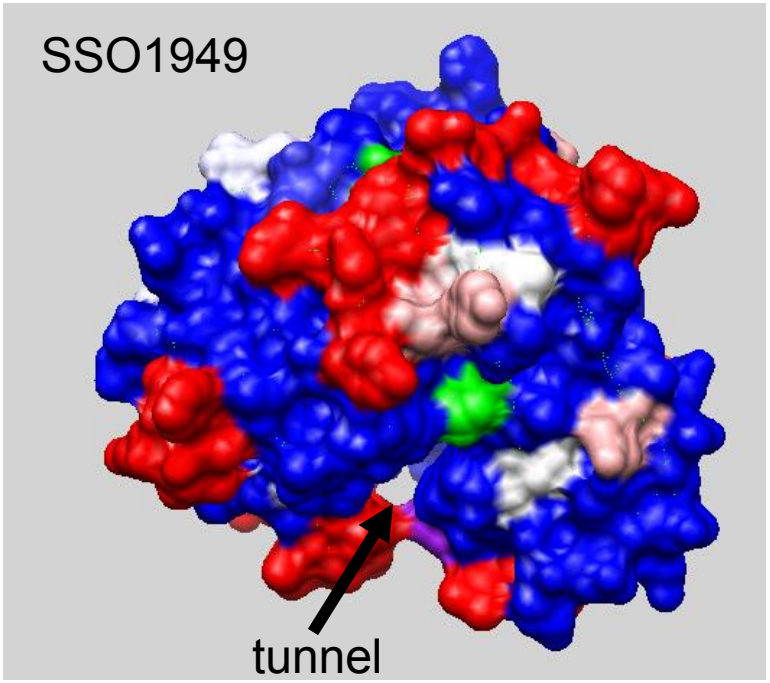


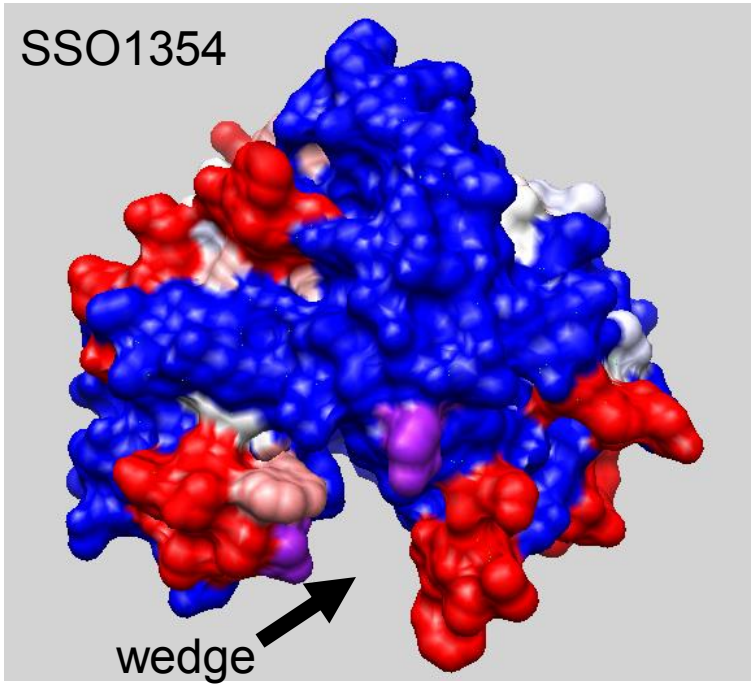
Growth of unadapted wild type S. solfataricus and

s.

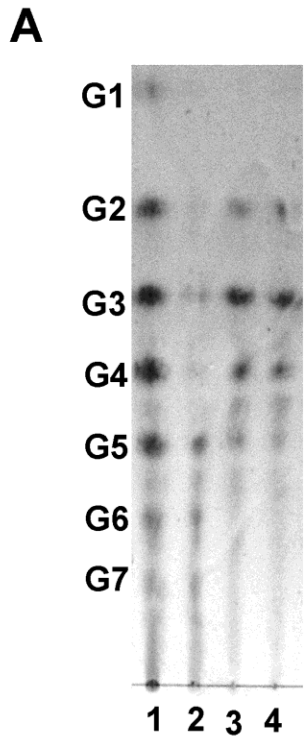


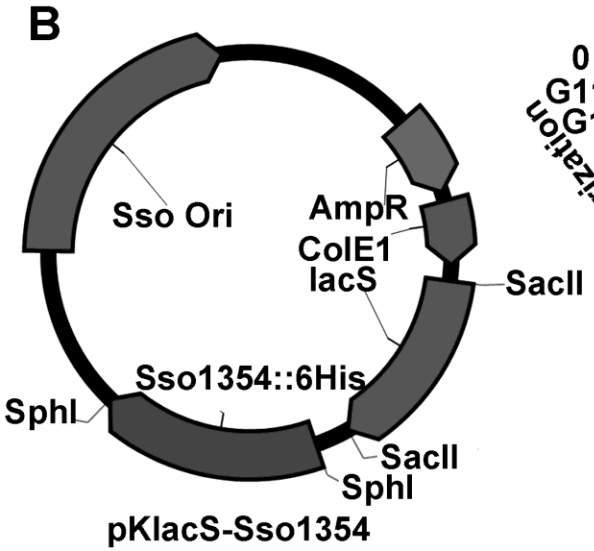
Saccharolytic Enzymes: Hot Acid Stable

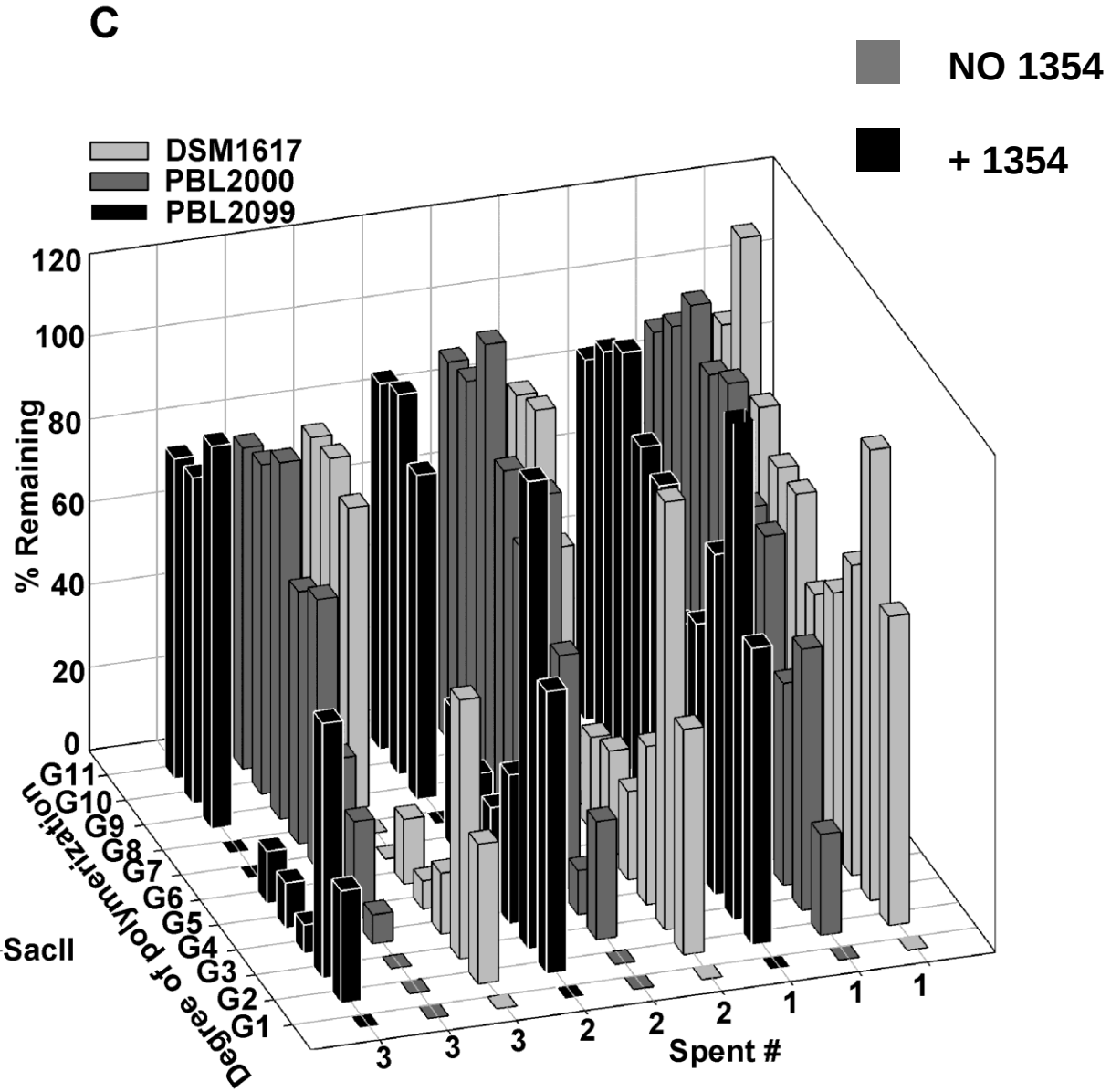




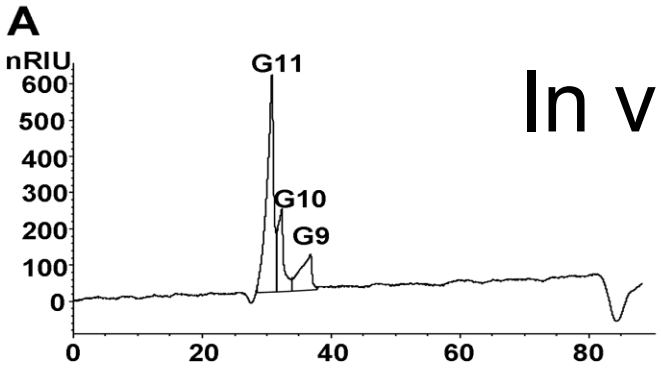
In vivo activity of Sso1354

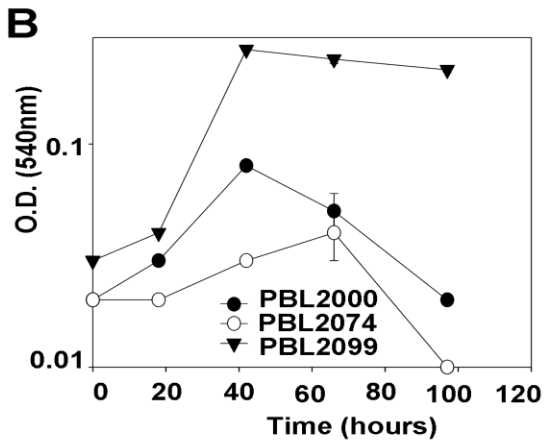


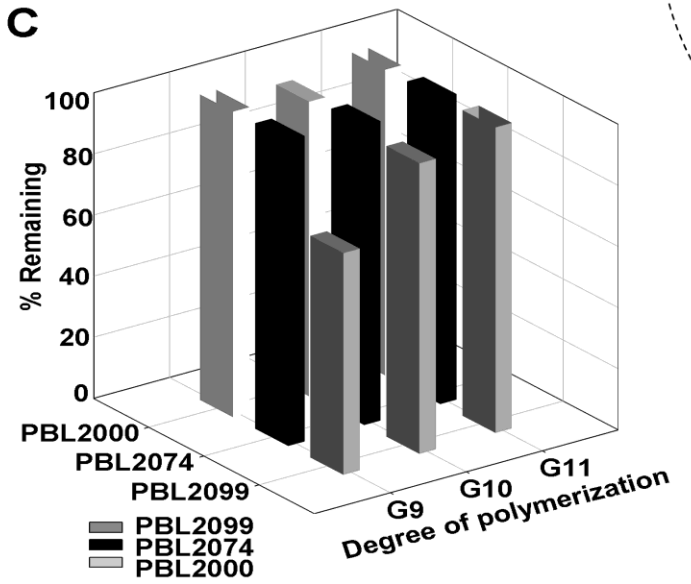


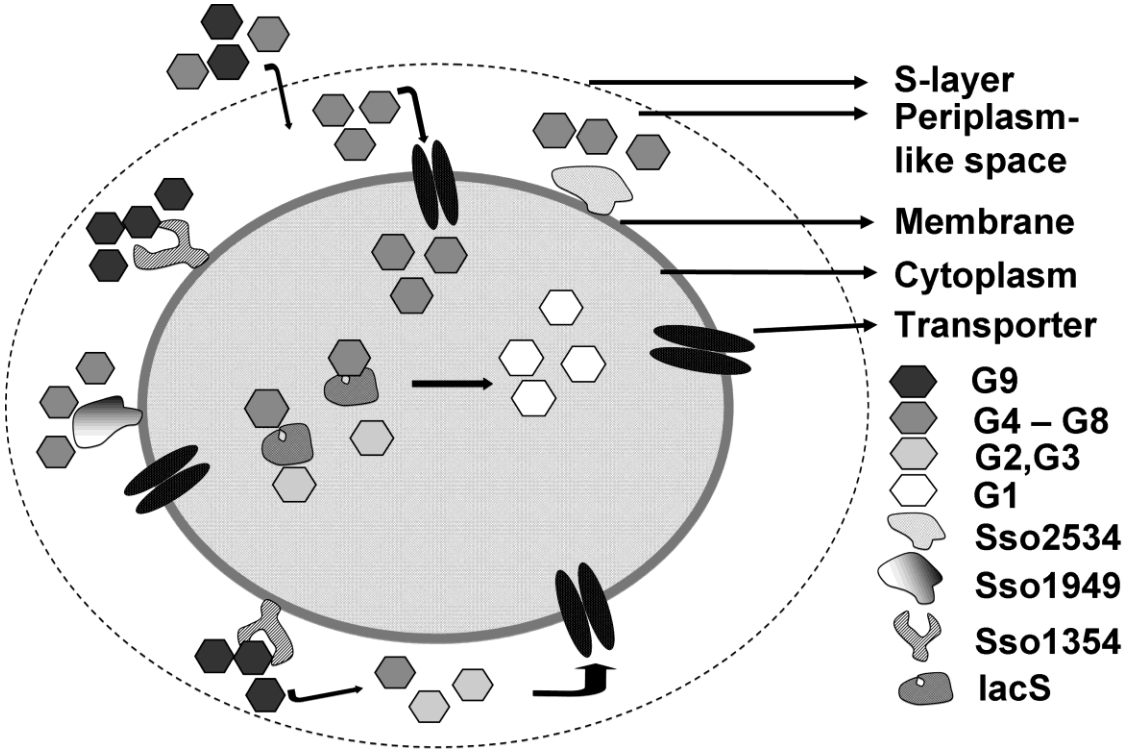


In vivo activity of Sso1354

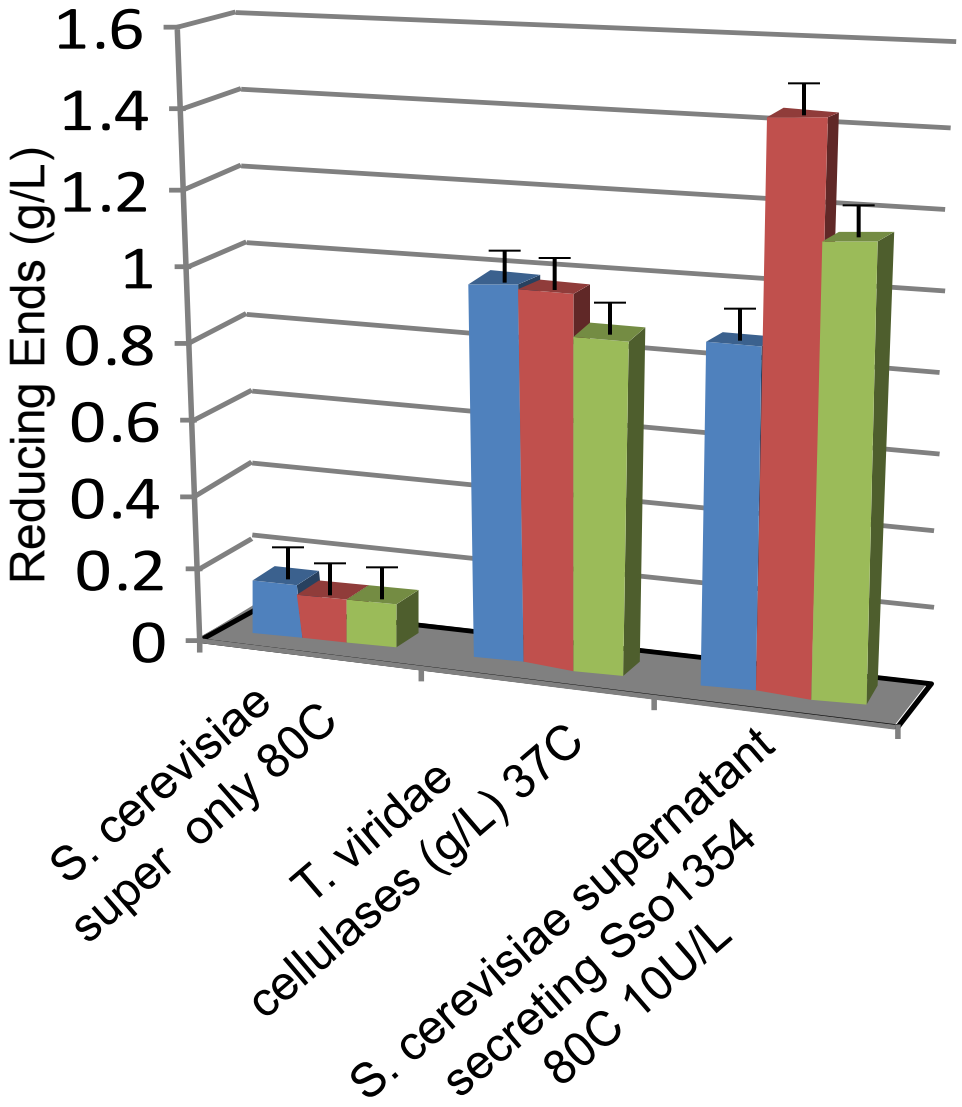








Heterologous Sso1354 Stover Hydrolysis



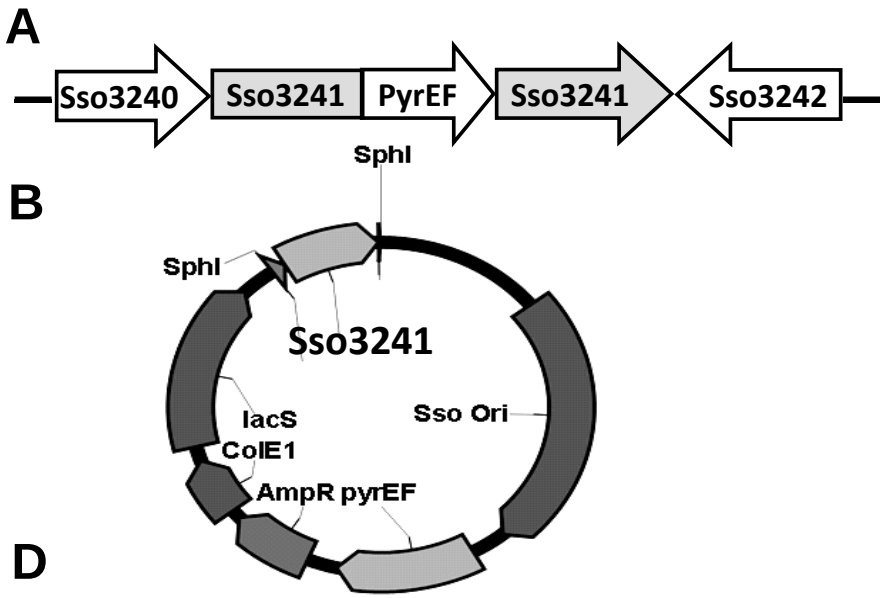
Corn stover B52 % (w/v)

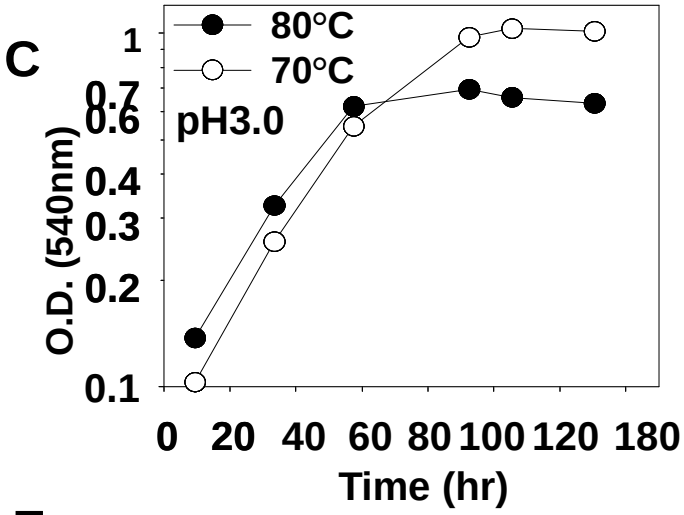
- 0.5
- 0.05
- 0.005

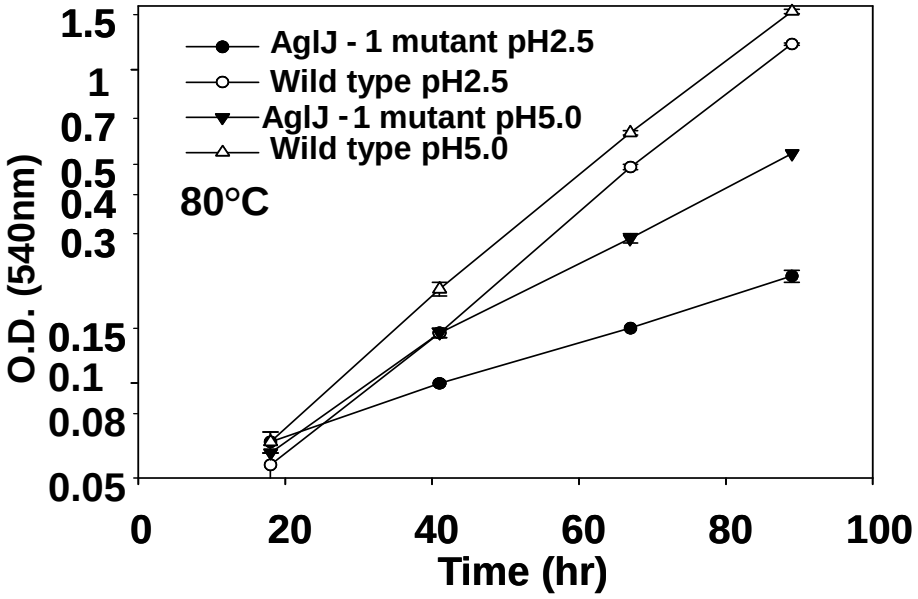
Pretreatment (pH 3, 120 PSI, 10 min)

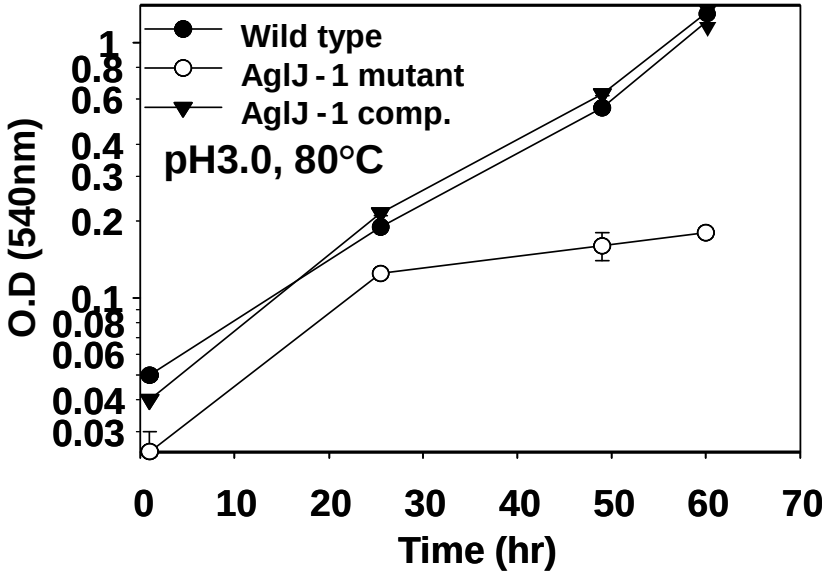
6 hr incubation under indicated conditions

Platform for Engineering Hot Acid Resistance









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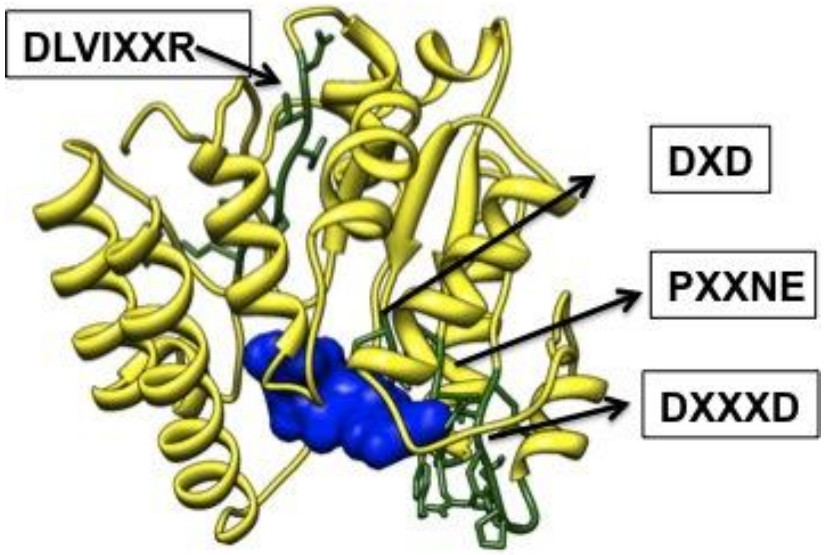


Endoglucanase

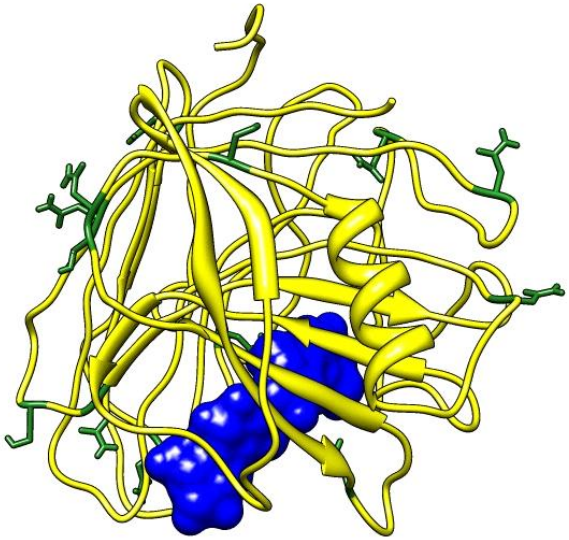
N-linked

Position	Sequon	1	2
98	NMW		
101	NAK		
104	NYN		
114	NPL		
155	NIF		
163	NMT		
174	NLT		
183	NFD		
273	NGY		
297	NYY		

O-linked

Position	1	2
S-181		
S-253		
T-272		
S-277		

Variation
No variation
Not modified



3 - Relevance

- **Publications:**
- Renewable Energy Global Innovations selected our recent publication Tevatia 2012 (see below) for summary presentation in their next series on the Energy Sector.
- Lalithambika S, Peterson L, Dana K, Blum P. 2012. Carbohydrate Hydrolysis and Transport in the Extreme Thermoacidophile Sulfolobus solfataricus. Appl. Environ. Microbiol. 78(22):7931-8.
- Maezato Y., Blum P. 2012. Survival of the Fittest: Overcoming Oxidative Stress at the Extremes of Acid, Heat and Metal. Life. 2(3):229-242.
- Tevatia, R., Y. Demirel, and P. Blum. 2012. Kinetic modeling of photoautotropic growth and neutral lipid accumulation in terms of ammonium concentration in Chlamydomonas reinhardtii. Bioresource Technology 119:419-424.
- Maezato, Y., K. Dana and P. Blum. 2011. Engineering Thermoacidophilic Archaea Using Linear DNA Recombination. Methods Mol Biol. 765:435-45.
- Friest. J. A., Y. Maezato, S. Broussy, P. Blum and D. B. Berkowitz. 2010. Use of a Robust Dehydrogenase from an Archael Hyperthermophile in Asymmetric Catalysis-Dynamic Reductive Kinetic Resolution Entry into (S)-Profens. J. Am. Chem. Soc. Apr 8. [Epub ahead of print].
- Chemical & Engineering News, 88(16), April 19, 2010 summarizes the process chemistry implications of Friest et al., 2010, arising from the use of hyperthermophilic enzymes from thermoacidophilic microbes for asymmetric synthesis.
- Miller PS, Blum PH. 2010. Extremophile-inspired strategies for enzymatic biomass saccharification. Environ Technol. 31(8-9):1005-15.

4 - Critical Success Factors

- Inquiries from cellulosic ethanol producers, grain ethanol producers, plant biotechnology companies, and enzyme producers
- Primary Challenges are to integrate all aspects of the technology into a single system.
- Technoeconomic analysis using ASPEN batch mode indicates savings at multiple stages of process development.

5. Future Work

- Through project completion, will work on integrating aspects of project technology.
- Refine technoeconomic implications.
- Search for partners to achieve process scale.

Gannt Chart

	PROJECT GO88055	BUDGET YEAR 5			
TASK	TOPIC				
		17	18	19	20
A.	**Screening thermoacidophilic taxa.**				
A.1	Single taxa				
A.1.1.	Assessment of action				
A.2	Community samples				
A.2.1	Assessment of action				
A.3	Community optimization				
A.3.1	Assessment of action				
A.4	Post-secondary fermentation and ethanol yields				
B.	**Inhibitors**				
B.1	Detection of lignocellulose deconvolution inhibitors				
B.2	Inhibitor intervention				
B.2.1	Detection of furfural and hydroxymethal furfural				
B.2.2	Inhibitor remediation				
B.3	Post-secondary fermentation and ethanol yields				
C.	**Sugar flux channeling and catabolic blocks.**				
C.1	Assessment of hydrolytic products				
C.2	Metabolic blocks for hexoses				
C.2.1	Imposition of hexose blocks				
C.2.2.	Assessment of hexose block				
C.3	Metabolic blocks for pentose				
C.3.1	Imposition of pentose blocks				
C.3.2	Assessment of pentose block				
C.4	Post-secondary fermentation and ethanol yields				
D.	**Addition of deconvolution traits.**				
D.1	Cellulases				
D.1.1	Heterologous expression of cellulose-binding cellulases				
D.1.2	Assessment on product formation				
D.2	Xylanases				
D.2.1	Induction of endogenous xylanases and assessment				
D.2.2	Heterologous expression of xylanases and assessment				
D.3	Post-secondary fermentation and ethanol yields				
H.	**Bioenergy Facility**				

Summary

- Consolidated bioprocessing with thermoacidophiles is possible and worth exploring further.
- Primary technical accomplishments include converting an extremophile into an industrial organism.
- Key relevance is the ability to achieve process simplifcation and cost reduction by integrating deconvolution and saccharification. This may also minimize costs associated with neutralization and water.
- Future objectives are project Integration and scale up.
- Technology transfer: IP available for licensing.