A novel strategy for fermentation of C5 and C6 sugars with native yeast

May 22, 2013

Biochemical Platform Review

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University of Toledo

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Goal Statement

- Goal
  - To develop cost-effective methods of increasing utilization of C5 and C6 sugars by <u>native</u> yeast in the conversion of lignocellulosic biomass to ethanol
- Alignment with DOE-BETO and industry goals and objectives
  - Efficient utilization of C5 sugars essential for ethanol process economics
    - these sugars are a major portion of sugars present in lignocellulosic biomass
  - Ability to employ proven native yeast from corn ethanol industry allows seamless transition from corn to cellulosic ethanol

Quad Chart Overview

Timeline

- Start date: 09/30/2008
- End date: 06/30/2013
- Percent complete: 95% (funds released in Feb. 2009)

Budget

- Total Project: $788,875
  - DOE: $499,784
  - UT: $289,091
- FY 11 funding: $ 99,652/$80,622
- FY 12 funding: $ 106,725/$21,555
- FY 13 funding: $ 165,694/$73,691
- No ARRA funding

Barriers

- Bt-J: Fuels Organism Development

Partners

- UT is coordinating research activities
- IP is licensed to SuGanit Systems, Inc.; SuGanit scale-up efforts are not part of this project

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NOTICE OF ALLOWANCE AND FEE(S) DUE

4859 7590 04/25/2013
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720 WATER STREET
TOLEDO, OH 43604-1619

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EXAMINER	
PYLA, EVELYN Y	
ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 04/25/2013

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/811,288	07/23/2010	Sasidhar Varanasi	1-29786	4864

TITLE OF INVENTION: Methods for Fermentation of Xylose and Hexose Sugars

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$890	$300	$0	$1190	07/25/2013

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

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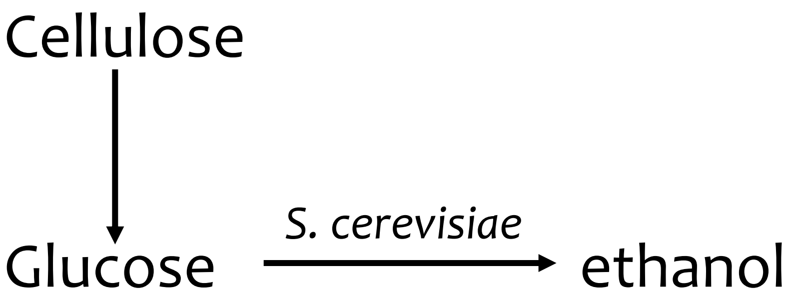
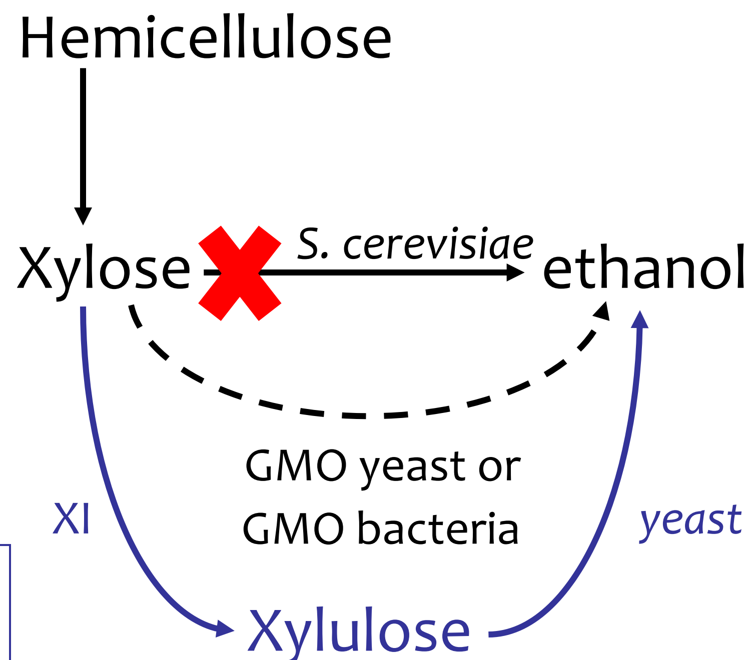
Project Overview
Our strategy for fermentation of sugars to ethanol





Advantages
- Control over the isomerization step

Issues with implementation
- Unfavorable xylose/xylulose equilibrium
- XI enzyme recovery and reuse

1. Approach

Strategy 1*: SIF in presence of soluble ketose binding agents

	Advantages	Special requirements
1a. Unfiltered hydrolysates	• In addition to SIF, also permits SSI • Hence, SSF becomes feasible	• Co-immobilized urease-and-XI pellets for isomerization at fermentation pH
1b. Filtered hydrolysates	• Permits SIF while allowing: • Recovery and reuse of the co-immobilized enzyme pellets • Recovery of lignin	• In addition to 1a, non-traditional fermentor configuration

Strategy 2**: Sequential I & F with immobilized ketose binding agents

Advantages	Special requirements
• Off-the-shelf XI/no co-immobilization with urease • Recovery and reuse of XI and ketose binding agent • Concentrate and purify xylulose	• pH adjustment between isomerization and fermentation

* S. Varanasi, K. Rao, P. Relue, and D. Yuan, “Methods for Fermentation of Xylose and Hexose Sugars,” US utility patent application PCT/US09/30033 filed Jan. 2, 2009.

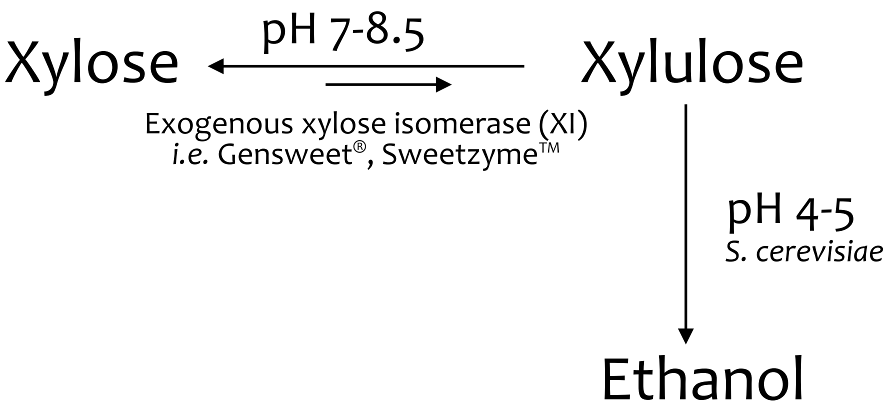
** Bin, Li, S. Varanasi, and Patricia Relue, “Aldose-Ketose Transformation for Separation and/or Chemical Conversion of C6 and C5 Sugars from Lignocellulosic Biomass Hydrolyzate, US Provisional Patent filed, Ser No:61/325,710, 04/2010

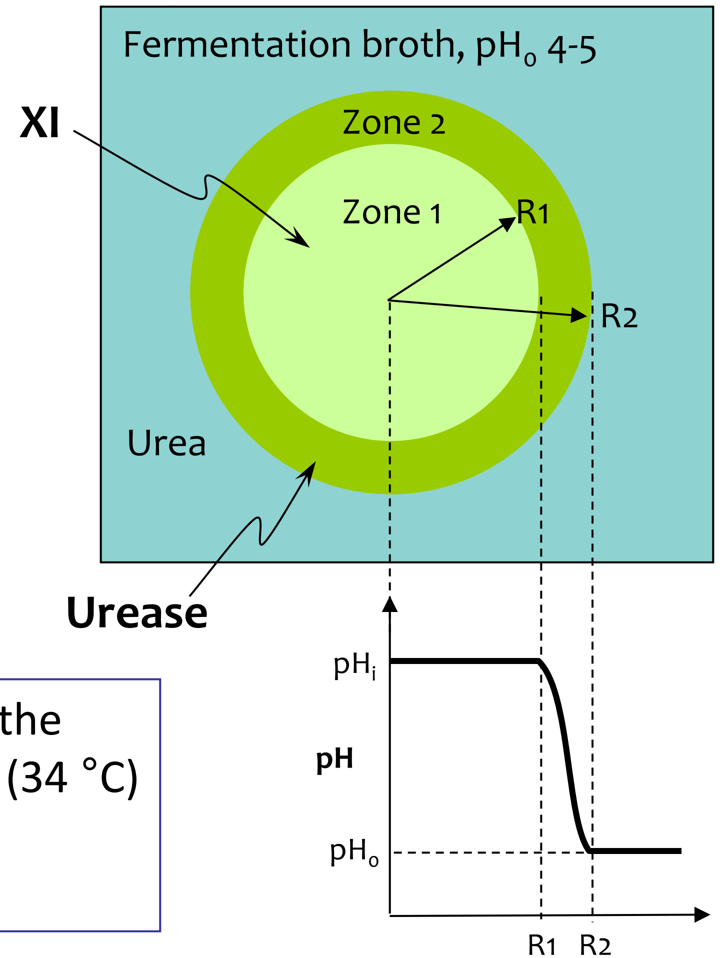
Project Approach (con't)
Milestones

- A: Co-immobilized enzyme pellet production
- B: Design of reactors
  - Packed bed immobilized enzyme pellet reactor designed & performance optimized
  - Fermentation modules designed and tested for SIF
- C: SSF with co-immobilized enzyme pellets and native yeast
- D: New ketose binding agents
  - Identify and test their performance
  - Develop strategies for their recovery and reuse

2. Technical Accomplishments/Results
Strategy 1: Simultaneous isomerization and fermentation (SIF)

Method for sustaining two pH microenvironments in a single vessel



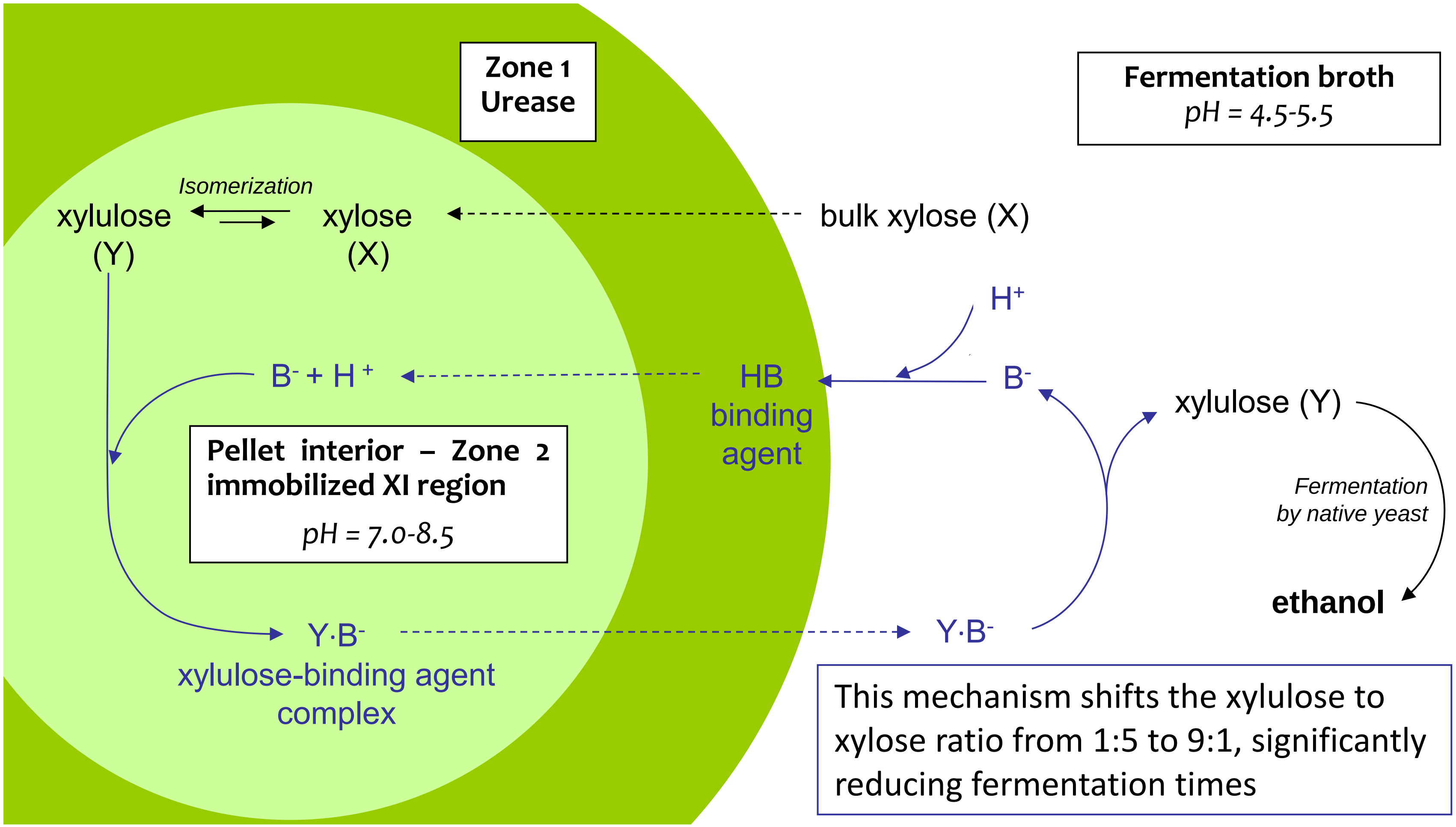


- While SIF drives the isomerization forward, the xylulose:xylose ratio is never greater than 1:5 (34 °C)
- Low xylulose concentration results in long fermentation times

S. Varanasi, K. Rao, P. Relue, and D. Yuan, "Methods for Fermentation of Xylose and Hexose Sugars," US utility patent application PCT/US09/30033 filed Jan. 2, 2009.

2. Technical Accomplishments/Results (con't)
Strategy 1: Simultaneous isomerization and fermentation (SIF)

Enhancing conversion of xylose to xylulose in co-immobilized enzyme pellets via addition of a soluble ketose binding agent (HB)

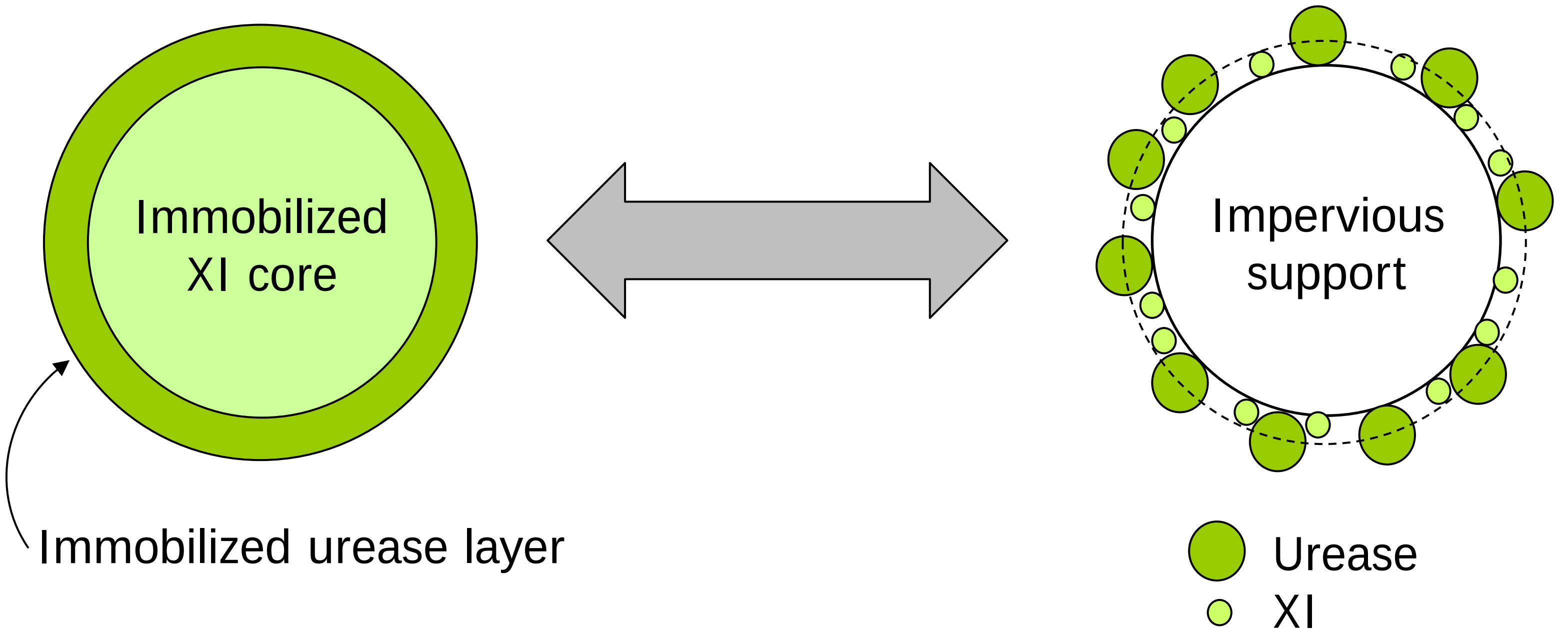


This mechanism shifts the xylulose to xylose ratio from 1:5 to 9:1, significantly reducing fermentation times

K. Rao; S. Chelikani; P. Relue; and. S. Varanasi, "A Novel technique for Optimizing the Simultaneous-Isomerization-and-Fermentation (SIF) Approach of Converting Xylose to Ethanol," *Applied Biochemistry and Biotechnology*, 146(1-3):101-117, 2008.

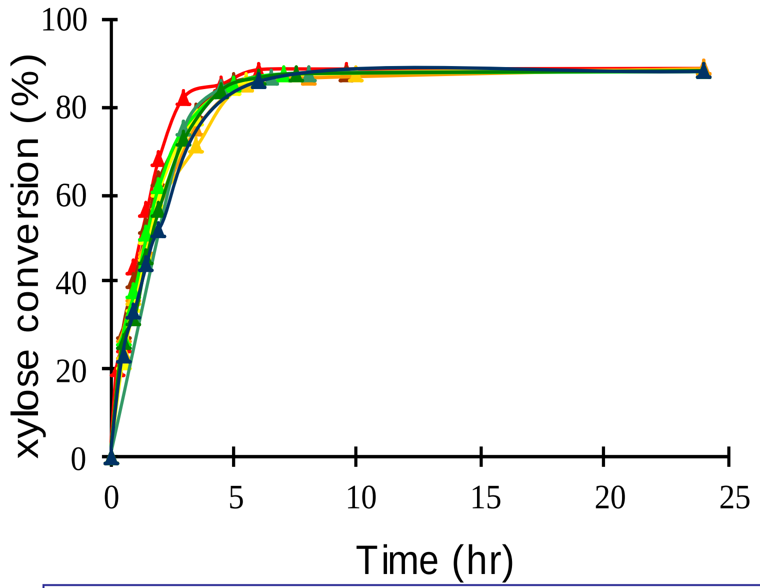
Bi-layered versus co-immobilized enzyme pellets

- Co-immobilized enzyme pellets can be made using commercial functionalized supports, much simpler to prepare
- Both methods of immobilization sustain the two-pH microenvironments

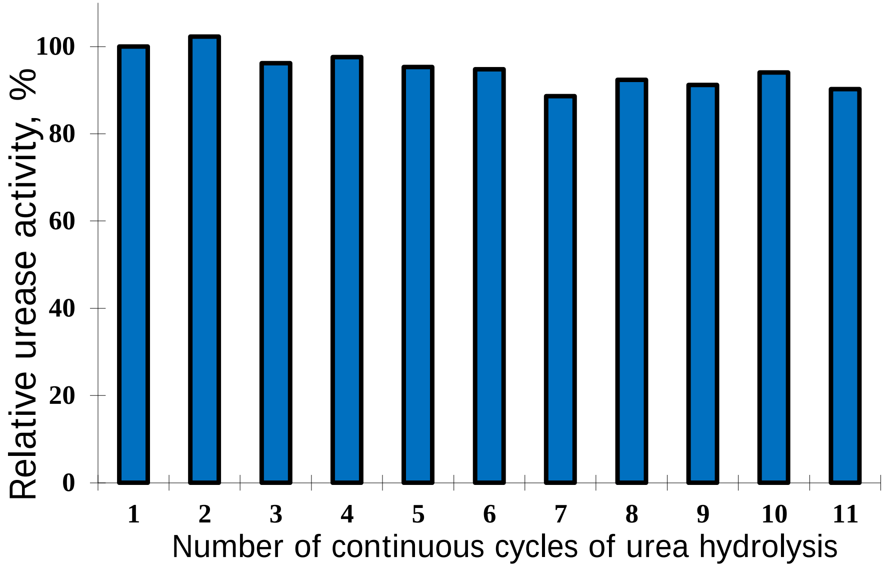


2. Technical Accomplishments/Results (con't)
Strategy 1: Biocatalyst development (Milestone A)

- We identified and tested 3 commercial functionalized supports (Eupergit® 250c, Sepabeads® HA, and Sepabeads® EP) for XI and urease immobilization
- We found good activity of both enzymes when immobilized on Sepabeads EP.



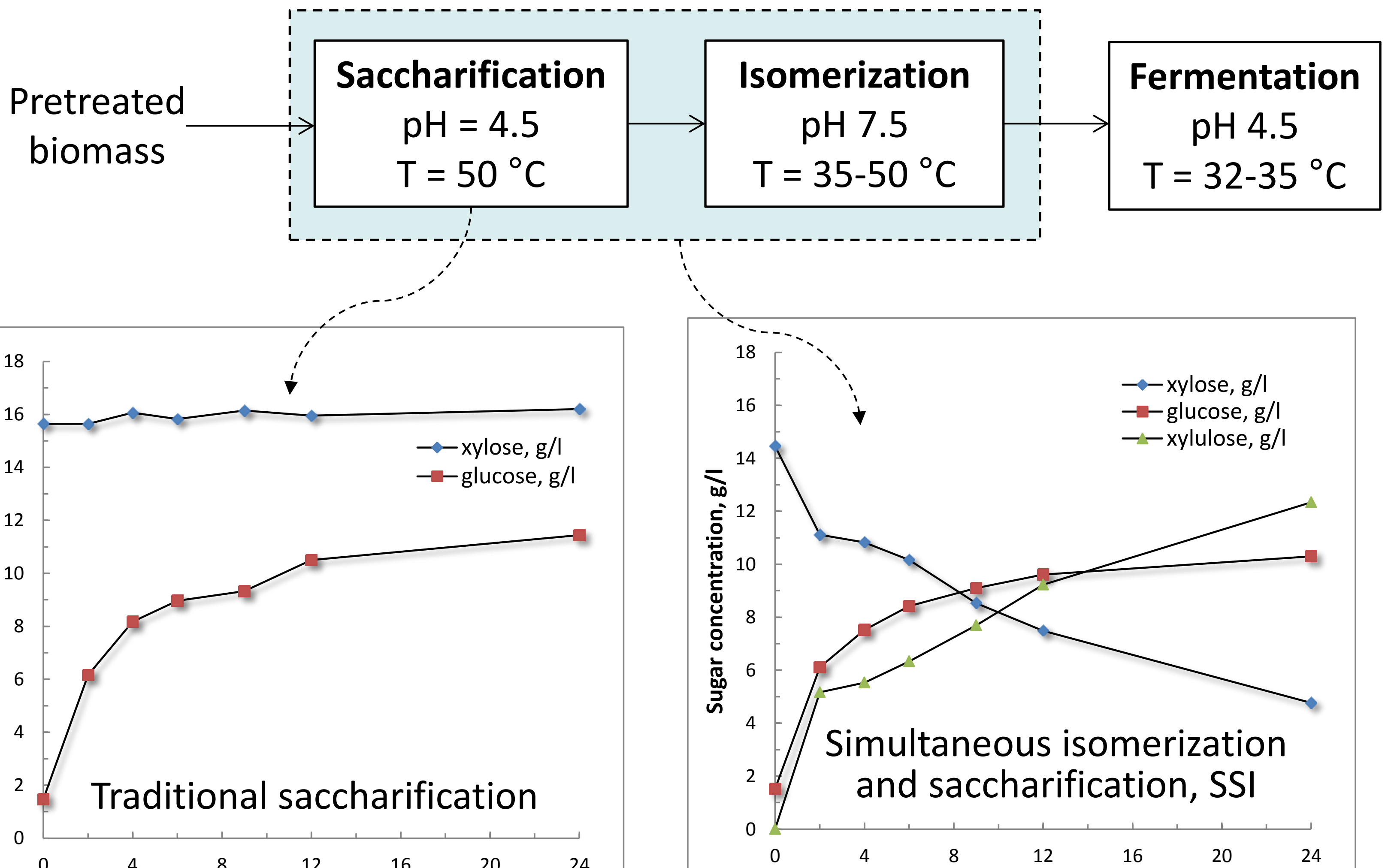
Immobilized XI active after 14 days of continuous xylose isomerization, pH 7.5



Immobilized urease activity for repeated cycles of urea hydrolysis, pH 7.5

2. Technical Accomplishments/Results (con't)

Strategy 1a: Unfiltered hydrolysate and soluble binding agent (SSI) (Milestone C)



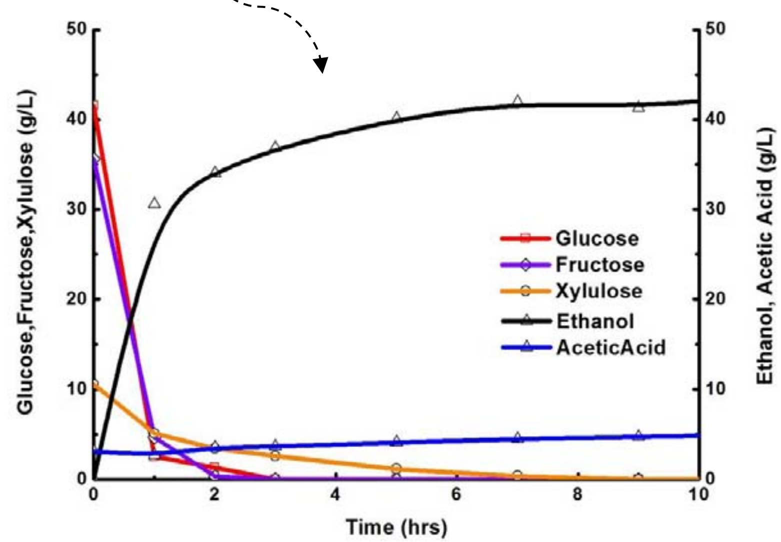
- Dilute acid pretreatment (1% H_2SO_4 with 7.5% solids loading at 120 °C for 60 min)
- Pretreated biomass saccharified using standard enzyme cocktail after pH adjustment (citrate buffer @pH 4.5)
- Simultaneous isomerization is effected by adding co-immobilized XI pellets, urea, and borax

2. Technical Accomplishments/Results (con't)

Strategy 1a: Unfiltered hydrolysate and soluble binding agent (SSF) (Milestone C)

Ionic-liquid Pretreated biomass → **Saccharification** pH = 4.5, T = 50 °C → **Isomerization** pH 7.5, T = 35-50 °C → **Fermentation** pH 4.5, T = 32-35 °C

- We successfully demonstrated that SSF is feasible with the co-immobilized enzyme pellets
- An added benefit of isomerization is that cellulase inhibition during saccharification may be aleviated



- Initial sugar concentrations: 77 g/l glucose and 12 g/l xylose
- Ethanol productivity is 0.43 g ethanol/g fermentable sugar

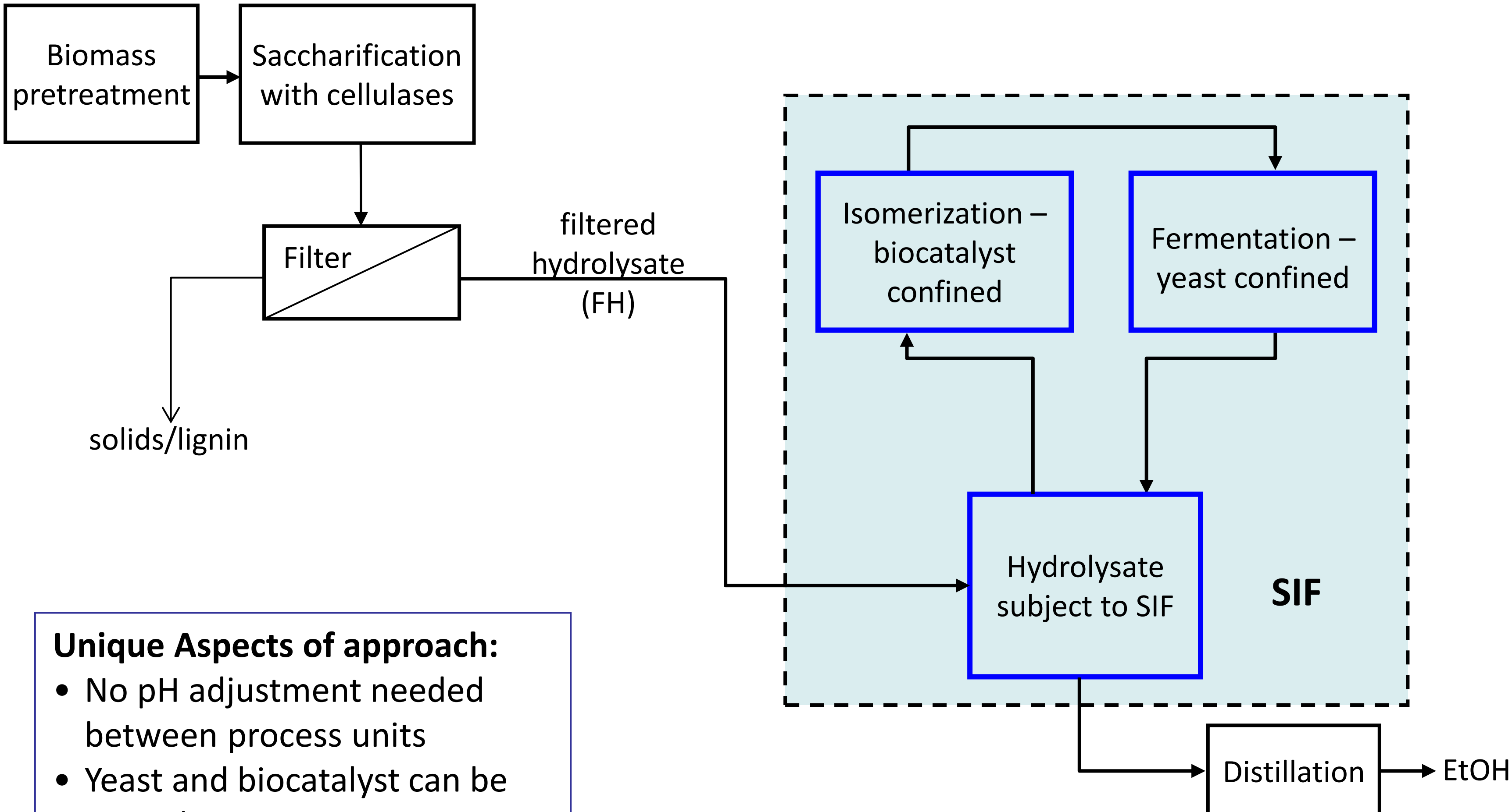
2. Technical Accomplishments/Results

Strategy 1a summary: Unfiltered hydrolysate and soluble binding agent

- SSIF with native yeast was successfully demonstrated for producing ethanol from major lignocellulose sugars
- In this approach, following separation of ethanol, residual is a complex mixture of yeast, lignin, borate, and saccharifying enzymes
- Process economics warrants approaches that are capable of recovering and reusing most of these components

2. Technical Accomplishments/Results (con't)

Strategy 1b: Filtered hydrolysate and soluble binding agent



Unique Aspects of approach:

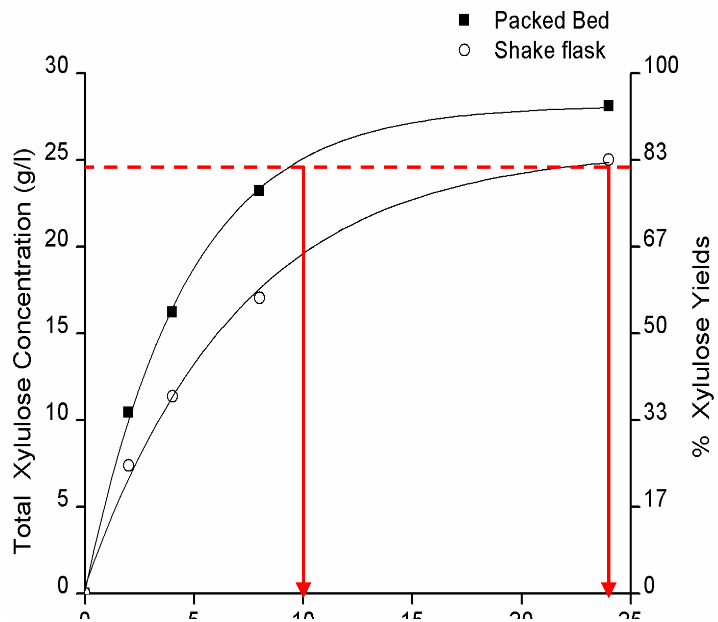
- No pH adjustment needed between process units
- Yeast and biocatalyst can be reused

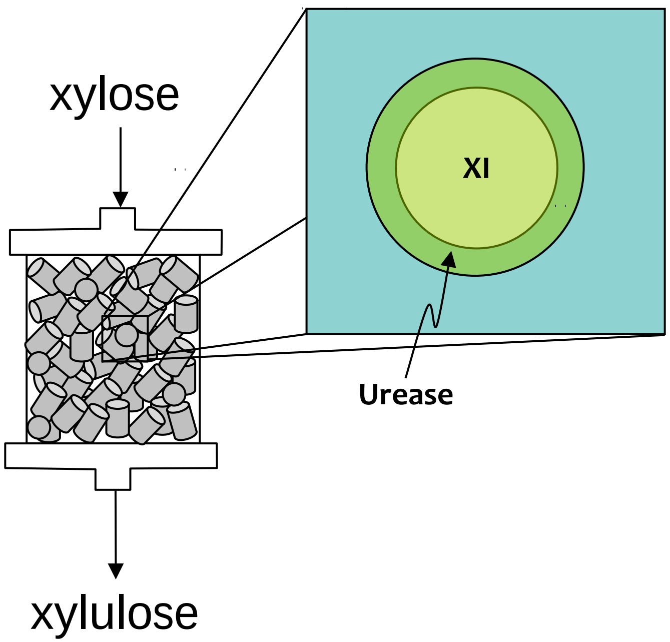
2. Technical Accomplishments/Results (con't)

Strategy 1b: Filtered hydrolysate and soluble binding agent (SIF)

Containment devises for reuse of enzyme pellets (Milestone B)

- Packed bed reactors designed and tested with pellets
  - Designed for ease of recovery and reuse of the pellets in SIF
  - Kinetics are significantly faster than in shake flask due to improved mass transfer (10 hrs vs. 24 hrs for 83% isomerization yield)



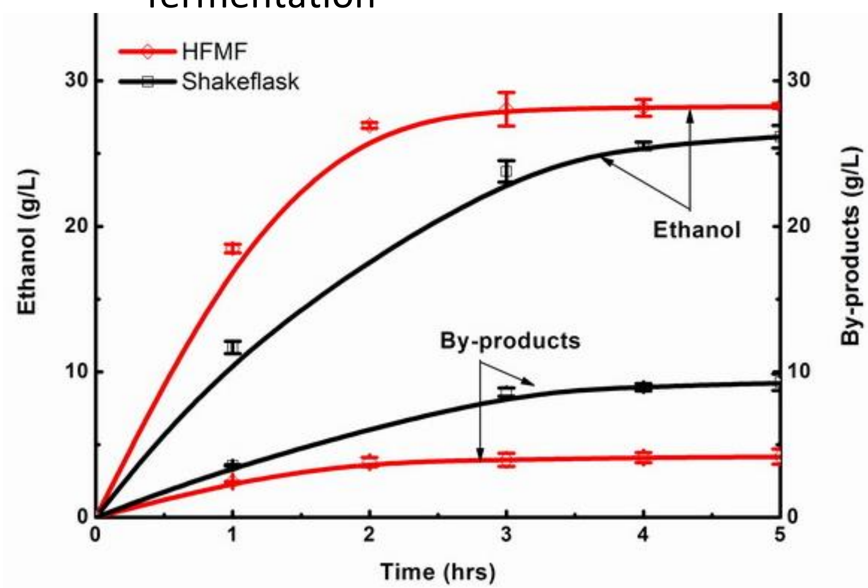


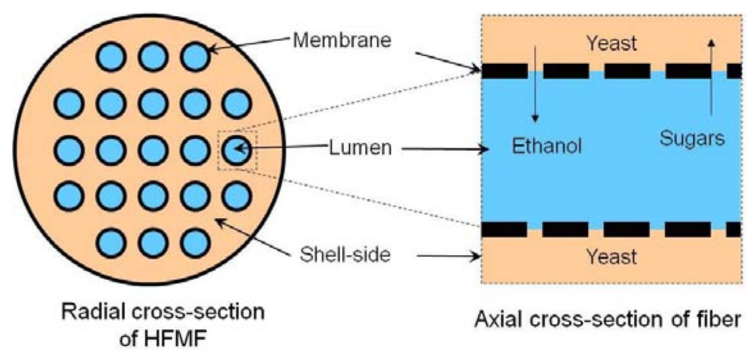
2. Technical Accomplishments/Results (con't)

Strategy 1b: Filtered hydrolysate and soluble binding agent (SIF)

Fermenter design for confinement of yeast (Milestone B)

- HFMF designed for rapid fermentation while preventing direct yeast and pellet contact
  - Enables reuse of yeast and pellets
  - Yeast used for sugar fermentation for up to 7 days with no reduction in yield (longest time tested)
  - Byproduct (glycerol and acetic acid) formation is low as compared to shake flask fermentation

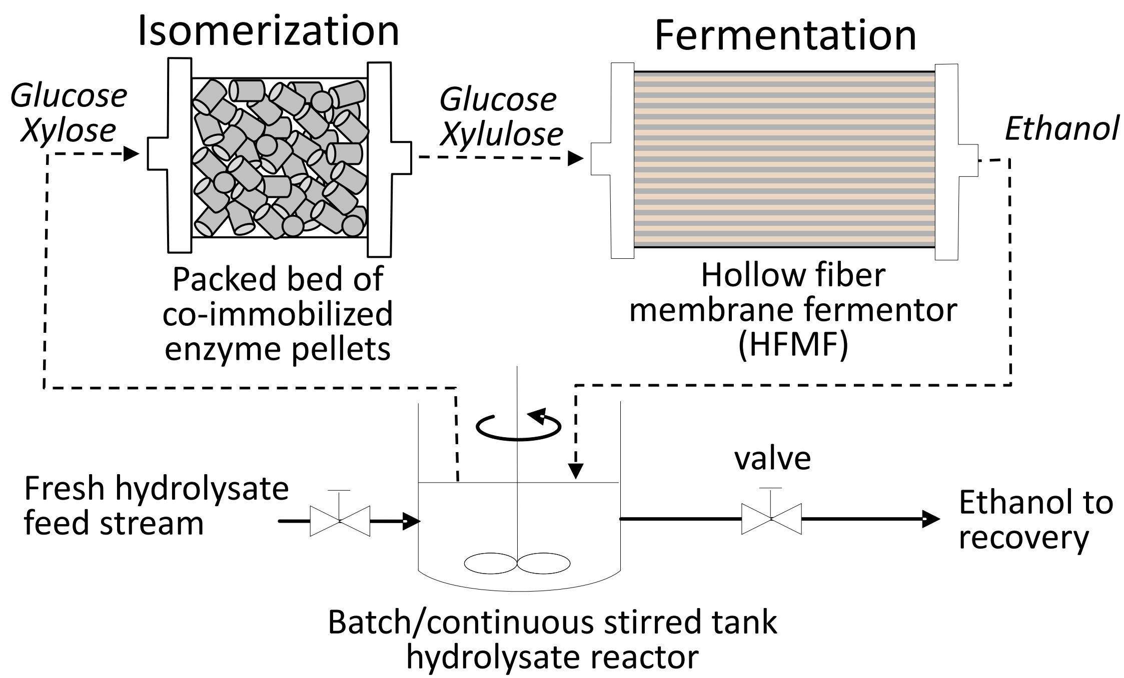




2. Technical Accomplishments/Results (con't)

Strategy 1b: Filtered hydrolysate and soluble binding agent (SIF)

SIF of filtered hydrolysate (Milestone B)



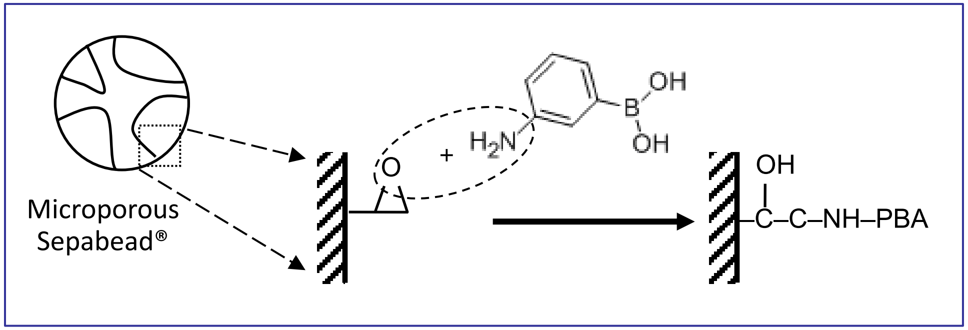
Mode of Operation (all data is at 24 hrs)	Ethanol (g/l)	Ethanol yield (g ethanol/ g sugar)	Sugar utilization (60 g G+30 g X)	Xylitol (g/l)	Glycerol (g/l)	Acetic Acid (g/l)
No isomerization of xylose	30.93	0.34	84%	2.75	5.32	1.94
SIF	37.22	0.41	84%	2.07	5.32	1.95
Pre-isomerization of xylose (to 50%) followed by SIF	41.22	0.46	98%	2.61	4.92	1.94

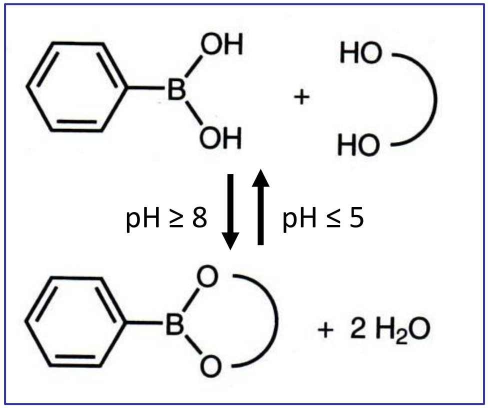
2. Technical Accomplishments/Results (con't)

Strategy 2: Sequential I & F with immobilized binding agents

Evaluation and immobilization of ketose binding agents (Milestone D)

- Economics/environmental concerns require recovery and reuse of the binding agent
- Suitable complexing agents were borate, germanate, phenylboronic acid (PBA), 3-amino-PBA, 4-carboxy-PBA, and Amino-Napthalene 2-boronic acid (N2B)
- The 3aPBA, 4cPBA and aN2B are easily immobilized to solid supports

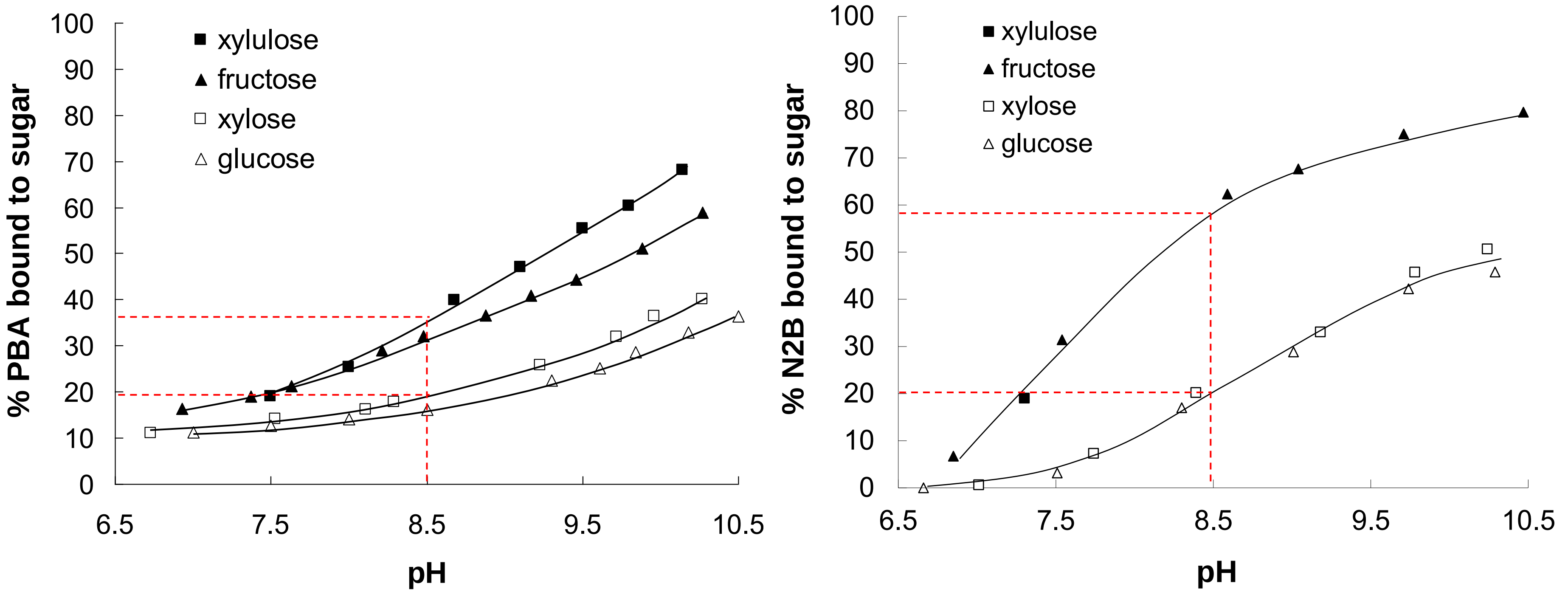




2. Technical Accomplishments/Results (con't)

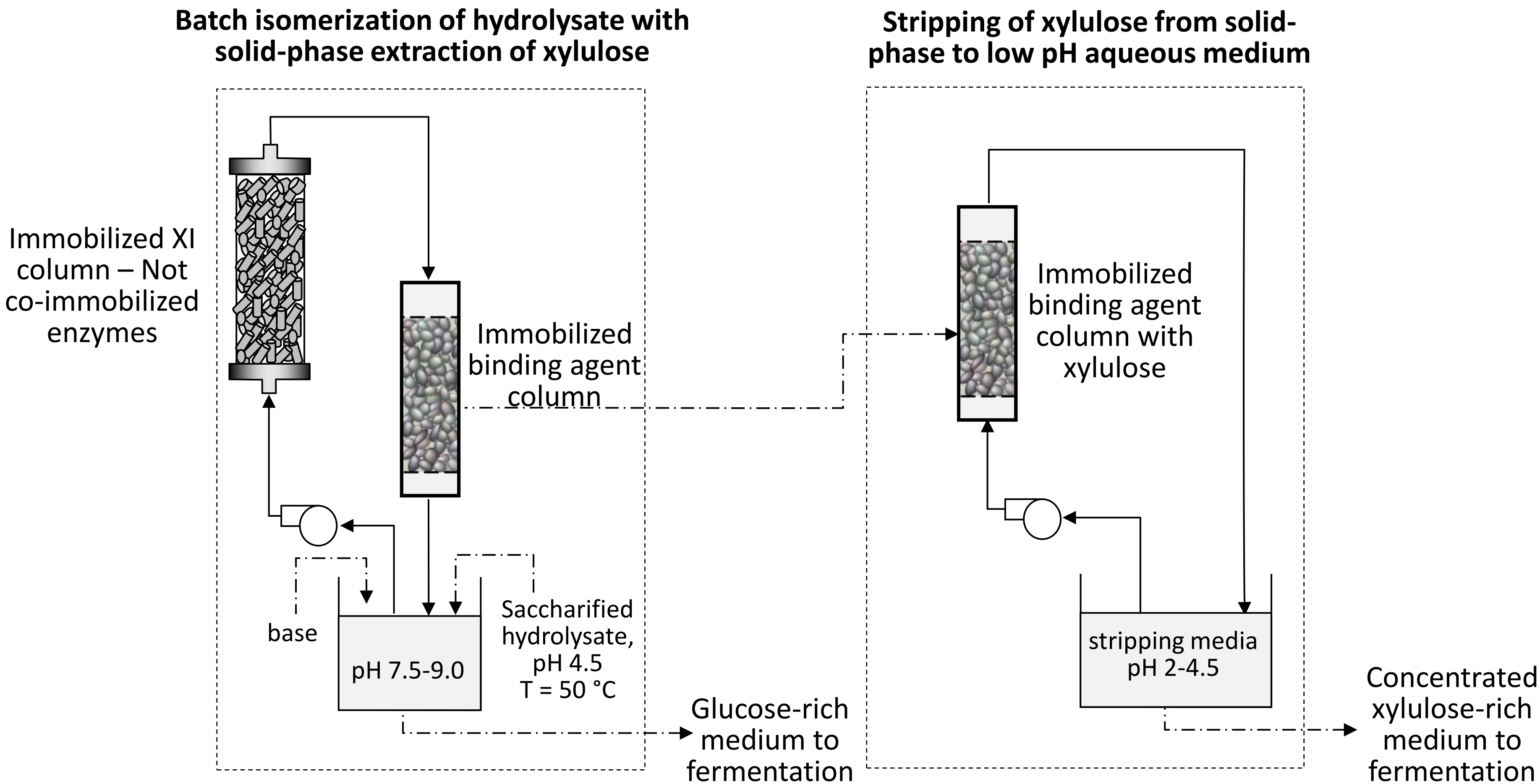
Strategy 2: Sequential I & F with immobilized binding agents

- Ketoses selectively complex with the binding agents
- N2B shows much higher selectivity than PBA at the pH of isomerization



2. Technical Accomplishments/Results (con't)

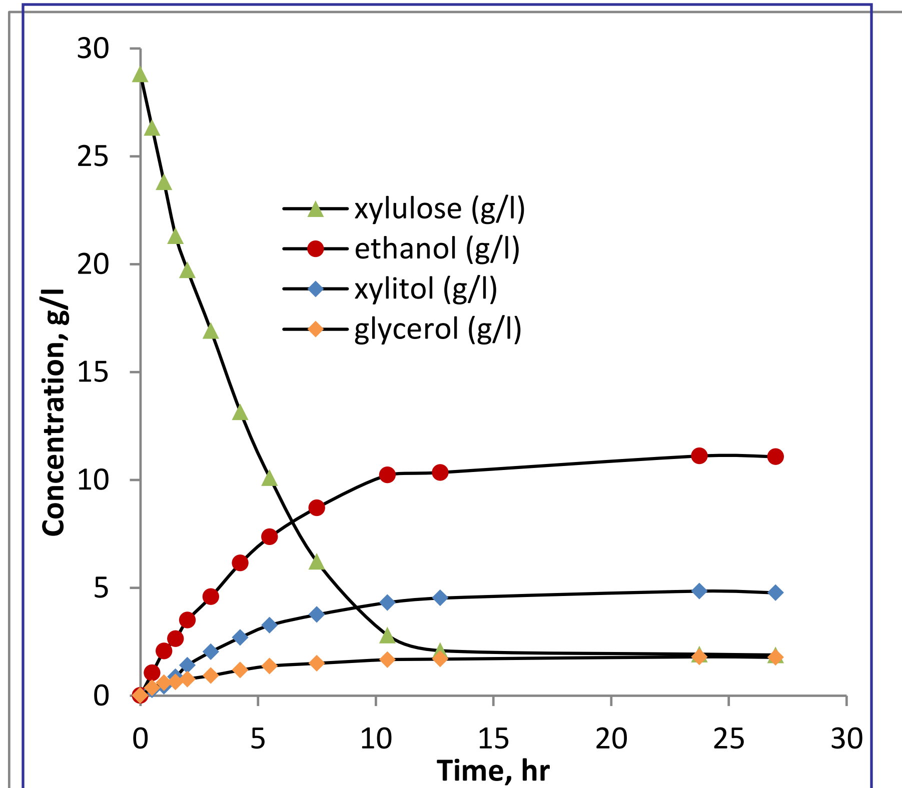
Strategy 2: Sequential I & F with immobilized binding agents



2. Technical Accomplishments/Results (con't)

Strategy 2: Fermentation of pure xylulose

- Fermentation can be done in a conventional fermentor
- No need for co-immobilized enzyme pellets
- Simpler to implement and easily scalable while retaining the advantages of non-GMO yeast
- Immobilized binding agent columns retain their viability indefinitely
- The sugars can also be converted to other more energy-dense fuels *i.e.* furans



Xylulose fermentation using native yeast at initial pH 4.5 and 34°C.

Relevance

- The goal of our research project is to enable conversion of both C5 and C6 sugars to ethanol, increasing utilization of the feedstock
- This goal is in-line with the MYPP 2012
  - R&D portfolio goal of technology development for producing fuels and bioproducts from lignocellulosic feedstocks
  - Conversion R&D goal to develop commercially viable technologies for converting biomass feedstocks via biochemical routes into energy-dense fungible liquid transportation fuels

Success factors and challenges

- Critical Success Factors
  - Minimize cost and maximize life of the co-immobilized enzyme pellets
  - Industrial collaborator anticipates pilot implementation within 12 months
- Advance the state of technology and positively impact commercial viability of biomass and/or biofuels
  - Added cost for xylulose fermentation must be less than the revenue generated from additional ethanol produced
  - (Biocatalyst + native yeast) cost vs. GMO licensing, propagation and nutrient cost

Possible future work
outside the scope of this project

- Fermentation of hydrolysates in SIF
  - Testing ethanol-tolerant/adapted yeast strains from corn-ethanol industry for ethanol productivity and sugar utilization
- Isomerization/fermentation of hydrolyzates from other pretreatments and other feedstocks
- Recovery of soluble binding agents used in Strategy 1b before/after fermentation
  - ion exchange resins work for borate, will they work for the other binding agents?

Summary

- This project has the potential to significantly increase the ethanol yield from lignocellulosic biomass
- Approach is to ***exogenously isomerize*** xylose to xylulose and ferment with other C6 sugars using ***native*** yeast
- By successfully executing all the proposed tasks and meeting all the project milestones, we have demonstrated
  - a ***viable*** technology for fermenting C6 and C5 sugars to ethanol using (i) abundantly available *Xylose Isomerize* enzyme, (ii) ketose-selective binding agent and (iii) **native** yeasts;
  - how the readily-available immobilized *Xylose Isomerize pellets* and ketose-selective-agents leads to ***high yield*** conversion of xylose to xylulose, while simultaneously concentrating the ketose sugar;
  - simple, robust, and scalable methods for producing immobilized biocatalyst and ketose-binding agent pellets, using commercially-available functionalized-supports;
  - biocatalyst and ketose-binding agent ***recovery and reuse*** strategies;
  - simultaneous saccharification and fermentation (***SSF***) by adding *XI* particles (with co-immobilizing urease) along with cellulases and native yeast to the pretreated-biomass;

Summary (con't)

- Further economic optimization can result through identification of
  - cheaper supports and methods of immobilization for *XI* and binding agents
  - yeast strains with higher ethanol tolerance
- Tangible outcomes of the project
  - Five peer-reviewed publications
  - Nineteen conference presentations
  - Two patents filed and licensed to SuGanit Systems, Inc.

Additional Slides

Responses to Previous Reviewers' Comments

- **Question 1:** The use of non-GMO co-fermenting organisms are always of interest for the program; however these systems should be streamlined for industrial purposes. The current project has considerable challenges ahead with regards to industrial applications. The economics of enzyme pellets and hollow fiber reactors as applied here (i.e., relatively low cost bulk commodity fuels industry) is questionable.
- **PI Response:** Immobilized XI pellets are used in the high fructose corn syrup (HFCS) industry on a commercial scale routinely. Based on this experience, we do not anticipate issues with the scaling-up of the enzyme pellet technology. The use of hollow fiber reactors for waste water treatment on a significantly larger industrial scale is common practice (see ZeeWeed membrane bioreactors, GE). Some of the perceived concerns with use of hollow fibers for hydrolysate conversion are already proven to be tractable through this proven technology. We revised the project goals to focus on further cost-improvements via recovery and reuse of the complexing agents. We are currently addressing simplification of the process to reduce the cost for scale-up.
- **Question 2:** A clever idea to promote fermentation of xylose by native [non-GMO] yeasts. The research represents an honest assessment of merits and demerits of this approach. Impediments to practical application include the inability to use unfiltered hydrolysates and need to capture and recycle complexing agents [e.g. borate].
- **PI Response:** The technology can indeed handle unfiltered hydrolysates. However, based on the value proposition offered by lignin, we focused on filtered hydrolysates and methods to improve the process economics for this mode of implementation. Recycling and reuse of borate (or whatever complexing agent is employed), although adding cost to the process, reduces the cost of the reagent replacement.
- **Question 3:** The experimental approaches being used to address the proposed research appear appropriate. It is the proposed research itself, the whole concept that seems to be a long-shot for providing the most economical route for xylose utilization.
- **PI Response:** At its heart, our process is not significantly different than commercially-implemented HFCS production connected in tandem with a corn-ethanol process. The fact that we reuse the XI pellets (up to 6 months), coupled with a non-GMO yeast fermentation, makes a compelling economic case.

Publications and Presentations

Publications:

- "High yield aldose-ketose transformation for isolation and facile conversion of C5 biomass sugar to furan," Bin Li, P. Relue, and S. Varanasi, *under review*.
- "Evaluation of complexing agents for shifting the xylose:xylulose equilibrium with commercial immobilized xylose isomerase," B. Li, D. Yuan, H. Shao, P. Relue, S. Varanasi, *in preparation* for *I&EC Research.*
- "Immobilization of urease for a microenvironmental pH control system for xylose isomerization: effect of media additives on enzyme activity," B. Li, D. Yuan, H. Shao, P. Relue, S. Varanasi, *in preparation* for *Enzyme and Microbial Technology.*
- "Simultaneous isomerization and reactive extraction of biomass sugars for high yield production of ketose sugars," Bin Li, P. Relue, and S. Varanasi, *Green Chemistry*, 14 (2012) 2436-2444.
- "A viable method and configuration for fermenting biomass sugars to ethanol using native Saccharomyces cerevisiae," Y. Dawei, K. Rao, P. Relue and S. Varanasi, *Bioresource Technology, 117(2012) 92-98.*
- "Fermentation of biomass sugars to ethanol using native industrial yeast strains," Dawei Yuan, Kripa Rao, Patricia Relue, and Sasidhar Varanasi, *Bioresource Technology,* 102 (2011) 3246–3253.
- "A Novel technique for Optimizing the Simultaneous-Isomerization-and-Fermentation (SIF) Approach of Converting Xylose to Ethanol," K. Rao, S. Chelikani, P. Relue, and S. Varanasi, *Applied Biochemistry and Biotechnology*, 146, (2008) 101-117.

Patents:

- Bin, Li, S. Varanasi, and Patricia Relue, "Aldose-Ketose Transformation for Separation and/or Chemical Conversion of C6 and C5 Sugars from Lignocellulosic Biomass Hydrolyzate, US Provisional Patent filed, Ser No:61/325,710, 04/2010
- S. Varanasi, K. Rao, and P. Relue, "A Novel technique that enables efficient fermentation of xylose and hexose sugars from biomass hydrolysates using native non-GMO yeasts," US Utility Patent filed, 01/02/09.

Publications and Presentations (con't)

Presentations:

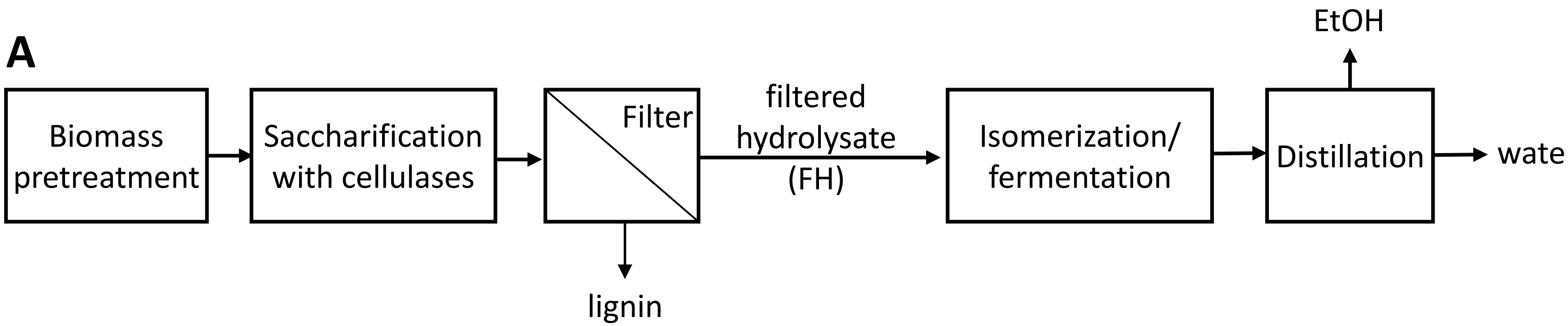
- "Mathematical modeling of furfural production from high purity xylulose," K. Marbaugh, B. Li, S. Varanasi and P. Relue, 35th Symposium of Biotechnology for Fuels and Chemicals, Poster 18-23, April 29-May 2, 2013, Portland, OR.
- "Furfural production from xylulose in a bi-phasic reaction system," B. Li, P. Zhang, S. Varanasi and P. Relue, 35th Symposium of Biotechnology for Fuels and Chemicals, Poster 18-24, April 29-May 2, 2013, Portland, OR.
- "2,3-Butanediol production from xylulose fermentation," P. Zhang, B. Li, H. Shao, S. Varanasi and P. Relue, 35th Symposium of Biotechnology for Fuels and Chemicals, Poster 2-14, April 29-May 2, 2013, Portland, OR.
- "Simultaneous isomerization and reactive extraction of biomass sugars for efficient furan production via high yield ketose intermediate," B. Li, P. Zhang, S. Varanasi and P. Relue, 35th Symposium of Biotechnology for Fuels and Chemicals, Poster 3-45, April 29-May 2, 2013, Portland, OR.
- "Fermentation of Biomass Sugars to Ethanol Using Native Yeast," B. Li, S. Varanasi and P. Relue, AIChE Annual Meeting, Pittsburg, PA, Oct, 2012.
- "Simultaneous aldose isomerization and separation/concentration of ketose by reactive extraction," B. Li, P. Relue and S. Varanasi, Session 13-33; 34th Symposium on Biotechnology for Fuels and Chemicals, April, 2012 New Orleans, LA.
- "Methods for simultaneous xylose isomerization and separation/concentration of xylose and xylulose by liquid-liquid extraction," B. Li, P. Relue, and S. Varanasi.; Paper 293a; 2011 AIChE Annual Meeting; October 16-21, 2011, Minneapolis, MN.
- "A novel SIF strategy for co-fermentation of C5 and C6 sugars with native yeast," P. Relue and S. Varanasi, DOE Office of the Biomass Program, Biochemical Platform Review Meeting, Denver, CO, February 16, 2011.
- "Evaluation of Complexing Agents for Shifting the Xylose:Xylulose Equilibrium with Immobilized Xylose Isomerase," Bin Li, Dawei Yuan, Heng Shao, Patricia Relue, and Sasidhar Varanasi, AIChE Annual Meeting, Salt Lake City, UT, November 7-11, 2010.

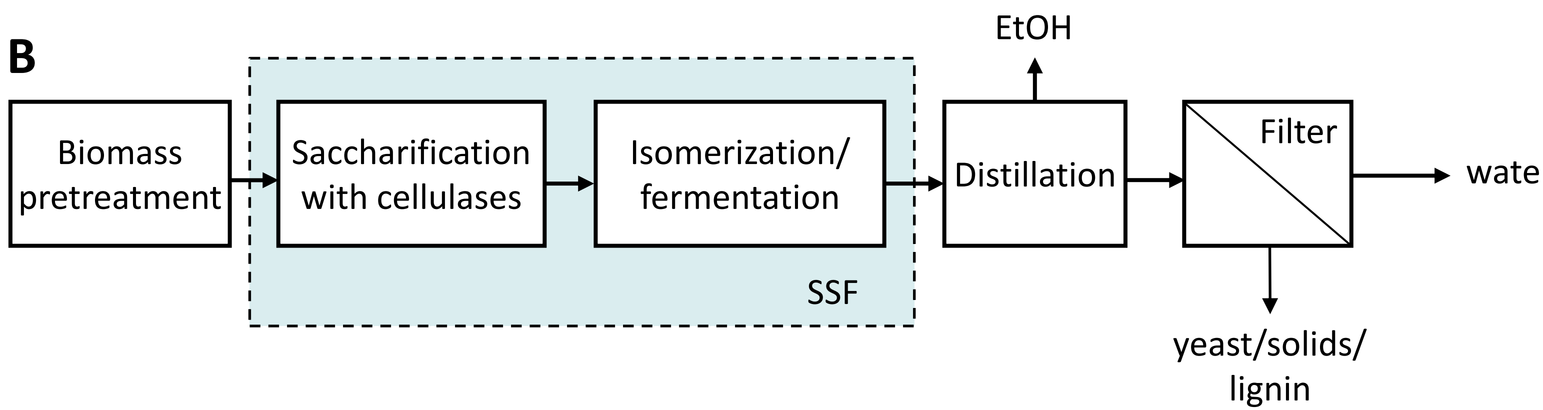
Publications and Presentations (con't)

Presentations:

- "Xylose isomerase catalyzed conversion of xylose to xylulose: role of sugar complexing agents," Bin Li, Dawei Yuan, Heng Shao, Sasidhar Varanasi, Patricia Relue, AIChE Annual Meeting, Salt Lake City, UT, November 7-11, 2010.
- "Adaptation of Native S. cerevisiae for Improved Xylulose Utilization," D. Yuan, B. Li, H. Shao, S. Varanasi and P. Relue, 32nd Symposium on Biotechnology for Fuels and Chemicals, Clearwater Beach, FL, April 2010.
- "Immobilization of Urease for Micro-environmental pH Control System: Effect of Media Additives on Enzyme Activity," B. Li, D. Yuan, H. Shao, S. Varanasi and P. Relue, 32nd Symposium on Biotechnology for Fuels and Chemicals, Clearwater Beach, FL, April 2010.
- "Techniques for dual-layer co-immobilization of xylose isomerase with urease," B. Li, P. Relue, and S. Varanasi, Midwest Graduate Research Symposium, Toledo, OH, March 6, 2010.
- "Simultaneous isomerization and fermentation of xylose to ethanol using native *S. cerevisiae*,"D. Yuan, P. Relue, and S. Varanasi, Midwest Graduate Research Symposium, Toledo, OH, March 6, 2010.
- B. Li, D. Yuan, P. Relue, and S. Varanasi, "Evaluation of Enzyme Immobilization Methods for Xylose-to-Xylulose Isomerization," *AIChE National Annual Meeting*, Nashville, TN, November 11, 2009.
- "A Native S. Cerevisiae Fermentation Methodology for Converting Glucose and Xylose From Biomass Hydrolysate to Ethanol," D. Yuan, B. Li, P. Relue, and S. Varanasi, *AIChE National Annual Meeting*, Nashville, TN, November 11, 2009.
- "A novel SIF strategy for co-fermentation of C5 and C6 sugars with native yeast," P. Relue and S. Varanasi, DOE Office of the Biomass Program, Biochemical Platform Review Meeting, Denver, CO, April 2009.
- "A Simultaneous Isomerization and Fermentation (SIF) Process for Co-fermentation of Glucose and Xylose," D. Yuan, P. Relue, and S. Varanasi, Midwest Graduate Research Symposium, Toledo, OH, *received 1st place poster award*, April 2009.
- "Xylose isomerization to xylulose with urease-coated xylose isomerase," B. Li, D. Yuan, S. Varanasi, and P. Relue, Midwest Graduate Research Symposium, Toledo, OH, *received honorable mention poster award*, April 2009.

A comparison of saccharification-followed-by-fermentation versus SSF-based processes





- A: Saccharification followed by isomerization/fermentation
    - Lignin valorization feasible; yeast reuse possible; simpler distillation
- B: SSF-based process
    - One less unit operation; recovery and reuse of isomerization enzyme, binding agent, and yeast not feasible; lignin valorization complicated by other solids