Corporate Overview

- **Biotech R&D Group**
  - Enabling Carbohydrate Technology
  - Platform of Proprietary Process Technologies and Know-How

- **Focus:**
  - Food Ingredients/Specialty Chemicals
  - Fine Chemicals/Pharma

- **Biotech Company**
  - Chicago - Headquarters
  - Peoria - Main Research Facility

- **Key Collaborators**
  - U.S. Department of Agriculture National Center for Agriculture Utilization Research (NCAUR)
Contributors

zuChem
- F. Mike Racine, Ph.D.
- Ryan Woodyer, Ph.D.
- Paul Taylor, Ph.D.
- Nathan Wymer, Ph.D.
- Shama Khan
- Trevor Christ

USDA - NCAUR
- Badal Saha, Ph.D.
- Yoshikiyo Sakaibura, Ph.D.
- Douglas Antibus, Ph.D.
- Greg Kennedy

University of Illinois
- Huimin Zhao, Ph.D.
- Ryan Sullivan, Ph.D.
- Nick Nair, Ph.D.

Other Collaborators/Consultants
- Ian Fotheringham, Ph.D. – Ingenza Ltd.
- David Dodds, Dodds & Associates
- Bill Dowd, Ph.D. – fmr VP R&D Dow
- David Ager, Ph.D. – Competence Mgr DSM

Funding

Biotechnology Research and Development Corporation a
U.S. Department of Energy – Biomass Program
National Science Foundation
Improving Economics of Ethanol Production

**INPUT**
- Inexpensive Biomass Feedstock

**OUTPUT**
- Ethanol
  - DDG, Fiber Bagasse
    - ~$300/ton
- Value Added Chemicals
  - e.g. Xylitol
    - >>$3000/ton

**BY-PRODUCTS**
Polyol Market

Specialty Sweeteners

- **Sorbitol**
- **Xylitol** - 3X growth projected
- **Mannitol**
- **Erythritol and Others**
Xylitol Production

Current Chemical Process

Purified Xylose

- expensive
- limited supply

Chemical Hydrogenation

Xylitol

Polyol contaminants & purification cost driven by xylose purity

zuChem Bioprocess

Hemicellulose

- inexpensive
- abundant supply
- Mixed C5/C6 is OK

Fermentation

Xylitol

No polyol contaminants
Simple purification
Challenges and Process Goals

- Little or no arabitol produced
- Overcome Problems with Fermentation Inhibition
  - Furfurals and other inhibitors
- Abundant Feedstock Supply
- Tolerant to Feedstock Variability
- Process Economics
  - Yield – 100 g/l
  - Purity – essentially free of sugar and polyol contaminants
  - Throughput - >3 g/L-h
  - Recovery - >85%
# Hemicellulose C5/C6 Composition

<table>
<thead>
<tr>
<th></th>
<th>C5</th>
<th>C6</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-Xylose</td>
<td>L-Arabinose</td>
<td>D-Glucose</td>
<td>D-Galactose</td>
</tr>
<tr>
<td>Birch Wood</td>
<td>89.3%</td>
<td>1%</td>
<td>1.4%</td>
<td>-</td>
</tr>
<tr>
<td>Rice Bran</td>
<td>46%</td>
<td>44.9%</td>
<td>1.9%</td>
<td>6.1%</td>
</tr>
<tr>
<td>Wheat Straw</td>
<td>65.8%</td>
<td>33.5%</td>
<td>0.3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Corn Fiber</td>
<td>48-54%</td>
<td>33-35%</td>
<td>-</td>
<td>5-11%</td>
</tr>
<tr>
<td>Sugar Cane Bagasse</td>
<td>80-90%</td>
<td>5-10%</td>
<td>0-5%</td>
<td>0-2%</td>
</tr>
</tbody>
</table>

Conversion of Xylose to Xylitol

D-Xylose

\[
\text{CHO} \quad \text{CH}_2\text{OH}
\]

\[
\text{H} \quad \text{HO} \quad \text{H} \quad \text{HO} \quad \text{H} \quad \text{CH}_2\text{OH}
\]

Xylose-Specific Xylose Reductase

Xylitol

\[
\text{CHO} \quad \text{CH}_2\text{OH}
\]

\[
\text{H} \quad \text{HO} \quad \text{H} \quad \text{HO} \quad \text{H} \quad \text{CH}_2\text{OH}
\]

Xylose Isomerase

Xylitol DH

D-Xylose

\[
\text{CHO} \quad \text{CH}_2\text{OH}
\]

\[
\text{H} \quad \text{HO} \quad \text{H} \quad \text{HO} \quad \text{H} \quad \text{CH}_2\text{OH}
\]

D-Xylulose

Xylitol

\[
\text{CHO} \quad \text{CH}_2\text{OH}
\]

\[
\text{H} \quad \text{HO} \quad \text{H} \quad \text{HO} \quad \text{H} \quad \text{CH}_2\text{OH}
\]

Glucose

Arabinose

Arabitol

Xylitol

Concentration (W/V)

0 20 40 60 80
Hours

0 1 2 3 4 5 6 7 8
Concentration (W/V)

0 0.5 1 1.5 2 2.5 3 3.5
Concentration (W/V)

0 10 20 30 40
Hours
Xylose Specific Selection Strain

Plate on L-arabinose/D-xylose

- XR

+ Xdh
+ araB

XR

L-arabitol + xylitol

\( \text{araB} \)

L-arabitol-5-phosphate

D-xylulose

+ xdh

XR'

xylitol

D-xylulose

Growth

XR - active on L-arabinose and D-xylose
XR' - D-xylose specific enzyme

LETAL

No growth

No growth
Xylose Specific Mutants

D-Xylose/L-Arabinose Rate Ration

- WT
- MUT1
- MUT2
- MUT3
- MUT4
Conversion of Arabinose to Xylitol

L-Arabinose $\rightarrow$ L-Ribulose $\rightarrow$ L-Xylulose $\rightarrow$ Xylitol

Diagram showing the conversion process with the involvement of Isomerase, Epimerase, and Reductase enzymes.
Testing on Hydrolysate

Fermentation Test On Unremediated Hydrolysates

- Slow growth on hydrolysates due to fermentation inhibition
## Remediation Tests

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative growth</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absorbance 600 nm</td>
<td></td>
</tr>
<tr>
<td>NaOH to pH 7</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂ to pH 7</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂ to pH 9.05</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>H₂SO₄ to pH 7</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂ to pH 10.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fermenter results confirmed that over-liming at high pH is best; charcoal also helps.
Xylitol Process Optimization

- Optimum pH (6.8) and temperature (37°C) were determined
- Optimal nutrients identified
- Fed-batch protocol developed
- Air/agitation requirements were determined
Met Goals of Process

- Conversion of xylose to xylitol was 98%.
- Volumetric productivity 3.1g /L-h at 32 hours
- Yield was >100 g/L
- Complete conversion of sugars
Summary

- ~8 patents on file in four patent families - First patents now issued.
- Piloting now underway with first Manufacturing Partner – Godavari Biorefineries
- Sampling to Customers Expected this Summer
- New Manufacturing facility expected to be operational by end of 2014
- Broad Licensing Strategy
Products to make life sweeter.