Enzymatic Process for High Glucose Production using Granular Starch

57th Detmold Starch Convention, Germany

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Today's Presentation

- Current Process for Glucose Production
- Brief History of Granular Starch Hydrolysis
- Granular Starch Hydrolyzing Enzymes (GSHE) For Glucose Production
- Proposed Process
- Summary and What is next?
Current Process for Conversion of Starch to Biochemicals

Current Process for Conversion of Starch to Biochemicals

Water → Liquefaction → Saccharification → Purification Ion Exchange Carbon

Wheat → Corn → Tapioca

Current Process

Starch Tank

Jet Cooker 108°C, 5-8 min.

95°C, 90 min.

60°C, 36-72 hrs.

Glucose

Specialty Chemicals

Amino Acids
MSG
Sorbitol
Citric
Ascorbic
Gluconic
Lactic
1,3 Propanediol
Antibiotics
Enzymes
Bio-pharmaceuticals
Liquefaction Process For Solubilizing Granular Starch Prior to Enzymatic Hydrolysis

Picture of a typical jet used in primary liquefaction

Drawings copied with permission of Hydrothermal Corp.
Energy Costs ($/MM BTUs)
Forecast Assumes 5% Increase Per Year

Source: Historical : DOE
Forecast: GCOR Estimate
April 1985, Yoshizumi, Matsumoto, Ukuda and Fukushi (4,514,496) describe a non-cook alcohol process using ground grains and cite energy savings.

Oct 1986 Dwiggins, Pickens and Niekamp (4,618,579) describe a multi-step process for the solubilization of starch slurries between 20-60% solids.

Sept. 1995, (Starch/Starke, 46, 1995) Yamada, Hisamatsu, Teranishi, Katsuro, Hasegawa and Hayashi report that the residual granules after partial A. niger enzyme digestion contain the same ratio of amylose/amylopectin as non-treated.

March 1996, Zhao, Madson and Whistler report that starch granules eroded with glucoamylase are open caverns suitable for the uptake of oils such as peppermint.
Figure 18 shows corn starch granules after being subjected to attack by glucoamylase (from *Aspergillus niger*) for 16 hours. The granules show two apparent forms of attack. In most granules, deep holes that often penetrate to the center of the granule are formed. Also, surface erosion occurs that exposes a layered structure due to some areas being more resistant to enzymic attack than others.

Glucose Production Without High Temperature Jet cooking

Granular Starch Hydrolyzing Enzymes

For

Glucose Production
Granular Starch Hydrolyzing Enzymes: Glucoamylases and Alpha Amylases

Granular Starch

Catalytic Domain

Endo-Acting Alpha Amylase

Exo-Acting Glucoamylase

Linker Region

Starch Binding Domain

GLUCOSE

Starch Binding Domain

SBD

Endo-Acting Alpha Amylase

SBD

Exo-Acting Glucoamylase
GSHE For Sweetener r-HGA/B.s. AA Application

- Effect on starch solubilization
- Effect of pH
- Effect of temperature
- Effect of enzyme ratio on the final glucose yield and sac. rate
- Effect of saccharification time, solubilization and final glucose yield
- Effect of dissolved solids
- Comparison of saccharification kinetics: Current VS GSHE
Solubilization and hydrolysis of different granular starch substrates by *Bacillus stearothermophilus* Alpha Amylase @pH 5.5, 60°C, 30% dsb. (0.6 AU/g ds)

Graph showing the solubilization and hydrolysis of wheat, tapioca, and corn starch substrates over reaction times ranging from 0 to 28 hours. The graph includes a table with data for different molecular weight fractions (DP1, DP2, DP3, DP4+) for each starch type.

<table>
<thead>
<tr>
<th></th>
<th>DP1</th>
<th>DP2</th>
<th>DP3</th>
<th>DP4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat</td>
<td>0.6</td>
<td>1.6</td>
<td>2.4</td>
<td>72.2</td>
</tr>
<tr>
<td>tapioca</td>
<td>1.3</td>
<td>11.5</td>
<td>14.1</td>
<td>73.1</td>
</tr>
<tr>
<td>corn</td>
<td>1.6</td>
<td>11.6</td>
<td>14.6</td>
<td>72.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>12</td>
<td>15</td>
<td>72.4</td>
</tr>
</tbody>
</table>
Solubilization and hydrolysis of different granular starch substrates by Bacillus stearothermophilus alpha amylase and r-HGA glucoamylase @pH 5.5, 60°C, 30% dsb.

<table>
<thead>
<tr>
<th></th>
<th>DP1</th>
<th>DP2</th>
<th>DP3</th>
<th>DP4+</th>
<th>AT 24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>97.4</td>
<td>2.1</td>
<td>0.2</td>
<td>0.2</td>
<td>Corn</td>
</tr>
<tr>
<td>Tapioca</td>
<td>96.5</td>
<td>1.6</td>
<td>0.4</td>
<td>1.5</td>
<td>Tapioca</td>
</tr>
<tr>
<td>Corn</td>
<td>97.3</td>
<td>2.3</td>
<td>0.2</td>
<td>0.2</td>
<td>Wheat</td>
</tr>
</tbody>
</table>

Reaction time (hrs)
Effect of pH on the solubilization of granular corn starch (32% dsb) @ 60 °C, blend of *B.s.* amylase and HGA
Effect of enzyme concentrations on the solubilization of granular corn starch (32% dsb), pH 5.5, 60°C

<table>
<thead>
<tr>
<th>Enzyme Concentration</th>
<th>B. stearothermophilus</th>
<th>r-HGA</th>
<th>% Relative Solubilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg/MT</td>
<td>GSHE U/g</td>
<td>3 hrs</td>
<td>6 hrs</td>
</tr>
<tr>
<td>0.1</td>
<td>0.5</td>
<td>54.3</td>
<td>72.9</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>66.9</td>
<td>79.5</td>
</tr>
</tbody>
</table>
Comparison of Glucose production from soluble and insoluble starch substrates

- Liquefied Starch at 32% Dry Substance, pH 4.2 and 60 °C. Dosed with *A. niger GA*
  - 61 Hours to maximum DP1
  - Saccharide Distribution

<table>
<thead>
<tr>
<th>DP1</th>
<th>DP2</th>
<th>DP3</th>
<th>DP4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>95.2</td>
<td>3.1</td>
<td>0.4</td>
<td>1.3</td>
</tr>
</tbody>
</table>

- Granular Starch at 32% Dry Substance, pH 5.5 and 60 °C. Dosed with *B. stearothermophilus and r-HGA*
  - 24 Hours to maximum DP1
  - Saccharide Distribution

<table>
<thead>
<tr>
<th>DP1</th>
<th>DP2</th>
<th>DP3</th>
<th>DP4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>96.8</td>
<td>2.8</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
MF GSHE (HGA+B. stearothermophilus) Corn Starch
Summary of MF GSHE Treated Corn Starch

- Large bored holes into granules visible
- Not all granules digested
- Some granules seem to be cut in half, revealing that some holes go all the way to the center
- Sample prep and imaging conditions were conducive to obtaining good images of granules
Solubilization and SEM of Rice Starch treated with H-GA+B. stea rothermophilus, pH 5.5, 60 °C
Process for Converting Starch to Biochemicals

Current Process

1. Liquefaction
   - Jet Cooker
   - 108°C, 5-8min

2. Saccharification
   - 95°C, 90min
   - 60°C, 36-72 hrs

3. Starch Separation
   - Purification
   - Ion Exchange
   - Carbon

4. Glucose

Specialty Chemicals

- Amino Acids
- MSG
- Sorbitol
- Citric
- Ascorbic
- Gluconic
- Lactic
- 1,3 Propanediol
- Antibiotics
- Enzymes
- Bio-pharmaceuticals
Process for Converting Starch to Biochemicals

Starch Tank

Water

Wheat

Corn

Tapioca

Glucose

GSHE For Sweetener

Low Energy Process

Amino Acids
MSO
Sorbitol
Citric
Ascorbic
Gluconic
Lactic
1,3 Propanediol
Antibiotics
Enzymes
Bio pharmaceuticals

Low Energy Process
GSHE Offers:

- Energy saving by elimination of jet cooking
- Simple: Single step VS multiple steps
- Single pH: Reduced Ion Exchange cost, reduced controls, reduced process water
- No calcium addition needed: reduced ion exchange cost
- No viscosity issues due to no gelatinization
GSHE Offers: continued

- Alpha amylase inactivation is not needed prior to saccharification process

- No iodine positive starch in saccharification: Starch is never solubilized

- Shorter saccharification time: capacity increase

- Less by-products formation at high temperature: i.e., maltulose, Maillard reaction, color etc.
Direct Conversion of Granular Starch to Glucose using GSHE

Precipitation
- DS: 25 – 40%
- pH: 5.0 – 5.5
- Temp: 55 – 65°C
- Time: 20-30hr
- >90% solubilization

Reaction
- >96% glucose

Microfilter
- pH adjustment

Fermentation
- Starch Processing
- Refining
- HFCS
- Dextrose Liquor
Conclusion -- or is this the Beginning

- Established foundation that we can build on
- Biotech solutions through enzymes enable achievement of targets
- Considerable advantages plus the imaginations and creative talent of many will accelerate development
- Through improved enzymes and process engineering this will become a commercially acceptable and economically viable system.
## Patent Information

**Title:** EXPRESSION OF GRANULAR STARCH HYDROLYZING ENZYMES IN TRICHODERMA AND PROCESS FOR PRODUCING GLUCOSE FROM GRANULAR STARCH SUBSTRATES

**Assignee:** Genencor International, Inc.

**Patent #:**
- WO 05052148
- US 20050136525

**PDF File:**
- WO05052148A2.pdf

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