The Effect of Protease on Ethanol Fermentation of Whole Ground Grains and Endosperm

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AGENDA

- Introduction
- Effect of Protease in Conventional Fermentation Processes using whole ground corn
- Future Ethanol Market Trend- Fractionated Grain
- Fermentation optimization using fractionated corn, endosperm
- Benefits of Protease in ethanol fermentation containing granular starch from whole ground corn and endosperm
- Summary
Agricultural Raw Materials For Fuel Alcohol Production

- Sugar Cane
- Wheat
- Triticale
- Rice
- Rye
- Barley
- Corn
- Sorghum
- Tapioca
- Sugar Beet
Global Ethanol Production From Grains: 2005

**United States**
- 2005: 16.28 Billion Liters
- 2008: 22 Billion Liters; 12% Annual Growth Rate
- Feedstock: Mainly Corn, Milo (Sorghum)
- 95 Plants + 31 Plants under construction

**Europe**
- 2005: 0.6 Billion Liters
- 2008: 4.4 Billion Liters
- Feedstock: Mainly Wheat, Rye, Triticale, Corn
- 10 Plants

**Asia**
- 2005: 0.75 Billion Liters
- 2008: 2.5 Billion Liters
- Feedstock: Mainly Sorghum, Corn, Rice, Tapioca
- 3 Plants in China

Source: Genencor; RFA’s Homegrown for the Homeland, Ethanol Industry Outlook, 2005
• Endosperm – This is the part where the starch comes from. Part of the gluten also comes from here. 60-70% of the kernel is comprised of this.

• Germ – this is the part of the corn that oil is made from. 5% of the kernel is comprised of germ.

• Pericarp – This is the outer layer of the kernel. It comprises about 5% of the kernel.

• Protein (Gluten) 8-9% of the kernel is this. Supplement in animal feed.

• Other components – Moisture comprises about 15% and ash and other solubles are about 6%.
Protease in Conventional Processes
Conventional Dry Grind Ethanol Process

Conventional Ethanol Production Process

- Thermo-stable Alpha Amylase
- Glucoamylase
- Yeast
- Alcohol Recovery

- Grinding
- Slurry Tank
- Jet Cooker >100°C 5-8 min.
- Liquefaction
- Saccharification
- Fermentation
- Distillation & Dehydration

* pH adjustment steps are not shown
Yeast require certain nutrients to grow and maintain their population in order to convert glucose into ethanol.

These may include the following:
- Free Amino Nitrogen
- Peptides and amino acids
- Vitamins and Minerals (Inositols, Zinc, etc.)

If yeast nutrition is not maintained, then the fermentation will suffer and result in lower rates and yield of ethanol formation.

Nitrogen sources such as Urea, Ammonia, etc. can be added. However, this tends to give only Free Amino Nitrogen.
Why Protease?

Protease: Enzyme that hydrolyzes proteins to peptides and/or amino acids.

The use of certain proteases in ethanol fermentation has been proven to improve fermentation in the following ways:

- Faster Fermentation Time
- Higher Ethanol Yields
- Enhanced Yeast Growth
- More Efficient Filtration and Evaporation in downstream process steps
- More consistent fermentation
- More carbohydrate fermented
- Reduced carbohydrate in thin stillage
FERMGEN™ : A Novel Protease For Alcohol Production.

A new novel Acid Fungal Protease (FERMGEN™) derived from Trichoderma reesei provides the following benefits:

1. Works at pH’s commonly found in existing processes (below 5.0).
2. Faster ethanol rates and yield for grain based substrates as compared to those without protease.
3. Provides essential yeast nutrients in the form of amino acids, peptides, and free amino nitrogen essential for yeast growth and maintenance.
4. Hydrolyzes the protein matrices in the kernel that bind the various fractions which releases “hard” to hydrolyze starch.
Protease addition to conventional ethanol systems

- FERMGEN™ addition substantially increased both rate and overall ethanol yield.
- GA/Protease blend has already been patented. (US 5,231,017)
- Commercially sold as FERMENTZYM®
Protease in Fractionated Endosperm Processes
## Composition of Corn and Endosperm

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Corn</th>
<th>Endosperm</th>
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</thead>
<tbody>
<tr>
<td>Starch</td>
<td>68-70%</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>Protein</td>
<td>8-9%</td>
<td>11-12%</td>
</tr>
<tr>
<td>Oil</td>
<td>4%</td>
<td>&lt;0.1%</td>
</tr>
<tr>
<td>Phytate</td>
<td>0.8-1.2 % ds</td>
<td>0.04 % ds</td>
</tr>
</tbody>
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Other processes – Fractionation of grains

- Fractionation of corn has been gaining momentum.
- There are a variety of systems for fractionation presently in the industry.
- Fermentation streams will mainly consist of endosperm and protein.
- Germ and fiber will be removed and sold as co-products.
Since grain is fractionated, important nutrients from the germ have been removed. Nutrients can include: Free Amino Nitrogen, albumin, trace minerals, and fat soluble vitamins.

Protease plays an important role in replacing these nutrients needed for yeast growth and viability.

Protease also may be able to break up more of the starch/protein matrix allowing for more starch to be available to be converted to ethanol.

Utilizing endosperm in a conventional process with protease will obtain similar results as previously shown.

However, there are other ways to ferment starch!
Another way to ferment without utilizing cooking step.

STARGEN™ enzyme system can take you from starch slurry to ethanol in one step. STARGEN™ consists of blend of alpha and glucoamylases designed to convert uncooked (raw) starch to ethanol.

Raw starch is converted to glucose and then fermented simultaneously. Only fermentation temperatures are needed.

NO COOK STEP NEEDED!

FERMGEN™ protease works in conjunction with STARGEN™ in a no cook process for a more robust system for fuel ethanol production.
Low Energy Ethanol Production Process

STARGEN™ and FERMGEN™

Milo
Corn
Wheat
Tapioca

Water

Slurry Tank

Saccharification & Fermentation

Alcohol Recovery

Distillation & Dehydration

Storage Tank

GSHE & Yeast

* pH adjustment steps are not shown
Protease Dose Response in a low energy process

- As the protease dose is increased, the amount of ethanol produced is also increased.
- Protease dose at 1.0 kg/MT is slightly better than 0.75.
- This is also dependent on GA dose, time in fermentor, % ds, etc.
- Increased protease addition beyond 1.0 kg/MT is not beneficial.
FERMGEN™ and STARGEN™ synergy

- As the STARGEN™ dose is increased, the rate changes significantly.

- The fermentation can be completed in 45 hours.

- As the protease is increased, it appears that the STARGEN™ dose can be reduced.
Addition of FERMGEN™ increased rate and overall yield of ethanol.

Urea helped but did not have the effect at the end of fermentation as protease.

Corn Steep Solids was added to simulate thin stillage. Did not do as well as FERMGEN™ or urea.
Glucose remaining is quite high without the addition of nutrients.

FERMGEN™ results in lower glucose levels. Helps to pull reaction faster.

Urea above 800 ppm resulted in slightly lower glucose numbers, but lower overall ethanol as well.
Not much difference in glycerol formation.

However, it indicates slightly less yeast stress with FERMGEN™ compared to the other nutrients.

Important in later stages of fermentation.
FERMGEN™ - Other Substrates

- FERMGEN™ also works with other substrates.
- Have conducted successful plant trials utilizing wheat and milo with similar success to corn in both commercial and raw starch processes.
The use of protease has been demonstrated to provide significant benefits in the production of ethanol.

Protease can improve overall process of fermentation through enhanced yeast nutrition, increased rate and yield of ethanol, and improved downstream operations.

Can be used in multiple processes such as conventional and raw starch conversion to ethanol.
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