New Enzymatic Advances in the Dry Grind (Grain) Ethanol Process

David Johnston
Crop Conversion Science and Engineering Research Unit
USDA-ARS-Eastern Regional Research Center
Wyndmoor, PA
Topics

- Corn Kernel Structure
- Ethanol Production Technologies and Products
- Conventional Enzymes
- Enzyme and Modified Corn Processing
- New Enzyme Use in Ethanol Production
- Enzymatic Germ/Fiber Recovery in a Dry Grind Ethanol Process
- Process Engineering and Cost Models being Used and Developed
Structure of the Corn Kernel

- Endosperm
- Pericarp
- Tip Cap
- Germ
Structure of Steeped Corn Kernel

- Pericarp
  - Cuticle
  - Epidermis
  - Mesocarp
- Cross & Tube Cells
- Aleurone Layer
- Starch Granules
- Endosperm
Corn Kernel Images

Confocal Laser Scanning Microscope

Scanning Electron Microscope
Corn Processing

- Dry Milling
- Dry Grind Processing
- Wet-Milling
Ethanol Production Technologies

Two Main Process for Fuel Ethanol Production

- Wet Milling
- Dry Grind Processing

- Wet-milling plants are capital intensive but produce high valued coproducts
- Dry grind plants cost less to build but lower valued coproducts
**Coproduts from the Corn Wet-milling Process**

- One bushel of Corn
  - 56 lbs
- Corn Wet-Milling Facility
- 2.5 gal of Ethanol
- CO₂
- 1.5 lbs of Corn Oil
- 12.4 lbs of Gluten Feed
- 3 lbs of Gluten Meal
- Livestock Feed
- Poultry Feed
Wet Milled Coproducts

- Germ
- Fiber
- Starch
- Gluten Meal
Coproducts from Corn Dry-grind Process

One bushel of Corn

Corn Dry-Grind Facility

56 lbs

CO₂

2.7 gal of Ethanol

17 lbs of DDGS

Livestock Feed
Why are the Ethanol Yields Different?

- Losses of starch to coproducts in the wet milling process:
  - Germ 1%
  - Corn Gluten Feed 1-2%
  - Corn Gluten Meal 2-3%

- Dry Grind processing has the “opportunity” to ferment all of the starch to ethanol.
Enzyme Advances

- Reduction of pH optimum
- Improved thermal stability
- Decrease of calcium requirement
- Minimization of maltulose formation
- Cost effective native starch degradation
Native Starch Hydrolysis in Germinating Corn
Conventional Dry Grind Corn Process

- Corn → Conventional Dry Grind Process
- Conventional Dry Grind Process → Ethanol
- Conventional Dry Grind Process → Carbon Dioxide
- Conventional Dry Grind Process → DDGS
Renessen’s Corn Fractionation Pilot Plant

[Diagram of the flow process]

From: Feedstuffs, Feb. 6, 2006, p. 17
Modified Dry Grind Processes

**Enzymatic Or Quick Germ/Fiber Process**

- Corn → Enzymatic Or Quick Germ/Fiber Process
  - Ethanol
  - Carbon Dioxide
  - DDGS
- Pericarp Fiber
- Germ

**Wet Fractionation Process**

**Dry Degerm Defiber Process**

- Corn → Dry Degerm Defiber Process
  - Ethanol
  - Carbon Dioxide
  - DDGS
  - Germ
  - Pericarp Fiber

**Dry Fractionation Process**
Enzymes in Modified Corn Processing

- **Wet Milling**
  - Protease

- **Enzymatic Germ/Fiber Recovery with Ethanol Production**
  - Protease
  - Starch hydrolyzing
  - Others
E-Milling: An Enzymatic Corn Wet Milling Process

- **Processing Steps**
  - Soaking (no SO$_2$ and no lactic acid)
    - 2-6 hr
  - Coarse grind
  - Incubation with enzyme
    (controlled pH and temperature)
    - 2-4 hr
  - Conventional wet milling fractionation

E-Milling  US Patent # 6,566,125
Conventional Corn Wet Milling Process

- Steeping: 24-36 hrs
- Corn + Water + SO₂ → Steep
- Grind → Hydrocyclone
- Sieve → Fiber
- Centrifuge
- Germ
- Protein
- Starch

Enzymatic Corn Wet Milling Process

- Preprocessing: 6-8 hrs
- Corn + Water → Soak
- Enzymes → Incubate
- Grind → Hydrocyclone
- Sieve → Fiber
- Centrifuge
- Germ
- Protein
- Starch
100 g Wet Milling Results

![Graph showing starch yield (%) for different samples with categories Chemical Control and Buffer Control. The sample with Proteases has a notable increase in starch yield compared to others.](chart.png)
GC 106 and Bromelain Versus Conventional at the 1 kg Scale
Optimization of Enzyme Use in Batch Processing

Conv. Steep  Bromelain Enzyme  GC106 Enzyme

5 g  1 g  0.25 g  0.25 g  10 ml  10 ml  5 ml  2 ml  1 ml

SO₂
500 ppm  200 ppm 300 ppm 300 ppm

Bromelain Enzyme

Wet-milling Ethanol Production

1. **Steeping**
2. **Grinding** (Degermination mill)
3. **Sieving**
4. **Fine Grinding** (Degermination mill)
5. **Centrifugation**
6. **Hydrocyclone**
7. **Germ Dryer**
8. **Gluten Dryer**
9. **Fiber Dryer**

**Products:***
- **Light Steep Water**
- **Heavy Steep Water**
- **Germ**
- **Fiber**
- **Starch**
- **Condensed Solubles**
- **CO₂**
- **Saccharification & Fermentation**
- **Yeast & Enzymes**
- **Liquefaction**
- **Dehydration column**
- **Ethanol**
- **Overhead product (Recycled back)**
- **Condensed Solubles**

**Inputs:**
- **Corn**
- **Gluten Feed**

**Other:**
- **Evaporator**
- **Stripping/Rectifying column**
- **Evaporator**
- **Overhead product (Recycled back)**
Benefits of E-Milling

- Reduced or eliminated use of $SO_2$
  - Many microorganisms are very sensitive
- Reduce processing time
- Potential reduction in water use
Degradation caused by inappropriate enzyme used during Enzymatic Milling

Bad Enzyme for E-Milling
Enzyme Addition During Fermentation

- Glucoamylase
- Protease
Dry-grind Ethanol Production

Corn

Grinding (Hammermill)

Water

Blending

Mash

Liquefaction

Yeast & Enzymes

Saccharification & Fermentation

CO₂

Overhead product (Recycled back)

Dehydration column

Ethanol

Stripping/Rectifying column

DDGS
Saccharification Controller

- Regulate the release of glucose during fermentation through the use of a dynamic controller
- Enzyme dosage is controlled during fermentation to match the yeast requirements
- Feedback data taken at intervals is used to adjust enzyme input rates
- Joint project between the University of Illinois and the USDA-ARS-ERRC
Fermentation Stressed Conditions

![Graph showing the concentration of glucose and ethanol over time with and without control.](image)
Fermentation Normal Conditions

![Graph showing concentration of glucose and ethanol over time with and without control.](image-url)
Glycerol Production

![Graph showing glycerol production over time with and without control.](image-url)
Non Traditional Enzyme Addition

- Protease
- Cell wall degrading
- Native starch degrading
Protease Addition in High Starch Fermentations

Final Ethanol Concentration (%v/v)

- Yeast A
  - Without Protease: 16%
  - With Protease: 17%

- Yeast B
  - Without Protease: 17%
  - With Protease: 18%

- Yeast C
  - Without Protease: 17%
  - With Protease: 19%

- Yeast D
  - Without Protease: 18%
  - With Protease: 20%
Protease Addition in High Starch Fermentations

Residual Glucose (%w/v)

Without Protease
With Protease

Yeast A
Yeast B
Yeast C
Yeast D
Amino Acid Profiles

Concentration (pmol/μL)

Aspartate
Glutamate
Asparagine
Serine
Glutamine
Histidine
Glycine
Threonine
Citrulline
Arginine
Alanine
Tyrosine
Valine
Methionine
Tryptophan
Phenylalanine
Isoleucine
Leucine
Lysine
Hydroxyproline
Proline

GC106 added
No GC106
## Results

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<tr>
<th>Enzyme</th>
<th>Liquid Weight (g)</th>
<th>Solid Weight (g dry wt)</th>
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<td>30.35</td>
<td>1.81</td>
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<tr>
<td>B</td>
<td>27.59</td>
<td>2.22</td>
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<tr>
<td>C</td>
<td>28.78</td>
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<td>D</td>
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<td>E</td>
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<td>1.97</td>
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<td>F</td>
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<td>O</td>
<td>29.43</td>
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<tr>
<td>Control</td>
<td>27.36</td>
<td>2.16</td>
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</table>
Results

Enzyme A  Control
Dry-grind Ethanol Production

Corn

Grinding (Hammermill)

Water

Blending

Mash

Liquefaction

Yeast & Enzymes

Saccharification & Fermentation

CO₂

Overhead product (Recycled back)

Dehydration column

Ethanol

DDGS

Stripping/Rectifying column
Degradation caused by enzymes used during Enzymatic Milling

Great Enzyme for Enzymatic Dry Grind Processing
Enzymatic Germ/Fiber Recovery in a Dry Grind Ethanol Process

- Modified dry grind ethanol process
- Utilizes enzymes to alter the specific gravity allowing physical separations
- Can be used to recover Germ or Germ and Pericarp fiber together
- Significant improvement over previous germ and fiber recovery processes
- Patent was allowed in November 2004
  US Patent #6,899,910
Enzymatic Germ and Fiber Recovery Process

**Enzymatic Preprocessing**
- Corn
  - Soaking
  - Incubation

**Germ & Fiber Process**
- Grinding (Degermination mill)
- Germ & Fiber Dryer
- Germ clones
  - Air
- Aspirator
- Germ
- Fiber
- Corn Fiber Oil
- Corn Fiber Gum
- Other Chemicals

**Protein Recovery**
- Fine Grinding (Degermination mill)
- Gluten Dryer
  - Gluten + Fine Fiber

**Yeast & Enzymes**
- Liquefaction
- Saccharification & Fermentation

**Overhead product**
- (Recycled back)

**Dehydration column**
- Stripping/Rectifying column
- Ethanol
- Solubles
Fermentation Profiles

Conv. - Conventional Corn Dry Grind Processing
QG - Quick Germ process
QGQF - Quick Germ and Quick Fiber
E-Mill - Enzymatic Germ and Fiber Recovery

Cereal Chemistry 82(2):187-190
# DDGS Nutrient Analysis

<table>
<thead>
<tr>
<th>%</th>
<th>Conv.</th>
<th>QG</th>
<th>QGQF</th>
<th>E-Mill</th>
<th>Soy Meal</th>
<th>CGM</th>
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<tbody>
<tr>
<td><strong>Crude Protein</strong></td>
<td>28.50</td>
<td>35.91</td>
<td>49.31</td>
<td>58.50</td>
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<td><strong>Crude Fat</strong></td>
<td>12.70</td>
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<td><strong>Ash</strong></td>
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<td>ND</td>
<td>ND</td>
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<tr>
<td><strong>Acid Detergent Fiber</strong></td>
<td>10.8</td>
<td>8.22</td>
<td>6.80</td>
<td>2.03</td>
<td>5.95</td>
<td>6.88</td>
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</table>

 Conv. - Conventional Corn Dry Grind Processing  
 QG - Quick Germ process  
 QGQF - Quick Germ and Quick Fiber  
 E-Mill - Enzymatic Germ and Fiber Recovery  
 CGM - Corn Gluten Meal  

*Cereal Chemistry 82(2):187-190*
Benefits of Germ and Fiber Recovery in a Dry Grind Ethanol Process

- Recovery of valuable coproducts: reduction in net corn cost
  - Corn germ
  - Corn fiber
    - Corn fiber oil
    - Corn fiber gum
    - Feedstock for fuels and chemicals
- Increased ethanol capacity
- Reduction in fouling of thin stillage evaporators
- Germ and Fiber dilute the protein content of DDGS
  - Removal increases the protein content of DDGS
Process Engineering & Cost Models

• Important to compare “Base Case” with the “Modified Process”.
  - Determine benefits and added costs.
  - Discover new areas for research.
  - Resolve problems
Dry Grind Process for Ethanol using Corn
Corn Cost Impact on Starch Production Costs

Cost of Corn $/Bu

- $0.03
- $0.04
- $0.05
- $0.06
- $0.07
- $0.08
- $0.09
- $0.10
- $0.11
- $0.12
- $0.13

Production Cost - Starch

Net Corn price

Gross Corn Price

Graph showing the relationship between the cost of corn and the production cost of starch.
Estimated Enzyme Cost Using Current Process Model Estimates

**Batch Process:**
- Amount of enzyme required: 1 mL/kg or 25.4 mL/bu
- Current Cost of enzyme: $15/kg or $15/850 mL
- Enzyme cost per bushel: $0.45 / bu

**Continuous Process:**
- Amount of enzyme required: 0.114 mL/kg or 2.9 mL/bu
- Current Cost of enzyme: $15/kg or $15/850 mL
- Enzyme cost per bushel: $0.05 / bu

Estimates calculated using current cost of enzyme.
No processing cost savings are included.
Pre-Commercialization

- E-Milling plant trial was conducted in the summer of 2005
  - (Starch recoveries were all higher than the conventional runs for each of the 4 enzymatic trials conducted)

- Pilot scale trial of the enzymatic germ and fiber recovery process was conducted in July at ERRC. Lab scale optimization completed.

- Economic Comparisons of each process with “Base Case” currently being done.
Wet Milling Plant Trial

1st Grind and Enzyme Addition

Gluten

Starch

Processing Team
Conclusions / Predictions

- Enzymes will continue to be an integral part of the ethanol process.
- Enzyme use will continue to expand in the ethanol industry as well as other commercial processes.
- New enzymes and applications will continue to be developed.
- The economics of enzyme use will continue to improve.
$340.00/Kg

$75.00 /Kg

$15.00 /Kg

$?
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