**Abstract and Research Motivation**

Corn biorefineries with diversified product portfolios offer great potential for corn growers and sugar producers by providing new, high margin market opportunities to capture added value and a higher return on investment. Conventional dry grind utilizes starch to produce ethanol, while leaving all other components (germ, pericarp) unutilized and mixed together as in distiller’s grains. Wet mill processes involve steeping at elevated temperatures. In this study, we present a new approach for disassembly rather than destruction of corn kernels into its components (starch, pericarp, and germ oil) by enzyme catalysis at temperatures of 50 to 60°C (Figure 1). The enzymes are formulated to separate pericarp from endosperm while leaving germ oil floating on the reaction solution at the end of the process. The process involves no chemical steeping of corn kernels prior to the enzymatic deconstruction and can be easily adapted to a conventional dry grind process. There is no mechanical grinding, while the tip cap is mechanically removed so that the process is initiated when enzyme is added.

Fractionation of pericarp and germ, followed by washing will generate a starch stream which is subsequently hydrolyzed to glucose by amylases. The enzymes are specifically formulated for this task by screening numerous commercially available enzymes that will disassemble corn kernels. To facilitate the enzyme penetration, the tip caps of kernels are removed. This process provides an alternative approach to fractionate corn kernels into components that are suitable for production of chemical building blocks for polymers, chemicals, and liquid fuels.

**Materials and Methods**

- **Substrate:** Dried yellow corn kernels (12% moisture), tip caps removed. 30% w/v slurry in pH 5.5 sodium citrate buffer.
- **Enzyme:** 2% v/v of Pectinase 162L (from Biocatalysts) (a peeling enzyme used to separate the skin from fruit prior to juicing)
- **Hydrolysis:** pH 5.5, 45°C, 250 rpm, 2 weeks
- **Enzyme Screening experiment:** Depol 692L, Depol 740L, Pectinase 162L, Pectinase 656L from Biocatalysts and Spezyme CP from Genencor were used for enzyme screening purpose. Hydrolysis conditions were the same as above except that corn kernel slurry was at 15% w/v. Enzymes were added at 5% wt of dry corn kernels.
- **Fractionate corn kernels into starch, pericarp, and germ using a mixture of enzyme only
- **No chemical soaking
- **Minimal power input
- **Allowing industries to maximize profits from each fractionated component**

**Diversified Product portfolio for Conventional Corn-to-Ethanol Industries**

- Corn biorefineries with diversified product portfolios offer great potential for corn farmers and sugar producers to capture added value, and a higher return on investment, while achieving energy and economic goals simultaneously [1].
- Sugar derived platform chemicals include
  - Hydroxymethylfurfural (HMF)
  - Furfural
  - Levulinic acid
  - γ-Valerolactone

**The key is to cost-effectively obtain relatively pure sugar stream in a concentrated form that is suitable for catalytic conversion to alkanes, and to precursor molecules for use in production of polymers, lubricants, and herbicides.**

**Results**

Table 1. Measured activities of commercial enzymes used for enzyme screening.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Cellulase (FPU/ml)</th>
<th>β-glucosidase (CBU/ml)</th>
<th>Xylanase (OXS/ml)</th>
<th>β-xylosidase (U/ml)</th>
<th>β-amylase (U/ml)</th>
<th>arabinofuranosidase (U/ml)</th>
<th>Ferulic acid esterase (U/g)</th>
<th>p-coumaryl esterase (U/ml)</th>
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<tbody>
<tr>
<td>Spezyme CP</td>
<td>C</td>
<td>30</td>
<td>128</td>
<td>2622</td>
<td>7.3</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Depol 740L</td>
<td>FAE</td>
<td>0.3 (nd)</td>
<td>9.1</td>
<td>88.5</td>
<td>30.3</td>
<td>9.9</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Depol 692L</td>
<td>F, X, FAE</td>
<td>5.9</td>
<td>11</td>
<td>1,510</td>
<td>18.2</td>
<td>354.5</td>
<td>38.7</td>
<td>102.7</td>
</tr>
<tr>
<td>Pectinase 162L</td>
<td>C, P</td>
<td>24.6</td>
<td>90.5</td>
<td>120</td>
<td>73</td>
<td>2,817.9</td>
<td>149.7</td>
<td>152.7</td>
</tr>
<tr>
<td>Pectinase 656L</td>
<td>P</td>
<td>0.03 (nd)</td>
<td>92.3</td>
<td>71.3</td>
<td>6.5</td>
<td>2,507.1</td>
<td>244.4</td>
<td>129.8</td>
</tr>
</tbody>
</table>

**Acknowledgments**

This work was supported by Indiana Corn Marketing Council (Sponsor Award Number: 11076424), Michael R. Ladisch is CTO at Mascoma Corporation.