STARGEN™ 002
Granular Starch Hydrolyzing Enzyme for Ethanol Production

DESCRIPTION

STARGEN™ 002 enzyme contains Aspergillus kawachi alpha-amylase expressed in Trichoderma reesei and a gluco-amylase from Trichoderma reesei that work synergistically to hydrolyze granular starch substrate to glucose. The endo-activity, alphaamylase and exo-activity, gluco-amylase catalyze the complete hydrolysis of granular starch under a variety of alcohol fermentation conditions.

TYPICAL CHARACTERISTICS

Activity: 570 GAU/g (minimum)
Appearance: Clear brown liquid
Specific gravity: 1.13 - 1.16 g/ml
pH: 4.0 - 4.5

UNIT DEFINITION

One Glucoamylase Unit (GAU) is the amount of enzyme that will liberate one gram of reducing sugars calculated as glucose per hour from soluble starch substrate under the conditions of the assay. A detailed assay method is available upon request.

PERFORMANCE BENEFITS

STARGEN™ 002 enzyme provides the following benefits to ethanol producers:

- Hydrolyzes granular starch without the need for starch gelatinization or liquefaction.
- Maintains high activity under simultaneous saccharification and fermentation conditions (pH and temperature) favoured by yeast for ethanol production.

When used in a well designed no-cook ethanol production process and compared to conventional liquefaction and saccharification enzyme system with a cook step it offers:

- Higher ethanol yield per unit of grain
- Reduced energy input per unit of ethanol produced
- Reduced unit operations (e.g. cook, liquefaction)
- Improved sustainability of the production process

APPLICATIONS

STARGEN™ 002 enzyme is used to saccharify un-cooked grain mashes from various sources including wheat, triticale, rye, barley and rice.

The resultant glucose is fermented by yeast or another appropriate microorganism to yield alcohol. STARGEN™ 002 may be added directly to the fermentor.

STARGEN™ 002 enzyme may be favourably combined with enzyme products like OPTIMASH™ to reduce mash viscosity or FERMGEN™ to provide amino acids and peptides as yeast food.

RECOMMENDED OPERATIONAL CONDITIONS

<table>
<thead>
<tr>
<th>pH</th>
<th>3.3-4.5 (To inhibit lactic acid bacteria a low pH might be preferred)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Solids</td>
<td>20-34% w/w</td>
</tr>
<tr>
<td>Simultaneous</td>
<td>Pretreatment: recommended for all grains to improve hydration and increase conversion rate. The recommended minimum temperature is 48°C (118°F) and is specific for the type of raw material. Fermentation: 24-76 hours at 20-40°C (68-104°F), with sufficient agitation to keep solids suspended.</td>
</tr>
</tbody>
</table>

DOSAGE GUIDELINES

The optimal usage level of STARGEN™ 002 enzyme in simultaneous saccharification and fermentation is dependant upon processing parameters such as type of raw material, DS (Dry Solids), fermentation time, pH and temperature. A minimum STARGEN™ 002 starting dose of 0.8 - 1.6 kg/MT (metric ton) of grain is recommended under most fermentation conditions. Grains like corn or milo might require a higher dose.
EFFECT OF GRAIN PARTICLE SIZE

Hammermills are typically used in dry milling of grains. The recommended screen hole diameter are specific for the raw material used.

<table>
<thead>
<tr>
<th>Screen Hole Diameter</th>
<th>Wheat/Triticale/Rye</th>
<th>Barley</th>
<th>Corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td>2.5</td>
<td>3.0</td>
<td>1.5</td>
</tr>
<tr>
<td>US mesh</td>
<td>8</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

IMPACT OF SLURRY TEMPERATURE

To improve hydration of milled grains and include hot plant effluents like condensates or thin stillage optimal mixing temperatures for each raw material needs to be adjusted.

<table>
<thead>
<tr>
<th>Grain</th>
<th>(°C)</th>
<th>(°F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye</td>
<td>49-51</td>
<td>120-124</td>
</tr>
<tr>
<td>Wheat</td>
<td>56-57</td>
<td>133-135</td>
</tr>
<tr>
<td>Triticale</td>
<td>56-57</td>
<td>133-135</td>
</tr>
<tr>
<td>Barley</td>
<td>56-57</td>
<td>133-135</td>
</tr>
<tr>
<td>Corn</td>
<td>62-63</td>
<td>144-145</td>
</tr>
</tbody>
</table>

ENZYMES ADDED TO THE MIXING STEP

To reduce mash viscosity and to activate the starch granules GC 626 (acid alpha amylase) and hemicellulases (OPTIMASH™) should be added to the mixing tank. The recommended pH during mixing is at 3.5-4.0.

<table>
<thead>
<tr>
<th>Grain</th>
<th>GC 626 Enzyme (kg/MT)</th>
<th>OPTIMASH™ Enzyme (kg/MT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye</td>
<td>0.13</td>
<td>VR 0.19</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.16</td>
<td>-BG 0.10</td>
</tr>
<tr>
<td>Triticale</td>
<td>0.13</td>
<td>-VR 0.10</td>
</tr>
<tr>
<td>Barley</td>
<td>0.13</td>
<td>-BG 0.20</td>
</tr>
<tr>
<td>Corn</td>
<td>0.16</td>
<td>none</td>
</tr>
</tbody>
</table>

EFFECT OF STARGEN™ 002 ENZYME CONCENTRATION

STARGEN™ 002 enzyme is a highly active product. As the amount of enzyme is increased, the rate of fermentation increases.
STORAGE & STABILITY

To ensure maximum retention of activity, store STARGEN™ 002 enzyme under refrigerated conditions with the container closed. Prolonged storage at elevated temperature should be avoided. For more information on the storage of this product, please contact your DuPont representative.

PACKAGING

STARGEN™ 002 enzyme is available in various packaging sizes. Please contact DuPont for detailed information.

SAFETY & ENZYME HANDLING

Inhalation of enzyme dust and mists should be avoided. In case of contact with the skin or eyes, promptly rinse with water for at least 15 minutes.

For detailed handling information, please refer to the appropriate Material Safety Data Sheet, the Enzyme Technical Association (ETA) handbook Working Safely With Enzymes, and the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) handbook Guide to the Safe Handling of Microbial Enzyme Preparations. All are available from DuPont.

TECHNICAL SERVICE

DuPont will work with customers to enhance processes and solve problems. Let us know what you need and we will assist you.

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REV0712 3054 STARGEN002 02