STARGEN™ 002 WB
Granular Starch Hydrolyzing Enzyme for Ethanol Production

DESCRIPTION

STARGEN™ 002 WB 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 WB 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 WB 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 WB may be added directly to the fermentor.

STARGEN™ 002 WB 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 Saccharification and Fermentation</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 WB 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 WB 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 WB ENZYME CONCENTRATION**

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

![Graph showing effect of STARGEN™ 002 WB enzyme concentration on fermentation rate]

Experimental conditions: Substrate: milled whole wheat, 28% DS, fermentation temperature: 32°C, 0.05 kg/MT FERMGEN™ protease, 400 ppm urea. Mash pretreated at 56°C for 90 minutes.

![Graph showing ethanol production from different STARGEN™ 002 doses]

- 0.80 kg STARGEN™ 002/MT
- 1.00 kg STARGEN™ 002/MT
- 1.20 kg STARGEN™ 002/MT

- 67 hrs ferm. time
- 52 hrs ferm. time

Experimental conditions: Substrate: milled whole corn, 28% DS, fermentation temperature: 32°C, 0.20 kg/MT FERMGEN™ protease, 400 ppm urea. Mash pretreated at 60°C for 90 minutes.
STORAGE & STABILITY

To ensure maximum retention of activity, store STARGEN™ 002 WB 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 WB 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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