

# ACCELLERASE® TRIO™

Optimized Cellulase, Hemicellulase and Beta-Glucosidase Enzyme Complex for Improved Lignocellulosic Biomass Hydrolysis

## Product Information

### DESCRIPTION

ACCELLERASE® TRIO™ is an enzyme complex designed specifically for use in the lignocellulosic biorefinery industries producing renewable fuels and chemicals. Lignocellulosic material is composed mainly of cellulose, hemicellulose, and beta-glucans which are associated with each other and also with lignin, pectins, proteins, starch, and lipids. ACCELLERASE® TRIO™ efficiently hydrolyzes lignocellulosic biomass into fermentable monosaccharides such as glucose and xylose. The newest addition to our Accellerase® product portfolio, ACCELLERASE® TRIO™ is an optimized enzyme complex designed to further enable the commercialization of second-generation biorefineries. Key benefits and properties of the proprietary enzyme complex include:

- Dose-efficiency and cost-effectiveness – often a two-fold dose reduction, compared to ACCELLERASE® DUET.
- All in one product with optimized composition of enzyme activities delivering higher biomass saccharification rates and increased conversion of both glucan and xylan, thus leading to higher ethanol yields when using microorganisms capable of utilizing six-carbon (C6) and five-carbon (C5) carbohydrates.
- Engineered β-glucosidase for increased glucan conversion and reduced product inhibition.
- Improved viscosity reduction enabling performance at high biomass solids loadings.
- Broader operating conditions for greater flexibility of process configuration including Saccharification; Sequential hydrolysis and fermentation (SHF); Hybrid saccharification and fermentation (HSF); and Simultaneous saccharification and fermentation (SSF).
- Wide feedstock and pretreatment flexibility.
- Whole broth formulation provides nutrients for fermentative organisms reducing the energy and chemical inputs in the product manufacturing cycle and lowers the chemical load introduced into our customers' processes.

ACCELLERASE® TRIO™ is produced using genetically modified *Trichoderma reesei*. The production host is inactivated at the end of the controlled fermentation.

### TYPICAL CHARACTERISTICS

ACCELLERASE® TRIO™ enzyme complex contains a potent combination of multiple enzyme activities including exoglucanase, endoglucanase, hemi-cellulases (including xylanases), and β-glucosidase. The activities of ACCELLERASE® TRIO™ enzyme complex are expressed in carboxymethylcellulase (CMC U) units, acid birchwood xylanase Units (ABXU), and pNP-glucosidase units (pNPG U). The biomass hydrolysis performance of this enzyme complex is a result of the synergistic effect of all the main and accessory activities and cannot be completely evaluated on the basis of the declared activities alone.

<i>Endoglucanase Activity:</i>	2000 – 2600 CMC U/g
<i>Xylanase Activity:</i>	> 3000 ABX U/g
<i>Beta-Glucosidase Activity:</i>	> 2000 pNPG U/g
<i>Appearance:</i>	Brown liquid, with suspended solids
<i>pH:</i>	4.3-4.5

One CMC unit of activity liberates 1 μmol of reducing sugars (expressed as glucose equivalents) in one minute at 50°C (122°F) and pH 4.8. One ABX unit is defined as the amount of enzyme required to generate 1 μmol of xylose reducing sugar equivalents per minute at 50°C (122°F) and pH 5.3. One pNPG unit denotes 1 μmol of nitro-phenol liberated from para-nitrophenyl-B-D-glucopyranoside per minute at 50°C (122°F) and pH 4.8. Detailed assay methods are available upon request.





## APPLICATIONS RECOMMENDATIONS

ACCELLERASE® TRIO™ enzyme complex will hydrolyze the lignocellulosic carbohydrates into fermentable monosaccharides as well as improve materials handling through liquefaction and viscosity reduction. ACCELLERASE® TRIO™ works on a wide range of feedstocks including paper pulp, corn stover and cob, corn fiber, sugarcane bagasse, wheat straw, wood chips, waste paper, rice straw, municipal solid waste (MSW), switchgrass and many others.

ACCELLERASE® TRIO™ is compatible with a variety of pretreatments including alkaline, ammonia fiber-expansion (AFEX), dilute acid, steam expansion and thermal/mechanical pretreatments. ACCELLERASE® TRIO™ works in all biomass conversion processes including saccharification only, sequential hydrolysis and fermentation (SHF), hybrid saccharification and fermentation (HSF), and simultaneous saccharification and fermentation (SSF).

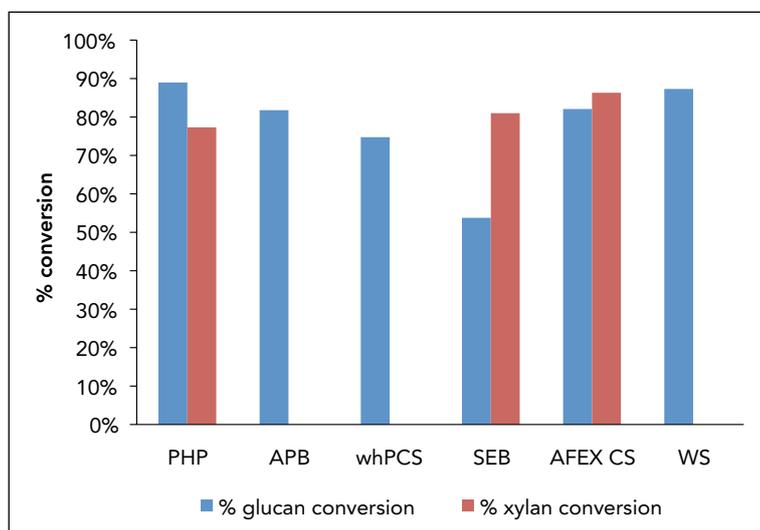
## DOSAGE GUIDELINES

The optimum dosage levels of ACCELLERASE® TRIO™ enzyme complex will vary considerably with different substrates and their associated pretreatments. Operating conditions such as pH, temperature and reaction time may also affect enzyme performance. **Please be aware that the pH and temperature stability optima and limits**

of the enzyme in use will depend upon the other operating parameters and your process configuration. Saccharification performance may be enhanced by the addition of other Genencor® enzymes depending on the type of pretreatment and/or composition of the pretreated feedstock.

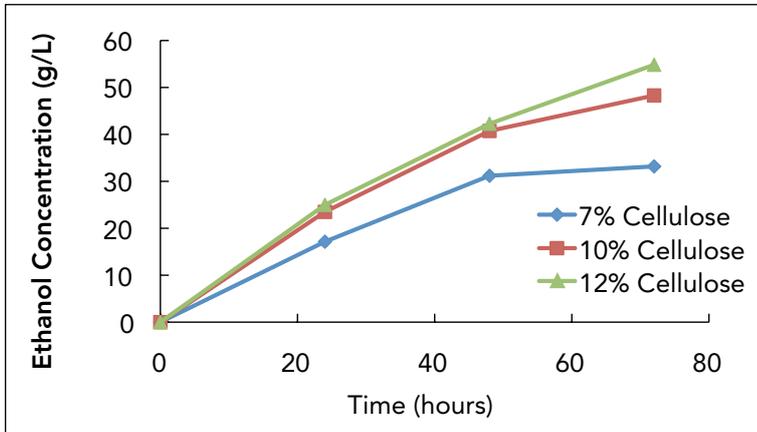
An ACCELLERASE® TRIO™ dosage rate of 0.05 - 0.3 mL per gram cellulose or roughly 0.03 -0.16 mL per gram of biomass (depending on biomass composition) is recommended as a starting point for optimization of enzyme dosage. ACCELLERASE® TRIO™ rapidly liquefies and hydrolyzes a variety of substrates within 6-24 hours. Extending the hydrolysis time may provide additional benefits. Small-scale experiments are recommended to determine optimum enzyme performance in each system. Figures 1-2 show the results of using ACCELLERASE® TRIO™ enzyme complex on pretreated hardwood pulp\* (PHP), acid-pretreated bagasse (APB), acid-pretreated whole hydrolysate corn stover (whPCS, NREL), sub-optimally steam pretreated bagasse (SEB), ammonia fiber expansion corn stover (AFEX CS), and pretreated wheat straw (WS) in saccharification only or in SSF process configurations (100g batch-scale experiments in 500mL shake flasks).

### Saccharification Only:



**Figure 1. Substrate Flexibility:** Percent glucan and xylan conversion of a pretreated hardwood pulp\* (PHP), acid-pretreated bagasse (APB), NREL acid-pretreated corn stover (whPCS), sub-optimally steam pretreated bagasse (SEB), ammonia fiber expansion corn stover (AFEX CS), and pretreated wheat straw (WS) using ACCELLERASE® TRIO™ at 0.22 mL/g cellulose, 7% cellulose loading, 50°C, pH 5.0, and in 3 days.

## Simultaneous Saccharification and Fermentation



**Figure 2. Simultaneous saccharification and fermentation (SSF) at high solids:** SSF of a pretreated hardwood pulp\* (PHP) at 7%, 10%, and 12% cellulose loading using ACCELLERASE<sup>®</sup> TRIO<sup>™</sup> at 0.22 mL/g cellulose, pH 5.0, and 37°C with THERMOSACC<sup>®</sup> DRY yeast (Milwaukee, WI), an industrial yeast only capable of utilizing C6 sugars. At 3 days, xylose concentrations of 16.6, 24.5, and 28.5 were observed, respectively, representing the potential for an additional 7.6, 11.2, and 13.1 g/L ethanol that would be produced if using micro-organisms capable of utilizing xylose and assuming 90% fermentation yield.

## Effect of pH and Temperature

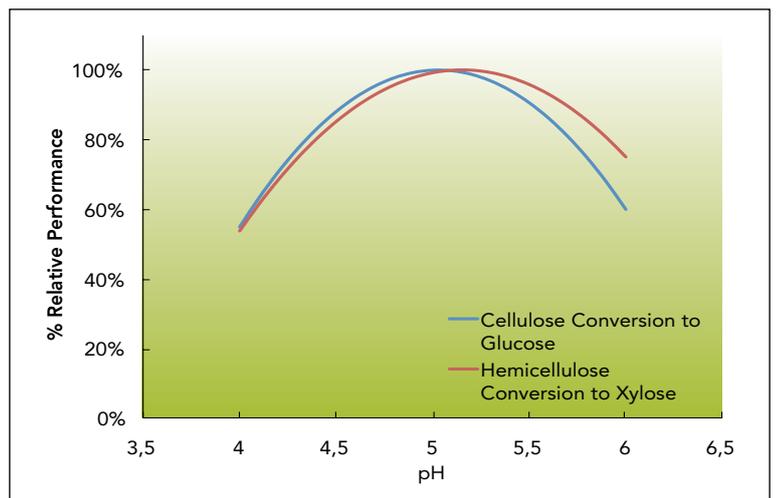
ACCELLERASE<sup>®</sup> TRIO<sup>™</sup> enzyme complex has the best operational stability in the following ranges:

pH: 4.0 – 6.0

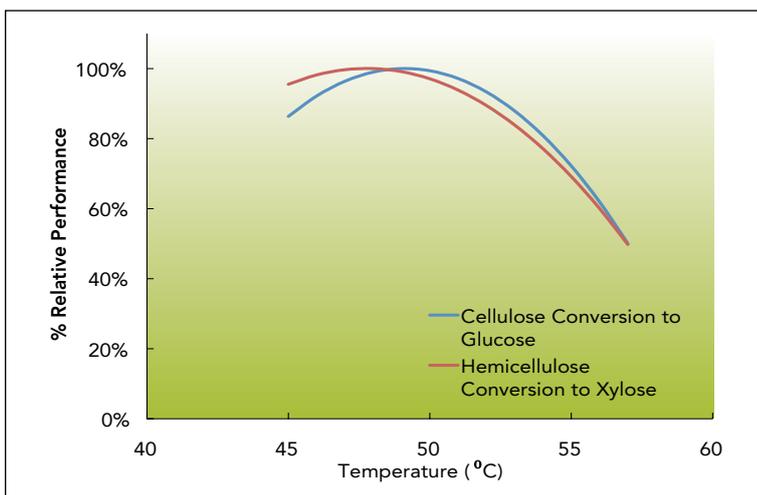
Temperature: 40 - 57°C (104 - 135°F)

ACCELLERASE<sup>®</sup> TRIO<sup>™</sup> enzyme complex can be inactivated at temperatures above 70°C (158°F) or at pH levels above 7.0 or below 4.0. Long term storage should be at 4°C (39°F).

\* Pretreated hardwood pulp was generated in research funded by Agence Nationale de la Recherche, France (ARN-05-BIOE-007) through L'Agence Nationale de l'Environnement et de la Maitrise de l'Energie (ADEME 0501 C0099).



**Figure 3. pH profile of ACCELLERASE<sup>®</sup> TRIO<sup>™</sup> enzyme complex at 50°C.** Activity measured over 24 hours using a pretreated hardwood pulp\* (75.1% glucan, 19.1% xylan) at 7.0% cellulose loading (9.32% dry solids).



**Figure 4. Temperature profile of ACCELLERASE<sup>®</sup> TRIO<sup>™</sup> enzyme complex at pH 5.0.** Activity measured over 24 hours using a pretreated hardwood pulp\* (75.1% glucan, 19.1% xylan) at 7.0% cellulose loading (9.32% dry solids).

## PACKAGING

ACCELLERASE<sup>®</sup> TRIO<sup>™</sup> enzyme complex is a bulk product sold only in totes (1000 kg) or at larger scale. Please consult your Genencor<sup>®</sup> representative for detailed information.

## STORAGE

It is advisable to store ACCELLERASE<sup>®</sup> TRIO<sup>™</sup> enzyme complex under refrigerated conditions of 4°C (39°F) and sheltered against direct sunlight. It may be stored at a controlled room temperature for up to 3 months. Storage above 25°C (77°F) should be avoided. The inactive production host in the product can settle gradually. This settling does not affect product performance, but large-scale storage with either gentle agitation or occasional pump recirculation is recommended.

## 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 (MSDS), 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 Genencor<sup>®</sup>.

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