

ACCELLERASE™ 1000

Cellulase Enzyme Complex for Lignocellulosic Biomass Hydrolysis

Product Information

■ DESCRIPTION

ACCELLERASE™ 1000 cellulase is an enzyme complex preparation intended specifically for the lignocellulosic biomass processing industries, including renewable fuels and chemicals. ACCELLERASE™ 1000 will facilitate process development and scale up in this emerging industry. Key features that are expected to be important at commercial scale biorefineries are already built in to this first generation product. Benefits observed with the proprietary enzyme complex and the unique product formulation of ACCELLERASE™ 1000 compared to conventional cellulases include:

- Enhanced saccharification performance on a variety of feedstocks.
- Ability to operate in simultaneous saccharification and fermentation (SSF) processes, two step sequential hydrolysis and fermentation (SHF) processes or hybrids of the two.
- High beta-glucosidase activity to minimize residual cellobiose, which may lead to higher rates of saccharification and ultimately to a faster ethanol fermentation. Yields may also be improved.
- Unclassified product. The remaining nutrients from enzyme production are available to the yeast in addition to the fermentable sugars produced by saccharification. This may lead to faster ethanol fermentations, reduce the cost of ethanol fermentation raw materials, and possibly even improve ethanol yields.
- Minimal formulation to ensure that enzyme formulation chemicals do not interfere with saccharification carbohydrate profile analysis or subsequent yeast fermentation.

ACCELLERASE™ 1000 enzyme complex contains a potent combination of enzymes which effectively modify and digest non-starch carbohydrates, the structural material of lignocellulosic biomass. 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. This product is capable of efficiently hydrolyzing lignocellulosic biomass into fermentable monosaccharides. ACCELLERASE™ 1000 contains high levels of beta-glucosidase to ensure almost complete conversion of cellobiose to glucose.

ACCELLERASE™ 1000 is produced with a genetically modified strain derived from *Trichoderma reesei*. The production host is inactivated at the end of the controlled fermentation.

■ TYPICAL CHARACTERISTICS

ACCELLERASE™ 1000 enzyme complex contains multiple enzyme activities; mainly exoglucanase, endoglucanase, hemicellulase and beta-glucosidase. The endoglucanase activity is standardized on the basis of its activity on carboxymethylcellulose (CMC). Beta-glucosidase activity is standardized on the basis of activity on pNP-glucoside. The

biomass hydrolysis performance of this enzyme preparation 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.

Endoglucanase Activity:	2500 CMC U/g (minimum)
Beta-Glucosidase Activity:	400 pNPG U/g (minimum)
Appearance:	Brown liquid
pH:	4.8 - 5.2

The activity of ACCELLERASE™ 1000 enzyme complex is expressed in carboxymethylcellulose (CMC U) activity units. One CMC U unit of activity liberates 1 μmol of reducing sugars (expressed as glucose equivalents) in one minute under specific assay conditions of 50°C (122°F) and pH 4.8. Beta-glucosidase is reported in pNPG units. One pNPG unit denotes 1 μmol of Nitrophenol liberated from para-nitrophenyl-B-D-glucopyranoside in 10 minutes at 50°C (122°F) and pH 4.8. Detailed assay methods are available upon request.

■ APPLICATIONS

ACCELLERASE™ 1000 enzyme complex will hydrolyze the non-starch carbohydrates in lignocellulosic biomass into fermentable monosaccharides as well as aid materials handling by liquefaction and viscosity reduction. Feedstocks including paper pulp, corn stover, sugar cane bagasse, wood chips, waste paper and many others can all be hydrolyzed using ACCELLERASE™ 1000. ACCELLERASE™ 1000 can work with a variety of pretreatments including dilute acid, AFEX and steam expansion. This can be done using simultaneous saccharification and fermentation (SSF) or in a two step sequential hydrolysis and fermentation (SHF) configuration. **Please be aware of pH and temperature stability optima and limits of the enzyme depending on your process configuration.** Saccharification performance may be enhanced by the addition of other Genencor® enzymes depending on the composition of the pretreated feedstock.

■ DOSAGE GUIDELINES

The optimum dosage levels of ACCELLERASE™ 1000 enzyme complex will vary considerably with different substrates and their associated pretreatment technologies and conditions. Operating conditions such as pH, temperature and reaction time may also affect enzyme performance. An ACCELLERASE™ 1000 dosage rate of 0.1 - 0.5 g (0.1 - 0.5 mL) per g cellulose or roughly 0.05 to 0.25 mL per g of biomass (depending on biomass composition) is recommended as a starting point for optimization of enzyme dosage. ACCELLERASE™ 1000 rapidly liquefies and hydrolyzes a variety of substrates within 24 hours, with some additional benefit by extending the time. Small-scale experiments are recommended to determine optimum enzyme dosage in each system. See Figures 1 and 2 for an example of such experiments.

Saccharification only:

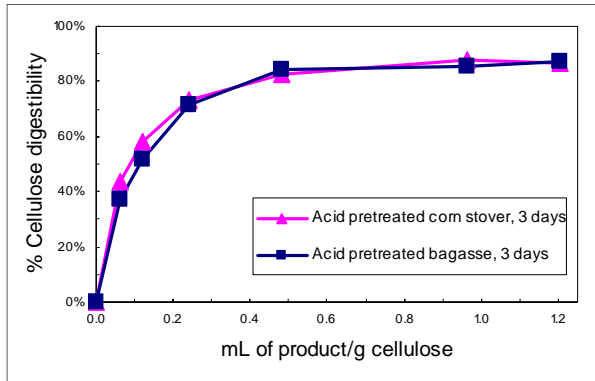


Figure 1: % Cellulose digestibility vs. mL of product per g cellulose for washed acid-pretreated corn stover and sugar cane bagasse using ACCELLERASE™ 1000 at 7% cellulose loading, 50°C, pH 5.0, and in 3 days.

Simultaneous Saccharification and Fermentation (SSF):

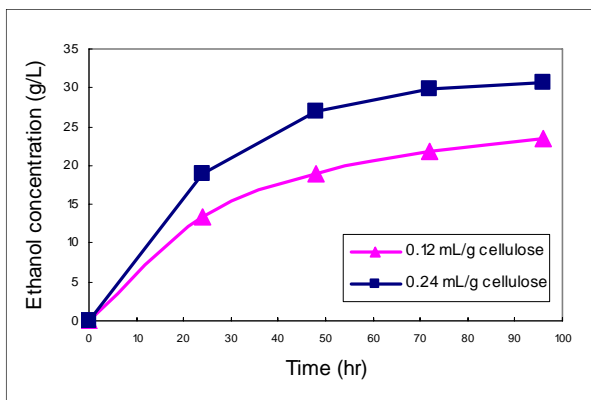


Figure 2: Ethanol concentration vs. time for two doses of enzyme (0.12 mL/g cellulose and 0.24 mL/g cellulose) in an SSF process. SSF of acid pretreated sugar cane bagasse using ACCELLERASE™ 1000 at 7% cellulose solids, pH 5.0, 38°C.

EFFECT OF pH AND TEMPERATURE

ACCELLERASE™ 10000 enzyme complex has the best operational stability in the following ranges:

Temperature: 50 - 65°C (122 - 149°F)

pH: 4.0 - 5.0

ACCELLERASE™ 1000 enzyme complex is easily 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).

PACKAGING

ACCELLERASE™ 1000 enzyme complex is available in various package sizes. Please consult your Genencor® representative for detailed information.

STORAGE

It is advisable to store ACCELLERASE™ 1000 enzyme complex under refrigerated conditions of 4°C (39°F) and sheltered against direct sunlight. Storage above 20°C (70°F) should be avoided. The product can settle gradually, so 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, 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®.

TECHNICAL SERVICE

Information covering specific applications of this product is available. Genencor® 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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