

# ACCELLERASE™ 1000

## Cellulase Enzyme Complex for Lignocellulosic Biomass Hydrolysis

### Technical Bulletin #1: Saccharification

#### ■ SUMMARY

ACCELLERASE™ 1000 cellulase is an enzyme 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 a higher rate of saccharification and ultimately to a faster ethanol fermentation. Yields may also be improved.
- Unclarified product. The remaining nutrients from enzyme production are available to the yeast in addition to the fermentable sugars produced by saccharification.
- Minimal formulation to ensure that enzyme formulation chemicals do not interfere with saccharification carbohydrate profile analysis or subsequent yeast fermentation.

Technical Bulletin #1 describes saccharification using ACCELLERASE™ 1000 enzyme complex. This bulletin first shows hydrolysis of a model cellulose (Phosphoric Acid Swollen Cellulose or PASC), and then discusses a more real-world application in the biofuel and biochemical industry where ACCELLERASE™ 1000 was used to hydrolyze dilute acid pre-treated biomass such as corn stover and sugar cane bagasse into fermentable glucose and xylose sugars. Enzyme hydrolysis experiments were performed on these two substrates at 7% cellulose loading (~13% solids) and the cellulose digestibility is reported.

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.

#### ■ SUBSTRATE INFORMATION

Substrates used in this work :

1. Phosphoric acid swollen cellulose (PASC) produced by a standard method (Wood, 1988) in which Avicel PH101 (Fluka 11365) was treated with 85% ortho-phosphoric acid, precipitated with acetone, washed and stored as a slurry. Although not an industrial lignocellulosic biomass substrate, this is a very easily digestible "amorphous" cellulose useful in demonstrating intrinsic stability and activity characteristics of cellulase preparations.
2. A dilute sulfuric acid pretreated corn stover (PCS) was produced and provided by NREL, as detailed in Schell et al 2003. The PCS was pretreated at a solids concentration of 20% (w/w), temperature of 165°C, 1.44% (w/w) acid and an approximate residence time of 8 minutes.
3. A dilute sulfuric acid pretreatment sugar cane bagasse (APB) produced in a similar manner to the PCS.

The cellulose composition of the corn stover and bagasse were 55.6% and 53.6% respectively. Compositional analyses were performed using the standard assays detailed in the NREL protocols for Standard Biomass Analytical Procedures.

[http://www1.eere.energy.gov/biomass/analytical\\_procedures.html](http://www1.eere.energy.gov/biomass/analytical_procedures.html)

#### ■ ENZYME PROPERTIES

ACCELLERASE™ 1000 enzyme complex contains multiple enzyme activities; mainly exoglucanase, endoglucanase, hemi-cellulase 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 its activity on para-nitrophenyl-B-D-glucopyranoside (pNPG). Detailed assay methods are available upon request. The product data sheet contains additional information on enzyme pH and temperature stability ranges. 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 only.

*Endoglucanase Activity:* 2500 CMC U/g (minimum)

*Beta-Glucosidase Activity:* 400 pNPG U/g (minimum)

*Appearance:* Brown liquid

*pH:* 4.8 - 5.2

#### ■ SACCHARIFICATION CONDITIONS

Enzymatic digestion studies were carried out based on NREL standard assay method LAP-009 "Enzymatic Saccharification of Lignocellulosic Biomass" (<http://devafdc.nrel.gov/pdfs/9578.pdf>), except for modification of enzyme and cellulose loading. The PASC hydrolysis was done at 1.2% cellulose and dosed with 0.2 mL product/g cellulose of ACCELLERASE™ 1000. pH optimum experiments were conducted at 2.5% PASC and dosed with 0.12 mL product/g cellulose. The acid pretreated corn stover and bagasse saccharification experiments were carried out at a higher 7% cellulose loading (~13% solids) at 50 °C and pH 5.0. The amount of enzyme dosed was 0.24 mL product/g cellulose. Samples of the hydrolysate were taken at various time intervals over 5 days and then analyzed for glucose concentration and cellobiose concentrations. All samples were performed in duplicate. The data reported was the average of the duplicates. The % cellulose digestibility was calculated by dividing the amount of cellulose digested by the amount of cellulose loaded. Glucose and cellobiose concentrations were determined using a Waters HPLC system (Alliance system, Waters Corp., Milford, MA). The HPLC column used for sugar analysis was purchased from BioRad (Aminex HPX-87P, BioRad Inc., Hercules, CA).

#### ■ RESULTS AND DISCUSSION

Figure 1A demonstrates the conversion of phosphoric acid swollen cellulose (PASC), loaded at 1.2% cellulose, over 6 hours. The optimal pH range for ACCELLERASE™ 1000 is shown to be 4.0 to 5.0 in Figure 1B. Figure 2 demonstrates that ACCELLERASE™ 1000 will rapidly hydrolyze acid pretreated sugar cane bagasse or corn stover into fermentable sugars within 24 hours at

the dosage level used, but that some additional benefit will be seen by extending the time. It is also shown that ACCELLERASE™ 1000 hydrolyses biomass at a significantly faster rate than Spezyme® CP, a common benchmark enzyme. Figure 3 shows the low residual cellobiose levels using ACCELLERASE™ 1000 versus Spezyme® CP. Most fermentation organisms, such as yeast, are unable to metabolize cellobiose. Additionally, the lower cellobiose levels may lead to a much faster hydrolysis during the first few days from reduced inhibition of the cellulases.

The optimum dosage levels of ACCELLERASE™ 1000 will vary considerably with different substrates and their associated pretreatment technologies and conditions. Operating conditions such as pH, temperature, and reaction time should also be optimized to improve cellulose digestibility.

An ACCELLERASE™ 1000 enzyme complex 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 the optimization of enzyme dosage. Small-scale experiments are recommended to determine optimum enzyme dosage in each system.

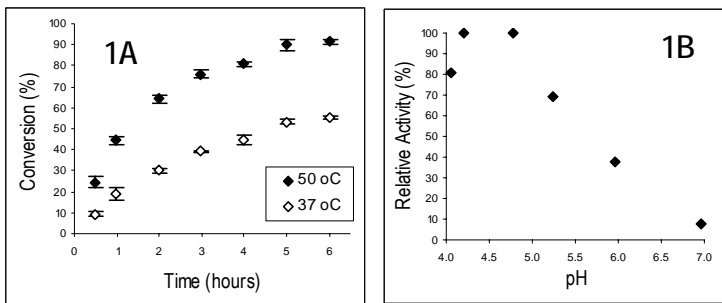


Figure 1A: % Cellulose digestibility using ACCELLERASE™ 1000 at 0.2 mL product/g cellulose on PASC at 1.2% cellulose loading, 37°C and 50°C, and pH 5.0. Note that the

Figure 1B: shows the pH optima to be 4.0 to 5.0 on 2.5% PASC after 7 hours at 25°C. Loading was 0.12 mL ACCELLERASE™ 1000/g cellulose.

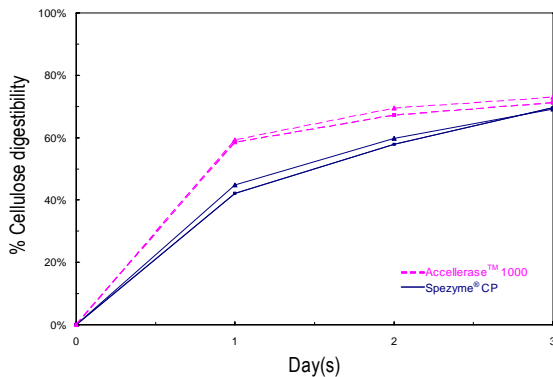


Figure 2: % Cellulose digestibility using ACCELLERASE™ 1000 at 0.24 mL product/g cellulose vs. an equivalent enzyme loading of Spezyme® CP on acid pretreated corn stover (▲) and sugar cane bagasse (■) at 7% cellulose loading, 50°C, and pH 5.0.

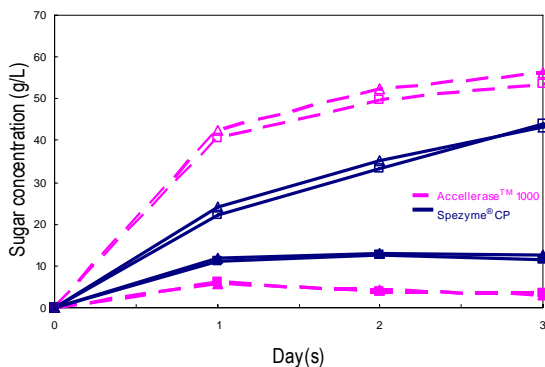


Figure 3: Low residual cellobiose (filled symbols) and higher glucose (open symbols) levels seen using ACCELLERASE™ 1000 at 0.24 mL product/g cellulose vs. the benchmark cellulase product Spezyme® CP on acid pretreated corn stover (▲) and sugar cane bagasse (■), 7% cellulose loading, 50°C, and pH 5.0.

## 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.

## REFERENCES

- Wood, T. (1988) in *Methods in Enzymology*, Vol. 160. Biomass - Part A: Cellulose and Hemicellulose (Wood, W. & Kellog, S. Eds.), pp. 19-25, Academic Press, San Diego, CA, USA
- Schell, D., Farmer, J. Newman, M., McMillan, J. 2003. Dilute-sulfuric Acid Pretreatment of Corn Stover in Pilot Scale Reactor; Investigation of Yields, Kinetics and Enzymatic Digestibilities of Solids. *App. Biochem. Biotechnol.* Vol. 105-108, 69-85.

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