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Inhibition of Cellulases by Impurities in Steam-Exploded Wood

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Abstract

Steam explosion of hardwood chips produces impurities that reduce the activity and stability of cellulases. Washing the exploded wood with water removes the inhibitors and permits hydrolysis equivalent to that with purified cellulose.

Index Entries: Inhibition, of *Trichoderma* cellulases; cellulases, inhibition of; impurities, inhibition of wood cellulases by; wood, inhibition of cellulases in.

Introduction

The search for renewable alternatives for fossil fuels has stimulated interest in wood as a feedstock for glucose and ethanol. The rigidity and durability that make wood a desirable construction material, however, also confer resistance to hydrolysis. The Iotech process uses steam explosion to break down wood. Sudden release of pressure after wood chips have been impregnated with steam disintegrates their structure and potentiates hydrolysis by cellulase enzymes. The main components of the exploded mixture are cellulose, lignin, and hemicellulose. Lignin is melted at the steaming temperature to form submicron-size balls; hemicellulose is partially hydrolyzed to sugars that can react further; the cellulose has a reduced degree of polymerization well-suited for enzymatic hydrolysis.

Unwashed exploded wood can be hydrolyzed directly, but the time required for complete reaction is prolonged. The research presented here develops explanations and ways to overcome this inferior performance.

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Materials and Methods

Exploded wood from mixed hardwood species was supplied by the Iotech Corporation of Ottawa. It is a coarse, dark brown powder with a faint odor indicative of furfural. Washing was conducted at room temperature using nine parts tap water to one part wood. The mixture was filtered and the solids were air dried.

Cellulase was estimated by assaying glucose formation from filter paper (filter paper units) (*1*). Endogluconase activity was estimated by viscosity decrease at 25°C resulting from the hydrolysis of 1–2 g/L of carboxymethylcellulose of medium viscosity grade (Sigma Chemical Co., St. Louis) (*2*). Beta-glucosidase activity was estimated at 25°C by glucose release from 2.0 μ*M* cellobiose (*2*). Enzyme used for the hydrolysis tests consisted of a mixture of one part, by volume, *Aspergillus niger* beta-glucosidase (Novo) to 74 parts *Trichoderma reesei* filtrate. The latter was supplied by the Iotech Corporation, and was prepared by fermenting washed, exploded wood with Rutgers C30 strain of *T. reesei*. The initial enzyme mixture was diluted 4.44-fold. Wood hydrolysis was conducted at 45°C with the pH held at 4.5 in 0.1*M* acetate buffer.

Results and Discussion

Initial reaction rates based on glucose kinetics and glucose formation for the hydrolysis of raw exploded wood and water washed wood are shown in Fig. 1. Unwashed wood gives a relationship of initial velocity to substrate concentration that is characteristic of substrate inhibition; little effect is seen at low substrate level, and powerful inhibition occurs when excess substrate is added. Similarly, glucose yield at a specified time of 120 h increases continually with the amount of

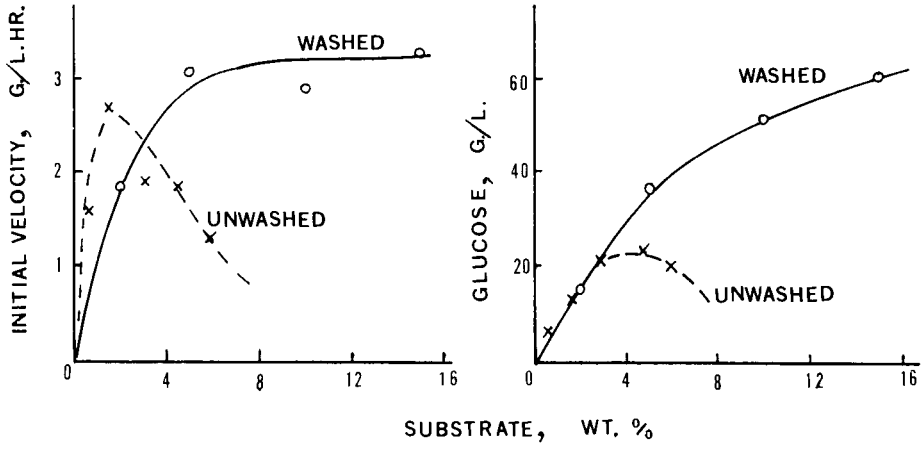

Fig. 1. Effect of substrate concentration on initial hydrolysis rate and glucose yield. Conditions: ten percent exploded wood with culture filtrate from *T. reesei* plus added beta-glucosidase (Novo). Net activities, 1 filter paper unit/mL; 2.25 U/mL endoglucanase; and 0.68 U/mL beta-glucosidase. Buffered at 25°C with gentle magnetic stirring at 45 C.

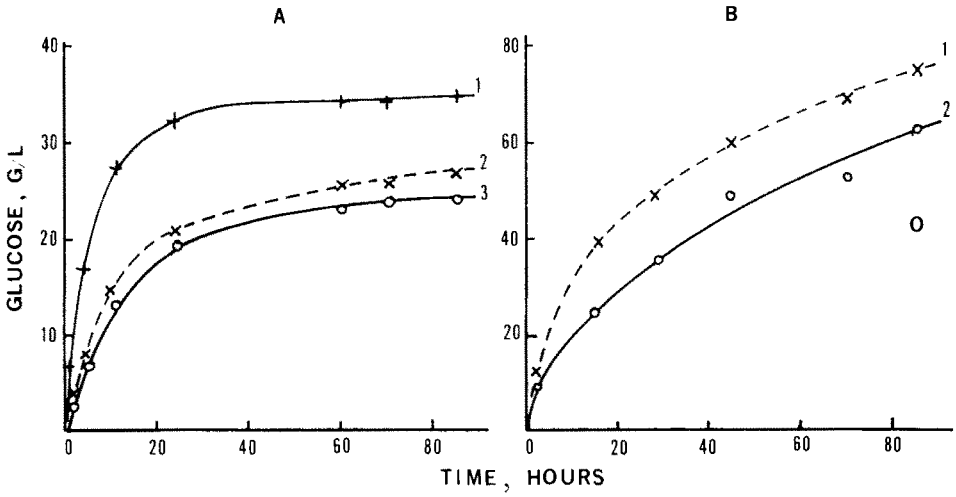

Fig. 2. Inhibition of hydrolysis by wash water from exploded wood. A1, washed exploded wood; A2, washed wood with washings added back; A3, unwashed exploded wood. Solids were 10 percent by weight. B. Solids were 10 percent by weight Solka Floc. 1, control; 2, with washings from exploded wood. All hydrolysis conditions were the same as for Fig. 1.

washed wood added. Unwashed wood has a poorer yield that peaks and then declines at higher levels of wood.

Inhibitors remain active in the wash water. Figure 2 shows hydrolysis of washed wood or of Solka Floc (a purified cellulose) when the wash water was added back. Unwashed wood and washed wood plus returned washings showed very similar hydrolysis behavior. Both are markedly inferior to that of washed wood alone. Solka Floc hydrolysis was also impaired when the wash water from exploded wood was present.

Although cellulases are a mixture of several activities, the inhibition does not appear to be specific for only one component. Both endogluconase and beta-glucosidase activities are affected, as shown in Table 1. Wash water destabilized beta-glucosidase to a considerable degree because its half-life in buffer at pH 4.5 at 44°C is roughly 100 h. Of course, part of the endoglucanase is adsorbed to the cellulose and leaves the solution. Beta-glucosidase has low affinity for cellulose, and loss of activity results from degradation or inhibition.

TABLE 1
Activity of Enzymes in Wash Water from Exploded Wood[a]

	Percentage of initial activity remaining	
Type of activity	After 10 min	After 100 h
Endoglucanase	48	39
Beta-glucosidase	20	6

[a]Conditions are the same as those for hydrolysis, but washings are without wood. Exploded wood was washed in 9 parts tap water at 25°C and filtered. Enzymes were buffered at pH 4.5.

Conclusions

Steam-exploded wood contains water-soluble impurities that inhibit enzymatic activity and destablize the enzyme mixture. The nature of the impurities has not been determined, but this is of more theoretical than practical interest. Furfural is a likely inhibitor; it is known to be present in exploded wood because of degradation of sugars from hemicellulose during steaming. Increases in both hydrolysis rate and enzyme stability result from washing exploded wood prior to hydrolysis.

References

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