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# Recovery of Enzymes from the Insoluble Residue of Hydrolyzed Wood

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## Abstract

During the enzymatic hydrolysis of steam-exploded wood, enzymes are adsorbed on both the cellulosic and noncellulosic components. Significant amounts of enzymes are recovered from the spent hydrolysis residue, which consists mostly of lignin, by neutralizing the pH from 4.5, the optimum for hydrolysis.

**Index Entries:** Recovery of insoluble wood enzymes; enzymes, recovery from insoluble wood residue; insoluble residue, of hydrolyzed wood, recovery of enzymes from; residue, of hydrolyzed wood, recovery of enzymes from; hydrolyzed wood, recovery of enzymes from; wood, recovery of insoluble enzymes from hydrolyzed residue of.

## Introduction

As technology advances, wood promises to become an important renewable feedstock for liquid fuels and chemicals (*1*). Sugars derived from wood can be fermented to ethanol or to other products equivalent to those now produced from fossil feedstocks. A method of steam explosion recently developed by the Iotech Corporation of Ottawa offers a feasible means to advance bioconversion techniques.‡ The procedure reduces wood chips to coarse granules, and disrupts the cellular structure so that cellulose is exposed and can be hydrolyzed enzymatically. Balances on the cellulase complex suggest that in the course of hydrolysis some en-

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‡Details of the process are proprietary. However, periodic reports have appeared in the *Alcohol Fuels Program Technical Review*, available from L. Douglas, SERI, 1617 Cole Blvd., Golden, Colorado 80401.


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zyme activities are adsorbed by the lignin-rich residue. Recovery of two such cellulases, endoglucanase and beta-glucosidase, is of importance because these enzymes are a major expense in the commercial hydrolysis of wood.

It has been shown that the adsorption of cellulosic enzymes by milled cotton linters can be reversed by altering the pH (*2*). A similar technique was used to recover endoglucanase and beta-glucosidase from the hydrolytic residue of steam-exploded wood.

## Materials and Methods

Wood cellulose was hydrolyzed both by column and by batch techniques. The resultant hydrolyzates were then assayed for endoglucanase and beta-glucosidase activities, and for glucose. In addition, the column hydrolyzate was also assayed for reducing sugars, and all residues were then treated to remove adsorbed enzymes.

In preliminary steps, steam-exploded wood of mixed hardwood species, provided by the Iotech Corporation, was washed with tap water, rinsed with distilled water, and then air-dried. Calculations were based upon a cellulose content of 56% as determined by Iotech. This is somewhat higher than raw wood contents of cellulose because steam explosion degrades some of the hemicellulose to volatile furfural. The lignin content of the sample, according to the suppliers, was 20%.

Cellulose hydrolysis was carried out by an initial enzyme mixture prepared with 44.4 mL of Rutgers C30 strain *Trichoderma reesei* filtrate, provided by Iotech, and 0.6 mL *Aspergillus niger* beta-glucosidase (Novo), yielding 11.9 mL of endoglucanase activity and 3.64 U/mL of beta-glucosidase activity.

All experiments were conducted at 23°C. Glucose was assayed by the glucose oxidase peroxidase method (*3*). Sugars were assayed by modification of the method of Nelson and Somogyi (*4*). Endoglucanase activity was determined by viscometry at 25°C using 1–2 g/L carboxymethylcellulose (*4*), and beta-glucosidase activity by paranitrophenol release from 0.5 m*M* *p*-nitrophenolglucoside (*4*).

### *Column Hydrolysis*

A column was packed with 6 g of steam-exploded wood, to a volume of 60 mL, and 13.33 mL of the initial enzyme mixture was added. When the meniscus of the enzyme reached the level of the wood, pH 4.5 acetate buffer (0.05*M*) was introduced and pumped continuously, at 0.78 mL/min, through the system for 130 h. The effluent was collected in 8 mL samples at intervals and assayed for sugars and enzymes. After 130 h, the volume of the column was approximately 30 mL. It must be noted that this cannot be a practical hydrolysis method because enzymes are washed out with the buffer, and the products are very dilute.

### *Batch Hydrolysis*

Batch hydrolysis was conducted by adding 135 mL of a 4.44-fold dilution of the initial enzyme mixture to 15 g of steam-exploded wood. Enzymes and sugars were assayed in 0.1 mL samples of hydrolyzate at intervals. After 130 h the residue was separated from the hydrolyzate by filtration.

*Elution of Adsorbed Enzyme*

Following cellulose hydrolysis, pH 7 phosphate buffer (0.5*M*) was pumped through the insoluble residue in the column, and the effluent was analyzed for endoglucanase and beta-glucosidase activities.

Damp residue (4.6 g) from batch hydrolysis was placed in a column, rinsed with 113 mL 0.1*M* acetate buffer (pH 4.5) to remove soluble or weakly adsorbed enzyme, and the effluent assayed for endoglucanase and beta-glucosidase. Neutral buffer was then added, and the effluent reassayed.

## Results and Discussion

Approximately 5.5 h were required for the enzyme mixture to pass through the column. The enzymes present in the effluent at 5.5 h represented those that were not adsorbed on the substrate. The results of column hydrolysis are shown in Fig. 1.

After 5.5 h, 60% of the original amount of beta-glucosidase had passed through in the hydrolysate. Endoglucanase activity in the hydrolyzate, on the other hand, represented only 27% of the initial amount. Clearly beta-glucosidase was less readily adsorbed than was endoglucanase. After the initial 5.5 h, the total conversion to sugars was 4.9%, and glucose was 4.2%.

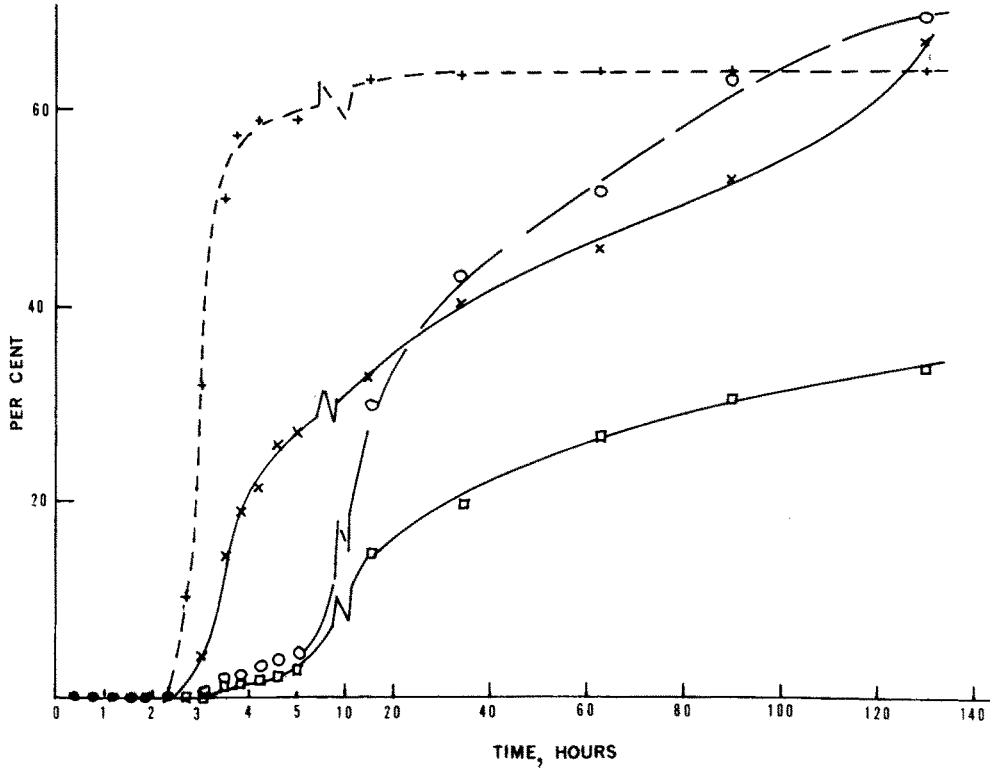


Fig. 1. Kinetics of cellulose hydrolysis in a column. x, endoglucanase; +, beta-glucosidase, ○ reducing sugars; □ glucose.

By 125 h no further sugars were produced and hydrolysis was at an end. At this time the yield of total sugars indicated 69% hydrolysis of cellulose, the yield of glucose was 34% or only about half of the total sugars produced. This finding is compatible with the change in relative composition of the two enzymes after 5.5 h.

Batch results confirmed that endoglucanase was more readily adsorbed than beta-glucosidase. After 1 h, 58% of the original amount of beta-glucosidase and 20% of endoglucanase activity were found in the aqueous fraction. Figure 2 shows that maximum batch hydrolysis was achieved by 130 h, with 85% conversion to glucose. At this time, endoglucanase activity in the aqueous fraction had risen to 30%. Beta-glucosidase had diminished to 17% of the initial amount.

Washing the column residue with pH 7 buffer resulted in recovery of an additional 20% of the original endoglucanase, but only 2.3% of beta-glucosidase. The batch filtrate gave similar results. The percentages of enzymes eluted and total enzyme recoveries are summarized in Table 1.

The decrease in beta-glucosidase activity in both systems suggests that the enzyme is either inactivated or bound very tightly to the residue. Because inactivation could be attributed to neither temperature nor pH, it is possible that it is brought about by prolonged contact with the substrate. The hypothesis of substrate inactivation was strengthened by repeating the column procedure, but minimizing the time of contact between beta-glucosidase and wood. By washing the substrate with pH 7 buffer immediately after the initial elution of enzyme, 100% of the original amount of beta-glucosidase was recovered.

Because of presumed inactivation, and the modest absorption of beta-glucosidase, it is impossible to recover significant amounts of this enzyme from either the hydrolyzate or the residue. However, a substantial amount of endoglucanase can be retrieved from the residue by changing the pH to 7.

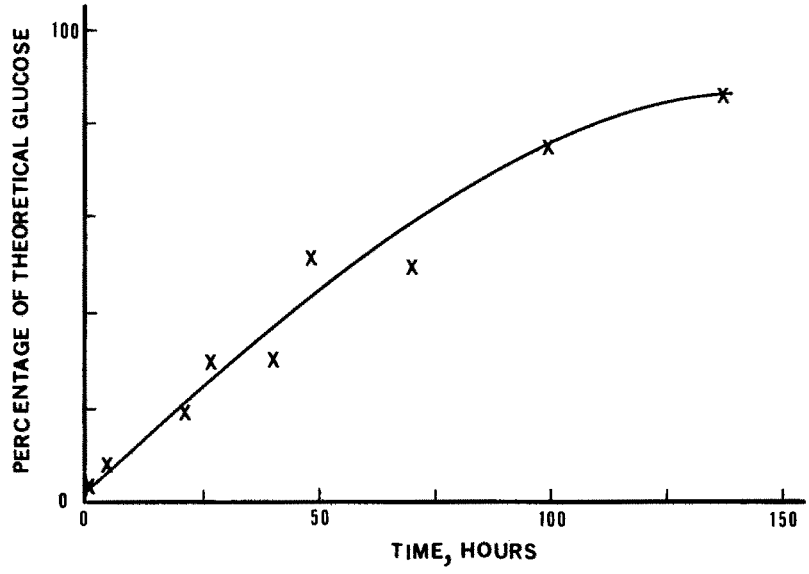


Fig. 2. Glucose formation in batch hydrolysis.

**Table 1**

| | Column | | Batch | |
|---|---|---|---|---|
| Total enzyme, % | Endo glucanase | Beta-glucosidase | Endo-glucanase | Beta-glucosidase |
| Recovered in hydrolyzate | 72 | 64 | 30 | 17 |
| Eluted at pH 4.5 | | | 12 | 16 |
| Eluted at pH 7 | 20 | 2.3 | 36 | 15 |
| Totals | 92 | 66 | 78 | 48 |

## Conclusion

The recovery of certain cellulases adsorbed on the solid residue from the hydrolysis mixture can be achieved through a simple pH adjustment to neutrality. Although these results are still to be verified at a larger scale, it appears that recovery and recycle of cellulases could have significant commercial potential.

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