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USE OF CHARCOAL TO MINIMIZE END PRODUCT INHIBITION IN ENZYMATIC HYDROLYSIS OF CELLULOSE

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SUMMARY

Tests made to improve saccharification of cellulose by Trichoderma cellulases showed that charcoal used as an adsorbent minimized the end product inhibition. Charcoal adsorbed both cellobiose and glucose and did not affect the enzymatic hydrolysis of cellulose. Results showed that charcoal is as effective as β-glucosidase in improving the enzymatic saccharification of cellulose.

INTRODUCTION

Enzymatic hydrolysis of cellulose eliminates the formation of unwanted by-products and it is more efficient than acid hydrolysis (Reese, 1959). However, products of cellulose hydrolysis, such as cellobiose and glucose, inhibit cellulolytic enzymes (Gong et. al., 1977; Maguire, 1977). As a result, the conversion efficiency of cellulose to sugars is low and the enzymes, the most expensive materials used in the process, are not utilized to their full potential. Also, because of end product inhibition, the substrate is only partially saccharified.

Extensive work has been carried out to minimize the inhibitory effects of end products on cellulolytic enzymes from Trichoderma species and their mutants. Cellobiose is a stronger inhibitor than glucose and with both sugars present, the extent of inhibition is increased (Howell and Stuck, 1975; Maguire, 1977). To lower the cellobiose concentration during saccharification, the Trichoderma cellulase system has been supplemented with β-glucosidase obtained from Aspergillus phoenicis (Sternberg et. al., 1977) or Botryodiplodia theobromae (Yamanake and Wilke, 1976). However, the presence of glucose also inhibits β-glucosidase (Gong et. al., 1977; Herr, D., 1980; Sternberg et. al., 1977). An alternate method of removing cellobiose utilizes cellobiose oxidase which converts cellobiose to cellobionic acid (Westermark and Eriksson, 1974). The removal of glucose by utilizing glucose isomerase, which converts glucose to fructose, has also been proposed (Woodward and Arnold, 1981). However, the use of additional enzymes is expensive.

This paper reports on the use of charcoal for the adsorption and removal of cellobiose and glucose in order to minimize their inhibitory effects on cellulases and consequently improve the saccharifying ability of Trichoderma cellulases.

MATERIALS AND METHODS

The organisms used were Trichoderma reesei Q.M. 9414 and Aspergillus phoenicis Q.M. 329. For this work, T. reesei Q.M. 9414 was selected because it produces only a small amount of B-glucosidase. For production of cellulases, T. reesei was cultivated in a medium containing cellulose (Whatman, CF-11) as the carbon source (Mandel and Andreotti, 1978). For production of β-glucosidase, A. phoenicis was cultivated in a medium containing starch as the carbon source (Allen and Sternberg, 1980). Enzyme production was carried out in shake flasks at 27°C. The concentration of these enzymes was carried out at room temperature at reduced pressure. Filter paper activity of the enzyme preparations were determined according to Mandels et. al. (1976), and β-glucosidase activity was determined by using cellobiose as a substrate (Khan and Lamb, 1984).

Cellulose hydrolysis was carried out using 5% cellulose (Whatman No. 1) suspended in 50 mM citrate-NaOH buffer at pH 4.8, and enzyme preparations containing 30-35 I.U. of filter paper activity / g of cellulose (Morisset and Khan, 1984). Saccharification was carried out under sterile conditions and a nitrogen atmosphere to minimize contamination. Incubation was done at 50°C. In these tests, granular charcoal (10-18 mesh, B.D.H. Chemical, Ltd., Poole, England) was used as an adsorbent for cellobiose and glucose. In tests containing charcoal, unhydrolyzed cellulose was separated from granulated charcoal by repeated decantation, filtered using preweighed filter paper and estimated by dry weight determination. Results obtained by this method were also checked by estimating adsorbed sugars. For this purpose, the adsorbed sugars were released by using an ethanol-water mixture (3:1, v/v). Total sugars were determined using dinitrosalicylic-acid reagent (Miller, 1959) and sugar composition by liquid chromatography (Guiliano and Khan, 1984). Protein content was estimated by Folin-Phenol reagent (Lowry et. al., 1951). All tests were made in triplicate, on two or three different occasions.

RESULTS AND DISCUSSION

Charcoal adsorbed both cellobiose and glucose under the test conditions (Table 1). Test conditions used in these tests were the same as those used for saccharifying cellulose. A number of adsorbents were tried in preliminary tests and granulated charcoal was selected because it did not affect the pH of the saccharifying mixture nor the saccharifying ability of the cellulase enzymes. Charcoal is relatively inexpensive, the adsorbed sugars can be readily eluted and the charcoal reused. The granulated material is also easy to separate from cellulose or partially digested cellulose because it has a relatively high density.

Table 1. Adsorption of sugars and protein by charcoal

Material	Adsorption[a] (mg/g)
Cellobiose	160 - 200
Glucose	50 - 90
Protein[b]	1 - 2

[a] Varied between different batches of charcoal.
[b] Cellulase from T. reesei.

Supplementing the saccharifying mixtures with charcoal or cellobiase obtained from A. phoenicis improved the hydrolysis of cellulose by Trichoderma cellulases (Table 2). For optimum saccharification, about a 1:1 ratio of filter paper activity and cellobiase activity was required. This indicates that 30-35 I.U. of cellobiase activity / g of cellulose are required for efficient hydrolysis. Similar observations have been reported by Chahal et. al. (1982).

Table 2. Saccharification by Trichoderma cellulases in the absence or presence of B-glucosidase or charcoal.[a]

Additions		Incubation Time	Saccharification
β-glucosidase (I.U./g cellulose)	Charcoal (g/g cellulose)	(days)	(% of initial cellulose content)
no	no	1	32
30	no	1	65
60	no	1	73
no	5	1	76
no	10	1	75
no	no	2	45
30	no	2	86
60	no	2	91
no	5	2	90
no	10	2	92

[a] Reaction mixture contained 5% cellulose and 30 I.U. of filter paper activity / g of cellulose.

Use of double the amount of β-glucosidase (60 I.U. / g of cellulose) improved saccharification only by 5 - 10 %. Comparable or better results were obtained by using of 5g of charcoal / g of cellulose. At this concentration, tests containing charcoal gave better results than tests containing 60 I.U. of β-glucosidase / g of cellulose. These beneficial effects of charcoal on saccharification may be due to the partial removal of glucose by charcoal, since glucose has been shown to inhibit cellulolytic enzymes (Howell and Stuck, 1975; Maguire, 1977). Charcoal is much cheaper than β-glucosidase and it can be reused after recovering sugars by ethanol-water extraction. Glucosidase, on the other hand, cannot be reused as it is difficult to recover. Charcoal may also help in concentrating sugar solutions by extracting absorbed sugars with a smaller volume of extractant than the saccharification mixture volume. Results reported here provide a direct evidence on the role of β-glucosidase in removing inhibitory cellobiose (Gong et. al., 1977; Maguire, 1977; Reese, 1956; Sternberg et. al., 1977; Westermark and Eriksson, 1974). Additionally, these results indicate the possibility of using charcoal as an alternative to the more expensive β-glucosidase or other enzymes for minimizing end product inhibition.

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