Purification and Characterization of Extracellular β -Xylosidase and β -Glucosidase from *Aspergillus fumigatus*

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A β -xylosidase (β -D-xyloside xylohydrolase, EC 3.2.1.37) and β -glucosidase (β -D-glucoside glucohydrolase, EC 3.2.1.21) extracted from a wheat bran culture of *Aspergillus fumigatus* were purified up to 90-fold and 131-fold, respectively, by ammonium sulfate precipitation, gel filtration, ion exchange chromatography, and hydroxylapatite chromatography. Molecular weights of the β -xylosidase and β -glucosidase were 360,000 and 380,000, respectively, each consisting of four identical subunits. The isoelectric points of β -xylosidase and β -glucosidase were at pH 5.4 and 4.5, respectively. The optimum temperature for the β -xylosidase was 75°C, being stable up to 65°C for 20 min and for the β -glucosidase was 65°C, being stable up to 60°C for 20 min. The optimum pH for both enzymes was about 4.5, being stable between 2 and 8 at 50°C for 20 min. Both enzymes were inhibited by Fe^{3+} , Cu^{2+} , Hg^{2+} , SDS, and *p*-chloromercuribenzoate. The apparent Michaelis constants of the β -xylosidase were 2.0 and 23.8 mM for *p*-nitrophenyl- β -xyloside and xylobiose, respectively, and those of the β -glucosidase were 1.4, 11.4, and 24.8 mM for *p*-nitrophenyl- β -glucoside, gentiobiose, and cellobiose, respectively. To produce xylose from crude xylooligosaccharides prepared by steam-explosion of cotton seed waste (DP $\leq$ 10, 53%, total sugars = 150 g/liter), the crude enzyme from *A. fumigatus* (β -xylosidase activity = 14.7 units/ml, xylanase activity = 20 units/ml) could hydrolyze the substrate at 55°C and pH 4.5 resulting in almost complete conversion to xylose (160 g/liter).

The overall conversion of xylan to xylose is achieved by a synergistic hydrolysis between endo- β -xylanase and β -xylosidase.^{1,2)} In a previous work,³⁾ *Humicola lanuginosa* and *Aspergillus fumigatus* were selected as potent producers of endo- β -xylanase and β -xylosidase, respectively. The mixture of crude enzymes from *H. lanuginosa* and *A. fumigatus* hydrolyzed a wood xylan to xylose. Further the characteristics of purified endo- β -xylanase from *H. lanuginosa* were studied and the mode of enzyme actions on xylan and xylooligosaccharides indicated that all the substrates except xylobiose were hydrolysed mainly to xylobiose with a trace amount of xylose.⁴⁾ On the other hand, during the course of purifica-

tion of β -xylosidase predominantly produced from *A. fumigatus*, it was found that a significant amount of β -glucosidase which may be responsible for the hydrolysis of xylooligosaccharides was also produced in wheat bran solid culture. This paper described the purification and characterization of a β -xylosidase and a β -glucosidase from the wheat bran culture of *A. fumigatus*. In addition, xylose was produced enzymatically from the crude xylooligosaccharides prepared by steam-explosion of cotton seed waste.

MATERIALS AND METHODS

Microorganism. *Aspergillus fumigatus* kept on potato

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dextrose agar was used throughout the experiment.

Crude enzyme. *A. fumigatus* was cultured at 40°C for 5 days in a wheat bran solid medium supplemented with a wood xylan.³⁾ The wheat bran culture was soaked in deionized water at 4°C for 3 hr and the soaked suspension was filtered with a gauze cloth. Then the filtrate was centrifuged at $4,500 \times g$ for 20 min and the supernatant was used as the crude enzyme solution.

Enzyme assay. β -Xylosidase and β -glucosidase activities were measured by *p*-nitrophenol liberated from *p*-nitrophenyl- β -xyloside (pNPX) and *p*-nitrophenyl- β -glucoside (pNPG), respectively.^{5,6)} The reaction mixture (1 ml), consisting of 0.1 ml enzyme solution and 0.9 ml of acetate buffer (0.05 M, pH 5.0) containing 10 mM of substrate, was incubated at 65°C unless otherwise stated for 20 min. The reaction was ended by the addition of 0.5 M Na_2CO_3 solution, and *p*-nitrophenol released from the substrates was measured spectrophotometrically at 400 nm. One unit of enzyme activity was defined as the amount of enzyme which produced 1 μmol of *p*-nitrophenol per min.

Xylanase activity was assayed on the basis of the measurement of reducing sugar cleaved from xylan.³⁾ One unit of enzyme activity was defined as the amount of enzyme which produced reducing sugar equivalent to 1 μmol of xylose per min.

Protein measurement. During the course of enzyme purification, the protein eluted from the column was measured by the absorbance at 280 nm and the protein in the pooled sample was measured by Lowry's method⁷⁾ using bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was done by the method of Davis⁸⁾ as described in our previous paper.⁴⁾

Isoelectric focusing. The isoelectric point of the enzyme was measured by the method of Horio *et al.*⁹⁾ with a 5% polyacrylamide gel (5 $\times$ 90 mm) containing 2% ampholine (pH 3.5–10.0). Isoelectric focusing was done at 200 V for 5 hr at 4°C. The *pI* marker proteins having *pI* 4.1, 4.9, 6.4, 8.3, 9.7, and 10.6 were used as references.

Molecular weight measuring. The molecular weight of the purified enzyme was estimated by the method of Andrews¹⁰⁾ by gel filtration on Sephacryl S-300 and by SDS-polyacrylamide gel electrophoresis by the method of Weber and Osborn.¹¹⁾

Analysis of xylooligosaccharides. Hydrolyzates from xylooligosaccharides with the enzymes were analyzed by thin layer chromatography using silica gel (60 F 254, Merck & Co., Inc., U.S.A.) in the same manner as described in our previous paper⁴⁾ and by high performance liquid chromatography (HPLC) on a Shodex S 801 col-

umn (50 cm $\times$ 0.8 cm i.d.) with a differential refractometer SE-11 (Japan Spectroscopic Co., Ltd., Tokyo, Japan).

Chemicals. *p*-Nitrophenyl- β -xyloside and *p*-nitrophenyl- β -glucoside were purchased from Koch-Light Laboratories Ltd. (Colnbrock, Berks, England). *p*-Nitrophenyl- α -glucoside, *o*-nitrophenyl- β -galactoside, methyl- β -xyloside, methyl- α -xyloside, methyl- β -glucoside, and gentiobiose were obtained from the Sigma Chemical Co. (St. Louis, U.S.A.). Sephacryl S-300, DEAE-Sephacryl CL-6B, SP-Sephadex C-50, and standard proteins for molecular weight measurement of the enzyme were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). The *pI* marker proteins were obtained from Oriental Yeast Co., Ltd., (Tokyo, Japan). Hydroxylapatite was obtained from Bio-Rad Laboratories (Richmond, U.S.A.). Authentic β -1,4 linked xylooligosaccharides (xylobiose, X2 to xylopentaose, X5) were given to us by Prof. T. Yasui (The University of Tsukuba, Ibaraki, Japan) and xylobiose, by Dr. K. Koga (Suntory Co., Ltd., Japan). Raw material for the xylooligosaccharides (total sugars: about 20%) prepared by steam-explosion of cotton seed waste was obtained from Dr. S. Inoue (Jujo Pulp Co., Ltd., Akita, Japan). The components of the xylooligosaccharides in the substrate were analyzed by HPLC with Shodex S 801 and S 802 columns (Japan Spectroscopic Co., Ltd., Tokyo, Japan) using the authentic xylooligosaccharides (X2–X5) and PEG 600, 1000, 1540, and 2000 as the index of standard molecular weights. The component of xylooligosaccharides in the substrate based on the degree of polymerization (DP) are as follows (%) DP > 10, 42.6; DP 10–7, 34.6; DP 6–2, 22.1; and xylose 0.7. Other chemicals used were of chemically pure quality.

RESULTS AND DISCUSSION

Purification of β -xylosidase and β -glucosidase

All the purification procedures were done at 4°C unless otherwise stated.

The crude enzyme solution extracted from the wheat bran culture was brought to 80% saturation by adding solid ammonium sulfate. The precipitate was harvested by centrifugation ($27,000 \times g$, 20 min) and then dissolved in 0.05 M acetate buffer (pH 5.0). The clear supernatant obtained by centrifugation was put on a column of Sephacryl S-300 (2.5 $\times$ 80 cm) and eluted with 0.05 M acetate buffer (pH 5.0) at a flow rate of 90 ml per hour with a fraction size of 6.2 ml. As β -xylosidase and β -glucosidase were eluted in the same fractions (data not shown), these were combined and put on a DEAE-Sephacryl CL-6B column (2.0 $\times$ 35 cm) previously equilibrated with

FIG. 1. Elution Pattern of β -Xylosidase and β -Glucosidase Activities from DEAE-Sephacrose CL-6B.

The active fractions from Sephacryl S-300 were put on a column of DEAE-Sephacrose CL-6B (2.0×35 cm). The column was eluted by a linear gradient of zero to 0.3 M NaCl solution in 0.05 M acetate buffer (pH 5.0) at a flow rate of 60 ml/hr with fraction size of 7.0 ml.

Δ , β -xylosidase activity; $\circ$, β -glucosidase activity; $\bullet$, absorbance at 280 nm; -----, NaCl concentration.

0.05 M acetate buffer (pH 5.0). Elution was done at a flow rate of 60 ml per hour with a fraction size of 7.0 ml. After eluting the non-adsorbed proteins (up to fraction 20), further elution was done by a linear gradient from zero to 0.3 M NaCl solution in the same buffer (1 liter) (Fig. 1). In Fig. 1, fractions 10 to 20 had the main β -xylosidase activity and fractions 88 to 96, which eluted at 0.15 M NaCl solution, had much β -glucosidase activity together with a trace of β -xylosidase activity.

The proteins in the non-adsorbed fractions (10 to 20) were used for further purification of β -xylosidase. These fractions were combined and dialysed against 0.05 M citric acid-phosphate buffer (pH 3.5). This was then put on a column of SP-Sephadex C-50 (2.0×30 cm) previously equilibrated with 0.05 M citric acid-phosphate buffer (pH 3.5) and eluted with a linear gradient from zero to 0.4 M of NaCl solution (1 liter) in the same buffer at a flow rate of 60 ml per hour with a fraction size of 7.2 ml. The active fractions which eluted at 0.4 M NaCl solution were combined and concentrated by ultrafiltration (cut-off molecular weight 10,000). The solution obtained was

FIG. 2. Polyacrylamide Gel Electrophoregrams of β -Xylosidase Fraction (A) and β -Glucosidase Fraction (B) Eluted from Hydroxylapatite Columns (see in Table I).

dialysed against 0.01 M phosphate buffer (pH 6.8). The clear enzyme solution was put on a column of hydroxylapatite (0.9×18 cm) previously equilibrated with 0.02 M phosphate buffer (pH 6.8) and eluted by increasing the buffer concentration stepwise (0.02, 0.05, 0.1, 0.2 and 0.3 M) at a flow rate of 60 ml per hour with a fraction size of 4.2 ml. The fractions with β -xylosidase activity were eluted at 0.2 M phosphate buffer.

The combined active fractions had a single band upon polyacrylamide gel electrophoresis (Fig. 2A). The summary of purification of β -xylosidase is shown in Table I indicating that the specific activity was increased 90-fold with a yield of 2.9%.

For the purification of β -glucosidase, fractions 88 to 96 (Fig. 1) with β -glucosidase activity were combined and concentrated by precipitation with ammonium sulfate (80% saturation), and the precipitate was dissolved in 0.05 M phosphate buffer (pH 6.0) and dialysed against the same buffer. The clear enzyme solution was then put on a column of hydroxylapatite (2.0×26 cm) previously equilibrated with 0.05 M phosphate buffer (pH 6.0). After non-adsorbed proteins were eluted, 1 liter of phosphate buffer (pH 6.0) was added as a linear gradient from zero to 0.5 M at a flow rate of 60 ml per hour with a fraction size of

TABLE I. SUMMARY OF PURIFICATION OF β -XYLOSIDASE AND β -GLUCOSIDASE FROM *A. fumigatus*

Step	Total protein (mg)	Total activity for		β -Xylosidase			β -Glucosidase		
		pNPX	pNPG	Specific activity (U/mg protein)	Yield (%)	Fold (-)	Specific activity (U/mg protein)	Yield (%)	Fold (-)
Culture filtrate	14,900	2,680	11,300	0.18	100	1	0.76	100	1
Ammonium sulfate fraction (80% sat.)	3,360	1,980	9,000	0.59	73.8	3.28	2.67	79.7	3.51
Sephacryl S-300	516	1,340	7,000	2.60	49.9	14.4	13.6	61.9	17.9
DEAE-Sepharose CL-6B fraction									
# 10~20	63.1	287	195	4.55	10.7	25.2	3.09		
# 28~36	30.4	77.5	32.9	2.55			1.08		
# 88~96	102	107	2,800	1.05			27.5	24.8	36.0
<hr/>									
For fraction # 10~20									
SP-Sephadex C-50	14.9	196	ND	13.3	7.3	73.5			
Hydroxylapatite	4.74	76.8	ND	16.2	2.9	90.1			
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For fraction # 88~96									
Hydroxylapatite	24.6	ND	2,450				99.6	21.7	131

ND = not determined.

7.5 ml. The enzyme with β -glucosidase activity was eluted at 0.3 M phosphate buffer and the combined fractions were a single band upon polyacrylamide gel electrophoresis (Fig. 2B). The summary of purification of β -glucosidase is shown in Table I indicating that the specific activity increased 131-fold with a yield of 22%.

Substrate specificity

Several α - and β -glycosides were tested to examine the substrate specificity of the enzymes (see Table II). The β -xylosidase was active on pNPX and xylobiose, with a little activity upon pNPG, but not on methyl- α -xyloside or cellobiose. In contrast with the β -xylosidase, the β -glucosidase acted on pNPG, cellobiose, and gentiobiose with a little activity upon pNPX and xylobiose. This was similar to the purified β -glucosidase from *Stachybotrys atra*¹²⁾ which acted on pNPX. Both enzymes had no activity on xylan or avicel (data not shown).

From their substrate specificity together with the results from Fig. 2, we concluded that the purified enzymes were a β -xylosidase and a

β -glucosidase.

Molecular weight

The molecular weight of the β -xylosidase was 360,000, and the β -glucosidase was 380,000 by gel filtration on Sephacryl S-300 (Fig. 3). The β -xylosidase differed from those of *A. niger* (76,000),¹³⁾ *Penicillium wortmanni* (100,000),⁵⁾ and *Emmericella nidulans* (240,000).¹⁴⁾ The molecular weight of the β -glucosidase was similar to those of other fungi such as *Trichoderma koningii* (398,000)¹⁵⁾ and *A. fumigatus* of Rudick *et al.* (340,000),¹⁶⁾ but differed from the three β -glucosidases of *A. aculeatus* (132,000~136,000).¹⁷⁾

As the molecular weight of the β -xylosidase by SDS-polyacrylamide gel electrophoresis was 90,000 (Fig. 4), it might consist of four identical subunits. This differed from the β -xylosidase of *P. wortmanni*, a monomeric enzyme,⁵⁾ and that of *Bacillus pumilus*, with two identical subunits.¹⁸⁾ Also by SDS-polyacrylamide electrophoresis, the molecular weight of the β -glucosidase was 95,000, suggesting that the enzyme might consist of four identical

TABLE II. SUBSTRATE SPECIFICITY OF PURIFIED ENZYMES

Substrate (10 mM)	Specific activity	
	β -Xylosidase	β -Glucosidase
	(units/mg protein)	
<i>p</i> -Nitrophenyl- β -xyloside	27.5	1.03
<i>p</i> -Nitrophenyl- β -glucoside	0.76	112
<i>p</i> -Nitrophenyl- β -galactoside	0	0.05
<i>p</i> -Nitrophenyl- α -glucoside	0	0
Methyl- β -xyloside	0.74	ND
Methyl- α -xyloside	0	ND
Methyl- β -glucoside	0	2.39
Xylobiose	15.6	0.29
Cellobiose	0	57.3
Gentiobiose	ND	34.4
Salicin	ND	4.33
Maltose	0	0
Lactose	0	0
Sucrose	0	0

The reactions for β -xylosidase and β -glucosidase were done in 0.1 M acetate buffer (pH 4.5) at the optimal temperatures of 75°C and 65°C, respectively.

ND = not determined.

FIG. 3. Estimation of the Molecular Weight of the Purified Enzymes by Gel Filtration.

Each enzyme solution was put on the Sephacryl S-300 column equilibrated with 0.05 M phosphate buffer (pH 6.8). The column size were, (A): 1.6 $\times$ 66.5 cm, (B): 2.6 $\times$ 75 cm. 1 = ferritin (440,000); 2 = catalase (232,000); 3 = aldolase (158,000); 4 = bovine serum albumin (67,000); $\triangle$ = β -xylosidase; $\bullet$ = β -glucosidase.

subunits (Fig. 4). This differed from the β -glucosidase of *A. fumigatus* of Rudick *et al.* which had two identical subunits.¹⁶⁾

Isoelectric point

The isoelectric points of β -xylosidase and β -

FIG. 4. Estimation of the Molecular Weight of β -Xylosidase and β -Glucosidase by SDS-Polyacrylamide Gel Electrophoresis.

1 = phosphorylase *b* (94,000); 2 = bovine serum albumin (67,000); 3 = ovalbumin (43,000); 4 = carbonic anhydrase (30,000); 5 = trypsin inhibitor (20,100). $\triangle$ = β -xylosidase; $\bullet$ = β -glucosidase.

FIG. 5. Effects of Reaction Temperature on the Activities of β -Xylosidase and β -Glucosidase.

The reaction mixture consisting of 0.9 ml of 10 mM of respective substrates in 0.1 M acetate buffer (pH 5.0) and 0.1 ml of each purified enzyme was incubated at each temperature for 20 min.

$\triangle$, β -xylosidase; $\circ$, β -glucosidase.

glucosidase were at pH 5.4 and 4.5, respectively. These values were comparable to the β -xylosidase from *P. wortmanni* (5.0)⁵⁾ and the β -glucosidase from *H. insolens* YH-8 (4.2)²⁰⁾ and *T. reesei* (4.4).²¹⁾

Effects of temperature and pH on the activity and stability

The β -xylosidase and β -glucosidase activities were measured at various temperatures from 45 to 85°C for a 20 min reaction. The maximum β -xylosidase activity was observed at 75°C, and that of β -glucosidase activity was

Fig. 6. Effects of Reaction pH on the Activities of β -Xylosidase and β -Glucosidase.

The reaction mixture consisting of 0.9 ml of 10 mM of respective substrates in 0.1 M various buffer solutions and 0.1 ml of each enzyme was incubated at 75°C for β -xylosidase and 65°C for β -glucosidase for 20 min. The buffer solutions used were citric acid-phosphate (pH 3.0~4.0), acetate buffer (pH 4.5~5.5), and phosphate buffer (pH 6.0~7.0).

Δ , β -xylosidase; $\circ$, β -glucosidase.

at 65°C (Fig. 5). The thermostabilities of both enzymes were investigated by measuring the residual activities after leaving the enzymes at various temperatures between 45 and 75°C for 20 min. The β -xylosidase and β -glucosidase were stable up to 65°C and up to 60°C, respectively (data not shown).

The optimal pH of both enzyme activities was searched at various pHs from 3.0 to 8.0. The highest activity for β -xylosidase was at 4.5 and for β -glucosidase, 4.0~4.5 (Fig. 6). Both enzymes were relatively stable between pH 2~8 at 50°C for 20 min (data not shown).

Effects of metal ions and inhibitors on the activity

The effects of various reagents at 2 mM on both enzyme activities were investigated. As shown in Table III, EDTA, Li^+ , Mg^{2+} , Ca^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+} , Sn^{2+} , and Ba^{2+} did not significantly influence the enzyme activities, whereas Fe^{3+} , Cu^{2+} , Hg^{2+} , *p*-chloromercuribenzoate (pCMB), and SDS inhibited the enzyme activities. The inhibition of pCMB was partially reversed by 2 mM dithiothreitol, suggesting that a sulfhydryl group ($-\text{SH}$) was

involved in the catalytic active center of both enzymes as observed in the β -xylosidase from *A. niger*¹³⁾ and the β -glucosidase from *S. atra*,¹²⁾ *Clostridium thermocellum*,²¹⁾ and *Botryodiplodia theobromae*.²²⁾

Kinetic properties

The K_m of purified β -xylosidase against pNPX and xylobiose were 2.0 and 23.8 mM, respectively. This value against pNPX was higher than 0.2 mM for the β -xylosidase from *A. niger*¹³⁾ and 0.12 mM from *P. wortmanni*.⁵⁾ The inhibition by xylose of the β -xylosidase was examined with pNPX as substrate and competitive inhibition was confirmed with a K_i of 4.5 mM.

For β -glucosidase, the K_m against pNPG, gentiobiose, and cellobiose were 1.4, 11.4, and 24.8 mM, respectively. Glucose inhibited the hydrolysis of pNPG competitively with K_i of 3.1 mM, similar to the values reported from *Lenzites trabea*,²³⁾ *A. terreus*,²⁴⁾ and *Dekkera intermedia*.²⁵⁾

Hydrolysis of xylooligosaccharides with the purified β -xylosidase and β -glucosidase

As mentioned before (see Table II), β -glucosidase in the crude enzyme from *A. fumigatus* hydrolysed xylobiose and pNPX, so the actions of purified β -xylosidase and β -glucosidase on the authentic xylooligosaccharides (X2~X5) were first investigated. The hydrolyzates of xylooligosaccharides from the thin layer chromatograms on silica gel (Fig. 7) showed that both enzymes hydrolyzed the substrates X2~X5 to form xylose consecutively. However, for the β -glucosidase, the reaction required a high concentration of the enzyme and long incubation time. Second, the raw material of xylooligosaccharides was hydrolyzed. As shown in Fig. 8, both enzymes could produce xylose from the substrate, but no xylobiose was accumulated in the reaction containing β -xylosidase, whereas xylobiose accumulated was found in the case of β -glucosidase suggesting that this might be due to the different affinity of the two enzymes to xylobiose (see Table II). However, it is expected

FIG. 7. Thin Layer Chromatograms of Hydrolyzates of Xylooligosaccharides (X2~X5) with Purified β -Xylosidase or β -Glucosidase.

Reaction mixture (200 μ l) contained 0.25 mg of each substrate in 0.1 M acetate buffer (pH 4.5) was incubated with each enzyme (see below) at 55°C.

A: 0.2 unit of purified β -xylosidase, 30 min incubation: B: 3.5 units of purified β -glucosidase, 4 hr incubation.

FIG. 8. Hydrolysis of the Mixture of Xylooligosaccharides from the Steam-explosion of Cotton Seed Waste (see MATERIALS AND METHODS) with the Purified β -Xylosidase or β -Glucosidase.

Reaction mixtures (5 ml) containing 27.5 mg of total sugars (based on the phenol-sulfuric acid method) in 0.1 M acetate buffer (pH 4.5), 1 mg of NaN_3 with each enzyme (see below) were incubated at 55°C.

A: 0.09 mg of β -xylosidase (1.0 unit of β -xylosidase activity).

B: 0.7 mg of β -glucosidase (equivalent to 1.1 units of β -xylosidase activity).

○, xylose; □, xylobiose.

that xylobiose does not accumulate, if the reaction contains a mixture of β -xylosidase and β -glucosidase. The xylooligosaccharides produced (X3~X5) at very low concentrations of the enzymes could not be detected under the analytical condition by HPLC due to overlapping by the solvent peak (acetate buffer, 0.1 M).

We concluded from Figs. 7 and 8 that both enzymes might be responsible for the hydrolysis of the xylooligosaccharides from cotton seed waste.

Xylose production from xylooligosaccharides

Xylose was produced from the xylooligosaccharides obtained from the steam-explosion of cotton seed waste using the crude enzyme of *A. fumigatus* (concentration by $(\text{NH}_4)_2\text{SO}_4$ precipitation). Although 100 units of xylanase was contained in the reaction mixture, the hydrolysis rate and extent increased with the β -xylosidase activity (Fig. 9). The xylobiose and xylotriose accumulated were gradually decreased with a concomitant increase of xylose. In Fig. 9C, about 160 g per liter of xylose was produced after 24 hr of incubation, indicating that all the substrate might be converted to xylose. An effective hydrolysis of the substrate depended on the activity of β -xylosidase. In addition, the effects of adding xylanase to the crude enzyme of *A. fumigatus* were studied using the xylanase from *H. lanuginosa*, a potent producer of xylanase.³⁾ The addition of xylanase did not enhance the rate or extent

FIG. 9. Hydrolysis of the Mixture of Xylooligosaccharides from the Steam-explosion of Cotton Seed Waste with the Crude Enzyme from *A. fumigatus*.

Reaction mixtures (5 ml) containing 770 mg of total sugars in 0.1 M acetate buffer (pH 4.5), 1 mg NaN_3 with the crude enzyme (see below) were incubated at 55°C. The reactions contained 100 units of xylanase activity and β -xylosidase activity as follows (units): (A) 14.5, (B) 36.3 and (C) 72.5. ○, xylose; ●, xylobiose; △, xylotriose.

TABLE III. EFFECTS OF METAL IONS AND INHIBITORS ON THE β -XYLOSIDASE AND β -GLUCOSIDASE ACTIVITIES

Reagent (2 mM)	Relative activity (%)	
	β -Xylosidase	β -Glucosidase
None	100	100
pCMB	75.1	66.7
SDS	0	15.8
EDTA	109	109
Li^+	99.3	99.6
Mg^{2+}	98.6	103
Ca^{2+}	101	98.2
Mn^{2+}	103	98.0
Fe^{3+}	82.4	55.7
Co^{2+}	101	99.4
Ni^{2+}	99.2	98.0
Cu^{2+}	17.0	81.4
Zn^{2+}	98.2	96.0
Sn^{2+}	108	98.2
Ba^{2+}	93.8	99.6
Hg^{2+}	0	29.0

The reactions for β -xylosidase and β -glucosidase activities were done in 0.1 M acetate buffer (pH 4.5) at the optimal temperatures of 75°C and 65°C for 20 min, respectively.

of the hydrolysis of the xylooligosaccharides, which was in contrast to that of the hydrolysis of insoluble wood xylan (data not shown). The

xylooligosaccharides from steam-explosion of cotton seed waste, if hydrolysed with the crude enzyme from *A. fumigatus*, might be a useful raw material for the production of xylose because they are short fragments of xylan.

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