http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (3): 1010-1016, 1999 Research Article

Characteristics of *Streptomyces Flavogriseus* β-xylosidase and its Use in Xylan Hydrolysis

El-Sawah, M.M.A., Ashour, Eman H. and Shady, T.S.M.*
Microbiology Department, Faculty of Agriculture, Mansoura Univerity, Mansoura, Egypt.
*Agricultural Microbiology Department, Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

Abstract

β-Xylosidase was induced when *Streptomyces flavogriseus* strain was grown in shake-culture of a mineral salt medium containing 1 per cent wheat straw hemicellulose for 3 days at 30°C. The intracellular β-xylosidase was separated by precipitation with acetone, ammonium sulphate and purified on Sephadex G-150. The enzyme had an apparent molecular weight of 85 Kda as determined by SDS-gel electrophoresis. β-Xylosidase showed maximum activity at pH 6.5 and temperature 40°C. It was stable at pH 6.0-8.0. Heat treatment to 45°C for 1 h did not cause any inhibition for enzyme activity. It was stable for 5 months at 4°C. It was also stable against freezing, thawing and lyophilization with 5.65 per cent loss per cycle. It hydrolyzed ρ-nitrophenyl-β-D-xylopyranoside readily with a K_m. of 1.1 mM. I_2, and $HgCl_2$ were the most potent inhibitors for β-xylosidase activity. Xylose was inhibitory towards the enzyme and the inhibition constant was 5.2 mM. The activity of β-xylosidase and xylanase acting together was greater than the sum of the individual activities, indicating the synergism between *St. flavogriseus* xylanolytic components in the hydrolysis of insoluble hemicelluloses.

Introduction

β-D-Xylosidase (1,4-β-D-xylan xylohydrolase, EC 3.2.1.37) hydrolyzes xylo-oligosaccharides to xylose. The enzyme is essential for the complete breakdown of xylans. The need for the enzyme is in increasing for investigating the carbohydrate to protein linkage in glycoproteins (Reese *et al.*, 1973 and Poutanen and Puls, 1988). β-Xylosidase has been isolated from several microbial hemicellulolytic systems, e.g., *Bacillus pumilus* (Kersters-Hilderson *et al.*, 1969), *Aspergillus niger* (Reese *et al.*, 1973 and Rodionova *et al.*, 1983), *Penicillium wortmanni* (Reese *et al.*, 1973 and Deleyn *et al.*, 1982), *B. coagulans* (Esteban *et al.*, 1983), *Trichoderma viride* (Matsuo and Yasui, 1984a), *Emericella nidulans* (Matsuo and Yasui, 1984b), *Chaetomium trilaterale* (Uziie *et al.*, 1985), *Thermomonospora* sp. (Ristroph and Humphrey, 1985), *Trichoderma reesei* (Poutanen and Puls, 1988) and *Thermomonospora fusca* (Bachmann and McCarthy, 1989). β-Xylosidase hydrolyzes xylobiose as well as xylooligosaccharides and has no action on xylan. The rate of hydrolysis of xylooligosaccharides by β-xylosidase is decreased with increasing the length of xylo-oligosaccharides chain (van Doorslaev *et al.*, 1985). Additions of exogenous β-xylosidase to the hydrolysis process of xylan by xylanases improve the yield of xylose (Dekker, 1983, Deshpande *et al.*, 1988 and Poutanen & Puls, 1988).

This work is a continuation of our studies of the factors responsible for increasing the yield of xylanolytic enzymes from *Streptomyces flavogriseus* (El-Sawah *et al.*, 1991 and El-Sawah, 1991). In the present paper the β-xylosidase of *St. flavogriseus* is characterized. The role of the enzyme in the hydrolysis of hemicellulose is discussed.

Materials and Methods

Culture used and the growth condition: The strain of *Streptomyces flavogriseus* was kindly provided by the Dept. of Plant, Fac. of science, Mansoura University. Media for stock culture maintenance and enzyme production, culture conditions as well as the sources of substrates and chemicals, have already been described by El-Sawah *et al.* (1991).

Enzyme preparation: The crude β-xylosidase was produced by growing *Streptomyces flavogriseus* in Sorensen (1957) medium. Several carbon sources (1% w/v) were tested. The pH of basal medium was adjusted to pH 7.0. The cultivation was performed with shaking (200 rev./min) at 30°C. The cultures were harvested on the 3rd day by centrifugation at 10000 rpm for 10 min. Harvested cells were washed three times and resuspended in 5 ml 10 mM-Tris/HCL, pH 7.0. Intracellular β-xylosidase activity was released by sonication for 10 min at 18 μm peak-to-peak on ice. The crude lysate was cleared by centrifugation (10 min at 10000 rpm). This crude enzyme preparation was subjected to purification.

Enzyme assays: β-Xylosidase activity was measured by assaying the amount of ρ-nitrophenol (ρNP) liberated from the substrate ρ-nitrophenol β-D-xylopyranoside (ρNPX Sigma Chem. Co.). The standard reaction mixture contained 10 mM-ρNPX dissolved in 50 mM-potassium phosphate buffer (pH 6.5) and diluted intracellular enzyme preparation in a total volume of 1.0 ml. After incubation at 40°C for 1 min, the reaction was stopped by the addition of 1 Na_2CO_3 and the absorbance at 420 nm was measured.

Analysis of the purified β-xylosidase by gel electrophoresis revealed one single protein band. The molecular mass of the β-xylosidase was 85 Kda. The estimated molecular mass of the *St flavogriseus* β-xylosidase is well within the range reported for other bacterial β-xylosidase (Claeyssens *et al.*, 1975; Lee and Forsberg, 1987; Panbangred *et al.*, 1984; Bachmann and McCarthy, 1989).

pH and temperature relationships of β-xylosidase activity: β-xylosidase activity reached its maximum at pH 6.5 (Fig. 2). However 85-100 per cent of its maximum activity were exhibited in the range pH 6.0-7.5. Actinomycete xylanolytic enzymes generally exhibit pH optima which are neutral to alkaline, in contrast to the acidic pH requirements of corresponding fungal enzymes (Ishaque and Kluepfel 1981; Nakanishi *et al.*, 1987; Backmann and McCarthy, 1989).

Table 1: Production of β-xylosidase by *St. flavogriseus* on various substrates.

Substrate	β-Xylosidase activity (U/ml)
Wheat straw UT	0.09
Corn cobs UT	0.13
Corn stalks UT	0.16
Flax shives UT	0.15
Barley straw UT	0.08
Larchwood xylan	0.19
Wheat straw hemicellulose	1.30
Xylose	1.11
Arabinose	0.09
Wheat straw T	1.25
Corn Cobs T	1.10
Corn stalks T	1.15
Flax shives T	1.13
Barley straw T	0.07

T, alkali treated; UT, untreated

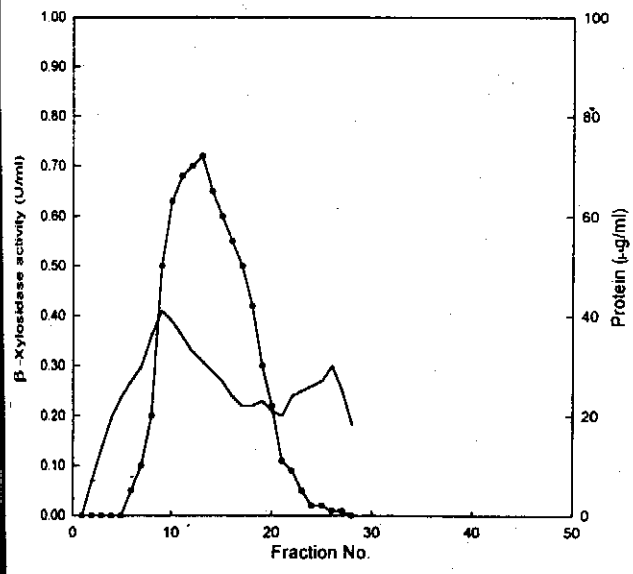

Fig. 1: Elution profile of β-xylosidase on Sephadex G-150. Protein (o) and β-xylosidase activity (•).

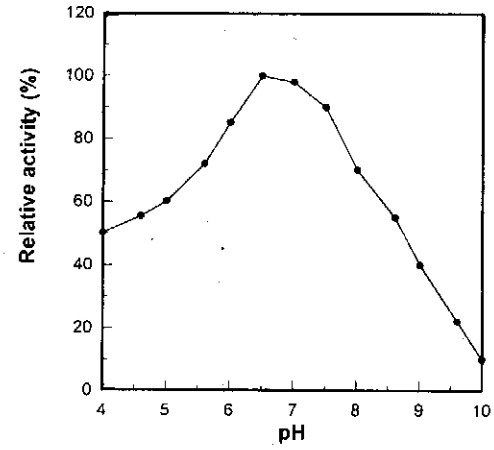

Fig. 2: Effect of pH on β-xylosidase activity. Optimum pH: The enzyme activity was assayed under standard conditions using various buffers.

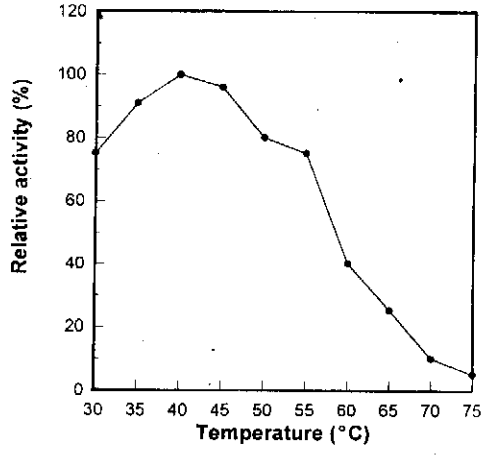

Fig. 3: Effect of temperature on β-xylosidase activity. Optimum temperature: The enzyme was assayed under standard conditions with varying temperatures.

The maximum activity of β-xylosidase was obtained at 40°C (Fig. 3). In the range of 30-55°C, β-xylosidase exhibited 75-100 per cent of its activity. In this connection Ishaque and Kluepfel (1981) and Nakanishi *et al.* (1987) reported maximum activity from *Streptomyces* sp. at 40-45°C. The β-xylosidase of *St. flavogriseus* was stable in the range of pH at 6-8 (Fig. 4). Most of the activity was lost below pH 5.0. *Streptomyces* sp. β-xylosidase was stable from pH 6.0 to 9.0 (Nakanishi *et al.*, 1987). The β-xylosidase was stable up to 45°C when incubated in phosphate buffer at optimum pH for 1 h (Fig. 5). Nakanishi *et al.* (1987) found that *Streptomyces* sp. β-xylosidase was stable at below 35°C.

unit (U) of β-xylosidase activity is defined as the amount that produced 1 μmol pNP/min under the above conditions, and specific activity is expressed as U/mg protein.
Xylanase activity was determined by measuring the release of reducing sugar from larchwood xylan as described by El-Sawah *et al.* (1991). One unit of xylanase activity is the amount of enzyme required to liberate 1 μmoll xylose/min at pH 7.0 and 45°C.

Purification of β-xylosidase: The crude enzyme preparation was concentrated, precipitated by acetone, saturation with ammonium sulphate up to 95 per cent. After centrifugation at 11000 rpm for 30 min at 4°C, the precipitated protein was dissolved in 10 ml phosphate buffer (pH 7.0) and examined for specific activity. The enzyme preparation was desalted using Sephadex G-25 in a Pyrex glass column (2.2 X 31 cm) and equilibrated with phosphate buffer (pH 7.0) for elution. The eluant was concentrated by using lyophilizer. Then dialyzed against distilled water for 24 h, then against phosphate buffer (pH 7.0) for 24 h, with several changes of the buffer solution. For fractionation, separation and purification of the concentrated dialyzed enzyme solution, Sephadex G-150 (Pharmacia Sweden) was packed in a column (2.5 X 50 cm) and equilibrated with phosphate buffer, pH 7.0. Elution was carried out at 6°C at flow rate (12 ml h^{-1}) and fractions of 2 ml were collected. The action fractions of enzyme were combined and lyophilized.

Electrophoresis: Disc gel electrophoresis was performed in 7.5 per cent polyacrylamide at pH 4.0, according to Davis (1964). Molecular weight of enzyme was estimated by sodium dodecyl sulphate gel electrophoresis according to the method of Weber and Osborn (1969). The low molecular weight calibration mixture LMW kit E (Pharmacia) was used as a molecular weight matter.

PH and temperature optima: For determination of the optimum pH, pNPX dissolved in 0.05 M acetate, phosphate and glycine NaOH buffers with pH values ranged between 4.0 and 10.0 and the incubation (10 min) was performed at 40°C. For determination of the optimum temperature, the incubation of the enzyme was performed at pH 6.5 at the temperatures ranged between 30-75°C.

pH and temperature stability: For determination of pH stability, the enzyme preparation in 0.05 M acetate, phosphate and glycine NaOH buffers, pH 4.0-10.0, was incubated at 4°C for 24 h. Samples were withdrawn and assayed for activity at pH 6.5. For determination of temperature stability, the enzyme preparation in 0.05 M phosphate buffer (pH 6.5) was incubated at different temperatures (30-80°C) for 1 h and the withdrawn samples were assayed for residual β-xylosidase activity.

K_m measurement: The K_m value of the enzyme preparations was measured using samples containing 0.02 mg protein/ml. The pNPX concentrations ranged from 0.05 to 10 mM at pH 6.5.

Inhibition kinetics: Inhibition kinetics were determined by the addition of 10-100 mM sugar to the reaction mixture. The K_i values were determined from Dixon plots (Roberts, 1977)

Protein determination: Protein was estimated by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma Chem. Co.) as standard.

p-Nitrophenol Determination: p-Nitrophenol was determined by the method of Okada *et al.* (1968).

Reducing sugars determination: Reducing sugars were determined by the method of Nelson (1944) as modified by Somogyi (1952), with D-xylose as standard.

Hydrolysis experiments: The hydrolysis was performed in 0.05M phosphate buffer (pH 6.5) at 40°C. Wheat straw hemicellulose prepared by the method of Chen and Anderson (1980), and larchwood xylan were used at concentration of 10 mg/ml. The hydrolysis percentage was determined.

Results and Discussion

β-Xylosidase production: The production of β-xylosidase by *St. flavogriseus* on various natural substrates was investigated previously (El-Sawah *et al.*, 1991). However moderate amounts of β-xylosidase were obtained. The aim of this paper is to increase the yield of xylanase. Therfore fourteen substances were tested as shown in Table 1. The results show that β-xylosidase activites were in the range 0.07-1.30 units/ml. In general, β-xylosidase activites were higher in the presence of treated substances than with untreated ones. *St. flavogriseus* produced the highest β-xylosidase activity in the growth medium containing wheat straw hemicellulose as substrate. Hemicelluloses are more readily degraded than cellulose, and this may partly explain improved production of β-xylosidase in cultures containing hemicellulose rather than cellulose (Kluepfel and Ishaque 1982 and Kluepfel *et al.*, 1986).

β-Xylosidase purification: The enzyme was concentrated and purified using gel filtration chromatography. The final purification was approximately 20 fold and the mean overall yield was 26.84 per cent. Specific activity was gradually increased with the progress of purification steps, which reached to 10.12 IU/mg protein (Table 2). The β-Xylosidase activity eluated as a single peak (Fig. 1). β-Xylosidase fractions were pooled from the first fractions. The component, clearly recognized in Fig. 1, includes one peak of protein activity at the respective fraction number. The enzyme started its activity at fraction no. 6, ended at fraction no. 27 and reached its peak at fraction no. 13 with a corresponding activity value of 0.72 U/ml.

Table 2: Purification of intracellular β-xylosidase from *Streptomyces flavogriseus*

Fraction	Total activity (IU)	Total protein (mg)	Sp. Act. (IU/mg)	Yield (%)	Purification fold
Crude enzyme	26.53	53.33	0.50	100.00	-
Acetone precipitate	14.46	5.02	2.88	54.50	5.76
$(NH_4)_2SO_4$ precipitate	10.84	2.13	5.10	40.85	10.20
Sephadex G-150	7.12	0.7	10.12	26.84	20.24

Table 3: Stability of β-xylosidase towards storing (at 4°C) and freezing, thawing & lyophilization.

Storing at 4°C		Freezing, thawing and lyophilization	
Storage period (monthly)	Residual activity(%)	Cycle	Residual activity (%)
1	100	1	94.7
2	100	2	94.0
3	100	3	90.0
4	100		
5	100		
6	100		

β-Xylosidase was completely stable (100% residual activity) with storing at 4°C for five months in 0.05M phosphate buffer, pH 6.5 (Table 3). The enzyme was also stable towards freezing, thawing and lyophilization (Table 3). The residual activity was 90.0 per cent after three successive cycles of freezing, thawing and lyophilization. The results are in line with Ghosh *et al.* (1980) and El-Sawah (1988, 1991).

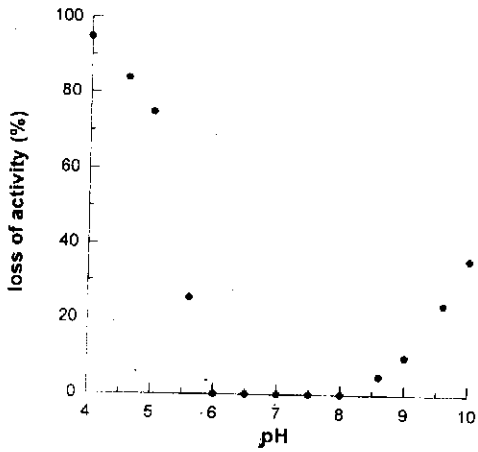

Fig. 4: Effect of pH on β-xylosidase stability. pH stability: The enzyme solution was brought to different pH values (pH 3-10) by addition of universal buffers and kept for 24h at 4°C. Activity of these pre-incubation samples were measured.

Kinetics of β-xylosidase activity: The affinity of β-xylosidase for pNPX was examined using a Lineweaver-Burk plot. The apparent K_m for purified β-xylosidase was 1.1 (Fig. 6).

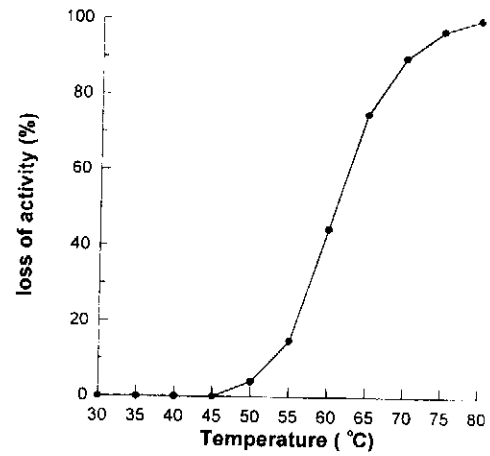

Fig. 5: Effect of temperature on β-xylosidase stability. Thermostability: The enzyme solution was kept without substrate aseptically in 0.05 M phosphate buffer, pH 6.5, for 1h at various temperatures (30-80°C) and residual enzyme activity was measured by the standard assays.

The results in (Table 4) show that although, Ca^{2+}, Co^{2+} and Na^{+} had no effect on β-xylosidase activity, iodine was complete inhibitory. Mn^{2+}, Zn^{2+} slightly reduced the enzyme activity 5 &18 per cent. By Ag^{+} and Fe^{2+}, the reduction of the β-xylosidase activity was 30 and 50 per cent, respectivily. Moreovere, Hg^{2+} was the most potent inhibitor of β-xylosidase activity. The inhibitory action, especially, of Hg^{2+} ions may be attributed to reaction with thiol groups. Hg^{2+} is known to react with peptide linkages and may be capable of coordinating with carboxyl and amino groups (Dekker and Richards, 1976).

Table 4: Effect of various reagents on β-xylosidase activity.

Reagents	Concentration (M)	Relative activity (%)
None	$1.0x10^{-3}$	100
$CaCl_2$	$1.0x10^{-3}$	100
$CoCl_2$	$1.0x10^{-3}$	100
NaCl	$1.0x10^{-3}$	100
$MnCl_2$	$1.0x10^{-3}$	95
$ZnSO_4$	$1.0x10^{-3}$	82
$AgNO_3$	$1.0x10^{-4}$	70
$FeSO_4$	$1.0x10^{-3}$	50
$HgCl_2$	$1.0x10^{-4}$	15
I_2	$1.0x10^{-4}$	00
SDS	$1.0x10^{-4}$	92

These results indicated that the enzyme is not metallo-enzyme or metal activated enzyme. Also, this protein is active against its substrate and does not need any activators. SDS resulted in partial inhibition of the enzyme. The results are similar to those obtained by El-Sawah (1991) on xylanase from the same organism.

β-Xylosidase activity was inhibited by D-xylose and L-arabinose, but not by D-glucose with pNPX as substrate (Table 5). Such inhibition was more pronounced with increasing concentration of the added sugar.

Table 5: Inhibition of β-xylosidase activity by sugars.

Sugar	Sugar concentration (%)				
	0.0	0.1	0.2	0.3	0.4
D-Xylose	0.0	20.75	31.05	60.55	80.60
L-Arabinose	0.0	11.00	15.00	25.70	35.50
D-Glucose	0.0	00.00	00.00	00.00	00.00

(extent of inhibition is expressed as a percentage)

Table 6: Synergism between the pure xylanolytic components from *S. flavogriseus* in hemicellulose or xylan hydrolysis (Enzymatic hydrolysis was carried out at pH 6.5 and 40°C for 6h on 10 mg hemicellulose or xylan).

Enzyme component	Hydrolysis (%)	
	Hemicellulose	Xylan
Endo β-1,4 xylanase (1U)	15	28
Endo β-1,4 xylanase (2U)	28	51
β-Xylosidase (0.03U)	10	12
β-Xylosidase (0.01U)	2	4
Endo β-1,4 xylanase (1U) + β-xylosidase (0.03U)	30	60
Endo β-1,4 xylanase (1U) + β-xylosidase (0.01U)	21	44
Endo β-1,4 xylanase (2U) + β-xylosidase (0.03U)	42	80
Endo β-1,4 xylanase (2U) + β-xylosidase (0.01U)	33	60

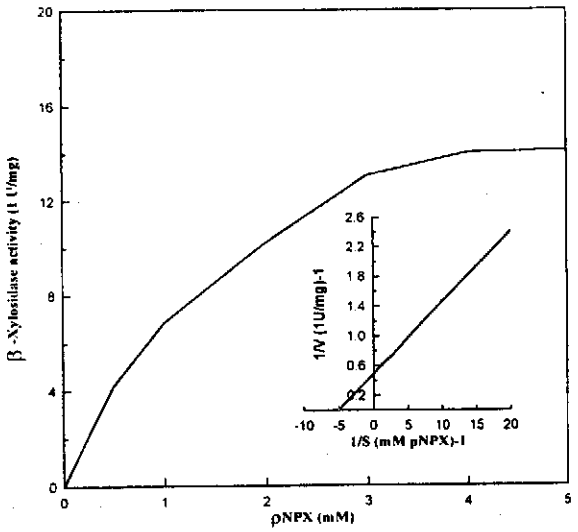

Fig. 6: Effect of pNPX concentration versus β-xylosidase activity.

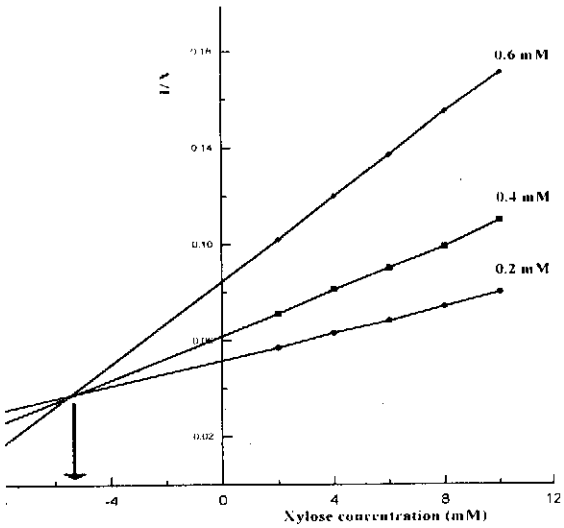

Fig. 7: Dixon plots of the effect of substrate and product concentrations on β-xylosidase activity at 40°C and pH 6.5.

The hydrolysis of 10 mM pNPX was reduced to 79.25 per cent and 19.4 per cent of its original value when 1.0 and 4.0 (mg/ml) of xylose was added. The results show that xylose is an end product inhibitor and inhibit β-xylosidase competitively, and the K_i as determined from a Dixon plot was 5.2 mM (Fig. 7). End-product inhibition of β-xylosidase activity by xylose or arabinose is important in limiting the rate of lignocellulose saccharification in any process, since it is likely that the action of β-xylosidase itself, in removing

oligosaccharides inhibitory to endoxylanase activity, would be a key regulatory mechanism (Bachmann and McCarthy, 1989).

Hydrolysis of xylan: The results in Table 6 showed that β-xylosidase plays an important role in both hemicellulose and xylan hydrolysis. β-xylosidase increased the substrate hydrolysis when added to the hydrolysis mixture containing xylanase. The hydrolysis process depended on the level of β-xylosidase than with that of xylanase. However, the activity of β-xylosidase alone on substrates was limited. It is apparent from the results that the β-xylosidase is a key enzyme for the hydrolysis of hemicellulose. In addition to the substituent-cleaving enzymes (Poutanen *et al.*, 1987 and El-Sawah, 1997), the synergistic action between β-xylosidase and xylanase for hemicelluloses hydrolysis was confirmed (Poutanen and Plus, 1988; Desphande *et al.*, 1988).

References

Backmann, S.L. and A.J. McCarthy, 1989. Purification and characterization of a thermostable β-xylosidase from *Thermomonospora fusca*. J. Gen. Microbiol., 135:293-299.

Chen, W.P. and A.W. Anderson, 1980. Extraction of hemicellulose from raygrass straw for the production of glucose isomerase and use of the resulting straw residue for animal feed. Biotech. Bioeng., 22: 519-531.

Claeyssens, M., E. Saman, H. Kersters-Hilderson and C.K. De Bruyne, 1975. β-D-Xylosidase from *Bacillus pumilus*: molecular properties and oligometric structure. Biochem. Biophys. Acta, 405: 475-481.

Davis, B.J., 1964. Disc electrophoresis. 11. Method and application to human serum proteins, Ann N.Y. Acad. Sci., 121:404-411.

Dekker, R.E.H., 1983. Bioconversion of hemicellulose: Aspects of hemicellulase production by *Trichderma reesei* QM 9414 and enzymatic saccharification of hemicellulose. Biotechnol. Bioeng., 30: 1127-1146.

Dekker, R.F.H. and G.N. Richards, 1976. Hemicellulases: their occurrence, purification, properties and mode of action. Adv. Carbohydr. Chem., 32:277-352.

Deleyn, F., M. Claeyssens and C.K. De Bruyne, 1982. β.D-Xylosidase from *Penicillium wortmanni*. Meth. Enzymol., 83: 639-644.

Desphande, V., A. Lachke, C. Mishra, S. Keskar and M. Rao, 1988. Mode of action and properties of xylanase and β-xylosidase from *Neurospora crassa*. Biotechnol. Bioeng., 28:1832-1837.

El-Sawah, M.M.A., 1988. Further studies on microbial decomposition of xylan, Ph.D. Thesis, Mansoura University, Mansoura, Egypt.

El-Sawah, M.M.A., 1991. Separation and some properties of an endo-1,4-β-D-xylanase produced by *Streptomyces flavogriseus*. Egypt. J. Appl. Sci., 6: 458-468.

El-Sawah, M.M.A., 1997. Evaluation of different xylanolytic systems in thermophilic *Bacillus* spp. with special reference to *B. stearothermophilus*. J. Agric. Sci., Mansoura Univ. (Egypt), 22: 1541-1556.

El-Sawah, M.M.A., A.E.I. Selim, M. Bayoumy, M. Samia and H. Affify Aida, 1991. Production of xylanase and β-xylosidase from *Streptomyces flavogriseus*. J. Agric. Sci., Mansoura Univ.(Egypt), 16: 2430-2439.

Esteban, R., A. Chordi and T.G. Villa, 1983. Some aspects of a 1,4-β-D-xylosidase and a β-xylosidase secreted by *Bacillus coagulans* strain 26. FEMS Microbiol. Lett., 17:163-166.

Ghosh, A.K., P.C. Banerjee and S. Sengupta, 1980. Purification and properties of xylan hydrolase from mushroom *Termitomyces clypeatus*. Biochim. Biophys. Acta, 612:143-152.

Ishaque, M. and D. Kluepfel, 1981. Production of xylanolytic enzymes by *Streptomyces flavogriseus*. Biotechnol. Lett., 3: 481-486.

Kersters-Hilderson, H., F.Q. Loontiens, M. Claeyssens and C.K. DeBruyne, 1969. Partial purification and properties of an induced β-xylosidase of *Bacillus pumilus* 12. Eur. J. Biochem., 7: 434-441.

Kluepfel, D. and M. Ishaque, 1982. Xylan-induced celluloytic enzymes in *Streptomyces flavogriseus*. Dev. Ind. Microbiol., 23: 389-395.

Kluepfel, D., F. Shareck, F. Mondou and R. Morosoli, 1986. Characterization of cellulase and xylanase activities of *Streptomyces lividans*. Appl. Microbiol. Technol., 24: 230-234.

Lee, S.F. and C.W. Forsberg, 1987. Isolation and some properties of a β-D-xylosidase from *Clostridium acetobutylicum* ATCC 824. Appl. Environ. Microbiol., 53: 651-654.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.L. Randall, 1951. Protein measurment with Folin-phenol reagent. J. Biol. Chem., 193: 265-276.

Matsuo, M. and T. Yasui, 1984a. Purification and some properties of β-xylosidase from *Trichoderma viride*. Agric. Biol. Chem., 48:1845-1852.

Matsuo, M. and T. Yasui, 1984b. Purification and some properties of β-xylosidase from *Emericella nidulans*. Agric. Biol. Chem., 48:1853-1860.

Nakanishi, K., K. Yokotsuka and T. Yasui, 1987. Induction of membrane-bound xylosidase in a *Streptomyces* sp. J. Ferment. Technol., 65: 1-6.

Nelson, N., 1944. A photometric adaptation of somogyi method for determination of glucose. J. Biol. Chem., 153: 375-380.

Okada, G., K. Nisizawa and H. Suzuki, 1968. Cellulase components from *Trichoderma viride*. J. Biochem., 63: 591-607.

Panbangred, W., O. Kawaguchi, T. Tomita, A. Shinmyo and H. Okada, 1984. Isolation of two β-xylosidase genes of *Bacillus pumilus* and comparison of their gene products. European J. Biochem., 138: 267-273.

Poutanen, K. and J. Puls, 1988. Characteristics of *Trichoderma reesei* β-xylosidase and its use in the hydrolysis of solubilized xylans. Appl. Microbiol. Biotechnol., 28: 425-432.

Poutanen, K., M. Rättö, J. Puls and L. Viikani, 1987. Evaluation of microbial xylanolytic systems. J. Biotechnol., 6:49-60.

Reese, E.T., Maguire, Anne and F.W. Parrish, 1973. Production of β-D-xylopyranosidases by fungi. Can. J. Microbiol., 19: 1065-1074.

Ristroph, D.L. and A.E. Humphrey, 1985. The β-xylosidase of *Thermomonospora*. Biotech. Bioeng., 27: 909-913.

Roberts, D.V., 1977. Enzyme kinetics. Cambridge Chemistry Tests, Cambridge.

Rodionova, N.A., I.M. Tavobilov and A.M. Bezborodov, 1983. β-Xylosidase from *Aspergillus niger* IS: purification and properties. J. Appl. Biochem., 5:300-312.

Somogyi, A.M., 1952. A new reagent for determination of sugar. J. Biol. Chem., 195:19-24.

Sorensen, H., 1957. Microbial decomposition of xylan. Acta Agric. Scand. Suppl. 1, Stockholm.

Uziie, M., M. Matsuo and T. Yasui, 1985. Purification and some properties of *Chaetomium trilaterale* β-xylosidase. Agric. Biol. Chem., 49: 1159-1166.

Van Doorslaev, E., H. Kerster-Hilderson and C.K. De Bruyne, 1985. Hydrolysis of β-D-xylo-oligosaccharides by β-D-xylosidase from *Bacillus pumilus*. Carboh. Res., 140: 342-346.

Weber, K. and M. Osborn, 1969. The reliability of molecular weight determination by dodecyl sulphate-polyacrylamide gel electrophoresis. J. Biol. Chem., 249: 4406-4412.