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PREPARATION AND PROPERTIES OF XYLANASE PRODUCTION BY *Aspergillus achuleatus* DSM 63261 AND ITS USES FOR SOME HEMICELLULOSIC SUBSTANCES DEGRADATION

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ABSTRACT

For their high economic value of xylanase in biotechnological application, sixteen fungal strains were screened for their xylanase potentiality and the results revealed that: *Aspergillus achuleatus* DSM 63261 was the highest xylanase producer. *Aspergillus niger* M2 and *A. terreus* were also highest xylanase production. Other strains were found in the moderate xylanase potentialities. 1% corn stalk conc. Was found as the best inducers between different cellulosic materials used for stimulating the enzyme biosynthesis. The addition of sugar cane molasse as carbon & energy source and the present of 0.042% of corn steep liquor and 0.2325% of KH_2PO_4 as nitrogen and phosphorus content in the production media were very necessary for highest xylanase secretion. pH 4.0 and 7.5% inoculum level to the volume of the production media, 35°C, 200 rpm as agitation rate, and 96 hours were found as the suitable factors supported the enzyme biosynthesis. Final purification procedure of *A. achuleatus* xylanase with sephadex G-100 revealed that, specific enzyme activity reached 455.5 units/mg protein with 10.6 purification folds and 21.2% yield. Optimum enzyme activity was observed at pH 7.0 and 50°C. The enzyme was stable up to 50°C for 1 hour and retained 95% from its maximum activity at 60°C and 45% at 80°C. Thus this enzyme protein is a thermolabial one. The enzyme was quite stable in the pH range of 6.0 – 8.0. At pH 5.0 the enzyme lost 51% from its maximum activity and only 23% at pH 9.0, these results show the neutrality nature of the enzyme protein. Only KCL between different matal salts induced the enzyme activity, but the other such as $FeCL_2$ and $HgCl_2$ were significantly inhibited the enzyme activity. *A. achuleatus* purified xylanase was highly specific for xylan hydrolysis, which the enzyme donot succeeded to hydrolyze other substrates tested. Rapid hydrolysis of xylan with *A. aculeatus* xylanase was present after one hour of hydrolysis. At which 30% from initial xylan weight in the reaction mixture was present. Finally, these results might show that *A. achuleatus* produced highly amount of xylanase under these conditions tested, which used significantly for xylan hydrolysed in different biotechnological applications.

Keywords: *Aspergillus achuleatus* DSM 63261, xylanase, biosynthesis, bioconversion, degradation, hemicellulosic plant raw materials.

INTRODUCTION

The enzymatic hydrolysis of lignocellulosic materials using cellulose alone is unlikely to be cost effective without the cocurrent use of hemicellulases to hydrolyze the hemicellulose content, which may represent up to 35% of the total dry weight. Xylan is a major component of hemicellulose, which is the second most abundant group of polysaccharides

in nature. It is a principle constituent of plant hemicelluloses and offers a potential feed stock for generating food and fuels (Dekker, 1985; Luthi *et al*., 1990, Abdel-Mohsen *et al*., 1996; Kadowaki *et al*., 1997; El-Sawah, 1997 and El-shafei & Rezkallah, 1998a). There is increasing interest in fungi, which can produce high levels of enzyme capable of degrading plant cell wall polysaccharides. Such enzymes are useful as specific tools for elucidating the structure of plant cell walls. They are also required for evolving biodegradative methods for conversion of biomaterials containing cellulose and hemicellulose into monosaccharides from which single-cell protein, single-cell oil or ethanol could be produced (Fall *et al*., 1984, El-Sawah, 1997 and El-Shafei and Rezkallah, 1998a).

Enzymatic conversion of xylans to fermentable sugars has gained importance in the last few years, mainly in response to increasing shortages in energy and neutral resources. Recent interest in xylanase production and application is a welcome development. Xylanase degrade plant xylans to xylose and xylo-oligosaccharides have received much attention on account of industrial use of xylose as a source of xylitol for use as a sweetner. Xylanase have been reported from bacteria, fungi, actinomycetes and yeasts. Xylanase was separated in the purified form as that obtained by *Aspergillus niger* (Shei *et al.*, 1985, Abdel-Naby, 1993, Ahmed *et al.*, 1997, Gawande and Kamat, 1999 and Radwan, 2001). Several fungi strains have been reported to produce xylanolytic enzymes. Strains of *Penicillium funiculosum* (Abdel-Fattah *et al.*, 1996), *A. terreus, A. niger* and *A. awamori* are known to produce xylanase (Siedenberg *et al*., 1997; Gawande & Kamat, 1999; Lemos *et al*., 2001 and Radwan, 2001).

In this communication, screening of different fungal strains for their xylanase potentiality were described. The conditions necessary for optimal production of the enzyme together with purification and characterization of the purified enzyme were also investigated. Also, the role of the enzyme in the hydrolysis of hemicellulosic substances is described.

MATERIALS AND METHODS

Microorganisms:

Aspergillus achuleatus DSM 63261, and *A. niger* DSM 823 were obtained from Deutsche Sammlung Von Mikroorganismen und Zell Kulturen, Mascheroder weg Ib, D-33, Braunschweig, Germany.

Aspergillus awamori NRRL 3126, *Fusarium culmorum* NRRL 32188, *Geotrichum candidum* NRRL Y–552 and *Aspergillus foetidus* NRRL 341 were obtained from NRRL ARS culture collection, Northern Regional Research Lab., Agric. Res. Service, Peoria, USA.

Aspergillus niger Nos. 36, 10, 11, 12, 26, 57, M 2, *A. terreus*, *A. wentii* 2001 and *Fusarium* sp. were obtained from Agric. Microbiol. Dept., Soil, Water and Environ Res. Institute, Agric. Res. Center, Giza, Egypt. These organisms were maintained on PDA medium at 4°C and subcultured monthly.

Media and culture conditions:

The fungal strains were cultured on basal nutrient medium (Kvachadze and Yashvili, 1996), which had the following composition (%): 1 microcrystalline cellulose, 0.13 $(NH_4)_2SO_4$, 0.68 KH_2PO_4, 0.05 $MgSO_4.7H_2O$, 0.02 $CaCl_2$, 0.15 peptone and 1.5 corn-steep liquor. The pH was adjusted to 4.5 before autoclaving. One ml of spore suspensions containing approximately 1 x 10^6 spores were used to inoculate 500 ml Erlenmeyer flasks containing 100 ml of the above liquid medium. Flasks were incubated at 40°C ± 1 for 4 days, at which time, maximum yield of enzyme was produced on a rotary shaker (200 rpm). Mycelia were harvested by filtration and the supernatant assayed for enzymatic activities.

Enzyme assay:

Enzyme activity was assayed by determination of reducing sugar liberated from xylan hydrolysis. The enzyme activity was determined by incubation 2ml of culture supernatant with 0.02g xylan in 2ml of phosphate buffer (pH 7.0, 10 mM) for 10 min at 40°C. The reaction mixture was then filtrated to remove insoluble xylan (Ahmed *et al*., 1997). The liberated reducing sugars were determined by Somogyi method (1952).

One unit of xylanase was defined as the amount of enzyme, which liberates 1 μ mole equivalent of xylose from xylan in one min.

Enzyme purification:

1. Acetone precipitation:

The culture supernatant was subjected to acetone precipitation using acetone concentration ranged from 30-80% (v/v), centrifuged at 8000 rpm under cooling. Then, the precipitate was collected and resuspended in 50 mM sodium phosphate buffer (pH 6.0) and dialized against the same buffer for 24 hr. This insoluble materials formed during the hydrolysis were removed by centrifugation (El-Shafei and Rezkallah, 1998a).

2. DEAE-Sephadex A-50 chromatography:

The dialyzed fraction was applied to a DEAE-Sephadex A-50 column (2.5 by 50 cm) equilibrated with 50 mM sodium phosphate buffer (pH 6.0). Elution of xylanase preparation was carried out using 0-0.5 M NaCl in the same buffer at a flow rate 20 ml/hr. The active fractions were cooled, lyophilized and dialyzed for 24 hr at 4°C (El-Shafei and Rezkallah, 1998a).

3. Sephadex G-100 gel filtration:

The dialyzed active fraction from the DEAE-Sephadex A-50 (30 mg of protein) were further purified on Sephadex G-100 column (2.5 by 50 cm) which was equilibrated with 50 mM sodium phosphate buffer (pH 6.0). Elution was carried out using the same buffer at a flow rate of 20 ml/hr (El-Shafei and Rezkallah, 1998a).

Protein determination:

This was done according to the method of Lowry *et al*. (1951) in comparison to bovine serum albumin (Fluka AD, CH-9470 Buchs) as standard.

Enzyme stability:

Thermal stability of the enzyme was investigated by determining the residual activity after incubating culture supernatants at different temperatures range for 1 hr in phosphate buffer pH 7.0.

pH stability of the enzyme was assayed by determination the residual activity after incubating the enzyme solution in an appropriate buffer (pH 3-9) at optimum temperature for over night.

Effect of various metal ions on xylanase activity:

Effect of metal ions, *e.g.*, KCl, $FeCl_3$, $HgCl_2$, $CaCl_2$, $FeSO_4$, $ZnCl_2$, $CuSO_4$ and $MgCl_2$ (0.2 M conc.) on enzyme activity in the reaction mixture was studied.

Substrate specificity:

Different substrates hydrolysis, *e.g.*, cellulose, carboxymethyl-cellulose, soluble starch and xylan was performed with 0.5% of the substrate which were dissolved in 50 mM phosphate buffer at the optimum pH of the enzyme. Incubated at the optimum temperature of the enzyme for 2 hours with 20 units of this enzyme. Reducing sugars as xylose were determined (Somogyi, 1952) and the phenol-sulphoric acid method (Hodge and Hofreiter, 1962). The degree of hydrolysis was expressed as the percentage of the reducing sugar against the total sugars x 100 (El-Shafei and Reskallah, 1998a).

Xylan hydrolysis:

Xylan (150 mg) was incubated with approximately 20 units of xylanase at optimum pH and temperature of this enzyme. At the times indicated aliquots were taken and the total sugars were determined.

The degree of hydrolysis was estimated from the decrease in dry weight (El-Shafei and Rezkallah, 1998b).

RESULTS AND DISCUSSION

I- Screening of some fungal strains for their xylanase activity:

The screening of sixteen fungal strains for their xylanase activity is presented in Table (1). It could be observed from the results that *Aspergillus achuleatus* DSM 63261 was the most active xylanase producer. The corresponding enzyme activity was found to be 3.9 U/ml/min. *Aspergillus niger* M 2 and *A. terreus* were found in the second order for xylanase production. Other fungal strains were varied considerably in its xylanase production and found in the moderate potentialities. But, *Geotrichum candidum* NRRLY-552 was found as the lowest one for xylanase biosynthesis. Therefore, *Aspergillus achuleatus* DSM 63261 was used for further studies until optimizing the conditions controlling its xylanase production.

Table (1): Enzyme activity of sixteen fungal strains through the fermentation time of corn stalk (for 4 days).

Fungal culture	Xylanase activity (U/ml/min)
Aspergillus niger DSM 823	1.5
Aspergillus niger No. 36	1.7
Aspergillus niger No. 26	2.0
Aspergillus niger No. 57	1.8
Aspergillus niger M2	3.7
Aspergillus terreus	2.9
Aspergillus awamori NRRL 3126	2.1
Aspergillus achuleatus DSM 63261	3.9
Fusarium culmorum NRRL 3288	1.0
Aspergillus wentii	1.9
Aspergillus niger No. 10	2.1
Aspergillus niger No. 11	2.4
Aspergillus niger No. 12	1.7
Fusarium sp.	1.5
Geotrichum candidum NRRL Y-552	1.0
Aspergillus foetidus NRRL 341	1.2

II- Factors affecting *A. achuleatus* xylanase production:

1. Effect of different cellulosic materials on enzyme production:

Data on the effect of different cellulosic materials on the production media of *A. achuleatus* xylanase are presented in Table (2). As the results indicate, the type of cellulosic materials greatly affected the enzyme activity. It is obvious from the data that corn stalk as the platfield waste allowed a highest production of the enzyme. Rice bran, barley flour and carboxy methyl cellulose also supported the enzyme formation, but found in the second order. Other materials gave moderate enzyme yield. The poor synthesis of enzyme was noticed when bagasse was present in the fermentation media. El-Sawah *et al*. (1991) reported that wheat straw powder was found as the best inducer for xylanase production. Patel and Ray (1994) and Hoq and Deckwer (1995) found that when wheat bran was replaced by corn cobs, xylanase production increased about three folds.Similar results were reported by Gawande and Kamat (1999).

2. Effect of corn stalk concentrations:

Data tabulated in Table (2) showed that corn stalk was found as the best favourable cellulosic materials for *A. achleatus* xylanase production. Therefore, results in Table (3) show the effect of corn stalk concentrations on enzyme activity.Corn stalk was added as the sole carbon source in concentration ranging from 1% to 5% (w/v). It is clear from the results that the increasing of corn stalk concentration resulting sharp reduction of enzyme biosynthesis. This means that, the increasing of corn stalk concentration above 1%, repressed the enzyme biosynthesis, which 1% was found as the suitable concentration and the best inducers for enzyme synthesis. At this concentration, enzyme activity reached 5.0 U/ml/min. These results reflect

that higher concentration of corn stalk repressed the synthesis of enzyme with feed back inhibition, as well as higher concentration of the released simple sugars. Similar results were reported by El-Sawah (1997) and Radwan (2001).

Table (2): Xylanase production through the fermentation of various cellulosic materials.

Added materials	Xylanase (U/ml/min)
Wheat bran	4.0
Rice bran	4.5
Bagasse	3.6
Rice straw	4.1
Banana waste	4.4
Carboxymethyl cellulose (CMC)	4.5
Filter paper	4.1
News paper	4.4
Magazine paper	3.9
Barley flour	4.5
Corn stalk* (Control)	4.9

1% of indicated material (corn stalk) plus 1% cellulose powder was added.

Table (3): Effect of corn stalk concentrations on enzyme biosynthesis.

Corn stalk conc. (%)	Xylanase activity (U/ml/min)
1.0*	5.0
2.0	4.6
3.0	4.5
4.0	4.0
5.0	3.6

*** Control.**

3. Effect of some carbon sources:

The results obtained on the effect of different carbon sources on the biosynthesis of *A. achuleatus* xylanase are given in Table (4). In this respect, cultures were grown in the basal medium supplemented with different carbon sources which they added individually at 1% (w/v) + 1% corn stalk. It is seen that the kind of carbon source greatly affected the yield of enzyme produced in the culture fluids. It is also evident that addition of sugar cane molasse to the production media induced the biosynthesis of xylanase activity. This is may be due to its content of some vitamins and minerals, which enhancing the synthesis of enzyme. Sucrose and glucose also highly induced the enzyme formation. Xylose and arabinose were repressed the enzyme production. Other carbon sources supported moderate enzyme secretion. El-Sawah (1997) found that xylan induced the enzyme formation. Similar results were obtained by Radwan (2001).

Table (4): Effect of some carbon sources on enzyme production.

Carbon sources	Xylanase activity (U/ml/min)
Corn stalk only (Control)	5.2
Glucose	5.5
Galactose	4.0
Lactose	3.9
Arabinose	3.5
Maltose	4.0
Fructose	3.6
Xylose	3.1
Sucrose	5.5
Soluble starch	4.1
Sugar cane molasses	5.6
Vinasse	4.1
Glucose syrup	4.1

These carbon sources (1%) were added to the production media containing 1% corn stalk.

4. Effect of medium composition:

Several different media were tested and these media contained corn stalk + sugar cane molasse as carbon and energy source. The results presented in Table (5) show that xylanase biosynthesis was affected greatly with the presence or absence of any of the ingredients of the fermentation media. The absence of KH_2PO_4 or corn steep liquor repressed greatly the enzyme production, which at this treatment, enzyme activity reduced sharply. The absence of any other ingredients also reduced this enzyme production but with negligible effect. In the other side, the presence of all ingredients carbon, nitrogen and phosphate were very necessary for the enzyme biosynthesis. This means that the presence of carbon, nitrogen, phosphorus and other growth factors in the production media were played an important role in the metabolism of any organism especially enzyme production. Mansour and Saber (2001) and Radwan (2001) reported similar results.

Table (5): Influence of medium composition on the biosynthesis of enzyme.

Ingredients						Xylanase activity U/ml/min
$(NH_4)_2SO_4$	KH_2PO_4	$MgSO_4.7H_2O$	$CaCl_2.2H_2O$	Peptone	CSL	
+	+	+	+	+	+	5.8
-	+	+	+	+	+	5.6
+	-	+	+	+	+	2.1
+	+	-	+	+	+	3.9
+	+	+	-	+	+	4.1
+	+	+	+	-	+	4.3
+	+	+	+	+	-	2.3

5. Effect of corn steep liquor concentration:

The medium was supplemented with corn steep liquor (CSL) at different concentrations ranged from 0.014% to 0.098% as nitrogen content. Results in Table (6) show that accompanied increase in enzyme productivity was found with the increasing of CSL up to 0.042% as nitrogen content. Above this concentration, enzyme synthesis repressed with higher amount. Mansour and Saber (2001) reported that CSL supported α-amylase and glucoamylase production. Radwan (2001) found that CSL at 0.70% as nitrogen content in the production media supported xylanase production.

Table (6): Effect of corn steep liquor concentration on enzyme production.

Corn Steep Liquor		Xylanase activity (U/ml/min)
N%	Ml%	
0.014	0.044	2.3
0.028*	0.088	5.8
0.042	0.132	6.8
0.056	0.176	4.3
0.070	0.220	3.2
0.084	0.264	2.7
0.098	0.308	2.3

*** Control.**

6. Effect of different phosphorus sources:

The effect of phosphate types on *Aspergillus achuleatus* xylanase production is shown in Table (7). Highest amount of enzyme was produced when KH_2PO_4 was used as phosphate source in the fermentation medium. Also, $(NH_4)_2HPO_4$ and NaH_2PO_4 were supported greatly the enzyme secretion. But, rock phosphate and $Ca_3(PO_4)_2$ reduced sharply the enzyme formation. These results mean that the form of phosphorus presented in the culture media was very necessary for the metabolic pathways in the organisms especially enzyme production. Similar results were also reported by Kvachadze & Yashvili (1996) and Siedenberg *et al*. (1997).

Table (7): Effect of some different phosphorus sources on the biosynthesis of enzyme.

Phosphorus source	Xylanase activity (U/ml/min)
KH_2PO_4 (Control)	6.9
K_2HPO_4	4.3
NaH_2PO_4	5.8
$(NH_4)HPO_4$	6.2
$Ca_3(PO_4)_2$	1.5
Rock phosphate	0.5

7. Effect of KH_2PO_4 level on enzyme production:

Table (7) shows clearly that the kind of phosphorus affected greatly the enzyme production. Also, its concentration was directly affected on the

rate of enzyme biosynthesis. The increasing in concentration of KH_2PO_4 up to 0.2325% was accompanied with an increase in xylanase production (Table 8). Above or below this concentration, enzyme biosynthesis was reduced greatly. Radwan (2001) found that 0.155% phosphorus is the best concentration for xylanase production.

Table (8): Effect of KH_2PO_4 levels on the biosynthesis of enzyme.

Phosphorus conc. %	Xylanase activity (U/ml/min)
0.0775	2.9
0.155*	6.9
0.2325	7.9
0.310	7.3
0.3875	5.2

*** Control.**

8. Effect of initial pH:

The results on the effects of different pH values on enzyme production are recorded in Table (9). It is seen that the pH greatly affected the xylanase activity of culture fluids. Therefore, the accumulation of xylanase was depended on the initial pH (Table 9). Maximum enzyme production was found at pH 4.0, thereafter, enzyme formation decreased sharply. The explanation of this observation might lie in the fact that acidity of the fluid by which microorganism is surrounded, profoundly affected them. El-Sawah (1997) and Radwan (2001) reported that pH 6.5 was the suitable pH for xylanase production.

Table (9): Effect of initial pH of the production media on the biosynthesis of enzyme during.

pH		Xylanase activity (U/ml/min)
Initial	Final	
4.0	4.0	8.3
4.5*	4.0	7.9
5.0	4.0	7.6
5.5	5.5	7.0
6.0	5.5	6.7
6.5	6.0	5.1
7.0	6.5	3.6

*** Control.**

9. Effect of inoculum level:

In this experiment, inoculum size was added to the production medium solution at 2.5% and increased to 15%. Data in Table (10) show that maximum yield of enzymes was obtained at 7.5% of inoculum level to the medium solution. Accompanied decrease in enzyme formation was found with the increasing or decreasing the inoculum level. This may be due to lowest concentration of cell concentration which responsible the enzyme production or absent one or more of the nutritional factors. These results are in agreement with those reported by Gawande and Kamat (1999), Mansour and Saber (2001) and Radwan (2001).

Table (10): Effect of inoculum level on the biosynthesis of enzymes.

Inoculum size Ml %	Xylanase (U/ml/min)
2.5	4.2
5.0*	8.3
7.5	8.7
10.0	6.3
12.5	6.0
15.0	4.2

***Control.**

10. Effect of agitation rate:

Data presented in Table (11) shows that highest enzyme activity was reached at 200 rpm/min as agitation rate, which the agitation rate ranged from 100 to 250 rpm/min. Above or below, enzyme secretion decreased sharply. These results are similar to those obtained by Mansour and Saber (2001) and Radwan (2001).

Table (11): Effect of agitation rate on the biosynthesis of enzyme.

Agitation rate (rpm)	Xylanase activity (U/ml/min)
100	0.5
125	1.0
150	2.9
175	6.5
200*	9.0
225	6.2
250	3.4

*** Control**

11. Effect of incubation temperature:

Results presented in Table (12) show that maximum enzyme activity was observed at 35°C. Thereafter, enzyme production decreased sharply. These results are similar to those obtained by El-Sawah (1997), Gawande and Kamat (1999) and Radwan (2001).

Table (12): Effect of incubation temperature on the biosynthesis of enzyme.

Temperature(°C)	Xylanase activity (U/ml/min)
20	1.8
25	4.3
30*	9.0
35	9.1
40	6.3
45	3.3

***Control.**

12. Time-course profile for enzyme formation:

The results achieved on the effect of time-course on xylanase production are summarized in Table (13). The results indicated that, the prolongation of incubation time up to 96 hours cause highest increasing in enzyme productivity and decreased gradually thereafter. Similar results were reported by El-Sawah *et al*. (1991); Kadowaki *et al.* (1997); Siedenberg *et al.* (1997), Gawande and Kamat (1999) and Radwan (2001).

Table (13): Effect of time-course on enzyme biosynthesis.

Time (hrs)	Final pH	Xylanase activity (U/ml/min)
24	6.0	1.2
48	4.8	7.7
72	4.5	9.5
96*	4.4	9.9
120	4.4	7.5
144	4.4	6.1
168	4.0	5.2

- **Control.**

III: Purification of enzyme:

About 1000 ml of the culture filtrate was collected and the steps of the enzyme purification procedure and the yield from each step are shown in Table (14). The results show that 50-60% acetone precipitation resulted 43.04 U/mg as xylanase activity with 1.6 fold increase in purity. Although DEAE-sephadex A-50 gave about 2.7 fold purification, which at, some contaminating proteins were found in the enzyme preparation. Therefore, a final separation was achieved by sephadex G-100 column chromatography. At this step, specific xylanase activity reached 455.4 units/mg protein with 10.6 purification fold and 21.2% yield. Similar results were obtained by Nakamura *et al.* (1993).

IV: Properties of the enzyme:

Effect of pH and temperature on enzyme activity:

The effect of pH and temperature on enzyme activity are shown in Figs. (1and2). The results show that optimum enzyme activity was observed at pH 7 and 50°C. These results are in harmony with those obtained by Christakopoulos *et al.* (1996); Kang *et al.* (1996); Bataillon *et al.* (2000) and Abd El-Nasser (2001).

Temperature and pH stability:

Enzyme solutions (20 units of enzyme) were incubated with 50 mM sodium phosphate buffer (pH 7.0) for 1 h at temperature ranging from 30-90°C, chilled on ice, and the activity was determined at 50°C. The results show that enzyme was stable up to 50°C. There was no loss of activity after 1 h at 50°C, whereas the enzyme retained 95% at 60°C and 45% at 80°C. But, 80% from its activity was lost at 90°C. These results revealed that this enzyme is a thermolabial one.

Table (14): Purification steps of *Aspergillus achuleatus* xylanase.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield %	Purification fold
Crude enzyme	9900	230	43.04	100	---
50-60% Acetone precipitation	7405	105	70.52	74.8	1.6
DEAE-Sephadex A-50	3270	28	116.8	33.0	2.7
Sephadex G-100	2095	4.6	455.4	21.2	10.6

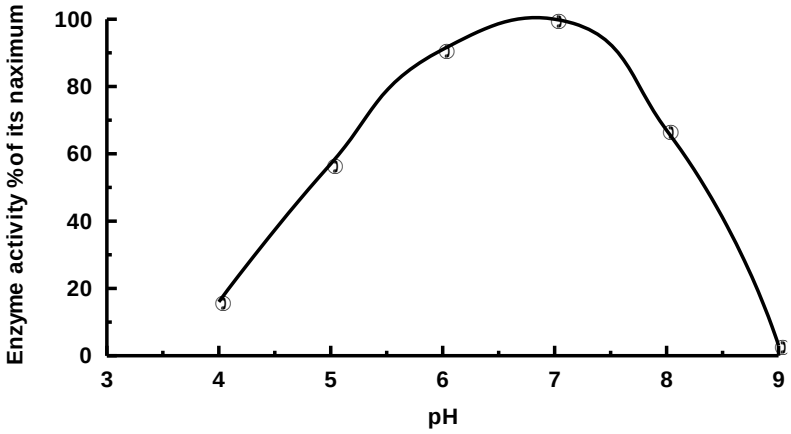

Fig. (1): pH profile of purified *A. achuleatus* xylanase.

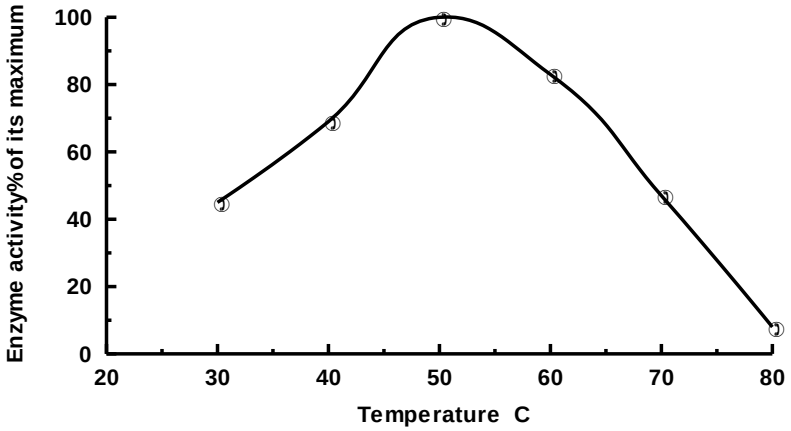

Fig. (2): Temperature profile of purified *A. achuleatus* xylanase.

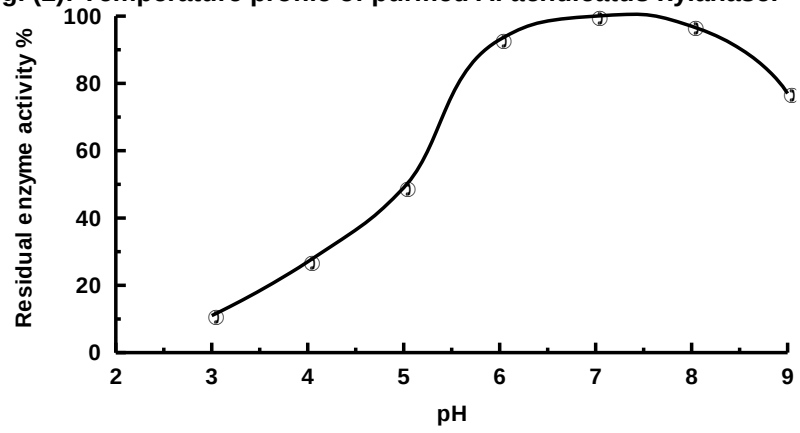

Fig. (3): Effect of pH on the stability of the purified *A. achuleatus* xylanase.

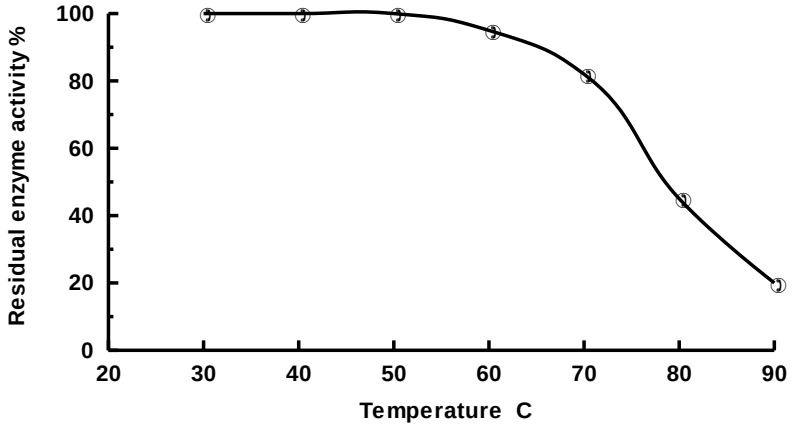

Fig. (4): Effect of temperature on the stability of the purified *A. achuleatus* xylanase.

Purified xylanase (20 units) was incubated at 4°C for 24 h in appropriate buffers to obtain a pH between 3.0 and 9.0. The enzyme was quite stable in the pH range of 6.0 – 8.0 approximately. But at pH 5.0, the enzyme lost 51% of its activity and only 23% at pH 9.0. The results also show that the enzyme lost 89% at pH 3.0. This means that the acidic region had most deleterious effects for the enzyme, at which enzyme protein was denaturated with highest degree. Similar results were obtained by Christakopoulos *et al.* (1995).

Effect of various metal ions on xylanase activity:

Effect of some metal ions on enzyme activity are shown in Table (15). None of the tested metal ions seemed to be markedly stimulate the activity of enzyme except KCL (111%). Whereas $FeCl_3$ and $HgCl_2$ inhibited the enzyme activity with much more inhibition. Also, significant inhibition was

also found with $CaCl_2$ and $CuSO_4$. Other ions had little inhibition for enzyme activity. These results are in agreement with those obtained by Berenger *et al.* (1985); El-Shafei & Rezkallah (1998b) and Bataillon *et al.* (2000).

Table (15): Effect of various metal ions on activity of the purified xylanase from *A. achuleatus*.

Salts	Concentration (M)	Enzyme activity % of its maximum
None	--	100
KCl	10^{-2}	111
$FeCl_2$	10^{-2}	10
$HgCl_2$	10^{-2}	12
$CaCl_2$	10^{-2}	39
$FeSO_4$	10^{-2}	95
$ZnCl_2$	10^{-2}	75
$CuSO_4$	10^{-2}	31
$MgCl_2$	10^{-2}	87

Substrate specificity:

The purified xylanase preparation hydrolyzed β-1, 4-xylan rapidly (Table 16). Other sbstrates used were not able to act as a substrate during incubation for 20 min at 50°C. This means that highest affinity between xylan and enzyme was present. But no affinity between each of other substrates and this enzyme. In other words, this enzyme was highly specific with xylan. Similar results were obtained by Raj and Chandra (1996) and El-Shafei and Rezkallah (1998a and b).

Table (16): Activity of *A. achuleatus* xylanase on some polysaccharides.

Substrate	Enzyme activity % of its maximum
Cellulose	---
Carboxy methyl-cellulose	---
Soluble starch	---
Chitin	---
Chitosan	---
Xylan	100

Xylan hydrolysis:

The time-course of hydrolysis of xylan by *A. achuleatus* xylanase is shown in Fig (5). Xylan hydrolysis was measured by reduction in dry weight of the substrate. These results show that rapid hydrolysis of xylan was present. This means that the enzyme was highly specific with xylan. After 1 h, only 30% remained and after 3 h, very little xylan (10%) was left. These observations were similar to those obtained by El-Shafei and Rezkallah (1998b).

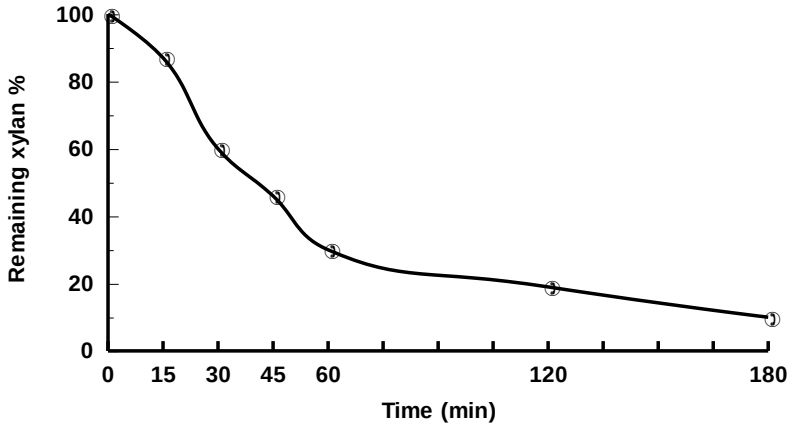

Fig. (5): Time course of enzymatic hydrolysis of xylan by *A. achuleatus* xylanase.

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تحضير وخواص إنزيم الزيلانيز المنتج بواسطة فطر الأسبرجلس أكيوليتس وإستخدامه فى تحليل بعض المواد الهيموسليلوزية

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نظراً للإستخدامات البيوتكنولوجية الهامة والعديدة لإنزيمات الزيلانيز الميكروبية وبصفة خاصة الفطرية منها وذلك لإستخدامها فى توفير مصادر الطاقة وكذلك توفير سكر السوربيتول الهامة فى صناعة الحلويات وكذلك إرتفاع معدل هضم المواد الهيموسليلوزية التى تدخل فى علائق الحيوان . فقد هدفت هذه الدراسة لتوفير الظروف المثلى التى تحث فطر الأسبرجلس أكيوليتس لإنتاج وفرة من إنزيم الزيلانيز ثم تنقية هذا الإنزيم وإستخدامه فى تحليل بعض المواد الهيموسليلوزية ، وقد أوضحت الدراسة النتائج التالية:-

١-تبين أن فطر الأسبرجلس أكيوليتس هو أفضل السلالات الفطرية المستخدمة فى إنتاج إنزيم الزيلانيز ، وقد أوضحت الدراسة أن فطريات الأسبرجلس نيجر والتيريس ذو كفاءة عالية فى إنتاج هذا الإنزيم أيضاً ، بينما كان هناك إنتاج متوسط من باقى السلالات .

٢-إضافة ١% من مطحون سيقان الذرة إلى بيئة النمو أدى إلى زيادة إنتاجية الإنزيم .

٣-إضافة ١% من مولاس سكر القصب كمصدر كربون و ٠,٤٢% كنسبة نيتروجين من منقوع الذرة و ٠,٢٣٢٥% كنسبة فوسفور من فوسفات البوتاسيوم ثنائية الأيدروجين كمصدر للنيتروجين والفوسفور فى بيئة النمو أدى إلى حث جيد لإنتاج الإنزيم .

٤- كانت درجة pH ٤,٠ و ٧,٥% لقاح من الأسبرجلس أكيوليتس إلى حجم بيئة الإنتاج و ٣٥°م و ٢٠٠ لفة/دقيقة كمعدل هز و ٩٦ ساعة هى الظروف المثلى لإنتاج أعلى كمية من الإنزيم .

٥- تنقية الإنزيم بالسيفادكس ج – ١٠٠ أظهر نشاط نوعى عالى للإنزيم وصل إلى ٤٥٥,٤ وحدة / مجم بروتين بمعدل تنقية وصل إلى ١٠,٦ مرة مع الحصول على ٢١,٢% من النشاط الكلى.

٦- كانت درجة pH ٧,٠ و ٥٠°م هما المثاليين لنشاط الإنزيم .

٧- أظهر الأإنزيم ثبات تجاه درجات الحرارة حتى ٥٠°م وإستعاد ٩٥% من نشاطه عند ٦٠°م و ٤٥% عند ٨٠°م مما يعنى أن هذا الإنزيم من النوع المتحمل للحرارة – أيضاً أظهر الأنزيم ثبات عالى تجاه درجات الـ pH بين ٦ – ٨ ولكن تناقص هذا الثبات بدرجة عالية عند درجة pH ٥,٠ حيث وصل النقص فى النشاط عند هذه الدرجة إلى ٥١% فى حين أنه فقد ٢٣% فقط من نشاطه عند درجة pH ٩,٠ . مما أظهر الطبيعة المتعادلة لبروتين هذا الإنزيم .

٨- أظهر كلوريد البوتاسيوم حث لنشاط الإنزيم وصل ١١١% من نشاطه فى حين ثبط كلوريد الحديدوز والزئبقيك نشاط الإنزيم بدرجة عالية .

٩- أوضح زيلانيز الأسبرجلس أكيوليتس النقى توافق كبير مع مادة تفاعله وهى الزيلان فى حين أنه فشل فى تحليل باقى مواد التفاعل المستخدم مما يعنى أنه إنزيم متخصص فى تحليل مواد تفاعله حيث أظهر تحليل سريع وعالى للزيلان وصل إلى ٧٠% من كمية الزيلان المستخدم فى مخلوط التفاعل الإنزيمى .

وبصفة عامة أظهرت الدراسة إنتاجية إنزيم الزيلانيز بواسطة فطر الأسبرجلس أكيوليتس بكميات عالية بإستخدام الظروف الغذائية والبيئية موضع الدراسة وقد أظهر أيضاً هذا الإنزيم المنقى كفاءة عالية فى تحليل الزيلان مما يعنى أهمية إستخدامه فى صناعات بيوتكنولوجية عديدة .