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Characterization of Xylanase Produced by *Aspergillus niger* Fermented on Corn Cob

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THE USE of waste as raw material is important for government economy and natural balance. This article investigates a strain of *Aspergillus niger* for xylanase production in solid state fermentation (SSF) using different agricultural residues without pretreatment. The organism produced 164.4 (µ mol/ ml/ min) of xylanase in static flask on corn cob, an inexpensive lignocellulosic biomass, without enrichment of medium (only distilled water). Using surfactant solution (Tween 80, 1%) for enzyme extraction from solid substrate increased the yield by 52.5 %. Maximum xylanase activity was recorded at 55°C, with high activity at wide range of temperature (50 - 60°C). The enzyme produced on corn cob showed good thermal stability with residual activity of 62.1 and 54.3 % after 2 hr incubation at 50 and 60°C, respectively. Addition of glycerol (50 %) improved stability against temperature by 19.9 % after incubation at 60°C for 1hr, and 17.8 % after incubation at 60°C for 2 hr. The optimum pH for the enzyme activity was 4.5, increasing pH causing decreasing in activity. Xylanase enzyme was stable at different pH's after 1 hr with remaining activity of 89.6 % and 55.0 %, respectively at pH 4.0 and 9.0. The enzyme possessed 70.6 and 37.6 % of its activity by storage at room temperature after 10 and 30 days. In presence of metal ions such as Na^{2+}, Ca^{2+} and protein disulphide reducing agents such as dithiothreitol (DTT), the activity of enzyme was increased by 34.9, 10.7 and 32.8 %, respectively. Sodium dodecyl sulphate (SDS) and Cu^{2+} ion strongly inhibited the enzyme by 56.8 and 23.7 %, respectively. Saccarification of different wastes by the enzyme was studied. The highest yield of reducing sugars (20.3 mg/ 200 mg dry weight substrate) was obtained from corn cob with maximum saccharification after 72 hr (71.8 %) as compared to other wastes.

Keywords: Lignocellulosic biomass, Xylanase, Stability, Saccharification.

The ability of some microorganisms to metabolize lignin and hemicelluloses make them potentially important to take advantage of agricultural residues. Agricultural and agro-industrial wastes, like sugar cane bagasse, wheat bran, rice peel, corn straw, corn cob, fruit peels etc. have increased as result of industrialization and thus becoming a problem regarding environmental pollution

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(da Silva *et al*., 2005). Academic and industrial researchers are putting more effort to reduce the amount of such wastes by finding alternative uses in order to take advantage of the nutrients found in residues matter (Herculano *et al.,* 2011). Hemicellulose is the second most abundant plant fraction available in nature. Agricultural residues contain up to 40% hemicelluloses formed by pentose sugars (Magge & Kosaric, 1985). Xylanases are responsible for xylan hydrolysis, which is the main polysaccharide component of hemicelluloses in hard wood and grasses (Farinas *et al*., 2011). Xylan has a linear backbone of β-1,4-linked D-xylopyranose residues (Anuradha *et al*., 2007). There is great interest in the enzymatic hydrolysis of xylan due to possible applications in feed stock, chemical production and paper manufacturing (Coughlan & Hazlewood, 1997). Recently, interest in xylanases has markedly increased due to the potential application in pulping and bleaching processes, animal feed, textile processes, enzymatic saccharification of lignocellulosic materials and waste treatment (Wong *et al*., 1988; Mechaly *et al*., 1997; Arabi *et al*., 2011 and Kaur *et al*., 2011). They can be used for reducing both juice viscosity and turbidity, hydrolysis of xylan into xylose to be converted biologically to single cell protein, fuels and chemicals (Lemos *et al*., 2000). Most of these processes are carried out at high temperatures, so that thermostable enzymes find applications (Sonnleitner & Fiechter, 1983). Several studies have shown that the xylanases are co-induced in response to xylan or natural substrates containing hemicellulose or even by pure cellulose (Ganju *et al*., 1989 and Kadowaki & Souza, 1997). Many filamentous fungi secrete hydrolyzing enzymes into their culture media and are employed for the hydrolysis of lignocellulosic materials (Okafor *et al*., 2007). Most studies on the production of xylanases have been investigated in submerged liquid culture. There have been few reports on xylanase production in solid state fermentation (SSF) using agricultural residues without chemical pretreatment or xylan addition (Couri *et al*., 2000; Souza *et al*., 2001; Yang *et al*., 2001 and Kaur *et al*., 2011). The cost of the enzyme is one of the main factors determining the economics of the process (Xu *et al*., 2005). SSF an environment friendly and cost effective technology which involves the growth and metabolism of microorganisms on most solids, generally cheap agricultural residues in absence or near absence of any free flowing water, which mimics the natural habitats of microbes has proven to be an efficient fermentation system in producing certain enzymes and metabolites including xylanases (Khandeparkar & Bhosle, 2006; Mohana *et al*., 2008 and Arabi *et al*., 2011). The aim of this work is to evaluate the potential use some agricultural residues as cost-effective substrates for xylanase production by *Aspergillus niger* and its properties.

Materials and Methods

Microorganism

Aspergillus niger was obtained from the Culture Collection of the National Research Centre, Dokki, Cairo, Egypt. It was maintained at 4°C on Potato Dextrose Agar (PDA) slants. Spores suspensions were prepared from 4-days-old

cultures that had been grown on PDA slopes at 37°C. Sterile distilled water was aseptically added to each slope and a suspension of the spores was made by lightly brushing the mycelium with a sterile wire loop. The spore concentration in the suspension was determined by counting the spores in a Neubauer chamber (Hemacytometer**)**. The inoculum volume used to inoculate the fermentation medium was calculated to obtain a final concentration of 10^8 spores/ ml.

Medium and cultivation

The medium used for xylanase production was composed (g/l): xylan (birch wood xylan), 7; peptone, 5; yeast extract, 1; NaCl, 5; K_2HPO_4, 1; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.1. The initial pH value of the medium was adjusted to 7.0 and the medium was sterilized at 121°C for 20 min. For inoculation 2.5 ml of spore suspensions was inoculated into 50 ml of production medium in a 250 ml-Erlenmeyer flask (final concentration 10^8 spores/ ml), and incubated at 37°C, 180 rpm for 7 days. At the end of fermentation, the mycelium was separated from the enzyme-containing broth by centrifugation at 10000 x g for 15 min at 4°C to obtain the crude enzyme.

Solid state fermentation

The agricultural residues (corn cob, saw dust, wheat bran, orange peels, rice straw and molukhiyah stem) were dried and powdered to a diameter of 1.0 mm. The solid media were prepared by mixing 5 ml of distilled water with 2 g of solid substrate and sterilized at 121°C for 20 min. For inoculum preparation, 2.5 ml of diluted spore suspension (final concentration 10^8 spores/ ml) of *Aspergillus niger* was inoculated into solid medium in a 250 ml-Erlenmeyer flask, and incubated at 37°C for 7 days under shaking (180 rpm) and static conditions.

Enzyme extraction and assay

Enzyme extraction was achieved by adding 50 ml of distilled water, acetate buffer (0.05 M; pH 5.0) or Tween 80 solution (1%) to each flask. The flasks were then placed on a rotary shaker 180 rpm at 30°C for 30 min. Crude extracts were centrifuged at 10000 x g for 20 min and then the supernatant was used for enzyme activities assays. Xylanase activity was assayed by measuring the reducing sugar released from xylan. The reaction mixture containing 0.5 ml of birch wood xylan (Sigma) solution 1% (w/v), in acetate buffer (0.05 M; pH 4.5) and were incubated at 45°C for 30 min. The reducing sugars released were determined by Somogyi (1952). The unit of xylanase activity (U) was defined as the number of µ mol of reducing sugars formed per ml per min under assay conditions.

Enzyme properties

Effect of temperature

The enzymatic reactions were carried out at different temperatures ranged from 30 to 75°C and the activity measurements were determined as described above.

Thermal stability

The thermostability of enzyme was determined by measuring the residual activity after incubating the enzyme at various temperatures ranging from 40 to 70°C for 30, 60, 90, and 120 min.

Stability at 50% glycerol

Glycerol was added to the enzyme solution at the beginning of the pre-incubation, at a final concentration of 50%. Measurements were carried out after pre-incubation at different temperatures (40, 50, 60, and 70°C) for interval times (30, 60, 90 and 120 min), and the residual activity was determined at 55°C.

Effect of pH and pH stability

Optimum pH was determined by measuring the activity at optimum temperature over the pH range of 3.5-10.0 using following buffers (0.05 M): Acetate buffer (3.5 - 4.5), Citrate buffer (5.0 – 6.0), Phosphate buffer (7.0 – 8.0), and Glycine-NaOH buffer (9.0 – 10.0). Xylanase stability was measured over pH range of 4.0-10.0 by incubating 0.5 ml enzyme and 1.5 ml of different buffers (as explained above) for 60 min at 30°C. After incubation, residual activity was determined under optimal assay conditions.

Storage stability

Residual activity was determined after incubating the enzyme at room temperature (~25°C) for several periods (1, 5, 10, 15, 20, 25 and 30 days).

Effect of chemical reagents on xylanase activity

The enzyme was incubated at 30 °C for 1hr with 10 mM solution of different chemical reagents like $CaCl_2$, KCl, NaCl, $BaCl_2$, $CuSO_4.5H_2O$, $MgSO_4.7H_2O$, dithiothreitol (DTT), and sodium dodecyl sulphate (SDS). Residual activity was estimated following the procedure described above.

Saccharification study

Natural untreated lignocellulosic substrate was saccarified using xylanase enzyme. The reaction mixture contained 0.2 g of substrate, 8.0 ml acetate buffer (0.05 M; pH 4.5) and 2.0 ml enzyme solution (100 U). After the incubation periods (6, 24, 48 and 72 hr) at 50°C, the reaction mixture was filtered to remove unreacted substances. The resulting filtrates were assayed for total reducing sugar at regular time intervals.

Saccarification % = [Reducing sugar (mg) / Total carbohydrates (mg)] X 0.9 X 100

Determination of total carbohydrates

Total carbohydrates were determined as xylose according to the method of Dubois *et al*. (1956).

Statistical analysis

Data were analyzed through least significant differences using the statistic analysis system SPSS software (Version 16). Statistical analysis for xylanase production by SSF and its properties are presented in Table 3.

Results and Discussion

Enzyme production

Xylanase from microbes are inducible enzymes which determine the great significance of nutrient composition. The high xylan content in some of the wastes like corn cob makes them an accessible and cheap source of inducers (Mohana *et al*., 2008). Crude enzyme in this study was produced using submerged fermentation (SmF) and solid state fermentation (SSF). Preliminary observation (Fig.1) have demonstrated that *Aspergillus niger* produces considerable levels of xylanase when grown in corn cob by SSF under static condition (164.4 U) but lower levels by SmF under shaking condition (27.9 U) with significant variation ($p< 0.05$). The enzyme production under static condition was 68-fold higher in SSF than that in SmF ($p< 0.05$). This may be explained because the growth conditions in SSF are very similar to natural habitat, which favors the spreading of mycelium and consequently, results in a larger production of enzymes and better growth (Leite *et al*., 2007 and Farinas *et al*., 2011). Many studies about the application of SSF are focused in adding value to agro industry residues, which have been extensively used as physical support or source of nutrients (Soccol & Van denberghe, 2003; Agnihotri *et al*., 2010 and Bokhari *et al*., 2010). Corn cob, the central wooden core of maize has recognized as a useful and cost-effective medium ingredient, because it is largely produced as a by-product during the corn processing (da Silva *et al*., 2005 and Xiros *et al*., 2008). In this work, it is interesting that corn cob residue without any pretreatment was used as excellent carbon source for enzyme production by fungi and the growth medium was not supplied with any nutrients or saline solution, only distilled water was used to humidify the solid substrates.

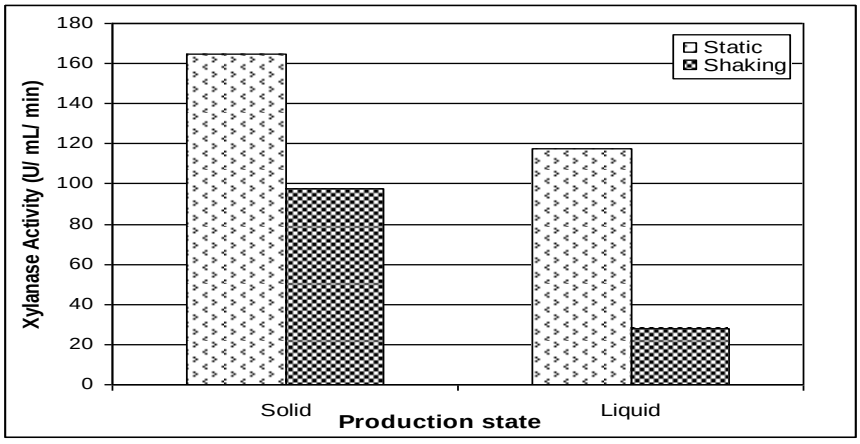

Fig. 1. Production of xylanase enzyme using different conditions.

Enzyme extraction

Some enzymes produced under SSF have been recovered from the solid phase by treatment with distilled water (Xiros *et al*., 2008)**,** surfactant Tween 80 (Silveira *et al*., 1997), or buffer solution (Couri *et al*., 2000). In this study, 50 ml of extraction systems employed, water, acetate buffer (0.05 M; pH 5.0), and Tween 80 (1%), were found to be efficient in recovering xylanase. The time of extraction was 30 min under shaking conditions (180 rpm) and temperature 30°C. The highest enzyme extraction (250.7 U) was obtained by Tween 80. This result is in agreement with Rezende *et al*. (2002), who found that Tween 80 (0.1%) was the most suitable for extraction of *Trichoderma harzianum* xylanase after 15 min. On the other hand, the lowest extraction of *Aspergillus niger* xylanase (96.6 U) was obtained by acetate buffer (data not shown).

Enzyme properties

Effect of temperature

Effect of the temperature in the activity of xylanase enzyme (Fig. 2), shows that the optimum temperature was 55°C yielding 269.7 U and statistically highly significant (p=0.00). This result is similar to that reported by Durand *et al*. (1984) and Agnihotri *et al*. (2010). On the other side the result is slightly higher than that found by Leite *et al*. (2007), who found that optimum temperature for *Aureobasidium pullans* xylanase was 50°C.

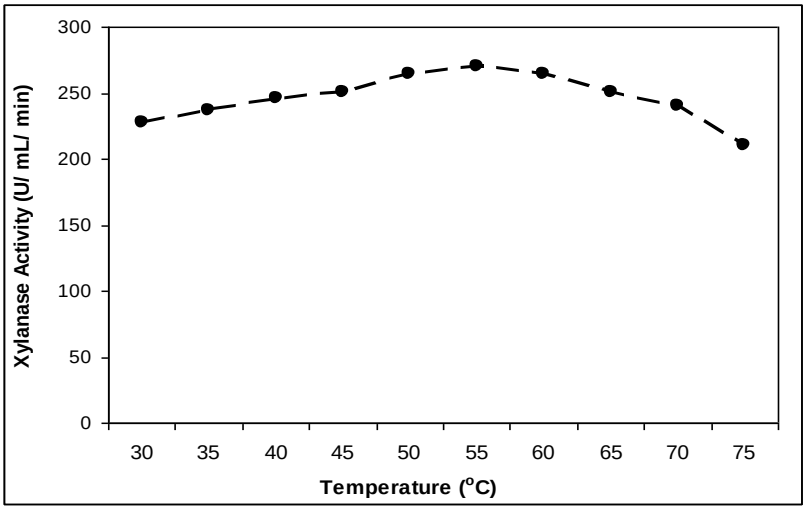

Fig. 2. Effect of temperature on the activity of xylanase enzyme.

Thermal stability

Enzyme stability to heat inactivation is important property due to its potential applications in several industrial processes (Table 1). The enzyme retained 64.3% and 60.2 % of its activity by pre-incubating at 50°C and 60°C, respectively, for 1 hr with high significant (p= 0.00). At 70°C the enzyme

retained 50.0 % of its activity after 2 hr. Mohana *et al*. (2008), pointed to the loss in activity of *Burkholderia* sp. DMAX xylanase (50.0 %) after heating at 60°C for 1 hr. The results in this study is superior to that reported by Li *et al*. (2007) on xylanase from *Penicilliu oxalicum* ZH-30, which was completely inhibited (only retained 0.9 %) within 15 min pre-incubating at 70°C. Kaur *et al*. (2011) reported that crude xylanase extracted from *Coprinopsis cinerea* HK-1 was retained 50 % of its activity when incubated at 55°C for 15 min. The use of thermostable enzymes to carry out hydrolysis at higher temperatures is advantageous because it increases the reaction speed and it prevents microbial contamination thus contributing to increase the economical and biotechnical viability of the process (Gomes *et. al*., 1994 and da Silva *et al*., 2005).

Stabilization by glycerol 50 %

In order to avoid thermal inactivation, the eventual protective effect of glycerol (at 50 %) in aqueous medium was investigated and the results are exhibited in Table 1, which xylanase stability could be improved by glycerol, and there were highly significant increases (p= 0.00) from 64.3 and 60.2 %, to 78.3 and 72.2 %, respectively, after 1 hr at 50 and 60°C. The phenomenon of protein stabilization by polyols may be explained by changes in the microenvironment of the enzyme, which result in a more rigid conformation of enzyme structure. Probably, the benefit of polyols is related to the effect they promote by increasing the degree of organization of water molecules, which in turn, intensify the hydrophobic interactions among non polar groups. Hydrophobic interactions appear to be the single most important factor to stabilize protein structure, therefore making the protein more resistant to unfolding (Klibanov, 1983 and Lemos *et al*., 2000). Angelo *et al.* (1997), found that the stability of xylanase from *Aspergillus* sp. was markedly improved by the addition of 50 % glycerol. Also, Duarte *et al.* (2000) reported that stability of *Bacillus pumilus* xylanase could be enhanced by glycerol and the increase was from 15 % to 36 %, after 2 hr at 50°C.

TABLE 1. Thermal stability of xylanase enzyme.

Time of heating (min) / Temperature (°C)	Residual activity (%)							
	without glycerol				with glycerol			
	30	60	90	120	30	60	90	120
40	96.20	94.0	91.3	89.8	100	100	100	100
50	66.30	64.25	63.81	62.09	80.01	78.32	74.48	70.02
60	61.67	60.16	56.88	54.31	74.40	72.18	67.18	63.99
70	58.90	55.78	52.96	50.00	68.40	66.10	63.13	61.35

Effect of pH

Enzyme activity is markedly affected by pH. This is because substrate binding and catalysis is often dependent on charge distribution on both, substrate and particularly enzyme molecules (Mohana *et al*., 2008). The optimum pH for xylanase activity was detected to be 4.5 yielding (269.7 U) with high significant variation (p= 0.00). As results in Fig. 3, the activity decreased by increasing of pH. Xylanase produced by *Aureobasidium pulluans* had a maximum activity at pH 5.0 (Leite *et al*., 2007).

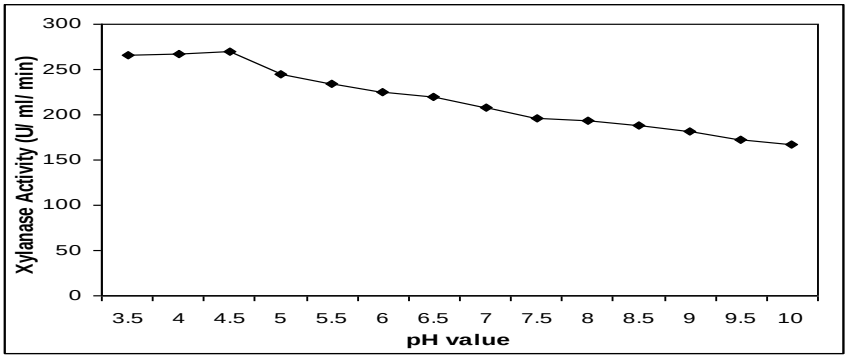

Fig. 3. Effect of pH on the activity of xylanase enzyme.

pH stability

The results obtained after incubation of the enzyme at different pH values for 1 hr pointed to high significant variation (p=0.00) and presented in Fig. 4. Xylanase retained 63.1 % of its activity after incubation at pH 7.0 However, at pH 10.0 the activity decreased rapidly and the enzyme retained 46.6 % of its activity. This result is in contrast with that obtained by Leite *et al*. (2007), who found that *Aureobasidium pulluans* xylanase maintained 53 % of its original activity after 1 day at pH 11.0.

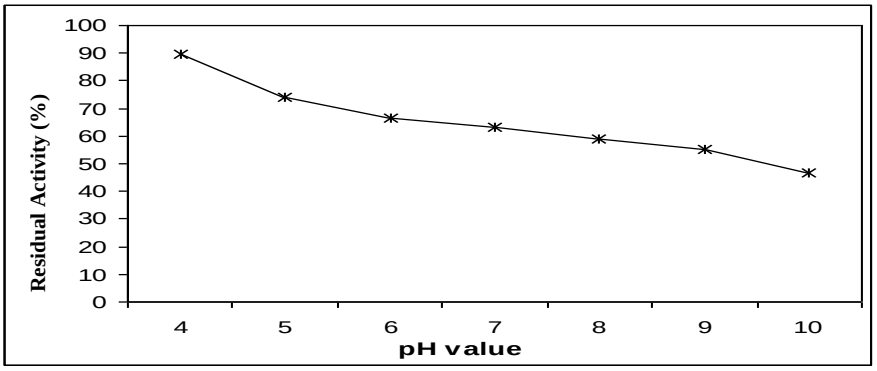

Fig. 4. pH stability of xylanase enzyme.

Storage at room temperature

Experiments were also carried out to estimate the enzyme stability concerning its storage at room temperature (~25°C). The retained activity obtained after 5 days was 89.3 %. The results in Fig. 5 pointed to high significant variation (p=0.00) and showed that xylanase enzyme seems to be resistant to storage for 15 days with residual activity 62.6 % and these results are interesting for application due to the ability of enzyme storage at room temperature and decrease the storage cost.

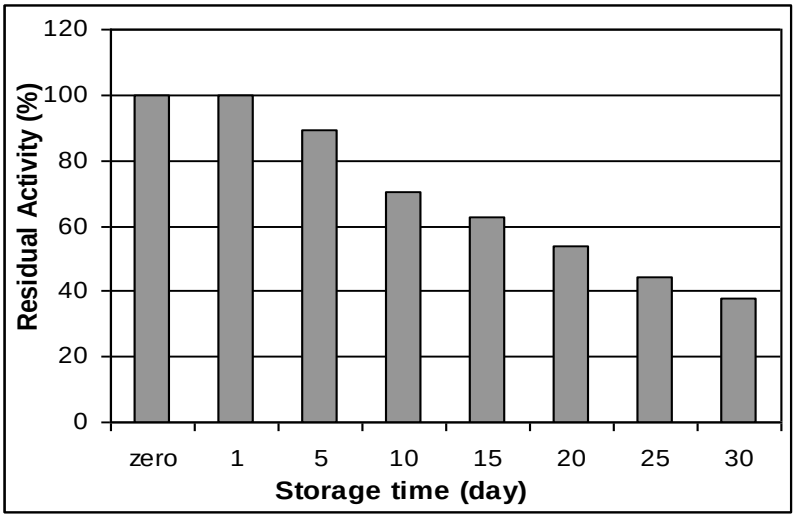

Fig. 5. Stabilty of xylanase enzyme at room temperature.

Effect of chemical reagents

The influence of different chemical reagents on xylanase activity is given in Fig. 6. Xylanase activity was stimulated with significant variation ($p< 0.05$) in the presence of $CaCl_2$, KCl, $BaCl_2$ and $MgSO_4.7H_2O$. NaCl caused highest increase in enzyme activity (134.9 %), perhaps due to alteration of enzyme conformation (Anuradha *et al*., 2007). Metal ions can be involved in enzyme catalysis in a variety of ways, they may : (1) Accept or donate electrons, (2) Themselves act as electrophiles, (3) Mask nucleophiles to prevent unwanted side reactions, (4) Bring together enzyme and substrate by coordinate bonds, (5) Hold the reacting groups in the required 3D orientation, and (6) Simply stabilize a catalytically active conformation of the enzyme (Palmer, 2001 and Mohana *et al*., 2008). The protein disulphide reducing reagent DTT caused high stimulation of enzyme (132.8 % relative activity), confirming the presence of a reduced thiol group of cysteine as a part of the catalytic site in the enzyme structure, such thiol compounds prevent oxidation of this group (Anuradha *et al*., 2007).

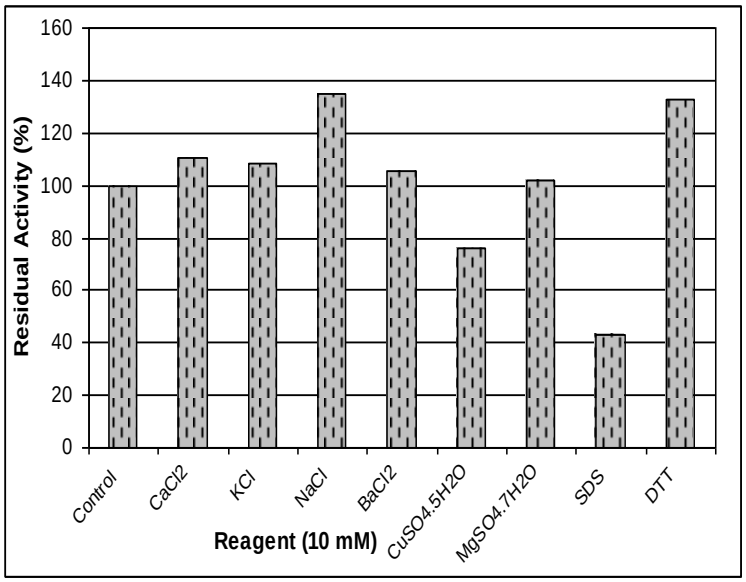

Fig. 6. Effect of some chemical reagent on xylanase activity.

Saccharification study

Results for saccarification of different agricultural residues using xylanase enzyme are depicted in Table 2. Corn cob was found to be more susceptible to hydrolysis than other substrates and statistically significant ($p< 0.05$) after 72 hr rendering (20.3 mg/ 200 mg dry weight of corn cob**)** fermentable sugars corresponding to 71.8 % of the saccharificable material. Prolonged subjection of the wastes to enzyme treatment for more than 72 hr, showed no significant enhancement in the liberation of reducing sugars (data not shown). Also, xylanase enzyme could hydrolysis wheat bran with high value of saccarification (70.6 %) after the same incubation period. Mohana *et al*. (2008) reported reducing sugar liberation by saccharification of lingocellulosic waste using crude xylanase from *Burkholderia* sp. DMAX. Also, Heck *et al*. (2006), pointed to the saccharification of agroindustrial waste by xylanase from *B. circulans*.

Statistical analysis

Statistical analysis results for xylanase production by SSF and its properties are presented in Table 3A, B .

TABLE 2. Saccharification study of some agricultural wastes.

Agriculture wastes	Total carbohydrate (mg)	Incubation period (hr)	Reducing sugar (mg)	Saccharification (%)
Saw dust	14.99	6	3.35	20.11
		24	5.04	30.26
		48	6.18	37.10
		72	6.75	40.53
Wheat bran	11.99	6	5.41	40.61
		24	8.59	64.48
		48	9.16	68.77
		72	9.40	70.56
Molukiyah stem	25.54	6	7.12	25.09
		24	8.46	29.81
		48	10.94	38.55
		72	12.31	43.38
Rice straw	28.22	6	3.71	11.83
		24	8.99	28.67
		48	15.03	47.93
		72	15.26	48.67
Corn cob	25.50	6	10.07	35.54
		24	15.19	53.61
		48	19.39	68.44
		72	20.34	71.79

TABLE 3A. Statistical analysis for xylanase production by SSF and its thermal stability.

Factor	t	P
Static/ Shaking	2.751	0.016
Solid/ Liquid	4.938	0.00
Static/ Shaking (in solid)	1.969E16	0.00
Static/ Shaking (in liquid)	2.858E16	0.00
Thermal stability (with/ without glycerol)	-1.885	0.05

High significant (P< 0.01); Significant (P< 0.05); Not significant (P> 0.05).

TABLE 3B. Statistical analysis for xylanase properties.

Factor	F	P
Temperature	1.691E3	0.00
pH	1.384E4	0.00
pH stability	546.554	0.00
Stability at room temperature	1.867E3	0.00
Metal ions	2.818E3	0.00
Saccarification	3.753	0.032
Reducing sugar	2.607	.0.87

High significant (P< 0.01); Significant (P< 0.05); Not significant (P> 0.05).

Conclusion

It was possible to obtain xylanase enzyme from *Aspergillus niger* using corn cob as cheap agricultural residue by SSF system. It is interesting to use corn cob without either pretreatment, or supplements, only distilled water was used to humidify the solid substrate. The results show good stability of the enzyme to heat inactivation and pH, which pointed to its application in industrial process. Glycerol enhanced thermal stability of xylanase enzyme by 21.90 % when incubated at 50°C for 1 hr. It was also possible to storage the enzyme at room temperature (~25°C) for a long time (10, 20 and 30 days) with high residual activity (70.62, 53.6 and 37.59 %, respectively). Xylanase enzyme was able to convert agricultural wastes to fermentable sugars with higher saccarification percentage compared to other studies (71.79 %).

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دراسة إنزيم الزيلانيـــز المنتج بتخمـــر قـــوالح الـــذرة بواسطة *Aspergillus niger*

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تهدف هـــذه الدراسة إلى محاوله إنتاج إنزيم الزيلانيـــز بواسطة تنمية فطـــــر *Aspergillus niger* على بعض المخلفات الزراعية كمواد خام منخفضة التكلفة إضافة إلى أهميتها للتوازن الطبيعي والاقتصاد القومى وذلك دون إجراء أي معاملات أولية للمواد الخام. وقد تم ذلك باستخدام تكنيك تخمـــر المـــواد الصلبـــة (Solid State Fermentation) . أظهر البحث القدرة العالية للفطر المستخدم على إنتاج إنزيم الزيلانيز (١٦٤,٤ ميكروموول/ مللي/ دقيقة) وذلك باستخدام المزارع الساكنة والتنمية على قوالح الذرة كمصدر رخيص للمواد السليوليزية الحيوية دون أي إضافات للبيئة المستخدمة (ماء مقطر فقط). عمل استخلاص الإنزيم من المادة الصلبة بمحلول Tween 80 (١٪) على زيادة النشاط بنسبة ٥٢,٥ ٪. كما أعطى الإنزيم أعلى نشاط عند درجة حرارة ٥٥ °م بالإضافة إلى إظهار نشاط عالي على مدى واسع من درجات الحرارة (٥٠- ٦٠ °م). كما أظهر الإنزيم درجة ثبات حراري عالية عند التعرض لدرجات حرارة وفترات زمنية مختلفة حيث أعطى ٦٢,١ ، ٥٤,٣ ٪ نشاط متبقي بعد التحضين لمدة ساعتين على ٥٠ ، ٦٠ °م. تم زيادة الثبات الحراري في وجود الجليسرول (٥٠ ٪) بحوالي ١٩,٩ ، ١٧,٨٪ بعد التحضين على ٦٠ °م لمدة ساعة، ساعتين على التوالي. أشارت الدراسة إلى أن الإنزيم يعطى أعلى نشاط له عند أس هيدروجيني ٤,٥ مع انخفاض تدريجي في النشاط بزيادة الأس الهيدروجيني. أشارت النتائج إلى ثبات إنزيـــم الزيلانيز عند التحضين لمدة ساعـــة على درجـــات أس هيدروجيني مختلفة (٤ ، ٩) مع الأحتفاظ بنشاط متبقي ٨٩,٦ ، ٥٥ ٪ على التوالي. تم دراسة ثبات الإنزيم عند التخزين على درجة حرارة الغرفة (~ ٢٥ °م) لفترات زمنية مختلفة وأظهرت النتائج احتفاظ الإنزيم بحوالي ٧٠,٦ ، ٣٧,٦ ٪ من النشاط الأصلى بعد ١٠ ، ٣٠ يوم مما يمكن من استخدامه في المجالات الصناعية لفترات زمنية طويلة. تحسن نشاط الإنزيم بنسبة ٣٤,٩ ، ١٠,٧ ، ٣٢,٨ ٪ في وجود أيونات بعض المعادن مثـل Na^{2+}، Ca^{2+} ، DTT على التوالـــي ، في حين تسبب كلا من SDS ، Cu^{2+} في تثبيط قوى للنشاط الأنزيمى يقدر بحوالي ٥٦,٨، ٢٣,٧٪ على التوالي. كما استهدفت الدراســة عملية التسكر Saccarification وذلك بهدف إنتاج بعض السكرات من المخلفات الزراعية (نشارة الخشب ، ردة ، سيقان الملوخية ، قش الأرز ، قوالح الذرة) وكان أعلى إنتاج من السكرات المختزلة (٢٠,٣ ملليجرام/ ٢٠٠ ملليجرام قوالح ذرة جافـة) بالإضافة إلى أعلى نسبة تسكر (٧١,٨ ٪) بعد ٧٢ ساعة مقارنة بالمخلفات الأخرى.