J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 8(2): 71 -78 ,2017

Optimization of Xylanase Production from Some Agro-lignocellulosic Wastes by *Aspergillus niger* Via Solid State Fermentation

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ABSTRACT

Extracellular xylanase production by *A. niger* was studied under solid-state fermentation using six agricultural wastes individually (wheat bran, rice husk, corn cobs, rice straw, clover straw and wheat straw) as well as fifteen treatment represent different combinations of the above agro-lignocellulosic wastes. The best substrate was the combination of wheat bran and clover straw. The initial moisture content, additive carbon source, additive nitrogen source, initial pH of the culture, incubation temperature and inoculum size affected the production of xylanase. The optimal fermentation medium was at 80% of initial moisture and composed of 5 g WB+CS (1:1 w/w) supplemented with 1% (w/w) of xylan and 1% (w/w) of $(NH_4)_3PO_4$ and inoculated by adding 1 ml of spore suspension containing $2x10^5$spores/ml. Xylanase production reached a peak of 1294.15 IU/gdw after 7 days of incubation at 35 °C under SSF. The produced enzyme has maximum activity at 70 ºC and pH 5.5. Addition of 10 mM $FeSO_4$ and 5mM of KCl, $CaCl_2$ and $MnCl_2$ to the reaction mixture enhanced xylanase activity to 127.46, 118.86, 115.76 and 111.15%, respectively. *A. niger* xylanase showed superior heat and pH tolerance, it remained 86.47 and 76% of its activity after 60 min of incubation at 70 ºC with and without 10 mM $CaCl_2$.The enzyme showed more stability in a pH ranged from 4 to 5 retaining more than 75% of its original activity, therefore it may have significant applications in biofuel and paper industries.

Keywords: xylanase, agricultural wastes, solid state fermentation.

INTRODUCTION

At the present time, xylanases, cellulases and pectinases participate about 20% of enzyme market of the world (Polizeli *et al.,* 2005). Low production yields and high production cost had cause restrict for applications of industrial enzymes (Kang *et al.,* 2004).On a commercial scale, most of xylanases and cellulases enzymes were produced by submerged fermentation (SmF). However, the filamentous fungi which considered as vigorous xylanases and cellulases producers prefer Solid-State Fermentation (SSF) where solid substrate is similar to fungal natural habitat (Mitchell, 2006).Furthermore, SSF has many advantages such as simple technique, superior productivity, higher product stability, reduced energy requirements, low wastewater output, low capital investment, improved product recovery and low contamination risk (Mitchell, 2006).In many cases, enzymes produced under SSF have better properties than enzymes produced by submerged fermentation such as thermal and pH stability (Saqib *et al*., 2010). Another advantage of SSF is the use of cheap solid agro-lignocellulose wastes which used as a carbon source and reduce the need of expensive medium (Ang *et al*., 2013).

Xylan is a main hemicellulosic component of soft wood and hard wood. After cellulose, xylan is the most renewable abundant polysaccharide. It is considered to be a potential resource for producing numerous valuable products (Pandya and Gupte, 2012). This compound of heteropolysaccharide is composed of a main chain of 1,4-β-D-xylose monomers and short chain branches consisting of O-acetyl, α-L-arabinofuranosyl and α-Dglucuronyl residues. Xylanases enzymes are produced by different microorganisms including yeast, filamentous fungi, and bacteria (Maheshwari *et al.,* 2000). Filamentous fungi are particularly interesting producers of xylanases since their enzyme levels are much higher than those of yeast and bacteria (Jeya *et al*., 2005)

Xylanases are group of enzymes consisting of endoxylanase (EC 3.2.1.8) which cleaves β-1,4 linked xylan backbone and β-xylosidase (EC 3.2.1.37) which converts xylooligomers to monomeric xylose subunit (Biely, 1985). Recently, xylanases have great interest because of its potential industrial application. This enzymes has many industrial applications ranging from bioethanol production, clarifying fruit juices, production of food additives and bioconversion of lignocelluloses biomass to sugars (Ferreira *et al.*, 1999).Using pure xylan as a substrate in xylanase production raises the cost. Therefore, efforts have been made to use available inexpensive renewable abundantly lignocellulosic materials instead of high cost pure xylan (Bakri *et al.,* 2003).

The present study was designed to investigate the potential use of some agro-lignocellulosic wastes as carbon sources for xylanase production by the tested fungi. The aim of this study is to produce an inexpensive and highly active xylanase lies in a combination of critical factors such as improved enzyme quality, enhanced enzyme productivity and yield, prolonged enzyme lifetime and minimal cost of media and substrate.

MATERIALS AND METHODS

Microorganism and culture condition:

The fungal isolate used in the present investigation was isolated from soil of the farm of Faculty of Agriculture, Mansoura University during a screening study for xylanase producers and identified as *Aspergillus niger* by The Regional Center for Mycology and Biotechnology, Al-Azhar University. It was grown on potato dextrose agar (PDA) for 7 days at 30 ºC and then stored at 4 ºC. Stock culture was transferred to fresh medium every 2 months and incubated under the same conditions.

Inoculum preparation: Spore suspension was prepared by addition of 10 ml of sterilized 0.9% sodium chloride to 7 days old PDA slant culture whose surface was gently scraped with a sterile wire loop. The spore

suspension was counted using Neubauer chamber and adjusted to obtain a concentration of 1 $X10^6$ spores/ml.

Xylanase production in SSF:

In an attempt to find out a cheap source of substrate for SSF which supports xylanase production, various agricultural wastes, including wheat bran(WB), rice husk (RH), corn cobs (CC), rice straw (RS), clover straw (CS) and wheat straw (WS), collected from local farms/markets- were screened individually as well as in combinations (mass ratio of 1:1 w/w). The basal medium used for xylanase production in SSF consists of 5 g of dry substrate in a 250-mL Erlenmeyer flask moistened with 5 ml distilled water. The contents of the flasks were autoclaved at 121 °C for 20 min. Each fermentation medium was inoculated using l ml of spore suspension at concentration of 1 $X10^6$ spores/ml and incubated at room temperature (30±2 ºC) for 7 days.

Xylanase extraction:

The crude enzyme was extracted from solid state cultivation media by shacking a fermented fungal substrate with 50 ml distilled water on a rotary shaker (150 rpm) at room temperature for 1 h. The slurry was squeezed through double gauze. Extracts were pooled and centrifuged at 5000 rpm for 20 min to separate cells, spores and small substrates particles. The clear supernatant was used as a crude enzyme for xylanase assays.

Enzyme assay:

Xylanase activity was determined by mixing 0.9 ml of 1% birchwood xylan prepared in 0.1 mM citrate buffer pH 5.5 with 0.1 ml of the appropriately diluted enzyme (Bailey *et al*., 1992). The reaction mixture was incubated at 50 ºC for 5 min. The released reducing sugars were determined using 3,5-dinitrosalicylic acid (DNS) method. The reaction was stopped by addition of 1.5 ml (DNS) reagent and the content was boiled for 5 min, xylose is used as standard for xylanase activity (Bailey *et al*., 1992). One unit of xylanase is defined as the amount of enzyme that liberates 1 μmol of xylose equivalents per minute.

Factors affecting xylanase production:

Optimization of initial moisture content:

Ten initial moisture contents of the basal medium were adjusted by addition of distilled water. Fermentation media were prepared with different values of moisture content ranging from 1:0.5 to 1:5(substrate: distilled water, w/v) at intervals of 0.5 moisture content. The xylanase activity was determined after 3, 5, 7 and 9days of incubation at room temperature (30±2ºc).

Effect of additional carbon sources:

Xylanase was produced by *A. niger* in the basal medium supplemented with different carbon sources (1% w/w) of xylan, cellulose, xylose, starch, mannitol, galactose, lactose, arabinose, maltose, fructose, sucrose and glucose. The flask without any additional carbon source was served as a control. The xylanase activity was determined after 3, 5, 7 and 9days of incubation at room temperature.

Effect of additional nitrogen sources:

The effect of nitrogen source on xylanase production was evaluated by adding ten various organic and inorganic nitrogen sources to the fermentation media at a concentration of 1% (w/w). Flasks were inoculated and incubated for 5, 7 and 9 days at room temperature. The flasks without addition of any nitrogen source were served as a control.

Optimization of incubation temperature:

Effect of different four temperatures ranging from 25 ºC to 40 ºC with 5 ºC interval was studied for the maximum xylanase production.

Optimization of initial pH:

For optimization of pH for maximum xylanase production, the *A. niger* was grown in the production medium having different pH ranging from 3.5 to 7.0 with 0.5 pH interval at 35ºC for 6 and 7 days.

Effect of inoculum size:

The effect of inoculum size was studied by inoculating the fermentation medium by 1 ml of the spore suspension of concentrations varies from 2×10^3 to 2×10^7 spores/ml and incubated for 6 and 7 days at optimum pH and temperature.

Factors affecting crude xylanase activity:

Optimum pH, it was determined at 50 ºC by conducting the enzyme assay at different pH using the following buffers: sodium citrate (pH 3 -6), sodium phosphate (pH 6.5–8).

pH stability, it was determined by diluting the crude enzyme two-fold by different buffers as previously mentioned followed by measuring the remaining activity at appropriate time intervals using standard assay methods.

Optimum temperature, it was determined at different temperatures ranging from 40 to 95°C for 5 min in 0.1 M sodium citrate buffer (pH 5.5).

Thermal stability, it was measured by incubating crude enzymes under 50, 60, 70 and 80ºC for appropriate time intervals. The residual activity was measured using standard assay method.

Effect of mineral salts:

Xylanase activity was determined using the enzyme assay described before in the presence of different metal salts ($CaCl_2$, NH_4SO_4, EDTA, $MnCL_2$, KCl, $FeSO_4$, $MgSO_4$, $NiSO_4$, $CuSO_4$ and $HgCl_2$) at different concentrations (2, 5 and10mM).

RESULTS AND DISCUSSION

Effect of substrate type on xylanase production:

Data presented in Fig.(1) show the variations of xylanase production using six agricultural wastes individually as well as in combinations as nutrient medium and anchorage for *A. niger* under SSF after seven days of incubation. Among the individual agroresidues, the WB was found to be the most effective. Maximum xylanase production (536.78 IU/gdw substrate) was achieved after seven days of incubation with WB. Also, CS produced 383.13IU/gdw substrate after 7 days of incubation and was found to be a good substrate. Many researchers reported the WB as being preferential substrate for production of xylanase as it is easily available cheap and does not require any pretreatment (Bakri *et al*., 2003; Antoine *et al*., 2010; Garai and Kumar, 2013). With RH, RS and CC the production of xylanase decreased to 229.86, 165.05and

132.61IU/gdw substrate respectively. On the other hand WS was the weakest xylanase producer which yielded 33.11 IU/gdw substrate.

The variations in the xylanase productivity of these different agroresidues were probably because of the differences in their degree of degradability and their composition, including the presence of some additional nutrients (Yang *et al.*, 2006).

Results in Fig. (1) also show that blending substrates together increase the production of xylanase particularly with WB and CS. Among all combinations, the WB+CS was found to be the best for xylanase production which yielded 857.91IU/gdw achieving 1.6 fold increase compared to WB and 2.24 fold increase compared to CS individually. Mixing WB with the other substrates also increase xylanase production compared to individual substrates after 7 days of incubation as well as mixing CS with other substrates increase xylanase production particularly with WS which yielded 612.72 IU/gdw achieving 1.6 fold increase compared to CS and 18.51 fold increase compared to WS individually as seen in Fig. (1).

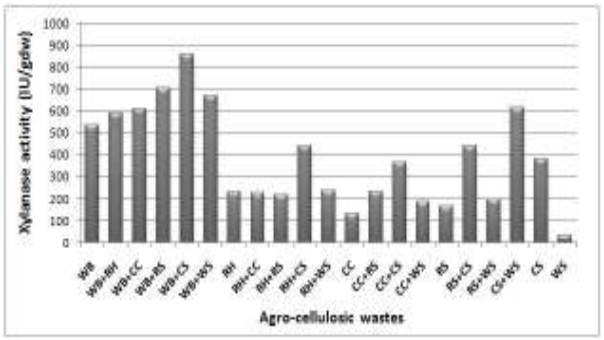

Fig. 1. Effect of different agricultural wastes and combinations between them as a nutrient medium and anchorage for xylanase production by *A. niger* via SSF technique after 7 days of incubation at room temperature (32±2 ºC).(LSD at 5% = 28.7)

Optimization of initial moisture content:

Moisture content is a critical factor in Solid State Fermentation that has influence on the microbial growth and as a result of that affect enzyme production (Sivaramakrishnan *et al.,* 2006).Fig.(2) shows the effect of moisture content on the production of xylanase obtained by *A.niger* under SSF containing WB+CS after 3, 5, 7 and 9 days of incubation. The enzyme production increased by raising moisture ratio from 1:0.5 (55%) and reached a peak at ratio 1 : 2 (80%) in 7 days but further increase in moisture ratio resulted in a gradual decrease in xylanase production. The maximum xylanase production (922.87 IU/gdw) was observed at 80% of initial moisture (substrate: distilled water ratio 1:2).

Results are in close agreement with those obtained by previous investigators. Mohamed *et al.,* (2013) reported that higher moisture content changes particle structure, reduces gas volume, decreases diffusion and decreases porosity, which results in decreased of transfer rate of oxygen. On the contrary, lower moisture content causes low degree of swelling, reduction in solubility of the nutrients of the substrate, and high water tension. Also, Antoine *et al*., (2010) and Pirota *et al*., (2013) reported that the higher xylanase activity from *Penicillium canescens* and *A. oryzae* , respectively, was obtained at moisture content of 80%.

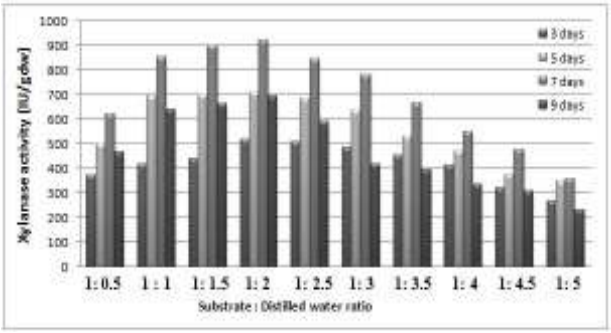

Fig. 2. Effect of moisture content on xylanase production from WB+CS combination as a nutrient medium and anchorage by *A. niger* via SSF at room temperature.(LSD at 5% = 27.48)

Effect of additional carbon sources:

Fig. (3) shows the effect of twelve additional carbon sources on xylanase production by *A. niger*. Xylan, starch and cellulose enhanced xylanase production from 921.48 to 1081.47, 964.59 and 950.45 IU/gdw respectively, while addition of the other sugars decreased the production of xylanase with respect to control. These results are in harmony with that obtained by Garai and Kumar, (2013) who reported that fungi produced lower xylanase by addition of glucose, xylose, sucrose, maltose and fructose. Liu *et al.*, (2008) reported that addition of xylan and carboxymethylcellulose showed high improvement in the production of xylanase by *Aspergillus niger* SL-05.

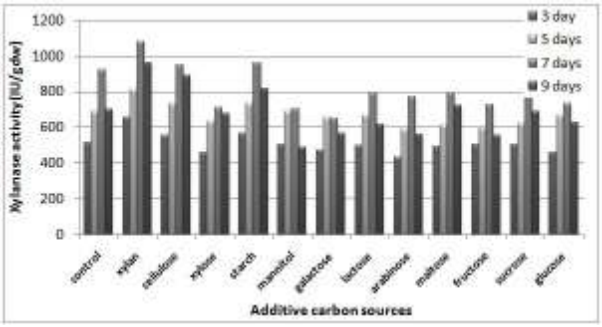

Fig 3. Effect of additional carbon sources (1% w/w) on xylanase production by *A. niger* via SSF at room temperature under initial moisture content of 80%.(LSD at 5% = 26.57)

Effect of additional nitrogen sources:

The availability of the nitrogen source has influences on the mechanism that regulate the extracellular enzyme formation. During fermentation process, the nitrogen source can significantly affect pH of the medium and in turn it may influence the enzyme production. Under SSF, addition of nitrogen source is not always essential, as it depends on the requirement of the microorganisms and the availability of nitrogen in the substrate (Pandya and Gupte, 2012).In the current study, different organic and inorganic nitrogen sources were added in a concentration of 1% (w/w) using WB+CS as a substrate. Results in Fig. (4) show that the xylanase production increased by addition of peptone,

beef extract, $(NH_4)_3PO_4$ and $(NH_4)_2SO_4$ while addition of NH_4Cl and $Ca(NO_3)_2$ caused insignificant increase. In contrast, supplementation with yeast extract, tryptone, proteose peptone and malt extract caused marginal decrease in xylanase production. The maximum xylanase production (1196.49 IU/gdw) was reached with $(NH_4)_3PO_4$, followed by Peptone (1169.22 IU/gdw) after 7 days of incubation.

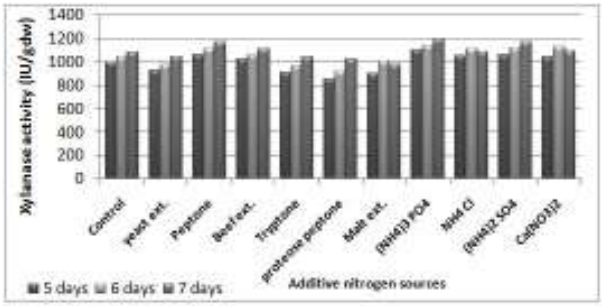

Fig 4. Effect of additional nitrogen sources(1% w/w) on xylanase production from WB+CS by *A. niger* via SSF, the media was supplemented with 1% xylan and incubated at room temperature under initial moisture content of 80%.(LSD at 5% = 30.59)

Also, it was observed that there is no significant difference in xylanase production between the organic or inorganic nitrogen sources and these results are in agreement with that reported by Kheng and Omar (2005). Senthilkumar *et al*., (2005) reported that the addition of $NaNO_3$ enhanced the production of xylanase produced by *Aspergillus fischeri* grown on wheat bran as substrate under SSF whereas yeast extract had no effect. Ncube *et al.*, (2012) found 13% increasing in xylanase production by *A.niger* FGSCA733 when the media of jatropha seed cake supplemented with ammonium chloride.

Optimization of incubation temperature:

Fig. (5) shows that the production of xylanase increased gradually by further incubation at different temperatures to reach a peak at 7 days except at the highest temperature at 40 ºC which caused insignificant decrease in the production by further incubation for 7 days. The maximum production of xylanase (1219.82 IU/gdw) was obtained at 35ºC. The second best temperature for xylanase production by *A. niger* was 40 ºC which achieved 1153.76 IU/gdw after 6 days of incubation.]]]

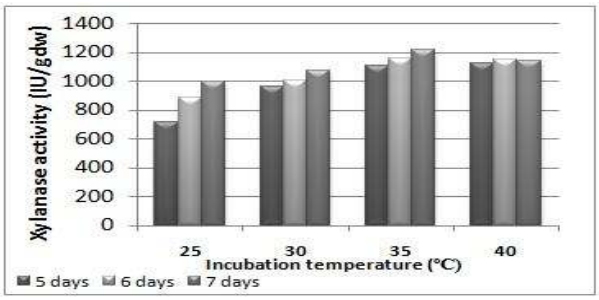

Fig. 5. Effect of incubation temperature on xylanase production from WB+CS combination by *A. niger* via SSF, the media was supplemented with 1% xylan, 1% $(NH_4)_3PO_4$ and incubated at room temperature under initial moisture content of 80%.(LSD at 5% = 28.95)

The obtained results are in close agreement with that obtained by Pal and Khanum (2010) who reported that the maximum production of xylanase by *Aspergillus niger* DFR-5 was obtained at 40 ºC after 6 days of incubation and a sharp reduction occurs at higher temperatures.

Optimization of initial pH:

Results in Fig. (6) show that a higher xylanase production was obtained at slightly acidic pH (pH5) than at neutral pH and at acidic pH. Maximum xylanase production was (1294.15IU/gdw) at pH 5 after 7 days of incubation, while the minimum was observed at pH 3.5 (945.49). The results are in line with Shah and Madamwar, (2005) who had obtained maximum xylanase production by *A. foetidus* MTCC at pH 5.0. Most investigators have reported an acidic pH to be the most appropriate for maximum xylanase production by fungi (Murthy and Naidu, 2012; Ncube *et al*., 2012; Ang *et al*., 2013).

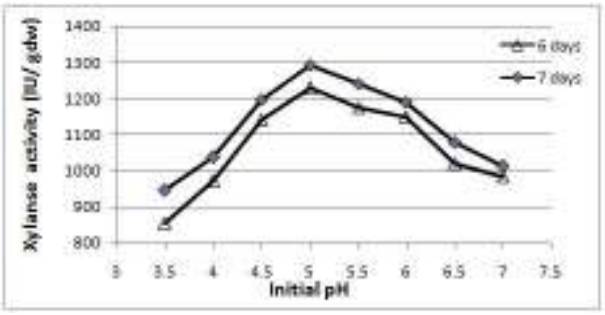

Fig. 6. Effect of initial pH on xylanase production from WB+CS combination by *A. niger* via SSF, the media was supplemented with 1% xylan, 1% $(NH_4)_3PO_4$ and incubated at 35 ºC under initial moisture content of 80%.(LSD at 5% = 29.62)

Effect of inoculum size:

To study the effect of the inoculum size on xylanase production 1 ml of different concentrations of spore suspension varied from $2X10^3$ to $2X10^7$ was inoculated to the fermentation medium. Xylanase production increased with increasing of inoculum concentration and reaches a peak of 1339.17 IU/gdw after 7 days of incubation at 35 ºC when the inoculum density was $2X10^5$, further increasing of the inoculum density led to decrease the xylanase production as shown in Fig. (7).

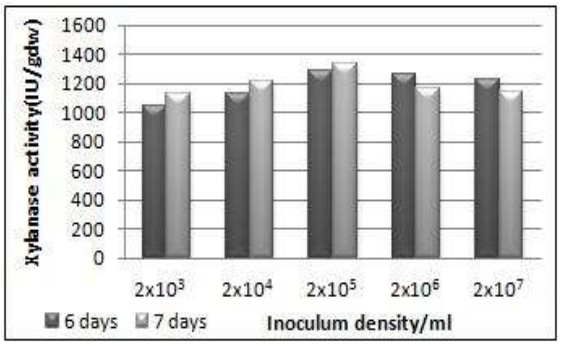

Fig. 7. Effect of inoculum density on xylanase production from WB+CS combination by *A. niger* via SSF, the media was supplemented with 1% xylan, 1% $(NH_4)_3PO_4$ and incubated at 35 ºC with initial pH of 5 under initial moisture content of 80%.(LSD at 5% = 22.65)

This data are in close agreement with that reported by Park *et al.,* (2002) who obtained maximum xylanase activity from *A. niger* mutant at inoculum size of $5X10^5$.

Factors affecting crude xylanase activity:

Effect of pH:

The activities of enzymes are obviously influenced by reaction mixture pH. This is because of the binding with substrate and catalysis is dependent on distribution of charge on both substrate and enzyme molecules (Shah and Madamwar, 2005). Results in Fig.(8) show that the suitable pH range for *A. niger* xylanase activity was 4.5–6.0, with optimum pH at 5.5 which was considered to be 100%. A considerable drop in the activity of xylanase was observed below pH 4.5 and above pH 6.0.The behavior of the enzyme shows that it is more suitable for any application in the pH range of 4.5 to 6.0 where it retained 81.07% and 90.86% of its activity at pH 4.5 and 6, respectively.

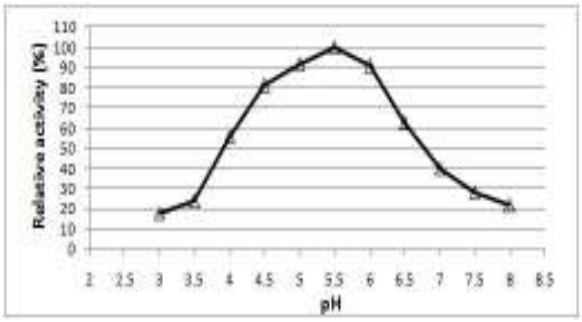

Fig. 8. Effect of the reaction mixture pH on *A. niger* xylanase activity in the pH range of 3 – 8, the reaction was done at 50 ºC for 5 min.(LSD at 5% = 2.82%)

Results are in agreement with those obtained from previous studies. Coral *et al*., (2002) obtained xylanase from *A. niger* with suitable pH range of 4–5. Shah and Madamwar, (2005) found that the favorable pH range for xylanase activity of *Aspergillus foetidus*, was 4.6 to 5.6, with optimum at pH 5.3. Also they reported that a remarkable drop was observed in xylanase activity below pH 4.6 and above pH 5.6. Also, Ncube *et al.*, (2012) reported that xylanase of *Aspergillus niger* was most active at pH 5.

Effect of temperature:

The effect of temperature on the produced xylanase activity is shown in Fig.(9). Results show gradual increase in xylanase activity by raising reaction temperature from 40 to 70 ºC, and 70 ºC was the optimum temperature which was considered to be 100%, but further increase of temperature decreased the activity. The enzyme do well in the range of 50 – 85 ºC and retained 82.76% and 61.41% of its activity at 80 ºC and 90 ºC, respectively which made it suitable for many industrial applications.

Optimum temperature at 65 ºC has been reported for the activity of xylanase from *A. terreus* FSS129, at 80 ºC the enzyme retained only about 22% of its activity (Bakri *et al.*, 2010). Ncube *et al.*, (2012) determined optimum temperature for the activity of *Aspergillus niger* xylanase, which found to be 50 ºC. Coral *et al.*, (2002); Shah and Madamwar, (2005) reported that xylanases from fungal sources have optimal temperatures between 40 and 60 ºC.

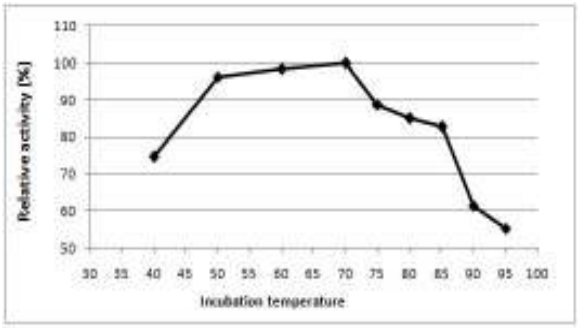

Fig. 9. Effect of the reaction mixture temperature on xylanase activity produced by *A. niger* in the temperature range of 40 – 95, the reaction was done at pH 5.5 for 5 min.(LSD at 5% = 2.26%)

Effect of different metal salts:

The effects of several mineral salts at different concentrations on xylanase activity were estimated, and the results are shown in Fig.(10). The xylanase activity was slightly decreased in the presence of NH_4SO_4 and $CuSO_4$ at a low concentration (2 mM) and the inhibition increased by raising concentrations up to 5 and 10 mM. For EDTA, the extent of the inhibition of xylanase activity was enhanced by increasing the concentration from 2 mM to 10 mM, While $HgCl_2$ caused a drastic effect over 2 mM. However, for other metal salts, the enzyme activity increased and then decreased with increasing concentration from 2 mM to 10 mM except $FeSO_4$ which caused gradual increase in the activity by increasing concentration to reach a peak at 10 mM achieving 127.46% relative activity.

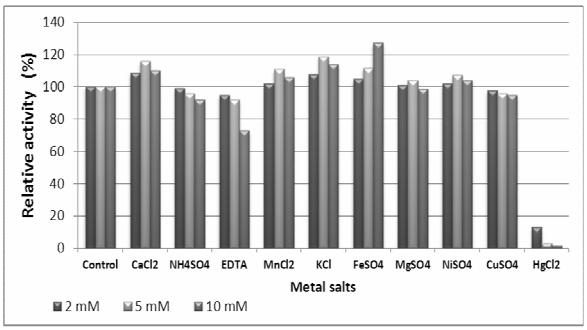

Fig. 10. Effect of different metal salts on the activity of xylanase produced by *A. niger* at concentrations of 2, 5 and 10 mM. The activities were measured at 70 ºC and pH 5.5 for 5 min. (LSD at 5% = 2.27%)

Results are in agreement with those obtained from previous studies. Shah and Madamwar, (2005) found that the presence of $CoCl_2$ activated the xylanases of *A. foetidus* MTCC 4898, while the presence of $CuSO_4$ or $HgCl_2$ causes greatly reduce in xylanase activity. Also, He *et al.*, (2015) reported that Mg^{2+}, Mn^{2+}, Ca^{2+} and K^+ enhanced xylanase activity, which produced by *A. oryzae* HML366.

Effect of temperature on xylanase stability:

To determine the stability of xylanase, the enzyme solution were exposed to different temperature ranged between 50 to 80 ºC with 10 ºC interval, for different appropriate interval times. The above procedures were designed also in the presence of 10 mM $CaCl_2$.Samples from incubated enzyme were taken at different appropriate interval times, then activities were tested as in usual manner except that the determination was performed at 70 ºC and pH 5.5 for 5 min.

From the illustrated data in Fig.(11), it could be stated that the addition of $CaCl_2$ gave the enzyme high thermal stability at all incubation temperature ranged from 50 to 80 ºC. It was more obviously particular at 70 ºC till the end of experiment. The enzyme lost 42.62, 46.28, 46.7 and 56.03% of its activity by holding it at 50, 60, 70 and 80 ºC for 120, 24, 3 and 0.5 h respectively. It is interesting to note that the loss of activity was pronounced in the first 12, 1, 0.5 and 0.25 h at 50, 60, 70 and 80 ºC when it lost 4.95, 4.38, 15.03 and 31.18% of its activity respectively.

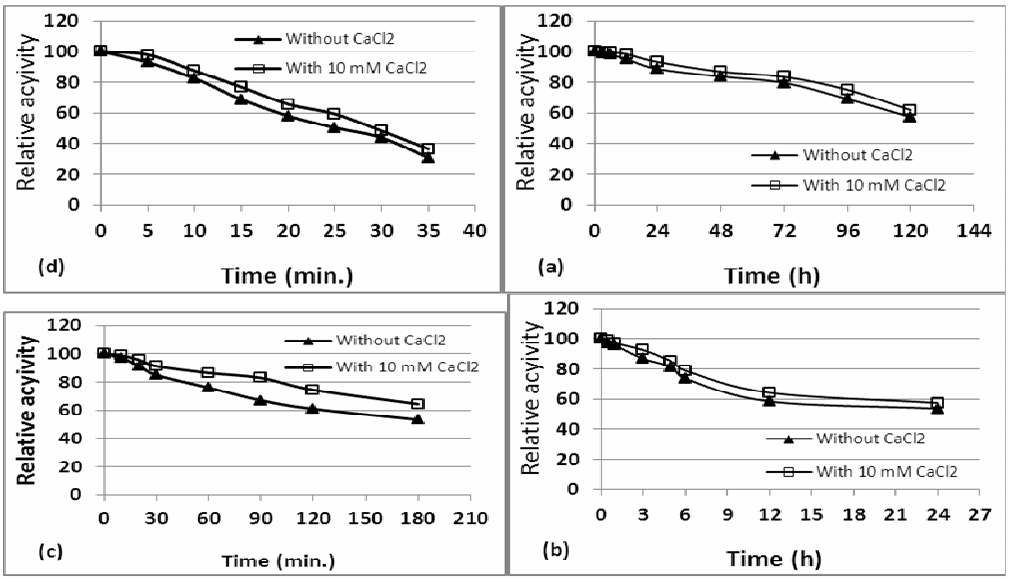

Fig. 11. Effect of storage at 50 (a), 60 (b), 70 (c) and 80 ºC (d) on stability of xylanase produced by *A. niger*. The residual activities were measured at 70 ºC and pH 5.5 for 5 min.(LSD at 5% = 2.95%)

Bakri *et al.*, (2010) reported that *A. terreus* FSS129 xylanase showed high thermal stability at temperature below 50 during 3 h of incubation while at 50ºC, the residual activity was 65.4%. The enzyme was sensitive at 55ºC, retaining only 10.5% activity after 3 h The residual xylanase activity was only 4.2% at 65 ºC after 3 h of incubation. Ang *et al.*,(2013) reported that *A. fumigates* xylanase showed lower thermal stability at temperature above 50 ºC which loss 75%, 85% and 99.5% of its activity after 2 h of incubation at 60, 70 and 80 ºC respectively.

Effect of pH on xylanase stability:

In this experiment one ml of the enzyme solution was added to one ml of each buffer at various pH values (ranged from 3 to 7), the mixtures were kept at 4 ºC for 72 h with 12 h interval. Aliquot of each solution was taken and assayed for enzyme activity at 70 ºC and pH 5.5 for 5 min.

From the illustrated data in Fig.(12), it could be reported that concerning the relationship between the time of incubation and the stability of the enzyme against pH changes, it could be noticed that the figure of all the five curves had the same trend with regard of the enzyme stability against pH changes till 72 h period.

The enzyme showed more stability at pH ranged from 4.0 to 5.0 retaining more than 75% of its original activity with maximum stability at pH 4 when retained 84.8% of its activity after 72 h of incubation. The minimum pH stability was at pH 7.0 where xylanase retained 61.85% of its activity after 72 h of incubation.

A. fumigatus SK1 crude xylanase was stable at pH 4–6 while the stability was rapidly lost in the first 10 min at 60 ºC when incubated in pH above neutral (Ang *et al.*, 2013). He *et al.*, (2015) observed 78% of the activity of *A. oryzae* HML366 xylanase was retained after incubation for 1 h at pH 4.0 and 76% at pH 10.0, and the xylanase showed stability within the pH range of 4.0–10.0.

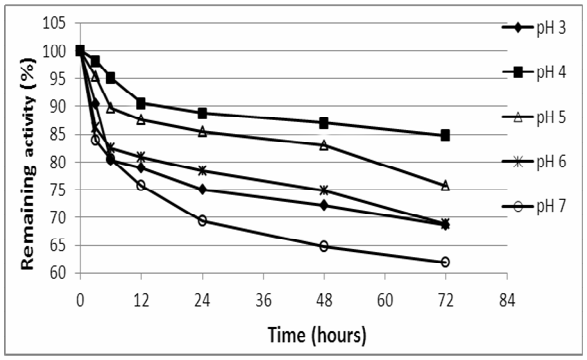

Fig. 12. Effect of pH on the stability profiles of xylanase produced by *A. niger*, the residual activities were measured at 70 ºC and pH 5.5 for 5 min. (LSD at 5% = 2.14%).

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تهيئة الظروف المثلى لإنتاج إنزيم الزيلانيز من بعض المخلفات الزراعية بواسطة فطر *Aspergillus niger* تحت ظروف التخمير الصلب

عبدالله العوضي سليم ،فتحي اسماعيل حوقة ، عايدة حافظ عفيفي و عبدالرحمن محمد العتابي
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تم دراسة إنتاج إنزيم الزيلانيز باستخدام عزلة محلية لفطر *Aspergillus niger* تحت ظروف التخمير الصلب و ذلك باستخدام ستة مخلفات زراعية – وهى ردة القمح ، رجيع الأرز، قوالح الذرة ، قش الأرز، تبن البرسيم وتبن القمح –حيث استخدمت هذه المخلفات بصورة فردية وفى خلطات فيما بينها (بنسبة 1:1). و كانت أفضل المواد لإنتاج الإنزيم هي الخلطة ما بين ردة القمح و تبن البرسيم. و في محاولة للوصول لأفضل ظروف للإنتاج تم دراسة كلا من نسبة الرطوبة الأولية للبيئة و كذلك إضافة مصادر كربون ، مصادر نيتروجين، درجة حرارة التحضين ، درجة الحموضة الأولية للبيئة و أخيرا حجم اللقاح. و قد وُجد أن بيئة التخمير المثالية تتكون من 5 جم من خلطة ردة القمح+تبن البرسيم (بنسبة 1:1) مع إضافة 1% من الزيلان ، 1% فوسفات أمونيوم و التي لقحت بواسطة 1 مل من معلق جراثيم يحتوي على 2×10^5 جرثومة/مل و كانت نسبة الرطوبة الأولية للبيئة 80%. بعد سبعة أيام من التحضين على درجة حرارة 35 °م وصل إنتاج الزيلانيز إلى 1294.15 وحدة دولية / جرام محققا أعلى إنتاجية. و قد وجد أن أعلى نشاط للإنزيم المنتج كان عند درجة حرارة 70 °م و درجة حموضة 5.5 . كما وجد أن كلا من كبريتات الحديدوز ، كلوريد البوتاسيوم ، كلوريد الكالسيوم و كلوريد المنجنيز زادت من قيم النشاط الإنزيمي إلى 127.46 ، 118.86 ، 115.76 و 111.15% على التوالي. إنزيم الزيلانيز المنتج أظهر تحملا جيدا لكل من الحرارة و الحموضة، حيث احتفظ الإنزيم بـ 86.47 و 76% من نشاطه بعد تحضينه لمدة 60 دقيقة على 70 °م مع و بدون 10 مل مول كلوريد كالسيوم على التوالي و كذلك أظهر الإنزيم ثباتا لدرجات الحموضة ما بين 4 ، 5 محتفظا بأكثر من 75% من نشاطه، مما يجعله مناسبا للتطبيقات الصناعية كصناعة الورق و الوقود الحيوي.