JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 161-166

Xylanase Production by *Aspergillus niger* KSU 23 using Corn Cobs

Abeer R.M. Abd El-Aziz[1]*, Monira R. Al-Othman[1], Mohamed A. Mahmoud[2] and Hesham A. Mohamed[2]

[1]Botany and Microbiology Department, College of Science, King Saud University, Riyadh 1145, Saudi Arabia.
[2]Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

(Received: 03 August 2014; accepted: 25 October 2014)

***Aspergillus niger* KSU 23 isolate was selected as a potential producer of xylanase via a submerged fermentation technique using corn cobs as substrate. The trials for xylanase production were conducted at two concentration levels (2.5 and 3.0) of corn cobs, four different fermentation temperatures (25.0, 27.5, 30.0 and 32.5°C) and four various initial pH levels (5.0, 5.5, 6.0 and 6.5) of the culture medium for a period of 96 h. The results revealed that the *A. niger* KSU 23 exhibited maximum enzyme activity (62.36±1.91 IU/mL) at 3.0 % corn cobs concentration followed by (60.12±1.09 IU/mL) at 2.5 % concentration respectively at 30°C, 6.0 pH after a period of 72 h of incubation. The comparison of the effect of different initial pH grades of culture medium exhibited that pH 6.0 had most potent role in xylanase production as compared to different other pH levels. Time scale analysis revealed that fermentation period of 72 h was the most suitable for obtaining maximum enzyme activity. Moreover a temperature of 30°C was found to be optimum for higher yields of xylanase.**

Key words: Xylanase, *Aspergillus niger*, corn cobs, production.

Microbial enzymes are a fast growing field in biotechnology. The global market of industrial enzymes was closed to a billion dollars in 1990 and crossed the 2.0 $ billion mark in 2005 the market has been estimated at 3.3 $ billion in 2010 and is expected to reach 4.4 $ billion by 2015 (Krishna, 2005). Endo-1, 4-β-xylanase (Endo-^{2}-1, 4-xylan, xylanohydrolase; EC. 3.2.1.8) commonly called xylanase is an industrialy important enzyme (Sharma and Kumar, 2013).

Xylan is the most abundant hemicellulose and a complex polysaccharide composed of a backbone of β-1, 4-glycoside-linked xylose residues. Several hydrolytic enzymes are required for complete xylan degradation. xylanase play a major role in the degradation of xylan by cleaving the xylosyl backbone and releasing short xylooligosaccharides, which are further hydrolyzed into xylose units by xylan 1,4-β- xylosidase (EC. 3.2.1.37) (Shallom and Shoham 2003).

Many microorganisms, including bacteria, fungi, and yeast, are known to produce xylanase (Beg *et al*. 2001). The filamentous fungi *Aspergillus* and *Penicillium* are particularly important xylanase producers because they excrete the enzyme into media at higher levels than other microorganisms (Chávez *et al*., 2006).

Aspergillus niger was used for production xylanase by using most of the agricultural waste materials/by products like corn cobs, sugar cane bagasse, rice husk, rice straw and oat straw (Siedenberg *et al*., 1998, Haq *et al*., 2002).

The xylanase has been used for a number of commercial applications 1) bioethanol production (Beg *et al*., 2001), 2) animal feedstocks, 3) paper industries (Tan *et al*., 2008), 4)

* To whom all correspondence should be addressed.
E-mail: aabdelaziz@ksu.edu.sa.com

Biobleaching (Birijlall *et al*., 2011), 5) baking technology Pariza and Johnson, 2001) and 6) Bioenergy (Chiranjeevi *et al*., 2012).

This work was aimed to optimize xylanase enzyme production by *A. niger* using corn cobs (two concentrations) and different pH grades and temperatures.

MATERIALS AND METHODS

Substrate

Using corn cobs like carbon source were utilized as substrate for the production of xylanase enzyme. Corn cobs were dried, ground to 40 mm mesh and treated with 2.0% NaOH. The prepared sample was stored in air tight container for additional utilization in the xylanase production.

Fermentative organism

The isolation, purifications and identification of *A. niger* isolate was obtained from the team of authors in this paper depend on experience in *Aspergillus* spp. for xylanase production.

Growth on PDA for sporulation

A. niger isolate was cultivated on the Potato dextrose Agar (PDA) as the spores were to be stored for longer period for the utilization of organism in different trials according to (Asghar, 2000).

Preparation of inoculum

The medium for inoculation was prepared, maintained at pH 5.5 and sterilized by autoclaving. The culture from the sporulation medium was transferred to the inoculation medium in 500 ml conical flask by using inoculation loop under sterilizer conditions. The inoculated medium was incubated at 37°C in an orbital shaker at 130 rpm for 3 days.

Enzyme production

After 72 h of incubation, 3% of the inoculum was added to each fermentation flask (250 ml) for xylanase production. The optimization of various culture conditions like temperature, pH and period of incubation for fermentation was carried out during the work.

Optimization conditions for xylanase production

Carbon source

Substrate of enzyme as corn cobs were used at 2.5, 3.0 and 3.5 % concentrations.

pH

Xylanase production was performed at different pH grades (5.0, 5.5, 6.0 and 6.5) using the corn cobs to find out the optimum pH worth for enzyme production.

Temperature

The production of xylanase was carried out at different temperatures (25, 27.5, 30 and 32.5°C).

Incubation time

To find out the optimum time required for maximum xylanase activity, samples were harvested at different time intervals; 24, 48, 72 and 96 h.

Sample harvesting

After specific interval of incubation, the biomass from the experimental flasks was filtered through Whatman filter paper No.1. The filtrate was centrifuged at 10,000 rpm for 15 min at 10°C in the centrifuge to remove the spores and mycelia of the organism. The supernatant was then carefully collected and stored at a refrigerated temperature in sterilized glass bottles.

Enzyme activity

Xylanase hydrolyzes the polymer xylan into the xylose monomers. The free xylose units produced as a result of xylanase activity react with 3-5 dinitrosalicylic acid (DNS) reagent, and form a colored complex that is measured by spectrophotometer at wavelength 550 nm.

Enzyme assay

The filtrate was assayed for xylanase activity, determined at 55°C using 0.6% (w/v) oat spelt xylan (Sigma) at pH 6.0. Reducing sugars were measured using DNS method (Miller, 1959; Carmona, 1998). Enzyme activity was expressed as IU/ml.

Unit of activity

According to the International Union of Biochemistry, one international unit of Xylanase (1 IU) corresponds to the amount of enzyme required to release 1 micromole of reducing sugar (xylose) in 1 min.

Estimation of activity

For the estimation of enzyme activity, 1.0 ml of enzyme filtrate was added in a test tube followed by 0.5 ml xylan (0.6%) along with 0.5 ml of distilled water. The test tube was incubated at 30°C for 30 min. Later, DNS reagent was added (2.0 ml) to the test tube kept in boiling water for 5 min and

cooled in ice water. A blank was also prepared in the same way as aforementioned, but without xylanase. The color intensity was estimated at 550 nm using spectrophotometer.

Statistical analysis

The data obtained were analyzed by Complete Randomized Design (CRD) and level of significance was determined by analysis of variance technique as described by Steel *et al*. (1997).

RESULTS

The production of xylanase by *A. niger* was determination by two corn cobs concentrations using four different pH levels (5.0, 5.5, 6.0 and 6.5) and four incubation temperatures (25.0, 27.5, 30.0 & 32.5°C) over a period of 96 h.

The mean values in Fig. 1 depict the effects of different pH grades on xylanase production at 2.5% corn cobs concentration and 25°C over an incubation period of 96 h. The enzyme production increased gradually up to 72 h and then a decreasing trend was observed. The highest level (46.36±1.12 IU/mL) of enzyme production was obtained at pH 6 after 72 h of incubation that decreased to 41.89±1.31 IU/mL at 96 h.

The production of xylanase was carried out by *A. niger* at 27.5°C, various pH grades and 2.5% concentration of corn cobs (Fig. 2). The enzyme production increased gradually up to 72 h and then a decreasing trend was observed. The highest level (50.21±1.56 IU/mL) of enzyme production was obtained at pH 6 after 72 h of incubation that decreased to 22.06±1.56 IU/mL at 96 h.

In another assay (Fig. 3) at the initiation of the assay, lower activities of xylanase production were observed at all pH grades, after that increasing trend in enzyme production up to 72 h then showed

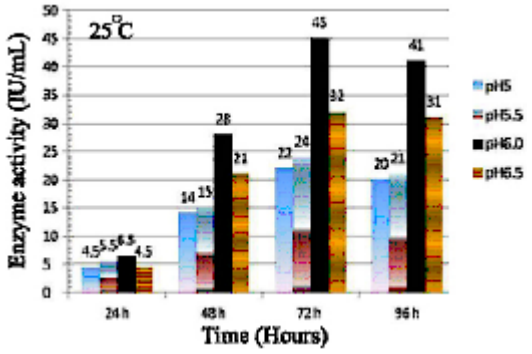

Fig. 1. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 25°C and 2.5% corn cobs concentration in culture medium at different pH levels.

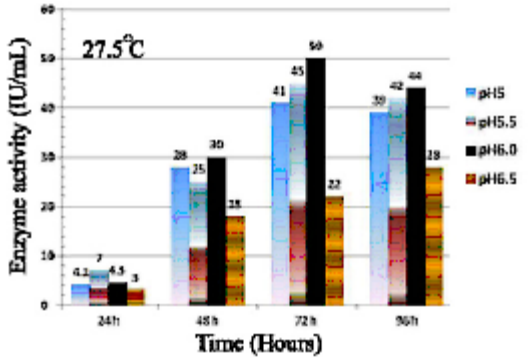

Fig. 2. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 27.5°C and 2.5% corn cobs concentration in culture medium at different pH levels.

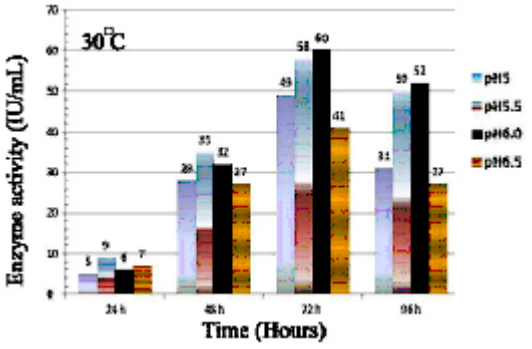

Fig. 3. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 30°C and 2.5% corn cobs concentration in culture medium at different pH levels.

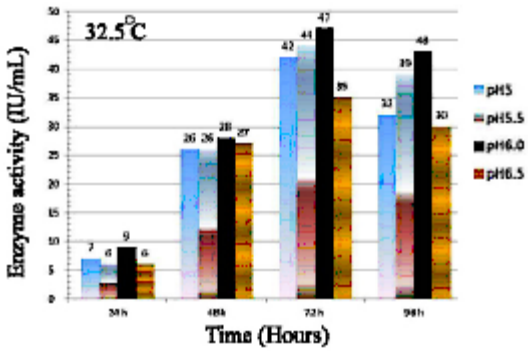

Fig. 4. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 32.5°C and 2.5% corn cobs concentration in culture medium at different pH levels.

a landing. The highest level (60.12±1.09 IU/mL) of enzyme production was obtained at pH 6 after 72 h of incubation that decreased to 42.11±1.34 IU/mL at 96 h.

The Fig. 4 explained the time course production of xylanase by *A. niger* at 32.5°C, selected pH grades. The fungus exhibited maximum enzyme production (47.19±1.41 IU/mL) after 72 h when the fermentation was carried out at pH 6 that decreased to 43.67±1.13 IU/mL at 96 h.

The mean values in Fig. 5 depict the effects of different pH grades on xylanase production at 3% corn cobs concentration and 25°C over an incubation period of 96 h. The highest level (51.89±1.26 IU/mL) of enzyme production was obtained at pH 6 after 72 h of incubation that decreased to 47.51±1.49 IU/mL at 96 h.

The production of xylanase was carried out by *A. niger* at 27.5°C, various pH grades and 3% concentration of corn cobs (Fig. 6). The enzyme production increased gradually up to 72 h and then a decreasing trend was observed. The highest level (56.74±1.38 IU/mL) of enzyme production was obtained at pH 6 after 72 h of incubation that decreased to 51.17±1.59 IU/mL at 96 h.

In another assay (Fig. 7) at 30°C, lower activities of xylanase production were observed at all pH grades, after that increasing trend in enzyme production up to 72 h then showed a landing. The highest level (62.36±1.91 IU/mL) of enzyme production was obtained at pH 6 after 72 h of incubation that decreased to 58.52±1.71 IU/mL at 96 h.

The Fig. 8 explained the time course production of xylanase by *A. niger* at 32.5°C, selected pH grades. The fungus exhibited maximum enzyme production (55.22±1.63 IU/mL) after 72 h when the fermentation was carried out at pH 6 that decreased to 51.19±1.83 IU/mL at 96 h.

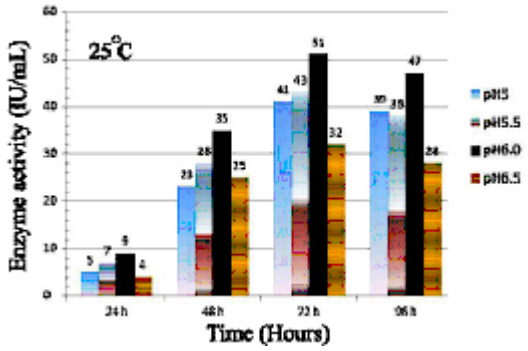

Fig. 5. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 25°C and 3% corn cobs concentration in culture medium at different pH levels.

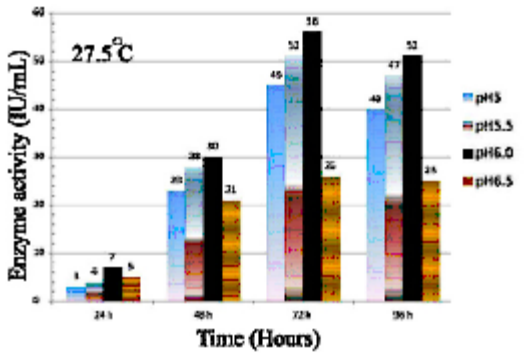

Fig. 6. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 27.5°C and 3% corn cobs concentration in culture medium at different pH levels.

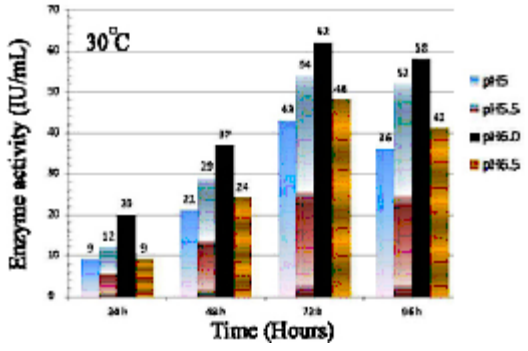

Fig. 7. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 30°C and 3% corn cobs concentration in culture medium at different pH levels.

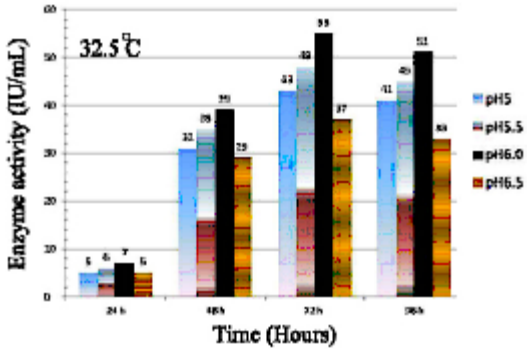

Fig. 8. Xylanase production by *A. niger* KSU 23 during 96 h fermentation at 30°C and 3% corn cobs concentration in culture medium at different pH levels.

DISCUSSION

The findings of the present study are in line with those of Ali *et al*. (2002) that 30°C is the best temperature for *Aspergillus* to produce maximum xylanase activity. In another investigation conducted by Chen *et al*. (1999), *A. niger* showed maximum production 357.2 IU/ml for the enzyme when grown in shake flask at 28 to 32°C for 60 hours.

Senthilkumar *et al*. (2005), who observed optimum xylanase recovery at 72 h of incubation. The findings are also supported by the results found by Camacho and Aguillar (2003), that when corn cob was used as carbon source for the growth of *Aspergillus* sp, it synthesized 37.0 and 39.5 IU/ml xylanase activity at an incubation period of 48 and 72 h, respectively. *Aspergillus niger* GCBT-35 was used for optimal production of xylanase (289.86 U/ml/min) was achieved 72 h after the conidial inoculation, when 1.0% (w/v) meat extract was used as a nitrogen source in the culture medium at an initial medium of 5.5 pH (HAQ *et al*., 2008). Optimum pH and temperature for xylanase activity were found to be 8 and 28°C, Thus the present study proved that the fungal strain *A. niger* used was potential and useful for xylanase production (Tallapragada and Venkatesh, 2011). *Aspergillus niger* was used for optimum production of xylanase production 3.0% sugarcane bagass or corn cobs were used in the culture medium having pH 5.5 for the period of 72 h at 30°C (Zulfiqar *et al*., 2013, Zulfiqar *et al*., 2012). Kohli *et al*. (2001) observed maximum enzyme production after 96 h, whereas Park *et al*. (2002) reported that the optimum xylanase production could be obtained after 5 days fermentation period. The contradiction may be due to the difference in fermentation conditions.

ACKNOWLEDGEMENTS

The authors would like to extend their sincere appreciation to the Deanship of the Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RGP-VPP-269.

REFERENCES

1. Krishna, C. Solid-state fermentation systems-an overview. *Crit.Rev. Biotechnol*. 2005; **25**:1-30.
2. Sharma, M. Kumar, A. Xylanases: An Overview. *British Biotechnology Journal*, 2013; **3**(1): 1-28.
3. Shallom, D, Shoham, Y. Microbial hemicellulases. *Curr. Opin. Microbiol*. 2003; **6**: 219-228.
4. Beg, Q.K., Bhushan, B., Kapoor, M., Hoondal G.S. Enhanced production of a thermostable xylanase from *Streptomyces* sp. QG-11-3 and its application in biobleaching of eucalyptus kraft pulp. *Enzyme Microb. Technol*. 2000; **27**:459-466.
5. Chávez, R., Bull, P., Eyzaguirre, J. The xylanolytic enzyme system from the genus *Penicillium*. *J. Biotechnol*. 2006; **123**:413-433.
6. Siedenberg, D., Gerlach, S.R. Schugerl, K. Guiseppin, M.L.F., Hunik, J. 1998. Production of xylanases by *Aspergillus awamori* on complex medium in stirred tank an airlift tower loop reactors. *J. Biotechnol*. 1998; **56**: 205-216.
7. Haq, I., Khan, A. Butt, W.A., Ali, S., Qadeer, M.A. Effect of carbon and nitrogen sources on xylanase production by mutant strain of *Aspergillus niger* GCBMX-45. *J. Biol. Sci*. 2002; **2**:143-144.
8. Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S. Microbial xylanases and their industrial applications: a review. *Appl Microbial Biotechnol*. 2001; **56**: 326-38.
9. Tan, S.S., Li, D.Y., Jiang, Z.Q., Zhu, Y.P., Shi, B., Li, L.T. Production of xylobiose from the autohydrolysis explosion liquor of corncob using Thermotogamaritima xylanase B (XynB) immobilized on nickel-chelated Eupergit C. *Bioresour Technol*. 2008; **99**:200-204.
10. Birijlall, N., Manimaran, A., Kumar, K.S., Permaul, K., Singh, S. High level expression of a recombinant xylanase by *Pichia pastoris* NC 38 in a 5 L fermenter and its efficiency in biobleaching of bagasse pulp. *Bioresour Technol*. 2011; **102**: 9723-29.
11. Pariza, M.W., Johnson, E.A. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. *Regul Toxicol. Pharmacol*. 2001; **33**:173- 186.
12. Chiranjeevi, T., Rani, G.B., Chandel, A.K., Sekhar, P.V.S., Prakasham, R.S., Addepally U.

Optimization of holocellulosic enzymes production by *Cladosporium cladosporioides* using Taguchi- L'16 orthogonal array. *J. Biobased Materials Bioenerg.* 2012; 6: 148-157.

13. Asghar, M., Yaqub, M., Sheikh, M.A., Barque, A.R. Optimization of cellulase production by *Arachniotus* sp. using corn stover as substrate. *J A P S.* 2000; **10**(1-2): 37-40.
14. Miller, G.L. Use of dinitrosalcyclic acid reagent for the determination of reducing sugars. *J. Anal. Chem.* 1559; 31: 426-429.
15. Carmona, E.C., Marcia, R.B.B., Aline, A.P.K. Jao, A.J. Purification and biochemical characterization of an endoxylanase from *Aspergillus versicolor*. *FEMS. Microbiol. Lett.*1998; **166**: 311-315.
16. Steel, R.G.D., Torrie, J.H., Dickey, D.A. Principles and procedures of statistics: A Biometric Approach.1997; McGraw Hill Book Inc., New York.
17. Ali S., Haq, I., Qadeer, M.A., Iqbal, J. Production of citric acid by *Aspergillus niger* using cane molasses in a stirred fermentor. *Electron J. Biotechnol.* 2002; **5**(3): 258-271.
18. Chen, H., Jiang, Z., Gaigin., L, Zizheng, Y., Shuzheng, Z. (1999). Screening of acidic xylanase producing strain and studies on its enzyme production condition. Wei. Sheng. Wu. Xue. Bao. 1999; **39**(4): 350-354.
19. Senthilkumar, S.R., Ashokkumar, B., Chandra, R.K, Gunasekaran, P. Optimization of medium composition for alkali-stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design. *Bioresour. Technol.*, **2005**; 96: 1380-1386.
20. Camacho, N.A., Aguilar, G.O. Production, purification and characterization of low molecular mass xylanase from *Aspergillus* sp. and its application in baking. Appl. Biochem. Biotechnol., 2003; **104**: 159-172.
21. Kohli, V., Nigam, P., Singh D., Chaudhary. K. Thermostable, alkalophilic and cellulose free xylanase production by *Thermactinomyces thalophilus* subgroup C. *Enzyme Microbial Technol*. 2001; 28: 606-610.
22. Park, Y.S., Kang, S.W., Lee, J.S., Hong, S.I., Kim, S.W. Xylanase production in solid state fermentation by *Aspergillus niger* mutant using statistical experimental designs. *Appl. Microbiol. Biotechnol.* 2002; **58**: 761–766.
23. Haq I., Hussain R., Hameed U., Javed M. 2008 selection of *Aspergillus niger* mutant using antimetabolite 2-deoxy D-glucose after N-methyl N-nitro N-nitroso guanidine (MNNG) treatment. *Pak. J. Bot.*, 2008; **40**(6): 2613-2623.
24. Tallapragada, P. and Venkatesh K. Isolation, identification and optimization of xylanase enzyme produced by *Aspergillus niger* under submerged fermentation. *J. Microbiol. Biotech. Res.*, 2011, **1** (4):137-147.
25. Zulfiqar, A., Butt, M.S., Nadeem M.T., Yasin, M. Optimization of cultural conditions for xylanase biosynthesis by *Aspergillus niger* using sugarcane bagasse. Pak. *J. Food Sci. 2013;* 23(2): 94-99.
26. Zulfiqar, A., Butt, M. S, Anjum, F M, Awan, M. S., Rathore, H. A., Nadeem, M. T, Ahmad, A., Khaliq, A. Effect of corn cobs concentration on xylanase biosynthesis by *Aspergillus niger. Afr. J. Biotech.* 2012; **11** (7): 1674-1682.