

Optimization of Cultural Conditions for the Production of Xylanase by Chemically Mutated Strain of *Aspergillus niger* GCBCX-20

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ABSTRACT

Present investigation deals with the studies on the cultural conditions for enhanced biosynthesis of xylanase by a chemically mutated strain of *Aspergillus niger* GCBCX-20. The effects of time course, incubation temperature, medium pH and volume of fermentation medium on the production of xylanase were studied. The maximum xylanase production (250 U mL^{-1}) was observed at an incubation temperature of 30°C after 48 h of incubation period. The optimum initial pH of the medium was 5.0 because maximum production of xylanase (260 U mL^{-1}) was observed at this pH. The production of enzyme was found to be maximum (265 U/ml) when the volume of fermentation medium was kept as 25 mL/250 mL conical flasks. All the fermentation batches were carried out in triplicates under submerged conditions.

Key Words: Xylanase; Fermentation; *Aspergillus niger*

INTRODUCTION

Xylanase (EC 3.2.1.8), an extracellular enzyme is the key enzyme for the break down of xylan, which is a polysaccharide composed of β , 1-4-linked xylopyranose units (Saha, 2003). It degrades xylan to short-chain xylo-oligosaccharides of varying lengths (Krengel *et al.*, 1996). Xylanases have increased their importance due to their potential application in biomass fuel, bio bleaching, wine industry, improving animal feed and production of ethanol (Tucker *et al.*, 1989; Xu *et al.*, 2000; Nunez *et al.*, 2001; Sudha *et al.*, 2003; Moers *et al.*, 2003). These uses have placed greater stress on the demand for increasing xylanase production (Bocchini *et al.*, 2003).

The production of xylanase is dependent on cultural conditions of the batch fermentation such as time course, incubation temperature, volume of fermentation media and initial pH. The optimum conditions for maximum xylanase production vary with the organism type and fermentation conditions. Time of incubation and incubation temperature plays an important role in the production of xylanase. Kansoh *et al.* (2001) produced maximum yield of xylanase after two days of incubation on a rotary shaker (180 rpm) at 30°C . Temperature between $25\text{--}30^\circ\text{C}$ was usually employed for culturing of *Aspergillus niger*. Youn and Rungyu (1999) studied the maximum xylanase production at 27°C by *Aspergillus niger* and the incubation temperature of 30°C temperature was reported as optimum by Deschamps *et al.* (1984), Smith and Wood (1991) and Lenartovicz *et al.* (2002). The production and stability of the xylanase in batch fermentation is very sensitive to pH of the medium. In shake flask culture, Ilieva *et al.* (1995) found a pH range of 3.5–4.0

as optimum for maximum xylanase production. Singh *et al.* (2000) reported xylanase to be stable upto 50°C between pH 5.5–9.0 for 30 minutes.

In the present study, chemically mutated *Aspergillus niger* GCBCX-20 was used for the optimization of cultural conditions such as time course, pH, temperature and volume of fermentation media for the enhanced production of xylanase in 250 mL cotton plugged shake flasks.

MATERIALS AND METHODS

Organism and culture maintenance. *Aspergillus niger* strain GCBCX-20 strain which was taken from the Biotechnology Research Centre G.C.U. Lahore was used in the present studies. The culture was maintained on potato dextrose agar (Merck, Germany) slants (pH 4.5) by weekly transfers onto fresh slants and was stored at 4°C in refrigerator.

Inoculum preparation. The conidial inoculum was used in the present study. Conidia from 5 days old slant cultures were used for inoculating the fermentation media in the flasks. The conidial suspension was prepared by adding 10ml of 0.005% Monoxal 0.T (Di-octyl ester of sodium sulpho succinic acid) to the slant having profused conidial growth on its surface. The inoculating needle was used to break the conidial clumps. The tube was shaken vigorously to make a homogeneous mixture of conidial suspension.

Fermentation technique. Shake flask technique was used for xylanase production in the present studies. The fermentation medium containing (g L^{-1}); wheat bran, 2.0; NaNO_3 , 0.1; NH_4Cl , 0.1; KH_2PO_4 , 0.1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.03; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 & Tween 80, 0.2 mL (pH 4.5) was used

for fermentation. Twenty-five milliliter of the said fermentation medium was transferred to each 25 mL Erlenmeyer flask, which was then cotton plugged. The flasks were sterilized in the autoclave at 121°C for 15 min (15 lbs inch⁻²). After cooling the medium at room temperature, 1.0 mL of the conidial suspension was transferred to each flask and the flasks were placed in the rotary incubator shaker (200 rpm) at 30°C for 48 h. After 48 h, the ingredients of the each flask were filtered and the filtrate was used for the estimation of extracellular xylanase.

Analytical method. The xylanase was estimated according to the method of Millar (1959). The color intensity was estimated at photoelectric colorimeter using green wratten filter of 546 nm. One unit of xylanase was defined as “the amount of enzyme which released one mg of reducing sugars per hour at pH 7.0 and 30°C”. Enzyme activity was expressed as U mL⁻¹ (Wong, 1988).

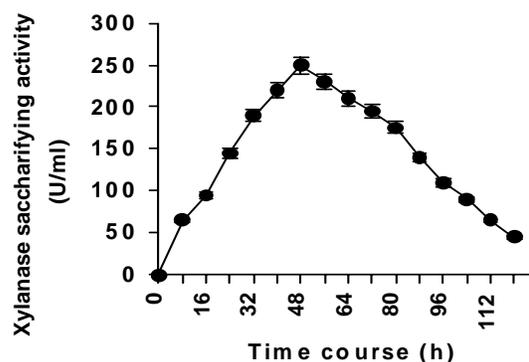
RESULTS AND DISCUSSION

Time course is a significant parameter in fermentation experiments because it actually determines the incubation period for any microbial culture. So, the rate of xylanase production by mutated strain of *Aspergillus niger* was studied (Fig. 1). The maximum xylanase saccharifying activity (250 U mL⁻¹) was observed after 48 h of conidial inoculation. Time course study revealed a significant increase in enzyme production with the increase in time, which was presumed to be due to rapid hydrolysis of xylan in the medium. Further increase in incubation period (after 48 h) resulted in the decreased enzyme production. The decrease in enzyme production might be due to the rapid digestion of susceptible portion of xylan molecules and then only crystalline portion was left behind, which cannot be used by the organism for the production of enzyme. This finding is in accordance with the work reported by Irwin and Wilron (1993).

Incubation temperature plays a critical role in enzyme productivity (Seyis & Aksoz, 2003). So, the enzyme production in present studies was investigated at different temperatures ranging from 20-55°C (Fig. 2). The *Aspergillus niger* GCBCX-20 gave promising results, producing maximum amount of xylanase (250 U mL⁻¹), when incubated at 30°C. When the temperature was increased or decreased from 30°C, the production of xylanase was gradually reduced. It might be due to that at high or low temperature than optimum, the growth of the fungus was inhibited and hence the xylanase production was also decreased (Yaun & Rangyu, 1999; Rahman *et al.*, 2003).

Productivity of the enzyme by mould culture is very specific to the pH of the medium (Kubackova *et al.*, 1975). The production of xylanase by *Aspergillus niger* GCBCX-20 was studied by varying the pH of the fermentation media from 4.0 to 7.5 (Fig. 3). Maximum production of enzyme (260 U mL⁻¹) was achieved when the initial pH of basal

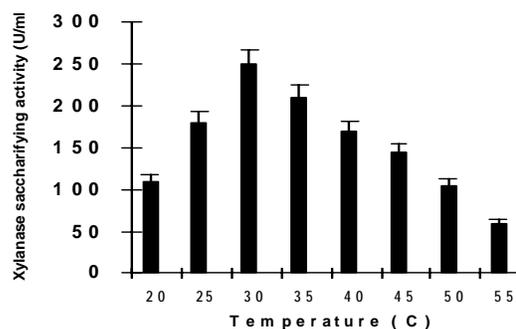
Fig. 1. Time course study for the production of Xylanase by *Aspergillus niger* GCBCX-20



Each value is an average of three parallel replicates. Y error bars indicate the standard error of mean.

Initial pH 4.5
Incubation Temperature 30 ± 1°C

Fig. 2. Effect of incubation temperature on the production of xylanase by *Aspergillus niger* GCBCX-20



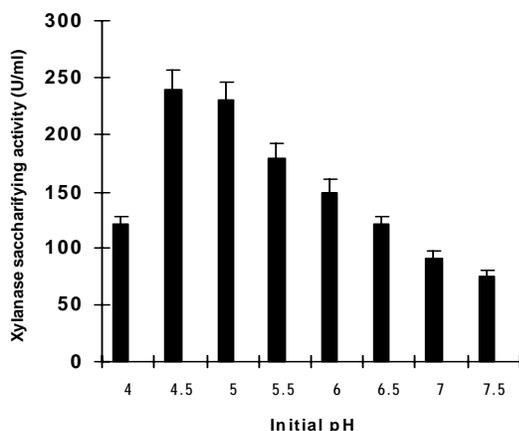
Each value is an average of three parallel replicates. Y error bars indicate the standard error of mean.

Incubation period 48h
Initial pH 4.5

medium was kept at 5.0. When the pH was increased or decreased from the optimum value, the production of xylanase was greatly decreased. It was because the organism required slightly acidic pH for the growth as well as enzyme production (Gomes *et al.*, 1994). On the other hand, alkalinity in medium had a strong inhibitory effect on the production of xylanase due to restricted growth of *Aspergillus niger* (Duarte *et al.*, 1999).

Optimization of the volume of fermentation medium is very important for air supply, nutrient supply, growth of microorganism and production of enzyme (Mimura & Shinichi, 1999; Ivanova *et al.*, 2001). The effect of different volumes of fermentation medium (15 to 50 mL) in 250 mL cotton plugged conical flask on the production of xylanase

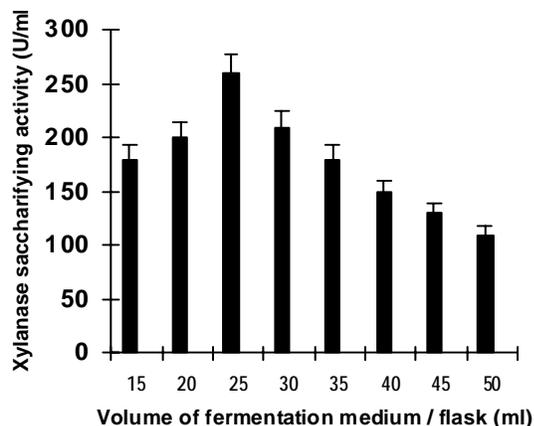
Fig. 3. Effect of different initial pH of medium on the production of xylanase by *Aspergillus niger* GCBCX-20



Each value is an average of three parallel replicates. Y error bars indicate the standard error of mean.

Incubation period 48h
Incubation Temperature $30 \pm 1^\circ\text{C}$

Fig. 4. Effect of different volume of fermentation medium on the production of xylanase by *Aspergillus niger* GCBCX-20



Each value is an average of three parallel replicates. Y error bars indicate the standard error of mean.

Incubation period 48 h
Incubation Temperature $30 \pm 1^\circ\text{C}$
Initial pH 4.5

by *Aspergillus niger* GCBCX-20 was investigated (Fig. 4). The maximum production of enzyme was obtained in 25 ml of the fermentation medium (265 U mL^{-1}) per 250 mL^{-1} conical flask. As the volume of the fermentation medium was increased above 25 mL/flask, the production of enzyme was decreased. It might be due to the reduction in the agitation of medium, decrease in air and mineral supply

and subsequent decreased growth of the organism. Similarly, at low level of fermentation medium (below 25 mL/flask, the production of enzyme was also decreased. It might be due to the nutrient present in the fermentation medium were not sufficient for good growth of the *Aspergillus niger* and hence enzyme formation.

CONCLUSION

From the present study it was concluded that maximum production (265 U mL^{-1}) of xylanase was observed after 48 h of inoculation at 30°C and pH 5.0 by mutant strain of *Aspergillus niger* GCBX-20. The optimum volume of fermentation medium per 250 ml shake flask was found to be 25 mL.

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