RESEARCH PAPER:

Xylanase production from *Aspergillus niger* using rice bran as a carbon source

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SUMMARY

The aim of this study is to produce the xylanase enzyme, (which plays a key role in paper and pulp industries) from *Aspergillus niger. Hence* in order to reduce the cost of the substrate for xylanase production, several agricultural residues such as rice bran, saw dust, pine apple fiber, coir pith, and orange peels, sweet lime peels were tested for the maximum xylanase production. Among the tested agricultural residues, rice bran was evolved as the best suitable substrate for maximum xylanase production. All the other agricultural residues were able to produced less amount of xylanase. The production of xylanase by *Aspergillus niger* was examined on rice bran under solid-state fermentation (SSF). The optimum pH and temperature for xylanase production by SSF was 6.5 and 35^{0}C respectively. Time course experiments indicated maximum xylanase production at 144 hours. Amendment of fructose and sodium nitrate in the medium proved suitable for higher xylanase production.

Key words : Solid state fermentation (SSF), Rice bran, Xylanase, pH, Temperature, *Aspergillus niger*

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Large quantities of agricultural residues accumulate every year which result not only in the deterioration of the environment but also in the loss of potentially valuable material which can be processed to yield a number of value added products such as food, fuel ,feed and a variety of chemicals (Someet *et al.*,2001).The increasing energy demands have focused world wide attention on the utilization of renewable resources, particularly agricultural wastes, the major components of which are cellulose , starch, lignin and xylan (Satyanarayana *et al*.,2004).Agricultural residues contain 20-30% hemicellulosic materials which can be utilized by microorganisms. The main carbohydrate constituent of the lignocellulosic material is cellulose,mannan and xylan. Xylan constitutes the major noncellulosic polysaccharides of primary cell wall of grasses and secondary wall of all angiosperms (Asbah *et al*.,2000; Diaz *et al*.,2004).

Xylan is heterogenous polysaccharide consisting of a homopolymeric backbone of1,4-linked –ß-D-xylopyranose units and short chain branches including *O*-acetyl -x -L-arabinofuranosyl,x-glucuronyl residues (Whistler and Richards 1970).The complexity of xylan requires the action of multiple xylanases with overlapping yet different specificities to effect extensive hydrolysis (Dekker and Richards,1976).Xylanases are the key enzymes for break down of xylan since they depolymerise the back bone. Strains of *Aspergillus* are known to produce xylanase on various lignocellulosic substrates (Labeille *et al.*,1999; Gawande and Kamat,2000). Xylanases have been isolated from diverse range of microorganisms including fungi and bacteria (Medeiros *et al*., 2000).Fungal xylanases can be produced using two main methods, solid-state cultivation systems and submerged liquid cultivation systems.

Most research has used submerged culture, which allows control of the degree of aeration, pH and temperature of the medium and the control of other environmental factors required for the optimum growth of microorganisms. However, solid-state fermentation (SSF) has gained renewed interest from researchers in recent years and has often been employed for the production of xylanases because of a number of economic and engineering advantages (Smith *et al.*,1977; Pandey,1994).These include the simplicity of the equipment and the low moisture content, which prevents other microbial contamination. The importance of xylanase (EC3.2.1.8) lies in the recycling of biomass and its wide usage in biotechnology, such as in pulp bleaching, baking industry and manufacturing of animal feed (Viikari *et al.*,1994; Poutaten,1997).Hence, an attempt was made to study the cultural conditions for

xylanase production by *Aspergillus niger* under solid state fermentation.

MATERIALS AND METHODS

Isolation of the microorganism:

Isolation of *Aspergillus niger* was made from coir pith soil sample collected from Pollachi, Coimbatore. It was cultured on Potato Dextrose Agar (PDA) medium performing constant replications in culture tubes and maintained at 4^0C. Constant replications were made twice in a month.

Chemicals used in the present study were of Analar grade. Dinitrosalicylic acid, Oat spelt xylan were purchased from Sigma Chemical Company (USA). Coir pith, orange peels, sweet lime peels, rice bran, pineapple peels were purchased locally.

Culture media:

Xylanase production was carried out by using various xylan rich substrates , mainly agricultural wastes .Xylan extraction was followed by the method of Panbangred *et al*. (1987).The substrates were uniformly mixed with mineral salts solution containing(g/litre): NaNo2 : 20g ; KCL : 0.5g;MgSo4:0.5g;K2HPo4:1.0g;FeSo4:0.01g.

Distilled water was added in such a way that the final rice bran to moisture ratio was 1:2.After sterilization in autoclave, the flasks were cooled and inoculated with 10^6/mlof spores for six days under static condition.

Enzyme extraction:

The method of Jain (1995) was followed for enzyme extraction. Fungi grown on the solid substrates in the conical flasks were flooded with 50 ml of 0.2M sodium phosphate buffer pH7.0 and kept at 4^0C for 4 hours. It was then filtered through muslin cloth and the filterate was centrifuged at 4,000rpm to get clear supernatant which was used as a source of crude xylanase.

Xylanase assay:

Xylanase assay was followed by the method of Bailey *et al*. (1992). Oat spelt xylan was used as the substrate for xylanase and the amount of xylose released was measured by DNS method Miller (1959).The xylanase activity is expressed in International units. One International unit of xylanase is the amount of enzyme required to liberate 1μ mol of D-xylose per min/mL.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Effect of agricultural residues on xylanase production:

Several workers have investigated the effect of various agricultural residues on the xylanase production. The xylanase has been produced from *Aspergillus tamari* by using rice bran as the solid substrate (Souza *et al*., 2001). Hence, an experiment was carried out to find the xylanase production by using various substrates. Xylan from all these agricultural residues were extracted by the method as mentioned in materials and methods. Among the substrates tested, rice bran supported maximum xylanase production than other agricultural residues (Fig.1). These results are closed to the findings of Virupakshi *et al*. (2005) in *Bacillus* sp.

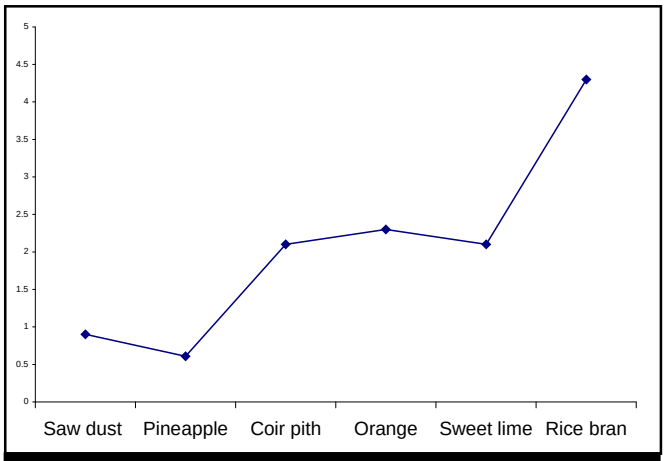

Fig. 1 : Effect of agricultural residues on the xylanase production by *Aspergillus niger* in solid substrate fermentation

Effect of initial moisture content on xylanase production:

Each organism prefers its own moisture content for maximum expression of xylanase. In *Flavobacterium*, increase in concentration of xylan increased the xylanase production (Bhatt *et al.*,1994). Solid substrates used in SSF are insoluble in water, therefore water will have to be absorbed on to the substrate particles, which can be used by the microorganisms for growth and metabolic activity (Pandey,1992). It is also expected that the rate of water absorbed by different substrates varies from one substrate to another. This is another possible explanation for the variation in the xylanase production using different substrates. Thus, it is concluded that the degree of hydration of the substrate plays an important role in the growth of the fungi and subsequently the enzyme production.

Water causes the swelling of the substrate and facilitates good utilization of substrates by the

microorganisms. Increasing moisture level is believed to reduce the porosity of substrate, thus limiting the oxygen transfer into the substrate (Feniksova *et al*., 1960; Raimbault and Alazard,1980). Likewise a lower moisture ratio leads to reduced solubility of the nutrients of the substrate, degree of swelling and a higher water tension (Ikasari and Mitchell,1994). Therefore, the effect of different concentrations of moisture content of rice bran on xylanase production by *A.niger* was studied. From (Fig. 2) it can be seen that 75% to100% moisture content promoted maximum xylanase production.

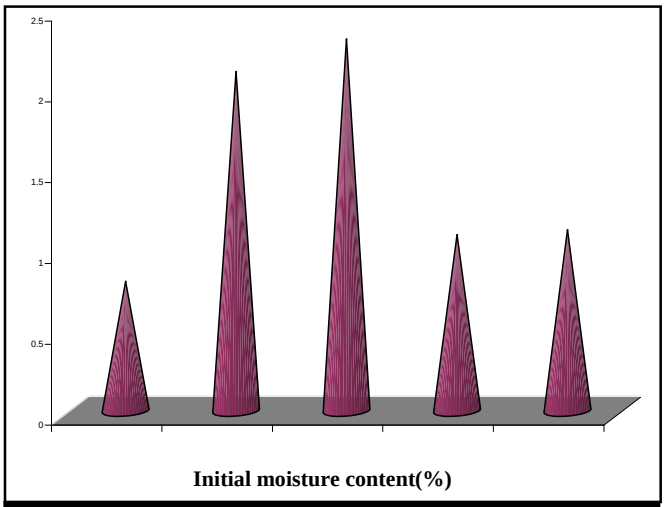

Fig. 2 : The effect of moisture content (%) in the production of xylanase by *Aspergillus niger* grown on rice bran under solid state fermentation

Time course of xylanase production:

Time course of xylanase production varies from organism to organism. In *A.niger* UV-45 maximum production occurred after 72 hours. While in *A.niger*, xylanase production reached a maximum at 60 hours (Gawande and Kamat, 2000). Concentration of reducing sugar showed a significant increase in enzyme production with increase in time, which was presumed to be rapid hydrolysis of xylan in the medium. Further increase in incubation period resulted in the decreased enzyme production.

The decrease in enzyme production may be because the susceptible portion of xylan molecules was rapidly digested and only crystalline portion left behind which cannot be used by the organism for the production of enzyme (Roose, 1993). Hence, an experiment was carried out to find out the maximum time for the production of xylanase (Fig.3). In the present study, the maximum xylanase production was noticed at 144hrs.After that the xylanase production declined.

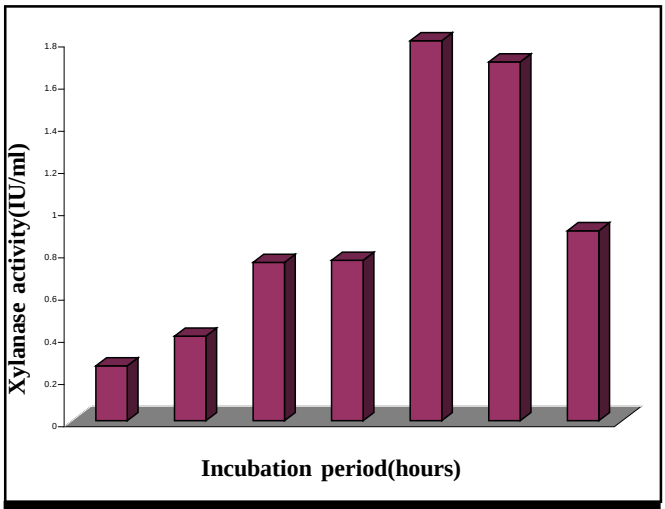

Fig. 3 : Time course of xylanase production by *Aspergillus niger* on rice bran medium under solid state fermentation

Influence of pH and temperature on xylanase production:

The production of xylanase is greatly influenced by different factors. Cai *et al.* (1997) optimized pH 4.2 for xylanase production from *A.niger*.The optimum pH of xylanase was 4.6 in shake flask culture (Chen *et al.*, 1999). In our study, the xylanase production was found to be maximum at 6.5 (Fig .4). Temperature is one of the important parameters that determines the success of SSF system. The optimum temperature for the xylanase production was similar to the optimum temperature for the growth of the fungus. This observation is reported by Sudgen *et al.* (1994). The production of xylanase was maximum at the ambient temperature (28± 30^{0}C).

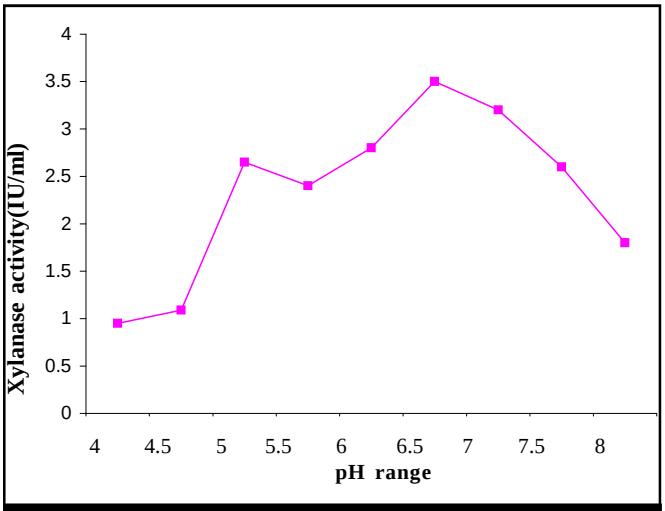

Fig. 4 : Influence of pH on xylanase production by *Aspergillus niger* under solid state fermentation

For *A.niger* USM A11,the ambient temperature which was the optimum temperature for xylanase production was similar to the temperature of the natural habitat of the fungus where it was initially isolated by Kheng and Omar (2005). In the present study, the xylanase production was seen at 30^0C,35^0C,40^0C,45^0C. From Fig .5, it can be noticed that xylanase production was not seen above 50^0C in *A.niger*.Maximum xylanase production was noticed at 35^0C.

Effect of carbon and nitrogen sources on xylanase production:

Enzyme production is also related to the type and

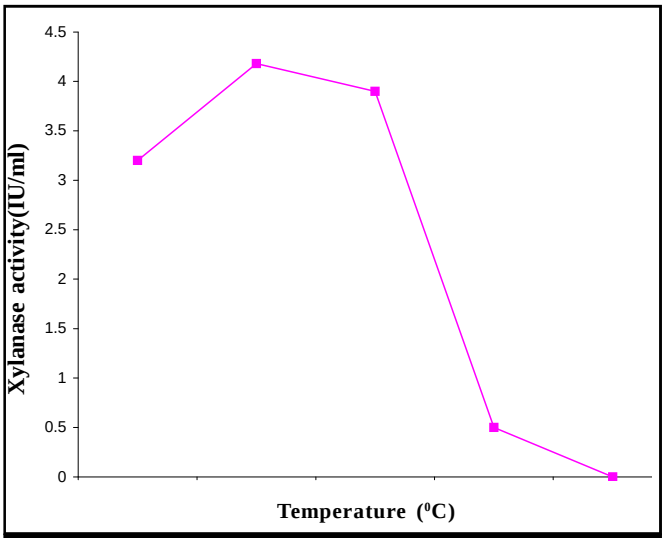

Fig. 5 : Influence of temperature on xylanase production by *Aspergillus niger* under solid state fermentation

concentration of carbon source used (Gawande and Kamat ,2000). Cho (1997) studied starch as the best carbon source for maximum xylanase production. The effect of different carbon sources at different concentrations were checked for the optimum xylanase production by using mutant strain of *Aspergillus niger* (Haq *et al.*, 2002). So, different carbon sources were used for xylanase production.

In the present study, the sucrose in the medium was replaced with carbon equivalents of glucose, fructose, xylose and maltose, galactose and sucrose. It was noticed that fructose promoted maximum amount of xylanase (Fig .6).

The production of xylanase was greatly influenced by the addition of different organic nitrogen sources (Kulkarni *et al.*, 1999). *Aspergillus niger* produced higher levels of extrabeta glucosidase and xylanase in submerged fermentation, when ammonium sulphate, ammonium dihydrogenorthophosphate and corn steep

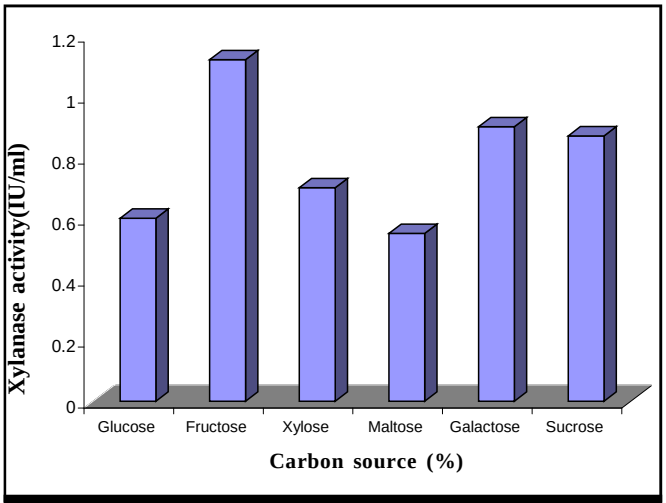

Fig. 6 : Influence of carbon source in the xylanase production by *Aspergillus niger* under solid state fermentation

liquor were used as nitrogen sources (Gokhale *et al.*, 1991). *A.niger* produced xylanase with undetectable amounts in submerged fermentation when a mixture of sodium nitrate and nitrite was used as nitrogen source (Gouda, 2000).

The $(NH_4)_2SO_4$ showed maximum xylanase production (Haq *et al.*, 2002) .In the present study sodium nitrate supported maximum xylanase production in *A.niger* (Fig.7).These results are similar to the findings of Gouda,(2000).

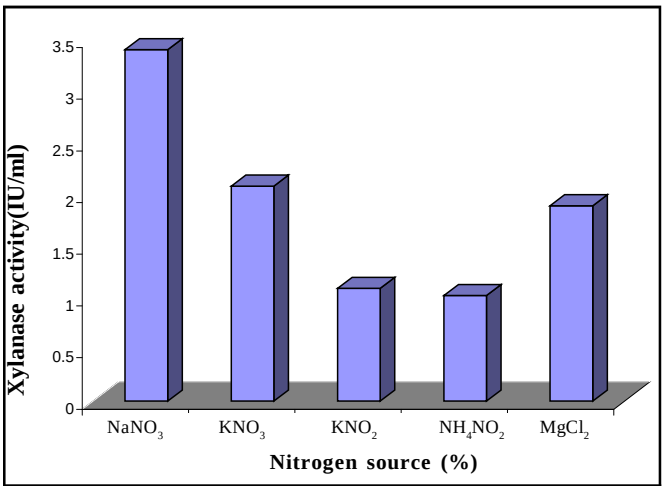

Fig. 7 : Influence of nitrogen source in the xylanase production by *Aspergillus niger* under solid state fermentation

Conclusion:

In the present study optimization of xylanase production by using agricultural residues wastes is considered. Rice bran, which is an inexpensive and

abundant, substrate supported the maximum xylanase production in solid-state fermentation, since pure xylan is presently too expensive to be used as an industrial substrate for the production of xylanase, the development of rice bran as a cheap alternative substrate is an attractive.

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