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Optimization of cultural conditions for the partial purification of xylanase from *Aspergillus flavus*

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SUMMARY

The fungus *Aspergillus flavus* was grown in czapek's dox medium containing wheat bran xylan as carbon source .The various cultural conditions namely pH, incubation period, temperature, time period for hydrolysis, concentration of wheat bran xylan, nitrogenous sources, bivalent ions were studied .The thermal stability of the crude enzyme was studied at 40 ºC for varied time intervals. SDS Poly Acrylamide Gel Electrophoresis was carried out for the crude protein obtained from *Aspergillus flavus*

Key words : Lignocellulose, Hemicellulose, Xylan, *Aspergillus flavus*

Hemicellulose is one of the major components of lignocellulosic biomass and consists mainly of xylan. Xylan is a polymer of xylose containing β-1 4 xylosidic linkages. It is found in large amounts in agricultural residues and as a component of hard wood and soft wood (Luthi *et al*., 1990). Due to its complex structure the biodegradation of xylan requires the synergistic action of several enzymes for efficient and complete break down. Xylanolytic enzymes are receiving increasing attention because of their potential applications in improving digestibility of animal feed and pulp bleaching (Biely, 1985).

Various microorganisms are actively involved in the degradation of hemicelluloses, particularly xylan. Xylanases have recently gained attention due to their potential application in the paper and pulp industry for replacing chlorine based bleaching processes and in food industry for the bio conversion of lignocellulose material into fermentation products (Lubek *et al*., 1997).The production of these enzymes is highly dependable on the cultural conditions for fermentation and simple inexpensive substrates. Thus, the present study aims in selecting a suitable pH, temperature and other important factors which favours the enzyme production.

MATERIALS AND METHODS

Microorganism:

Aspergillus flavus, Link was obtained from the laboratory of PSGR Krishnammal College for Women, Peelamedu, Coimbatore. The cultures were maintained in Petriplates using potato dextrose agar medium.

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Chemicals:

All the chemicals used were of analytical grade. Oats spelt xylan from Sigma Chemicals. Co. / USA was used as a substrate for xylanase assay.

Media used:

Czapek's dox medium was used with sucrose replaced by 3% wheat bran xylan.

Preparation of inoculum and cultivation conditions:

The culture broth consists of 50 ml of czapek's medium with wheat bran xylan as carbon source in a 250 ml conical flask. Each flask was inoculated by an actively sporulating mycelial disc. Cultures were incubated for 5 days at 40º C.

Determination of protein concentration:

The protein of the samples was estimated using Bradford method with Bovine serum albumin as the protein standard.

Xylanase assay:

The xylanase activity was determined using Di Nitro 8Salicylic acid (DNS) method, Miller (1959) by measuring the amount of reducing sugars released during 10 minutes in a reaction mixture containing 1%w/v oat spelt xylan and 0.05m potassium phosphate buffer at 50ºC. One unit of enzyme activity was defined as the amount of enzyme required to release 1µ mol of D xylose per min per ml.

SDS –PAGE:

SDS PAGE was carried out using 12 %

polyacrylamide gel according to a standard protocol Laemmli (1970).The gel was fixed in an acetic acid – methanol mixture and stained with silver nitrate in an alkaline medium in the presence of formaldehyde Merrie *et al*. (1983). Standard molecular weight markers proteins were used for determining molecular mass of protein.

RESULTS AND DISCUSSION

Aspergillus species were extensively studied by number of workers for cellulase and xylanase production (Takenishi and Tsujisaka, 1975). Most of the organism prefer an acidic pH for growth and xylanase production. Each organism may prefer its own pH for maximum xylanase production .In the present study also *Aspergillus flavus* produced maximum xylanase at pH 5.8 (Fig. 1) among the varied concentration studied.

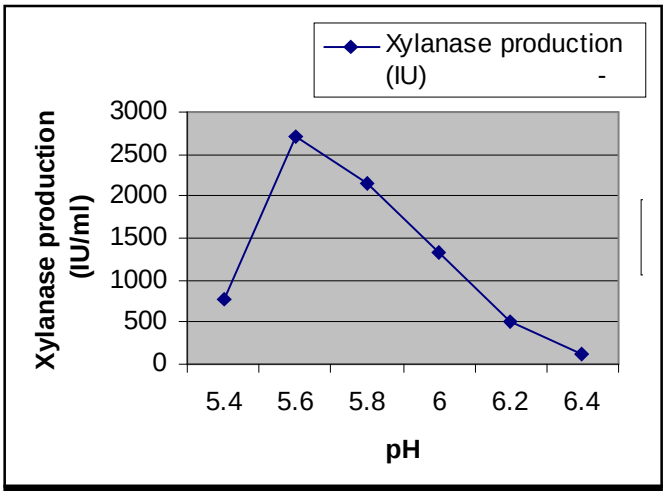


Fig. 1 : Effect of pH on the xylanase production by *Aspergillus flavus*

In order to find out a suitable incubation period for maximum xylanase production, the different incubation hours were studied and 120 hours of incubation proved to be the best (Fig. 2). Similarly result was reported by Poutanen *et al*. (1987) in *Fusarium oxysporum*. The enzyme activity was highly influenced by temperature. The fungus showed a luxurious growth in 40º C when compared to 30ºCand 35ºC (Fig. 3). Dubey and Johri (1987) also reported similar results.

Maheswari and Kamalam (1985), Biswas *et al*. (1986) and Tan *et al*. (1986) and Dubey and Johri (1987) incubated the reaction mixture for 30 minutes at 50ºC for maximum hydrolysis .It was noted that there was a gradual increase in the hydrolysis of the enzyme and at 60 minutes duration, maximum hydrolysis took place (Fig. 4).

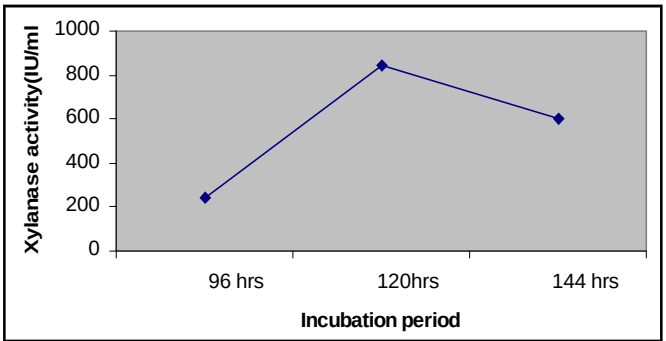


Fig. 2 : Effect of incubation period on the xylanase production by *Aspergillus flavus*

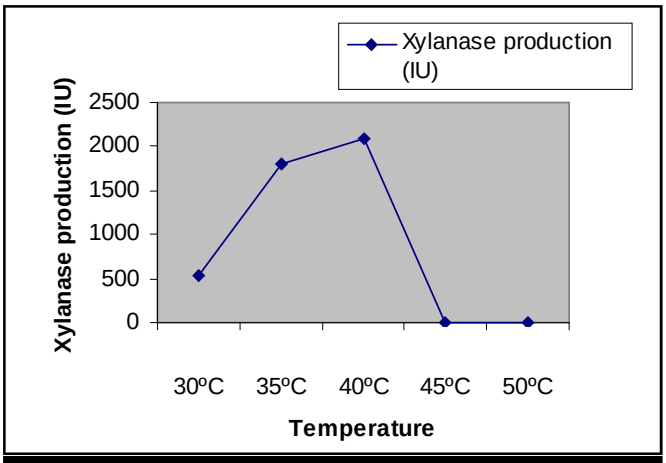


Fig. 3 : Effect of temperature on the xylanase production by *Aspergillus flavus*

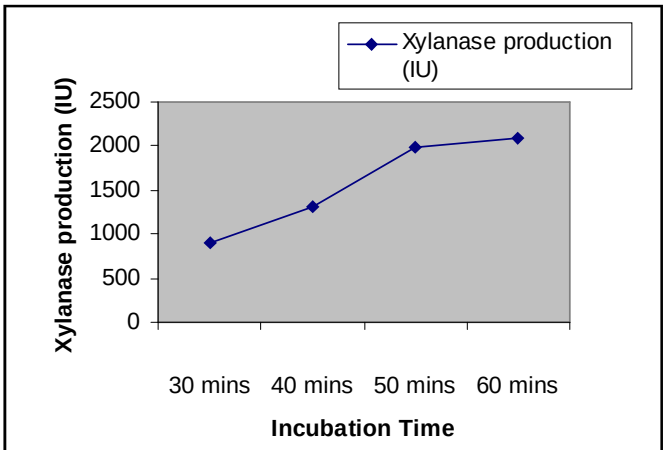


Fig. 4 : Effect of incubation time on the xylanase production by *Aspergillus flavus*

The effect of increasing concentration of wheat bran xylan for the maximum production of xylanase was studied with varying concentrations. 0.3% level of substrate concentration in accordance to Dubey and Johri (1987) was found to be the best (Fig. 5).

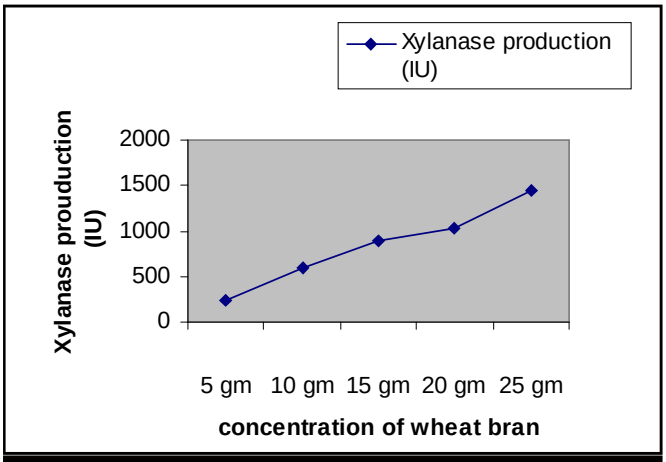


Fig. 5 : Effect of different conc. of wheat bran xylan on the xylanase production by *Aspergillus flavus*

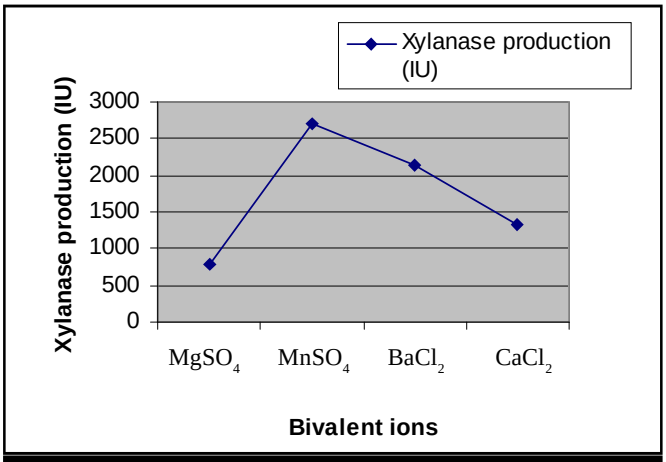


Fig. 7 : Effect of bivalent ions on the xylanase production by *Aspergillus flavus*

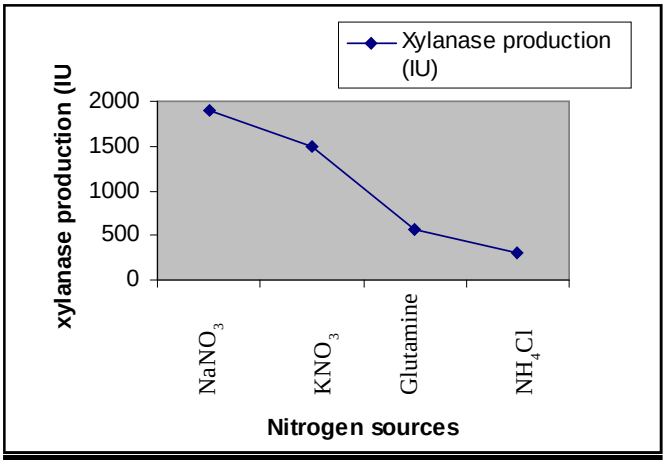
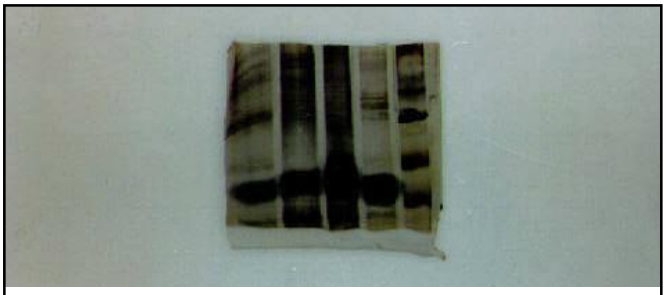


Fig. 6 : Effect of nitrogen sources on the xylanase production by *Aspergillus flavus*



Lane 1 - crude enzyme
Lane 2 - Acetone extract
Lane 3 - 50% ammonium sulphate precipitation
Lane 4 - 70% ammonium sulphate precipitation
Lane 5 - Standard molecular weight markers *E. coli* galactosidase

Plate 1 : Protein profile at the different stages of partial purification is depicted

Various nitrogen equivalents were tested for their maximum expression of xylanase,of which sodium nitrate in the Czapek's dox wheat bran xylan medium was found to be the best (Fig. 6). *Melanocarpus albomyces* when grown in medium containing sodium nitrate showed maximum growth and production of xylanase, Maheswari and Kamalam (1985).They also reported that the ammonium compounds responded to poor growth.

Sreenath and Joseph (1982) reported that the effect of metal ions on *Streptomyces exfoliates* showed calcium, barium stimulated the high xylanase activity. In the present study, and magnesium sulphate strongly activated the production of xylanase (Fig. 7).

Crude enzyme obtained from *Aspergillus flavus* was incubated at 40 ºC with varying time intervals. The enzyme activity was found to be maximum at 60 minutes. Thermal denaturation of the crude enzyme was noticed with the increase in the incubation time.

The effect of various inhibitors like $MnCl_2$, $HgCl_2$, $CdCl_2$, Sodium azide showed complete inhibition of xylanase as reported by Biswas *et al*. (1989)

SDS page of protein profile at the different stages of partial purification was depicted in Plate 1. Which indicated the presence of a major protein band in the molecular weight ranging from 13000-17000 Da conformed the earlier published literature of the molecular weight on the xylanase protein.

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