

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

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Accepted : April, 2009

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 °C 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,

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Peelamedu, Coimbatore. The cultures were maintained in Petriplates using potato dextrose agar medium.

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 40° 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 50°C. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μ mol of D xylose per min per ml.

SDS -PAGE:

SDS PAGE was carried out using 12 %