



## Original Research Article

### Production and purification of enzyme Xylanase by *Aspergillus niger*

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#### ABSTRACT

##### Keywords

Xylan,  
Xylanase,  
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Xylan is the next most abundant and widely distributed carbohydrates after cellulose. Xylanase (1, 4,  $\beta$ -D - xylan xylanhydrolase EC 3.2.1.8) catalyse the hydrolysis of xylan to xylooligosaccharides and xylose. Contemporary interest in xylan-degrading enzymes is due to new applications in the pre-bleaching of craft pulps and to their possible use in recovering fermentable sugars from hemicellulose. Xylanase producing *Aspergillus niger* was isolated from soil sample collected from garden. The maximum release of the sugar into the medium was analysed under different conditions like variation in temperature, pH, substrate, agitation. Heavy growth and high enzyme activity was found at pH 8.0, incubated for three days at 30°C in minimal broth kept under agitation. The stability of the enzyme at different temperature and pH was also noted, by analyzing the hydrolytic action of xylanase on xylan. The enzyme was found to be stable at 40°C and pH 8.0.

## Introduction

Xylan is the most abundant non cellulosic polysaccharide present in both hardwoods and accounts for 20-30% of the total dry weight in tropic biomass. In temperate softwoods xylans are less abundant and may comprise about 8% of the total dry weight. Xylanolytic enzymes are a group of enzymes that hydrolyse xylan and arabinoxylan polymers (Biely 1993). Xylanolytic enzymes are major focus of applied research and of great interest for biotechnology applications. A number of xylanolytic enzymes from various sources and especially from microorganisms, have been studied to understand their physical and biochemical characteristic (Sunna and Antranikian, 1999).

The enzymatic hydrolysis of xylan is accomplished by the action of endo-1,4- $\beta$ -xylanase (EC 3.2.1.8) and  $\beta$ -D-xylosidase (EC 3.2.1.37). The first enzymes acts on xylan by the endo hydrolysis of 1,4 - $\beta$ -xylosidic linkages to generate a small xylo oligosaccharide, which are further hydrolysed to D-xylose by the second enzyme which removes successive D-xylose residues from the non-reducing ends.

## Materials and Methods

### Isolation and identification of the organism degrading xylan

The organism was isolated from the soil

sample collected from garden in the Women's Christian College campus on Potato Dextrose agar. The identification of the organism was carried out by morphological identification using microscope.

### **Standardisation of growth of organism and production of enzyme**

The organisms were cultivated on media with different pH, substrates, variation in the temperature of incubation and agitation. The enzyme produced was estimated by the amount of sugar released using 3, 5-dinitrosalicylic acid enzyme assay.

### **Purification of the enzyme and immobilisation**

The enzyme was produced under the standardised conditions subjected to ammonium sulphate precipitation, dialysis and native gel electrophoresis. The purified enzyme was immobilised using sodium alginate and enzyme activity was assayed.

### **Solid State Fermentation**

The rice straw was used for solid state fermentation and spores of *Aspergillus niger* was inoculated on the straw. The flasks were incubated at 40°C for four days and pH was maintained at 8.0. After four days the xylanase production obtained was estimated.

## **Results and Discussion**

### **Isolation and identification of the organism degrading xylansolation of organism**

The soil sample was collected from garden and it was inoculated on Potato dextrose agar, black colored colonies and sponges were observed after three days of

incubation. Based on the colony characteristic and morphological appearances the organism was identified as *Aspergillus niger* spp (Fig I). The organism also produced xylanase when the assay was done.

A standard graph was plotted with glucose as standard ranging 50 to 250 micrograms. Within the standard graph the unknown values were extrapolated to calculate the amount of reducing sugar (glucose) released under varying condition like pH, temperature, agitation was detected. The organism growth and the production of enzyme was standardized by varying the pH of the medium using hydrochloric acid and sodium hydroxide. The medium filtrate was assayed for the amount of sugar. There was considerable increase in the growth of the organism from pH 6.0 to 8.0 above which there was a slight decrease in growth. Hence the optimal pH required for the growth of the organism was found to be 8.0. similar to studies on better optimal pH for xylanase production was found to be around pH 6.0 (G.M. Maciel *et al* 2008) Fig II

The organism growth and the production of enzyme was standardized by varying the temperature by incubating at different temperature medium was filtered and assayed for the amount of glucose. An increase in growth was observed at the temperature increased at 40°C above which the growth decreased. The optimal temperature for the growth of the organism was found to 40°C. Similar to that xylanase degrading enzyme from soil the temperature was optimized for the better production of enzyme, like white fungi also needed 30°C (G.M.Maciel 2008) Fig III.

The xylanolytic action for the enzymes and its maximum was determined by inoculating the organism in minimal broth containing

xylan with different concentration (0.5, 0.75, 1.0, 1.5, 1.25). and the incubation was carried out at optimum temperature (40°C) and pH (8.0).The amount of the reducing sugar released in the broth was calculated by using DNSA reagent where the OD is read at 540 nm using a colorimeter. At the result, the amount of the reducing sugar released in the concentration at 1.25 and the maximum activity can be detected at 96 hours.The activity of the enzymes was determined at certain condition like agitation, to analyse the maximum release of the sugar in the medium. To detect the presence of reducing

sugar in the medium, a comparative analysis was made between the organism inoculated in minimal broth with xylan, one kept at room temperature and another flask, incubated at 28°C, inside the shaker, under agitation conditions, the amount of the reducing sugar, released in the medium is estimated by using DNSA reagent and is calculated from the standard calibrated graph of glucose sugar Fig IV. Best result (release of reducing sugar) was obtained, when the organism was kept under agitation.

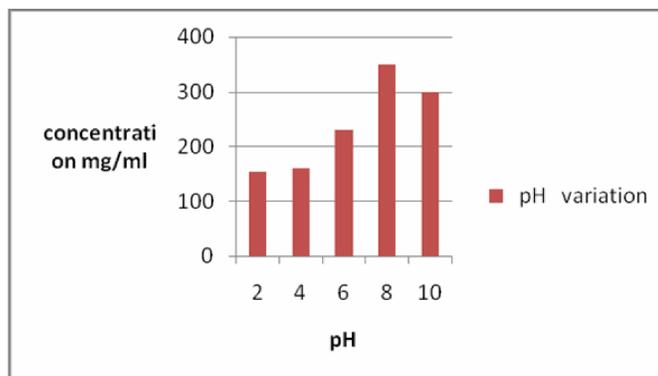


*Aspergillus niger*

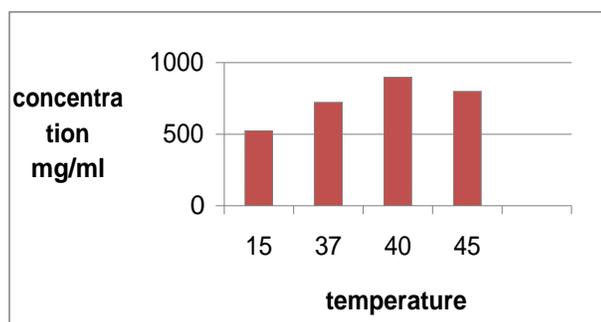


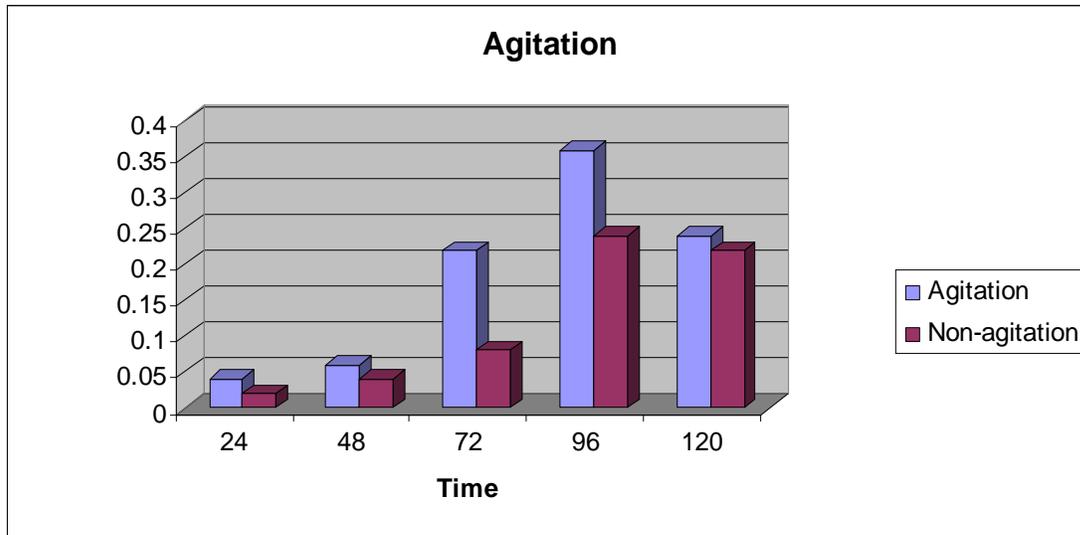
Microscopic view

**Fig II pH Variation**



**Fig III Temperature Variation**





**Fig.V Enzyme Immobilization by sodium alginate**

**Purification of the enzyme and immobilisation**

The crude enzyme was obtained by Ammonium sulphate precipitation was further purified by dialysis. Native page was performed using 10% acrylamide gel and the protein were stained with coomassive brilliant blue. Single band was obtained for the enzyme xylanase approximately 29.8 kDa. The purified enzyme was stored at 4°C and immobilised using sodium alginate. Fig V.

**Solid State Fermentation**

Solid-state cultivation offers advantages over liquid cultivation, especially for fungal cultures. Solid-state fermentation

(SSF) has considerable economical potential in producing products for the food, feed, pharmaceutical and agricultural industries. The rice straw were taken in the flask as the solid medium and mix it well with water. The sterile medium was inoculated with a spore suspension of organism. After mixing, the flasks were incubated at 40°C for four days and pH was maintained at 8.0. After four days the readings were taken, as a result which showed the best xylanase production was obtained at this temperature. Xylan-degrading enzymes are important due to their applications in the pre-bleaching of craft pulps and to their possible use in recovering fermentable sugars from hemicellulose. Xylanase can be applied in other industries also.

## References

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