



Original Research Article

Xylanase production from *Aspergillus niger* by Solid State Fermentation using Agricultural waste as substrate

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ABSTRACT

Keywords

Aspergillus niger;
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xylanase.

Xylanase production by *Aspergillus niger* via Solid State Fermentation was carried out using various substrates in an economically low operational cost in solid state cultivation offers advantages over liquid cultivation, especially for fungal cultures. The aim of this work is to enhance xylanase production using *Aspergillus niger* by optimization of some physical and chemical parameters involved in solid state fermentation. The raw materials such as wheat bran, paddy straw, sugarcane bagasse and saw dust are used in SSF. The highest xylanase production of 1.4 U/ml was obtained in wheat bran as substrate. By optimizing the pH at 6 and temperature at 30°C, xylose and NaNO₃ as carbon and nitrogen source, it yield high xylanase production.

Introduction

Agro industrial and food-processing wastes are available in staggering quantities all over the world, which largely become a source of health hazard. The majority of these wastes contain cellulose (30-40%), hemicelluloses (xylan 20-40%), and lignin (20-30%). The utilization of these wastes for the production of strategic chemicals and fuel requires hydrolysis of all the components. Because xylan is the second most abundant polysaccharide, xylanases and the microorganisms that elaborate them could be used in food processing and paper and the pulp, sugar, ethanol, feed, and agro fiber industries

(Gomes *et al.*, 1993). For most bioconversion process, xylan must first be converted to xylose or xylooligosaccharides.

Xylan is a heteropolysaccharide containing substituent groups of acetyl, 4-o-methyl-D-glucuronosyl and α -arabinofuranosyl residues linked to the backbone of β -1,4, - linked xylopyranose units and has binding properties mediated by covalent and non covalent interactions with lignin, cellulose and other polymers. Lignin is bound to xylans by an ester linkage to 4-o-methyl -D- glucuronic acid

residues. The depolymerization action of endo-xylanase results in the conversion of the polymeric substance into xylooligosaccharides and xylose. Xylanases are fast becoming a major group of industrial enzymes finding significant application in paper and pulp industry. Xylanases are of great importance to pulp and paper industries as the hydrolysis of xylan facilitates release of lignin from paper pulp and reduces the level of usage of chlorine as the bleaching agent.

Xylan, one of the major components of hemicelluloses found in plant cell wall is the second most abundant polysaccharide next to xylose. The term hemicelluloses refer to plant cell wall polysaccharides that occur in close association with cellulose and glucans. In fact, the plant cell wall is a composite material in which cellulose, xylan and lignin are closely linked. Xylan, having a linear backbone of β -1,4-linked xyloses is present in all terrestrial plants and accounts for 30% of the cell wall material of annual plants, 15-30% of hard woods and 7-10% of soft woods.

Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicelluloses, one of the major components of plant cell walls.

Xylanases are extracellular enzymes produced by micro organisms such as Bacteria (saprophytic and phytopathogenous), Mycorrhizic fungi and some yeasts. The enzyme is also found in protozoa, insects, crustaceans, snails, seaweed and also seeds of plants during the germination phase in the soil.

A complete and efficient enzymatic hydrolysis of this complex polymer

depends mainly on two types of enzymes;

Endo-1,4- β -xylanases, which hydrolyze the xylanopyranose of the central chain.

β -xylosidases, which hydrolyze xylobiose and other xylooligosaccharides resulting from the action of endoxylanases,

Many environmental factors affect microbial metabolic activity which can induce or repress enzyme biosynthesis such as, the substrate used, pH, temperature, cultivation time, inoculum concentration.

The xylanolytic enzymes are also employed for clarifying juices and wines, for extracting coffee, plant oils and starches, for improving the nutritional properties of agricultural silage and grain feed. Xylanases are also having application in rye baking where the addition of xylanase makes the doughs soft and slack. Xylanases are used as dough strengtheners' since they provide excellent tolerance to the dough towards variations in processing parameters and in flour quality. They also significantly increase volume of the baked bread. Sugars like xylose, xylobiose and xylooligomers can be prepared by the enzymatic hydrolysis of xylan.

Bioconversion of lignocelluloses to fermentable sugars has the possibility to become a small economic prospect. It is because massive accumulation of agricultural, forestry and municipal solid waste residues create large volume of low value feedstock.¹⁴⁹ If the feed stock is variable, a complete xylanolytic system would appear desirable to ensure maximal hydrolysis. Such an enzyme system would include xylanases, β -xylosidases, and the various debranching enzymes. Production

of environmentally friendly fuel is gaining great importance as the energy sources are shrinking. There are reports regarding the production of ethanol from the agro wastes by incorporating xylanase treatment.

Materials and Methods

Isolation of xylanolytic fungi

The isolation of fungi was carried out on Potato Dextrose Agar (PDA) using the samples obtained from agricultural soil. 1.0 gram of soil samples was suspended in 10 ml distilled H₂O and shake vigorously for 10 min. Then 0.5 ml diluted suspension was spread on PDA using an L-rod and incubated at 37°C for 5 days. Then the individual colonies were observed under the identify the specific species based on the following identification and characteristic features described by sundararaj manual. The fungal isolates formed were sub cultured to purify and examined for xylanolytic activities (Plate.1).

Screening of xylanolytic activities

Screening for xylanolytic activities was performed on Malt Extract Agar (MEA) containing 0.1% Wheat Bran. Plates were incubated at 29°C for 48 hrs after, which they were stained with Iodine solution for 15min. Positive xylanolytic isolates were detected based on clear zones of hydrolysis.

Solid state fermentation (SSF)

Cultivation of fungus was performed in 250 ml Erlenmeyer flask containing 10g of solid substrate such as wheat bran, sugarcane baggase, saw dust and paddy straw with the addition of 15 ml of Mandel's medium. The medium was

autoclaved and inoculated with 1×10^6 spores/ml of the moistening agent and incubated for 5 or 7 days at the ambient temperature $28 \pm 30^\circ\text{C}$.

Enzyme extraction

70 ml of cold water (4°C) was added to the SSF medium (10g substrate) after cultivation. The mixture was centrifuged at 5000 rpm for 20 min. The solid biomass residues were separated from the suspension by filtration through Whatmann paper. The cell free supernatant was used as the source of crude enzyme preparation.

Xylanase assay

The supernatant was used as source for enzyme sample. Xylanases activity was measured using different substrate. The reducing was determined by the Dinitrosalicylic acid Method described by Miller (1959) with xylose as standard. The released xylose was measured spectrophotometrically at 540 nm.

Optimization of temperature

Temperature is one of the parameters that determine the success of optimization system. Therefore, the effect of temperature on Xylanases production by *Aspergillus niger* was examined at various temperature ranges 25°C, 30°C, 35°C, 40°C and 45 °C.

Optimization of pH

The initial pH of medium was adjusted to variable pH range by 0.1N HCl. Enzyme purified was tested in the pH range (pH 5 to 9).

Effect of carbon source

Flask containing production media supplemented with carbon sources such as Glucose, Xylose, Lactose, Maltose, etc., was tested for the influence of carbon at 1% concentration.

Nitrogen source

Nitrogen sources such as Peptone, Urea, Yeast extract, NaNO_3 were tested for the influence of Nitrogen sources at 0.075% concentration.

Protein assay

Protein contents of the culture supernatants were assayed by the Folin Ciocalteu method of Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as standard.

Czapek-Dox broth containing saw dust, wheat bran, paddy straw and sugarcane baggase were inoculated with *Aspergillus niger* and incubated. For every 24 hours the culture cell free supernatant were examined for protein assay by Lowry method. To the 1ml of culture filtrate, 5ml of alkaline copper reagent was added and allowed to stand at room temperature for minutes. To this 0.5ml of Folin's phenol reagent was added and incubated at room temperature for 3min and measured at 650nm.

Results and Discussion

Selection and screening of isolates

Based on screening programme, the isolates were capable of exhibiting xylanolytic activities on Malt Extract Agar (MEA) with the diameter of the clear zones ranging from 35-45mm. These

isolates were identified based on the structural morphologies and observed that they possess distinct conidiophores terminated by a swollen vesicle bearing flask shaped Phialides. Growth on MEA showed that the initial white mycelia turned yellow and finally black upon maturation (Plate. 2).

Effect of substrate

Several agricultural wastes consisting of wheat straw, sugarcane baggase, Paddy straw and saw dust were examined as substrates for the growth and xylanase production by *Aspergillus niger*. The results after 5 days incubation at ambient temperature showed that wheat straw remained the best substrate for xylanase production by *Aspergillus niger* (Figure-1).

Effect of temperature

To estimate the optimum temperature of an enzyme, the activity was determined at several temperatures between 25°C - 45°C. The production of xylanase was maximum at the ambient temperature with an activity of 1.72 U/ml on wheat straw as substrate by *Aspergillus niger* as shown in Figure -2 (a).

Effect of pH

The initial pH of the medium was adjusted to variable pH range by adding the 0.1N HCl. Enzyme purified was tested in the pH range (pH 5-9). The production of xylanase was found to be the best at pH 6 of 1.48 U/ml on Wheat straw by *Aspergillus niger* as shown in Figure-3(a).

Effect of supplemented carbon sources

The supplementation of sugars, which may

act either as carbon sources or inducers. As shown in Figure - 4, the addition of xylose resulted in an increment of all substrates for xylanase production when compared to the cultivation in the absence of xylose.

Effect of supplemented nitrogen sources

The effect of nitrogen source supplementation on the production of xylanase by *Aspergillus niger* was also examined. The results obtained using various substrates were shown in the Figure-4 (a). Among the nitrogen sources tested, NaNO_3 was found to enhance the production of xylanase by *Aspergillus niger*.

Protein assay

The protein released by *A. niger* in the media containing the respective carbon sources are shown in Figure 5. Cultures containing the agro-wastes gave higher protein levels. The highest value was obtained with wheat bran cultures which gave a maximum protein concentration of 1.14 mg/ml at 96 h.

The results showed that *A. niger* produces xylanase enzyme when cultured in media containing the different agro-wastes (sawdust, sugarcane baggase, and paddy straw and wheat bran) as substrates. Most members of the *A. niger* group are notable producers of extracellular enzymes including important plant cell-wall hydrolyzing enzymes such as xylanases. The result after 5 days incubation at ambient temperature (30°C), revealed that wheat bran remained the best substrate for xylanase production by *A.niger* in SSF with the enzyme production of 1.42 U/ml. The sugarcane baggase, saw dust and

paddy straw ,although supporting the growth it produces enzyme activity as 0.54;0.98 and 0.61(U/ml).Higher xylanase production was also reported from *A.niger* using SSF at optimum temperature(Pang Pei Kheng *et al.*,2005).The same kind of xylanase production was also reported from *Bacillus spp* using SSF(Gessesse and Mamo,1999).

It was found that the xylanase of *Aspergillus niger* exhibited the activity at acidic pH range. The optimum pH of the enzyme was found to be 6.0 with an activity of 1.48U/ml in wheat bran. However certain xylanases from *Aspergillus* and *Penicillium* exhibit an optimum pH more on acidic side (pH 2.0 - 6.0) (Funaguma *et al.*,1991; Ito *et al.*,1992). The production of primary metabolites by microorganisms are highly influenced by their growth which is determined by the availability of the nutrients in the substrates .Therefore , it is expected that nutritional value of substrates by the supplementation of carbon and nitrogen source will also improve the growth of *A.niger* and subsequently the enzyme production.

Supplementation of sugars, which may act either as carbon sources or inducers. In addition of xylose, maltose, lactose and glucose, the xylose resulted in an increment in xylanase production (1.68U/ml) by SSF. Similiarly, in addition of peptone, urea, NaNO_3 and yeast extract, the NaNO_3 produces high yield of xylanase production (1.65U/ml) by SSF. Cultures containing the agro-wastes gave higher protein levels. The highest value was obtained with wheat bran cultures which gave a maximum protein concentration of

Figure.1 SSF cultivation system by *Aspergillus niger* on various agricultural wastes as substrate

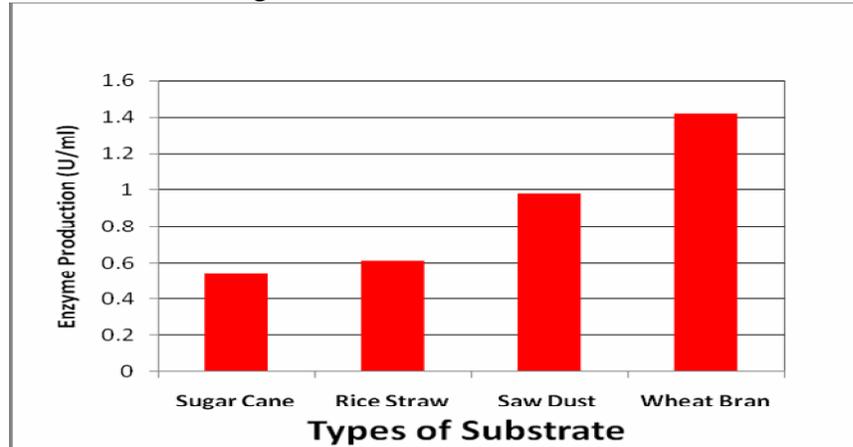


Figure.2 Xylanase enzyme activity by *A.niger* on agro waste substrates at different temperature by SSF system.

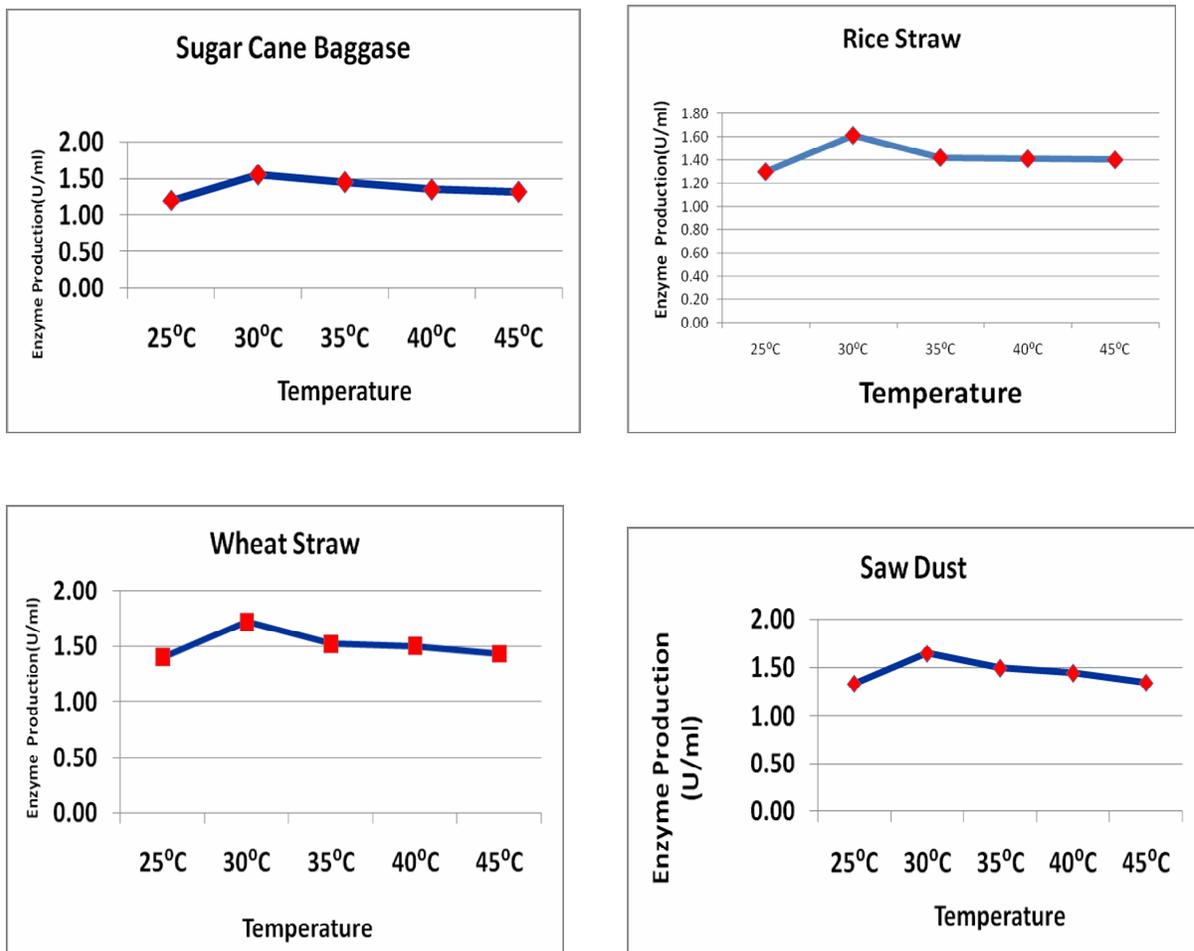


Figure.3 Xylanase enzyme activity by *A.niger* on agro waste substrates at different pH by SSF system.

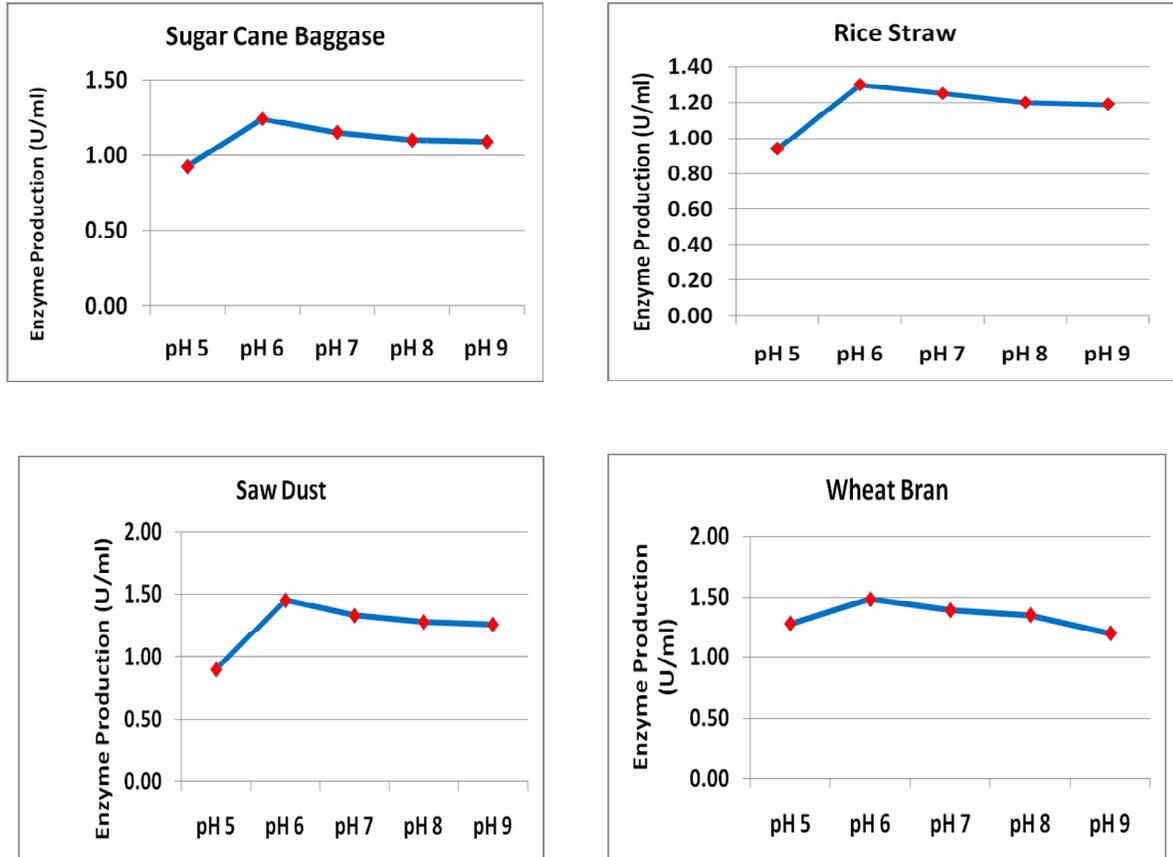


Figure.4 Effect of Carbon Sources supplemented to the SSF by *A.niger* for Xylanase Production.

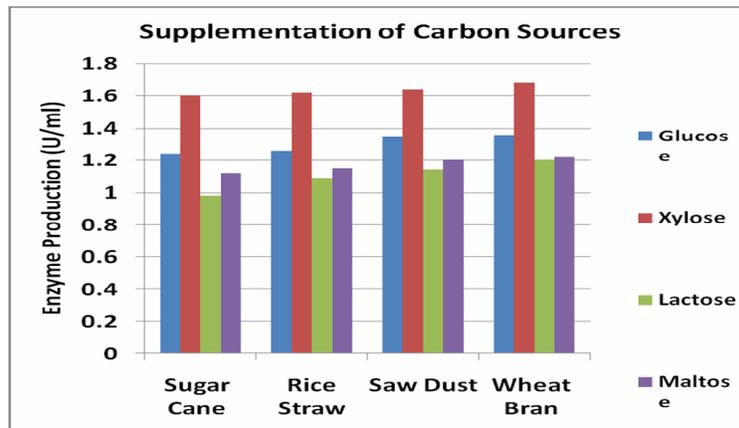


Figure.4 Effect of Nitrogen Sources supplemented to the SSF by *A.niger* for Xylanase Production.

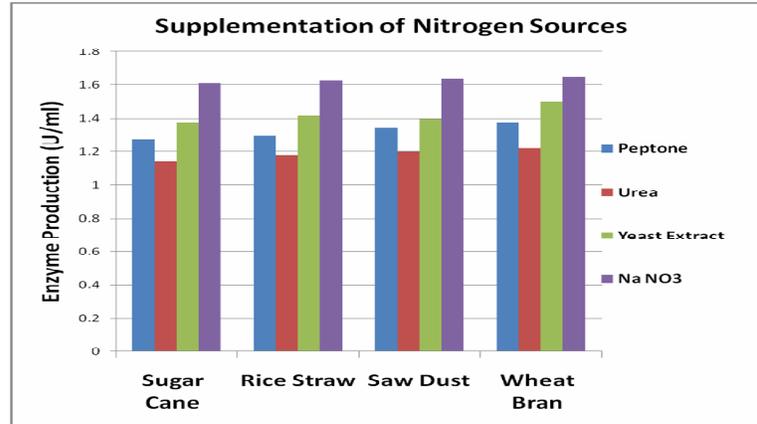
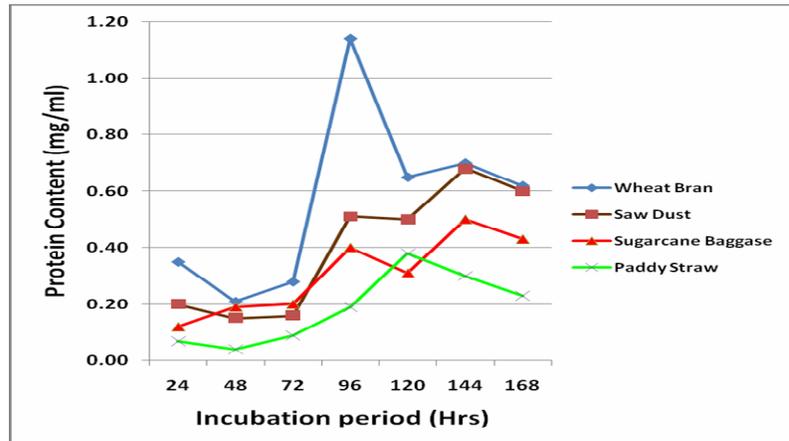


Figure.5 Protein content of culture supernatant of *Aspergillus niger* using substrate.



1.14 mg/ml at 96 h. Protein peaks of 0.68 and 0.50 mg/ml were obtained respectively from cultures containing sawdust and sugarcane baggase at 144 h. The least protein peak value of 0.38 mg/ml was obtained from cultures containing paddy straw at 120 h. This shows that of all agro wastes, wheat bran is the best prospective carbon source for the production of the enzyme (Okafor *et al.*, 2007).

High cost of production of plant cell-wall hydrolyzing enzymes is a limiting factor in their commercial production and industrial applications (Spano *et al.*, 1978). One area currently considered as cost-reduction

strategy is the use of waste plant materials as carbon sources for the production of the enzymes. In conclusion, our data has shown that the *A. niger* can produce extracellular proteins with significant xylanase activity when cultivated in media containing different agro-wastes as sole carbon sources. It also reveals that wheat bran, compared to the other agro-waste materials studied, is a very promising substrate for xylanase production. The use of agro-wastes in the production of such enzymes as xylanases will ultimately bring down their production cost and at the same time reduce environmental pollution due to the wastes.

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