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Research Article

PRODUCTION OF XYLANASE FROM LOW COST WHEAT BRAN, CORN COBS AND PIGEON PEA PODS WASTE BY ISOLATED FUNGI UNDER SOLID STATE FERMENTATION

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ABSTRACT

Agro industrial and food-processing substrates are considered as best cheap carbon source for enzyme production by SSF. The cost of enzyme production by submerged fermentation (SmF) is higher as compared to solid state fermentation (SSF). In this work we have used wheat bran, corn cob, and pigeon pea pods wastes for production of industrially important enzyme xylanase. It is important enzyme in paper and pulp and in food industry. The production of xylanase was investigated in SSF of *Aspergillus niger*. The parameters like pH, temperature, moisture content, incubation period, and nitrogen sources were optimized on wheat bran waste. The optimum condition for maximum biosynthesis of xylanase by *A. niger* (in term of xylose production) were shown to be at pH 6.0, temperature 40 ^{0}C, moisture 1:2.5 (w/v), incubation period 4 days, and in peptone with yeast extract as nitrogen source. By using optimized conditions corn cobs waste and wheat bran wastes were found to be best carbon source for xylanase production followed by pigeon pea pod waste.

INTRODUCTION

Agro industrial and food-processing wastes available in large quantities all over the world, which largely become a source of health hazard. The majority of waste 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 (Kanimozhi K. and Nagalakshmi P.K.2014).

Xylan hydrolyzing enzymes are called xylanases (Motta *et al.,* 2013; Corral and Ortega, 2006; Butt *et al.,* 2008).Xylanase degrades the linear polysaccharide beta 1, 4 xylan into xylose, by breaking down hemicelluloses which is one of the major components of plant cell wall.

Xylanases have commercial value in food industries, juice industries for clarification. It is used for oil extraction and waste paper recycling (Sharma and Kumar, 2013).They have application in improving digestibility of animal feed (Wong KKY *et al*. 1998), pulp bleaching (Rifaat HM *et al*., 2005), and bioconversion of lignocelluloses into feed stock and fuels(Kim JH *et al* 2000). Xylanases find specific application in jute fibre upgradation also (Cobley & Steele, 1976).

Xylanases are normally produced by using submerged fermentation. But solid state fermentation (SSF) has gained deep interest and attention of researchers due its high percentage conversion rate of biomass to energy, waste treatment and its production of secondary metabolites.

In SSF process, substrates in solid forms not only provides essential nutrients for the growth of microorganism in the culture, but it also serve as a support for the microbial cells or body. Therefore enzyme production using waste as substrates under SSF conditions have provided quit a lot of advantages.

The cost of enzyme production by submerged fermentation is higher as compared to SSF. Since the production of xylanase enzyme is a major factor in hydrolysis of xylan material, it is important to make process economically viable; this study therefore investigated on the bioconversion of wheat bran waste, corn cobs, and pigeon pea pod waste into more useful product xylanases using *Aspergillus niger*.

MATERIAL AND METHODS

Isolation and Screening of Aspergillus niger

Xylanase producing fungi was isolated from both outer dry and the inner fleshy scales of the onions on Sabouraud dextrose

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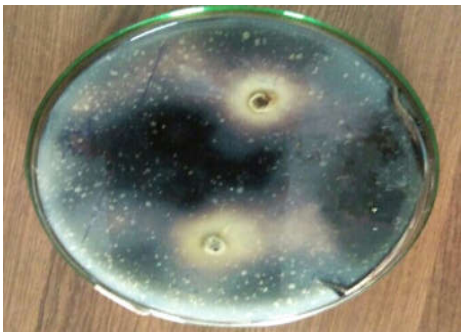
agar. The plates were incubated for 5-7 days at 25 ^{0}C. The isolation of xylanase producing strain was carried out on Sabouraud dextrose agar fortified with 1% w/v of xylan. The sterilized xylan containing SDA agar was poured into sterilized plates. Surface was dried in a hot air oven set at 45 ^{0}C. After drying, 5 mm size holes were made on the agar surface using sterile cork borers. The plates were aseptically inoculated with the fungal isolates on the holes made with cork borers (Spot Inoculation), and incubated at 25 ^{0}C for three days. The plates were flooded with grams iodine. The clear zone around the inoculated holes indicated the hydrolysis of xylan.



Photograph: Clear zone by hydrolysis of xylan

Development of inoculum. The selected fungal colonies were individually maintained on Sabouraud dextrose agar slant at 25^{0}C for 48 hours. The selected fungal cultures were inoculated in basal medium (xylan; 2.5 g, peptone; 0.6 g, beef extract; 0.6 g, KH_2PO_4 ; 0.1 g, $MgS0_4$. $7H_2O$; 0.01 g at pH 6 in 100 ml) and incubated at 37 ^{0}C for 48 hours which was used throughout the experiment.

Pre-treatment of wheat bran, corn cobs and pigeon pea pod waste

The substrates used for this work were first ground to fine powder, and then it was individually treated with 1 % (w/v) NaOH solution in the ratio 1:10 (substrate: solution) for 1 hour. This was then brought to neutral pH by washing thoroughly with distilled water. The pre-treated wastes were dried at room temperature, and further made on to powder form in an electric blender.

Solid state fermentation cultivation for xylanases production

Solid state fermentation was carried out in 250 ml Erlenmeyer flask that contain 10 grams of treated , dried, powdered, waste was mixed with 25 ml of basal medium. After sterilization and cooling 1 % inoculums was added, and incubated in humidified incubator at 40^0 C for 4 days.

Enzyme extraction

After incubation 25 ml phosphate buffer (pH 6) was added to inoculated substrate after 4 days of incubation period, and vigorously shaken in rotary shaker for 15 minutes at 120 rpm. The mixture was filtered through cheese cloth, and filtrate was centrifuged at 2000 rpm for 15 minutes. The supernatant was used as source of crude enzyme. It was stored at refrigerated temperature for further use.

Enzyme activity

The xylanase activity was determined by Dinitrosalicylic acid (DNS) method (Miller GL, 1959). 1.8 ml of 1% xylan solution was placed in a test tube, and 0.2 ml of crude enzyme extract was added. The reaction mixture was incubated at 40 ^{0}C in a water bath for 15 minutes. The reaction was terminated by adding 3 ml of 3, 5 dinitrosalicylic acid (DNS) reagent, and heated in boiling water bath for 5 minutes. Allowed to cool the test tube at room temperature. Optical density was read at 540 nm in colorimeter.

Optimization of process parameters

All the parameters were optimized using wheat bran waste

Effect of Ph

Solid state fermentation investigated the effect of pH on xylanase enzyme production by adjusting pH of basal medium to 5.0, 6.0, 7.0, 8.0., 9.0 and 10.0.The substrates were then incubated at 40 ^{0}C for 4 days.

Effect of Temperature

The effect of temperature on xylanase production was investigated by solid state fermentation in wheat bran waste and incubated at 30 ^{0}C, 40 ^{0}C, 50 ^{0}C, 60 ^{0}C, 70 ^{0}C at pH 6.0 for 4 days.

Incubation Period

The effect of incubation period on xylanase enzyme production was investigated by checking the enzyme activity from 1 to 8 days of incubation at pH 6.0 at 40 ^{0}C.

Moisture Content

Solid state fermentation was investigated the effect of moisture content on xylanase production by varying the volume of basal salt solution to 15 ml,20 ml, 25 ml, and 30 ml. The substrate was then incubated for 4 days at 40^0 C in incubator.

Nitrogen sources

The effect of nitrogen sources on xylanase production was studied by replacing the nitrogen source peptone with beef extract with 1.2 grams of NH_4Cl, NH_4NO_3, and whey in basal medium at pH 6.0, and incubated at 40^0 C for 4 days.

Xylanase production using wheat bran, corn cobs and pigeon pea pods waste

Solid state fermentation was carried out at optimum growth conditions for maximum xylanase production using wheat bran waste, corn cobs wastes, and pigeon pea pod wastes.

RESULTS

Solid state fermentation

The enzyme xylanase was produced by *Aspergillus niger* using agroindustrial and food processing wastes. The media and culture conditions like pH, temperature, incubation period, moisture content and nitrogen source were optimized for maximum production of xylanase. Using optimized condition solid state fermentation was carried out using wheat bran waste, corn cobs waste and pigeon pea pod waste. After incubation optical density of unknown xylanase enzyme was calculated from standard xylose graph (fig.1)

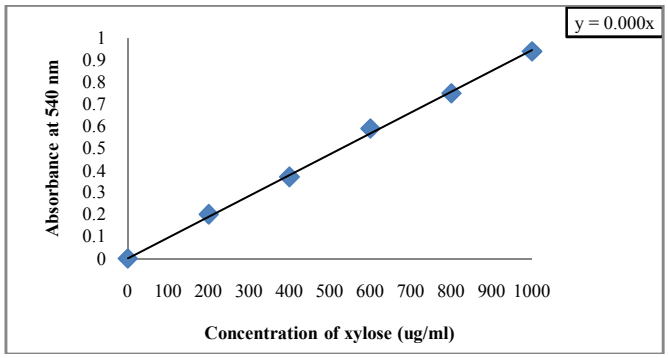


Fig 1 Standard xylose concentration curve

Effect of pH

Aspergillus niger was allowed to grow in a media at different pH ranging from 5.0-10.0. The pH change observed during the growth of microbes affect the product stability in the medium. Optimum pH for maximum xylanase production (in terms of xylose concentration) was found to be 6.0(fig.2).

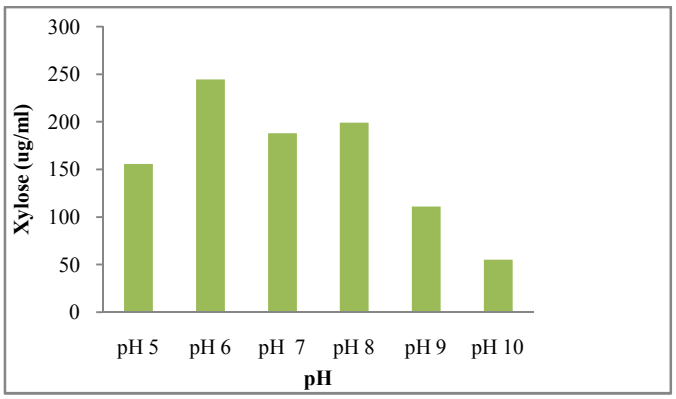


Fig 2 Effect of pH on xylanase production

Effect of temperature

Incubation temperature plays an important role in the metabolic activities of micro-organisms. In the present study enzyme activity was recorded at different temperatures revealed that A.niger shown maximum xylose concentration on wheat bran at 40^{0} C (fig.3).

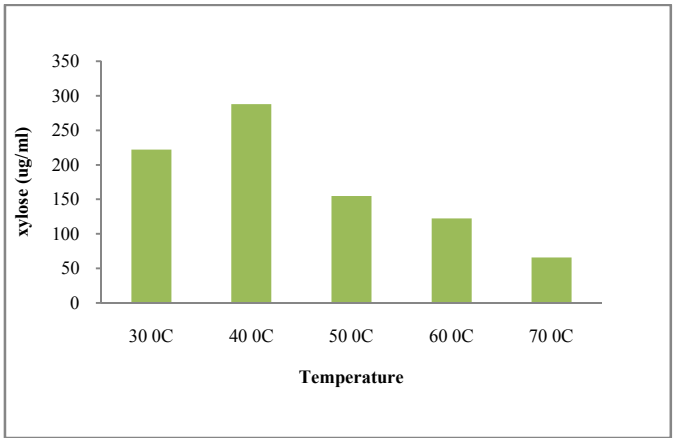


Fig 3 Effect of temperature on xylanase production

Effect of incubation period

After incubation period it was found xylose concentration was increased from 3 to 6 days of incubation. The maximum xylose production was observed on 4^{th} day of incubation. The xylose concentration was decreased after 6 days due to depletion in nutrients or accumulation of other byproducts in fermentation or basal media (fig.4).

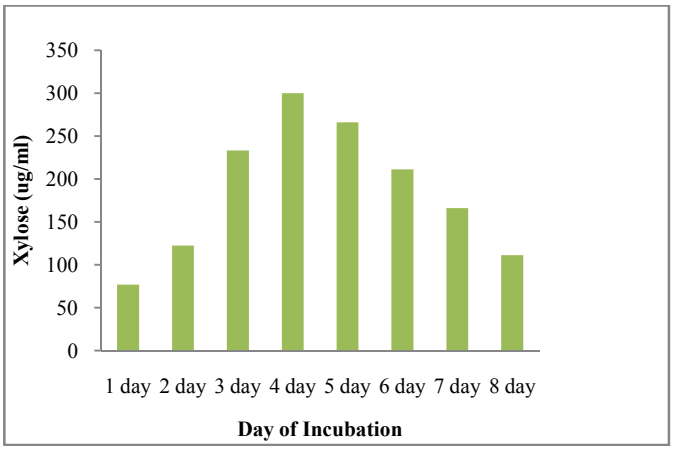


Fig 4 Effect of time of incubation on xylanase production

Effect of moisture content

Moisture content is a critical factor in solid state fermentation because this variable has influence on growth, biosynthesis and secretion of enzyme. In the present study *Aspergillus niger* was allowed to grow with different volumes of basal media ranging from 15 ml to 30 ml. The maximum xylose concentration was observed when volume of media was maintained at 25 ml. Any further decrease or increase in moisture ratio decreased the xylose concentration (fig.5.0).

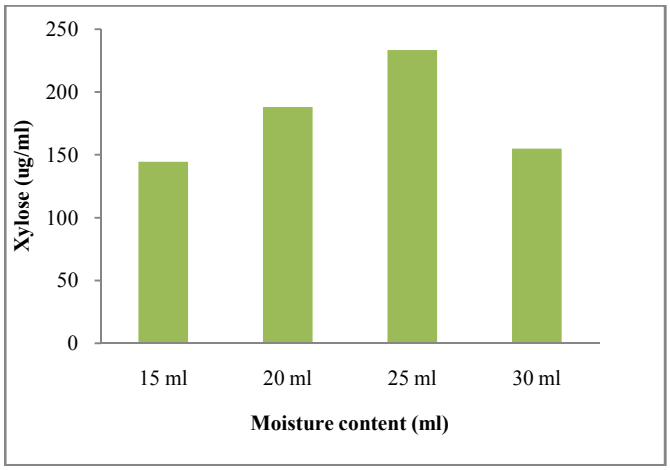


Fig 5 Effect of moisture content on xylanase production

Effect of nitrogen source

The effect of nitrogen sources was studied in the growth medium, where peptone with yeast extract was replaced with ammonium nitrate (NH_4NO_3), ammonium chloride (NH_4Cl), and whey. Among the entire nitrogen source, it was found that peptone with beef extract was the best nitrogen source for xylanase production (fig.6).

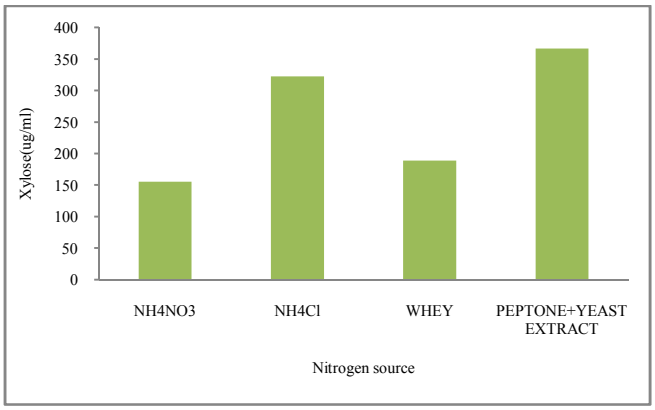


Fig 6 Effect of nitrogen source on xylanase production

Effect of different solid substrate

Effect of xylanase production using different solid substrates; wheat bran, corn cob, and pigeon pea pods waste was studied using optimum growth conditions. Corn cobs waste and wheat bran wastes are the best sources for xylanase production followed by pigeon pea pod waste (fig.7).

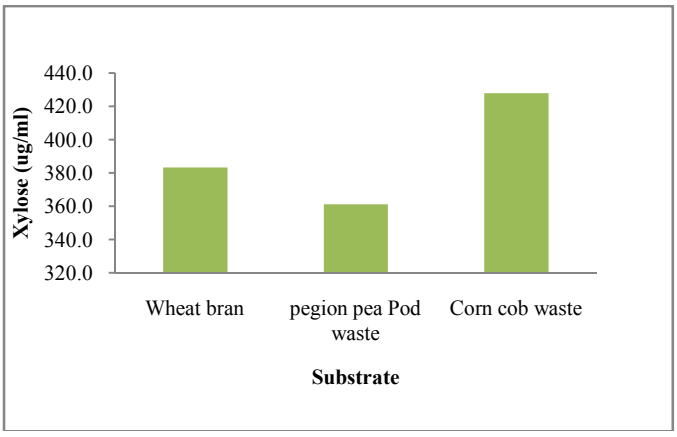


Fig 7 Effect of different substrate on xylanase production

DISCUSSION

An enzyme remains a major cost factor for the lignocellulosic fuels and chemical industries. In the recent study most of the enzymes are produced from lignocellulosic material by fermentation processes. An important consideration in fermentation processes is the cost of carbon source, which contributes significantly to overall production expenses. Accordingly, wheat bran, corn cob and pigeon pea pods waste which contain hemicellulose was examined as substrate to xylanase production by *Aspergillus niger.*

Aspergillus niger grown rapidly on wheat bran waste utilizing it as a source of carbon, and give promising level of xylanase enzyme until 7 days incubation period. Maximum xylanase production was found at 4^{th} day, and after 4^{th} day there is slow decrease of xylanase production upto 6^{th} day of incubation, where culture reaches towards the death phase and xylanase production goes on decreasing. Roy *et al.,* (2013) worked on xylanase production through solid state fermentation using wheat bran as sole carbon source, and the yield of enzyme was 236.4 unites/ml within 5 days of fermentation.

The effect of variation of pH, temperature, moisture content, and different nitrogen sources on the production of xylanase was also evaluated during cultivation of *Aspergillus niger* by SSF on wheat bran waste. The xylanase production was maximum when culture was allowed to grow in the optimized condition. The optimum condition such as pH 6, temperature 40^0 C, moisture content 25 ml, and nitrogen source peptone with beef extract was found to be responsible for maximum growth production of xylanase from wheat bran waste. Any further variation in pH, temperature, and moisture content and nitrogen source decreased the production of xylanase. In a report by Coral *et al*., (2002), a strain of *A.niger* released a xylanase with a pH 7.5 and temperature 60^oC. Christina Vafiadi *et al.,* (2010) reported that enzyme, stxyn1 and stxyn2, was optically active at pH 5, and at 60^0 C. Cai *et al*., (1998) studied the potential of *A.niger A3* for the production of xylanase in Solid state fermentation with initial pH 4.6, and temperature 28^0 C.

The effect of different solid substrates i.e. wheat bran, corn cobs, and pigeon pea pods waste (using optimum conditions) for maximum xylanase production showed that ; the production of xylanase in corn cob was more followed by wheat bran, and pigeons pea pods waste respectively. Corn cob is a rich source of xylan (Uma Gupta and Rita Kar 2009). K. Kanimozhi, K. and Nagalakshmi R.K (2014) used different agriculture wastes; wheat bran, paddy straw, sugarcane bagasse and saw dust for xylanase production.

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