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XYLANASE ACTIVITY OF *ASPERGILLUS NIGER* AT DIFFERENT ECOLOGICAL CONDITIONS

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ABSTRACT

Maximization of xylanase activity at different media, temperature, pH and salt concentration has been presented in this paper. YpSs, Czapek dox and Malt extract medium were taken for evaluation of optimum growth and activity. Amongst all tested media, YpSs showed the highest growth. Three different natural carbon substitute i.e., wheat husk, rice husk and sugarcane baggase were used for xylanase activity. Maximum enzyme activity was observed in test fungus at rice husk. Production and maximum xylanase activity at rice husk has been observed at different temperatures, pH and Salt concentrations. The highest xylanase activity has been observed on day 5 at temperature 32° C, pH 6.5 and salt concentration of 2%.

Keywords: Baggase, Rice and Wheat husk, Xylanase activity, Environmental factors etc.

INTRODUCTION

Agriculture waste is one of the major environmental wastes and caused maximum pollution. However agricultural wastes at the same time are one of the cheap natural resource which may be used for various applications. Agricultural waste in India produced per year is approximately 998 million tonnes. The aim of this research is to utilise agro-waste (a cheap raw material) for the production and activity of industrially important enzyme xylanase. Agricultural waste contains Lignocellulose which includes cellulose, hemi-cellulose, lignin and many other components. Hemi-cellulose consists of xylan and enzyme xylanase is one of the important enzymes to recycle it. Xylanase has many applications which include both scientific as well as social utility (Mukherjee *et al.*, 1991). Xylan is a hetero-polymer characterized by the backbone of (1-4) linked Xylose units as the major component. Xylan accounts for 30-40% of the weight of agriculture residues (Sayed *et al*., 2014). Xylanase could be used in following areas (Mukherjee *et al.,* 1991):-

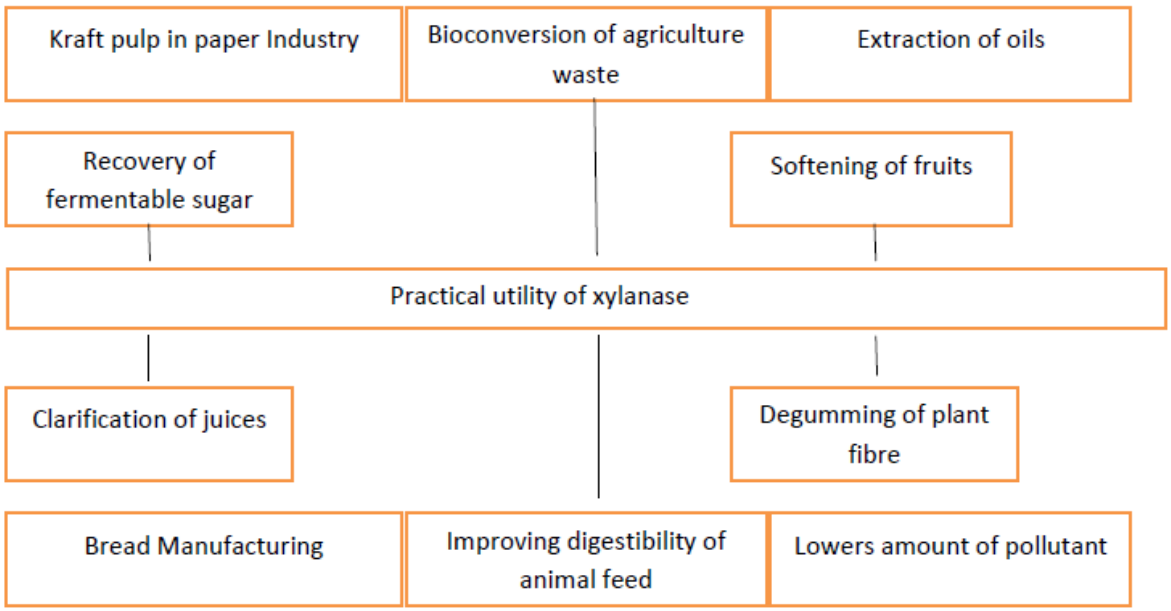


Fig. 1 : Applications of Xylanase

Therefore, common agricultural wastes have been used to ferment by the most commonly isolated fungus *A.niger* from Agricultural field for the production of xylanase and its activity was optimized at different ecological parameters (Solimon *et al.*, 1994).

MATERIAL AND METHODS

Aspergillus niger was isolated most frequently from samples collected from Agricultural field, and has been used for present study. Samples used for isolation of this fungus were also evaluated for their physio-chemical parameters like; pH, temperature, EC, TDS and salinity. Isolated *A. niger* was screened for optimum growth and activity at different medium like Malt extract, YpSs and Czapek 'dox.

Dry weight measurement and colony diameter methods (Goswami and Seema Rawat, 2015) were used to observe the periodic growth at every 24 hrs. 20ml of liquid medium and solid medium of each kind was poured in 100ml Erlenmeyer flasks and petriplates respectively. Both liquid and solid media were inoculated with test fungus and incubated for different periods both under stationary and rotatory incubators at 30° as well as at 45°C to observe its nature (Shukla and Jaitly, 2003). Flasks were kept for three different days i.e. 3, 5 and 7 day. Each flask was taken in triplicate. After incubation flasks were taken out, filtered on pre-weight (w_1) Whatman filter paper No.1. Fresh weight and dry wt. were taken (w_2) after incubation period. Difference in weight was taken for fungal biomass of the test fungus. Two colony diameters at right angle to each other were taken for calculating average diameter at every 24 hrs on solid medium for seven days.

Xylanase activity of *Aspergillus niger* at different ecological parameters like Temperature, pH & salinity): Fermentation of agricultural waste; wheat husk, rice husk, and sugarcane baggase was taken for growth and xylanase production by test fungus. Fungus was grown on YpSs broth medium supplemented with 1% above, as a carbon source for five days. Xylanase activity in culture filtrate was tested at different temperature ranging from 20-50°C at a difference of 5°C each (Haltrich and Nidetzyk, 1996). Further the effect of pH and salinity on xylanase activity was undertaken at different pH ranged from 5.5 to 8.0 with a difference of 0.5 in the medium with phosphate buffer. Similarly medium with different salt was prepared to test effect of salt on enzyme production. The enzyme activity was tested by method used by Khan (Haltrich and Nidetzyky, 1996).

RESULT AND DISCUSSION

The pH, moisture, EC, TDS and salinity content in the samples were found to range from 5.2-8.5, 20-70 %, 235-428 µS, 412-915 ppm and 103-410 ppm**.** Results of growth of *A. niger* on different medium have been presented in Fig.1. It is apparent from the tabulated data that the highest growth occurred on YpSs broth medium on day 5 and similarly on solid medium also it was on day 5. These results are important for further physiological experiments on tested fungus. Other researchers have also found the similar results (Okafor *et al.*, 2007) (Yuan *et al*., 2005). Fermentation ability of the fungus was further evaluated on YpSs medium supplemented with 1% agricultural waste has been presented in table 1.2, in place of carbon content. It has been found that rice husk was the best source for optimum growth and xylanase activity of the test fungus followed by wheat husk and sugarcane baggase (Solimon, 1994). These results are important in recycling of rice husk with the use of test fungus and shall be a milestone in reducing pollution by burning rice husk.

DNS Method of Xylanase: Xylanase activity was determined by DNS method. The mixture of 0.2 ml enzyme and 1.8 ml of 1% Xylan as substrate was incubated for 30 min at 50°C. 1ml from this reaction mixture was taken and 3ml of DNS was added, boiled for 5 min. It was allowed to cool and read against spectrozero at 540 nm. Enzyme activity was calculated by extrapolating with the concentration of Xylose from standard graph prepared with different concentrations of Xylose. Enzyme activity was calculated with the following formula:

Enzyme activity = OD X 1/enzyme volume X 1/ substrate volume X 1/ Incubation time X Retention coefficient X DF

Test fungus (*A. niger*) showed the maximum enzyme activity at 30° while the lowest at 50°C (Table 2) Similar results were also obtained by other workers (Cunha *et al*., 2018) (Karunakaran *et al.,* 2014). Maximum xylanase activity was obtained at pH 7.0 and the least at 5.5 pH. It was also observed that pre-experiment pH was different than that of the from post experiment pH which clearly indicate that test fungus has the potential to change the pH of the substrate (Table 3) (Karunakaran *et al.,* 2014) Salinity is also one of the important factors for the enhancement of xylanase activity of the fungus (Table 5). *Aspergillus niger* showed the highest xylanase activity at 2.0% salt level (Karunakaran *et al.,* 2014).

The result showed that ecological parameters i.e., temperature, pH and salt concentrations have increase xylanase activity of the test fungus by 3%. Results are also important in recycling of agricultural waste especially rice husk and in reducing environmental pollution caused by its burning. It has been reported that *Aspergillus niger* is an important fungus for conversion of lignocellulosic waste into useful products (Nazish and Jaitly, 2020)

Table 1 : Growth of *Aspergillus niger* on different medium on different days under stationary and rotatory conditions

Medium / Days	Growth of *Aspergillus niger* on different medium on different days under stationary and rotatory conditions											
	Czapek dox				Malt extract				YpSs			
	Solid (cm)	State	Liquid (gm)		Solid (cm)	State	Liquid (gm)		Solid (cm)	State	Liquid (gm)	
			FW	DW			FW	DW			FW	DW
3rd	4.1	R	5.945	1.821	5.1	R	6.258	1.885	4.7	R	6.178	1.564
		S	2.516	1.467		S	3.041	1.507		S	4.185	1.571
5th	7.8	R	7.976	2.079	8.9	R	8.44	2.142	9.3	R	10.206	2.235
		S	4.017	1.673		S	3.942	1.631		S	4.823	1.736
7th		R	10.467	2.423		R	9.589	2.997		R	11.769	3.254
		S	4.871	1.762		S	4.527	1.786		S	4.996	1.781

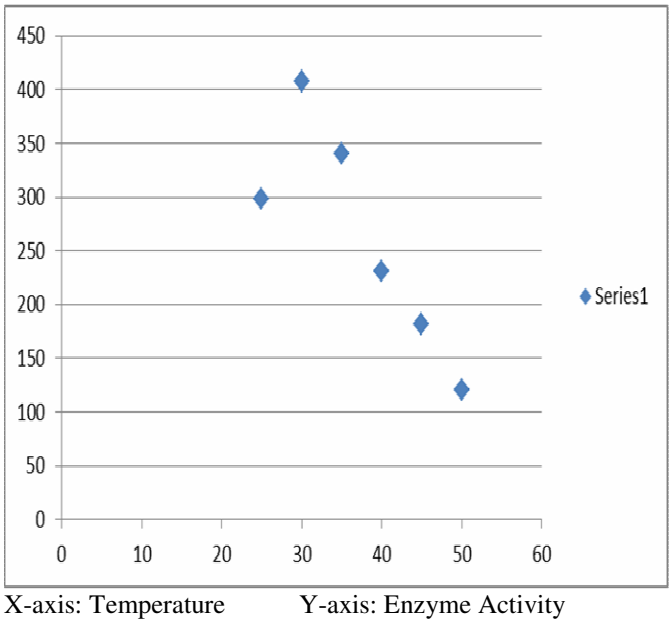
R= Rotatory State; S= Stationary State; FW= Fresh Weight; DW= Dry Weight

Table 2 : Growth and Xylanase Activity of fungus *Aspergillus niger* on YpSs medium supplemented with 1% different agricultural waste

S. No.	Substrate	Growth (gm)	Xylanase activity
1	Rice husk	9.685	387.52 IU/ml
2	Wheat bran	8.771	345.71 IU/ml
3	Sugarcane baggase	5.493	272.15 IU/ml

Table 3: Temperature optimization for xylanase activity/ production by *Aspergillus niger*

S.No	Temperature	Xylanase Activity
1	25°C	298.38 IU/ml
2	30° C	407.52 IU/ml
3	35° C	340.19 IU/ml
4	40° C	231.54 IU/ml.
5	45° C	181.64 IU/ml
6	50° C	120.98 IU/ml

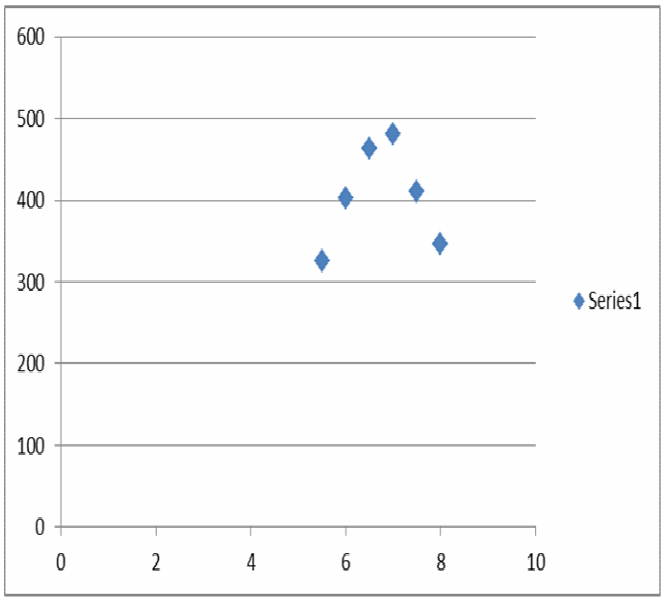


X-axis: Temperature Y-axis: Enzyme Activity

Fig. 2 : Temperature optimization for xylanase activity

Table 4: Effect of pH on xylanase activity of *A. niger* at optimum temperature

S. No.	Pre-experimen pH	Post-experiment pH	Xylanase activity
1	5.5	4.4	324.91 IU/ml
2	6.0	4.2	401.69 IU/ml
3	6.5	4.3	463.34 IU/ml
4	7.0	4.1	480.21 IU/ml
5	7.5	4.6	410.17 IU/ml
6	8.0	5.2	346.01 IU/ml

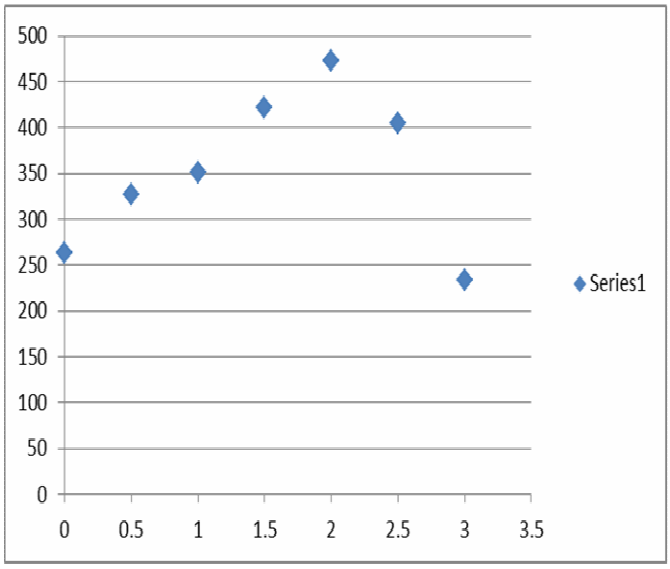


X-axis: pH Y-axis: Enzyme activity

Fig. 3: Effect of pH on xylanase activity

Table 5: Effect of salt concentration on xylanase activity of *A. niger* at optimum temperature and pH

S.No.	Salt concentration	Xylanase activity
1	0%	263.84 IU/ml
2	0.5%	327.43 IU/ml
3	1.0%	351.38 IU/ml
4	1.5%	422.40 IU/ml
5	2.0%	473.61 IU/ml
6	2.5%	405.06 IU/ml
7	3.0%	234.11 IU/ml



X-axis: Salinity Y-axis: Enzyme activity

Fig. 4: Effect of salinity on xylanase activity

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