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# FERMENTATION OF AGRO WASTE UNDER DIFFERENT ECOLOGICAL CONDITIONS FOR THE PRODUCTION OF XYLANASE BY *ASPERGILLUS NIGER*

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**ABSTRACT**

One of the most frequently isolated fungus form agricultural field, *Aspergillus niger* was grown on three different media for its growth and activity. Out of three media i.e., Malt extract, YpSs and Czapek 'dox, the YpSs was found as best medium for growth. Xylanase activity of this fungus was further tested on YpSs broth medium supplemented with three agricultural wastes i.e. rice husk, sugarcane bagasse and wheat husk. Out of these rice husk was found as the best substrates for xylanase activity. Effect of temperature, pH and salinity for the xylanase activity was also explored. The highest activity of xylanase was observed on 5th day at temperature of 32° C, pH 7.0 and salinity 2.0%.

## INTRODUCTION

Agriculture waste is one of the major environmental waste and pollutant. At the same time, it is 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. Rice husk, Sugarcane bagasse and wheat husk are the major agriculture waste comprises of lignin, hemicelluloses, and cellulose. Many microorganisms possess an efficient hydrolytic system which secretes different enzymes such as cellulase, hemi-cellulase and beta-glycosidase. These enzymes convert lignocellulosic materials to essential metabolites. Hemi-cellulose consists of xylan and xylanase have the capability to degrade it. Xylan is a hetero polymer characterized by a backbone of (1-4) linked Xylose units as the major component. Xylanolytic enzymes have huge applications and are produced by several microorganisms.

The aim of this research is to utilise agro-waste, a cheap raw material to produce industrially important enzyme xylanase. Agricultural waste contains Xylan accounts for 30-40% of the weight of agriculture residues. Xylanase could be used in following areas which includes kraft pulp in paper industry, in extraction of oils, softening and clarification of fruits, bioconversion of agriculture waste, recovery of fermentable sugar, bread manufacturing, degumming of plant fibre, Improving and digestibility of animal feed and also in lowering amount of pollutants produced as a result of residues from agricultural field. Therefore, common agricultural waste are used to ferment by the most commonly isolated fungus A.niger for the production of xylanase and its activity was optimized on different ecological parameters.

## MATERIAL AND METHODS:

*Aspergillus Niger* was isolated most frequently from samples collected from Agricultural field. This fungus was used in the study. Three different media i.e., Malt extract, YpSs and Czapek 'dox were used to observe the maximum growth of *A. niger*. Growth on different media was under stationary and rotated stage to plan further physiological studies. Dry weight measurement and colony diameter methods (Jaitly, 1991) 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 flask and petriplates, respectively. Flasks were inoculated with inoculum of the fungus while petriplates were inoculated with single point inoculum. Flasks and petriplates were kept in the incubator under stationary and rotatory conditions at 29± 1 C for three different days i.e.,3, 5 and 7days. Each flask was taken in triplicate.

After fix incubation flasks were taken out filtered on pre-weight ($w_1$) Whatman filter paper No. 1. Fresh weight after air dry and dry wt. after drying at 60° C for 24 hrs, were taken ($w_2$). Difference in weight was taken for fungal biomass of the fungus. Two diameters at right angle to each other were taken for colonies grown into petriplates for calculating average diameter at every 24 hrs on solid medium for seven days.

Fermentation of agricultural wastes: wheat bran, rice bran, and sugarcane bagasse were used for growth and xylanase production by *A. niger*. Fungus was grown on YpSs broth medium supplemented with 1% Agricultural waste (give above) as a carbon source for five days.

**Standard of xylanase enzyme:**

**Assay for xylanase enzyme:** 1% xylanase was prepared in acetate phosphate buffer with pH 6.0 and it was used as a substrate for enzyme assay.

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

**Spectrozero:** 0.9 ml of buffer and 0.1 ml of substrate was incubated at 50 C for 30 min in test tube. 3 ml of DNS reagent was added and boiled for 5 min.

**Reaction:** 0.2 ml enzyme and 1.8 ml substrates was taken in a test tube and kept at 50 C for

**Standard:** Standard curve was prepared using 1 mg/ml of Xylose solution. Different concentrations of Xylose solutions were prepared from stock solution of Xylose in different test tubes and boiled after adding 3ml of DNS reagent. OD was taken at 540 nm against spectrozero after cooling it.

**Xylanase form *Aspergillus niger* on different ecological parameters (Temperature, pH and salinity):**

Fungus was checked for xylanase activity in broth culture using rice bran (an agriculture waste) supplemented in YpSs broth medium using method of Khan (1969) at different temperatures ranging from 25° to 50°C at a difference of 5 C each; pH ranged from 5.5 to 8.0 with a difference of 0.5 and salt concentration varied from 0% to 3% with a difference of 0.5% each. Xylanase activity was calculated by extrapolating activity with standard graph of Xylose at 540nm of wavelength.

## RESULT AND DISCUSSION:

Growth of *Aspergillus niger* on different medial in solid and broth condition was undertaken. In broth condition rotatory and stationary state of medium were also undertaken and result are given in Table 1

**Table 1.** Growth of *Aspergillus niger* on different media on different days under stationary and rotatory conditions

| Medium Days | 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

Tabulated data showed the highest growth on YpSs medium both in solid and broth conditions on 7th day, colony on solid medium over the petriplates in all the media so data on 5th day has been undertaken. Under broth condition highest growth was observed under rotation on YpSs medium [Table1]. These results are important for further physiological experiments on tested fungus. Other workers also found the similar results [1][3].Further fermentation ability of the fungus was evaluated by growing on YpSs medium supplemented with 1% agricultural waste in place of carbon content (sugar). Three common agricultural wastes i.e., Rice husk, wheat husk and sugarcane bagasse were tested (table 2). It has been found that rice husk was the best source for optimum growth and xylanase activity of the test fungus followed by wheat bran and sugarcane bagasse. These results are important in recycling of rice husk by the fungal enzymes in various useful products and could be a milestone in reducing pollution by burning rice husk and thus improving economic conditions of farmers.

**Table 2.** Growth and Xylanase Activity of fungus *Aspergillus niger* on YpSs medium supplemented with 1% different agricultural waste as carbon source on 7th day as maximum growth earlier was observed on this day.

| S.no | Substrate | Growth (gm) | Xylanase activity |
|---|---|---|---|
| 1 | Rice husk | 9.685 | 387.52 IU/ml |
| 2 | Wheat husk | 8.771 | 345.71 IU/ml |
| 3 | Sugarcane bagasse | 5.493 | 272.15 IU/ml |

Six different temperatures .i.e., 25°C to 50°C with a difference of 5°C each were taken to prepared enzyme (as given before) at their optimum day of enzyme production (table 6) and pH 6.0. Enzyme activity was evaluated at their respective optimum temperature as stated.

**Table 3:** Temperature optimization for xylanase activity 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 | 390.19 IU/ml |
| 4 | 40° C | 231.54 IU/ml. |
| 5 | 45° C | 181.64 IU/ml |
| 6 | 50° C | 120.98 IU/ml |

*Aspergillus niger* (407.52 IU/ml) showed the maximum activity at 30°C which showed that it is mesophilic in nature. At 30°C, Xylanase activity in Aspergillus niger was checked at six different pH ranging from 5.5-8.0. It was obtained from this that maximum xylanase activity (480.69IU/ml) was obtained at pH 6.0.

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

| S.no | Pre-experiment pH | Post-experiment pH | Xylanase activity |
|---|---|---|---|
| 1 | 5.5 | 4.4 | 324.91 IU/ml |
| 2 | 6.0 | 4.2 | 480.69 IU/ml |
| 3 | 6.5 | 4.3 | 463.34 IU/ml |
| 4 | 7.0 | 4.1 | 410.21 IU/ml |
| 5 | 7.5 | 4.6 | 382.17 IU/ml |
| 6 | 8.0 | 5.2 | 346.01 IU/ml |

Tested fungus was further evaluated for their optimum salt condition at their optimum temperature and pH. 25 ml of culture media prepared at required pH of the test fungus in acetate phosphate buffer were further supplemented with 0-3 % concentration of salt with a difference of 0.5% each. The flask inoculated with test fungi were incubated at their optimum temperature. After 5 days flask were taken out and enzyme activity was evaluated as stated above. Results so obtained have been tabulated in table 5.

**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% | 493.61 IU/ml |
| 6 | 2.5% | 405.06 IU/ml |
| 7 | 3.0% | 234.11 IU/ml |

Tabulated data revealed that tested fungus exhibited different salt optima for their maximum Xylanase Activity. The optimum activity of *A. niger* (493.61 IU/ml) was at 2.0% The above result clearly indicated that variation in ecological parameters fungal forms exhibited different maximum enzyme activity.

**Growth of fungus on YpSs medium using rice husk as substrate**

*A.niger* showed the maximum enzyme activity at 30° while, the lowest was at 50°C (Table 3)[Yuan et.al.,2005][Luana 2009]. Similarly, maximum xylanase activity was obtained at pH 7.0 and the least at 5.5 pH. (Table 4) 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 4)(Haltrich et.al 1996)Salinity was also one of the important factors for the enhancement of xylanase activity of the fungus. *Aspergillus niger* showed the highest xylanase activity at 2.0% salt level[Luana Cunha 2009]

The result showed that ecological parameters i.e., temperature, pH and salt concentrations caused increase in xylanase activity by the fungus which may play an important role in recycling of rice bran and in reducing environmental pollution caused by burning of rice bran.

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