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RESEARCH ARTICLE

Effect of various physical parameters for the Production of the enzyme Xylanase from mixed culture of *Bacillus polymyxa* and *Cellulomonas uda*

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ABSTRACT

In the last decade, Xylanases have extended their use from the paper and pulp industries to newer needs such as biofuel production. The enzyme xylanase was produced by utilizing cheaper substrates like grass extract and sugarcane sheath leaf extract supplanted with nutrient sources in Submerged Fermentation using a mixed culture of *Bacillus polymyxa* and *Cellulomonas uda*. A fermentation time of 6 days was required to obtain maximum xylanase activity.High activities of enzyme (14.662 IU/ml) were obtained with sugarcane sheath leaf extract as the major carbon source. The optimum pH and temperature for maximum production of xylanase were found to be 8 and 50°C respectively. Additionally the effect of various inducers were also studied and it was found that xylan(0.2%) could induce xylanase production resulting in an enzyme activity of 44.773 IU/ml.

Keywords: Xylanases, submerged fermentation, mixed culture, sugarcane sheath leaf extract.

1. INTRODUCTION

Xylanases (EC 3.2.1.8) are a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants. Many bacterial and fungal species can produce a mixture of xylanase, β-xylosidase and accessory side-group cleaving enzymes in order to utilize xylan(1). While many applications of enzymes in paper industries are still in the research and developmental stage, several applications have found their way into the mills in an unprecedented short period of time in the last decade (2). In the last decades, it has been emphasized that the use of xylanolytic enzymes could greatly improve the overall economics of processing lignocellulosic materials for the generation of liquid fuels and chemicals (3). In large scale processes the carbon source has been estimated as the major cost factor in enzyme production. A reduction in the production costs can be achieved by the usage of inexpensive waste materials, such as grass extracts and sugar cane sheath leaf that are available in abundant amounts. The purpose of this research is to evaluate the production of xylanase using a mixed culture of *Bacillus polymyxa* and *Cellulomonas uda* and to optimize some parameters such as pH, temperature and effect of various inducers.

2. MATERIALS AND METHODS:

2.1. Materials

All chemicals were of analytical grade and procured from Merck India Ltd. Media for growth and production were purchased from HiMedia.

2.2. Microorganisms

The microorganisms namely *Bacillus polymyxa* (NCIM 2539) and *Cellulomonas uda* (NCIM 2353) which were used were obtained from National Collection Of Industrial Microorganism, Pune. The culture was maintained on Nutrient Agar slants and stored at 4°C.

2.3. Growth curve

Both the microorganisms were inoculated in nutrient broth in conical flasks and incubated in a shaker at 120rpm

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and 50°C. Absorbance of the culture was read at 600nm periodically at an interval of every two hours for 10 days. A graph was drawn with time in x-axis and absorbance in Y-axis.

2.4. Xylanase production

The lyophilized cultures were sub cultured in Nutrient Agar and GPY (Glucose, Peptone, Yeast extract) medium periodically. The seed medium, which was used, was same as growth medium without agar. 100 ml of the seed medium was taken and a loop full of culture was inoculated into the flasks. The culture was incubated in a shaker at 120 rpm at 50ºC for 3 to 4 days.

100ml of Grass extract and sugarcane sheath leaf extract were used for production along with nutrient solution (Yeast extract, peptone, NaCl), which contains the basic requirements for the growth of the organism. The extracts were prepared by heating finely cut leaves grounded in Phosphate Buffer Saline. After inoculation, the flasks were incubated at 50ºC in a rotatory shaker at 150 rpm. At each sampling time, the culture medium was filtered using a 0.22µm syringe filter and the filtrate was used for further enzymatic assays. During the cultivation, two flasks or more were sampled daily.

2.5. Enzyme assays

Xylanase was assayed by the optimized method described by Damaso *et al.* (4), using birchwood xylan as substrate. The solution of xylan and the enzyme at appropriate dilution were incubated at 75ºC for 3 minutes and the reducing sugars were determined by the dinitrosalicylic acid procedure (5), with xylose as standard. The released xylose was measured spectrophotometrically at 540 nm. One unit of enzyme activity was defined as the amount of enzyme capable of releasing 1 µmol of reducing sugar per minute under the assay conditions. Protein concentration was measured by the method of Lowry et al. (6) with bovine serum albumin as a standard.All experiments were performed in duplicate or triplicate, and the analytical measurements were performed at least in triplicate.

2.6. Substrate optimization

For xylanase production, economical substrates that contain xylose sugar were chosen (Grass and Sugarcane sheath). Here graphs werw plotted to analyze the growth of a mixed culture of *Bacillus polymyxa, Cellulomonas uda* in three different substrates (Grass, Sugarcane sheath and mixed substrate of ratio 1:1) and the results were plotted.

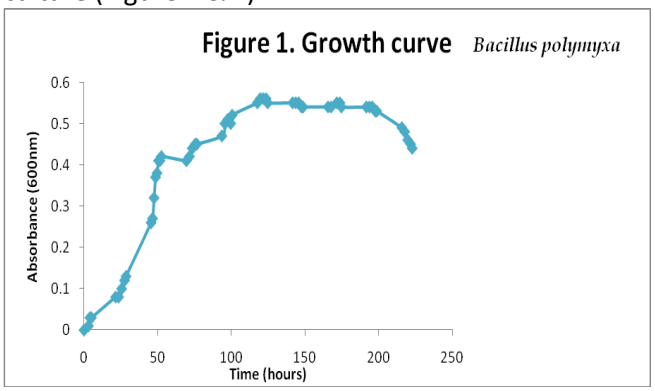
2.7. Effect of pH, temperature and inducer on xylanase production

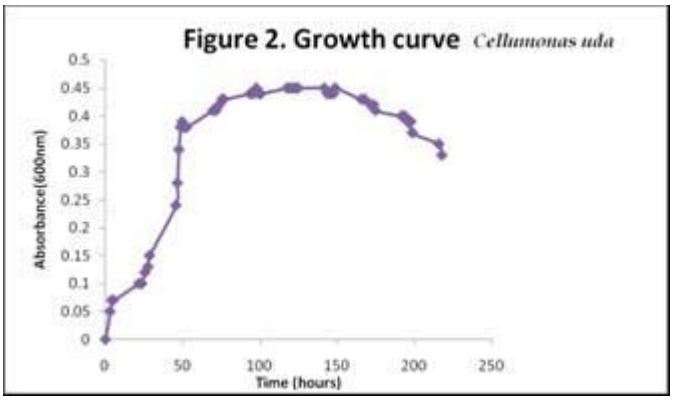
The effect of pH and temperature on xylanase production was determined during the maximum production phase of the mixed culture. The enzyme activity was measured at different pH and a range of temperature and results were plotted. Two different inducers at various concentrations were used to induce the production of xylanase and enzyme activity was determined periodically during production.

3. RESULTS

3.1. Growth kinetics

Enzyme production showed a cell growth associated profile hence the growth characteristics for both the microorganisms were determined and the different phases of growth were identified in order to estimate the time of maximum xylanase production in the mixed culture (Figure 1 & 2).





3.2. Substrate optimization

Grass extract, sugarcane sheath leaf and a 1:1 ratio of both, were provided as substrates for the production of xylanase. High activities (13.587, 14.662 and 14.125 IU/ml) were obtained using sugarcane sheath leaf extract compared to the other substrates, indicating that this agricultural waste can be utilized as substrate for an economical large scale production of xylanase. There is no production cost attached to it although its seasonal availability may restrict its use for a continuous process. (Table 1)

3.3. Optimization of pH and temperature for production of xylanase

It was analyzed from previous works(7) that a near neutral pH was necessary for the production of xylanase hence a pH range of 6-8 was chosen and it was found that maximum xylanase production occurred at pH 8(Figure 3, 4). The investigation of the temperature profile for

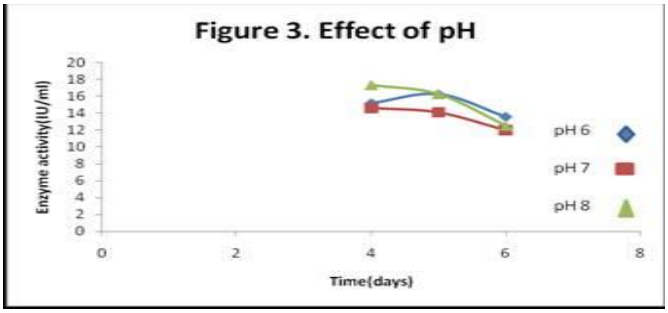
production of xylanase reveals that maximum xylanase activity is obtained at 50°C (Figure 4) which is in agreement with previous works.

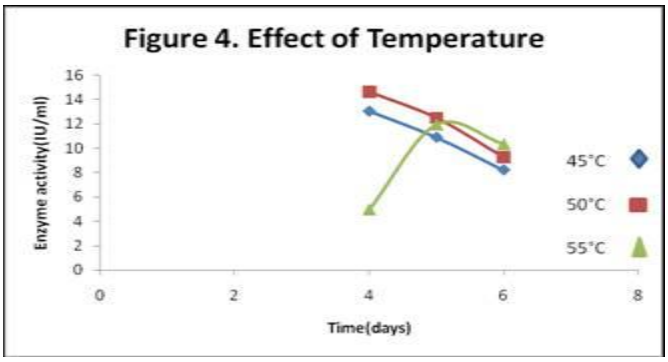
3.4. Effect of Inducers on xylanase production

Xylan and Maltose were examined for their ability to induce xylanase production at different concentrations. These inducers were added during the log phase at low concentrations. It was seen that xylan (0.2%) induced maximum production of xylanase resulting in activity of 44.773 IU/ml on the 5th day(Table 2).The media can be Supplemented with cheaper sources of xylan like wheat straw(8) for better yield of xylanase economically. (Table 2)

TIME (Days)	GRASS	SUGARCANE	MIXED
4	11.43665	13.58741	12.51203
5	14.66278	14.66278	14.1251
6	14.1251	14.1251	13.58741

Time(days)	Xylan(0.05%)	Xylan(0.1%)	Xylan(0.2%)	Maltose(0.5%)	Maltose(1%)	Maltose(2%)
4	29.71808	36.17035	37.78342	24.87888	28.10501	26.49195
5	35.63266	42.62262	44.77338	31.33115	33.48191	32.40653
6	29.18039	34.55729	36.17035	24.34119	27.02964	25.95426





4. DISSCUSION:

pH 8 and temperature 50ºC were found to be good physical parameters for the production of enzyme Xylanase using *Bacillus polymyxa* and *Cellulomonas uda* as mixed culture and xylan has a significant influence on the Xylanase production level. Various other agricultural wastes could be examined as alternative carbon sources for xylanase production and further work is recommended to purify and assess the enzyme in terms of its properties to degrade lignocellulosic biomass for production of biofuels.

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Conflict of Interest: None Declared

