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Optimization of Solid State Fermentation Conditions for the Production of Cellulase by Using *Trichoderma Reesei*

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ABSTRACT

*The enzyme cellulase is produced by utilizing contemptible substrates like rice bran and corn straw supplanted with nutrient sources in Solid State Fermentation using the fungal strain Trichoderma reesei (MTCC *164). A fermentation time of 6 days is required to obtain maximum Cellulase and β-glycosidase activity. The activity decreases with further increase in fermentation period. The optimum rice bran- corn straw ratio, pH and temperature for maximum production of Cellulase are found to be 5:5, 5 and 28°C respectively. The crude enzyme is partially purified by Ammonium sulphate Precipitation, Dialysis and finally Ion Exchange Chromatography. The effect of temperature, pH and substrate concentration were also studied in the purified sample and the optimum condition for enzyme activity is found to be pH 5.0, temperature 50° C.*

Keywords: *Trichoderma reesei*, Solid State Fermentation, Cellulase, rice bran, corn straw.

INTRODUCTION

Cellulases(E.C.3.2.1.4) are a class of enzymes produced chiefly by fungi, bacteria and protozoa that catalyze the hydrolysis of cellulose. Cellulase breaks down cellulose to beta-glucose. It is produced mainly by symbiotic bacteria in the ruminating chambers of herbivores. *Trichoderma* sp. are fungi that are present in nearly all soils and other diverse habitats. In soil, they frequently are the most prevalent culturable fungi. *Trichoderma reesei* has a long history of safe use in industrial scale enzyme peoduction.Applications of Cellulases and xylanases produced by this fungus are found in food, animal feed, pharmaceutical, textile and pulp and paper industries. *Trichoderma reesei* is non-pathogenic to manand it has been shown not to produce fungal toxins or antibiotics under conditions used for enzyme production. Solid State Fermentation is the cultivation of microorganisms on moist solid raw materials, such as grains, beans or wheat bran. Cellulases are being extensively used in the pulp and paper industry, textile industry, in wool finishing, in the laboratories for isolation of protoplasts, for clarification of fruit juices and as a component of detergents. The purpose of this research is to produce cellulase using Trichoderma reesei by Solid State Fermentation under optimized condition and to purify the crude enzyme and to study the effect of pH, temperature and substrate concentration on enzyme activity.

MATERIALS AND METHODS

Microorganism

The organism selected for the production of Cellulase was *Trichoderma reesei* (MTCC *164), which was obtained from MTCC.

Chemicals

All the chemicals utilized were of analytical grade and were procured locally.

Cultivation

The culture was maintained on Malt Extract Agar slants and stored at 4°C. The culture was grown under aerobic condition at a temperature of 25°C for about 7 days and was periodically sub-cultured. The seed medium, which was used, was same as growth medium without agar. About 100 ml of Malt Extract liquid medium was taken and sterilized at 1.5 kg/cm^2 gauge pressure at 121°C for 15 minutes and then cooled to room temperature. Loop full of spores of *Trichoderma reesei* was inoculated into 100 ml of the seed medium in a 250 ml conical flask. The culture was incubated in a shaker at 150 rpm at 30°C for 2 days. This culture was then cultured in 250ml Erlenmeyer flasks on medium containing rice bran, corn straw and nutrient solution (KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, Urea and Yeast extract). The rice bran and corn straw were pretreated to remove lignin and to expose the inner cellulose fibers to cellulolytic attack by the microorganism. The pretreatment process was carried out by adding 1L of 1% NaOH to 100 g of rice bran and corn straw and autoclaved at 121°C and at 15 PSI for 30 minutes. The pretreated substrates were allowed to cool and subsequently filtered and washed to neutral pH. Then it was air dried at 60°C in an oven for 12 hr. the dried substrate was milled and sieved to obtain 50 mesh sizes and kept ready for SSF. Different ratios of rice bran and corn straw (1:4 , 2:3 , 2.5:2.5 , 3:2 and 4:1) were used for the production of Cellulase. The best ratio of rice bran and corn straw was selected for further experimental work.

Analytical Procedures

The fermented medium of Solid State Fermentation from each flask (originally 10g of substrate) was mixed with 100 ml of water. The mixture was kept in shaker for 1 hour at 200 rpm. It was then centrifuged at 10,000 rpm for 10 minutes. The supernatant was used for enzyme determination. 44.2g of Ammonium sulphate is dissolved in 100ml of extract to achieve 70% saturation. When all the ammonium sulphate is dissolved, the solution is stirred for another 2 hours to allow complete equilibration between the dissolved and aggregated proteins. The sample is centrifuged at 10,000 rpm for 10 minutes to pellet the precipitated protein containing the enzyme. The supernatant is decanted and the pellet is dissolved in 100mL of 50mM Tris-HCl buffer (pH 5).The sample is dialyzed using cellulose acetate membrane at 4°C overnight for the process to continue. The dialyzed sample of enzyme is allowed to flow through the prepared ion exchange column and left undisturbed for 10 minutes. After 10 minutes the extract is filtered down through the column. The gradient solution Tris NaCl (25:25) is allowed to flow through the column one after another and is left undisturbed for 2 to 3 minutes and the elute is collected followed by next elutant. Elutes are assayed for protein and Cellulase activity.

Enzyme Assays

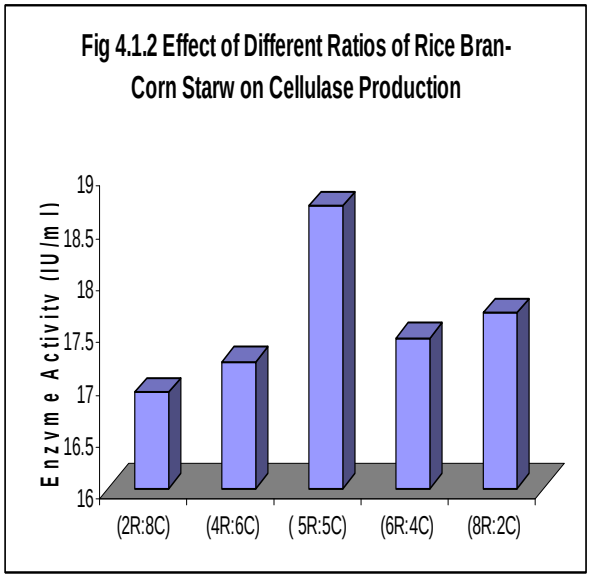
Filter paper Cellulase activity (FPU) was determined by measuring the increase in reducing sugars liberated from the hydrolysis of Whatman No.1 filter paper as described by Mandels *et al*. The procedure has been designed to measure Cellulase activity in terms of "filter-paper units" (FPU) per milliliter of original (undiluted) enzyme solution. The value of 2.0 mg of reducing sugar as glucose from 50 mg of filter paper (4% conversion) in 60 minutes has been designed as the intercept for calculating filter paper Cellulase units (FPU). The β- glycosidase activity was determined with the help of the salicin assay. The total protein content was estimated by Lowry's method using bovine serum albumin as standard.

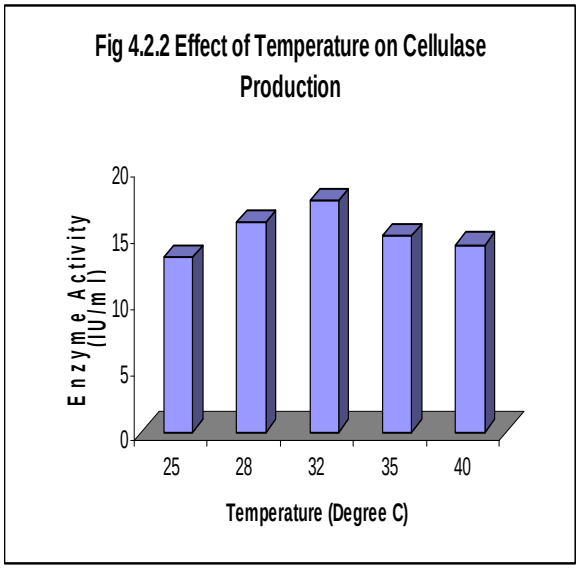
RESULTS AND DISCUSSION

The optimal pH and temperature were determined by assaying the enzyme at various pH (pH 3-10), in varying buffers – 50mM citrate buffer (3-6), Tris-HCl (7-9), carbonate-bicarbonate (10) and temperature ranging from 30° C to 70° C. the pH stability was investigated by incubating the enzyme at different pH for 24 hr at 50° C.

Optimization of medium composition

The effect of different ratios of rice bran and corn straw (2:8, 4:6, 5:5, 6:4, 8:2) on production of Cellulase by Solid State Fermentation using *Trichoderma reesei* was studied in a batch reactor (fig 4.1.1). The superior effect of natural carbon sources in enzyme production may be due to the presence of growth promoters in enough amounts covering the requirements of fungal growth and enzyme production. Rice bran and corn straw ratio of 5:5 gave the maximum production of Cellulase on the 6th day (18.5 IU/ml) and it was used for further studies (fig 4.1.2).





Optimization of temperature and pH

The effect of temperature on cellulase production using Trichoderma reesei was studied by varying the temperature range from 25°C to 40°C (fig 4.2.1). The maximum production of cellulase was obtained at 28°C (fig 4.2.2). The rate of enzyme catalyzed reactions increase with temperature up to a certain limit. Above a certain temperature enzyme activity decreases with increase in temperature because of enzyme denaturation. The optimum pH for the growth of the organism leads to reduction in unwanted extra-cellular proteins other than the required product. The

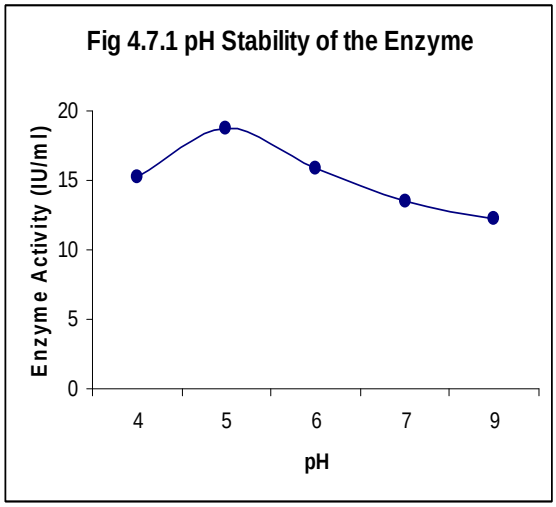
effect of various pH (4-8) on production of Cellulase using *Trichoderma Reesei* was studied (fig 4.3.1). The maximum production of Cellulase was obtained at pH5 (fig 4.3.2).

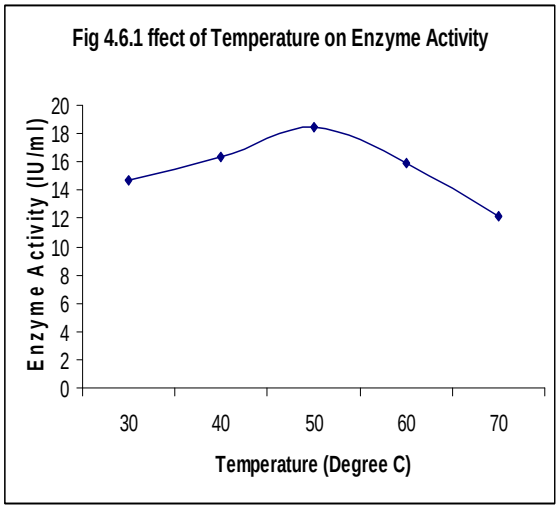
Effect of temperature on enzyme activity

The effect of temperature (30-70°C) on enzyme activity was determined (fig 4.6.1). The maximum activity of enzyme was obtained at 50°C. Above a certain temperature enzyme activity decreases with increase in temperature because of enzyme denaturation.

Effect of pH on enzyme activity

The effect of pH (4-9) on enzyme activity was determined Changes in pH may also alter the three dimensional shape of the enzyme. Variations in pH of the medium results in the ionic form of active site of the enzyme and change the activity of the enzyme and hence the reaction rate. The enzyme exhibited 82% activity at pH 4, 100% activity at pH 5 and 83% activity at pH 6, after 24 hrs of incubation.





Solid State Fermentation for Cellulase production was carried out by using *Trichoderma reesei*. A fermentation time of 6 days was required to obtain maximum Cellulase (19.3 IU/ml) and β- glycosidase (21.8 IU/ml) activity using *Trichoderma reesei* utilizing Rice Bran – Corn Straw as substrates. The Cellulase activity increases with further increase in fermentation period. Then according to the results obtained the optimum Rice Bran – Corn Straw ratio, pH and Temperature for maximum production of Cellulase was found to be 5:5, 5.0 and 28°C respectively and the optimum condition for enzyme activity was found to be pH 5.0 and Temperature 50°C.

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