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Solid state fermentation of wheat for the production of cellulase

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Abstract

Solid state fermentation of wheat straw for cellulase production by *Phanerochaete chrysosporium* has been studied. The most optimum moisture level for solid state fermentation was found to be 40% while the best enzyme yield was obtained at an initial pH of 4.5 and incubation temperature of 30 ^{0} C. Supplementation @ 0.2% (W/W) was found to have enhanced the yield of the enzyme by three folds. With all conditions standardized an enzyme yield of 78 IU pergds could be obtained.

Key Words: ***Cellulase ,Phanerochaete chrysosporium, Fungus***

Introduction

Utilization of lignocellulosic wastes is gaining considerable interest in most developing countries due to their availability in abundance. Almost five decades have passed since the pioneering work of Reese on fungal degradation of cellulosic substrates (Mandels and Sternberg, 1976, Esterbrauer *et al*. 1991). In view of relative abundance of wheat straw, its biodegradation has been carried out by several fungi (Zadrazil and Brunnert, 1980: Zafar *et al.* 1989). *Phanerochaete chrysosporium* has been reported to be a potent lignin degrading white rot fungi (Faison and Kirk 1985). In the present investigation an attempt has been made to utilize wheat straw for the production cellulose by solid state fermentation.

Materials and Methods

Organism and cultural conditions

Phanerochaete chrysosporium (HHB 103737 S) was obtained from Prof. Kirk's laboratory of Forest Research Laboratory, Canada. It was grown and maintained on malt extract agar slants and repeated transfers were performed at 30 days intervals. The culture was preserved in refrigerator at 4^0C. The medium for cultivation was prepared according to Kirk *et al.* (1978) with 0.22 g per litre of ammonium tartarate as the nitrogen source. As per the requirements the Erlenmeyer flask of 100 ml capacity was added with 4.5 ml of the basal medium containing 30 mm 2,2- dimethyl succinate to buffer the cultures at 4.3-4.5 and 0.5 ml of fungal mycelium. The fungal mycelium was obtained from a Roux bottle run in batch. Such an inocculums was used to obtain rapid primary growth of the culture (Ulmer *et al*. 1983).

Preparation of Starter culture

Starter culture of the test mould was prepared according to the standard method as described by Tao and Zuohu, (1997). Conidiospores were aseptically harvested from a 15-day old culture slant by suspending in 10 ml sterile distilled water containing 0.01% Tween 80. The conidiophores suspension was suitably diluted to obtain a population of approximately 10^7 per ml One ml culture was used per 100 g substrate for all experimental purposes.

Solid state Fermentation

All fermentations were carried in autoclavable LDPE bags (30*25 cm : Himedia). The bags were perforated at 1.5 cm distance throughout and filled with the test materials. The raw materials viz. wheat straw was ground in Waring blender and 100 g of each were then loodely filled in the perforated autoclavable LDPE bags in triplicate. All the bags were then autoclaved at 15 psi for 30 minutes.

Optimization for moisture content

Moisture levels in solid state fermentation depends upon the nature of substrate, the organism and the type of end product (Ramesh and Lonsane1990). Since the nutrient concentration is inversely proportional to the quantity of water present in the substrate, an increase in the salt concentration resulting in high osmotic potential, will have an adverse effect on the growth and productivity. This preliminary experiment was thus undertaken to optimize the moisture content for each organism and substrate used in this study.

Fifty gram of substrate was transferred into the each three perforated autoclavable LDPE bags (Himedia, India) for testing of each substrate as well as organism. Each of the three bags were added with water at the rate of 25, 50, and 75 ml water. The bags were then autoclaved at 15 psi for 30 minutes. After cooling to room temperature the materials were inoculated with 2.5 ml of starter culture containing 1.0 X 10^7 cells or spores per ml. The bags were then incubated at 28^0 C. The growth of the organism was measured by standard plate count method.

Effect of Nitrogen supplementation

Carbon: Nitrogen ratio is an important factor in determining the growth behaviour and productivity. Thus nitrogen in form of ammonium, nitrate and protein were supplied through addition of ammonium sulphate, urea, sodium nitrate and casein. The test substrate was tested for effect of nitrogen supplementation on enzyme yield. Three concentration viz. 0.2, 0.5, 1.0 and percent (as N) were added to the substrates and were inoculated with the test microorganisms. A control (without N) was also run simultaneously.

Effect

For the optimization of pH the wheat straw was soaked in water of different pH of raw material were 3, 4.5 6.0 and 7.0. The material were then loosely packed in auloclavable LDPE bags and inoculated. Other fermentation conditions were same as for other experiments.

Optimization of incubation temperature

The experimentation for incubation temperature, the inoculated bags were incubated at different temperatures viz. 25^0, 30^0,37^0 and 40^0 C.

Determination of Cellulose activity

Sample extraction and analysis

One gram samples in triplicate were withdrawn aseptically at 5 days intervals and extracted in 10 ml citrate buffer (0.05 M; pH 4.8) using pestle mortar. The content was shaken for 10 min on a rotatory shaker and finally centrifuged. The supernatant was collected and the residue was washed twice and again centrifuged. All the supernatant were finally pooled and cellulase activity determined.

Filter Paper (FPase) Activity

FPase activity was determined using 1.0 cm X 6.0 cm of Whatman No. 1 filter paper strip (in 1 ml of 100 mM sodium citrate buffer, pH 5.0) as substrate and incubating it with 1.0 ml of enzyme extract for 1 hr at 500^{o} C. The reaction was terminated by boiling the contents for 2 min. The released reducing sugar content was measured by Nelson's (1944) using glucose as standard.

Carboxy Methyl Cellulose (CMCase) Acidity

CMCase activity was determined by using the IUPAC method (IUPAC, 1987). One ml of extract was added with 1 ml of Carboxymethyl cellulose (1% prepared in 0.05N; pH 4.8). The contents were incubated at 500^{o} C for 30 min. and finally the reaction was terminated by adding 3 ml of 1 mM sodium carbonate solution. The released reducing sugar was estimated by Nelson's method (1944).
One unit of cellulase activity was CMCase expressed as micromoles of glucose released per min per gram of culture.

Determination of reducing sugars

Two gram samples were extracted in ten ml of 70% aqueous ethanol by finely grinding in pestel mortar. The contents were then centrifuged at 5000 rpm for 10 minutes. The pellets were washed thoroughly in same solution twice and again followed by centrifugation. All the supernatants were pooled and evaporated to dryness using flash evaporated. The contents were re-dissolved in 5 ml of 70% aqueous ethanol and used for estimation of carbohydrates using the standard method according to Nelson (1944).

Results and Discussion

Moisture levels in solid state fermentation depends upon the nature of substrate, the organism and the type of end product (Ramesh and Lonsane, 1990; Smits, *et al.* 1997). The results show that the most optimum moisture level was 40% . Since the nutrient concentration is inversely proportional to the quantity of water present in the substrate, an increase in the salt concentaration resulting in high osmotic potential, will have an adverse effect on the growth and productivity. Our results are in accordance with those of Jha *et al.* (1999) who have also shown 40% as the most optimum initial moisture content for the cellulase production from soyahulls. Zafar *et al.* (1989) and Smits et *al,* 1997 have however reported a minimum moisture of 60% in solid state fermentation. It may however be mentioned that lower initial moisture is always better as it greatly facilitates handling and extraction of the enzyme at pilot scale.

While studying the physiological characters of *Phanerochaete chrysosporium* grown on wheat straw it was seen that the high cellulase yield of 31.5 lU per g dry (FPase) substrate was obtained at an initial pH of 4.5. of pH Further, the temperature optimization experiment indicated that 25^0 C was the most optimum temperature for best cellulase activity.

Effect of supplementation of various nitrogen viz. Ammonium sulphate, sodium nitrate, urea and casein was also studied. The results indicate that all the compounds used showed stimulatory effect on the cellulase activity. Among different nitrogen sources supplemented, urea was found to be the best nitrogen source for cellulase production. Our results are in accordance with that of Nigam *et al.* (1988) who have also shown urea to be the best nitrogen sources in the fermentation of sugarcane bagasse and soyabean huls, respectively.

The over all results indicate that under all conditions standardized, the cellulase activity of 74.9 lU per g dry substrate could be obtained in 6 days and thus concludes that wheat straw can be successfully used for the production of cellulase at pilot scale.

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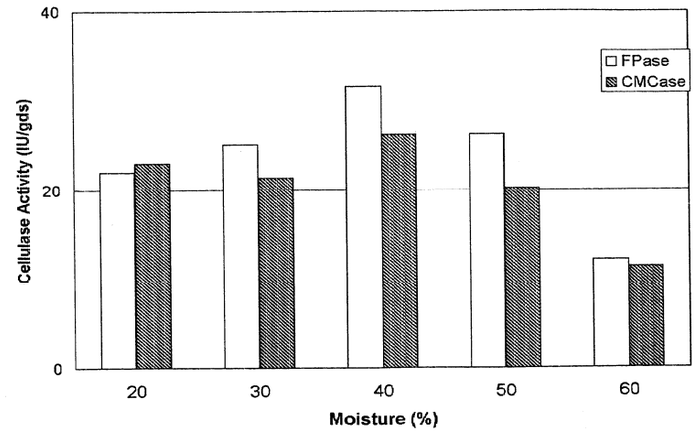

Fig.1 Effect of moisture level on the production of cellulase

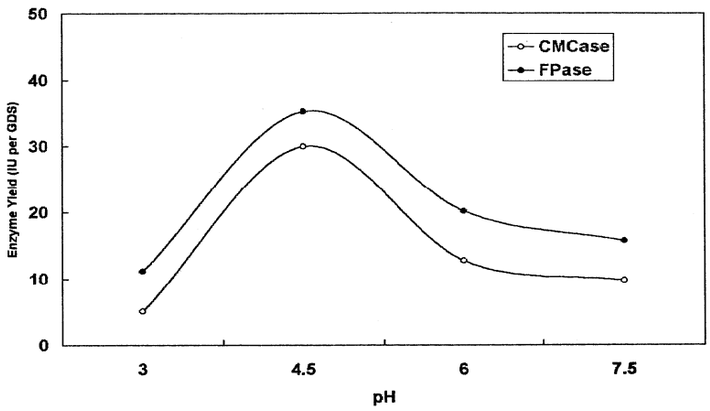

Fig. 2. Effect of pH on the Cellulase Production

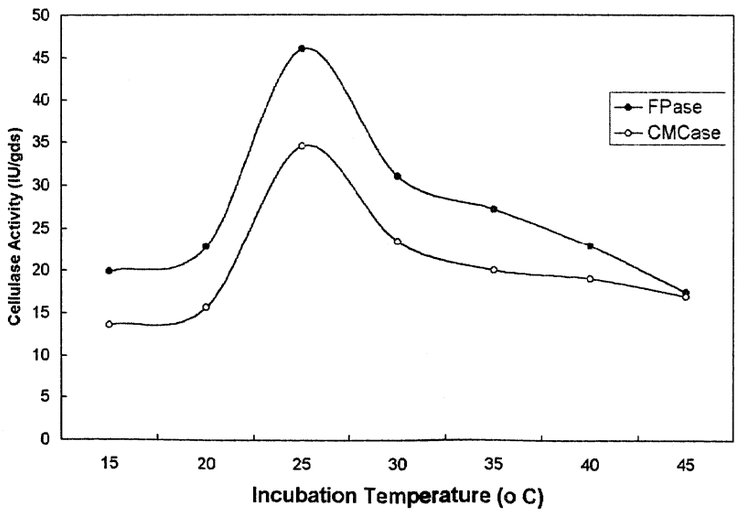

Fig. 3. Effect of Incubation temperature on cellulase activity

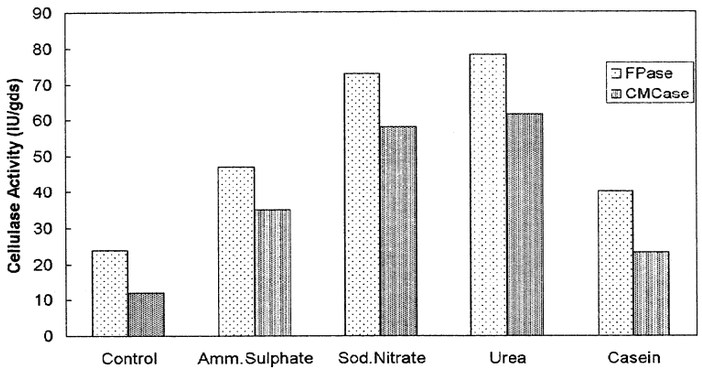

Fig 4. Effect of Nitrogen sources on Cellulase activity