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Production of cellulase by different co-culture of *Aspergillus niger* and *Penicillium chrysogenum* from waste paper, cotton waste and baggase

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Cellulases are a group of hydrolytic enzymes capable of degrading cellulose to the smaller glucose units. These enzymes are produced by fungi and bacteria. The solid waste of sugar, paper and industry using baggase, paper waste and cotton waste was fermented by *Aspergillus niger* and *Penicillium chrysogenum* in solid state fermentation. There is attempts to transfer the various industrial carbon waste to veterinary proteins depend on microorganisms by using of chemical process. The study indicates that the cellulases obtained from compatible mixed cultures simultaneous mixing of both fungi have more enzyme activity as compared to their pure cultures and other combinations. The fermentation experiments were performed in solid stat fermentation (SSF). Incubation time, carbon sources and initial pH of fermentation medium was optimized with simultaneous mixed culture. It was revealed that the newspaper at pH = 5 and 40°C was the best source of carbon for the enhanced production of cellulase in the compatible mixed culture experiments after 8 days of incubation. Based on the reported results, it may be concluded that industrial carbon waste can be a potential substrate for production of cellulase, incorporation of co-culturing *A. niger* and *P. chrysogenum*. The aim of this work is to produce cellulase from waste paper and reduce the pollution.

Key words: *Aspergillus niger, Penicillium chrysogenum*, cellulase, culture.

INTRODUCTION

Cellulose is commonly degraded by an enzyme called cellulase. This enzyme is produced by several micro-organisms, mainly by bacteria and fungi. Although a large numbers of microorganisms are capable of degrading cellulose, only few of these produce significant quantities of cell free enzymes capable of completely hydrolyzing crystalline cellulose *in vitro*. Fungi are the main cellulase producing microorganisms, although a few bacteria and actinomycetes have also been reported to yield cellulase activity.

Biotechnology is finding its applications in four major industrial domains, including health care, crop production and agriculture, non food (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels, enzyme etc.) and environmental uses. In view of rapidly growing economic demand, it is imperative to exploit all indigenous resources at their maximum to attain the goal. The production of value added products from industrial wastes can reduce to some extent the cost of production (Durrani et al., 2002). Cellulose is carbo-hydrate that is the essential an characterstic structural substance of the plant world. Not only is cellulose present in the more elongated fibrous cells, which are the usual interest of the industrialist , but also it is present in the thin walled, pichy, more or less round cells, with juicy contents which are the food material of herbivore and other animal species .

Nitrocellulose is used as explosive and also as lacquera. In plastic masses; acetylcellulose is used for injection and other moulding. nitrocellulose acetate. Masahiko et al. (1991) reported the uses of cellulose in

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foods, pharmaceuticals and cosmetics that is, as an anticholesterimic, hypolipemic, oil absorber, or moisturing agent. Cellulose source includes pulp and parenchyma – cell fibrous cellulose fibrous. Fungal genera like *Trichoderma* and *Aspergillus* and *Penicillium chrysogenum* are taught to be cellulase producers. Crude enzymes produced by these microorganisms are commercially available for agricultural and industrial uses. In general, bacterial cellulases are constitutively produced, whereas fungal cellulases are produced only in the presence of cellulose. Filamentous fungi, particularly *Aspergillus* and *Trichoderma* spp. are well known efficient producers of cellulases. Several studies were carried out to produce cellulolytic enzymes from biowaste degra-dation process by many microorganisms including fungi such as *Trichoderma, Penicillium, Aspergillus spp*. etc.

Cellulosic biomass constitutes the most abundant organic molecules on earth and is continually replenished by carbon dioxide fixation via photosynthesis. All cellulosic materials, including the agro-industrial wastes can be converted into commercially important products such as ethanol, methane, glucose syrups and single cell proteins. The products of degradation constitute both nutrients for growth and regulators of the production of lignocellulolytic enzymes (de Vries et al., 1999; Peij et al., 1998) Bioconversion, particularly enzymatic hydrolysis, of these cellulosic materials into simple sugars, has been a subject of intensive research . The development of an industrial process for cellulose bioconversion would help alleviate shortages in food and animal feeds and also reduce the problems of urban waste disposal and overdependence on fossil fuels. Successful utilization of these renewable resources is dependent on the development of an economically viable process which would include the production of cellulases required for the enzymatic hydrolysis of cellulosic materials.

Cellulase, a group of hydrolytic enzymes which hydrolyze the glycosidc bonds of native cellulose and related cellooligosaccharides, is the key enzyme of potential use for industrial saccharification of cellulosic materials into simple sugars. Cellulase production was found to be the most expensive step, accounting for about 40% of the total cost, during the production of ethanol from cellulosic biomass. Cellulase production from various waste cellulosic materials using different cellulolytic microfungi is being vigorously studied for cost reduction strategies. Although a large number of microorganisms (fungi, bacteria and actinomycetes) are capable of degrading cellulose, only a few of them produce significant quantities of cell-free enzyme fractions capable of complete hydrolysis of cellulose *in vitro*. Among the cellulolytic mircofungi, the genera *Trichoderma* and *Aspergillus* are notable cellulase producers. Cellulase preparations from species such as *T. viride* and *Aspergillus niger* and of several species of *Penicillium* have also been purified and studied extensively. In this study, we examined the relative potentials of common waste cellulosic materials; Paper waste cotton waste, and sugarcane pulp (Bagasse) - as microbial substrate for cellulase production using wild strains of *A. niger, P. chrysogenum.* Akiba S, Kimura Y, (1995) Purification and characterization of a protease-resistant cellulase from *A. niger.*

Plant cell walls are composed of chiefly Polysaccharides and lignin. Mostly a rigid complex matrix like structure which is an outcome of an interaction between diferulic acid and hemicellulose or between lignin and hemicellulose molecules which serve as an effective layer to prevent microbial invasion. The products of degradation constitute both nutrients for growth and regulators of the production of lignocellulolytic enzymes. Trichoderma spp. and Aspergillus spp. are well known for their secretory activities and amount of lignocellulolytic enzymes produced. Grasses on the other hand contain hydroxycinnamate esters which are linked with polycarbohydrate units and ferulic acid helps to bind polysaccharides. Mostly the cotton strip assay plays an important role to determine the interaction of cross-linkages and microbial population in the soil. The cotton strip assay was put into practical use before it was properly evaluated and before the relationships, if any, between tensile strength change of cotton cloth and soil processes relevant to soil research programmes were examined (Walton and Allsopp, 1977). Cellulaseas form an important part of food, animal and textile industry, they are also used in waster resource recycle management and in anti-pollution treatment (Tarek and Nagwa, 2007).

MATERIALS AND METHODS

General

All chemicals used from SITM biotech laboratory, Lucknow.

Substrates

Waste paper, cotton waste and Baggase waste was obtained from news paper vendor and sugarcane, industry respectively.

Microorganisms

A. niger and P. chrysogenum procured from pure culture were maintained on Potato dextrose ager (PDA) slants ager as shown in (Figure 1). All cultures were subcultured every 4 weeks, incubated at 30°C for 7 days and subsequently stored at 4°C for inoculums preparations.

Conidial count

The conidial count was made on a Haemacytometer slide bridge. (Sharma, 1989).

Culture media

A suspension containing *A. niger* and *P. chrysogenum* were used to initiate growth in 250 ml conical flask supplemented with $K_2H_2PO_4$ (0.20 g/L), KH_2PO_4 (0.18 g/L), $NaHPO_4$ (2.00 g/L), $NaN0_3$

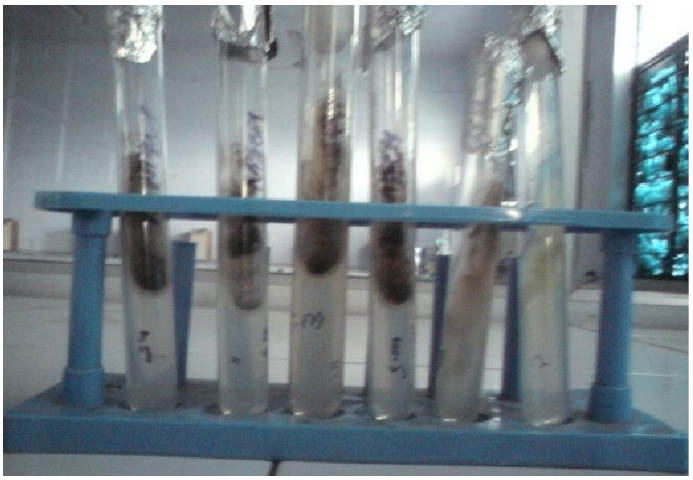

Figure 1. Pure culture of *A. niger* and *P. chrysogenum* on PDA slant

Figure 2. Culture media for *A. niger* and *P. chrysogenum.*

(3.80 g/L),H_3BO_3 (0.057×10-^{3}g/L), $MnSO_4.H_2O$ (5.5×10^{-6} g/L), $CuSO_4.7H2O$ (2.5×10^{-6} g/L), $ZnSO_4.7H_2O$ (0.5×10^{-3} g/L), $Fe(SO_4)_3.6H_2O$(5×10^{-6} g/L),$MgSO_4.7H_2O$ (5.5×10^{-6} g/L), NH_4NO_3 (0.60 g/L), $(NH_4)_6Mo_7O_{24}$ (0.025×10^{-3} g/L) as shown in Figure 2.

Fermentation Procedure

Using for cellulase production in 250 ml conical flask (and addition of 100 ml of media culture with 50 gm of carbon source (Paper). After the inoculation, the flasks were sterilized by using of autoclave at 121°C for 15 min. After cooling at room temperature was then added density with 2 × 10^{8} of fungi (*A. niger* and *P. chrysogenum* and both in different flask respectively), and incubated for 7 days. The supernatant was estimated with Whattman filter papers and saccharifying activity of cellulases.

Determination of optimum carbon source

To determine the optimum carbon source, 50 g of (paper waste and cotton waste) act as culture medium at 30°C for 6 days.

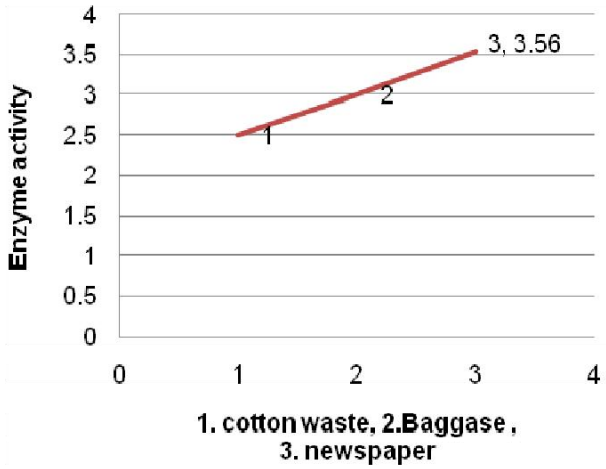

Figure 3. Enzyme acivity vs. sources

Effect of pH

The working pH of co-cultured media (*A. niger* and *p. chrysogenum*) was from 3 to 6 using phosphate buffer. The optimum pH was evaluated.

Effect of temperature

The working temperature of the co-cultured medium was varied from 20 to 50°C (+5)

Determination of enzyme activity

The determination of enzyme activity was done by using Mandels method. Incubation of 0.9 ml of substrate solution with enzyme extract at 45°C for 1 h, then added 1 ml of DNS solution. The mixture was then heated at water bath for 5 min. Then let it to cool and then added 10 ml of distilled water. The equivalent solution was prepared by added 1 ml of DNS to 0.9 ml of substrate then added 0.1 ml of enzyme solution. The determination of reduction saccharides was done by using o Mandels method and then calculates the enzyme activity. (Mandels and Andreotii, 1976)

RESULTS AND DISCUSSION

The highest enzyme activity by using of co-culture media (*A. niger* and *P. chrosogenum*) and it raised 3.56 unit per ml in (Figure 3) and it was very high as compared with another co-culture medias and that because of The three carbon sources news paper (paper waste) and cotton waste and baggase were optimized. Among them, in Figure 3, Newspaper waste was proved to be the best for cellulase production and it was 3.56 unit per ml and it was batter from the other wastes (that use in this paper,) due to high percentage of cellulose production and the easy of breaking bonds of waste paper, but Baggase and cotton waste have other substances like lignin and that is difficult to analyzed by enzyme. The effect of the H+ concentration on the activity and stability of the cellulose.

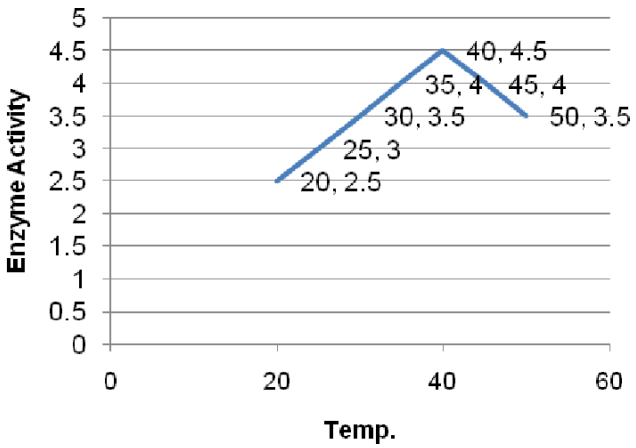

Figure 4. The Enzyme activity vs. temperature

Hydrolysis of was confined to acid media.

The enzyme activity being maximal (4.54) at pH 5 .In contrast with the sharp pH optimum seen in the activity profile, the cellulase was stable in the absence of substrate, over a wide range of pH values. Initial pH has a direct effect on the uptake of mineral nutrients, which are present in the fermentation medium. So, the effect of different pH (3.0 - 6.0) of fermentation medium on the enzymes production was also investigated with simultaneous co-culturing of *A. niger* and *T. viride.* High acidic and high basic pH, both showed negative effects, but a medium with low acidic pH 5 was ideal for enzyme fungal cultures require slightly acidic pH for their growth and enzyme biosynthesis (Haltrich et al., 1996). In the present study, a temperature of 20 - 50°C (+5°C) was used in the standard assay; however, the temperature of maximum activity under these conditions proved to be 40°C (Figure 4). Incubation temperature plays an important role in the metabolic activities of microorganism. The maximum enzyme activity at the 8th day and it was 6.10 units per ml.

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