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# Production of Cellulase enzyme by submerged fermentation process from *Penicillium variabile*

KEYWORDS: cellulase, *Penicillium variabile*, submerged fermentation

| Bhaskar Chandra Das | Manas Das | Pronoy Sinha |
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| Department of Chemical Engineering, University of Calcutta | Retired Professor & Former Head, Department of Chemical Engineering, University of Calcutta | Department of Chemical Engineering, University of Calcutta |

**ABSTRACT** *Studies on the environmental parameters of Penicillium variabile (IJMARI 121) for production of cellulase enzyme under submerged condition revealed that this strain behave differently from the conditions of surface fermentation. Enzyme production reached maximum on the fifth day of incubation in shake flask, but on fourth day in the fermentor. The optimal pH, temperature and inoculum volume were 4.0, 28°C and 5% respectively. The maximum production of enzyme in the fermentor was obtained with 10% wheat bran as substrate and V/2V aeration rate.*

**Introduction**

Cellulose is one of the most abundantly available organic compounds in the biosphere. Agricultural product introduces large amount of cellulosic biomass as solid pollutant. When burned for disposal it introduces secondary pollutant, particularly it forms $CO_2$ causing global warming. From the compositional studies it has been established that it could be transformed into sugar unit which can be subsequently processed to get a large number of useful products like biofuels, alcohols, chemicals, pharmaceutical products, industrial solvents etc. Various methods are available to transform cellulosic biomass into value added product, which basically needs hydrolysis. This hydrolysis process can be done by acid and /or enzyme. Enzymatic hydrolysis process is much more eco-friendly. The availability of a high active cellulase is the prime requirement for a successful process of enzymatic conversion of cellulose into useful product. This depends on proper selection and improvement of suitable strains for enzyme production and development of the process for production of enzyme of high quality.

The objective of the present work was to develop a suitable process for producing cellulase enzyme from low cost waste material.

Various investigators are working in the field of the enzymatic sacharificatlon of cellulose but the industrial production of cellulase is not encountered with complete success. The detail know-how for the industrial production of this enzyme is also not available.

A number of cellulolytic enzymes, specially cellulases produced by fungi and bacteria, have been isolated and characterized (Tomme, 1995). Due to the vast usefulness of cellulase several project has been implemented to isolate cellulase producing bacteria in order to apply for the degradation of cellulose found in agricultural waste( Camassola & Dilon, 2007). A cellulase producing bacterium was isolated from soil and identified which utilized chlorella powder as substrate for growth and subsequently produce high level of cellulase by submerged fermentation (Li-Jung, Poshin & Hsin-Hung, 2010). Some reports have appeared on the influence of different environmental conditions on the surface culture production of cellulase from *Penicillium variabile* (Pal, & Ghosh,1965; Basu & Ghose,1960) having high activity. But the data for the submerged fermentation is not available in the literature with this strain. In earlier report a suitable medium was suggested for submerged fermentation of *Penicillium variabile* enzyme (Das, Das & Sinha, 2009). But the environmental parameters were not optimized.

The optimum environmental parameters and the influence of other operating variables for the production of enzyme in the Laboratory fermentor are described in this paper.

**Materials and Methods**

*Penicillium variabile* (IJMARI 121) was obtained from Indian Jute Industries Research Association, Kolkata. The strain was maintained on malt agar slants (2.5% malt extract solution in 2% agar with pH 7) at 32-35°C for 5 days. The inoculum was prepared by adding 5 ml of spore suspension from fresh culture to 20 ml of medium containing 2.5% malt extract and incubated for 24 hrs.

Fermentations were carried out in 100ml conical flask containing 20 ml of specified medium (Das, et al., 2009) in a rotary shaker operated at 170rpm with the variation of the inoculum volume, incubation time, temperature and initial pH of the medium. The effect of temperature was studied in a thermostatic controlled bath. The initial pH of the medium was adjusted with 0.1N hydrochloric acid or 0.1N sodium hydroxide.

The content of the flask was centrifuged to separate the enzyme. The culture filtrate was the source of cellulase enzyme. The activity of enzyme was expressed in $C_x$ unit which is defined as that amount of enzyme which in 10 ml of assay solution (1% CMC) produces 0.1mg of reducing sugar as glucose in 1hr at 50°C. Reducing sugar produced due to the enzymatic action was measured by the Nelson-Somogyi's method (Nelson, 1944).

Newbrunswick Laboratory fermentor of capacity 5 litre was used for our purpose. The fermentor was fitted with a stirrer and an air pipe (Spurger). Fiber glass wool packed in a long glass tube fitted with an inlet and a delivery line was used as the air filter. A properly sealed conical flask with sterile distilled water serves as a humidifier to saturate the air and placed between the fermentor and the air filter. A rotameter was placed before the air filter to measure the air velocity.

Fermentor with 2 litre of medium, air filter and the humidifier were properly sterilized at 15 psig for 20 minutes. After sterilization the fermentor was cooled to the incubation temperature. Sterile air was then introduced into the fermentor and it was then inoculated with the seed culture. Silicon oil was used as antifoaming agent.

The fermentor was operated with different concentrations of wheat bran starting from 12.5%. Aeration rate was adjusted based on the volume of medium handled. Rates of aeration

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were controlled throughout the fermentation process from an average of 2100cc/min to 6500 cc/min. The agitator speed was adjusted at 170 rpm. Samples were taken out intermittently for estimation of enzyme concentrations.

**Results**

The production of enzyme gradually increased with the days of fermentation and reached the peak point after 5 days. Thereafter it decreases slowly as shown in Fig.1.

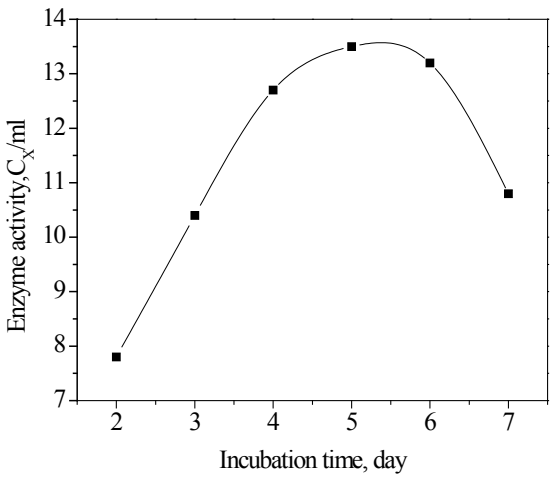


**Fig.1. Incubation period for Cellulase production**

The adjoining Fig.2 shows the relationship between inoculum volume and enzyme production after 5 days of incubation. From the above results the optimum inoculum volume is found to be 5%. The effect is very sharp specially in the region of optimum volume of inoculum.

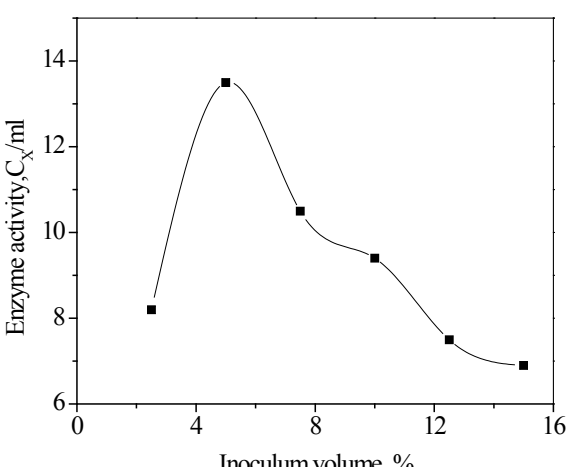


**Fig.2. Inoculum volume for Cellulase production**

From the results (Fig.3) it is quite clear that the maximum production is obtained at the temperature of 28°C in the temperature range tested. It is interesting to note from these experiments that the effect of temperature on the production of enzyme is different from that of the mycelia growth rate. The optimum temperature for growth i.e. 32°C is consistent with that which other workers suggested. But the enzyme production temperature is 28°C in contrast to 32-35°C as suggested for surface fermentation.

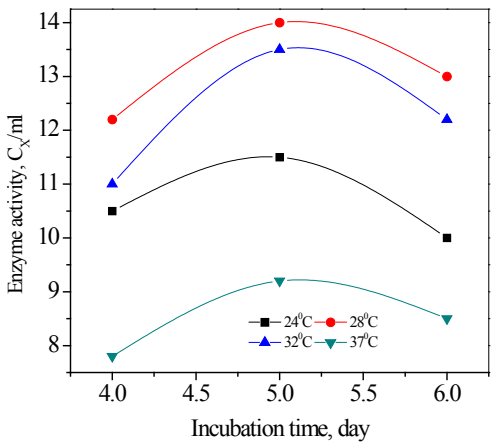


**Fig.3. Effect of temperature on enzyme production**

From the results (Fig.4) it may be inferred that optimum initial pH of the medium should be 4.0 to have highest activity of the enzyme-cellulase.

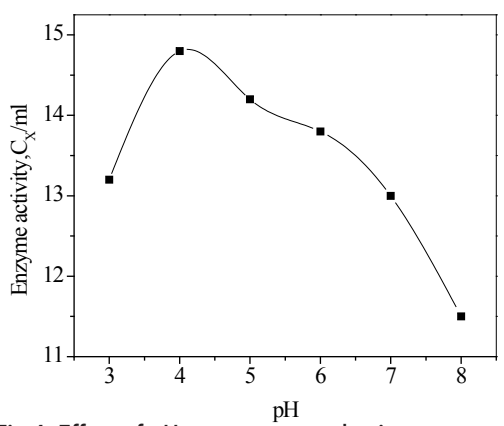


**Fig.4. Effect of pH on enzyme production**

With the above conditions the fermentation was conducted in the Laboratory fermentor. Unexpectedly the production in the fermentor was markedly decreased from that of the shake flask containing 12.5% wheat bran as substrate. This may be due to the difficulty in keeping the medium homogeneous throughout the experiment because the wheat bran with this percentage is difficult to agitate. Accordingly the substrate concentration was changed for proper mixing of the substrate. The results (Fig.5) indicate that the optimum substrate concentration in the fermentor should be 10%.

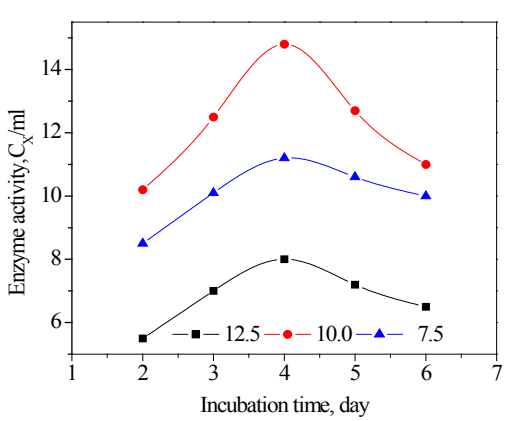


**Fig.5. Effect of substrate on enzyme production in fermentor**

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Since the fermentation was under aerobic condition hence the optimum aeration rate was determined. The air flow rate was based on the volume of the medium. From the results (Fig.6) it is found that with V/V aeration rate i.e. 2100 cc/min the enzyme activity is 14.8 $C_X$/ml in contrast to 15.8 $C_X$ /ml in the case of V/3V aeration rate. But the highest enzyme activity is 16.4 $C_X$/ml when the aeration rate is V/2V. Therefore it is concluded that the maximum production of enzyme is obtained after 4 days of fermentation with 10% wheat bran as substrate and V/2V aeration rate.

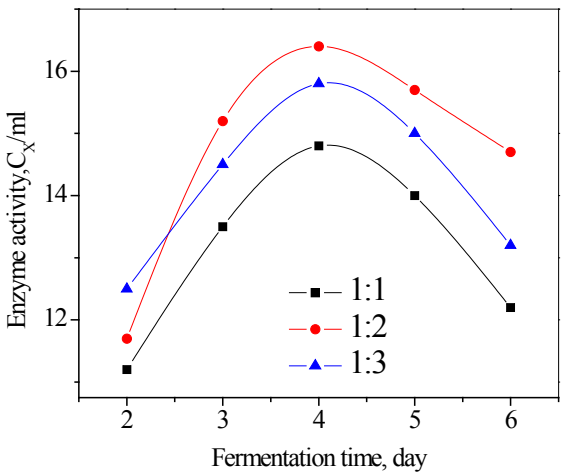


**Fig.6. Dependence of enzyme production on aeration rate**

**Discussions**

A great reduction in time is obtained for submerged fermentation from 10 days to 5 days. It is very interesting to note that when the enzyme is produced in the fermentor maximum production is reached in lesser time i.e. 4 days instead of 5 days. The *Penicillium variabile* is an aerobic organism. In shake flask and fermentor due to the agitation greater amount of oxygen is available for the submerged fermentation than surface fermentation and the production reaches to its maximum level quickly. Under the optimized conditions the enzyme activity was increased from 13.2 to 16.4 $C_X$ / ml i.e. 24% with a reduction of time in the order of a day which certainly minimize the cost of production.

**Acknowledgement**

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