Production of Multi-enzyme System by Isolated Aspergillus sp Under Solid State Fermentation and Submerged Fermentation

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Abstract-Cellulose is the most abundant organic compound on earth and has received much attention as a substrate for production of biofuels, single cell proteins and a variety of other chemicals through enzymatic degradation by cellulases. The conversion of cellulosic biomass to fermentable sugars requires action of cellulolytic enzymes like β-1,4 endoglucanase ,β-1,4 exoglucanse. The present study involves a fungal species isolated from soil collected from saw mill which was evaluated for production of cellulolytic enzymes under submerged fermentation (Smf) and solid state fermentation (SSF) using czapek-dox broth and potato peel respectively as media for the fermentation processes. Encouraging results were obtained when the fermentation was carried out at 30oC with a 6 day old culture on the 6th day of fermentation under SmF(29U/ml for CMCase and 3.1U/ml for FPase). On the other hand Potato peel was found to be well suited for the potent organisms growth and it was found to produce appreciable amount of cellulases like CMCase ,FPase by SSF. Evaluation of environmental parameters under SSF demonstrated that maximum amount of cellulases were produced at 30oC ,on the 7th day of fermentation utilizing a 7 day old culture (59.1U/gds and 7.8 U/gds for CMCase and FPase respectively).SSF resulted in 7.1 fold increase in CMCase production and 3.12 fold increase in FPase production compared to control while SmF resulted in 4.8 fold increase in CMCase and 2.21 fold enhancement in FPase production compared to control.

Keywords- SSF, SmF,CMCase, FPase

I. INTRODUCTION

Cellulases are the most commercially important of all the enzyme families. This group of enzymes catalyzes the hydrolysis of cellulose and related oligosaccharide derivatives, and is considered a prospective tool for industrial saccharification of cellulosic biomass [1]. An economic process for its production is thought to be decisive for the successful utilization of cellulosic materials [2-4].Fermentation is the primary technique for the production of various enzymes which biologically converts complex substrates to simple compounds with the help of various microorganisms. The techniques such as Solid State Fermentation (SSF) and Submerged Fermentation (SmF) has lead to industrial-level production of bioactive compounds and have been further developed based on various parameters such as the substrates used, environmental parameters and the organisms used for fermentation. Fermentation has been classified into SSF and SmF mainly based on the type of substrate used during fermentation.[5]. Cellulases are produced using the submerged fermentation (SmF) method traditionally, in which the cultivation of microorganisms occurs in an aqueous solution containing nutrients. An alternative to this conventional SmF method is the solid state cultivation (SSC) method, which involves the growth of microorganisms on solid materials in the absence of free liquids [6].Since SSC involves relatively little liquid when compared with SmF, downstream processing from SSC is theoretically simpler and less expensive. The SSF is generally favored as it offers many advantages such as higher concentration of the product in the medium, direct use of air-dried solids as substrates which cuts the production cost to a larger extent. The production of cellulase has been reported from a wide variety of microorganisms. However, filamentous fungi are preferred for commercial enzyme production, because the level of the enzymes produced by these cultures is higher than those obtained from yeast and bacteria [7]. Almost all fungi of genus *Aspergillus* synthesize cellulase, therefore this genus has the potential to dominate the enzyme industry.

II. MATERIALS AND METHODS

A. Microorganism

Aspergillus sp isolated from soil sample collected from saw mill was used for the production of Cellulases. The strain was maintained on PDA at 4 ◦C.

B. Pre-treatment of substrates

The procured cellulosic substrate potato peel was dried at 70 ^{o}C for 5hrs and was ground to fine powder. For SmF Czapek- dox broth was used and CMC was used as sole source of carbon.

C. SSF

Solid state fermentation was carried out in 100 ml Erlenmeyer flasks that contained 5 g of potato peel and 5 ml of distilled water (moistening agent). The flasks were sterilized for 15 min at 15lb/inch^2 pressure and cooled to room temperature. About 5ml of inoculum was added, mixed well and incubated at 30°C in a humidified incubator.

D. SmF

Submerged fermentation was carried out in 100 ml Erlenmeyer flasks containing 30 ml of fermentation medium. The composition of the medium contained the following g/l of distilled water. CMC-30, $NaNO_3$-2, K_2HPO_4-1, $MgSO_4$-0.05, KCl-0.5, $FeSO_4$-0.01. pH of the medium was adjusted to 5. The medium was sterilized by autoclaving at 121°C for 15 min. Each flask was inoculated with 3ml of the above inoculums containing 10^9/ml spores . The cultures were incubated on a rotary shaker 120 rpm at 30°C [8].

E. Enzyme Extraction

At the end of the fermentation the culture broth from submerged fermentation was centrifuged at 6000 rpm for 15 min and the supernatant was used as a source of extracellular enzyme. In SSF the enzyme was extracted by mixing homogenously potato peel with (1:10 w/v) distilled water and agitated at 120 rpm with a contact time of 1h. Dampened cheese cloth was used to filter the extract and pooled extracts were centrifuged at 6000 rpm for 15min and the clear supernatant was used as a source of extracellular enzyme[8].

F. Enzyme Assay

The filter paperase (FPase) and carboxymethylcellulase activity (CMCase) were determined by the method of Mandels and Weber [9] and Ghose [10] respectively. For both the cases the reducing sugar liberated was estimated spectrophotometrically at 540 nm after addition of DNS. One Filter paper unit (FPU) is defined as amount of enzyme in the filtrate releasing 1 µmol of reducing sugar from filter paper/mL/min [11]. Carboxymethycellulase (CMCase) is expressed in terms of units. One unit is the amount of enzyme releasing 1 µmol of reducing sugar from carboxymethyl cellulose/mL/min [11]. The enzyme activites was expressed as U/gds (i.e. Unit per gram dry substrate) for SSF and in U/ml for SmF. Dry weight of the samples was determined by drying them in a hot air oven at 105°C to a constant weight.

G. Protein estimation of Crude enzyme extract

An aliquot of this culture filtrates was used for estimation of extracellular protein content according to the method of Lowry [12]. Bovine serum albumin was used as protein standard.

H. Biomass Estimation

Glucosamine content was estimated [13] in case of SSF and for Smf , following centrifugation of fermentation medium cell pellet was collected on previously weighed aluminium cups, washed with distilled water and kept in hot air oven at 70^{o}C until contant weight.

I. Optimization of Process parameters in SSF and SmF

To find the optimum physical conditions for cellulase production we measured the activity on the pH range from 3 to 6.2 and the temperature range from 23 to 50°C for SmF and SSF . Determination of optimal age of inoculum was done by carrying out the fermentation process using 0 day -8 day old culture. For determination of optimal incubation period the fermentation was carried out at different time interval. The crude enzyme extracted were analyzed for cellulase activity.

J. Comparative evaluation of SmF and SSF systems for enzyme production

The enzyme yields were evaluated for SmF and SSF in comparison with their respective controls. [8, 14]

III. RESULTS AND DISCUSSION

3.1 Optimization of Process parameters in SSF and SmF

A. Temperature

Incubation temperature plays a significant role in the metabolic activities of a microorganism. In the current study the optimum temperature for maximum enzyme production was recorded at 30ºC at the levels of 26.8 and 2.9U/ml for CMCase and FPase respectively under SmF and under SSF maximum production was observed at the levels of 47.3 and 5.7 U/gds for CMCase and FPase respectively (Fig 1a,1b) .Earlier maximum cellulase production was reported at 30°C when *Trichoderma reesei* was used as the cellulase producer under SSF[15]

B. Time of Fermentation

In the present study cellulase activity increased steadily and reached maximum at 6th day of incubation for SmF and 7th day of incubation in SSF (Fig 2a,2b).However, further increase in the incubation time reduced the enzyme production. It might be due to the exhaustion of macro and micronutrients in the fermentation medium with time, which stressed the fungal physiology and resulted in the inactivation of secreting machinery of the enzymes [16].

C. Age of inoculums

The study indicated that highest cellulase production was seen when a 6day old culture was used for SmF at the levels of 29 and 3.1 U/ml for CMCase and FPase respectively, while maximum cellulase production was seen with 7day old culture for SSF at the levels of 59.1 and 7.8 U/gds for CMCase and FPase respectively.(Fig3a,3b)

D. pH

Among physical parameters, pH of the fermentation medium plays an imperative role by inducing morphological changes in microorganism and in enzyme secretion.[17].Maximum celluase production was seen at pH=5 (Fig4a, 4b)both in case of SSF (52 and 6.3 U/gds for CMCase and FPase respectively) and SmF (26 and 2.8 U/ml for CMCase and FPase respectively).

E. Comparative evaluation of SmF and SSF systems for enzyme production

Production of cellulases before and after optimization was compared with control for both SSF and SmF. It was observed that SSF resulted in 7.1 fold increase in CMCase and 3.12 fold increase in FPase production compared to control whereas SmF resulted in 4.8 fold increase in CMCase and 2.21fold increase in FPase production compared to control.(Fig5).The results were similar to the results obtained when production of cellulases were studied under SSF and SmF using coir waste as substrate[8].

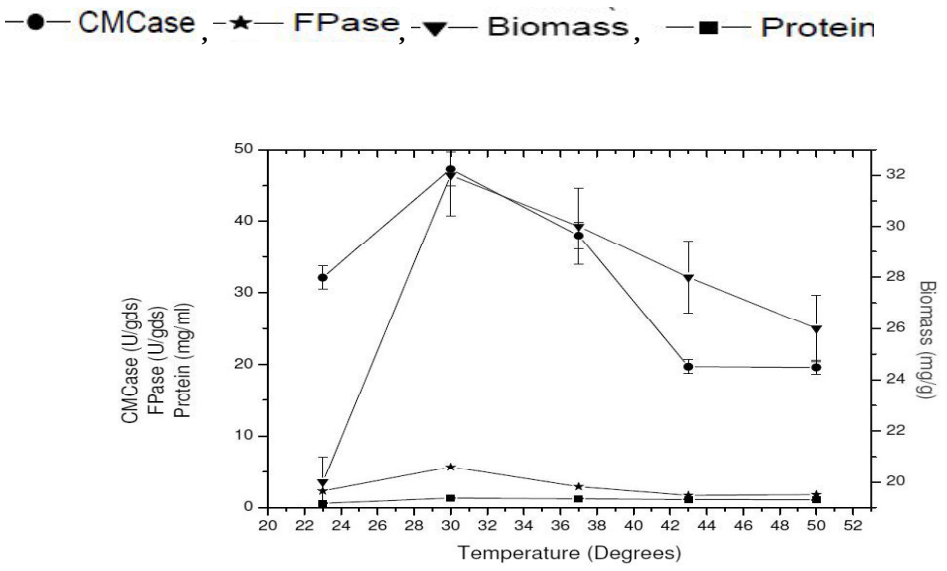


Fig1a Effect of temperature on production of cellulase by SSF

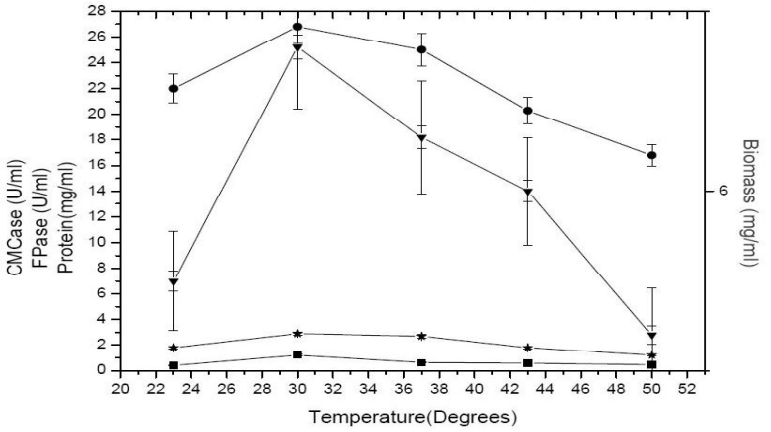


Fig1b Effect of temperature on production of cellulase by SmF

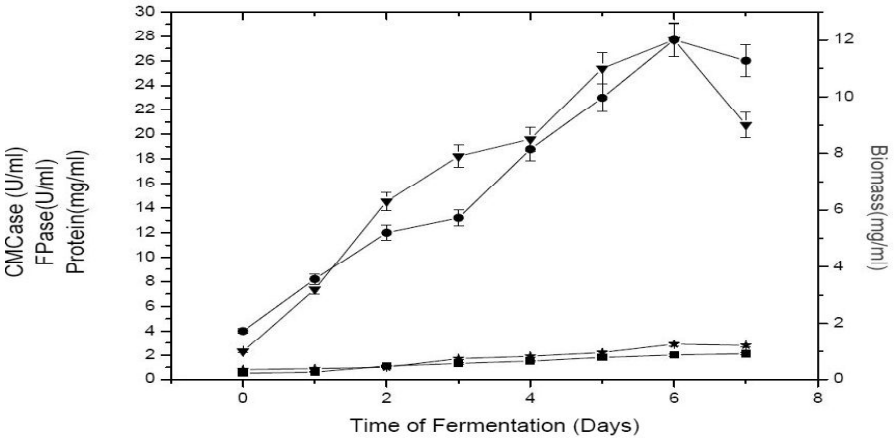


Fig2a Effect of time of fermentation on production of cellulase by SmF

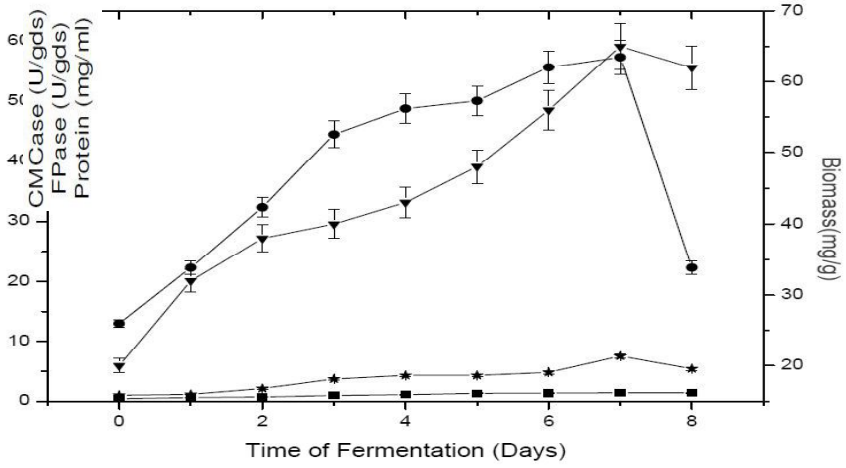


Fig2b Effect of time of fermentation on production of cellulase by SSF

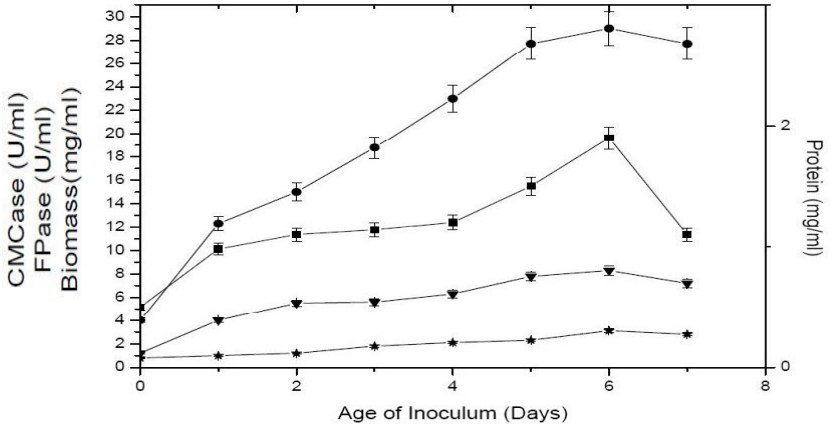


Fig3a Effect of age of inoculum on production of cellulase by SmF

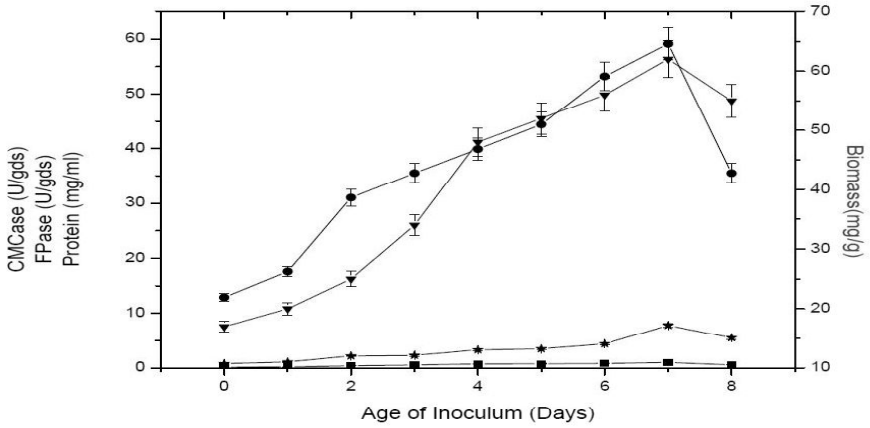


Fig3b Effect of age of inoculum on production of cellulase by SSF

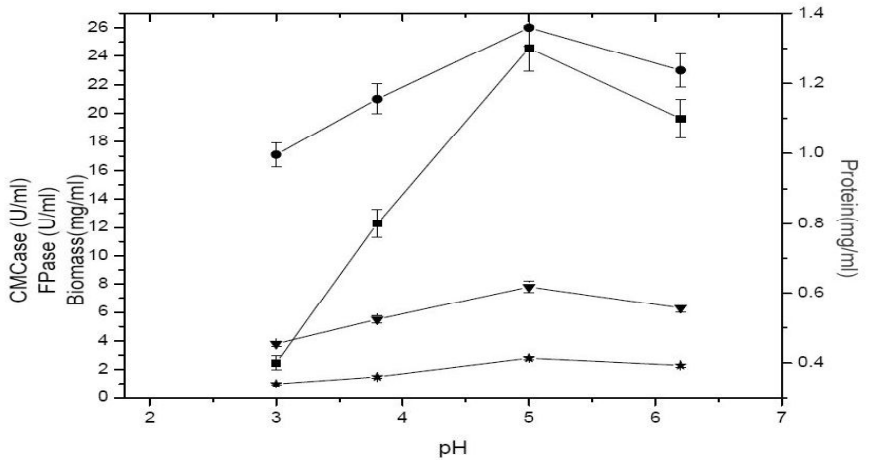


Fig4a Effect of pH on production of cellulase by SmF

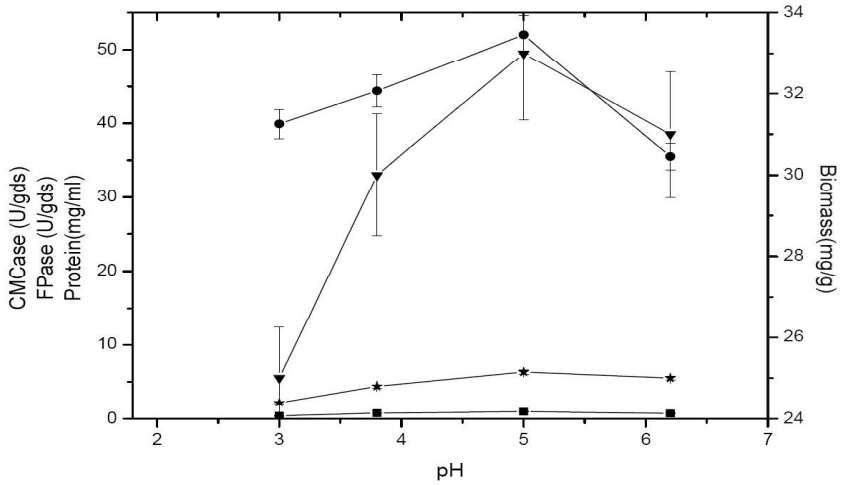
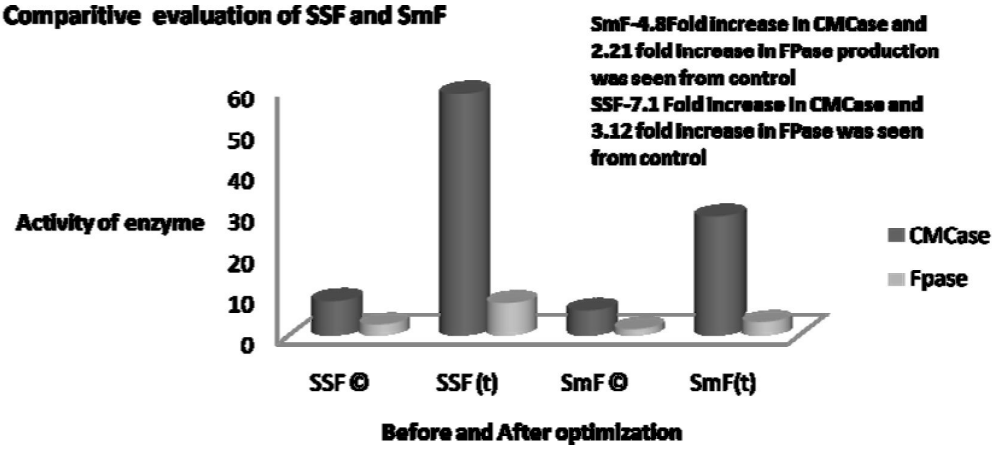


Fig4b Effect of pH on production of cellulase by SSF



IV. CONCLUSION

The present study revealed that potato peel ,the agricultural waste could proficiently increase CMCase production by 7.1 fold and FPase by 3.12 fold, consequently this substrate can be used for cost effective production of cellulase which is one of the most industrially significant enzyme.

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