Production of Cellulolytic Enzymes by *Aspergillus niger* on Solid and Submerged State Fermentation

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Microbial production of cellulolytic enzymes by *Aspergillus niger* in solid state fermentation (SSF) and submerged state fermentation (SSF) in laboratory scale was compared. Capecok Dox liquid broth amended with cellulose (0.5%) was used for cultivation in SF, whereas base rice bran was used as a solid support, moistened with cellulose, amended Capecok Dox broth for growth in SSF. The production of Carboxymethyl cellulase, Filter paperase and β-Glucosidase was monitored at regular intervals. The peak production of the enzymes occurred within 3 days of incubation in SSF as against ≥ 7 days in SF. SSF gave higher yields of enzymes in comparison to SF. Maximum titres of 0.40, 0.62 and 0.013 U/ml in respect of Fase, CMCase and β-glucosidase in SSF were recovered as against 0.13, 0.06 and 0.0013 U/ml in SF respectively, at their respective peak time intervals. Hence, SSF appeared to be a better choice for production of cellulolytic enzymes by *Aspergillus niger*.

**Key words:** *Aspergillus niger*, submerged state fermentation, solid state fermentation, filter paperase, carboxymethyl cellulase, β-glucosidase

**Introduction**

Cellulose is the most abundant polymer in the biosphere and comprises about 40% of the dry weight of the most plants [3]. Cellulose-rich plant biomass is one of the foreseeable sources of fuels, single cell protein and feed stock to chemical synthesis due to its continuous synthesis from CO₂ and solar energy at annual rate of 72×10⁹ tons through photosynthesis by plants [12]. Ability to utilize cellulose is restricted to certain bacteria and fungi [1,2,18] due to liberation of cellulolytic enzymes. Cellulose utilizing microorganisms present in the soil and water bodies is responsible for one of the largest material flows (carbon flux) in the biosphere at both local global scales. Cellulolytic enzymes include cellubiohydrolases or exoglucanases (E.C. 3.2.1.91), endoglucanases or CMCases (E.C. 3.2.1.4) and β-glucosidases (E.C. 3.2.1.21), which act synergistically to convert lignocellulosic biomass [10] in to glucose in saccharification which can be subsequently utilized for different purposes [5]. However, high cost of production of cellulolytic enzymes limit their industrial use in production of soluble sugars from cellulosic materials [9]. One of the approaches in improving saccharification is to search for highly efficient cellulolytic microorganisms with production of cellulase in copious amounts and to explore on cultural practices these organisms for enhanced rate of enzyme production. Hence, kinetic parameters of production of cellulase with a local potential isolate of *Aspergillus niger* by SF and SSF methods was compared in the present laboratory investigation.

**Materials and Methods**

Microorganism and preparation of inoculum

*Aspergillus niger* was isolated from the soils contaminated with the effluents of Cotton ginning mills, Nandyal, Andhra Pradesh [15]. It was maintained on Capecok Dox medium, its spore suspension was prepared by adding 2 ml of sterile distilled water to 7 day old slants.

Fermentation method in both SF and SSF

The spore suspension was used to inoculate at the spore density of 2×10⁶ spores/ flask, in 50 ml Capecok Dox broth
amended with 0.5% of cellulose in 250 ml Erlenmeyer flasks, which were incubated (30°C, 200 rpm). Ten grams of rice bran in 250 ml Erlenmeyer flask was used as solid support. Ten milliliters of the Czapek Dox broth amended with 0.5% cellulose was only once added to all matrices in the flasks, moistened the rice bran for growth in solid state fermentation (SSF). The above spore suspension was also added to solid medium at the spore density of 2×10⁶ spores/flask. At desired intervals, 250 ml Erlenmeyer flasks with growing culture of *Aspergillus niger* of both fermentations were withdrawn during the course of experiments. In SSF, fungal mass was mixed with 20 ml of acetate buffer (0.2 M, pH 5.0), the slurry was filtered through nylon cloth and the filtrate centrifuged (10,000 rpm, 20 min) at 4°C [19]. The 50 ml of broth derived from submerged fermentation (SF) was filtered on Whatman No.1 filter paper. The filtrate was used for enzyme assays. Each sample was monitored for pH, protein, Filter paperase (FPase), Carboxymethyl cellulase (CMCase) and β-Glucosidase activity.

**Estimation of protein**

Aliquots of *Aspergillus niger* culture filtrates with appropriate dilution were used for estimation of soluble protein content according to the method of Lowry et al. [11].

**Enzyme assays**

Filter paper assay method [13] was employed to measure total cellulase activity in the culture filtrate derived from SF and SSF. Activity of cellulase was expressed in filter paper units (FPU). One unit is the amount of enzyme that released 1 μmole of reducing sugar from filter paper per min. Activity of endoglucanase in the culture filtrate was quantified by Carboxymethyl cellulase method [4]. One unit of endoglucanase activity is defined as the amount of enzyme releasing 1 μmole of reducing sugar from Carboxymethyl cellulase per minute. β-Glucosidase activity in the culture filtrate was determined according to the method of Herr [8]. One unit of β-glucosidase activity is defined as the amount of enzyme liberating 1 μmole of p-nitrophenol per minute.

**Results**

**Production of filter paperase in SSF and SF**

The culture of the local isolate of *A. niger* was grown in a time-course study to compare production of cellulolytic enzymes and production of extracellular protein by both fermentation methods. The SSF yielded maximum titre of FPase with 0.396 FPU/ml within 24 hours incubation, whereas SF gave maximum titre of 0.127 FPU/ml at the end of 1st week (Fig. 1).

**Production of carboxymethyl cellulase in SSF and SF**

Maximum titre of carboxymethyl cellulase with 0.620 U/ml was recovered from the SSF at second day, as against 0.06 U/ml in SF at fourth week (Fig. 2).

**Production of β-glucosidase in SSF and SF**

Higher production of β-glucosidase in SSF was recorded in the second day filtrate with 0.01288 U/ml, whereas in

![Fig. 1. Production of Filter paperase by *A. niger*. Values in the figure are means of 3 replicates ± standard deviation.](image1)

![Fig. 2. Production of Carboxymethyl cellulase by *A. niger*. Values in the figure are means of 3 replicates ± standard deviation.](image2)
Fig. 3. Production of β-Glucosidase by *A. niger*. Values in the figure are means of 3 replicates ± standard deviation.

Fig. 4. Production of extracellular protein by *A. niger*. Values in the figure are means of 3 replicates ± standard deviation.

SF, maximal activity of β-glucosidase in the filtrate at 3rd week, didn’t exceed 0.00124 U/ml (Fig. 3).

Protein determination in SSF and SF

Protein content was more in the filtrate of 1st day SSF with 6.6 mg/ml and there onwards declined (Fig. 4). In contrast, the reverse trend was noticed in SF with recovery of maximum protein content (1.5 mg/ml), at 4th week.

Higher production of enzymes by *Aspergillus niger* was correlated to production of protein. During the course of growth in both fermentation, pH changes occurred in the media within a range of 3.28 to 7.20 in SF whereas in SSF, it was 4.48 to 5.28.

Discussion

Though there are many publications for production of cellulolytic enzymes using SF, very few reports are available on SSF. *Aspergillus niger* culture exhibited all three enzyme activities in the present study. Different cellulolytic enzymes reached peak production at different time intervals. Titres of all recovered enzymes at peak production interval in SSF were higher than that of respective enzymes at peak production interval in SF. Similarly, activities of cellulolytic enzymes were demonstrated with number of fungal cultures in different studies [6]. Majority of these studies on production of cellulolytic enzymes were conducted with either SSF or SF. The present study compared both methods. In SSF the higher production of cellulolytic enzymes occurred within 3 days of incubation. Whereas increase of SF maximum production of cellulolytic enzymes occurred longer incubation (weeks) time than SSF. From this study, it is clear that SSF has a distinct advantage than SF for the production of cellulolytic enzymes because of its increased yield and if the process is optimized it could be of great significance for the production of cellulolytic enzymes at a commercial scale. SSF culture conditions are reportedly more suitable than liquid for the growth of filamentous fungi [16] and might be the reason behind our findings of higher enzyme production in SSF than SF. However, the yields of cellulase production in the present study were low in comparison to the results reported by others [14,17,20-22] but were higher than titres obtained in a most recent study with *A. niger* [7]. Difference in titres of production of cellulolytic enzymes in different studies could be attributed to a variety of cultural patterns of organisms and of raw materials used in the studies.

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References

5. Gregg, D. J. and J. N. Saddler. 1996. Factors affecting cel-
초록: 액체와 고체 발효 조건에서 Aspergillus niger의 설루로오스계 효소 생산

수보쉬 친드리1,2, 라자세카르 레디1,2, 화용락6,6

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Aspergillus niger가 액체발효(SF) 와 고체발효의 시험 규모 조건에서의 설루로오스계 효소의 생산을 비교하였 다. 액체배치는 0.5% 설루로오스가 함유된 Czapex Dox를 사용하였고, 고체 지지체로 사용한 재료는 설루로오스 로 적시고 SSF 발효를 위하여 Czapex Dox 배지를 천거하였다. CMCase, 엘리아 펜타세, 그리고 β-Glucosidase의 생산을 정시적으로 측정하였다. SSF 배양에서의 3일간의 효소 생산량은 SF에서의 7일간 배양과 같았다. 따라서 SSF 조건이 SF 배양 조건보다 많은 효소를 생산할 수 있었다. SSF 조건에서 FFase, CMCase 및 β-glucosidase의 최적 활성은 0.40, 0.62 및 0.013 U/mL 였으나, SF 조건에서는 0.13, 0.06 및 0.0013 U/mL의 활성을 나타내 었다. 결론적으로 Aspergillus niger에 의해 생산되는 설루로오스계 효소의 생산을 위해서는 SSF 발효 조건의 선택 이 유리하다는 것을 알 수 있었다.