International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Comparative Study of Cellulase Production by *Aspergillus niger* and *Trichoderma viride* Using Solid State Fermentation On Cellulosic Substrates Corncob, Cane Bagasse and Sawdust

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Abstract: ***The enzyme activities of Trichoderma viride and Aspergillus niger grown on various waste cellulosic materials such as corncobs, saw dust and sugarcane baggase were tested for a period of 192 hrs. The maximum activity of 0.33 mg/min/ml & 0.026 mg/min/ml of cellulase enzyme were found to be produced on saw dust after 144 hrs by Aspergillus niger & Trichoderma viride, respectively. Corncob showed the highest activity at 120 hrs of 0.22 mg/min/ml & at 96 hrs of 0.0246 mg/min/ml by Aspergillus niger & Trichoderma viride, followed by sugarcane with the highest activity of 0.026 mg/min/ml & 0.0256 mg/min/ml 120 hrs by Aspergillus niger & Trichoderma viride, respectively. From the above results, Aspergillus niger produced the highest amount of cellulase activity with sawdust as substrate followed by Trichoderma viride by solid state fermentation.***

Keywords: *A niger, T viride,* SSF, Cellulase, cellulosic's

1. Introduction

The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria. Cellulases have a wide range of enormous potential applications in microbiology. Some of the most important applications of cellulases are in food, brewery& wine, biofuels, animal feed, textile & laundry, paper & pulp industry, as well as in agriculture & research puposes. (Y.M. Galante, A.Deconti, and R. Monteverdi. 1998)[1], and (R. K. Sukumaran *et. al.* 2005)[2]. Cellulase belongs to a class of enzymes produced chiefly by fungi, bacteria, and protozoans. It is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds. Cellulases hydrolyze cellulose (β-1,4-D-glucan linkages) & produce as primary products glucose, cellobiose & cello oligosaccharide. There are three major types of cellulase enzymes. Several different kinds of cellulases are known, which differ structurally and mechanistically (Chellapandi and Jani, 2008)[3].

Cellulase production on a commercial scale is induced by growing the fungus on solid cellulose or by culturing the organisms in the presence of a disaccharide inducer such as lactose. However, on an industrial scale, both methods of induction result in high costs. Since the enzymes are inducible by cellulose, it is possible to use cellulose containing media for production. The ability to secrete large amount of extracellular protein is characteristic of certain fungi & such strains are most suited for production of higher level of extracellular cellulases. One of the most extensively studied fungi is *Trichoderma reesei*, which converts native as well as derived cellulose to glucose. Most commonly studied cellulolytic organisms include fungal species- *Trichoderma, Humicola, Penicillium*, & *Aspergillus.* While several fungi can metabolize cellulose as an energy source, only few strains are capable of secreting a complex of cellulase enzyme, which could have practical application in the enzymatic hydrolysis of cellulose. Besides *T. ressei,* other fungi like *Humicola, Penicillium* & *Aspergillus* have the ability to yield high levels of extracellular cellulases. (Fan *et al*., 1987)[4].

The selection of substrates for enzyme production in a solid state fermentation process upon several factors, mainly related to cost & availability of substrate, the substrates that provides all the needed nutrients to the microorganisms growing in it should be considered as the ideal substrate. Thus, the solid-state fermentation (SSF) offers a low-cost alternative for producing cellulases using natural polymers derived from agro industrial residues (Milala *et al.* 2005)[5].

2. Materials and Methods

All chemicals, media and reagents used were purchased from Hi-media (Mumbai) and were of analytical grade.

2.1. Fungal strains used in cellulase production

Strains of *Aspergillus niger* and *Trichoderma viride* maintained as laboratory stock cultures, on PDA slants at 4^{o}C, subcultured every week were used in the present investigation.

2.2. Collection and Preparation of cellulosic waste

Corn cob waste and Sugarcane bagasse was collected as a throw away waste, and saw dust was obtained from saw mills during the period of investigation. The preparation of cellulosic substrate for production of cellulase enzyme was done according to D. S. Syawala *et al* (2013)[6] with slight modification. The cellulosic material such as corncob, and sugarcane bagasse was chopped and sundried for period of 48 hrs. For first delignification, corncob and sugarcane bagasse was first grounded to yield a fine powder. This powder was stored at 5^{o}C for further experiments. About 100 gm each of the above cellulosic powdered waste was soaked using 0.1N NaOH at room for five hours with a ratio of 1:8

(materials:solvent). Second delignification was carried out using 2 N NaOH at room temperature for 24 hours with a ratio of 1: 4 (materials:solvent). The resultant corncob and sugarcane bagasse powder was subjected to acid hydrolysis. The delignified corncob, sugarcane bagasse and saw dust was moistened with water in ratio of 1:5. About 0.5 N hydrochloric acid was added to adjust the pH- 4.5. The substrate was hydrolyzed by heating at a temperature of 115 ° C for 60 minutes using autoclave. The hydrolyzed substrate was dried at 40°C for solid state fermentation (SSF).

2.3. Inoculum preparation

Both fungal strains were grown on PDA slant until heavy sporulation (at 30°C for 5 days) occurred. Ten ml of sterile distilled water was added. The spores were gently scraped off with the help of a sterile needle and this inoculum was used to seed the production medium for fermentative cellulase production on cellulosic waste substrate supplemented with mineral salt medium.

2.4. SSF for production of cellulose

About 5gm of the powdered corncob, sugar bagasse and wheat bran was mixed with 100ml of mineral salt medium containing $NaNO_3$ .3, KCl- 0.5, $MgSO_4.7H_2O$ -0.5, KH_2PO_4- 1.0, $FeSO_4.7H_2O$ - 0.01, and $ZnSO_4.7H_2O$- 0.1 g/l. The pH was adjusted to 5.6. 500 ml conical flasks containing 100 ml of respective medium containing corncob, bagasse and sawdust, respectively was autoclaved at 121° C for15 minutes & inoculated with 5 day old culture of *Aspergillus niger* & *Trichoderma viride.* The flasks were incubated in dark at 25° C for seven days. The cells were harvested at 48 hrs interval by centrifugation at 6000 rpm for 15 minutes at 4°C using cryocentrifuge. The cell free supernatant were pooled and used as source of extracellular cellulase enzyme.\

2.5. Cellulase assay

Cellulase activity was assayed by a modification of Dintrosalicylic acid (DNS) method as described by Khan (1980)[7] using carboxymethyl cellulose (CMC) as substrate. The reaction mixture contained 2 ml of 1.0% (w/v) CMC in 0.1 M solution of sodium acetate buffer, pH 5.0 & 2ml of the cell free culture supernatant. The reducing sugar released by enzyme was measured as glucose equivalent. One unit of enzyme activity (EA) is defined as the amount of enzyme required to liberate mg of glucose per ml of enzyme solution per minute under standard assay conditions.

Calculation

$$\text{EA (mg/ml/min)} = \frac{\text{Absorbance of enzyme solution x}}{\text{Time of incubation (min)}}$$

Whereas, standard factor (SF)

$$\text{S F} = \frac{\text{Concentration (mg/ml) of standard glucose}}{\text{Absorbance at 540 nm}}$$

3. Result and Discussions

3.1. Fungal strains used in cellulase production

Solid state fermentation is preferable for cultivation of filamentous fungi because the hyphal mode of fungal growth & their good tolerance to low water activity & high osmotic pressure conditions make fungi efficient & competitive in natural microflora for bioconversion of solid substrates. (Milala *et al.* 2005). The ability to secrete large amount of extracellular protein is characteristic of certain fungi & such strains are most suited for production of higher level of extracellular cellulases. It has been reported that fungal cellulases are well-studied enzymes and are used in various industrial processes. The enzymatic depolymerization of cellulosic material has come from *Trichoderma* cellulase system. Species of *Trichoderma* can produce substantial amounts of endoglucanase and exoglucanase but very low levels of β-glucosidase. This deficiency necessitates screening of fungi for cellulolytic potential. (Fan *et al*.1987) and (Sohail M., R. Siddiqi. 2009)[8].

The present investigation is therefore an attempt using the laboratory maintained strains for production of higher yields of cellulase enzyme by *Aspergillus niger & Trichoderma viride* using cheap and available cellulosic substrates such as Corncob, sawdust, & sugarcane bagasse as major waste substrates using solid state fermentation. SSF of using *A niger* and *T viride* on corncob, sugarcane bagasse and sawdust is depicted in photolates 1 to6.

Plates 1: Production of cellulase by using *Aspergillus niger* with corncob.
Plates 2: Production of cellulase by using *Aspergillus niger* with bagasse.
Plates 3: Production of cellulase by using *Aspergillus niger* with sawdust.

Plates 4: Production of cellulase by using *Trichoderma viride* with corncob
Plates 5: Production of cellulase by using *Trichoderma viride* with sawdust.
Plates 6: Production of cellulase by using *Trichoderma viride* with sugarcane baggase.

3.2. Cellulase assay

The cell free supernatants subjected to enzyme assay yielded the following results. Figures 1 and 2 shows the comparison of the enzyme activities of *Trichoderma viride and Aspergillus niger* grown on various waste cellulosic materials such as corncobs, saw dust and sugarcane baggase for a period of 192 hrs. The organisms have different periods for optimal cellulase yield. Depression in cellulase activities was observed after the 196 hours for both the organisms. This is as expected for enzymatic reactions that may be

prone to post- reaction accumulation of hydrolytic by-products (Howell, 1978)[9].

It was found that the maximum activity 0.33 mg/min/ml & 0.026 mg/min/ml of cellulase enzyme were found to be produced on saw dust as substrate after 144 hrs by *Aspergillus niger & Trichoderma viride,* respectively. Corncob showed the highest activity at 120 hrs of 0.22 mg/min/ml & at 96 hrs of 0.0246 mg/min/ml by *Aspergillus niger & Trichoderma viride,* followed by sugarcane with the highest activity of 0.026 mg/min/ml & 0.0256 mg/min/ml 120 hrs by *Aspergillus niger & Trichoderma viride,* respectively. From the above results, *Aspergillus niger* produced the highest amount of cellulase activity with sawdust as substrate followed by *Trichoderma viride* by solid state fermentation. Our results are consistent with those reported by (Juwaied, *et al.* 2008) [10].

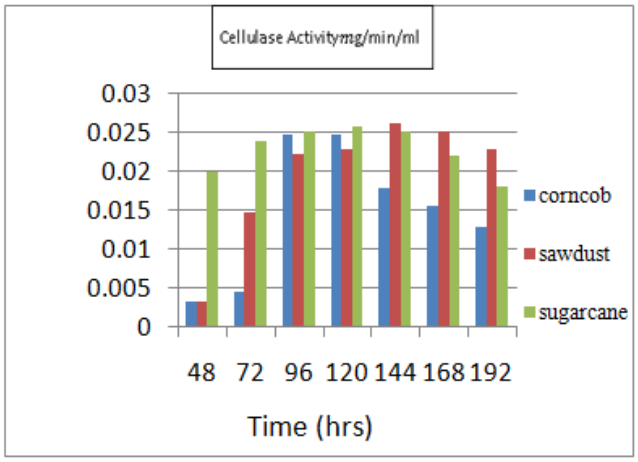

Figure 1: Comparison of enzyme activity between different substrates by using *Trichoderma viride.*

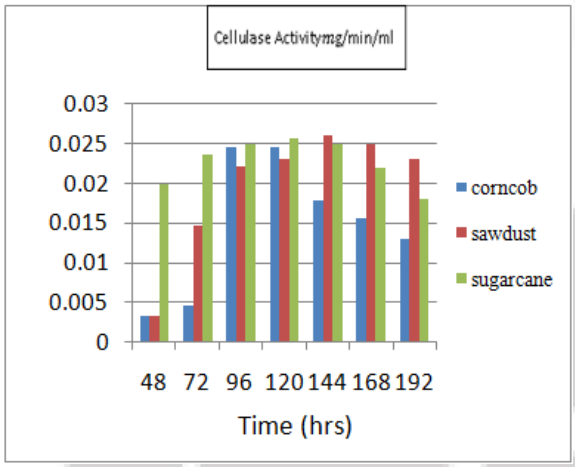

Figure 2: Comparison of enzyme activity between different substrates by using *Aspergillus niger*

4. Summary

The aim of present investigation "Comparative studies on cellulase production by *Aspergillus niger* & *Trichoderma viride* " was carried out *using* different cellulosic waste material. The cellulase production was demonstrated from various waste materials such as corncob, sugarcane, sawdust using fungi like *Aspergillus niger* & *Trichoderma viride* by solid state fermentation. From the observation it was found that the enzyme activity was most from *Aspergillus niger* using sawdust. The present study suggests that sawdust, the by-product of saw-mill operations, is available in large amount and therefore could be a suitable low-cost substrate for cellulase production using strains of *Aspergillus niger* & *Trichoderma viride.*

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