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# Production and viscometric assay of endoglucanse by *Aspergillus niger*

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## ABSTRACT

In this study, a fungal strain Aspergillus *niger* was isolated from soil contaminated with effluents of cotton ginning mill for cellulase production. The cellulolytic property of fungal culture was confirmed by Plate screening method. Formation of clear yellow zone of inhibition on Congo-red agar medium is an indication of endoglucanase activity of fungal strain. The endoglucanse activity was measured by viscometric method. The maximum reduction in Carboxy Methyl Cellulose viscosity with 98.77 percentages at 70 minutes is an indication of improved endoglucase activity of *Aspergillus niger*. 

## KEYWORDS

*Aspergillus niger*;
Endoglucanse;
Viscometric determination.

## INTRODUCTION

In recent years, the interest in cellulases has increased due to many potential applications for example, in the production of bio-energy and bio-fuel, in the textile industry and in the pulp and paper industry[1,2, 3]. The growing concerns about the shortage of fossil fuels, the emission of green house gasses and air pollution by incomplete combustion of fossil fuel have also resulted in an increasing focus on production of bioethanol from lignocelluloses[4] and especially the possibility to use cellulases to perform enzymatic hydrolysis of the lignocellulosic materials[5]. In production of bioethanol, the cost of the enzymes used for hydrolysis needs to be reduced and the enzyme's efficiency needs to be improved in order to make the process economically feasible[6]. The enzyme production costs are tightly connected with the productivity of enzyme-producing microbial strain and the final activity (protein) yield in the fermentation broth[7] The Enzymatic hydrolysis of cellulose to soluble sugars is a complex process requiring the concerted action of several enzymes with different substrate specificity[8]. Endo-1,4-β-D-glucanases (or 1,4-β-D-glucan 4-glucanohydrolases, EC 3.2.1.4) hydrolyze β-1,4-glucosidic linkages of the cellulose chain in a random manner, releasing cellodextrins with different degrees of polymerization, and thus providing substrates for exoglucanases. The standard substrate for characterizing endoglucanases is a soluble cellulose derivative, the sodium salt of carboxymethylcellulose, CMC. The course of the reaction is followed either by reduction in viscosity or by formation of reducing end-groups[9] Many cellulolytic organisms produce cellulases under suitable conditions that perform cellulolysis nec-

essary for cell growth. Fungi constitute a most fascinating group of organisms and are most common industrial source for hemicelluloses such as glucanases, xylanases, and galactanases. The viscometric method is considered standard for evaluating endoglucanases since it is highly sensitive and specific for enzymes that hydrolyze internal bonds within a polymer molecule[10]. The change in viscosity is primarily due to the change in the degree of polymerization of CMC that is the result of cleavage of the glucosidic linkages remote from the chain end. In contrast, exoglucanases, which act on CMC near the chain end, give little change in viscosity while releasing significant amounts of reducing end-groups. In this study an attempt was made to isolate and screen fungal culture for cellulase (endoglucanase) production, and determine the endoglunase activity by cost-effective and convenient viscometric method.

## MATERIALS AND METHODS

### Organism and growth conditions

The fungal culture used in this study for endoglucanse production was isolated from soil contaminated with cotton ginning mill effluents and the fungal isolate was identified as *Aspergillus niger* based on its macroscopic and microscopic observations and values matching these characteristics recorded with those listed in standard reference book entitled Compendium of Soil Fungi, Vol. 1 Domsch *et al.*,[11]. The identified fungal culture was further purified by sub-culturing number of times in Potato Dextrose Agar (PDA) plates for further experiments

### Screening of *Aspergillus niger* for cellulase production

The cellulolytic nature of *Aspergillus niger* was confirmed by plate screening method[12] For this 1% Carboxy Methyl Cellulose was amended with Czapeck -Dox agar media and the pH was adjusted to 5. The media was poured into sterile Petri dishes, after solidification of media a small hole was made on the centre of Petri dish aseptically and the *A.niger* spores were added to the well made centre of medium. The plates were incubated for 3days at $30^0$ C and 2days at $50^0$ C. After incubation the plates were stained with 1% Congo red solution for 15 minutes, after that the Congo red stain was neutralized with 1M Nacl solution. The yellow color zone formation concern the ability of substrate utilization and CMCase (endoglucanse) enzyme activity of fungal culture.

### Cultivation of *Aspergillus niger* for endoglucase production

For cellulase production, *A.niger* was cultivated in basal medium[13] with following ingredients in g/L. $KH_2PO_4$, 2.0; $MgSO_4.7H_2O$, 0.3; $CaCl_2.2H_2O$, 0.3%; $NaNO_3$, 1.4; $NH_4H_2PO_4$, 0.8; Protease peptone, 0.5; yeast extract, 0.3; $NaNO_3$ 1.4; Casamino acids, 0.4; Tween-80, 1 ml and trace elements (mg/L), $FeSO_4.7H_2O$, 5.0; $MnSO_4.4H_2O$, 1.6; $ZnSO_4.2H_2O$, 1.4 and $CuSO_4.5H_2O$, 1.0. The pH of the medium was adjusted to 5.0. Sterile fifty milliliters of the above growth medium in 250 ml Erlenmeyer flasks were amended with 0.5% W/V cellulose. Inoculums' of mycelial suspension was prepared by flooding the slant with 2 ml of sterile distilled water and was used to inoculate basal medium in the flasks. Inoculated fresh fungal cultures were incubated at 28 ± 20°C on a rotary shaker (180 rpm). The filtrate obtained after removal of mycelial mat by filtration through Whatman Filter paper No. 1, was used as an enzyme source.

### Determination of endoglucanase activity by viscometric method

The fungal strain *Aspergillus niger* endoglucanase activity was measured by Viscometric method[14]. Five grams of carboxymethyl (CMC) cellulose was slowly dispersed into 500 ml hot water (80°C). The solution was cooled to room temperature after complete solubilization of CMC and volume was made up to one liter with 0.1 M sodium acetate buffer pH 5.0 and stored at 4°C as stock solution until usage. CMC hydrolysis was performed in 50mM acetate buffer, pH 5 at 40°C. Fifteen milliliter aliquots of carboxymethyl cellulose stock solution were dispersed into 25 ml screw cap tubes followed by 5 ml of acetate buffer. Tubes were divided in two groups. Five milliliters of diluted enzyme culture filtrate was added to tubes of the first group whereas only 5 ml of acetate buffer was added to tubes of the second group (control) instead of enzyme. These tubes were equilibrated in water bath at 40°C. All tubes of both groups except two tubes from each group were

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incubated in a water bath at 40°C immediately after addition of enzyme source/acetate buffer. Two tubes from each group were withdrawn immediately and viscometric measurement was made. The reaction mixture in the tubes was separately transferred to Ubbelohde capillary tube of viscometer. The mixture was immediately made to flow down between two predetermined points in the viscometer and the flow rate was measured at zero time.

Two tubes from each group at the end of desired period of incubation were withdrawn and enzyme activity was determined by measurements of flow rate in the same fashion as described above. The same process was repeated with water in the place of enzyme. Per cent loss of carboxy methyl cellulose viscosity was separately calculated by the following formula in respect of enzyme treated carboxymethyl cellulose and untreated carboxymethyl cellulose (control) at any given time interval

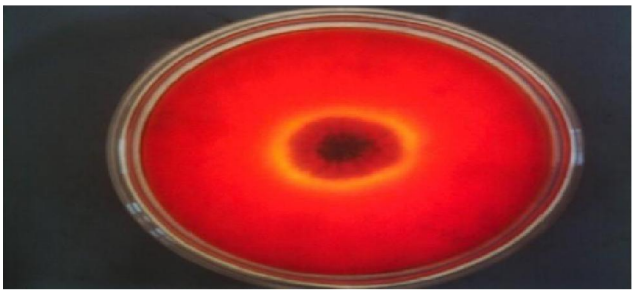
$$V = \frac{V_T - V_{T_0}}{V_T - V_{H_2O}} \times 100$$

Where V = loss of carboxymethyl cellulose viscosity; $V_T$ = flow time of CMC in seconds; $T_O$ = flow time in seconds at zero time (control); $V_{H_2O}$ = flow time of water in seconds

Difference between these two values represents net per cent loss of viscosity of carboxymethyl cellulose that is used to express the activity of endoglucanase.

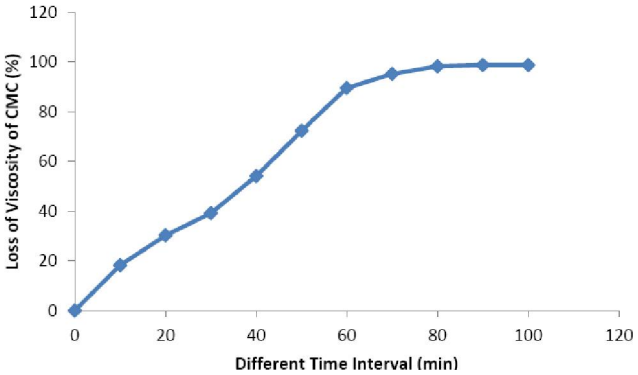
## RESULTS AND DISCUSSIONS

The cellulolytic activity of fungal striain *Aspergillus niger* was confirmed by plate screening method and formation of a clear yellow zone of hydrolysis concerns its ability of cellulolytic property (Figure 1).



***Values represented in figure are mean triplicates**

**Figure 1 : *Aspergillus niger* showed clear yellow zone of hydrolysis which indicates CMC degradation**




***Loss of viscosity of carboxy methyl cellulose in percentage; *Values represented in figure are mean triplicates**

**Figure 2 : Endoglucanse activity of *Aspergillus niger***

Similarly Sadaf Jahangeer *et al.*[15] observed the cellulolytic activity of *P.tigrinus*, *P.ostreatus*, *F.fomentarus* and *A.terreus*. The CMCase activity of mutant *Aspergillus niger* confirmed by plate screening method[16]. The endoglucanase activity *of A.niger* was determined by viscometric method[14] and the activity was measured in terms of loss in CMC viscosity in percentage with increasing the time interval. With increasing the time interval the loss of carboxy methyl cellulose (substrate) increased upto 90min. and constant at further intervals ( Figure 1). For instance the endoglucanse activity of *A.niger* at 0-10 mints is 18.5 of loss of CMC viscosity percentages whereas at 90 minutes this loss was tremendously increased up to 98.77 percentage and further intervals the activity was constant. Similar observation made Ortega[17] Nóra Szijártó[18] isolated the thermostable endoglucanse from *Thermoascus aurantiacus* and maximum activity was measured even at higher temperature.

## CONCLUSIONS

The fungal culture *Aspergillus niger* isolated from soil contaminated with effluents of cotton ginning mill was used for Cellulase (endoglucanse) production. The endoglucanase activity was confirmed by Plate screening method with formation of clear zone of inhibition on Congo red agar medium. The endoglucanase activity was determined by viscometric method. Maximum of loss of Carboxy Methyl Cellulose viscosity (98.77%) at 70 minutes of time interval is an indication higher endoglucanse (CMCase) activity of fungal culture *Aspergillus niger*.

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