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Production Of Cellulase From Corn Cobs By *Aspergillus niger* Under Submerged Fermentation

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Abstract: Corn cob is an agricultural lignocellulosic residue which has a high cellulosic content. This cheap biomass can be used for the production of Cellulase enzyme by cellulose degraders *Aspergillus niger*. A comparative study was done using corn cobs and wheat bran to find the best biomass source for production of cellulolytic enzyme. The biomasses were seperately pretreated with mild sodium hydroxide solution at a temperature of 80 degree C for 3 hours. After pretreatment, washed biomasses were separately inoculated with *Aspergillus niger* along with mineral salts. After 5 days of submerged fermentation, the protein concentration of crude extract from corn cob was 0.26 mg/ml and for wheat bran it was 0.178 mg/ml. The glucose estimation by CMC assay for corn cob was 0.370 mg/ml and for wheat bran was 0.253mg/ml. The protein estimation and glucose estimation of corn cobs crude enzyme was relatively higher than Wheat bran's extracellular enzyme.
Key words: Lignocellulose, Cellulase, Aspergillus *niger,*, Submerged fermentation, corn cobs.

Introduction:

A promising approach relies on the production of bioethanol from the abundant and renewable lignocellulosic biomass. Cellulose, the most common natural renewable biopolymer, is commonly degraded by the hydrolytic action of a multicomponent Cellulase enzyme which plays an active role in converting cellulose to glucose.[1] Cellulases has a broad industrial and commercial applications. Potential applications of cellulases are in food, animal feed, textile, fuel, chemical industries, paper and pulp industry, waste management, medical/ pharmaceutical industry, protoplast production, genetic engineering, and pollution treatment.[2] A cellulolytic enzyme is a complex system of enzymes, comprising endoglucanase (endo-1,4-β-D-glucanase, EC 3.2.1.4), exo-glucanase (1,4-β-D-glucan-cellobiohydrolase, EC 3.2.1.91) and β-glucosidase (β-D-glucoside glucano hydrolase, cellobiase, EC 3.2.1.21), which act synergistically to degrade cellulosic substrate.[3] The exoglucanase (CBH) acts on the ends of the cellulose chain and releases *β*-cellobiose as the end product; endoglucanase (EG) randomly attacks the internal *O*-glycosidic bonds, resulting in glucan chains of different lengths; and the *β*-glycosidases act specifically on the *β*-cellobiose disaccharides and produce glucose.[4] Cellulolytic microbes are primarily cellulose degraders but generally do not utilize lipids or protein as energy source. Fungi can grow and utilize agro-industrial residues better than other microbes as it closely resemble to their natural habitat. Filamentous fungi are well known as a cost effective resource for industrial cellulases. Several fungi have been extensively employed for commercial production of cellulases depending upon their ultimate application. [5]Among that, most commercial cellulases are produced by *Trichoderma ressei* and β-D-glucosidase is produced from *Aspergillus niger*. Industrially important enzymes have traditionally been obtained from submerged fermentation (SmF) because of the ease of handling and greater control of environmental factors such as temperature and pH.[6]

The most important thing is lowering enzyme production cost. Producing cellulolytic enzymes from inexpensive lignocelluloses is a preferred choice. Corncob is the central core of a maize ear and is an agricultural residue which is abundant in many corn-producing parts of the world. Corncob had been demonstrated to be an excellent carbon source for cellulse production[7]. This paper demonstrates the, comparative study done for Corn cob and wheat bran which is also a lignocellulosic biomass for production of cellulase enzyme by *Aspergillus niger* under submerged fermentation

Materials and Methods:

Organism:

The fungi *Aspergillus niger* was obtained from a research lab at Trichy, Tamil nadu. .The organisms were maintained on potato dextrose agar slants at 4 °C till required.

Inoculum Preparation:

The inoculum was prepared by growing the organism in 250 ml Erlenmeyer flask with 100 ml of Potato Dextrose broth. The medium was inoculated with inoculum from potato dextrose agar slants and incubated at room temperature for 5 days.

Biomass Preparation:

Corn cobs were collected from nearby shops and local markets. Wheat bran was purchased from a cattle feed shop. Cobs were chopped into small pieces and grinded. Both corn cob powder and wheat bran were dried in hot air oven at 50 degree C overnight for the removal of excess of moisture residue. Dried biomass were screened to obtain the average particles of size 0.500 mm (30 BSS) and it is stored separately in air tight containers for further use and to avoid microbial degradation

Pretreatment:

In case of lignocellulosic biomass, pretreatment is needed to remove the lignin barrier and release the cellulose. Among various pretreatment, Sodium hydroxide solution is an effective method leads to removal of the lignin barrier, disruption of structural linkages, reduction of cellulose crystallinity and decrease in the polymerization[13] 5 grams of screened corn cobs and wheat bran were soaked in 100 ml of 2% Sodium hydroxide solution in two seperate 250 ml conical flask at 80 degree C for 3 hours in hot air oven.

Detoxification and Neutraliation:

After being soaked in the Sodium hydroxide solution, solids of the slurry were separated using a muslin cloth. First it is washed with tap water and then with distilled water for removal of toxins present in the biomass which may affect the microbial growth and as well as for neutralizing the pretreated biomass. The biomass is continuously washed until the pH of the wash liquid became approximately 6.5–7.0. Once after washing the biomass, it is immediately used for hydrolysis or stored in a air lock covers at 4 degree C

Submerged Fermentation:

Submerged fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml of fermentation medium. The composition of the medium contained the following g/l of distilled water. Corn cob/ wheat bran, 20; Na_2HPO_4, 5; $MgSO_4$, 2; K_2HPO_4, 2; $CaCL_2$, 2; protease peptone, 7.5; $FeSO_4$, 5; $MnSO_4$, 1.6; $ZnSO_4$, 1.4.Before sterilizing, the pH of the media is adjusted to 4.8, so that fungus can have a better growth. The medium was sterilized by autoclaving at 121˚C for 15 min. Sterilized flask was inoculated with 1 ml of the above said inoculum. The culture was tightly plugged with cotton and incubated on a rotator shaker (150 rpm) at 30˚C for 5 days.

Enzyme Extraction:

At the end of submerged fermentation, the culture broth was filtered using a muslin cloth and the filterate was centrifuged at 6000 rpm for 20 min and the supernatant was used as a source of extracellular enzyme.

Estimation of protein in crude extract by lowry's method

Concentration of protein in crude extracellular extract was determined by Lowry's method.[8] Different concentrations of BSA (Bovine Serum Albumin) as standard protein in a range of 0.02- 0.2mg/ml, were reacted with Lowry's reagent C and D and the absorbance at 660nm were read. A standard graph was plotted between concentration of protein in X axis and absorbance at 660nm in Y axis. 100 µl of extract was reacted with Lowry's reagent C and D and absorbance at 660nm was read. This absorbance was compared with the standard graph in order to get the concentration of protein in crude extracellular extract.

Assay for cellulase enzyme

Endoglucanase activity- carboxyl methyl cellulase (CMCase):

Crude cellulase activity was determined by the CMC method of the International Union of Pure and Applied Chemistry.[9] Reaction mixture comprised of 0.5 ml carboxyl methyl cellulose, in 0.05 M citrate buffer at pH 4.8 and 0.5 ml culture filtrate in test tubes. Mixture was incubated at 50 °C for 15min. After incubation 1ml of dinitro-salicylic acid (DNS) reagent was added to stop the reaction. The reactants in test tubes were boiled for 5min in a boiling water bath and transferred to cold water bath[10] Absorbance was measured at 540 nm in calorimeter. Amount of reducing sugar was read off from a curve obtained by plotting value of absorbance against concentration of glucose

Results and Discussion:

Compositional Analysis:

The compositions of the Corn cob and wheat bran were then analyzed by National Renewable Energy Laboratory (NREL)[11] and AOAC[12] method to evaluate cellulose lignin and protein amount.

Protein estimation in crude enzyme by Lowry's method:

Concentration of protein in the extracellular crude extract of corn cobs and wheat bran were determined by comparing the absorbance readings of the test sample with the standard graph. It was found that crude enzyme from corn cob has 0.268 mg/ml of protein concentration. Whereas crude enzyme from wheat bran has 0.178 mg/ml of protein concentration. Figure 1, given below is the standard graph for protein estimation of Lowry's method. Table 2, given below shows the results of protein estimation of corn cob and wheat bran's crude extract.

Estimation of Cellulase enzyme using Carboxy Methyl Cellulase (CMCase) Assay:

After CMC assay, O.D of reaction tube containing crude enzyme from corn cob was 0.38 and wheat bran crude enzyme was 0.26. Thus the liberated concentration of glucose for cob was 0.370 mg/ml and for wheat bran was 0.253 mg/ml which was calculated according to standard graph. Results of the same are depicted in Table 3 and figure 2 represents the standard graph for glucose estimation.

In both Lowry's method and CMC assay, crude enzyme from corn cob has gained better result when compared to wheat bran. This may be due to variation in cellulose composition. In the Table 1, it was clearly depicted that corn cob possess 66% of cellulose whereas wheat bran contains 48% of cellulose only. Enzyme Activity of corn cob was calculated to be 0.0040 U/ml/min. Total activity of enzyme was 0.3825U and specific activity was 0.004U/mg.

Table 1: shows the Cellulose, lignin and protein composition of corn cob and wheat bran

Composition	Corn Cobs	Wheat Bran
Cellulose	66%	48%
Lignin	15.06%	12.8%
Protein	13.2%	11.3%

Figure 1: shows the standard graph for Protein estimation by Lowry's method

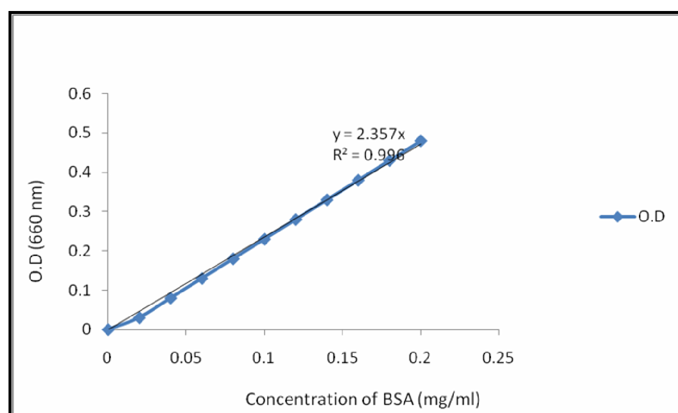


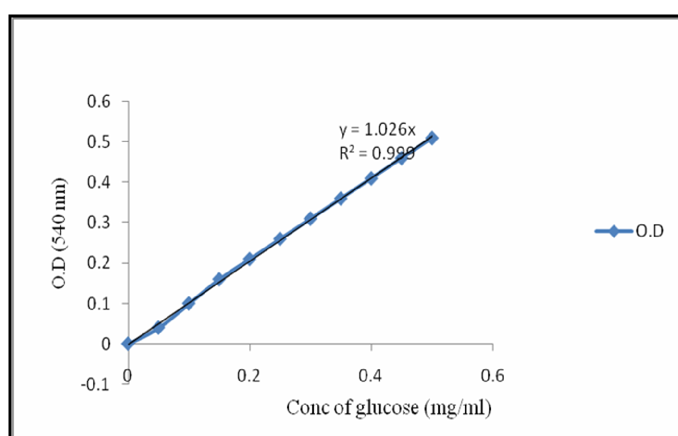
Table 2: shows the corn cob and wheat bran crude enzyme estimation by Lowry's method

<table>
<tr><th>Test tube</th><th>Crude enzyme (ml)</th><th>Dis water (ml)</th><th>Reagent C (ml)</th><th rowspan="4">Incubate for 10 min at 30 degree C</th><th>Reagent D (ml)</th><th rowspan="4">Incubate for 30 min at 30 degree C</th><th>O.D (660 nm)</th><th>Conc of protein (mg/ml)</th></tr>
<tr><td>Blank</td><td>0</td><td>1</td><td>5</td><td>0.5</td><td>0</td><td>0</td></tr>
<tr><td>Cob crude sample</td><td>0.5</td><td>0.5</td><td>5</td><td>0.5</td><td>0.53</td><td>0.268</td></tr>
<tr><td>Bran crude sample</td><td>0.5</td><td>0.5</td><td>5</td><td>0.5</td><td>0.42</td><td>0.178</td></tr>
</table>

Table 3: shows the estimation of Cellulase activity by CMC assay

<table>
<tr><th>S.NO</th><th>1% CMC (ml)</th><th>Vol of Crude enzyme (ml)</th><th rowspan="4">Incubate at 37 degree C for 15 mins</th><th>Vol of DNS (ml)</th><th rowspan="4">Boil the test tubes for 10 mins</th><th>O.D (540 nm)</th><th>Conc of giucose (mg/ml)</th></tr>
<tr><td>1</td><td>0.5</td><td>0.5 (Dis.Water)</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>2</td><td>0.5</td><td>0.5</td><td>1</td><td>0.38</td><td>0.370</td></tr>
<tr><td>3</td><td>0.5</td><td>0.5</td><td>1</td><td>0.26</td><td>0.253</td></tr>
</table>

Figure 2: shows the standard graph for glucose estimation by CMC assay



Conclusion:

From the present study, it was understood that Cheap source of lignocellulosic biomass will result in good yield of celluase enzyme. Corn cob and wheat bran, an agricultural lignocellulosic residues can be used for producing celluloytic enzymes. Among both, corn cob gave a better yield of cellulase enzyme. Hence it is concluded that *Aspergillus niger* induced sodium hydroxide pretreated corn cob under submerged fermentation gives better yield of Endo-glucanase, i.e. Carboxy Methyl Cellulase.

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