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Studies on Production of Cellulase from *Aspergillus niger* as Influenced by different Concentration of Glucose

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AUTHORS CONTRIBUTION

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ABSTRACT

A laboratory experiment was carried out at Department of Biochemistry and Molecular Biology, MGM College of Agricultural Biotechnology, Aurangabad to study the production of cellulase from *Aspergillus niger* as influenced by different concentrations of Glucose (0.25 to 1.50%) under in vitro condition. The experiment was laid out in completely randomized design with six treatments. The amount of protein produced after 21 days of incubation was estimated and Treatment T4 (1% Glucose) shown highest amount of protein (198.3 µg/ml) as compared to other treatments. The activity of cellulase enzyme was highest (103.80 U/ml) at 1% of glucose concentration and found highly significant over rest of treatments.

Keywords : Cellulase, *Aspergillus niger*, Protein, Enzyme activity

CELLULASE breaks down the cellulose molecule into monosaccharides, polysaccharides and oligosaccharide. The cellulose produced mainly by symbiotic bacteria in the ruminating chambers of herbivores that allows them to digest the cellulose from their vegetable diet. Cellulases are also produced by a few other types of organisms, such as some termites. Cellulase is used for commercial food processing in coffee. It performs hydrolysis of cellulose during drying of beans. It is widely used in textile industry and a laundry detergent. They have also been used in the pulp and paper industry for various purposes and they are even used for pharmaceutical application (Abubakar *et al.,* 2013). Cellulose is used in the fermentation of biomass into biofuels. Most fungal cellulases have a two-domain structure, with one catalytic domain and one cellulose binding domain that are connected.

Cellulose is the most abundant and renewable natural product in the biosphere with its estimated synthesis rate of 10^{10} tonnes per year. It is estimated that approximately 20 per cent of the >1 billion US dollars of the world's sale of industrial enzymes consists of cellulases, hemicellulases and pectinases. Since the production of cellulase enzyme is a major process and economically viable, much work has been done on the production of cellulases from lignocellulosics. About 2.9 × 103 million tons of lignocellulosic residues are produced from cereal crops and 3 × 103 million tons from pulse and oil seed crops. In addition, 5.4 × 102 million tons is produced annually from crops worldwide (FAO, 2006) and these materials accumulate in enormous amounts (GOP, 2009).

In many bacteria, cellulases are complex enzyme structures organized in supra molecular complexes, the cellulosomes. They contain roughly five different enzymatic sub units representing namely endocellulases, exocellulases, and oxidative cellulases (Acharya *et al.,* 2008).

Cellulases have been used and studied for most of 20th century and are the most commercially important of all enzyme families. The enzyme activities were increased about 30-80 per cent when produced by SSF (Solid-state fermentation) in comparison with conventional SmF (Submerged fermentation) enzyme

production. Cost of cellulose production may be brought down by multifaceted approaches which include the use of cheap lignocellulosic substrates and the use of cost efficient fermentation strategies like solid state fermentation

The agricultural wastes are composed essentially of cellulosic or lignocellulosic matter. These are considered to be the cheapest source for the production of different utilizable products throughout the world. Cellulose is commonly degraded by enzyme called cellulase. Complete enzymatic hydrolysis of cellulose requires synergistic action of 3 types of enzymes, namely cellobiohydrolases, endoglucanases or carboxymethyl cellulase (CMCase) and B-glucosidases (Bhat, 2000). Cellulases are enzymes which break down cellulose to β–glucose. Cellulases are one of the most useful enzymes in wide range of industrial applications. Cellulases are generally produced by fungi, bacteria or actinomycetes but the most common producer for industrial application is fungi. The cost of cellulases is high due to the high cost of substrates used in production and the slow growth rate of fungi.

Major constrains in enzymatic hydrolysis of cellulosic materials for the production of fermentation sugar are low productivity and the cost of cellulases. The most abundant renewable carbon source is the cellulosic material. An agricultural waste is a cheap source of cellulose for the production of different useful products all over the world. Cellulase production from agrowastes is economical as compared to production from pure cellulose. The hydrolysis of cellulose can be done by using enzymes to produce glucose, which can be used for the production of ethanol, organic acids and other chemicals (Ali *et al.,* 2010). Other applications include cotton processing, paper recycling and as animal feed additives. It is also used for deinking of fiber surfaces in paper industries and to enhance pulp drainage in textile industries.

Material and Methods

The details of materials used and methods adopted for conducting the present investigation is described below.

Table 1
Treatment details

Symbol	Concentration of Glucose % (w/v)
T_1	0.25
T_2	0.50
T_3	0.75
T_4	1.00
T_5	1.25
T_6	1.50

Materials

Aspergillus niger culture, Distilled water, Potato dextrose broth (PDB), NaNo3, KH2PO4, MgSO4.7H2O, KCl, Sodium salt, Peptone, Agar, Congo red solution, NaCl, beakers, Measuring cylinder, Erlenmeyer flasks, pH meter, Incubator, Laminar Air Flow, Autoclave, Weighing balance, Shake flask, Filter paper, Petri plates, etc.

Culture

A fungus *A. niger* was collected from Department of Biochemistry and molecular biotechnology of MGM college of Agricultural Biotechnology, Gandheli, Aurangabad.

Enzyme Production

The fermentation was carried out in 150 ml Erlenmeyer (conical) flask containing 50 ml of basal medium with different concentrations of Glucose (%w/v). The cotton plugged flask was subjected to sterilize in an autoclave at a pressure of 15 lbs inch (121°C) for 15 min. The production medium was cooled at room temperature and spore suspension is to be prepared in sterile distilled water from 6 days old culture of *A. Niger* grown on potato dextrose agar slants. The flask was inoculated for 3 weeks at 28°C on a rotary shaker (180 rpm).

Enzyme Extraction

Crude enzyme was extracted from fermented media by adding 100ml of 100mM Tris buffer having pH 6.2 and agitating the flask in shaking incubator at 180 rpm for 1 hours. The mixture was then filtered and centrifuged at 8000 rpm at 4°C for 5 min. The

supernatant was collected and used for further procedure.

Quantification of Enzyme - The Quantification of total Enzyme was done by F. C. Method

Reagent :

1. Reagent A- 2 per cent sodium carbonate in 0.1N sodium hydroxide
2. Reagent B- 0.5 per cent copper sulphate ($CuSo_4$, $5H_2O$) in 1 per cent potassium sodium tartrate
3. Reagent C- Alkaline copper solution - Mix 50ml of A and 1 ml of B prior to used
4. Reagent D-Folin-cicaltaeu's reagent, Protein working solution

Procedure - 0.2 - 1 ml of standard working solution was pipetted out into series of test tubes. Then 0.1 ml and 0.2 ml of the sample extract was pipetted out into different test tubes. The volume in all test tubes was made 1 ml with distilled water. A tube with 1 ml of water serves as blank. Then 5 ml alkaline copper reagent was added to each tube including the blank. It was mixed well and allowed to stand for 10 min. Then 0.5 ml of FC reagent was added in each test tube and mixed well. All test tubes were incubated at room temperature, in dark for 30 min, blue colour was developed. The optical density was measured at 540 nm by using spectrophotometer. A standard graph was drawn and the amount of total enzyme in the sample was calculated.

***Determination of Enzyme Activity* :- The Enzyme Activity Measured by DNS Method**

Chemicals Required : Phosphate buffer, Cellulose (1% w/v), Dinitrosalicylic acid (DNS), Glucose solution.

Procedure

The dry test tubes were taken and marked them. In each test tubes 0.5 ml enzyme and 0.5 ml of substrate was added. Then test tubes were incubated for 5 min at room temperature. After 5 min the reaction was arrested by pipetting 1 ml of DNS solution in all the tubes and mixed well. Then test tubes were incubated in boiling water bath for 15 min. After 15 min of incubation 5 ml of distilled water was added in all the test tubes and O.D was taken at 540 nm. The readings were tabulated and using standard glucose curve and the activity of cellulase was determined.

Results and Discussion

The extraction, quantification and effect of different Glucose concentrations on production of cellulase enzyme was determined. By using glucose as standard, OD were taken from spectrophotometer at 540 nm and standard glucose calibration curve was prepared.

Estimation of Protein

F.C. is sensitive method of estimating protein. Here, a complex reagent known Folin reagent was used and it contains phosphomolybdic acid and tungstate. The reaction takes place with tyrosine and tryptophan residue of protein and form a blue colour. This colour was enhanced by cupric ions.

The amount of protein produced after 21 days of incubation is presented in Table 2. Treatment T4 (1% Glucose) shown highest amount of protein (198.3 µg/ml) as compared to other treatments.

Table 2

Estimation of protein by Lowry's method

Treatment	Amount of protein (µg/ml)
T_1	147.1
T_2	154.9
T_3	177.7
T_4	198.3
T_5	171
T_6	149.8

Activity of cellulase Enzyme

Enzyme activity is a measure of substrate lost or product gained per unit time. Cellulase is an enzyme that cleaves the glycosidic bond in a substrate cellulose and yield glucose as product. The extent of cleavage *i.e.,* the activity of cellulase enzyme was determined by using glucose calibration curve.

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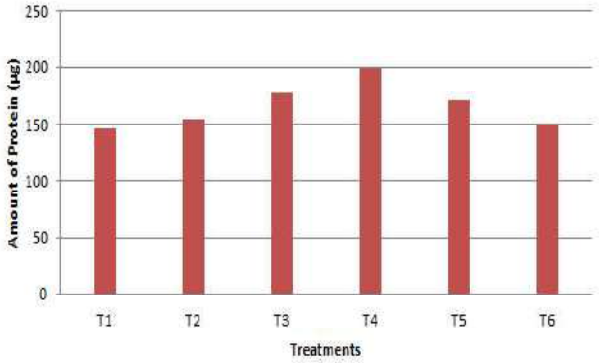

Fig. 1 : Amount of protein (µg/ml) produced after 21 days of incubation

Data presented in Table 3 would indicate that the activity of cellulase enzyme influenced significantly due to different concentrations of Glucose. The activity of cellulase enzyme was highest (103.80 U/ml) at T4 (1%) and found highly significant over the rest of treatments. The treatment T3 (86.20 U/ml) and T5 (84.66 U/ml) are significantly superior over the treatments T2, T6 and T1. However, treatment T2 (71.36 U/ml) and T6 (71.76 U/ml) are significantly superior over T1. The treatment T1 (53.76 U/ml) did not differ significantly.

TABLE 3

Effect of different Glucose concentrations on cellulase enzyme activity

Treatment (Glucose conc)	Activity of Enzyme (U/ml)
T_1 (0.25%)	53.76
T_2 (0.50%)	71.36
T_3 (0.75%)	86.20
T_4 (1.00%)	103.80
T_5 (1.25%)	84.66
T_6 (1.50%)	71.76
Mean	78.59
CD	4.86
SE ±	1.57

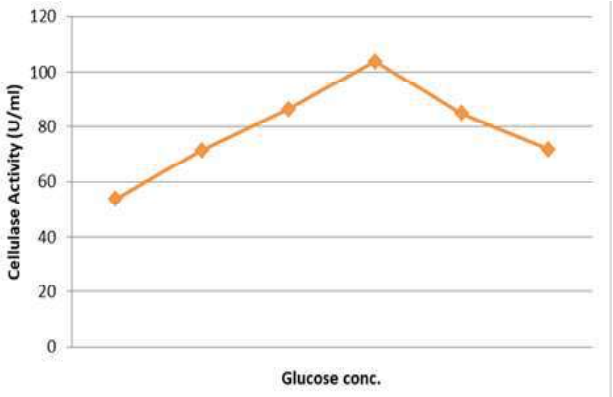

Fig. 2 : Effect of different glucose concentrations on activity of Cellulase enzyme

From the above results it can be revealed that the activity of cellulase enzyme increases with increase in concentrations. The activity of enzyme was maximum at (1%) of Glucose concentration. The activity of enzyme decreased with increase or decrease in Glucose concentration of 1 per cent.

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