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Cellulase Production from *Aspergillus niger* using Paddy Straw as a Substrate and Immobilization

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ABSTRACT

Conversion of lignocellulosic waste to sugars is important application of microbial cellulases. In objective of the present study is isolated the Aspergillus niger from spoiled coconut and produce cellulase enzyme using paddy straw as the substrate. The activity was evaluated using CMC method, the growth of the fungi was high at 100 mg/ml glucose concentration (0.65 at 540 nm) and Optimization of cellulase production was done by using various physical parameters (Temperature, pH). Cellulase production was maximum at the temperature 20°C and minimum at 40ºC. The optimal pH for the cellulase production was observed maximum in 5.5 and minimum in 7.0. Enzyme was immobilized using different size of alginate beads. The efficiency of enzyme high in 3.0 mm (90.93%) size of the beads.

Key words: *Cellulase, Aspergillus niger, Immobilization, Lignocellulose.*

INTRODUCTION

Agriculture wastes and all lignocellulosic materials can be converted into products that are of commercial interest such as ethanol, glucose, and single cell protein. Lignocelluloses are abundant sources of carbohydrate, continually replenished by photosynthetic reduction of carbon dioxide by sunlight energy[1]. Cellulose is the major component of biomass which is abundantly available in the form of municipal, agricultural and forest wastes, is promising as a renewable source of many important products. The bioconversion of cellulosic materials has been receiving attention in recent years. In India, 140 million tonnes of paddy straw which contain huge amount of lignocellulosic content are produced annually (FAO, 2016), half of the paddy straw are burned by farmers it cause the environment illness. Cellulase is an enzyme, which acts serially to decompose cellulosic material into simple sugar. Microbial cellulase have potential application in various industries, including Pulp and Paper, Textile, Laundry, Biofuel Production, Food Industry, Brewing and Agriculture.

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Cellulase are produced mainly by fungi because bacteria utilize cellulose by cell bound enzymes, but fungi secrete cellulase into the growth medium.

Most commonly studied cellulolytic fungi are *Trichoderma, Humicola, Penicillium* and *Aspergillus*[7]. Paddy straw consist the suitable solid state substrate for *Aspergillus niger*. The immobilisation is an enzyme attached to an inert, insoluble material such as calcium alginate (produce by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to change in conditions such as pH or temperature. Enzymes stabilization (immobilization) is important for many industrial applications of enzymes[6] . In lieu of the above justification, the present endeavour was to isolate the enzyme, optimization of enzyme in different culture condition and immobilization of enzyme.

MATERIALS AND METHODS

Isolation and identification of fungi:

A loopful of the fungal mycelium was taken from the spoiled coconut and inoculated on the potato dextrose agar medium and incubated at room temperature for three successive days. After the incubation period the fungal mycelium was taken by inoculating loop and placed on a clean glass slide. A drop of Lactophenol cotton blue strain was added to the mycelium and covered with a cover slip. Then observed under microscope.

Inoculums preparation:

The composition of production medium used was paddy straw-5g, KCl-2g, KH2PO4-1g, MgSO4-0.05g, FeSO4-0.02g and Distilled water-1000ml. The pH was adjusted to 6.5 and the media was sterilized in an autoclave for 15 min at 121°C. The media were inoculated with a loop full of spore suspension of *Aspergillus niger* and then incubated in 30ºC in an orbital shaker set at 100 rpm for 72 hours. The media were centrifuged at 5000 rpm for 15 minutes to obtain crude enzyme solution.

Cellulase assay:

Cellulase activity was determined using 1% (w/v) CMC as a substrate. A reaction mixture was composed of 0.45 ml of 1% (w/v) CMC in 0.1 M acetate buffer (pH 4), 0.15 ml of acetate buffer and 0.4 ml of culture supernatant, was incubated at 37 °C for 30 min. The reducing sugar was determined by Nelson Somogyi's method using glucose as standard.

Optimization of culture condition:

The factors such as pH, temperature production of cellulase were optimized.

pH:

To study the pH stability of the cellulase enzyme production, an experiment conducted in a 200ml Erlenmeyer flask containing production medium, and the pH was adjusted at different conditions (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0) and then the *Aspergillus niger* culture was inoculated. After 24 hours, the Optical Density (O.D) was taken at 540nm and the OD values were noted.

Temperature:

To study the heat stability of the cellulase enzyme production, an experiment conducted in a 200ml Erlenmeyer flask containing production medium. After sterilization by autoclaving, the flask were cooled and inoculated with *Aspergillus niger* culture and maintained at different temperature (20ºC, 25 ºC, 30ºC, 35 ºC and 40ºC). The Optical Density (O.D) was taken at 540nm after 24 hours and the OD values were noted.

Immobilization of enzyme

An equal volume of enzyme and 4% sodium alginate was mixed. The mixture was extruded drop wise through a burette, into 0.2M $CaCl_2$ at 4ºC from a height of 2cm, the beads were left for 20min and efficiency of enzyme was evaluated with different bead size such as 2.0, 2.5, 3.0[6]

RESULT AND DISCUSSION

Table 1: Absorbance of glucose at 540nm

Sl.no	Glucose concentration mg/ml	O.D
1	10.00	0.08
2	20.00	0.15
3	30.00	0.21
4	40.00	0.28
5	50.00	0.32
6	60.00	0.41
7	70.00	0.43
8	80.00	0.47
9	90.00	0.58
10	100.00	0.65

The fungi *Aspergillus niger* was isolated and identified from the spoiled coconut. Cellulase activity was determined at 37°C by using carboxymethyl cellulose as a substrate and the results were showed in Table-1. The growth of the fungi *Aspergillus niger* was high at 100 mg/ml glucose concentration (0.65 at 540 nm) and very low at 10 mg/ml glucose concentration (0.08 at 540 nm). The growth of the fungi was increased when the concentration of glucose was increased.

Table 2: optimization of growth pH for Cellulase production

PH value	Enzyme activity (IU/g-ds)
4.0	0.06
4.5	0.09
5.0	0.13
5.5	0.18
6.0	0.10
6.5	0.05
7.0	0.04

The cellulase enzyme activity was carried out under different optimization conditions. The table -2 indicates the activity of cellulase was high at pH 5.5. The optimum enzyme activity was 5.0 to 6.5.The lowest activity recorded in pH 7.0. Acharya *et al.*[4] and Gupta *et al.*,[3] have reported the maximum cellulase production at pH 4.5 and 6.0 respectively by *A. niger*

Table 3: optimization of growth temperature for Cellulase production

Temperature	Enzyme activity(IU/g-ds)
20.00	0.18
25.00	0.17
30.00	0.15
35.00	0.10
40.00	0.09

The effect of temperature on the activity of the cellulase was determined at various temperatures ranging from 20, 25, 30, 35 and 40°C and the results were showed in table-3. The cellulase activity was maximum at 20°C and minimum at 40ºC[5] .

Table 4: Immobilized enzyme efficiency at different bead size

Bead diameter (mm)	Efficiency (%)
2.0	83.58
2.5	86.15
3.0	90.93

Table -4 shows that the efficiency of enzyme increased with increasing the size of the beads. The enzyme efficiency was high in 3.0 mm (90.93%). Tran Quoc Viet *et al.,* 2013 study revealed that the enzyme efficiency increase with size.

SUMMARY

Most of the microorganisms can capable to produce various extracellular and intracellular enzymes using various cheap sources. The effect of enzyme activity is higher than the plant derived enzymes. Cellulase is an extracellular enzyme, which is produced from various organisms including bacteria, fungi, and also some Actinomycetes. Research on cellulase has progressed very rapidly over the last five decades and potential industrial applications of the enzyme especially in solid waste management have been identified. In the present study the enzyme was isolated and optimized in different parameters. Stabilization of enzyme is most important to increase the stability of enzyme.

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