Production of Cellulases by Aspergillus Heteromorphus from Wheat Straw under Submerged Fermentation

Anita Singh, Namita Singh and Narsi R. Bishnoi

Abstract—To investigate the production of cellulases from *Aspergillus heteromorphus*, submerged fermentation was performed using wheat straw as substrate. Optimization of saccharification conditions like pH, temperature and time were studied. Highest reducing sugar was released on 5th day at 5 pH, 30°C temperature. When *A. heteromorphus* was grown on wheat straw in submerged fermentation after 5 days incubation at 30°C, 3.2 IU/ml and 83 IU/ml filter paper activity and CMCase activity respectively.

Keywords—Aspergillus heteromorphus, Wheat Straw, Submerged Fermentation, Production of Cellulases

I. INTRODUCTION

LIGNOCELLULOSIC biomass is the most abundant organic raw material in the world [1]. Lignocelluloses constitute a major portion of agricultural wastes and forest wastes. Thus they are the most promising feedstock for the production of energy, food and chemicals and their utilization could allow self-sustainable processes and products. The utilization of cellulolic biomass continues to be a subject of worldwide interest in view of fast depletion of our oil reserves and food shortages [2]. The bioconversion of the agro waste material into fuel has received considerable interest during recent years. This lignocellulosic material was in abundant and available in free of cost. Enzymatic hydrolysis of cellulolic biomass is considered as the most efficient and least polluting methods for generating glucose from lignocelluloses, but the production economics of bioethanol is largely dependent on cost of cellulases [3]. Cellulases comprise a complex of enzymes involved in the natural degradation of cellulose, the major polysaccharide of plant cells. The enzymatic complex can convert the cellulose to oligosaccharides and glucose. Microorganisms such as fungi and bacteria are important producers of cellulases. Substrate costs account for a major fraction of the costs of cellulase production, and the use of cheap biomass resources as substrates can help to reduce cellulase prices [4]. The use of agro-industrial residues as the basis for cultivation media is a matter of great interest, aiming to decrease the costs of enzyme production and meeting the increase in awareness on energy conservation and recycling. The conversion of cellulolic mass to fermentable sugars through biocatalyst cellulase derived from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce use of fossil fuels and reduce environmental pollution [5], [6]. Cellulase production by different organisms in submerged state fermentation has received more attention and is found to be cost-prohibitive because of high cost of process engineering. India is an agricultural country and wheat is one of the most important agricultural crop in India. Every year nearly 78.4 Million Tones wheat is produced. With the processing of wheat grains a large amount of straw is produced which can be used as a substrate for ethanol production. Currently a most important application of cellulases and hemicellulases in the pulp and paper industry is the biobleaching of pulp, the production of dissolving pulp, the treatment of wastewater and the deinking of recycled paper. The potential of enzymatic treatments has been assessed and the processes have proved successful [7], [8].

In the present study the production of cellulolytic enzymes by a local isolate of *A. heteromorphus* on lignocelluloses in submerged fermentation is reported.

II. MATERIAL AND METHODS

A. Microorganism and Material

*Aspergillus heteromorphus* MTCC 8624 was used for the present study. The strain was cultivated on potato dextrose agar (PDA) at 28°C for 7 days. Wheat straw was used as a substrate for enzyme production. All the experiments were performed in triplicates and the average values were calculated.

B. Fermentation

2 g wheat straw, 50 ml of Mandel and Sternberg’s mineral media [9]: 1.4g/l (NH₄)₂SO₄, 2.0 g/l KH₂PO₄, 0.3 g/l CaCl₂, 0.3 mg/l MgSO₄·7H₂O, 7H₂O, 5.0 mg/l FeSO₄·7 H₂O, 1.6 mg/l MnSO₄·7H₂O, 1.4 mg/l ZnSO₄·7H₂O, 2.0 mg/l CoCl₂·6H₂O. The initial pH of the medium was adjusted to 5 before being autoclaved at 121°C for 15min. After cooling the flasks were inoculated with an amount initially containing 2 X 10⁸ spores per gram of substrate and the contents, after mixing, were incubated at 30°C on an orbital shaking bed at 120 rpm for 7 days and removed after 0.2,3,4,5,6,7 days.

C. Standardization of Conditions for Saccharification

To determine the optimum saccharification conditions of...
pH, temperature and time, the above mention reaction mixture was incubated from 3 to 8 pH, from 20 to 45 °C temperature and for 10 days at 115 ± 5 °C rpm. After this reducing sugar was analyzed by DNS method [10] for optimum conditions.

The percentage saccharification was calculated as:

\[
\text{Saccharification} \% = \frac{\text{Glucose (mg/ml)}}{\text{Substrate (mg/ml)}} \times 100
\]

D. Enzyme Assays

Filter paper assay was used to estimate total cellulase activity and endoglucanase activity (CMCase activity) [11] in the crude enzyme preparation as given below. For filter paper activity Whatman no. 1 filter paper strip of dimension 1.0 × 6 cm (50 mg) was placed into each assay tube. The filter paper strip was saturated with 1.0 ml of Na–citrate buffer (0.05 M, pH 4.8) and was temperate for 10 min at 50 °C. Half milliliter of an appropriately diluted (in Na–citrate buffer, 0.05M; pH 4.8) enzyme was added to the tube and incubated at 50 °C for 60 min. In case of endoglucanase activity half milliliter of 1 % carboxymethyl cellulose in 0.05M Na–citrate buffer, pH 4.8 was temperate for 10 min at 50 °C. After that half milliliter of an appropriately diluted enzyme was added to the tube and incubated at 50 °C for 30 min. Appropriate controls were also run along with the test. At the end of the incubation period, tubes were removed from the water bath, and the reaction was stopped by addition of 3 ml of 3, 5-dinitrosalicylic acid reagent per tube. The tubes were incubated for 5 min in a boiling water bath for color development and were cooled rapidly. The reaction mixture was diluted appropriately and was measured against a reagent blank at 540 nm in a UV-VIS spectrophotometer. The concentration of glucose released by enzyme was determined by comparing against a standard curve constructed similarly with known concentrations of glucose. One unit of enzyme activity was defined as the amount of enzyme required for liberating 1 μM of glucose per milliliter per minute and was expressed as U/ml.

III. RESULT AND DISCUSSION

The recent thrust in bioconversion of lignocellulosic biomass to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by bacteria and fungi. Though the growth period of bacteria is shorter than that of fungi, their half- backed cellulase system makes them less useful in the industrial production of cellulase. However, high cost of cellulases production hindered use of this enzyme in industry. For the utilization of lignocellulosic biomass, it is necessary step to enhance the cellulose production and reduces its production cost. The use of purified cellulolases as substrate is uneconomical for large scale production of cellulases. Therefore cheaply available agricultural lignocellulose waste materials as solid matrix, different cultural practices and different organisms. The decrease in both cellulase activities may be due to the accumulative effect of cellulobiose. Cellulobiose is a dimer of glucose which is known to inhibit both endoglucanase and glucosidase. The time of the highest cellulase activity depends upon the substrate and fungus used as studied by Ojumu [18] and Alam [19]. But in time, it decreases until the end of the fermentation period. As discussed by Hatakka [20] delignification produces aromatic water soluble products which may repress the cellulolytic action of the enzyme.

There were some applications of cellulases and hemicellulases. Considine [21] used the enzyme extracts of solid state fermentation of P. capsulatum for the saccharification of beet pulp. According to studies of Gonzalez [22], higher enzymatic activities (FPA and β-
glucosidase) were obtained with growth of *Trichoderma reesei* GM 9414 on wheat straw rather than on solka-floc as carbon source. Okeke and Obi [23] reported that the highest degree of hydrolysis was observed with melon seed shells in the study of saccharification of agro-waste materials by cellulases and hemicellulases. So the possibility of the use of cellulases produced by *A. heteromorphus* and wheat straw as substrate will be vast.

![Figure 1](image1.png)

**Fig. 1** Effect of Time (Days) on saccharification of Wheat straw: (♦) Release sugars (mg/ml); (●) % Saccharification

![Figure 2](image2.png)

**Fig. 2** Effect of pH on saccharification of Wheat straw: (♦) Release sugars (mg/ml); (●) % Saccharification

![Figure 3](image3.png)

**Fig. 3** Effect of Temperature (°C) on saccharification of Wheat straw: (♦) Release sugars (mg/ml); (●) % Saccharification

![Figure 4](image4.png)

**Fig. 4** Time course profile of enzyme production by *A. heteromorphus* on wheat straw under SSF condition (♦) CMCase (IU/ml); (●) FPase (IU/ml)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Enzyme activities (IU g-1)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Rice straw</td>
<td>0.96 0.606</td>
<td>Narasimha et al. 2006</td>
</tr>
<tr>
<td><em>T. reesei</em></td>
<td>Saw-dust</td>
<td>1.52 0.627</td>
<td>Muthuvelayudham and Viruthagir, 2006</td>
</tr>
<tr>
<td></td>
<td>Sugarcane bagasse</td>
<td>5.66 68</td>
<td>This work</td>
</tr>
<tr>
<td><em>Aspergillus heteromorphus</em></td>
<td>Rice straw</td>
<td>5.26 44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat straw</td>
<td>3.2 83</td>
<td></td>
</tr>
</tbody>
</table>

Filter paperase (FPase) is expressed in terms of filter paper units. One unit is the amount of enzyme in the culture filtrate releasing 1 μmole of reducing sugar from filter paper per min.

Carboxymethyl cellulase (CMCase) is expressed in terms of units. One unit is the amount of enzyme releasing 1 μmole of reducing sugar from carboxymethyl cellulose per min.

**IV. CONCLUSION**

The production of cellulases on wheat straw under submerged fermentation was studied by *Aspergillus heteromorphus*. Wheat straw is a cheap residue which can be used as a substrate for enzyme production which reduces the cost of enzyme production and enzymatic conversion of carbohydrate part of wheat straw into fermentable sugar.

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**REFERENCES**


