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Full Length Research Paper

Exploration of indigenous agrowastes for cellulase production by *Aspergillus niger*

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Regional agrowastes such as *Vigna mungo, Saccharum spontaneum* and *Brassica campestris* were collected and biohydrolysis of these substrates for cellulase production were carried out by *Aspergillus niger*. Proximate composition of each agrowastes was analyzed based on dry weight, to have an insight view of their chemical composition. Cellulose content of *S. spontaneum* and *B. campestris* was calculated as 43.1 ± 2.8 and 39.4 ± 3.1%, respectively, while cellulase activity of *A. niger* was found to be higher on *S. spontaneum.* Low lignin to cellulose ratio of *S. spontaneum* made it a preferred substrate for cellulase production. However, high lignin content of *B. campestris* made the cellulose inaccessible and resulted in poor yield of enzyme. Therefore, *S. spontaneum* has a great potential to serve as a cheaper, easily available and reasonable substrate for cellulase production.

Key words: Agrowastes, cellulase, indigenous, *Vigna mungo, Saccharum spontaneum*, *Brassica campestris*.

INTRODUCTION

Agrowastes are the most abundant and renewable material produced on earth. Large quantities of agro-wastes are obtained from forests, agricultural practices, and industrial processes, particularly from agro-allied based industries such as breweries, paper and pulp, textile and timber industries. These wastes generally accumulate in the environment as pollutants (Abu et al., 2000). About 2.9×10^3 million tons of lignocellulosic residues are produced from cereal crops and 3×10^3 million tons from pulse and oil seed crops. In addition, 5.4×10^2 million tons are produced annually from crops worldwide (FAO, 2006) and these materials accumulate in enormous amounts (GOP, 2009). Enzyme production from lignocellulosic biomass through the biological route seems to be very attractive and sustainable due to several reasons, the major being the renewable and ubiquitous nature of biomass and its non-competitiveness with food crops (Singhania et al., 2010). The poly-saccharide component of agrowastes includes cellulose and hemicellulose. Cellulose is produced in copious amounts in biosphere (100 billion dry tons/year). It is a linearly condensated polymer consisting of D-anhydroglucopyranose joined by β-1, 4- glycosidic bonds (Zhang and Lynd, 2004).

The cellulose molecule is very stable, with a half-life of 5 to 8 million years for β-glucosidic bond cleavage at 25°C (Wolfenden and Snider, 2001). Its degradation represents a major carbon flow from fixed carbon sinks and is very important in several agricultural and waste treatment processes (Schloss et al., 2005). Cellulases are the third largest industrial enzyme in the world, which is also gaining rejuvenated interests due to its applications (Singhania et al., 2010).

In the present studies, the potential and utility of local agrowastes were investigated for cellulase production in solid state fermentation by *Aspergillus niger*.

MATERIALS AND METHODS

The residual parts of *Vigna mungo, Saccharum spontaneum* and *Brassica campestris* were collected from Gujrat, Pakistan. The raw materials were sun-dried, chopped and materials were ground individually in a hammer mill, and then sieved by maintaining 2 mm mesh size. Sieved samples served as substrate for enzyme production. The proximate analysis including ash, moisture, cellulose, hemicelluloses, and lignin content of each substrate was

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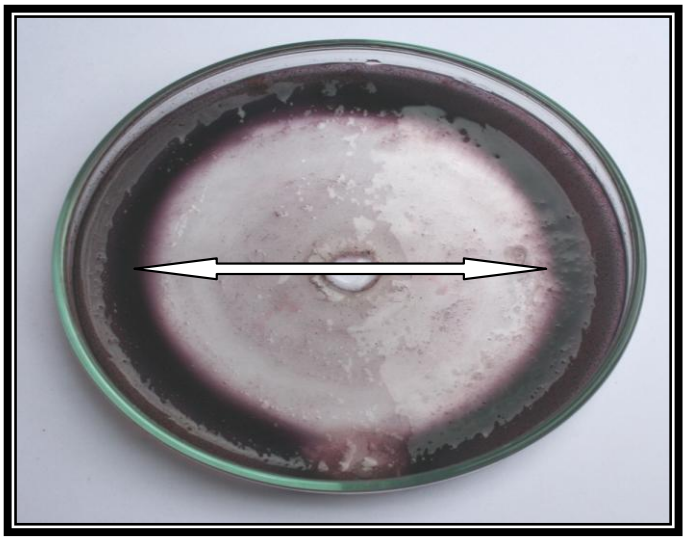

Figure 1. The halo zone (indicated with arrow head) evidence of cellulolytic ability of *A. niger* on screening media.

Table 1. Comparative Proximate Analysis of Substrates.

Parameter	Comparative proximate composition (%)		
	V. mungo	***S. spontaneum***	***B. campestris***
Ash	5.1 ±1.2[b]	6.2 ±0.9[a]	4.2 ±0.7[c]
Cellulose	26.8 ±2.3[b]	43.1 ±2.8[a]	39.4 ±3.1[a]
Lignin	23.14 ±2.1[a]	21.34 ±2.4[b]	24.58 ±2.8[a]
Hemicellulose	32.48 ±3.0[a]	20.41 ±2.6[b]	22.4 ±3.2[b]
Ether extract	2.83 ±1.4[b]	2.5 ±1.5[c]	4.2 ±1.2[a]
Crude protein	16 ±0.8[a]	11.82 ±0.5[b]	10.2 ±0.6[b]

analyzed by following AACC, 2000. Crude protein was estimated by using Kjeldahl's method (Hiller et al., 1984). The lignin content was determined by following the procedure of Gopal and Ranjahan (1980). Soxhlet apparatus as given in AACC (2000) was used to measure the fat content.

A. niger was isolated from corn cob and maintained on PDA slants at 4°C after its purification and identification. The culture of *A. niger* was identified on the bases of its morphological and microscopic characters as described by Raper and Fennel (1965). The cellulolytic ability of *A. niger* was confirmed on carboxymethylcellulose agar (CMC) medium as described by Onsori et al. (2005). 4 µl of spore suspension (~1 × 10^3 cells/ml) of *A. niger* were added in the small well of solidified screening plate and incubated for 48 h at 28°C. Afterwards, it was stained with 1% Congo red for 15 min and then de-stained with 1 M NaCl solution for 10 min. A clarified zone, which appeared in the center of screening plate (Figure 1), indicated cellulolytic ability of *A. niger.* Salt medium with composition as described by Juhasz et al. (2005) was used. Non-optimized solid state fermentation was carried out with 5 g of each substrate separately in Erlenmeyer flasks (250 ml), moistened with 5 ml of salt medium and 10 ml of distilled water to give 80% moisture at initial and autoclaved at 121°C for 30 min. The pH of fermentation medium was adjusted to 6 prior to sterilization. Autoclaved flasks were inoculated with 1 ml of spore suspension (~1 × 10^6) of *A. niger* and incubated for six days under static condition at 28 ± 2°C.

The crude enzyme was extracted by adding 5 ml of distilled water per gram of fermented substrate and stirred at 100 rpm for 60 min. This was then filtered and centrifuged at 13,000 rpm for 10 min at 4°C. Carboxymethyl cellulase (CMCase) activity of supernatant was determined following the method of Acharya et al. (2008). The amount of reducing sugar released was calculated by using 3,5-dinitrosalicyclic acid (DNSA) (Miller, 1959). One IU (International Unit) was defined as the amount of glucose (mM) released/min/ml of enzyme solution. The data were statistically analyzed by analysis of variance (ANOVA) and Duncan's multiple range tests using software package Co-stat version 3.03 at the significance level P = 0.05.

RESULTS

Various regional agrowastes were screened for their ability to serve as substrate for cellulase production in solid state fermentation by *A. niger* and the comparative proximate composition on dry weight basis of the collected substrates, *V. mungo, S. spontaneum* and *B. campestris* is illustrated in Table 1. The data demonstrate that *S. spontaneum* and *B. campestris* had higher cellulose content; 43.1 ± 2.8 and 39.4 ± 3.1%, as

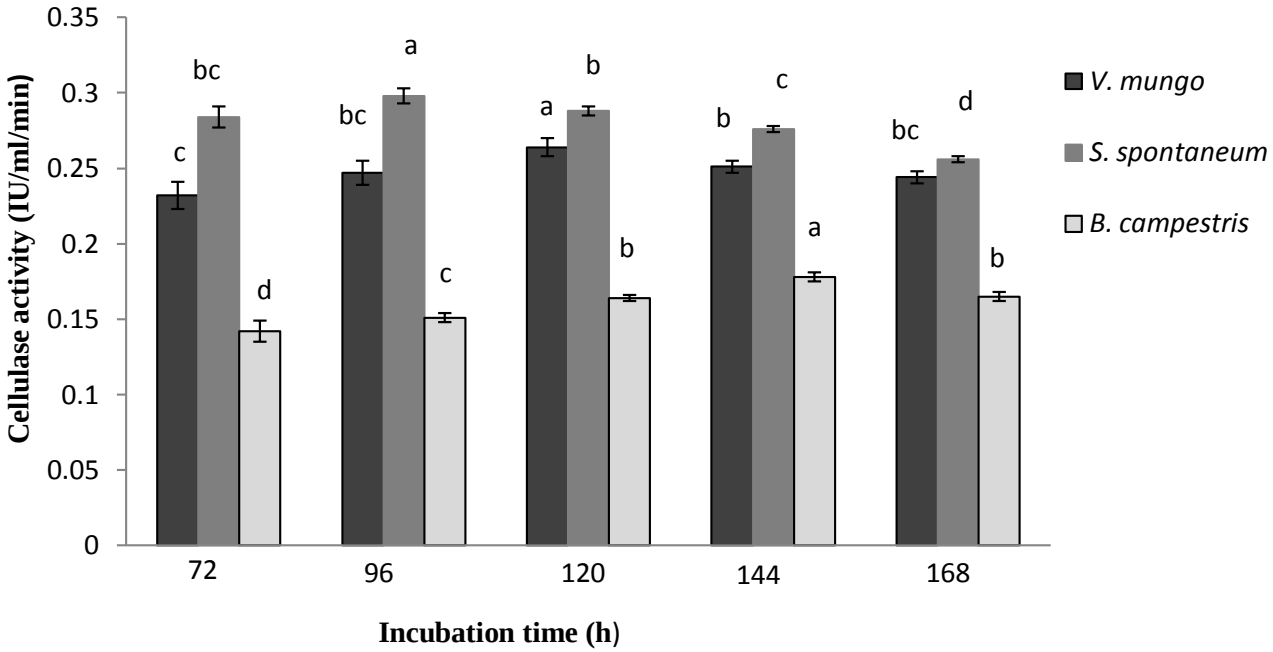

Figure 2. Screening of different substrates by action of *A. niger* for their cellulolytic potential.

compared to *V. mungo* (26.8 ± 2.3%). Least lignin content was obtained in *S. spontaneum* (21.34 ± 2.4%) among the tested substrates. However, lignin content of *B. campestris* and *V. mungo* was not significantly different. Moreover, the highest content of crude protein and hemicellulose was found in *V. mungo* as 16 ± 0.8 and 32.48 ± 3.0%, respectively. All collected substrates were evaluated separately to screen out the best substrate for cellulase production by *A. niger.* Maximum cellulase activity of *A. niger* was found on *S. spontaneum* with optimum incubation of four days as shown in Figure 2. However, enzyme activity of *A. niger* on *V. mungo* was highest on the 5th day of incubation. Biohydrolysis of *B. campestris* gave maximum cellulase activity (0.780 IU/ml/min) after six days of fermentation, while maximum activity was found on *S. spontaneum* (0.298 IU/ml/min) (Figure 1).

DISCUSSION

The indigenous agrowastes such as residual parts of *V. mungo* (common name: Maash daal, family: Fabaceae), *S. spontaneum* (common name: Surkanda, family: Poaceae) and the remains of *B. campestris* (common name: Sarsoon, family: Brassicaceae) were collected as substrate because they were in abundance and easily available. These were ground into particle form to provide a larger surface area for microbial attack and better aeration. Proximate analysis of *S. spontaneum* was calculated in percentage (%) as cellulose (43.1 ± 2.8); lignin (21.34 ± 2.4); ash (6.2 ± 0.9); protein (11.82 ± 0.5%). The obtained data for proximate composition of *S. spontaneum* showed similarities with the reports of Chandel et al. (2009) and Deka et al. (2002). However, a little change in proximate composition of plants belonging to same species, but from different localities might be due to variation in soil and environmental conditions.

Biohydrolysis of agrowastes for cellulase production was carried out with *A. niger.* The maximum cellulase activity by *A. niger* was found on *S. spontaneum* in comparison to *V. mungo* and *B. campestris.* Moreover, same isolate of *A. niger* gave different enzyme activities on different substrate, which indicated that the difference in substrates chemistry is also an important parameter of solid state fermentation. Proximate analysis gives an insight picture of chemical composition of plant material. Generally, the production of cellulases and hemi-cellulases have been shown to be inducible and effected by the nature of substrate (Kang et al., 2004). However, cellulose content was found to be higher in both *S. spontaneum* and *B. campestris*, although high lignin content in *B. campestris* (24.58 ± 2.8%) made cellulose inaccessible for the action of cellulase. Lignin provides compressive strength, stiffens the cell wall and protects the carbohydrates from chemical and physical damage (Saheb and Jog, 1999).

Conclusion

The results highlight that *S. spontaneum* has potential to be an indigenous source of cellulase production. Low lignin but high cellulose content makes it an ideal

substrate for cellulase production, although *V. mungo* with high hemicellulose content (32.48 ± 3.0%) might serve as a good substrate for hemicellulases production. Hence, there is need to optimize the physicochemical parameters for the enhanced production of cellulase from *A. niger* using *S. spontaneum* as sole carbon source in solid state fermentation.

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