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RESEARCH ARTICLE

BIODEGRADATION OF LIGNIN AND CELLULOSE IN SOLID STATE FERMENTATION OF SUGAR CANE BAGASSE BY AN ASCOMYCOTA FUNGUS

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ABSTRACT

Sugarcane bagasses are rich in cellulose and lignin content whose degradation is the main problem for industries utilizing them for various purposes. The present investigation is based on application of fungi in degradation of lignin and cellulose from sugarcane bagasse. Degradation was estimated after 3 weeks of solid state fermentation of sugarcane bagasse by *Aspergillus niger*. Fermentation process was divided into two parts i.e., culture media fortified with different concentrations of nutrient supplements and not fortified with any kind of supplements. The result reveals that maximum loss of cellulose was 14.2% (without fortification) and of lignin was 26.2% (with 5% glucose fortification). The initial cellulose and lignin content was reported to be 58.5 and 18.4 respectively.

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INTRODUCTION

Sugar cane bagasse is a secondary by product of sugar cane mills. They are used in the manufacture of pressed fibrous woods, paper pulp and as fuel (Barnes, 1980). Considering the world economy that has been dominated by technologies that depend on fossil energy, (Hartman and Meshbesher, 2009) are continuously depleting the naturally stored form of this energy. Biomass is a potential renewable energy source that could replace fossil energy. Lignocellulosic biomass is one among them which can be used to produce alternative liquid fuel sources. However, the production cost of liquid fuel such as ethanol from lignocellulosic biomass is higher, primarily because of high cost for cellulosic separation and hydrolysis. (Buswell and Odier, 1987). The structure of the lignocellulosic biomass is the main problem which composes mainly of cellulose, hemicellulose and lignin (Fox *et. al*., 1987; Sun and Cheng, 2002). The cellulose fibers are mainly embedded in an amorphous matrix of hemicellulose and lignin (Pareek, 2000). A large number of bacteria, actinomycetes, and filamentous fungi possess the ability to degrade cellulose and lignin. These organisms have in common the ability to produce extra cellular hydrolytic enzymes that attack the cellulose polymer. *Aspergillus* species is one of the most common fungi in man's environment. Some selected species of *Aspergillus* capable of utilizing an enormous variety of substrates for food because of the variety of enzymes they produce (Alexopoulos and Mims, 1979). The fungi has been reported for enzyme production to its good fermentation capabilities, high levels of protein secretion, production of a wide range of enzymes for degradation of plant cell wall polysaccharides (de Vries and Visser, 2001). This work has been done to see the degradation - effect of *Aspergillus niger*, a member of Ascomycetes on sugarcane bagasse with and without exogenous nutrient supplements.

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MATERIALS AND METHODS

Microorganisms Culture

Aspergillus niger belonging to Ascomycota was obtained from Dept. of Microbiology, I.A.R.I., New Delhi, India. The typed cultures of fungus were sub-cultured on Potato Dextrose Agar (PDA) slants and stored at 4^0C until required for study.

Inoculum Preparation

To prepare suspension, spores of *Aspergillus niger* were washed from seven day agar slant culture with 10ml sterile distilled water.

Substrate Treatment

Bagasse was kindly provided by the Simbhaoli Sugars Factory, Ghaziabad. Bagasses were chopped into 4-5 cm pieces. They were soaked into boiling water for 10 minutes, and excess water was drained off. For biodegradation substrate, 25g of moistened bagasse was transferred to Erlenmeyer flasks. To each flask was added 10ml culture medium (2g KH_2PO_4, 0.3g $MgSO_4.7H_2O$, 0.4g $CaCl_2$.2H2O, 0.1g yeast extract made to 1 litre by adding Distilled H_2O), containing different concentrations (5, 10 & 15%) of Glucose, Sucrose and Glucose plus Ammonium Sulphate as nutrients

lbs pressure for 15 minutes. To each flask added 2ml of the suspension (10^7 spores/ml). Culture medium containing bagasse not fortified with either of the nutrients are served as the control substrate. The inoculated flasks were incubated at 30^0C and 200rev/min for 3 days and further incubated for 3 weeks.

Table 1: Biodegradation of sugarcane bagasse with and without exogenous supplementation of nutrients during solid state fermentation by *Aspergillus niger*

SUBSTRATE	FORTIFICATION	BAGASSE ANALYSIS gm/100gm		LOSSES IN CONSTITUENTS (%)	
		CELLULOSE	LIGNIN	CELLULOSE	LIGNIN
Sugar Cane Bagasse	Fresh (Not Fermented)	58.5±0.3	18.4±0.2	--	--
	No Fortification	50.6±0.2	16.3±0.4	14.2	13.3
	5% Glucose	53.1±0.3	13.0±0.3	8.4	26.2
	10% Glucose	52.9±0.4	13.7±0.2	9.6	25.2
	15% Glucose	52.6±0.3	13.8±0.4	10.3	25.2
	5% Sucrose	51.4±0.2	14.7±0.2	12.6	20.1
	10% Sucrose	50.5±0.3	15.0±0.2	13.0	19.2
	15% Sucrose	50.6±0.4	15.2±0.3	13.6	18.9
	5% Sucrose + 0.21% $(NH_4)_2SO_4$	52.6±0.3	16.6±0.4	10.2	9.8

Analytical Procedures

The fermented bagasses and untreated bagasses were oven dried at 100-105^{0}C, and finally ground to less than 1mm.

Determination of Cellulose

One gram of oven dried sample was taken and to it added 15ml of 80% acetic acid and 1.5ml concentrated nitric acid. The content was refluxed for 20 min, then filtered. Collected residue was washed with ethanol, dried in oven at 100-105^{0}C, finally weighed and labeled sample A. Then sample A was incinerated at 540^{0}C and labeled sample B. Cellulose content was determined by the method of Goering and Van Soest (1970).

$$\% \text{ cellulose} = \frac{\text{sample A} - \text{sample B}}{\text{weight of initial sample}} \times 100$$

Determination of Lignin

One gram of oven dried sample was taken and to it added 70ml of 1.25% sulfuric acid. The content was refluxed for 2 hours, then filtered. Collected residue was washed with water. To this material was added 30ml of 72% H_2SO_4 and allowed-to stand for 4 hours with occasional stirring. Then filtered, washed and dried in oven at 100-105^{0}C, finally weighed and labeled sample A. Then sample A was incinerated at 540^{0}C and labeled sample B. Lignin content was determined by the method of Goering and Van Soest (1970).

$$\% \text{ lignin} = \frac{\text{sample A} - \text{sample B}}{\text{weight of initial sample}} \times 100$$

Statistical Analysis

All the experiments were done in three replicates and only the mean data of the obtained results has been incorporated in the tables. The results obtained were analysed statistically

1978).

RESULT AND DISCUSSION

The results of cellulose and lignin analysis in sugarcane bagasse and their biodegradation are presented in Table 1. The cellulose and lignin content of untreated sugarcane bagasse was 58.48 and 18.42% respectively. Generally plants contain 50-60% cellulose and 20-30% lignin. (Cullen and Kersten, 1992). Fermentation with *Aspergillus niger* for 3 weeks, resulted in loss of 14.2 % cellulosic content band 13.3% lignin contents. It was observed that on supplementing the sugarcane bagasse exogenously with different concentrations of sugars (5, 10 & 15%) varied results were obtained. It was observed that there was reduction in loss of cellulose on addition of 5% glucose to the culture medium which indicates that the organism utilized glucose instead of cellulose as a source of energy and for the production of hydrolytic enzymes. As a result there was increase in loss of lignin but decrease in loss of cellulose. Similar results of factors controlling and /or effecting lignocellulosic degradation has been observed on biodegradation of poplar wood by white rot fungi (Odier and Roch, 1983) and lignin by wood rotting fungi *Phanerochaete chrysosporium* and *Coriolus versiclor*. (Kirk *et al*., 1976). Further increase in exogenous glucose concentration from 10-15%, did not show much significant decrease in lignin content and percentage loss which further confirms that there is decrease in dependency of cellulose as energy source when other energy sources are available for microorganisms, glucose in this case (Ander and Eriksson, 1977; Ander *et al.,* 1983; Agosin and Odier, 1985). On replacing the exogenous supplement by 5% sucrose resulted in an increase in the loss of cellulosic content from 8.4 % (in glucose) to 12.6% and decrease in loss of lignin from 26.2% (in glucose) to 20.1%. Further increasing the sucrose concentration in culture media did not show any significant loss of lignin. The obtained results can be further summarized that the lignin degrading activity was suppressed in the presence of sucrose and hydrolysis of cellulose was enhanced in presence of sucrose. The result was supported by work of Hossain and Anantharaman, 2008 that more complex carbohydrate did not stimulate the break down of wheat straw. The result further concludes that the rate of cellulose utilization during fermentation had no quantitative correlation with lignin degradation. On addition of 0.21% ammonium sulfate to the culture medium containing 5% glucose showed noticeable reduction in lignin hydrolysis, the drop was observed fro 26.2% to 9.8%. (Table 1). It is evident that addition of ammonium sulphate had adverse effect on delignification

and *Polyporus tulipiferae* on addition of 0.5% ammonium sulphate (Levonen *et al*., 1983). Other supplements such as urea, casemic acid plus ammonium sulphate also gave the lowest result for lignin degradation (Ander and Eriksson, 1977).

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