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SHORT COMMUNICATION

COMPARISON OF LIGNOCELLULOSE BIODEGRADATION IN SOLID STATE FERMENTATION OF SUGARCANE BAGASSE AND RICE STRAW BY *ASPERGILLUS TAMARII*

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ABSTRACT

The present study examines the lignocellulose degradation in solid state fermentation in sugarcane bagasse and rice straw by *Aspergillus tamarii* over a period of 21 days. Lignocellulose degradation in sugarcane bagasse and rice straw, fortified with glucose was studied with and without ammonium sulphate. In bagasse fermentation, when the substrate initially containing 17.4% lignin was fortified with 5% glucose in the absence of ammonium sulphate, a maximum lignin loss of 24.7%, was recorded. This maximum lignin loss was accompanied by a minimum cellulose loss of 3%. In rice straw, fermentation under the same conditions resulted in a maximum lignin loss of 45.5% and 2.8% loss of cellulose. Efficiency of solid state fermentation using the two different substrates indicates that lignin loss is high in comparison to cellulose loss.

Key words: lignin, cellulose, fortification, white rot fungi

INTRODUCTION

Lignocellulose compounds are the most abundant agricultural residues in the world. They are constantly being replenished by photosynthesis. The, micro-organisms that are able to degrade these compounds include fungi as well as *Actinomycetes* and other bacteria. They have an important role in the biodegradation of lignocellulose (Holker *et al*., 1999).

Agricultural crop wastes such as bagasse and rice straw consist mainly of cellulose and hemi cellulose while, the rest comprise of lignin, nitrogenous compounds and ash (Abdullah and Zafar, 1999). Even though bagasse and rice straw contain enough cellulose to make them excellent sources of energy for ruminants, they are poor quality feeds due to low digestibility, poor palatability, low protein content and bulkiness.

Solid state fermentation (SSF) processe can be defined as the "growth of microorganism (mainly fungi) on moist solid materials in the absence of free-flowing water". This processes has been used for the production of food, animal feeds and both pharmaceutical and agricultural products (Cannel and Moo-Young, 1980; Moo-Young *et al*., 1983).

The main advantage of SSF is its low technology and high volumetric productivity, thus reducing downstream processing costs. For long, it had been thought that the main advantages of SSF is due to water limitation of the system so that a higher product concentration is attained. An additional but less investigated advantage of SSF may be the enhanced physiological process in cell adhesion or biofilm formation that is characteristic of SSF.

Solid state fermentation of lignocellulose materials by white-rot fungi is receiving attention, primarily because of the possibility of converting these materials into more digestible feedstuffs for ruminants (Reid and Seifert, 1982).

Furthermore, new applications of SSF have been suggested for the production of antibiotics (Barrios-Gonzales *et al*., 1988) secondary metabolites (Trejo-Hernandez *et al*., 1992) and enriched food stuffs (Senez *et al*., 1980). This paper describes the efficiency of solid state fermentation and lignocellulose degradation in sugarcane bagasse and rice straw.

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MATERIALS AND METHODS

Fungal strains and inoculum preparation

Aspergillus tamarii was isolated from marine drift wood pieces and was identified using Kolhmeyer manual (1979). The culture was maintained on PDA at 5-7 °C. Inoculum preparation and substrate inoculation were carried out as described by Abdullah and Zafar (1996 and 1989).

Substrate preparation and fermentation

Sugarcane bagasse and rice straw were chopped into 30-50 mm pieces and were separately soaked in boiling water for 10 min and the excess water was drained. About 25 g of moistened bagasse and rice straw were separately transferred into 1000 ml Erlenmeyer flasks and used as the biodegradation substrate.

To each flask was added, 10 ml of nutrient medium containing 5% glucose or glucose and 0.21% ammonium sulphate. Culture medium not fortified with glucose and without ammonium sulphate served as the control substrate. Flasks contain the substrate were autoclaved at 120° C and 1.06 kg cm^{-2} for 15 min and the fermentation was carried out at 25° C for 21 days. The fermented and untreated substrates were oven dried at 100-105° C, and ground to less than 1 mm pieces for determination of cellulose and lignin.

Determination of cellulose

To 1 g of dried sample, 15 ml of 80% acetic acid and 1.5 ml of conc. nitric acid were added and refluxed for 20 min. It was filtered and the residue was washed with ethanol, dried in an oven at 100-105° C and weighed (Material A). It was then incinerated at 540°C (Material B). Cellulose content was determined according to the method of Van Soest and Wine (1967). All experiments were carried out in triplicates.

Percentage of Cellulose = (Material A) −(Material B)/Weight of the sample x 100

Determination of lignin

To 1 g of dried sample, 70 ml of 1.25% sulphuric acid was added. The mixture was refluxed for 120 min, filtered and washed in water. To this was added 30 ml of 72% sulphuric acid and the material was allowed to stand for 4 h with occasional stirring. It was then filtered, washed and dried at 100-105° C (Material A) and incinerated at 540° C (Material B). Lignin content was determined according to the procedure of Van Soest and Wine (1967). All experiments were carried out in triplicates.

Percentage of Lignin = (Material A) −(Material B)/Weight of the sample x 100

Statistical analysis

The data in triplicates were tabulated and mean values calculated according to Steel and Torrie. 1986. Cellulose and lignin losses were determined by TAPPI (Technical Association of the Pulp and Paper Industry, USA) standard methods T223 and T224. The percent loss of cellulose and lignin was calculated as follows:

U1= (Go Co–Gt Ct)/Go Co ×100

Where Go and Gt are the dry weights of substrate in grams at the beginning and end of the fermentation respectively, and Co and Ct are the dry weights of the components (cellulose and lignin) at the beginning and end of the fermentation, respectively.

RESULTS AND DISCUSSION

Cellulose and lignin content in untreated sugarcane bagasse was 56.2% and 17.4% respectively (Table 1) and in untreated rice straw the values were 41.4% and 4.4% respectively (Table 2). Fermentation of unfortified baggase by *Aspergillus tamarii* for 21 days resulted in 12.63% of cellulose and 13.2% of lignin. Fermentation of unfortified rice straw for 21 days resulted in 6.2% of cellulose and 29.5% of lignin.

Supplementation of sugarcane bagasse with 5% glucose resulted in a decrease of 3.0% in cellulose and 24.7% of lignin. For rice straw, there was a 2.9% reduction in cellulose and 45.5% reduction in lignin content. Addition of sugar helped in the hydrolysis of lignin. A reduction in the loss of cellulose when 5% glucose was added to the fermentation medium indicated that *A. tamarii* utilized glucose instead of cellulose as a source of energy for fungal metabolism including the production of hydrolytic enzymes. These results agree with Odier and Roch (1983), who reported that degradation of poplar wood, by white rot fungi, was stimulated by reduced glucose concentration.

Addition of 0.21% ammonium sulphate to the medium containing 5% glucose and sugarcane bagasse showed a reduction of 8.89% cellulose and 6.9% of lignin. Fermentation of rice straw under similar conditions resulted in a reduction of 3.4% cellulose and 11.4% lignin.

Table 1. Lignocellulose degradation of sugarcane bagasse with nutrient medium fortified with nitrogen and carbon sources during solid-state fermentation by *Aspergillus tamarii* for 21 days at 25 °C.

Substrate fortification	Bagasse analysis g/100g		Loss in constituents (%)	
	Cellulose	Lignin	Cellulose	Lignin
Fresh bagasse not fermented	56.2±0.5	17.4±1.5	-	-
5% Glucose	54.5±1.0	13.1±0.5	3.0	24.7
5% Glucose + 0.21% $(NH_4)_2SO_4$	51.2±0.8	16.2±1.1	8.9	6.9
Not fortified	49.1±0.3	15.1±1.0	12.6	13.2

Culture medium consists of 0.3 g $MgSO_4.7H_2O$, 2g KH_2PO_4, 0.4 g $CaCl_2.2H_2O$, 0.1 g yeast extract made to 1litre.

Table 2. Lignocellulose degradation of rice straw with nutrient medium fortified with nitrogen and carbon sources during solid-state fermentation by *Aspergillus tamari* for 21 days at 25 °C.

Substrate fortification	Rice straw analysis g/100g		Loss in constituents (%)	
	Cellulose	Lignin	Cellulose	Lignin
Fresh bagasse not fermented	41.4±0.4	4.4±0.4	-	-
5%Glucose	40.2±1.2	2.4±0.5	2.8	45.5
5%Glucose + 0.21%$(NH_4)_2SO_4$	39.8±0.8	3.9±1.2	3.4	11.4
Not fortified	38.8±0.5	3.1±0.6	6.2	29.5

Culture medium consists of 0.3 g $MgSO_4.7H_2O$, 2 g KH_2PO_4,, 0.4 g $CaCl_2.2H_2O$, 0.1 g yeast extract made to 1litre.

Ander and Eriksson (1997) also reported that urea, casemic acid and ammonium phosphate gave the lowest lignin degradation. Sources of nitrogen have been reported to repress the degradation by *Ph. chrysosporium* (Fenn and Kirk, 1981). The ability of basidiomycetes to recycle their own nitrogen has been reported by (McDonald *et al*., 1977).

The most extensive lignin degradation was observed when glucose was supplied to nitrogen starved condition where *Termitomyces sp.* preferentially degraded lignin in the presence of 5% glucose. Similar results have been reported previously (Jalk *et al*., 1998).

Results reported here were in agreement with those of Odier and Roch (1983) who reported that degradation of C14 labeled poplar wood, by several white rot fungi, was stimulated at limiting glucose concentration. Abdullah *et al.* (2004) indicated that lignin hydrolysis was best at a marginal carbon/energy source for maintenance of metabolism.

Decomposition of lignin was reported to take place after profuse microbial growth. It was further reported that no microorganism could grow on carbohydrate-free medium and decomposition of lignin was difficult in the absence of readily degradable, high-energy source (Costa *et al*., 2002). In conclusion, rice straw, the major agricultural by-product of South Asia, is high in lignin and cellulose than sugarcane bagasse following degradation through solid state fermentation.

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