
Compositional changes of two lignocellulosic materials during degradation by *Pleurotus* spp. in solid state fermentation

MALAY GHOSH AND B. NANDI

Department of Botany, The University of Burdwan,
Burdwan, India

Pleurotus ostreatus and *P. sajor-caju* were grown in solid state fermentation with biomass of water hyacinth and banana pseudostem. Both the fungal species efficiently degraded the plant tissue constituents viz., holocellulose and lignin. Degradation of the polymeric components and release of breakdown products increased with the time of incubation. The rate of formation of breakdown products and their utilization by the mycelia of the test fungi varied thus resulting differences in the net available nutrients in the substrate enriched by mycelial proteins.

Key words : Biodegradation, cellulose, lignin, carbohydrate, nitrogen, solid state fermentation, *Pleurotus ostreatus*, *P. sajor-caju*, water hyacinth, banana pseudostem

INTRODUCTION

Cellulose and lignin are vital components of biospheric carbon cycle and ranked first and second in abundance as the naturally occurring biopolymers (Crawford and Crawford, 1976). Decomposition of lignocellulosic biomass by white rot fungi results in their improved digestibility of the ruminants (Mc Queen and Reade, 1983). In the southern Chili wood, decomposed by a consortium of fungi (primarily *Ganoderma applanatum* and *Armillaria* sp.), is used as a highly digestible feed known as 'Palo Podrido' (Zadrazil and Brunnert, 1982). Biological waste containing lignocellulose can thus be upgraded as feed by solid state fermentation which requires less energy input than liquid fermentation and hence of greater interest for its potential application.

The edible oyster mushroom *Pleurotus* spp. seems to be of particular promise as a bioconversion organism. Its colonization on beechwood, saw-dust, rape and sunflower straws (Zadrazil, 1980), wheat straw (Zadrazil, 1977; Zadrazil and Brunnert, 1980; Lindenfelser *et al.*, 1977; Zadrazil, 1985; Tsang *et al.*, 1987), sugarcane bagasse (Mitra and Nandi, 1992; Ortega *et al.*, 1993), jute stick (Mitra and Nandi, 1992), and cotton stalk (Platt *et al.*, 1983; Kerem *et al.*, 1992) significantly increases the digestibility of these materials.

The present investigation was designed to study the degradation of holocellulose, lignin,

total carbohydrate, soluble carbohydrate, soluble nitrogen in water hyacinth and banana pseudostem in solid state fermentation using two edible white rot species of *Pleurotus* which commonly grow on straw compost.

MATERIALS AND METHODS

The organisms *Pleurotus ostreatus* (Jacqu ex Fr.) Kummer and *P. sajor-caju* (Fr.) Singer, used in this study, were obtained from Mushroom Research Centre, Government of West Bengal, Chinsurah.

Water hyacinth (*Eichhornia crassipes* Solms.) were collected from local ponds after discarding the roots, which were rich in toxic substances like copper and lead, (Wolverton, 1978). Banana (*Musa paradisiaca* L.) pseudostems were collected from local gardens around Burdwan. After chopping these, plant pieces were dried in oven at 60°C to constant weights, ground in a Buhler mill and sieved through nylon net (40 mesh).

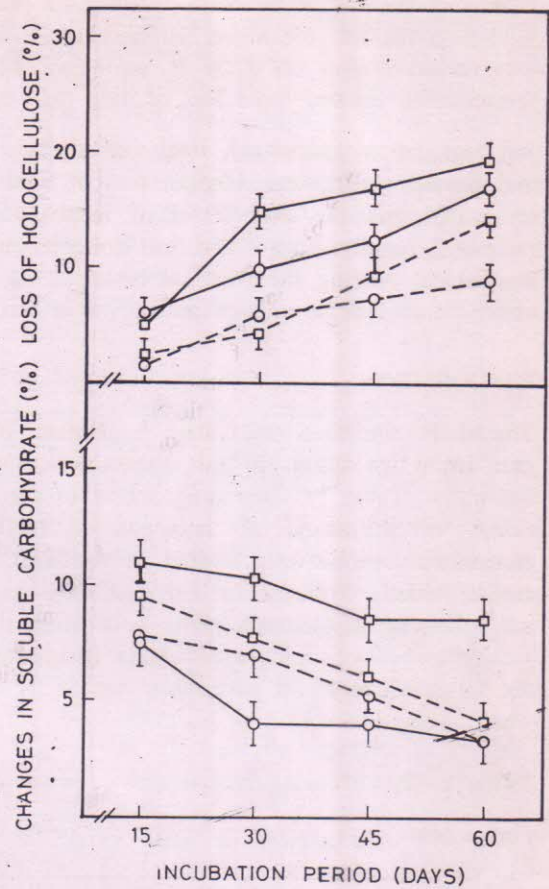
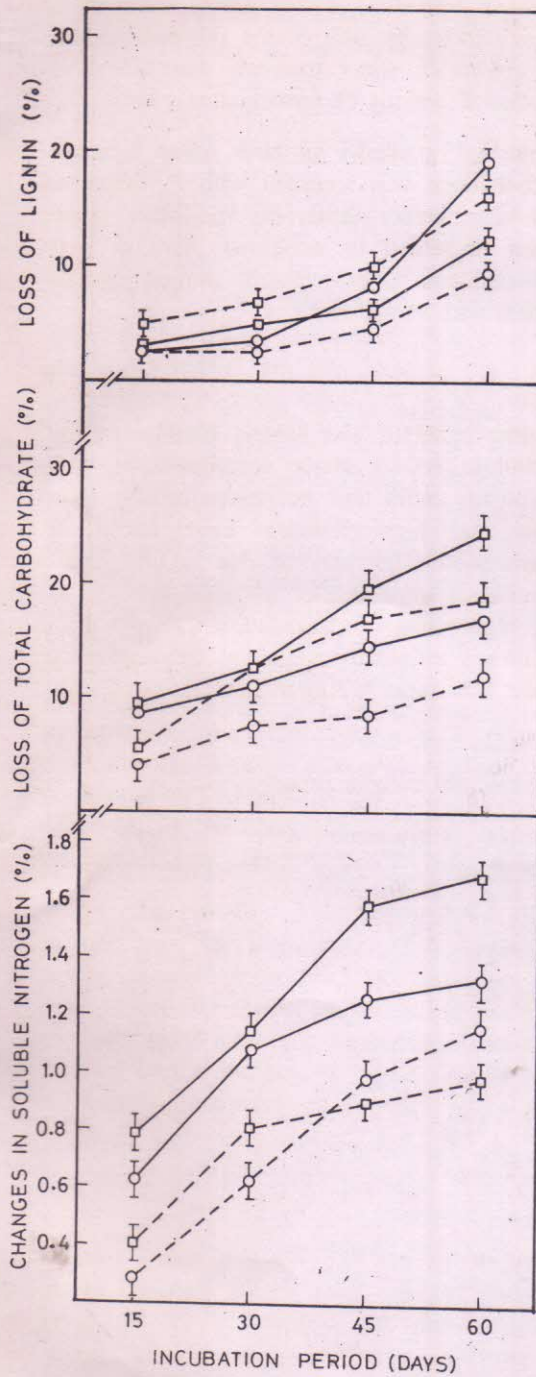
Stock cultures of the test organisms were maintained on 2% malt agar slants at 4°C. Mycelium from stock cultures was inoculated on 2% malt agar in petridishes and incubated for 4-5 d at 26 ± 1°C.

Throughout the study Erlenmeyer flasks (250 ml) with 4 g of substrate and 20 ml of modified salt solution I of Hofsten and Ryden (1975) as a basal medium (containing 0.2% phosphoric acid and 0.02% magnesium sulphate) were used. The pH of the medium was adjusted to 6.2 with 2(M) KOH solution. Flasks were autoclaved in 121°C for 20 minutes, cooled and incubated with 4 agar discs (7 mm in diam.) colonized by fungal mycelia from the edge of the actively growing colonies of the test fungi in Petridishes. The inoculated flasks were incubated separately as before, keeping 3 replicates of each set. Flasks were recovered at 15 d intervals, their contents were dried at 50°C. These were then ground to powder (40 mesh) and their different constituents were estimated as follows.

Holocellulose was determined following mainly Seifert (1983). Acid insoluble lignin content was estimated quantitatively following Saeman *et al.* (1954) by removing the total carbohydrate by 72% H₂SO₄ treatment from the powdered material. Total carbohydrate was estimated quantitatively following colorimetric method of Saeman *et al.* (1954). Soluble carbohydrate was estimated following Viles and Silverman (1949) and Mc Cready *et al.* (1950). Soluble nitrogen was estimated colorimetrically following Vogel (1961). The C:N ratios of the substrates were recorded by estimating total carbon and total nitrogen following Jackson (1973) and Vogel (1961) respectively.

RESULTS

Growth of *P. ostreatus* resulted in the disappearance of 19.5% and 14.5% of initial holocellulose counts from water hyacinth and banana pseudostem respectively within 60 d of fermentation. Holocellulose being degraded at rapid rate from 15 d to 30 d of that fermentation. *P. sajor-caju* degraded 9% and 17% of holocellulose when grown on banana pseudostem and water hyacinth biomass respectively at the end of the experiment. Of these two substrates holocellulose was utilized more efficiently from water hyacinth than that from banana pseudostem.



— Water hyacinth
 --- Banana pseudostem
 □ *Pleurotus ostreatus*
 ○ *P. sajor caju*

Loss of holocellulose, lignin, total carbohydrate and change in soluble carbohydrate and nitrogen during degradation of water hyacinth and banana pseudostem biomass by test fungi after different periods of incubation.

Lignin of both water hyacinth and banana pseudostem decreased slowly during fermentation with both the test organisms. Highest lignin loss amounting to 18% of the original amount was recorded after 60 d by *P. sajor-caju* when grown on water hyacinth. Last 15 d of fermentation showed rapid loss of lignin than the other 3 periods of fermentation.

As degradation progressed, total carbohydrate decreased gradually in both water hyacinth and banana pseudostem. Highest loss of total carbohydrate was recorded with *P. ostreatus* on water hyacinth after 60 d of incubation. Soluble carbohydrate, on the other hand, increased rapidly upto 15 d of fermentation then decreased or remained more or less unchanged. Among the four combinations of fermentations water hyacinth showed highest soluble carbohydrate content after 15 d of incubation with *P. ostreatus*.

DISCUSSION

The study compares solid state fermentation of water hyacinth and banana pseudostem by two white rot fungi. In this experiment holocellulose is the major constituent of both substrates (Table 1). Degrading ability of the organisms could thus be determined by their extent of utilization of holocellulose (cellulose and hemicellulose). First 15 d of fermentation shows only a little holocellulose degradation. This may be due to the simple sugars initially available in both test fungi as is evident from smaller mycelial growth. At the following acceleration phase both fungi show higher rate of degradation upto 30 d of incubation. Holocellulose of banana pseudostem is not efficiently degraded by neither of the fungi which could have been due to its higher C:N ratio (82:1) in contrast to that of water hyacinth (27:1).

Table 1. Data showing composition of water hyacinth and banana pseudostem

Components	Dry matter (%)*	
	Water hyacinth	Banana pseudostem
Holocellulose	53.7 $\pm$ 0.9	45.72 $\pm$ 1.2
Lignin	15.2 $\pm$ 0.6	11.52 $\pm$ 0.6
Total carbohydrate	57.9 $\pm$ 0.4	52.2 $\pm$ 0.7
Soluble carbohydrate	2.85 $\pm$ 0.3	3.2 $\pm$ 0.2
Total nitrogen	1.16 $\pm$ 0.02	0.43 $\pm$ 0.03
Soluble nitrogen	0.5 $\pm$ 0.03	0.12 $\pm$ 0.02
C:N ratio	27:1	82:1

* Mean $\pm$ standard errors.

In solid state fermentation (SSF) of lignin oxygen is not the limiting factor during degradation in contrast to submerged culture where oxygen may be a limiting factor (Leisola *et al.*, 1983; Ander *et al.*, 1981; Kerem *et al.*, 1992). Most of the lignin is released as CO₂ during degradation by the growing organisms. The rate of lignin degradation reported to be slower than that of any carbohydrates (Chopra and Somal, 1981). In this experiment ligninolytic activity appears from the very first phase. Ortega *et al.* (1993) reported lignin degradation at the initial 10 d to be 8% in SSF of sugarcane straw with *P. ostreatus*.

Generally ligninolytic activity appears only after the primary growth phase when carbon, soluble nitrogen and sulphur become limited in the medium (Waldner *et al.*, 1988). Tsang *et al.* (1987) reported smaller amount of lignin decomposed by *P. sajor-caju* than *P. ostreatus* after first flush. However after second flush total lignin degradation was more or less equal in the two species when grown on wheat straw under mushroom growing conditions. The present findings is in conformity and reports *P. ostreatus* to show higher ligninolytic activity during the first 30 d while *P. sajor-caju* after 60 d. Here *P. sajor-caju* proves to be the most efficient lignin degrader when grown on water hyacinth. Ortega *et al.* (1993) reported no clear connection of enzyme production with lignin degradation. Evidently the role of laccase is an indirect one even though it is essential for lignin degradation (Glenn and Gold, 1983).

Insoluble carbohydrates which constitute the major part of both substrates serve as the prime carbon source to the degraders. Changes in the level of carbohydrates during the process of degradation is considered an important index. As degradation proceeds loss of total carbohydrate increases steadily through combined degradation of cellulose, hemicellulose and lignin.

Soluble carbohydrates increase after 15 d of fermentation evidently from rapid breakdown of cellulose and hemicellulose when the rate of utilization of breakdown products is less than the rate of degradation by the test fungi. However, both the fungi utilize most of the soluble sugars as degradation further proceeds. Kerem *et al.* (1992) reported that the glucose content increased rapidly after 6 d during degradation of cotton stalk by *P. ostreatus* and remained more or less at the same level upto 30 days. This was due to higher cellulase activity at the first phase of degradation followed by more or less equal rate of production of glucose and its utilization in the metabolic process of the fungi. Lena and Quanglia (1992) reported increased reducing sugar in liquid medium in the first phase (8 d) followed by a depletion in the second phase when *P. florida* was grown in submerged culture with sugarbeet pulp.

In both the plant biomass nitrogen is normally present in very limited amount. Soluble nitrogen contents also increased in both of the substrates following degradation. Zadrazil (1985) concluded this to be dependent not only on the differences in the rate of breakdown and rate of utilization of carbohydrates but also on the amount of carbon that was lost as CO₂ during degradation and thus resulting relative increase in nitrogen level. Increase in the soluble nitrogen in the degraded substrate was reported by Jilek *et al.* (1978) and Burrows *et al.* (1979).

High digestibility of lignocellulosic components by the test organisms is the important findings of the present experiment. The results presented here show that both the fungal species are fairly efficient degraders during the course of solid state fermentation, but it is too premature to predict which one of the tested fungi and which of the substrates can be used for large scale production of fermented feed for ruminants. Many of these fungi have promising characteristics for the proposed process but only a scale up process and feeding experiments can give a definite answer.

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