CONTRIBUTION OF BASIDIOMYCETE FUNGI IN THE NATURAL PROCESS OF BIODEGRADATION OF WOOD IN FOREST STANDS.

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Introduction

The role of white rot basidiomycetes in the biodegradation of lignocellulosic materials is now well established (Janshekar and Fiechter, 1983; Kirk, 1984). Accordingly, numerous studies have been carried out on the comparative ligninolytic activities of a number of such basidiomycetes when grown on a variety of substrates under different cultural conditions (Golovlev *et al.*, 1983; Levonen-Munoz *et al.*, 1983; Zadrazil and Brunnert, 1980). Biodegradation of lignin in the nature, furthermore, is of particular importance because of its high recalcitrance (Reddy, 1984) and protection of cellulose from attack by various cellulolytic microbes (Fan *et al.*, 1982) thereby preventing bioavailability of as much as half the photosynthethically produced high energy carbon. Though lignocellulosic biodegradation under laboratory conditions has been well investigated, reports on this aspect under natural conditions are limited (Zadrazil *et al.*, 1982). Such studies are necessary not only for an understanding of the degradation process but have the applied potential to optimize production of feed and feed stack from lignocellulosics. In the present paper has been presented biochemical composition of *Dalbergia sissoo* degraded by various brown and white rot fungi in the natural habitat, their cow reticulo-rumen digestibility, and isolated *D. sissoo* lignin biodegradation by some basidiomycetes under cultural conditions.

Materials and Methods.

Biodegraded Wood: Dalbergia sissoo wood degraded under natural conditions was collected from Changa Manga plantation and placed in polyethylene bags. Fungal species were identified by fruiting bodies emerging from the decayed wood in accordance with Ahmad (1972).

Analytical Methods: Unless otherwise specified AOAC (1984) procedures were followed for analysis. Decayed wood was air dried and ground to less than 1mm for further analysis and the reticulo-rumen digestibility trials. Ash was determined by incineration at 450—500°C for 8 hrs. The percentage of water soluble substances was determined as the dissolved materials in extracts from 1g ground wood sample incubated for 3 hrs in 100 ml water at 80°C. Nitrogen was determined by the method of Kjehldahl and the values of crude protein were computed from these determinations by a multiplication factor of 6.25 (AOAC, 1984). Cellulose was determined according to Kurschner and Hanak (1930) and lignin by using the procedure of Khudyakove (1984).

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Reticulo-rumen Digestibility Trials: In vivo digestibility trials were carried out on a dry Sahiwal cow as described earlier (Zafar *et al.*, 1981). Reticulo-rumen digestibility was determined after a 48 hr incubation of the degraded wood samples.

Biodegradation of Isolated Lignin Under Cultural Conditions: Crude lignin was isolated from *D. sissoo* wood by decellulolization and washing according to Khudyakove (1984). To each 250-ml capacity Erlenmever flask was added 5 g of this crude lignin as degradation substrate for various basidiomycetes. To each substrate flask was added 2 ml of culture medium containing 25 g glucose, 2.1 g $(NH_4)_2$ SO_4, 2.0 g KH_2PO_4, 0.3 g $MgSO_4.7H_2O$, 0.4 g $CaCl_2.2H_2O$, 0.1 g yeast extract, and distilled water to a volume of 1 litre. Inoculum was prepared and substrate inoculated as described earlier (Zafar *et al.*, 1989). Cultures were incubated at 25°C for 21 days.

Results and Discussion

Composition of Biodegraded Wood: Composition of non-decayed *D. sissoo* wood and that decayed by various brown and white rot pathogens has been presented in Table 1.

The pH of water extract of the non-decayed wood was 8.9, whereas a lowering in the pH was observed in various decayed wood samples ranging between 5.5 and 7.3. It was further noted that in those host-pathogen interactions wherein cellulose values were relatively lower and that of lignin higher, as in the case of wood decayed by *Fomitopsis annosus, Ganoderma lucidum* and *Polyporus biennis,* the pH respectively was 7.3, 6.5 and 6.0 and thus higher than those wherein cellulose values were higher and that of lignin lower, as in the interactions involving *Lenzites betulina, L. platyphylla* and *Coriolus versicolor,* with the pH values respectively being 5.5, 5.6 and 5.5. On the basis of these observations it may be concluded that growth of these basidiomycetes leads to acidic conditions, which are more pronounced in the predominently ligninolytic than in the cellulolytic interactions.

Non-decayed *D. sissoo* wood had 11.1% water soluble substances (WSS). An appreciable increase in WSS was observed in the wood decayed by *F. annosus,* which was only slight in the wood decayed by *G. lucidum* and *P. biennis.* WSS in the decays involving *L. platyphylla, L. betulina* and *C. versicolor* respectively were 11.1, 9.8 and 7.5 showing a trend in their decrease with the degree of lignin degradation. It is interesting to point out that a correlation is evident between the higher WSS and pH values (as in *F. annosus*) with the overall trend of decrease in WSS and the lowering of pH culminating in the extreme case of wood decayed by *C. versicolor.*

Ash content was found to show increase from 2.5 times in *L. platyphylla* to 11.2 times in *P. biennis* that of 0.83% in the non-decayed wood. Upto 10 times increase was observed by Zadrazil *et al* (1982) in *Eucryphia cordifolia* wood decayed by *G. applanatum.*

All the decayed wood had appreciably higher values of nitrogen ranging from 0.3 to 0.82% as compared to 0.06% in the non-decayed wood. This increase may be interpreted in the light of claims that certain higher fungi are capable of fixing atmospheric nitrogen

into proteinous nitrogen.

(Ginterova and Maxianova, 1975; Rangaswami *et al.*, 1975) or that the wood rotting basidiomycetes are capable of recycling proteinous nitrogen present in the wood (Merril and Cowling, 1966). No definite pattern in this increase was, however, evident as was noted in the case of changes in pH and WSS contents which had a corresponding correlation not only between themselves but also with the changes in cellulose and lignin. This variability may be explained to be due to the different capabilities of the different wood rotting fungi under study to fix atmospheric nitrogen or recycle the wood nitrogen or both.

Wood samples decayed by *F. annosus, G.lucidum* and *P. biennis,* respectively containing 26.9, 33.6 and 30.8%, were found to have substantially decreased level of cellulose as compared with non-decayed wood having 51.9% cellulose. Lignin in these woods was accordingly higher, respectively being 38.5, 28.1 and 32.0%, as compared with non-decayed wood having 21.3%. Woods decayed by *L. platyphylla, L. betulina* and *C. versicolor* with 20.0, 18.0 and 16.4% lignin, respectively, had lower content than the non-decayed wood (21.3%). Corresponding values of cellulose in these woods, with 54.2, 57.3 and 52.0%, respectively, were only slightly higher than the non-decayed wood. This may be explained by assuming that lignin degradation is accompanied by utilization of some cellulose by the rot fungi to meet their respective metabolic energy needs. On the basis of these observation, the studied rot fungi, under natural conditions, may be grouped as *F. annosus, G. lucidum* and *P. biennis* as preferrential cellulose degraders and *C. versicolor, L. betulina* and *L. platyphylla* as preferrential lignin degraders in the descending order.

Reticulo-rumen Digestibility: The digestibility of various decayed wood samples has been presented in Table 2. Highest co-efficient of digestibility was observed in the wood decayed by *C. versicolor* followed by *L. betulina* and *L. platyphylla* respectively being 28.8, 26.1 and 24.2% as compared with 14.0% of the non-decayed wood. These levels of digestibility are in agreement with the higher cellulose and lower lignin contents noted in these when compared with the non-decayed wood (Table 1). Digestibility of the *G. lucidum* and *P. biennis* decayed woods were some what similar to that of the non-decayed wood. Higher digestibility of the *F. annosus* decayed wood as compared with that of the non-decayed wood, despite a rather high lignin and low cellulose contents, may be attributed to the exceptionally high value of WSS.

Biodegradation of Isolated Lignin Under Cultural Conditions: Results of degradation of the isolated *D. sissoo* lignin by some basidiomycetes have been presented in Table 3. In a 21-days period *C. versicolor* degraded 15.1% and *Pleurotus ostreatus* 11.9% lignin. In an earlier study *C. versicolor* was observed to degrade 18.1% lignin in 14 days when whole bamboo wood was subjected to solid state fermentation (Zafar and Abdullah, 1989). On a comparative basis *C. versicolor* was found to have more ligninolytic activity than *Pleurotus ostreatus* when wheat straw was used as the lignocellulosic substrate (Zafar *et al.*, 1989). Observations on the biodegradation of isolated lignin are, therefore, in agreement with those reported earlier. *Termetomyces* sp. did not grow on isolated wood lignin though with

studies on sugarcane bagasse the fungus has been found to have appreciable ligninolytic activity (unpublished data). No explanation at this stage can be afforded although it may be mentioned that isolated wood lignin is a much different substrate than sugarcane bagasse, which among other ingredients contains high proportions of cellulose and simple sugars.

The overall progression of wood decay and the associated biochemical changes under natural conditions are rather complex involving a number of biotic and climatic interactions and successions. Therefore, the data presented in the foregoing study and that by Zadrazil *et al* (1982) are at present too small for the elucidation of the intricate process of biodegradation of wood lignin in the nature. The present study, however, attempts at identifying the indicators and trends that may be involved in the biochemical ecology of wood decay as associated with the reported basidiomycete fungi. Evidently, nonetheless, more studies are needed to explain the mechanism (s) involved in the natural recycling of energy-rich carbon trapped in lignocellulosics for its efficient utilization.

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TABLE 1

Composition of *Dalbergia sissoo* wood decayed in nature by various white rot/brown rot pathogens.

Wood Decay Pathogen	pH	Water Soluble Substances (%)	Ash (%)	Nitrogen (%)	Crude Protein (%)	Cellulose (%)	Lignin (%)
Coriolus versicolar	5.5	7.5	7.0	0.6	3.8	52.0	16.4
Fomitopsis annosus	7.3	24.5	4.8	0.65	4.1	26.9	38.5
Ganoderma lucidum	6.5	11.8	4.3	0.3	1.9	33.6	28.1
Lenzites betulina	5.5	9.8	4.1	0.82	5.1	57.3	18.0
Lenzites platyphylla	5.6	11.1	2.1	0.3	1.9	54.2	20.0
Polyporus biennis	6.0	11.3	9.3	0.53	3.3	30.8	32.0
Undecayed Healthy Wood	8.9	11.1	0.83	0.06	0.4	51.9	21.3

TABLE 2

Co-efficient of reticulo-rumen digestibility of *Dalbergia sissoo* wood decayed in nature by various white rot/brown rot pathogens.

Wood decay pathogen	Reticulo-rumen digestibility (%)
Coriolus versicolar	28.8
Fomitopsis annosus	18.8
Ganoderma lucidum	14.2
Lenzites betulina	26.1
Lenzites platyphylla	24.2
Polyporus biennis	13.8
Non-Decayed Host Plant	14.0

TABLE 3

Degradation of the isolated *Dalbergia sissoo* lignin by various basidiomycetes after solid state fermentation for 21 days at 25°C.

Basidiomycete Organism	Lignin Before Degradation (%)	Lignin After Degradation (%)	Percent Loss in Lignin
Coriolus versicolor	98.1	83.3	15.1
Pleurotus ostreatus	98.1	86.4	11.9
Termetomyces SP.	98.1	No Fungal Growth	