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BIODEGRADATION OF UREA FORMALDEHYDE MODIFIED SAW-DUST BY CELLULOLYTIC FUNGI I

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ABSTRACT

1.0 : 0.75, 1.0 : 1.0 and 1.0 : 1.5 mole ratios of urea formaldehyde have been employed for the modification of saw-dust. Degradation of these modified saw-dust samples was carried out using three strains of cellulolytic fungi, *Phoma sp, Curvularia sp* and *Trichoderma viride* as well as their mixed cultures, in order to assess their effectiveness as well as their competitive ability over incubation period of 60 days.

Results show that samples of both unmodified and the urea formaldehyde (UF) modified saw-dust were degraded by the various fungi as well as their mixed cultures to varying degrees as was evident from increases in weight loss, carbon loss and nitrogen content with incubation time. This degradation was more pronounced with the UF modified saw-dust than the unmodified (pure) saw-dust. Whereas *Curvularia sp* showed the highest loss values (7.32 g) at 1.0 : 0.75 UF mole ratio modification, the mixed culture exhibited the highest loss in % carbon (56.13%), with *Trichoderma* showing the highest % nitrogen (13.26%) content with same mole ratio as compared to other mole ratios employed. Generally, there was a steady decrease in C : N ratio with increasing incubation period for both the unmodified and modified saw-dust and for all the fungi species used.

KEY WORDS: BIODEGRADATION, UREA-FORMALDEHYDE, SAW-DUST, CELLULOLYTIC FUNGI

INTRODUCTION

The use of fungi for the production of commercially important products has increased rapidly over the past half century. The exploitation of fungal activity by man is not a recent phenomenon . On the contrary, numerous examples are known which indicate that man has been aware of the value of fungi since the dawn of civilization. The fermentation of alcoholic beverages, practiced in the days of Pharaoh, is the best known example of the exploitation of the biochemical activities of a fungus by early man. The use of yeast to leaven bread also dates back to biblical times. The production of alcoholic beverages, biomass and the manufacture of therapeutic compounds together with the production of simple organic compounds still remains the major fields in which fungal activities are exploited by man (Smith and Berry, 1974). Although fungal activities have in some cases been used advantageously, several reports show that their activity could be disadvantageous as some of them have been known to act as biodeteriogen (Ogbonna and Pugh, 1982) and mycotoxins (Mannon and Johnson, 1985).

Until fairly recently only a few groups of fungi were known with certainty to degrade lignin (Kirk, 1971; Ander and Eriksson, 1978; Milgram, 1985; Rosenberg, 1978). However, several reports are now available regarding cellulose and lignocellulose degradation by cellulolytic and lignolytic fungal activity of micro-organism in compost and soil (Gascoign and Gascoigne, 1960; Hungate, 1966; Reese and Levinson, 1952; Siu, 1951; Crawford and Crawford, 1980; Njoku and Antai, 1987; Mishra et. al., 1981). However, it does appear that no report is available on the microbial degradation of modified cellulose using pure cultures.

Although the *Ascomycetes, Deuteromycetes* and *Phycomycetes* have been known to be responsible for the degradation of cellulose in varying degrees (Crawford and Crawford, 1980; Njoku and Antai, 1987), nothing has been reported on their ability to act on urea formaldehyde modified cellulose. The present work reports on the activity of three cellulolytic fungi, *Phoma species, Curvularia species* and *Trichoderma viride* and their mixed cultures on urea formaldehyde modified sawdust.

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The process of biodegradation of natural polymers by micro-organism has been known to release bioactive substances. It is in the light of this that natural polymer as sawdust is subjected to chemical modification with a nitrogen rich bioactive substance as urea through a chemical modifier, formaldehyde. It is envisaged that biodegradation through cellulolytic fungal activity may enhance decomposition of this modified natural polymer, hence necessitating the slow release of soluble bioctive substances suitable for subsequent plant utilization as nutrients.

MATERIALS AND METHODS

Materials: The sawdust used was of the mahogany type collected from Katako wood market in Jos, Plateau State, Nigeria. These were screened and sieved to obtain the required mesh size of < 0.25 mm with a Haver and Broekner EML 200 sieving machine running at 5 rpm. The mesh size was then washed twice with methanol and distilled water, dried in the oven at 105 °C for 2 hours and stored in a dessicator.

The urea, formaldehyde, sodium hydroxide, methanol and sulphuric acid used were products of BDH chemicals, Poole, England and were used as supplied.

Preparation of Urea-Formaldehyde Modified Sawdust

1.0 : 0.75 (60.06 g : 49.65 ml); 1.0 : 1.0 (60.06 g : 66.20 ml) and 1.0 : 1.5 (60.06 g : 76.13 ml) mole ratio of urea to formaldehyde were each prepared as described in our previous work (Nwufo and Tyuulugh, 1989) by dissolving urea into 37% w/v formalin. The dissolution was carried out in a beaker containing formalin with continuous heating at 40 °C and stirring for 30 minutes. During this period, the pH of the solution was adjusted to 8.0 with 1.0 M NaOH. 200 g of sawdust was gradually added to each of the prepared resin. This was done with continuous stirring until a smooth paste was obtained in each case. The resultant paste was allowed to cool and then spread thinly on a tray and dried in an oven at 60 °C for 6 hours. The dried material was then ground and stored for use. It should however be noted that because of the amount of urea formaldehyde modified sawdust required, the quantity of urea and formaldehyde used in the preparation of each UF mole ratio was doubled.

Preparation of the Fungi Cultures

Pure strains of each fungus used in this study was obtained from the Botany Department, University of Jos. These were *Phoma species, Curvularia species* and *Trichoderma viride.* Each of these strains was subcultured onto petri dishes containing nutrient medium made from strained, boiled and autoclaved Irish potato powder and glucose. They were then allowed to incubate.

Introduction of Fungi into Modified Sawdust

sixty (60) flasks containing modified sawdust were grouped into 5 sets of 12 flasks each. Each set contained four (4) flasks of 1.0 : 0.75 UF modified sawdust, four (4) flasks of 1.0 : 1.0 UF modified sawdust and four (4) flasks of 1.0 : 1.5 UF modified sawdust. The five (5) sets were labelled A, B, C, D and E with eacn flask having 10 g of the modified sawdust. The fungi were then introduced separately to each set as follows:

Set of 12 flasks A	-	*Phoma* sp
Set of 12 flasks B	-	*Trichoderma viride*
Set of 12 flasks C	-	*Curvularia*
Set of 12 flasks D	-	Mixed cultures of the fungi
Set of 12 flasks E		Mixed culture of the fun with 10 ml of nitrogen free water

Individual flasks in each set was inoculated with 25.0 ml nutrient solution and 2.0 ml of fungi spore suspension. They were then properly covered with aluminium foil, weighed in the dark, before incubating at 30 °C for 15, 30, 45 and 60 days. At the end of these periods three flasks from each group were harvested and the loss in weight of the UF modified sawdust during the degradation were determined gravimetrically after the flasks had been removed from the incubator, dried at 105 °C for 24 hours in the oven. The dried materials were then subjected to further analysis such as the determination of carbon loss and nitrogen contents. The organic matter was determined by weight loss on ignition of 1.0 g of the degraded UF modified sawdust at 550 °C for 6 hours. The carbon values were obtained by dividing organic matter values by 1.724. The total nitrogen in UF modified sawdust after degradation/decomposition was determined by micro Kjeldahl method (Welcher, 1975). Finally the C : N ratio of the degraded UF modified sawdust was obtained. The results are shown on Tables 1, 2, 3 & 4 respectively.

RESULTS AND DISCUSSION

The degradation of cellulose is often discussed in connection with the action of fungal parasites (Reese and Levinson, 1952; Siu, 1951; Crawford and

Crawford, 1980 and Garret, 1963). The factors affecting the activity of the fungal parasites include pH, temperature and moisture. The importance attached to the biodegradation of lignocellulose materials in relation to plant growth has been highlighted by most researchers (Agboola, 1986; Allison, 1973; Nwufo, 1997; Jekinson, 1971; Larson et. al., 1978; Parr and Papendick, 1978; Sanchez and Miller, 1986; and Singh, 1986). These researchers have shown that plant residue such as sawdust in addition to being sources of nutrients for plant and microorganisms, a factor in fertilizer use efficiency and a starting material for microbially produced organic and humus substances which influence mineral solubilization, soil aggregation and structure, can also be of significance in reducing erosion, runoff, sediment transport, in maintenance of infiltration rate, in prevention of surface crusting and transport and retention of water, heat and air in the soil.

Although plant residues may contain all the nutrients needed by plants, they are often found to be inadequate especially in nitrogen content because of its critical nature in terms of adequate availability for plant growth. It is on the basis of this that sawdust, a naturally occurring plant residue of lignicellulose origin has been chemically modified with such nitrogen rich compound as urea through a formaldehyde linkage and subsequently subjected to various fungal activity over a period of 60 days.

The results obtained for weight losses, % carbon, % nitrogen and C : N ratios for all the fungal activities on the varying mole ratios of UF modified sawdust at various incubation times are shown in Tables 1 - 4. These results show that during the incubation periods, all fungi examined caused weight losses to the unmodified (control) and modified sawdust samples respectively, with the degradation being more pronounced with the modified sawdust than the unmodified (pure) sawdust. (Compare Table 1 with Tables 2, 3, and 4). There was an increase in weight loss with increase in incubation period. This is in agreement with the findings of Mishra *et. al.*, (1981). The maximum cellulolytic activity was observed at 60 days with *Curvularia speices* on 1.0 : 0.75 UF modified sawdust. The mixed cultures did not appear to significantly cause higher weight losses than single cultures. This agrees with the results obtained by Asiodu and Rogers (1973) and Mishra et. al.,(1981). The implication of this is that fungal individualism do exist and can be identified within natural population (Todd and Rayner, 1980).

Results on Tables 1 - 4 also show a gradual decrease in % carbon content with increased incubation period. The least % carbon of 56.13 was obtained for mixed culture at 1 : 0.75 UF modification followed by *Curvularia species*, *Phoma species* mixed culture with 10 ml N_2 free H_2O and *Trichoderma viride* in ascending

Table 1: Biodegradation of Unmodified Saw-dust by Cellulolytic Fungi

Fungi	Weight Loss (g) Incubation Period (Days) 15	30	45	60	% Carbon Incubation Period (Days) 15	30	45	60	% Nitrogen Incubation Period (Days) 15	30	45	60	C/N Ratio Incubation Period (Days) 15	30	45	60
Phoma	0.49	0.51	0.56	0.61	71.2	69.8	69.0	68.5	3.50	3.71	4.00	4.31	20.3	18.8	17.3	15.9
Trichoderma viride	0.51	0.55	0.58	0.63	70.39	69.5	68.3	67.7	3.60	3.84	4.12	4.53	19.6	18.1	16.6	15.0
Curvularia sp.	0.48	0.70	0.88	0.93	71.5	70.3	69.4	68.7	3.42	3.80	4.01	4.22	20.9	18.5	17.3	16.3
Mixed culture	0.53	0.63	0.71	0.84	70.1	68.3	66.9	66.0	3.81	4.11	4.96	5.20	18.4	16.6	13.5	12.7
Mixed culture + 10 ml N_2 free H_2O	0.72	0.77	0.91	1.02	67.3	66.9	65.4	64.0	3.96	4.53	5.26	6.24	17.0	14.8	12.4	10.3

Unmodified sawdust has 71.82% C; 3.25% N; C : N ratio of 22.10 : 1.00

Table 4: Biodegradation of 1 : 1.5 UF Modified Saw-dust by Cellulolytic Fungi

Fungi	Weight Loss (g) Incubation Period (Days) 15	30	45	60	% Carbon Incubation Period (Days) 15	30	45	60	% Nitrogen Incubation Period (Days) 15	30	45	60	C/N Ratio Incubation Period (Days) 15	30	45	60
Phoma	2.379	3.106	5.290	5.856	57.48	57.37	57.07	56.70	12.04	12.30	12.59	12.90	4.77	4.66	4.53	4.40
Trichoderma viride	0.969	3.289	3.332	4.717	57.66	57.49	57.40	57.20	11.89	12.32	12.53	12.74	4.85	4.67	4.58	4.49
Curvularia sp.	1.455	2.962	4.075	4.781	57.53	57.38	57.12	57.00	12.11	12.45	12.53	12.68	4.75	4.61	4.56	4.50
Mixed culture	1.191	4.065	4.708	5.256	57.22	57.27	56.92	56.57	12.01	12.45	12.64	12.78	4.76	4.60	4.50	4.43
Mixed culture + 10 ml N_2 free H_2O	1.433	2.466	4.824	5.628	57.62	57.63	57.42	57.27	11.99	12.01	12.23	12.62	4.81	4.80	4.70	4.54

1 : 1.5 UF modified saw-dust has 58.21% C; 11.72% N; C : N ratio of 4.97 : 1.00

Table 3: Biodegradation of 1 : 1 UF Modified Saw-dust by Cellulolytic Fungi

Fungi	Weight Loss (g)				% Carbon				% Nitrogen				C/N Ratio			
	Incubation Period (Days)				Incubation Period (Days)				Incubation Period (Days)				Incubation Period (Days)			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
Phoma	1.759	3.455	4.112	5.713	57.43	57.36	57.29	57.14	11.92	12.35	12.78	12.82	4.82	4.65	4.48	4.46
Trichoderma viride	1.213	3.746	4.524	5.389	57.54	57.45	57.23	57.11	12.12	12.48	12.70	13.01	4.75	4.60	4.51	4.39
Curvularia sp.	2.816	3.408	4.422	5.866	57.58	57.50	57.26	57.03	12.37	12.40	12.45	12.52	4.66	4.64	4.60	4.56
Mixed culture	1.774	4.534	5.147	5.701	57.52	57.41	57.20	56.97	12.14	12.58	12.60	12.74	4.74	4.56	4.54	4.47
Mixed culture + 10 ml N_2 free H_2O	2.209	3.836	4.477	6.957	57.65	57.50	57.36	57.12	12.08	12.12	12.45	12.85	4.77	4.74	4.60	4.45

1 : 1 UF modified saw-dust has 58.01% C; 11.31% N; C : N tario of 5.13 : 1.00

Table 2: Biodegradation of 1 : 075 UF Modified Saw-dust by Cellulolytic Fungi

Fungi	Weight Loss (g)				% Carbon				% Nitrogen				C/N Ratio			
	Incubation Period (Days)				Incubation Period (Days)				Incubation Period (Days)				Incubation Period (Days)			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
Phoma	1.615	3.286	3.751	5.238	57.43	57.34	57.03	56.53	12.07	12.24	12.54	12.97	4.75	4.69	4.55	4.36
Trichoderma viride	1.466	3.073	3.752	5.064	57.55	57.45	57.37	57.16	12.16	12.23	12.33	13.26	4.73	4.70	4.65	4.31
Curvularia sp.	1.797	4.135	4.777	7.320	57.34	57.32	57.04	56.31	12.92	11.99	12.42	13.12	4.44	4.78	4.59	4.29
Mixed culture	2.263	4.047	4.630	7.186	57.27	57.22	56.07	56.13	12.03	12.25	12.45	12.81	4.76	4.67	4.50	4.38
Mixed culture + 10 ml N_2 free H_2O	2.276	3.789	5.576	6.326	57.67	57.30	56.14	56.87	12.02	12.08	12.36	12.95	4.80	4.74	4.54	4.39

1 : 075 UF modified saw-dust has 57.91% C; 11.87% N; C : N tario of 4.88 : 1.00

order, while the highest % carbon of 57.27 was observed with mixed culture containing 10 ml N_2 free H_2O followed by *Trichoderma, Curvularia, Phoma* and finally mixed cultures at 1.0 : 1.50 UF modification, in that order. The implication of this is that 1.0 : 0.75 UF modification appears highly suited for cellulolytic activity as more carbon is lost by biodegradation. Also it would appear that at 1.0 : 075 UF modification, the reaction between urea and formaldehyde produced the monomethylol urea as product for the modification of sawdust rather than the dimethylol urea as would be observed in the other reactions of urea and formaldehyde in the presence of more formaldehyde (Pnina, 1979). In all cases, there does not appear to be any consistency as regards fungal activity with respect to the increased formaldehyde used. This may be attributed solely to internal biological factors, as it has been observed that the rate of decomposition cannot be assumed constant (Chapman and Lynch, 1983).

For the % nitrogen content, results on Tables 1 - 4 show that the % nitrogen content of the various UF modified sawdust degraded by various fungi increased significantly. This increase in N_2 was due to loss of carbon, This is also in agreement with the findings of Mishra et. al., (1981). In all cases, the sawdust modified with the least UF mole ratio (1.0 : 0.75) showed the highest % increase in total N_2 content for all the fungi studied. This was followed by sawdust modified with 1.0 : 1.0 UF mole ratio and lastly by 1.0 : 1.50. Evidently, there appears to be no extraordinary increase in fungal activity with increased number of fungal species. This can be attributed to the antagonistic nature observed amongst fungal colony particularly as one of the number is known to produce antibiotics. *Trichoderma viride* produces viridine and by its growth and general antagonism is known to suppress a number of soil fungi (Garret, 1963). Incidentally, *Trichoderma viride* showed the highest % N_2 content after 60 days of incubation on 1.0 : 0.75 UF modified sawdust.

The increase in % nitrogen with increasing incubation time was closely related with decrease in C/N ratio. The decrease in C/N ratio was maximum at 1 : 0.75 UF modified sawdust with *Curvularia species* followed by *Trichoderma viride, Phoma species*, mixed culture and finally mixed culture with 10 ml N_2 free H_2O. The C/N ratio is the most important factor in defining the plant residue quality. High quality (Narrow C : N ratio) will lead to greater mineralisation of nutrients while low quality (wider C : N ratio) plant residue will contribute to more soil aggregate stability.

CONCLUSION

From the foregoing therefore, it has been established that urea formaldehyde could be used to modify sawdust adequately in order to improve on the fertilizing efficiency of this very abundant plant residue. This improvement in the fertilizing efficiency is achieved through fungal or microbial activity. This is evident from the degradation of UF modified sawdust by fungal activity in which the C : N ratio of all the samples

studied was narrowed. Apart from enhancing mineralization of the valuable plant nutrient to the advantage of the plant, sawdust as a plant residue will assist in the maintenance of soil humus and therefore soil aggregate stability. Based on these findings, UF modified sawdust could serve as an adequate slow-release N-fertilizer.

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