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Conversion of Industrial solid wastes to value added products by Filamentous Bacteria

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Abstract : Lignin biodegradation by *Streptomyces* is remarkable than basidiomycetes ligninolysis by the fact that degradation of lignin by *Streptomyces* results in the formation of value added intermediaries and products like APPL, low molecular weight phenols, humic like polycondensates. In the present investigation, composting of industrial wastes by *Streptomyces* showed that the pH of final compost was near neutral and a gain in all major plant nutrients, secondary nutrients as well as micronutrients was evident. The wide initial C/N ratio was radically reduced to around 20 per cent indicating good decomposition. Humic acid content was also substantially higher. Analysis of the water extract of compost sample showed that the compost had low A_{465}/A_{665} ratio indicating the formation of a high molecular weight humic fraction. Polymerization studies with laccase on humic acid fraction of the compost, elucidated that a high molecular weight compound was formed as a product of lignin degradation.

Key words : Industrial solid wastes, Composting, C/N ratio, Streptomyces,

Introduction

Lignins constitute the second most abundant group of biopolymers in the biosphere. It is estimated that the planet currently contains about $3x10^{11}$ metric tons of lignin with an annual biosynthetic rate of approximately $2x10^{10}$ tons (Argyropoulos and Menachem, 1997). Thus their biodegradation occupies an important position in the global carbon cycle. Lignocellulosic material is obtained in large quantities as residual waste in agriculture and forestry, which constitutes an abundant but underutilized source of renewable biomass. A major part comes as straw from barley, corn, paddy, wheat; coir pith and from sugarcane bagasse (Zimmermann and Broda, 1989). Studies of lignin biodegradation are also of great importance for possible biotechnological applications, since lignin polymers are a major obstacle to the efficient utilization of lignocellulosic materials in a wide range of industrial processes (Eggert *et al.*, 1996). And the last two decades has evinced much interest in lignin and its biodegradation because of the substantial potential application of bioligninolytic systems in paper pulping, bleaching, converting lignin to value added products and treating wastes (Kirk and Farrell, 1987). Ligninolytic microorganisms produce various isoforms of extracellular oxidases and peroxidases, which are involved in the degradation of lignin. These enzymes are nonspecific and oxidize a broad spectrum of structurally different substrates, such as highly toxic phenolic compounds, azo dyes and Polycyclic Aromatic Hydrocarbons (PAHs).

Composting is a microbial conversion of organic matter in the presence of suitable amounts of air and moisture into humus like product. Industrial and agro solid wastes are sources of nutrients and though the nutritive value is not complete in a single waste, a judicious combination and composting of the wastes could generate a healthier manure. The simultaneous advantage of the soil conditioning effects due to biological activity has a direct impact on the sustainability of soil health. The process uses very little external energy and as the organic matter is oxidized by microorganisms, it release metabolic heat that hastens the degradation process and also eliminates the pathogens from the raw material that is composted.

Materials and methods

Composting experiment was carried out at M/s Seshashayee Paper and Boards Pvt. Ltd, Cauvery. The waste materials accrued from Seshashayee Paper Boards Pvt. Ltd, Cauvery and Ponni Sugars Pvt. Ltd., were used as substrates for composting with the mass multiplied cultures. The compost beds were prepared from the substrates *viz.*, wet pith (bagasse), MLSS (sludge from secondary clarifier after centrifuging) and press mud in the ratio of 2:1:1 respectively with alternating layers of wet pith or MLSS and press mud were used for composting with a bed size of 4 x I x 1 m with a top width of 0.7 m. About 5 per cent inoculum was used for treatments.

In another experiment conducted at M/s Abdul Azeez Co., Erode, waste materials from tannery was used as substrates for composting. The compost beds were prepared using substrates viz., coir pith, soaking sludge, lime sludge and flesh waste at a ratio of 47.5:32.5:12.5:7.5 respectively with a bed size of 5 x 1 x I m with a top width of 0.75 m.

In both the experiments, the beds were watered periodically maintaining 60 per cent moisture level. And the beds were turned up at fortnightly intervals for aeration. Suitable replications were maintained.

Physico - chemical analysis of compost

Parameter	Method	Reference
Temperature	Using digital thermometer	Davis *et al.* (1992)
Moisture	Drying the sample at 105°C to constant weight	Mooijman and Lustenhouwer (1987)
pH	Compost water suspension at 1: 5 ratio by using pH meter	Falcon *et al.* (1987)
Electrical Conductivity	Compost water suspension at 1: 5 ratio by using conductivity bridge	Falcon *et al.* (1987)
Nitrogen	Microkjeldahl's method	Jackson, (1973)
Phosphorus	Vanadomolybdate yellow colour method	Jackson, (1973)
Potassium	EIL Flame photometer	Jackson, (1973)
Calcium and Magnesium	Tri acid extract	Tandon, (1995)
Total micro nutrients (Cu,Zn, Mn & Fe)	Atomic Absorption spectrophotometer	Lindsay and Norvell, (1978)
Organic carbon	Chromic acid wet digestion method	Walkley and Black, (1934)
Compost maturity	Measuring Humic acid content Qualitative tests H_2S production Starch Iodine test	Sequi *et al.* (1986)

Parameter	Method	Reference
Soluble sugars	Arsenomolybdate reagent method	Somogyi, (1952)
Cellulose	Colorimetric method	Updegraff, (1969)
Hemicellulose	Colorimetric method	Goering and Vansoest, (1970)
Lignin	Biopolymer fractionation method	Chesson, (1978)
Protein	Dye binding assay	Bradford, (1976
Phenols	Folin-Ciocalteau reagent method	Bray and Thrope, (1954)

Results and discussion

The C/N ratio is considered as the conventional and reliable index to establish the maturity of compost. Usually a narrow C/N ratio of 25-30 is favourable for rapid composting, but this again depends on the nature of substrate being composted. Organic carbon serves as an energy source and to build protoplasm and greater amount of carbon is assimilated than nitrogen (Gaur, 1982) and Borkar *et al.* (1991) reported that an increase in carbon dioxide evolution indicated higher degradation of organic carbon. A higher reduction in organic carbon content was observed in treatments inoculated with microbial accelerators. There has been a steady and significant decrease in the C/N ratio as the composting proceeded in both the experiments. Generally in both the experiments, microbial inoculant introduced compost beds showed a precipitous decrease in C/N ratio. Significant difference was observed between treatments. In composting of paper and sugar wastes, there was a reduction of 87.5 and 78 per cent reduction in C/N in treatments with *S. violaceusniger* and *Streptomyces* sp. respectively (Table 1). Similarly in composting of agro and tannery wastes, the reduction was 89 and 65 per cent (Table 2). The reduction in C/N ratio during composting is due to the conversion of carbonaceous materials in to cell biomass, CO_2, water and humus like substances (Kalaichelvi and Ramasamy, 1996). In confirmation the-results of the study indicated that composting narrowed down the CN ratio to optimal margin wherever elite microbial inoculants were introduced.

The soluble sugar and protein are important for the growth and maintenance of microbial cells during fermentation process. A decrease in soluble sugar content is evident from the study due to utilization by the microbial cultures. With respect to composting of paper and sugar industry wastes, soluble sugars though present at high levels during initial stages of composting, through the course of decomposition process it followed a decline phase (Table 1). The decrease in soluble sugar content was much pronounced in treatment with bioinoculants; 32.7 and 26.1 per cent in *S. violaceusniger* and *Streptomyces* sp. inoculated bed respectively. Significant results were obtained with respect to period of composting. However in treatments without wet pith the reduction of sugar content was lesser (18 per cent). In case of composting agro and tannery industry wastes, compost beds prepared of double dose of soaking sludge showed better reduction in sugar content (22 per cent). Treatment with *S. violaceusniger* and *Streptomyces* sp. bioinoculants was statistically at par. The maximum reducing sugars content was noted in inoculation with *S. violaceusniger* (622 mg^{-1}) noted during the first week, which subsequently reduced to 441 mg g^{-1} in 65 days

(Table 2). In paper and sugar industry waste composting trial, the maximum initial phenol content was observed in treatment without wet pith as a substrate (T4) (5.23 mg g^{-1}) (Table 1). There was significant difference between the treatments and the period of composting. Treatment of compost beds with S violaceusniger caused an increase in total phenol of 4.21 to 6.36 mg. g^{-1} (51 per cent). With regard to composting of agro and tannery wastes, the maximum initial phenol content was observed in the treatment with *Streptomyces sp.* (T3) (6.12 mg g^{-1}), which raised to 8.75 mg g^{-1} (42 per cent) in 65 d of composting (Table 2). Generally, in both the composting experiments, results from the phenol content of the treatments showed an increasing trend from the first to the end of composting. The phenol content during the course of composting increased, which may be due to the depolymerization of lignin modifying enzymes produced by the streptomycetes. However, the total phenol content was low which indicated that most of the phenols produced are released in to water.

One of the most difficult aspect of C determination is the evaluation of what fraction of the total C is readily available carbon for microorganisms. Hemicellulose, cellulose and lignin were recalcitrant and inoculation with specific consortia increased the rate of degradation of these substances. Earlier Inoko *et al.* (1982) showed that inoculation of microorganisms resulted in higher decrease in organic carbon content in garbage and paper compost and a constant value was reached in five weeks of incubation. The results of fractionating the organic matter of the samples into their fundamental components showed that cellulose and hemicellulose fractions were higher. In general, the hemicellulose content of the treatments decreased over time period of decomposition. Treatment with *S. violaceusniger* reduced the hemicellulose content to 53 per cent and 47 per cent in paper and sugar wastes and agro-tannery wastes respectively (Table 1 and Table 2). Significant reduction in hemicellulose content was recorded between treatment and the period of composting in both the experiments. In compost trial with paper and sugar industry wastes, a reduction in cellulose content was observed in treatments with microbial inoculants (T2, T3 and T5). Control treatment had an initial cellulose content of 211.61 mg g^{-1}. The maximum initial content of cellulose was recorded in treatment with S. violaceusniger (236.73 mg g^{-1}), which was degraded to a level of 123.86 mg g^{-1}) (47.68 per cent) in 65 days period. In the composting process, cellulose reduction to an extent of 25 - 48 per cent was observed on 65th day of sampling (Table 1). In compost beds prepared with agro and tannery wastes as substrates, the per cent cellulose degraded at the end of eight weeks was maximum with bioinoculant *S. violaceusniger* (45.38 per cent). However when the dose of soaking sludge was doubled in the compost beds a reduction in utilization of cellulose was noticed as observed by the reduction of cellulose utilization to 26.6 per cent (Table 2). Lignin content reduced over time irrespective of the treatments and waste substrates. In compost beds with paper and sugar industry wastes, maximum reduction of lignin (33.15 per cent) was observed in compost beds with out wet pith and inoculated with *S. violaceusniger* (T5) followed by beds with wet pith inoculated with *S. violaceusniger* (T2) (32.6 per cent) (Table 1). The lignin degradation was more in the first week itself (up to 8 per cent) when compost beds are inoculated with *S. violaceusniger* (T2) as compared to uninoculated beds (T1) (2 per cent). With regard to compost beds prepared of agro and tannery wastes, the per cent of lignin reduction in case of beds inoculated with *S. violaceusniger* was 23 (Table 2). The content of lignin decreased with increase of time irrespective of treatments. Water-soluble components were more labile and are readily used

by microorganisms for their nutrition and cell build-up, which in turn helped in higher degradation of polymer substances (Ramasamy *et al*., 1994). The decrease in soluble sugars and other water-soluble substances during the course of composting could be related to this phenomenon.

The maturity and stability of the compost is crucial, since the adverse effects of immature composts are evident during storage and application. The maturity of compost is critical and the criteria for mature compost reflect achievement of the objectives of composting. In the present investigation, apart from the gaze of brown colour and lack of noxious smell, the final composts gained appreciable nutritive value including the micronutrient content. One critical point about C/N ratio of mature compost is that further decomposition of the product in soils does not require soil N, but releases mineral N into the soil. So the acceptable limit of C/N ratio 20 was achieved in the present study. Also the pH and temperature attained safe levels. The maturity parameters of composts obtained with inoculation of *S. violaceusniger* were analyzed and are presented

Table 1. Dynamics of carbon substrate degradation during the composting of sugar and paper industry solid wastes

Treatment	C/N per cent reduction	Cellulose degradation (%)	Hemi-cellulose degradation (%)	Lignin degradation (%)	Soluble sugars (% decrease)	Total phenol (% increase)
T_1	52.13	24.96	22.76	12.22	15.27	20.86
T_2	87.51	47.68	53.11	32.61	32.76	51.07
T_3	78.32	35.17	32.14	28.58	26.14	37.26
T_4	51.62	27.60	24.58	18.81	18.32	48.95
T_5	77.46	36.38	50.04	33.15	29.60	42.11

T_1 : Wet pith + MLSS + press mud 2:1:1
T_2 : Wet pith + MLSS + press mud 2:1:1 + *Streptomyces violaceusniger*
T_3 : Wet pith + MLSS + press mud 2:1:1 + *Streptomyces sp.*
T_4 : MLSS + press mud 1:1
T_5 : MLSS + press mud 1:1 + *Streptomyces violaceusniger*

Table 2. Dynamics of carbon substrate degradation during the composting of agro and tannery solid wastes

Treatment	C/N per cent reduction	Cellulose degradation (%)	Hemi-cellulose degradation (%)	Lignin degradation (%)	Soluble sugars (% decrease)	Total phenol (% increase)
T_1	61.50	23.05	21.72	11.45	22.36	21.47
T_2	85.73	45.38	46.98	22.93	33.40	36.11
T_3	69.52	40.24	41.12	16.00	31.78	42.98
T_4	78.05	26.63	37.14	27.94	22.17	27.61

T_1 : Coir pith + Soaking sludge + Lime sludge + Flesh waste 47.5: 32.5: 12.5: 7.5
T_2 : Coir pith + Soaking sludge + Lime sludge + Flesh waste 47.5: 32.5: 12.5:.7.5 + *S. violaceusniger*
T_3 : Coir pith + Soaking sludge + Lime sludge + Flesh waste 47.5: 32.5: 12.5: 7.5 + *Streptomyces sp.*
T_4 : Coir pith + Soaking sludge + Lime sludge + Flesh waste 17.5: 62.5: 12.5: 7.5

by microorganisms for their nutrition and cell build-up, which in turn helped in higher degradation of polymer substances (Ramasamy *et al*., 1994). The decrease in soluble sugars and other water-soluble substances during the course of composting could be related to this phenomenon.

The maturity and stability of the compost is crucial, since the adverse effects of immature composts are evident during storage and application. The maturity of compost is critical and the criteria for mature compost reflect achievement of the objectives of composting. In the present investigation, apart from the gaze of brown colour and lack of noxious smell, the final composts gained appreciable nutritive value including the micronutrient content. One critical point about C/N ratio of mature compost is that further decomposition of the product in soils does not require soil N, but releases mineral N into the soil. So the acceptable limit of C/N ratio 20 was achieved in the present study. Also the pH and temperature attained safe levels. The maturity parameters of composts obtained with inoculation of *S. violaceusniger* were analyzed and are presented

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T_1	61.50	23.05	21.72	11.45	22.36	21.47
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in Table 3. The composts were having optimal major and secondary plant nutrients as well as enriched with micronutrients. Humic acid content was higher in the range of 14.8 - 16.5 per cent in composts. Qualitative analyses of compost like production of odouriferous compounds or H_2S, indicated that the composts were devoid of noxious smell and the colour was tea brown. And the starch-iodine test revealed that the composting of polymers is complete. Analysis of the water extract of compost sample showed that the compost had a water soluble phenol content of 0.038 - 0.064 per cent. Another important parameter determining compost maturity is the spectral characteristics. The ratio of optical densities of humic acid at A_{465}/A_{665} is considered to reflect the degree of condensation of aromatic nucleus of humus or its maturity and also the ratio was related to molecular weight of humic substances; a low ratio indicates high molecular weight and vice versa (Chen et al., 1977). The study recorded a low A_{465}/A_{665} ratio of 10.28 and 11.69 indicating that, the humic fraction is of high molecular weight compounds (Table 3).

Table 3. Characteristics of the composts obtained from *Streptomyces violaceusniger* inoculated beds

Parameter	Compost from Sugar industry solid wastes	Compost from tannery solid wastes
Colour	Brown	Dark brown
Smell	No noxious smell	No noxious smell
PH	6.8	7.05
C/N ratio	20.55	21.27
Temperature (°C)	32.0	34.5
Water holding capacity (per cent)	24.6	28.0
Respiration rate (mg CO_2 C. g compost^{-1} C. day^{-1})	5.45	4.85
Humic acid content (per cent)	14.8	16.5
N content (per cent)	1.52	1.14
P content (per cent)	2.24	1.86
K content (per cent)	0.88	0.62
Ca (per cent)	0.64	0.79
Mg (per cent)	0.36	0.42
Micronutrient content :		
Fe (μg g^{-1})	1820	365
Mn (μg g^{-1})	290	58
Zn (μg g^{-1})	164	42
Cu (μg g^{-1})	52	84
Analysis of water extract:		
Water soluble phenols (per cent)	0.038	0.064
Spectral characteristic of alkali extract (A_{465}/A_{665} ratio)	10.28	11.69

by microorganisms for their nutrition and cell build-up, which in turn helped in higher degradation of polymer substances (Ramasamy *et al*., 1994). The decrease in soluble sugars and other water-soluble substances during the course of composting could be related to this phenomenon.

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Table 2. Dynamics of carbon substrate degradation during the composting of agro and tannery solid wastes

Treatment	C/N per cent reduction	Cellulose degradation (%)	Hemi-cellulose degradation (%)	Lignin degradation (%)	Soluble sugars (% decrease)	Total phenol (% increase)
T_1	61.50	23.05	21.72	11.45	22.36	21.47
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