Composition and characterization of humic substances extracted from effluent-based pressmud composts

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Abstract: Humus production is the end result of composting and the value of compost can be based upon its humus value. The objectives of this study were to measure and quantify the humic substances (HS) produced during the composting of pressmud with other biodegradables and to characterize the HS extracted from matured composts for their elemental composition, functional groups and total acidity. The composts, which received inorganic additives, enhanced the formation of both HA (Humic Acid) and FA (Fulvic acid) compared with compost without additives. The carbon (C) and nitrogen (N) contents in HA were higher than in FA whereas oxygen (O) and hydrogen (H) contents were higher in FA than HA samples. The HS extracted from enriched composts were higher in oxygen and low in carbon content which suggests more labile nature of these fractions. The N/C and O/C ratios were considerably high in both HA and FA extracted from enriched composts than that from un-enriched composts. The content of carboxyl and phenolic hydroxyl groups, and total acidity were larger in HA's than in those from FA's. The E_4/E_6 ratios of FA's were higher than HA's and enriched composts exhibit considerably higher ratios compared to un-enriched composts indicating more of aliphatic nature of the fractions.

Additional key words: *Pressmud, windrow composting, humic acid, fulvic acid*

Introduction

The recycling of renewable organic wastes and industrial by-products as fertilizer for maintenance of soil health by hygienic methods is vital for increasing crop production. India is the second largest sugar producing country in the world. Sugar industries generate large quantities of liquid and solid wastes. The industries release annually about 7.5 and 5.0 million tones of molasses and pressmud, respectively as wastes. The substantial quantities of mineral nutrients as well as humus in these wastes are of great importance for maintaining soil organic matter especially in semi-arid tropics of India. Composting is

the most widely applicable process of handling these diverse wastes as a means of converting raw waste organic matter into usable humus. Humus production is the end result of composting and the value of compost can be based upon its humus value. The beneficial effect of humified organic materials on the physical and chemical properties of soil and nutrient uptake by roots has been increasingly recognized. Though the value of humus is more difficult to determine, appropriate techniques of composting organic wastes with suitable additives can greatly improve humus as well as fertilizer value of the compost. When pressmud is used as base material, the precursors for humic synthesis will be pre-dominantly

cellulose and lignin. The release of humic substances may also be favoured if lignin structure is weakened by composting through polymerization or condensation reaction of the phenolic compounds (Singh and Amberger 1990). No extensive research has been carried out on the characterization of humic substances extracted from distillery effluent-based pressmud composts. Keeping this in view, the present investigation was undertaken to quantify and characterize humic fractions extracted from these composts for better understanding.

Materials and Methods

The basic organic material used for composting was pressmud. Rock phosphate and micronutrients *viz.*, $ZnSO_4$ and $FeSO_4$ were used as inorganic additives. The organic additives used with pressmud were yeast sludge, bagasse, coirpith, water hyacinth and sugarcane trash and their initial properties are shown in table 1. There were six treatment combinations containing pressmud, organic and inorganic additives (Table 2).

Pressmud windrows of 2 tonnes capacity, which were 4.5 m in length, 1.5 m in width at the base and 1.5 m in height were constructed on the cement platform of Compost Yard, M/s Sakthi Sugars Ltd., Sakthinagar, Tamil Nadu, India as outlined by

Kuhlman (1990). The organic additives were added, each at 2% of the fresh weight of pressmud and mixed thoroughly using aerotiller. Then, weighed quantities of inorganic additives *viz.*, rock phosphate as Mussoorie rock phosphate at the rate of 5 per cent and micronutrients [$ZnSO_4$, $7H_2O$ and $FeSO_4$, $5H_2O$ each at 1.5 % (w/w basis)] were spread over the windrows and again mixed well. After two days, composite microbial culture at 0.1 % level was added and turnings were given to facilitate for complete contact with the decomposing materials. From the fifth day onwards, primary treated effluent at 1:3 (w/v) pressmud-effluent ratios was uniformly added for all the treatments at weekly intervals up to 90 days. After each addition of effluent to the windrows, the materials were mixed thoroughly by mechanical means using aerotiller and aeration was achieved by turning over the compost mass. Moisture level was maintained, during the whole bio-oxidative phase, between 40 and 60%, which was considered the optimum range (Spohn 1978).

Samples were collected from the left, middle and right side of the windrows at 30, 60, and 90 cm depths periodically during the composting process at an interval of 15 days up to 120 days. Samples from each side of the windrows at 3 depths were combined and

Table 1. Physico-chemical properties of raw materials used for composting process

Parameters	Pressmud	Coirpith	Yeast sludge	Bagasse	Sugarcane trash	Water hyacinth
pH (1:10 w/v)	7.18	5.91	5.45	4.74	6.95	6.95
EC (dSm^{-1})	2.96	1.43	9.30	0.33	0.63	6.15
Organic carbon (%)	32.60	37.26	9.52	37.98	36.00	27.67
Nitrogen (%)	1.20	0.42	1.67	0.45	0.44	0.81
C:N ratio	27.17	88.71	5.70	84.40	81.82	34.16
Phosphorus (%)	1.15	0.02	0.78	0.06	0.17	0.07
Potassium (%)	0.62	0.54	4.48	0.14	0.46	1.43
Calcium (%)	4.14	0.46	7.66	0.70	0.24	1.76
Magnesium (%)	1.10	0.31	0.77	0.30	0.18	1.40
Lignin (%)	16.40	49.80	5.31	20.82	12.20	28.24
Cellulose (%)	13.52	34.90	7.00	21.00	20.20	24.35
Hemicellulose (%)	19.38	7.30	6.80	16.00	8.00	10.61
Total phenols (mg 100g^{-1})	23.30	116.70	22.20	62.00	89.60	83.20

mixed to generate a single composite. The resulting three composite samples were air-dried and ground in a hammer mill followed pulverization using a vibrating cutter, to pass through a 1-mm sieve and utilized for analysis of various parameters. Temperature was monitored at weekly intervals using a digital thermometer probe (± 0.1 °C) inserted at different locations and depths in windrows (Bhoyar et al. 1979).

The pH and electrical conductivity (EC) were determined in a 1:10 compost-water suspension (Jackson 1973). Organic carbon was determined by chromic acid wet digestion method (Walkley and Black 1934) and total nitrogen by Kjeldahl micro-analysis (Piper 1966).

Extraction and composition analysis of humic substances

Humic and fulvic acids from compost samples were extracted with 0.1 N NaOH by using the method of Schnitzer and Skinner (1968). The alkali extraction was repeated thrice for complete extraction of humic acid. The pooled alkali extract (soluble humic substances) was acidified to pH 2.0 with 2N HCl, stirred well and allowed to stand at room temperature for 24 hours. The precipitated humic acid fraction was separated by centrifugation. Precipitation and centrifugation were repeated to attain partial purification of humic acid fraction as described by Stevenson (1981). The fractions were further purified by treating with HCl-HF mixture (5ml of each HCl and HF acids were dissolved in 990 ml of double distilled water) for 24 hours and this acid mixture was separated by centrifugation. The residue so obtained was thoroughly washed with distilled water, freeze dried, weighed, and expressed as a percentage of dry weight.

The soluble fulvic acid from coagulate was separated by centrifugation and purified by the modified procedure as given by Wander and Traina (1996). The aqueous solution obtained after centrifugation was passed through exchange resin (Seralite SRC-120) in the H^+ form. For this, adsorption columns of 20 cm length with a porcelain perforated bed were

used. Over this, a glass wool packing of 0.5 cm was placed. Then, the resin was uniformly packed up to 15 cm height using a wet packing method. Over the column, glass wool packing (0.5 cm) was again placed. The aqueous solutions were eluted through this column four times and the eluted fulvic acid fraction was directly transferred to 100 mwco dialysis bags and dialysed against double distilled water for 24 hours. The dialysed fraction was evaporated under low temperature, freeze dried, weighed, and expressed as a percentage of dry weight.

Characterization of humic substances

Humic and fulvic acids extracted from composts after 120 days of decomposition, were subjected for elemental analysis using procedures outlined by Schnitzer (1982). The carbon, hydrogen and nitrogen content of the humic and fulvic acids were estimated by dry combustion method using CHN analyser (Carlo Erba Strumetazione – MOD 1106). The oxygen content was considered as the difference between the sum of the C, H, and N percentages and a hundred. The molar ratios of elements were computed by dividing the content of elements in percentage obtained from analysis by their atomic mass (Orlov et al. 1992).

Total acidity of humic substances was estimated from the reaction with barium hydroxide and the carboxyl (CO_2H) groups from the reaction with calcium acetate (Wright and Schnitzer 1959). The amounts of phenolic OH groups were calculated in the following manner:

Phenolic OH group (meq g^{-1}) = Total acidity (meq g^{-1}) – CO_2H group (meq g^{-1})

The E_4/E_6 ratios of humic and fulvic acids, extracted from matured composts were determined by the procedure as described by Schnitzer and Khan (1972).

Humic acids extracted from matured composts were subjected to infrared spectrophotometric analysis (Tan 1996). Potassium bromide (KBr) pellets were prepared by mixing 1.0 mg of humic acid with 400 mg of dry KBr and pressed in a suitable dye under vacuum

at a pressure of 7,500 kg cm^{-2} for 20 min. These KBr pellets were used for analysis and the spectra were obtained in the region 400 to 4000 cm^{-1} frequency.

Results and Discussion

Changes in carbon to nitrogen (C/N) ratio during the composting

The rate of mineralisation of organic matter during composting was measured by determining the C/N ratio of the decomposing mixture. The C/N ratios of the mixtures were high at the initial stages, decreased substantially up to 90th day, and became comparatively stable later on (Fig. 1). The composts which received rock phosphate alone, and with organic additives showed higher potentialities in narrowing the C/N ratio of the composts. Similar observations were made by Bhanawase *et al.* (1994) who reported that the addition of RP resulted in lower C/N ratios in wheat straw compost. The incorporation of yeast sludge (rich in N) and bagasse (substrate for microbes) as additives might have also helped in increasing biological activity. In addition, coir pith might have provided aeration for proliferation of aerobic microbes. In this respect, the reduced loss of NH$_3$ in the presence of these additives has been attributed to a high content of easily decomposable cellulose, which forms a readily available energy source for microorganisms to multiply and immobilize nitrogen.

Composition of the humic substances

The changes in humic and fulvic acid contents during the composting process are shown in table 2. The percentage of the different fractions of humic substances varied and was dependent upon the organic materials in which polymerization and humification have taken place. The humic acid content increased considerably by the 60th day; subsequently the increase was gradual up to 120th day. Fulvic acid contents in compost mixtures was increased in the first 60 days of decomposition in all the treatments, and decreased during remaining period of the composting. This suggests that during early stage of composting, a

Table 2. Changes in humic and fulvic acids contents (%) at different periods of composting of pressmud and other biodegradables

Treatment	Composting period (days)			
	30	60	90	120
	Humic acid (% of dry matter)			
C1 -Pressmud (PM) only	10.61	13.00	14.45	14.72
C2 -PM+Organic Additives (OA)	10.80	14.24	15.00	15.20
C3 -PM+OA+Rock phosphate (RP) @5%	9.95	14.00	15.15	15.70
C4-PM+OA+RP@5%+ ZnSO$_4$ & FeSO$_4$ each@1.5%	10.00	14.10	15.33	15.75
C5 -PM+OA+Rock phosphate (RP) @10%	9.25	13.50	14.70	15.40
C6-PM+OA+RP@10%+ ZnSO$_4$ & FeSO$_4$ each@1.5%	9.45	14.10	14.79	16.30
LSD (P≤0.05): composts 0.34; days 0.28; composts x days 0.68.				
	Fulvic acid (% of dry matter)			
C1 --Pressmud (PM) only	12.00	19.30	15.40	14.75
C2 -PM+Organic Additives (OA)	13.62	22.71	16.53	15.64
C3 -PM+OA+Rock phosphate (RP) @5%	15.11	25.85	17.10	15.85
C4-PM+OA+RP@5%+ ZnSO$_4$ & FeSO$_4$ each@1.5%	16.22	26.50	20.50	16.40
C5 -PM+OA+Rock phosphate (RP) @10%	15.30	23.50	17.20	16.90
C6-PM+OA+RP@10%+ ZnSO$_4$ & FeSO$_4$ each@1.5%	16.25	27.80	20.70	17.13
LSD (P≤0.05): composts 0.57; days 0.46; composts x days 1.13.				

All the treatments received treated distillery spentwash at 1.3 (w/v) pressmud-effluent ratio; LSD-Least significant difference at the 5% probability level.

partial acid co-precipitation of incompletely humified components of organic matter could have occurred, because of the dependence of oxidation rate on the chemical nature of the organic compounds (Moré et al. 1987). Further, the compost mixtures might have contained a mixture of both humic and non-humic components. Some of these are of an aromatic nature (phenolic acids, benezene carboxylic acids, lignin-derived products) and others of an aliphatic nature (fatty acids, decarboxylic acids etc.), which are considered to be humic in nature (Inbar et al. 1990). Non-humic substances such as polysaccharides might have also formed from the beginning of composting. These usually exist in the fulvic acid-like fraction bonded to form highly complex polymers of an aliphatic nature with a very low degree of aromaticity

(Vila et al. 1982). Hence, as the decomposition proceeded with time, these are converted gradually into humic acids.

The results also show that the relative percentages of fulvic acids in all the composts were higher than humic acids, indicating fulvic acid formation through lower polymerization. The composts. which received inorganic additives, enhanced the formation of both humic and fulvic acids compared to compost without additives. Adequate degradation of ligno-cellulosic materials, as discussed earlier, might be a factor in increasing the formation of humic substances through condensation and polymerization reactions (Stevenson 1982).
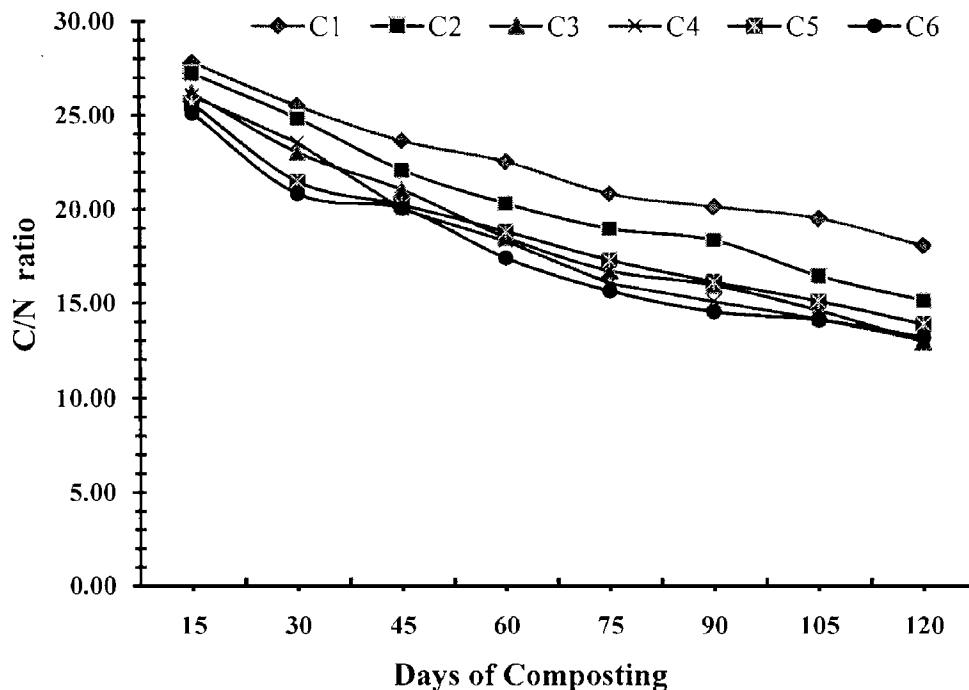

Fig. 1. Changes in C/N ratio during the composting process of pressmud and other biodegradables. C1- Pressmud (PM) alone; C2- PM+Organic additives (OA); C3- PM+OA + Rock phosphate (RP) @ 5%; C4-PM+OA+RP @ 5%+ZnSO₄ & FeSO₄ each @ 1.5%; C5- PM+OA+Rock phosphate (RP) @ 10%; C6 - PM+OA+RP @ 10%+ ZnSO₄ & FeSO₄ each @ 1.5%. Plotted values are means of three replicates.

Table 3. Elemental composition of humic acid extracted from matured composts

Composts	Contents of elements (%)				Molar ratios of elements		
	C	H	N	O	H/C	N/C	O/C
C1	46.36	6.05	4.30	43.29	1.57	0.080	0.700
C2	43.22	2.13	4.35	50.04	0.59	0.085	0.868
C3	43.91	3.91	4.59	47.59	1.07	0.090	0.813
C4	45.27	2.87	4.43	47.43	0.76	0.084	0.786
C5	42.68	3.20	4.38	50.26	0.90	0.088	0.883
C6	45.00	3.25	4.40	47.35	0.87	0.084	0.789

Table 4. Elemental composition of fulvic acid extracted from matured composts

Composts	Content of elements (%)				Molar ratios of elements		
	C	H	N	O	H/C	N/C	O/C
C1	34.50	5.61	2.52	57.37	1.95	0.063	1.25
C2	34.18	5.03	2.77	58.02	1.77	0.070	1.27
C3	32.80	4.51	2.71	59.98	1.65	0.071	1.37
C4	32.28	4.68	2.70	60.34	1.74	0.072	1.40
C5	31.68	4.86	2.83	60.63	1.84	0.077	1.44
C6	32.30	5.00	2.77	59.93	1.86	0.074	1.39

Characterization of humic substances

Elemental composition

The humic and fulvic acids extracted from matured composts i.e., 120 days of decomposition, were used to study the elemental analysis and their molar ratios and the results are presented in tables 3 and 4. The carbon and nitrogen contents were found higher in humic acid fractions than in fulvic acids while in case of hydrogen and oxygen, fulvic acid samples contained higher amounts than humic acids. Among the composts, C1 compost had highest carbon and hydrogen contents in both humic and fulvic acids. The lowest carbon content was recorded in humic and fulvic acids of C5 compost and that of hydrogen in humic acid of C2 compost (2.13 %) and fulvic acid of C3 compost (4.51 %). The highest nitrogen content was observed in humic acid of C3 compost (4.59 %) and fulvic acid of C5 compost (2.83 %) while the highest oxygen content was noticed in both humic and fulvic acids of C5 compost. The humic and fulvic acids extracted from enriched composts (C3 to C6) were found to contain higher oxygen and lower carbon contents, suggesting the more labile nature of these fractions and high degree of humification.

The results of the molar ratios of elements suggest the stoichiometric relationships that exist among the elements. The N/C and O/C ratios were considerably high in both humic and fulvic acids extracted from enriched composts than that from unenriched composts. This indicated that the content of acid-insoluble humic nitrogen increased considerably during composting which might have enriched the humus when they contained large amount of carbohydrates. Further, the lower H/C ratio in these fractions suggest that polymerization and/or condensation take place well, due to the introduction of carbohydrate and oxidation of the phenolic compounds with methoxyl groups and /or aliphatic side chain in the HA's, which occur during degradation of ligno-cellulosic materials present in compost mixtures (Vila et al. 1982).

Functional groups and E_4/E_6 ratios

The results of functional groups revealed that the content of carboxyl and phenolic hydroxyl groups, and total acidity were higher in HA's than in those from FA's (Table 5) which further confirm the above findings. The introduction of carbohydrate by

Table 5. Functional groups and E_4/E_6 ratio of humic and fulvic acids extracted from matured composts

Composts	Humic acid				Fulvic acid			
	Total acidity (meq g^{-1})	Carboxyl group (meq g^{-1})	Phenolic OH (meq g^{-1})	E_4/E_6 ratios	Total acidity (meq g^{-1})	Carboxyl group (meq g^{-1})	Phenolic OH (meq g^{-1})	E_4/E_6 ratios
C_1	10.24	6.14	4.10	4.85	7.50	5.50	2.00	8.19
C_2	10.46	7.11	3.35	5.34	7.90	5.10	2.81	8.34
C_3	10.71	7.50	3.21	6.78	8.27	6.56	1.71	9.00
C_4	11.10	7.61	3.49	6.91	8.30	6.52	1.78	9.07
C_5	10.68	7.48	3.20	7.15	8.32	6.48	1.84	9.23
C_6	10.75	7.56	3.19	7.03	8.29	6.55	1.74	9.14

degradation of ligno-cellulosic materials reflected in the high content of alcoholic OH, and the conversion of these alcoholic OH to carboxyl and phenolic OH groups in the HA's might have occurred by an oxidation reaction (Kakezawa et al. 1992). Higher content of carboxyl groups in both HA's and FA's than the phenolic hydroxyl groups suggest that the carbohydrates and phenolic compounds produced in these substances were easily degradable and thus readily converted to carboxyl groups on subsequent oxidation. The higher acidity or exchange-capacity of these humic substances could be attributed to the occurrence of ionisable H⁺ ions of carboxyl and hydroxyl groups found in aliphatic chains or aromatic rings of molecules (Schnitzer 1982). Relatively high content of oxygen and low content of carboxyl groups and total acidity determined in FA's than in HA's may be fictitious, caused by the lability of humic substances under alkaline conditions. Similar results have also been reported by Pandeya (1992) and Gundappa (1999). These authors reported that almost all possible functional groups (Carboxyls, phenolic, enolic and alcoholic hydroxyls; quinones, hydroxyquinones, other carbonyls, esters, lactones, ethers) are conclusively O-containing groups but there is no agreement on their amounts or even on their very existence. From 6 to 46% of the oxygen is not accounted for in the functional groups of various humic substances and it is also not known how much of this oxygen is in linkages, as structural element (Dubach and Mehta 1963).

The E4/E6 ratio is a valid and informative index for the characterization of humic substances with respect of aromaticity (Kononova 1966). In the present study, the E4/E6 ratios of fulvic acids were found higher than humic acids (Table 5). Enriched composts showed considerably higher ratios of both HA's and FA's than unenriched composts indicating more of aliphatic nature of the fractions (Garcia et al. 1991). Further, it is frequently suggested in the soil science literature (Schnitzer and Khan 1972 and Schnitzer 1976) that the magnitude of the E4/E6 ratio of humic substances is related to the relative concentration of condensed aromatic rings in these materials. Therefore, the high E4/E6 ratios of humic substances of enriched composts in the present study, supposedly indicates a relatively low concentration of condensed ring structures, which reflects a low degree of aromatic condensation and thus, infers the presence of relatively large proportions of aliphatic structures. Similar observations were also made by Pandeya (1992) and Kadalli et al. (2000).

Infrared spectra of humic acid of matured composts

Infrared spectra of humic compounds are mainly used to get valuable information on the structure including the distribution of functional groups. The absorption band in the 2950-2900 cm^{-1} and 3400 cm^{-1} region was decreased in all the samples except in humic acid of C1 compost where the absorption band in 3400-3300 cm^{-1} region was increased slightly (Fig. 2). The absorption in the 3400-3300 cm^{-1} region is mainly due to -OH and -NH stretching vibrations
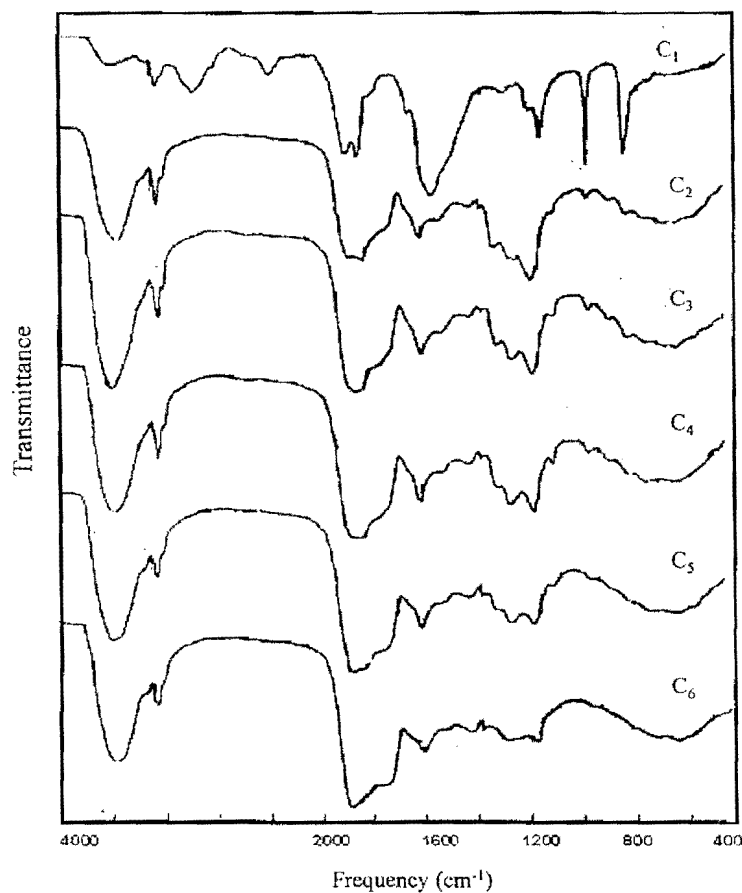
Fig. 2. Infrared spectra of humic acid extracted from matured pressmud composts

(Schnitzer 1971). More widening was observed in humic acids isolated from C2 to C6 composts, probably, due to high proportion of OH bond whereas the less absorption band in C1 compost may be due to lower proportion of OH bond. The decrease in absorption band in the region 2950-2900 cm^{-1} frequency indicates the aliphatic C-H stretching of the molecule. Similar observations were reported by Gundappa (1999). The difference in intensity of the absorption band in the 1700-1600 cm^{-1} region may be due to the more free COOH groups in the samples probably by incorporation of micronutrients or substrate rich in cations, as stated by Banerjee and Mukherjee (1972).

Thus, the foregoing results conclude that inorganic additives can be added for composting of pressmud with other biodegradables at the beginning of the composting itself for increasing humus content.

Incorporation of micronutrient cations such as Fe and Zn to compost mixers would get ionized and complexed with humic fractions released during decomposition of pressmud.

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