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Original Research Article

Composting of Pressmud Using Microbial Inoculants Isolated from Earthworm Gut

B. Rajesh[1], J. Arockia John Paul[2], N. Karmegam[3]*

[1]Shivashakti Bio Technologies Ltd, Rudrapur- 263 153, Uttarakhand, India
[2]Department of Zoology, Arumugam Pillai Seethai Ammal College, Tiruppattur– 630 211, Tamil Nadu, India
[3]Department of Botany, Government Arts College, Salem-636 007, Tamil Nadu, India

**Corresponding author.*

Abstract

Earthworm gut microbial isolates as inoculants for composting (windrow method) of pressmud along with the application of spent wash as a primary source of moisture has been studied. The changes physicochemical changes accompanying the composting process of pressmud were observed. Composting cycle took nine weeks, during the process every 15 days, physicochemical properties of pressmud compost piles, temperature, moisture, pH, total organic carbon, and total nitrogen, phosphorus and potassium (NPK) were analyzed. The NPK contents increased towards the end of composting process. The bacterial (B) isolate B2 showed narrow C:N ratio and superior compost quality parameters within 60 days of composting in windrow method than the bacterial (B1, B3 and B4) and fungal (F5, F6 and F7) isolates. The duration of composting with bacterial isolate B2 (*Pseudomonas* species) was shorter than normal (un-inoculated) composting and composting with other isolates.

Keywords

Biocomposting
Eudrilus eugeniae
Gut microflora
Pressmud
Spent wash

Introduction

Sugar industry in India is the second largest agro processing industry after textiles. A typical sugar factory generates large quantity of by products like bagasse, pressmud and molasses. The environmental problem associated with distillery effluent could be mitigated by chemically or biologically stabilized soluble nutrients through aerobic composting process. Composting of sugar factory pressmud and distillery spent wash could convert soluble nutrients to more stable organic forms. Chemical and physical properties of the compost have been well characterized (Hue and Liu, 1995). Composting is one of the ways of managing organic wastes involves biological aerobic transformation of organic wastes into stabilized organic product.

Earthworms are the unheralded soldiers of the soil. Vermicomposts are produced from organic wastes through interactions between earthworms and microorganisms, and can be utilized as plant growth media or soil amendments (Edwards and Arancon, 2004). During vermicomposting, temperature and moisture can act synergistically (Gunadi and Edwards, 2003). Solutions to the problems of disposal of animal solid waste require complex environmental control. Recycling of different organic wastes within short period of time than composting can be achieved by utilizing earthworms. The literature shows that the pressmud can be converted to vermicompost by using exotic earthworms, *Eisenia fetida* and *Eudrilus eugeniae* successfully (Ranganathan, 2006).

But the scientific knowledge on the availability of the microorganisms which can convert complex substances, cellulose, lignin and hemicellulose and their utilization for the enhancement of composting and vermicomposting are lacking. The present study was carried out with the following objectives of isolating and identify cellulose, lignin degrading and phosphate solubilizing microbes from the gut of *E. eugeniae* and to apply the microbial isolates as inoculants for composting (windrow method) of pressmud along with the application of spent wash as a primary source of moisture and to study the changes accompanying the composting processes.

Materials and methods

The raw material, pressmud (PM) was collected from M/S. The Rampur Distilleries Ltd., located in Rampur, Uttrapradesh. The earthworm, *Eudrilus eugeniae* (Kinberg.) collected from culture bank of the Department of Biology, Gandhigram Rural University, Tamil Nadu, India was mass multiplied in cowdung and used for the study. The cultures of *E. eugeniae* were acclimatized to the laboratory conditions using the culture medium, pressmud. After acclimatization, the worms were mass multiplied in the laboratory in plastic troughs of 45×30×15 cm size.

The study has been conducted in the Organic Manure Production Unit located in the premises of Rampur Distilleries Limited, Rampur, Uttar Pradesh State. The distillery units of liquid waste like spent wash and pressmud collected, these wastes are converted into humus containing organic material called bio-compost advantageous for crop production. The experimental details of the treatments implemented are given below.

T1: 1 Ton / m^2 PM + SW + B1 bioinoculum.
T2: 1 Ton / m^2 PM + SW + B2 bioinoculum.
T3: 1 Ton / m^2 PM + SW + B3 bioinoculum.
T4: 1 Ton / m^2 PM + SW + B4 bioinoculum.
T5: 1 Ton / m^2 PM + SW + F5 bioinoculum.
T6: 1 Ton / m^2 PM + SW + F6 bioinoculum.
T7: 1 Ton / m^2 PM + SW + F7 bioinoculum.
T8: 1 Ton / m^2 PM + SW (Control).
(PM- pressmud; SW-spent wash; B-bacterial isolates; F-fungal isolates)

A composting cycle took nine weeks to complete, during the process, every 15 days once physico-chemical properties like temperature, moisture, pH, organic carbon, organic matter, nitrogen, phosphorus and potassium were analyzed by adopting standard methods.

Pressmud was collected and transported to the compost yard provided with concrete platform that permits heavy equipment operation. Using a front end loader, eight compost piles were formed with pressmud. Each pile had 1 ton of pressmud. The compost piles were formed in a triangular shape with a dimension of 3 to 3.5 meters at the base and 1 to 1.5 meters height. In order to bring down the initial moisture content of pressmud from seventy percentage to fifty percentage, the piles were turned using the aerotiller (Fig. 1-A) on the sixth or seventh day after formation and then retrimmed. The pressmud piles were applied with microbial cultures (Fig. 1-B) isolated from earthworm gut. The dosage of the starter culture was one liter per ton of pressmud was applied on the pressmud compost piles to accelerate the decomposition process. All isolated microorganisms had the cell count of 5×10^9 cells/ml.

Application of spent wash on the compost piles was done based on the temperature prevailing inside the compost heaps. This was monitored regularly by measuring the temperature of the compost pile (Fig. 1-C). During active composting period, the temperature of the pressmud piles was maintained between 50 and 70°C.

Compost piles were sprayed with spent wash to attain desired moisture level. The total volume applicable each week was sprayed three to four times during the week based on the temperature. Application of spent wash was stopped during twelfth week. Spent wash application was stopped during the eight week, and the compost was allowed to cure and age. At this stage water was applied and aerotilling was continued as scheduled. The physical appearance of final compost is shown in Fig. 1-D.

Aerotilling was continued until the moisture stabilizes at 30 - 35%. Compost was powdery and free flowing. The maturity of the final product was evaluated to assess the state of decomposition and the suitability of the organic manure as a medium for plant growth. Compost samples were collected at different depths and from different locations from the compost piles. These samples were mixed together to form composite samples and stored in refrigerator in polythene bags. Analyses such as pH values, and moisture content were done on moist samples, nutrient contents (Organic carbon, NPK) were made with air-dried samples using standard procedures as given in Table 1. Samples were dried at 35°C in the shade, then ground to pass through a 2 mm sieve and stored in screw capped jars.

Table 1. Methods adopted for analyzing various physico-chemical parameters of the composts.

Parameter analyzed	Methodology adopted	Reference
pH	Digital pH meter (Elico)	Jackson (1973)
Electrical Conductivity (E.C.)	Conductivity Bridge (Elico)	Jackson (1973)
Total organic carbon	Potassium dichromate oxidation method	Walkley and Black (1934)
Total nitrogen	Micro-Kjeldhal method	Tandon (1993)
Total phosphorus	Spectrophotometric method	Tandon (1993)
Total potassium	Flame photometric method	Tandon (1993)

Fig. 1: Pressmud composting in the field: A-Aerotilling; B- Application of microbial culture; C-Temperature checking; D-Final compost.

Results

The results of the total microbial population (bacteria and fungi) in gut of *E. eugeniae* were isolated and identified (data not shown) at the 60th day of inoculating the worms into pressmud. Totally four bacterial species namely, B1-*Bacillus* species, B2-*Pseudomonas* species, B3-*Bacillus* species and B4-*Bacillus megaterium*, and three fungal species namely F5- *Aspergillus flavus,* F6- *Trichoderma viride*, F7-*Aspergillus niger* were isolated and they were cultured, sub-cultured and stored at refrigerated condition. Physical and chemical parameters of pressmud and spent wash used for composting process are given in the Table 2. The pressmud possessed above 70% moisture with 35.8% total organic carbon with a good amount of NPK. The spent wash showed 2,20,000 mg/l and 1,80,000 mg/l total solids and total dissolved solids respectively. The macro- and micro-nutrients in spent wash as shown in Table-2 is in good proportion.

Table 2. Physico-chemical characteristics of pressmud and spent wash used in the study.

Pressmud		Spent wash*	
Parameters	**Values**	**Parameters**	**Remarks**
Total organic carbon	35.8%	Specific gravity	1.06 g / m^3
Moisture	70.4%	Total solids	2,20,000 mg / l
Total nitrogen	1.02%	Total dissolved solids	1,80,000 mg / l
Total phosphorus	2.35%	Total volatile solids	90,000 mg / l
Total potassium	0.58%	pH	4.5
Calcium	0.98%	EC	45.2 dSm^{-1}
Magnesium	0.15%	Nitrogen	4,200 mg / l
Sulphur	0.22%	Phosphorus	3038 mg / l
Copper	45 mg/kg	Potassium	18,263 mg / l
Zinc	67 mg/kg	Calcium	7,000 mg / l
Manganese	263 mg/kg	Magnesium	2,100 mg / l
Iron	2250 mg/kg	Sodium	1,200 mg / l
pH	6.7	Chlorine	10,000 mg / l
* Colour – reddish brown; Odour – smell of burn sugars;		Sulphate (SO_4)	8,000 mg / l
		BOD	96,000 mg / l
		COD	2,00,000 mg / l

Physical parameters of biocompost

The pressmud was initially muddy brown in colour. The colour was changed into tea brown or black colour at the end of the composting process except control (T8). During the initial stages of composting process, odour was generated. After the completion of composting reactions, the mature compost had pleasant earthy smell but control remained with foul smell. The initial moisture content of the pressmud was between 61.68% and 68.25% in the treatments. After the addition of compost starter culture, the moisture content drastically reduced in T2. After drastic reduction from its initial moisture level of 68.25 to 56.80%, T2 recorded very slow reduction in its moisture percentage during the active composting process up to ninth week. Treatments T1, T3, T4, T5, T6, T7 and T8 showed gradual reduction in moisture percentage during the active composting period. The moisture content of the piles in all the treatments was below fifty percent after ninth week. The observed results are furnished in Fig. 2.

The initial temperature of the compost piles in all the treatments was between 49.16°C and 53.63°C. After the addition of compost starter culture, T2 recorded a gradual rise in temperature whereas T1, T3, T4, T5, T6 and T7 recorded the peak temperatures during sixth week and T3 during the fifth week. T2 recorded the maximum temperature of 63°C followed by T1 (61°C), T5 (60°C), T5 (59°C) and T3 (58°C). The pile temperature was above 50°C in T1, T2, T3, T4, T5 and T6 up to 8th week whereas it was below 50°C in T7 and T8. The observed results are furnished in Fig. 3.

Chemical parameters of biocompost

The initial pH of the pressmud was between 5.23 and 5.74 in all the treatments. Except T1, all the other treatments recorded a slight reduction in pH during second and third week of composting from the initial level. After the third week, there was a drastic increase in pH observed in T5 whereas pH increase was gradual in T1 and T2.

Fig. 2: Change in moisture level in compost pile during pressmud composting process.

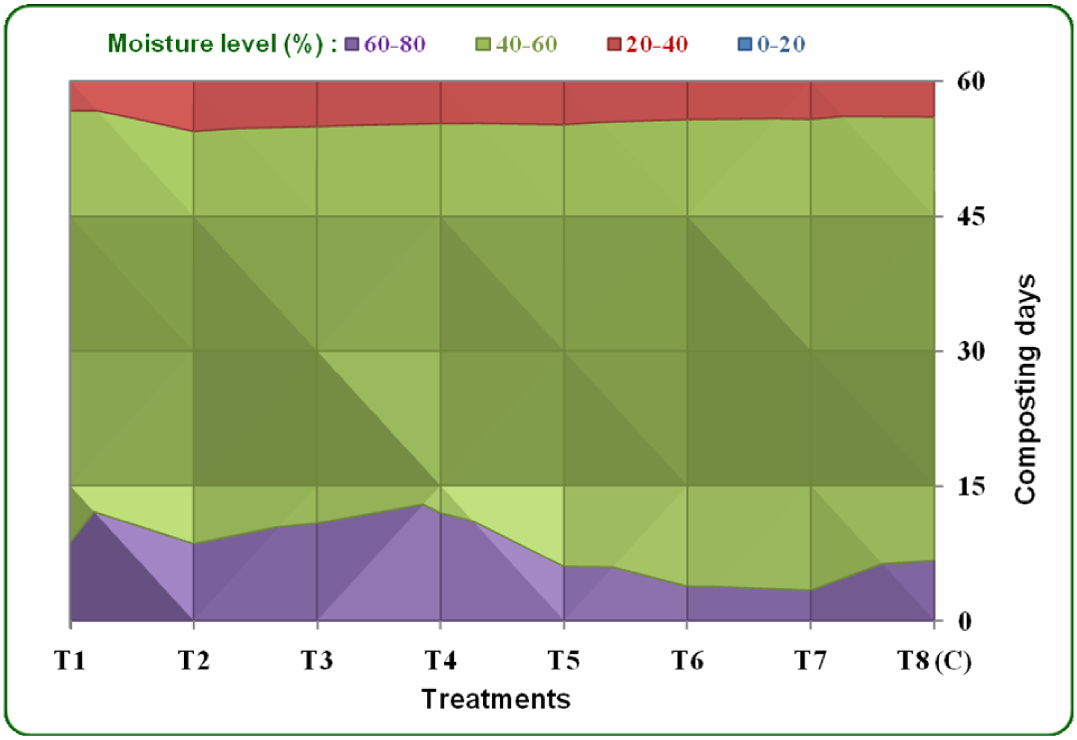
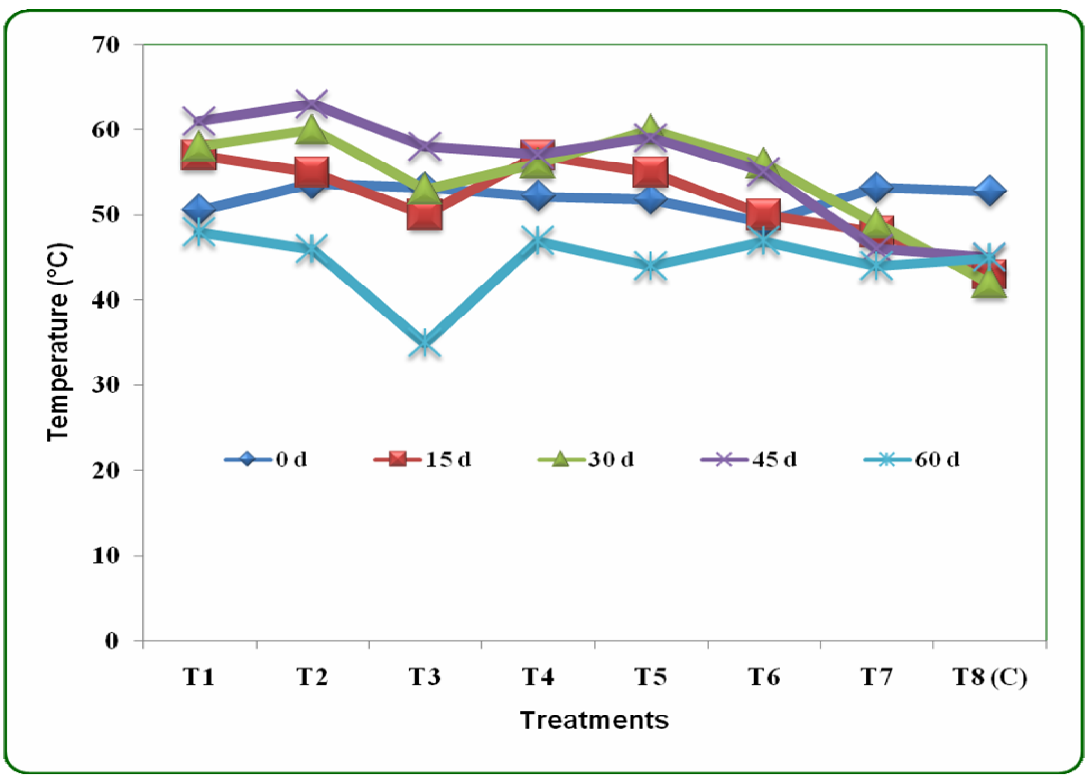


Fig. 3: Changes in temperature (°C) of compost pile during pressmud composting process.



The pH stabilized at neutral level during the 8th week. T5 recorded neutral pH only during the 8th week. During 9 weeks composting process, the pH increased from 5.65 to 7.26 in T1, 5.69 to 7.40 in T2, 5.72 to 7.32 in T3, 5.67 to 7.16 in T4 and 5.69 to 7.20 in T5, 5.74 to 6.38 in T6, 5.23 to 6.94 in T7, and 5.45 to 5.86 in T8.

The pH of the final compost was between 6.5 and 7 in T1 to T5. The observed results are furnished in Table 3. The initial organic carbon content of the pressmud was between 35.2 to 36%. After the addition of starter culture the organic carbon content was found to reduce drastically in treatments T1 and T5 during the second week whereas all other treatments showed a gradual reduction. T2 reached the desired level of organic carbon i.e. 17.91% during the 8th week whereas T1 and T3 reached the desired level of organic carbon *viz.*, 24.4% and 25.3% during the 9th week. Treatments T4 and T5 recorded the desired level of organic carbon percentage only during the 9th week (Table 3).

Table 3. Changes in pH and total organic carbon content of compost pile during pressmud composting.

Days	T1	T2	T3	T4	T5	T6	T7	T8 (C)*
pH								
0	5.65	5.69	5.72	5.67	5.69	5.74	5.23	5.45
15	6.21	5.63	5.44	5.20	5.65	5.33	5.41	5.32
30	6.28	6.69	6.12	6.20	5.96	5.86	5.94	5.26
45	6.24	6.80	6.89	6.73	6.32	6.35	6.24	5.40
60	7.26	7.40	7.32	7.16	7.20	6.38	6.94	5.86
Total organic carbon (%)								
0	35.20	35.26	35.28	35.63	35.27	35.32	35.82	35.71
15	33.13	33.43	33.42	34.92	34.62	33.43	34.60	35.04
30	30.80	29.53	29.85	29,95	30.15	30.53	30.78	33.84
45	27.70	23.17	29.19	28.95	28.92	25.16	26.17	32.83
60	24.40	17.91	25.30	19.49	20.09	22.26	21.12	30.85
(*C-control)								

Table 4. Changes in total NPK contents of compost pile during pressmud composting.

Days	T1	T2	T3	T4	T5	T6	T7	T8 (C)*
Total N (%)								
0	1.03	1.06	1.03	1.04	1.05	1.03	1.04	1.02
15	1.40	1.43	1.42	1.34	1.03	1.33	1.30	1.03
30	1.50	1.80	1.61	1.68	1.54	1.48	1.50	1.10
45	1.68	1.80	1.71	1.62	1.64	1.53	1.48	1.14
60	1.74	2.10	1.78	1.85	1.84	1.82	1.86	1.20
Total P (%)								
0	2.81	2.94	2.86	2.95	2.98	2.8	2.85	2.83
15	2.68	2.87	2.89	2.86	2.91	2.85	2.83	2.86
30	2.33	2.61	2.42	2.43	2.52	2.48	2.34	2.71
45	2.29	2.06	2.16	2.07	2.13	2.16	2.02	2.62
60	2.03	1.82	1.84	1.82	1.86	1.83	1.90	2.31
Total K (%)								
0	0.73	0.75	0.82	0.79	0.81	0.76	0.74	0.73
15	0.73	0.76	0.75	0.82	0.78	0.82	0.80	0.77
30	1.62	1.72	1.59	1.72	1.76	1.85	1.82	1.84
45	2.24	2.31	2.65	2.53	2.41	2.52	2.47	2.42
60	2.83	2.87	2.78	2.76	2.74	2.79	2.81	2.82
(*C-control)								

The initial nitrogen content of the pressmud was between 1.02 and 1.06% in all the treatments. The nitrogen content increased from 1.03 to 1.74% in T1, 1.06 to 2.10% in T2, 1.03 to 1.78% in T3, 1.04 to 1.85% in T4, 1.05 to 1.84 in T5, 1.03 to 1.82 in T6, 1.04 to 1.86 in T7 and 1.02 to 1.2% in T8. The results are furnished in Table 4.

The initial phosphorus content in the pressmud was between 2.8 and 2.98% in all the treatments. At the end of the composting process after 9 weeks, the phosphorus content decreased from 2.81 to 2.03% in T1, 2.94 to 1.82% in T2, 2.86 to 1.84% in T3, 2.95 to 1.82% in T4, 2.98 to 1.86% in T5, 2.8 to 1.83% in T6, 2.85 to 1.90% in T7 and 2.83 to 2.31% in T8. The observed results are furnished in Table 4.

In contrary to this, the initial potassium content of pressmud was between 0.73 and 0.82 % in all the treatments. At the end of the composting process after 9th weeks, the potassium content increased to 2.83 from 0.73% in T1, 2.87 from 0.75% in T2, 2.78 from 0.82% in T3, 2.76 from 0.79% in T4, 2.74 from 0.81% in T5, 2.79 from 0.76% in T6, 2.81 from 0.74% in T7, 2.82 from 0.73% in T8 (Table 4).

Discussion

Microbial decomposition is known to occur simultaneously with deliberate vermicomposting. The consortium of earthworms, the microflora living in their intestines, and those in the growth medium, enhance the decomposition process of the substrate. The intestinal mucous which consists of easily metabolizable compounds is considered to result in a priming-effect of earthworms to microbial decomposition (Vinceslas-Akpa and Loquet, 1997). The efficiency of vermicomposting may, therefore, depend on number and types of microorganisms in the substrate (Hand et al., 1988). To optimally integrate the microbial action and vermicomposting action, knowledge of C-to-N ratio requirements of both processes is vital. The importance of C-to-N ratio with respect to population and distribution of earthworms in their natural ecology has been well reviewed by Lee (1985).

Ascomycetes and to a lesser degree, basidiomycetes, were more abundant and more varied in vermicompost. This too, could be caused by different composition of the two composts, or to preferential grazing by earthworms on fast-growing fungi (such as zygomycetes and mitosporic fungi), rendering them less competitive and conferring an advantage for fungi (basidiomycetes and some ascomycetes) (Moody et al., 1992). Gut passage stress and the establishment of unfavorable microniches in the compost following the direct and indirect action of earthworms also would explain why the perfect states of *Pseudallescheria boydii* and *Corynascus sepedonium* were found only in vermicompost.

Zygomycetes diversity was lower in vermicompost, as observed by Brown (1995) and Tiunov and Scheu (2000). Another point is the isolation of a low number of potentially phytopathogenic species from both composts, particularly vermicompost with its significantly lower *Fusarium* load. These data are supported by the absence of phytotoxicity in these composts shown by the results of seed germination, root elongation and vegetative tests (shoot and root dry weight, shoot height and other growth parameters) (Caccavo 2002). Widespread application of these composts as fertilizers can be recommended.

The results of the study conducted by Jayakumar et al. (2009) showed that the vermicasts of *E. eugeniae*, *L. mauritii* and *P. ceylanensis* recorded higher values for electrical conductivity, total nitrogen, phosphorus and potassium than worm-unworked pressmud (WUP). Among the three species of earthworms, *E. eugeniae* recorded maximum percentage decrease of C/N, (144.73) followed by *P. ceylanensis* and *L. mauritii* respectively. The activity of the enzymes, amylase, cellulase, invertase, phosphatase and protease in vermicasts were higher than that of WUP. The gut enzyme activities are higher in clitellate worms (Karmegam et al., 2012). The majority of the gut enzyme activities are due to the presence of different types of microorganisms as evidenced from the above literature. In the present study, the bacterial and fungal organisms showing effective degradative abilities have been isolated and screened for various activities. As a result, effective degradative bacteria, *Pseudomonas* species has been isolated.

The ordinary composting in any method and vermicomposting process differs mainly in duration of composting. However, the phases of composting processes are more or less similar and the changes in physico-chemical parameters occur in the same way excepting the speed and quantum of mineralization. The higher percentage increase of NPK in vermicomposts in the present study may be attributed to the mineralization process caused by earthworm action along with microorganisms on organic materials. Increased level of P during vermicomposting is due to earthworm gut derived phosphatase activity and also increased microbial activity in the cast (Lee and Foster, 1991).

Krishnamoorthy (1990) reported that the rise in the level of P content during vermicomposting is probably due to mineralization and mobilization of P due to bacterial and faecal phosphatase activity of earthworms. The elevated level of Zn and Fe in vermicompost indicates accelerated mineralization with selective feeding by earthworms on materials containing these metals. The increased level of macro and micronutrients in the vermicomposts were in conformity with the results of earlier works (Parthasarathi and Ranganathan, 1999; Suthar, 2007). The results of the present study showed that the pressmud is rich in nutrients, suitable to be used as organically rich source of biofertilizers for any crop.

The microbial respiration may lead to rapid carbon loss through CO_2 production and also, digestion of carbohydrates, lignin, cellulose and other polysaccharides from the substrates by inoculated earthworms may cause carbon reduction during the decomposition of organic waste. Some part may be converted to worm biomass through the assimilation process, which consequently reduces the carbon budget of composted wastes (Suthar, 2009).

In the present study, the inoculation of microbial inoculants enhanced the level of nitrogen, phosphorus and potassium during windrow composting of pressmud. The rapid mineralization is the major process accompanied by *Pseudomonas* species isolated in the present study resulted in the effective composting of pressmud within 60 days.

Conclusion

The isolate B2 (*Pseudomonas* species) isolated from the gut of *E. eugeniae* showed narrow C:N ratio and superior compost quality parameters within 60 days of pressmud composting in windrow method than other microbial isolates. The duration of composting with B2 isolate was shorter than normal (un-inoculated) composting, and composting with other isolates.

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