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ORIGINAL ARTICLE

Vermistabilization and nutrient enhancement of anaerobic digestate through earthworm species *Perionyx excavatus* and *Perionyx sansibaricus*

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Abstract The aim of the study was to examine safe reuse and recycling of organic waste digestate obtained from a biogas plant (5 % total solid) with enhanced nutrient value through vermitechnology. Two indigenous epigeic earthworm species *Perionyx excavates* and *Perionyx sansibaricus* were tried individually for this purpose. The results clearly show a significant increase in pH values from 6.5–7.4, electrical conductivity (21.3–21.7 %), total N (84.5–94.6 %), total P (35.9–47.1 %), total K (49.8–52.6 %), Ca (41.9–41.9 %) and a significant decrease in total organic C (17.1–22.4 %), C/N ratio (7.2–6.9), C/P ratio (20.3–20.6), COD (51.9–55.7 %), BOD (85.5–91.2 %). Similarly, indicator organisms (fecal coliforms and fecal streptococci) showed decrement at the end of the composting period (60 days). Fecal Coliforms reduce to nil, while in fecal streptococci a 6 log reduction was observed. Oxygen uptake rate dropped to 67.4–70 % for vermireactors. Overall, the aforementioned findings highlighted that the indigenous earthworm species could enhance the nutrient value of the anaerobic digestate, which could be utilized as an efficient soil conditioner.

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Keywords Earthworm · *Perionyx excavatus* · *Perionyx sansibaricus* · Anaerobic digestate · Vermicomposting · Nutrient enhancement

Introduction

It is an issue of concern that the digested waste, sludge and effluents from various developmental activities are frequently disposed of on agricultural lands as fertilizer and irrigation water, respectively, due to their nutrient contents, especially N and P without any treatment, but they may induce plant and soil toxicity, and may have suppressive effects on the metabolism of soil microorganisms [1]. Stabilization involves the decomposition of organic materials to the extent of eliminating hazards [2]. Moreover, stabilization of any digested waste (sludge) reduces the amount of volatile organic matter, which produces unpleasant odours that are a source of attraction for vectors, and to limit the density of pathogenic microorganisms [3]. Digestate from biogas plants is a residual semisolid material generated in the anaerobic digester, which is sent to a sludge drying bed and sold as manure. The fertilizer properties of the dry sludge are limited as the concentration of nitrate is comparatively low. In good fertilizer, the concentration of nitrate is high, as this form of nitrogen is readily absorbed by plant roots. Therefore, there is a need for ecologically sound technologies, which are not only cost-effective, but also sustainable in terms of possible recovery of recyclable constituents from anaerobic digestate as they are rich in nutrients and have higher organic content.

Vermicomposting is an eco-friendly sustainable approach to handle the digestate and its conversion to a useful recyclable product produced on-site, making it an economically

viable method for sludge management [4–7]. It is easy to operate and can be conducted in a contained space to produce a good-quality product called vermicompost [8]. During vermicomposting earthworms act as the carrier of microbes prevailing under mesophilic conditions. They feed on the organic materials and convert them into a stable casting (excreted matter), which are rich in plant nutrients like nitrogen, phosphorous and potassium [9–12]. Vermitechnology has attracted much attention for anaerobic digestate stabilization worldwide due to simple, natural and low cost technology [13–15]. The composting potential of a substrate is directly proportional to the quantity of biodegradable organic matter contained in the substrate. The substrate will not be stabilized until all the biodegradable organic content is converted to a stabilized form, which is odorless and pathogen free, and is a poor breeding ground for flies and other insects. Even if the compost is stable, care should be taken in applying it to soil because the biological processes will continue and can strip the nutrients from soil [16].

During the study period, the efficiency of vermitechnology for composting was studied for stabilizing anaerobic digestate obtained from the biogas plant without pretreatment. The change in values such as total organic carbon (TOC), total nitrogen (TN), chemical oxygen demand (COD), biochemical oxygen demand (BOD), ammonical nitrogen (NH_4–N), nitrate nitrogen (NO_3–N), total phosphorous (TP), oxygen uptake rate (OUR), total potassium (TK) and calcium (Ca) were monitored to determine the efficiency of the system. Moreover, this study emphasizes the comparative study between the potential of two indigenous species (*P. excavates* and *P. sansibaricus*) for enhancing the nutrient value of anaerobic digestate.

Experimental methods

Earthworms and substrate for experiment

Two indigenous earthworm species, *P. excavates* and *P. sansibaricus*, were chosen from the study area. These species were cultured with cattle dung as culturing media in an environmental laboratory at the Indian Institute of Technology, Roorkee (IITR), India and randomly picked for further use in the experiments. Vegetable waste mixed with cattle dung was used as a bulking agent for bench scale a biomethanation plant situated at IITR laboratory. Anaerobic digestate was obtained from this biomethanation plant for vermistabilization. The raw digestate with high water content (95 %) was directly applied on the vermibed (without dewatering) for vermicomposting. Also, there was no need for extra water sprinkling during the process. The study was conducted for a period of 60 days. The digestate had initial physico-chemical characteristics of pH 6.51 ± 0.14, EC (dS/cm) 5.16 ± 0.09, TOC (%) 36.4 ± 1.01, TN (%) 1.88 ± 0.06, TP (%), 0.89 ± 0.02, C/N 19.4 ± 1.01, C/P 40.9 ± 1.03, NH_4–N (%) 0.52 ± 0.09, Na (%) 0.36 ± 0.07, K (%) 0.62 ± 0.08, Ca (%) 1.82 ± 0.2, BOD (mg/l) 854 ± 9.01, COD mg/l 1292 ± 15.1, TC (MPN/100 g) $2.4 \times 10^8 \pm 750$, FC (MPN/100 g) $9.3 \times 10^6 \pm 540$ and FS (MPN/100 g) $9.3 \times 10^7 \pm 719$.

Experimental setup

The experiments were replicated thrice for each set of reactors. A total three sets of reactors, two vermireactors were used for the study designated as T_1 (with *P. excavates*), T_2 (*P. sansibaricus*) and the last T_3, acting as the control (without any earthworm). Each set of reactors was used in triplicate. All the sets of reactors were of 6 L capacity and kept in the dark under identical ambient conditions (room temperature 25 ± 3 °C, relative humidity 60–80 %). The reactor was punched with 2 mm holes on the bottom and sides for leaching of extra water and aeration. Half depth (15 cm) of each reactor was filled with fully stabilized vermicompost free of earthworms and cocoons, which acted as the initial habitat of earthworms followed by 2 L of anaerobic digestate [17]. Each reactor was inoculated with 50 g of the respective earthworm's species and number was also counted.

Compost analysis

Homogenized wet samples (free from earthworms, hatchlings and cocoons) in 110 g amounts were collected in the beginning, i.e., on the 0th day (t_0), 15th (t_{15}), 30th (t_{30}), 45th day (t_{45}) and 60th day (t_{60}). Out of 110 g, 10 g of the wet sample was used for BOD, COD and bacteriological analysis while rest (100 g) of the sample was oven dried at 110 °C, ground in a stainless steel blender, passed through a 0.2 mm sieve and stored in plastic bags for further physico-chemical analysis. Each dried sample was analyzed for the following physico-chemical parameters: pH using a pH meter and electrical conductivity (EC) using a conductivity meter (1:10 w/v waste: water extract), total Kjeldahl nitrogen using the Kjeldahl method, NH_4–N and NO_3–N using KCl extraction, total organic carbon (TOC) determined by Shimadzu (TOC-V_{CSN}) solid sample module (SSM-5000A), total phosphorus (acid digest) using the stannous chloride method, potassium, calcium and sodium (acid digest) using a flame photometer. Indicator organisms such as fecal coliforms (FC) and fecal streptococci (FS) were analysed by the multiple fermentation method using lactose broth. Biodegradable organic matter measured as BOD by the dilution method and COD by the dichromate method. Stability parameters like OUR were measured on a liquid suspension of vermicompost (8 g of

compost in 500 ml of distilled water added with $CaCl_2$, $MgSO_4$, $FeCl_3$ and phosphate buffer at pH 7.2, made up according to the standard methods BOD test procedures incubated at 24 ± 2 °C). The DO probe was placed in the sample bottle, its sensor being at a depth of 5–7 cm below the water surface. The suspension was continuously stirred by means of a magnetic stirrer. The O_2 concentration was measured continuously and this value is written as OUR in mg of O_2/g volatile solids (VS)/day. These all tests were carried out as per standard methods [18].

Statistical analysis

All the reported data are the mean of the sample from three replicated reactors. One way analysis of variance (ANOVA) was performed to determine significant difference ($P < 0.05$) between all reactors (with and without earthworms) and parameters analyzed during study and Tukey's HSD test was used as a post hoc analysis to compare the means (SPSS Package, Version 16).

Results and discussion

The initial pH of the anaerobic digestate was 6.5 which increased to 7.4, 7.4 and 7.9 in T_1, T_2 and T_3 (control), respectively, at the end of vermicomposting period as shown in Table 1. Similar trends of pH increments have been reported earlier [19, 20]. The initial increase in the substrate pH might be attributed to the fact that initially microbes participate in the degradation representing aerobic metabolism. As a result, basic hydroxides are formed in the presence of sufficient moisture in the initial phase of decomposition [21] causing rise in pH. It is also reported that an earthworm's calciferous glands secrete NH_4^+ ions which reduces H^+ ions and thus increases the pH. However, the decrease in pH values at the end was related to the loss of ammonia, producing the stabilised vermicompost [22]. A gradual increase in EC was observed with time in all the reactors (Table 1). The increase in EC might have been due to the loss of weight of organic matter and release of different mineral salts in available forms (such as phosphate, ammonium, potassium) as reported by other researchers [23, 24]. The pattern of increase in initial EC observed was in the order: T_1 (103 %) >T_2 (102 %) > T_3 (26 %). The variation in EC was significant for both vermireactors ($P < 0.05$) as per the ANOVA.

Nutrient enhancement and microbiological changes during vermicomposting process

The role of organic carbon and inorganic nitrogen for cell synthesis, growth and metabolism is important in all living

Table 1 Physico-chemical characteristics of compost during vermicomposting on T_{0-60} days

Days	pH			EC (dS m^{-1})			TOC (%)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
0	6.5 ± 0.17a	6.5 ± 0.1a	6.5 ± 0.15a	2.74 ± 0.07a	2.74 ± 0.07a	2.74 ± 0.09a	36.4 ± 1.01a	36.4 ± 0.7a	36.4 ± 0.9a
15	6.6 ± 0.19a	6.7 ± 0.15a	6.5 ± 0.1a	2.95 ± 0.09bc	3.23 ± 0.06a	2.84 ± 0.05a	34.7 ± 0.9a	35.1 ± 0.8a	35.8 ± 1.04a
30	7.0 ± 0.1ab	7.1 ± 0.1ab	6.8 ± 0.2bc	4.00 ± 0.03bc	4.03 ± 0.07ab	2.93 ± 0.07b	31.3 ± 1.3c	31.2 ± 0.8ac	33.7 ± 1.09ab
45	7.5 ± 0.2ab	7.6 ± 0.1ab	6.9 ± 0.1bc	4.34 ± 0.03 cd	5.32 ± 0.08ab	3.08 ± 0.09ab	30.17 ± 0.8ac	28.23 ± 1.02ac	32.68 ± 0.8ab
60	7.4 ± 0.15ab	7.4 ± 0.16ab	7.9 ± 0.13d	5.57 ± 0.0 cd	5.55 ± 0.05ab	3.45 ± 0.04b	28.5 ± 0.08ac	27.6 ± 0.07ac	31.5 ± 0.09ab

Values followed by the same letter within each column are not significantly different and all data represents average of triplicates (mean ± SD, n=3)

organisms. The total organic carbon reduced during the process of vermicomposting as shown in Table 1. Earthworms fragment and homogenize the ingested material through the muscular action of their foregut, which results in an increasing surface area for microbial action, whereas, microorganisms biochemically degrade the organic C and provide some extra-cellular enzymes which are required for organic waste decomposition within the worms' gut [25]. This biological mutuality results in C loss from substrates through respiration and thus TOC was found to be lower in the final product as compared to initial values. The maximum reduction was recorded from 36.4 to 27.6 % in T_2 followed by 28.5 % in T_1 and the minimum reduction was observed in the case of the control (31.5 %). A significant variation ($P < 0.05$) was observed for vermireactors as per one way ANOVA analysis.

Nutritional value of the anaerobic digestate especially nitrogen and phosphorus content is important. The total nitrogen content increased (Fig. 1a) for all the reactors, a maximum increase of 98.9 % were observed for reactor T_1, 95.7 % for T_2 and 33.5 % was observed for T_3.

In general, nitrogen enrichment pattern and mineralization activities mainly depend upon the total amount of N in the initial waste material (e.g., anaerobic digestate) and on the earthworm activity in the waste decomposition subsystem, in addition to release of N from compost material, earthworms also enhance nitrogen levels by adding their excretory products, mucus, body fluid, enzymes, etc. to the substrate [8, 26].

A maximum increase in TP occurred in T_1 (57.3 %) followed by T_2 (50.5 %) and T_3 (30.3 %) (Fig. 1b). Increase in TP during vermicomposting is due to

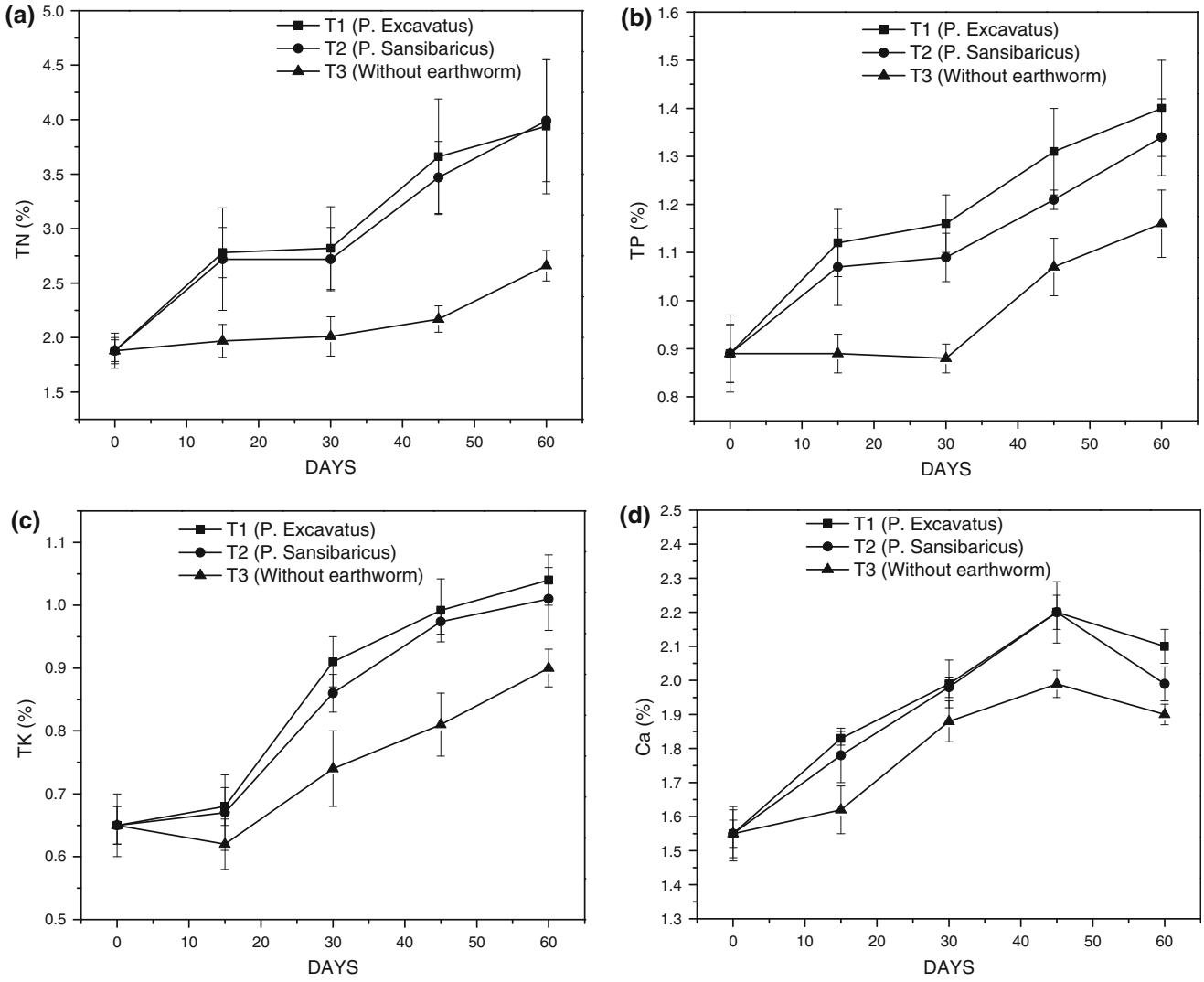

Fig. 1 Variation in parameter during the process **a** TN, **b** TP, **c** TK and **d** Ca profile during process

mineralization and mobilization of phosphorus by microbial activity of earthworms also due to release of TP during vermicomposting partly by earthworm gut phosphatases, and by the P-solubilizing microorganisms living in worm casts [15]. Earthworms also host millions of decomposer microbes in their gut and excrete them along with nutrients, nitrogen and phosphorus in their excreta [27]. A significant variation ($P < 0.05$) was observed for TP as per one way ANOVA for vermireactors. The nitrogen and phosphorus were further used by the microbes for multiplication and enhanced action.

The initial concentration of total potassium (TK) in the substrate was 0.65 % which had subsequently increased for all reactors. The pattern of TK increase was recorded in the order: T_1 (60 %) > T_2 (55.4 %) > T_3 (38.5 %) as shown in Fig. 1c. Acid production by the microorganisms is the major mechanism for solubilization of insoluble potassium. The enhanced number of microflora present in the gut of earthworms results in increased potassium as compared to control [28]. However, the increase in concentration was nominal when compared with the findings of earlier researchers [29]. A high significant difference ($P < 0.05$) was observed for K as per one way ANOVA. The initial concentration of Ca was 1.55 %, which subsequently increased for all reactors: up to 2.1 % for T_1, 1.9 % for T_2 and 1.9 % for T_3 (Fig. 1d). A high significant variation ($P < 0.05$) was observed for Ca as per one way ANOVA.

The exchangeable NH_4–N in the vermicompost was always greater than the NO_3–N during the course of experiment. A decrease in NH_4–N was observed in corresponding increase in NO_3–N at the end of the vermicomposting process. However, the rapid decrease in NH_4–N during composting did not coincide with a rapid increase in NO_3–N. The difference between various forms of N might be due to immobilization/denitrification or both. The loss in NH_4–N was in the order: T_1 (44.23 %) = T_2

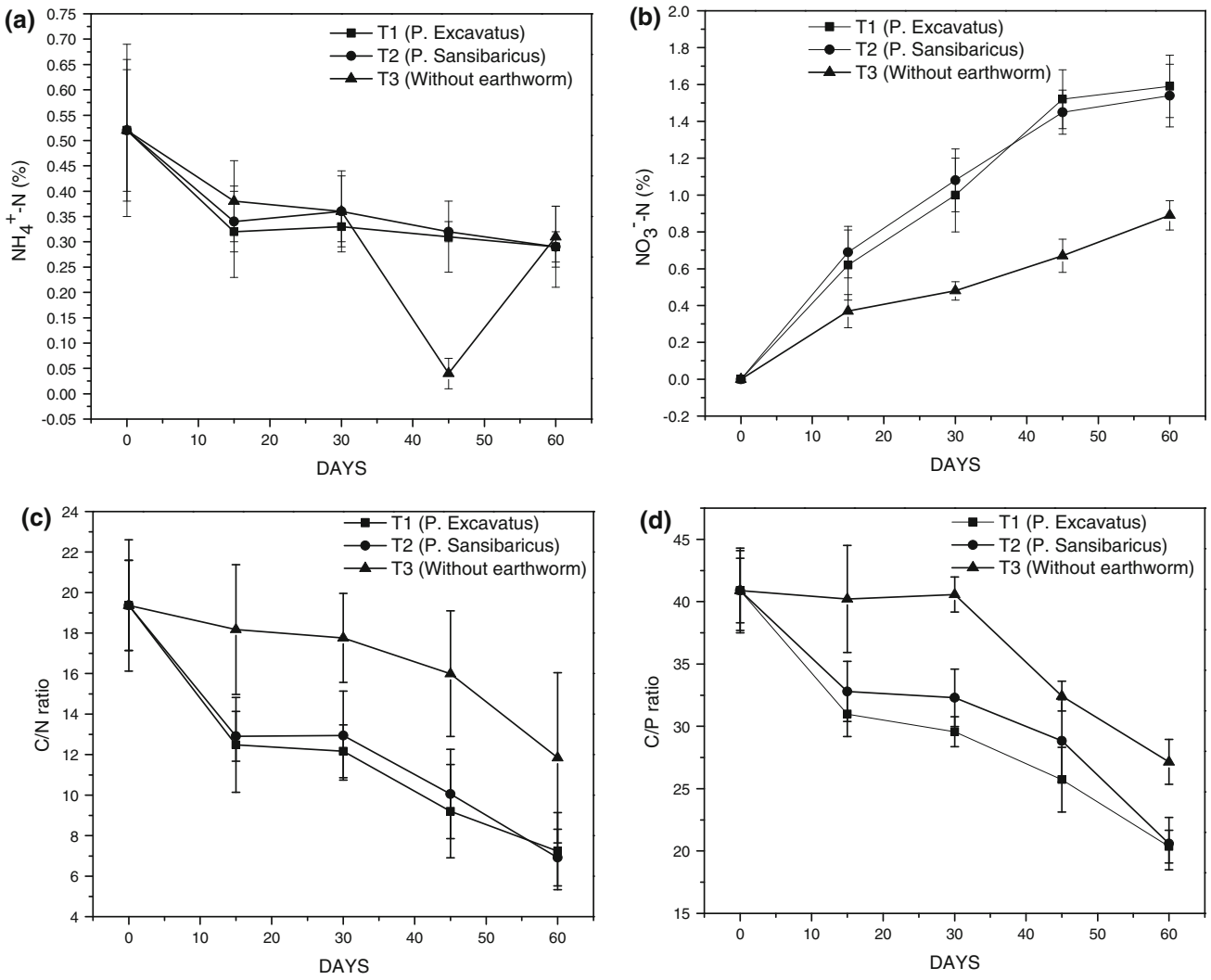

Fig. 2 Variation in parameter during the process **a** NH_4^+–N, **b** NO_3–N, **c** C/N and **d** C/P profile during process

(44.23 %) > T_3 (40.3 %) and NO_3–N gain in the order: T_1 (159 %) > T_2 (154 %) > T_3 (89 %) as shown in Fig. 2a, b. High significance ($P < 0.05$) was observed in vermireactor for NH_4–N and NO_3–N as per one way ANOVA.

The change in C/N and C/P ratios reflects the degree of organic waste mineralization and stabilization rate during the process of composting and vermicomposting; in addition, the plants cannot assimilate minerals N and P unless these ratios are of the order of 20: 1 and 15: 1, or less respectively [12]. The C/N ratio below 20 is an indicative of an advanced degree of stabilization and acceptable maturity, while a ratio of 15 or less is preferable for agronomic use of compost [29, 30]. The C/N ratio of the final vermicompost decreased considerably as compared to the initial value in anaerobic digestate for all the experiments. The initial C/N value of the substrate was 19.4, which had subsequently reduced to 7.23, 6.92 and 11.84 for T_1, T_2 and control, respectively, similarly C/P reduced to 20.36, 20.6 for T_1, T_2 and 27.16 for control subsequently

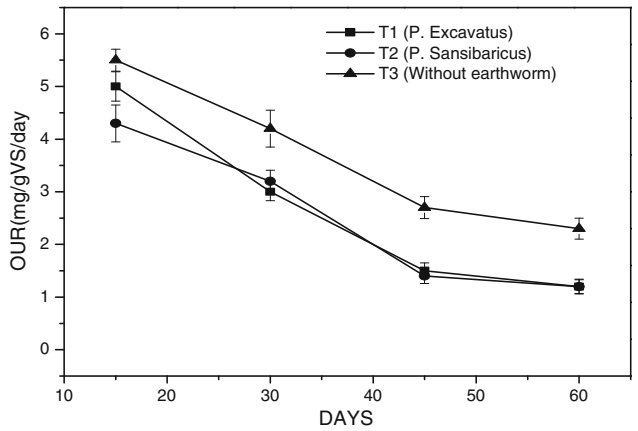

Fig. 3 Variation in OUR (mg/gVS/day) profile during process

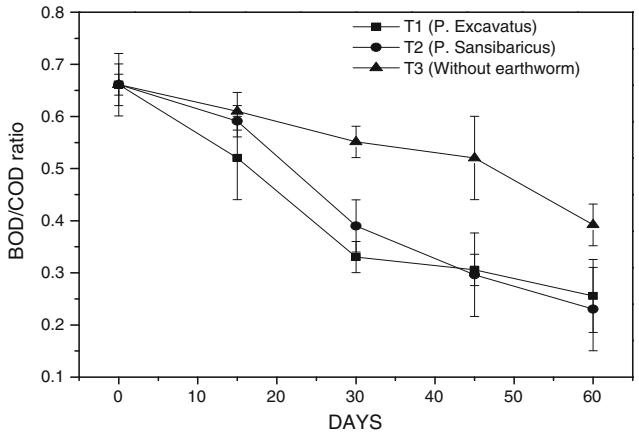

Fig. 4 Variation in BOD/COD profile during process

Table 2 Microbiological characteristics of compost during vermicomposting on $T_{0–60}$ days

Days	TC (MPN/g)			FC (MPN/g)			FS (MPN/g)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
0	230000000 ± 630a	230000000 ± 410a	230000000 ± 400a	930000 ± 240a	930000 ± 220a	930000 ± 180a	93000000 ± 410a	93000000 ± 310a	93000000 ± 370a
15	930000 ± 270be	430000 ± 190a	43000000 ± 510b	23000 ± 170a	93000 ± 120a	930000 ± 180a	43000 ± 120ab	23000 ± 140ac	230000 ± 160bc
30	23000 ± 120a	93000 ± 90a	4300000 ± 330d	9300 ± 90ab	2300 ± 80a	430000 ± 90ab	23000 ± 110ab	9300 ± 100b	930000 ± 130cd
45	430 ± 34a	930 ± 54a	930000 ± 180d	43 ± 6ac	93 ± 19b	230000 ± 170ab	150 ± 29b	930 ± 73d	150000 ± 120cd
60	150 ± 19a	150 ± 27a	930000 ± 170b	Nil	Nil	43000 ± 150b	24 ± 2c	93 ± 17d	24000 ± 120d

Values followed by the same letter within each column are not significantly different and all data represents average of triplicates (mean ± SD, n=3)

Table 3 Earthworm biomass growth and reproduction from vermicomposting on T_{60} days

Reactors	Earthworm	Mean weight of EWs in g		Live biomass % change	Cocoons (worm^{-1} day^{-1})	Juvenile hatched (worm^{-1} day^{-1})	Maximum growth rate (g wt worm^{-1} day^{-1})
		Initial	Final				
T_1	*P. excavatus*	50 ± 1.08a	71 ± 5.57ab	42 ± 3.1a	0.41 ± 0.05b	0.48 ± 0.04a	6.9 ± 1.47ab
T_2	*P. sansibaricus*	50 ± 1.80b	68 ± 2.65bc	36 ± 4.36b	0.46 ± 0.03b	0.55 ± 0.03ab	6.1 ± 2.55b

All data represents average of triplicates (mean ± SD, n=3)

from the initial value of 40.9 as shown in Fig. 2c, d. The percentage reduction in C/N from the initial ratio was observed in the order: T_1 (62.6 %) > T_2 (64.2 %) > T_3 (38.8 %) and percentage reduction in C/P was observed in the order: (50.2 %) > T_2 (49.6 %) > T_3 (33.5 %). The decrease in C/N ratio over time can be attributed to rapid decrease in organic carbon due to increase in oxidation of the organic matter. A significant difference ($P < 0.05$) was observed for C/N as per one way ANOVA.

OUR is the most direct technique of the measurement of compost stability, used for the determination of microbial activity of materials [31]. It directly correlates the aerobic respiration and in turn aerobic biological activity. OUR reduction was observed in the order: T_1 (70 %)> T_2 (67.4 %) > T_3 (50.9 %) as shown in Fig. 3. OUR varied significantly for all the reactors ($P < 0.05$) during vermicomposting. In all reactors BOD and COD had shown a trend of reduction during the course of vermicomposting. The initial COD of the substrate was 1292 mg/l, which reduced to 489 mg/l for T_1, 269 mg/l for T_2 and 1056 mg/l for T_3. Similarly, the BOD reduced for all reactors from initial value of 854 mg/l to 125 mg/l for T_1, 62 mg/l for T_2 and 414 mg/l for T_3 (Fig. 4). One way ANOVA showed high significance level for COD and BOD in all reactors ($P < 0.05$).

The presence of coliform bacteria is often used as an indicator of overall sanitary quality of the compost. The vermicomposting process caused significant reduction in all the microbiological parameters and the pathogens were well below the class A and B limits of biosolids set by the standard method [32] making the vermicompost suitable for agronomic application. Fecal streptococci are commonly considered to be the best indicator of fecal population. They are more resistant to different environmental factors than the coliforms. As shown in Table 2, FS showed a reduction from initial 9.3×10^7 to 24 ± 2, 93 ± 17 and 2.4×10^4 ± 120 for T_1, T_2 and T_3 respectively and FC reduced from initial 9.3×10^5 to nil for T_1, T_2 and 4.3×10^4 ± 150 for T_3, respectively. The reduction was presumably because of the elimination of coliforms as they entered the food chain of the earthworms. The coliforms varied significantly for all vermireactors ($P < 0.05$). The pathogen reduction during vermicomposting could be attributed to various earthworm actions like intestinal enzymatic action, secretion of coelomic fluids having antibacterial properties and selective grazing [33, 34].

Earthworm biomass (growth and reproduction)

Earthworms are bisexual animals and reproduce rapidly. The growth rate (g weight gained worm^{-1} d^{-1}) is considered as a good index to compare the growth of earthworms in varying conditions [14]. The data revealed that the initial weight increased by 42 % (T_1) > 36 % (T_2), in addition, 0.41 and 0.46 cocoons per worm per day and 0.48 and 0.55 juvenile hatchlings per worm per day were observed in T_1 and T_2, respectively, in the end product as shown in the Table 3. No mortality was observed in any reactor during the entire vermicomposting periods. The maximum growth rate (g wt worm^{-1} day^{-1}) was observed as 6.9 ± 1.47 for *P. excavates* (T_1) and 6.1 ± 2.55 for *P. sansibaricus* (T_2).

Conclusions

This study demonstrated the usefulness of vermicomposting technology for the recycling and safe reuse of anaerobic digestate. It was concluded from the study that the indigenous available earthworm species *P. excavates* and *P. sansibaricus* enhance the decomposition and mineralization rate thus increasing the nutrient value of the anaerobic digestate without dewatering. Hence, both *P. excavates* and *P. sansibaricus* have the same potential for rearing and mineralize anaerobic digestate from the biogas plant.

On the basis of observations, *P. Sansibaricus* performed better than *P. excavates* in terms of good quality compost but comparatively, biomass production was less. These earthworm species were found to destroy the pathogens thus making the compost hygienic and safe. The vermicompost finally obtained was more mature and stabilized, when compared to anaerobic digestate as demonstrated by OUR and significant decrement in C/N and C/P ratio. Moreover, anaerobic digestate was found to be ideal for the growth and reproduction of indigenous earthworm species.

Overall, *P. excavates* and *P. sansibaricus* appeared as a potential tool for the onsite treatment of anaerobic digestate to convert it into value added, hygienic and stable fertilizer, i.e. vermicompost. In India the farmers have their own biogas plants based on cattle manure hence they can get better fertilizer using this technology.

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