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Evaluation of anaerobic digestate as a substrate for vermicomposting

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Abstract: Vermicomposting is an established process to convert organic wastes into vermicastings suitable for plant growth. This research investigated the vermicomposting of anaerobic digestate with four different ratios of sawdust as a bulking material, for 75 days. The optimum proportion of anaerobic digestate to sawdust was identified as 70:30 based on worm growth and reproduction. Vermicomposting process increased the conversion of ammonium into nitrate when compared with control (p = 0.05). Vermicastings produced at the end of this experiment had significantly high N, NO_3-N, P and K than the control (p = 0.05). There was significant reduction in pathogen levels by the worms (99%), and also a germination test undertaken showed an 83% increase in radish seed germination after vermicomposting when compared to raw digestate. The results indicated that vermicomposting with *Eisenia fetida* is a sustainable technology to convert the anaerobic digestate into nutrient-rich, safe to handle vermicastings, which otherwise is a secondary pollutant.

Keywords: pre-stabilisation; anaerobic digestate; *Eisenia fetida*; germination index; sawdust; vermicomposting.

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Richard Bell is a soil fertility and land management specialist with particular interests in diagnosis and prognosis of mineral disorders of plants, plant adaptation to mineral stress, conservation agriculture, and land rehabilitation. He is the author of nine book chapters and 176 refereed papers, and Editor of nine books. He has led and coordinated collaborative international studies with: Thailand (1984–2012), China (1992–1997), Cambodia (2004–present), Bangladesh (2006–present) and Vietnam (2007–present). He is the supervisor of ten current and 47 completed post-graduate students.

1 Introduction

Anaerobic digestion is a promising technology to treat organic wastes due to the potential for energy generation, and its capacity to operate at various scales. The solids that remain as sludge after anaerobic digestion are usually referred to as anaerobic digestate. During the treatment process, most of the original nutrients present in the feed stock will be present in the digestate, and therefore could contribute to plant nutrition or enhance soil fertility (Salminen et al., 2001). The use of untreated digestate however, may pose several health problems; create unpleasant odour and environmental pollution. During anaerobic digestion, a major proportion of organic nitrogen is converted into ammonium which is phytotoxic, if present at high concentrations (Salminen et al., 2001). If the pH is greater than 7.5, then ammonium (NH_4) converts to ammonia (NH_3) and exists as a dissolved gas which is more toxic to plants than NH4, and the intermediate organic acids produced during anaerobic digestion are also potentially phytotoxic (Salminen et al., 2001). Further, there may be possible risk of pathogens if the digestion process was conducted under mesophilic conditions (Smith et al., 2005).

The digestate should be treated to convert potentially toxic compounds to plant-available forms that can be used as a fertiliser or soil conditioner. It is recommended to treat the digestate produced aerobically before land application (Hobson, 1990). The treatment of digestate for land application not only closes the loop of waste management but also avoids greenhouse gas emissions from the disposal of digestate to land-fill. However, there is a need to consider alternative, cost-effective and sustainable options to treat the digestate. In this study, vermicomposting with *Eisenia fetida* was investigated to treat the anaerobic digestate.

Dewatering the digestate is a primary process of treatment but the dewatered digestate in this case still had high water content which is not ideal for composting (Table 1) and therefore needs to be mixed with dry bulking materials (Banegas et al., 2007). The addition of bulking materials is also vital for vermicomposting because it: provides an appropriate degree of sponginess, and aeration that makes the substrate acceptable to worms; lowers the concentration of any unfavourable compounds in the feed materials; reduces the excess moisture content in the feed (Suthar, 2007a); adjusts the C:N of feed to acceptable levels (Nair et al., 2006); influences the mineralisation of organic wastes and worm biomass production and; improves the final vermicompost quality (Suthar, 2009).

Table 1 Characteristics of de-watered anaerobic digestate and of sawdust bulking agent

Parameters	*Anaerobic digestate*	*Sawdust*
pH (1:10 w/v)	8.42 ± 0.12	5.7 ± 0.1
EC (1:10 w/v) (dS/m)	2.18 ± 0.12	0.1 ± 0.08
Water content (%)	88	9.53
Total carbon (g/kg)	188 ± 17.8	647 ± 12
Total nitrogen (g/kg)	10.1 ± 0.1	1.84 ± 0.11
C:N	18.7 ± 2.03	353 ± 20.05
Phosphorus (g/kg)	3.99 ± 0.09	-
Potassium (g/kg)	4.46 ± 0.45	-
Ammonium nitrogen (g/kg)	1.59 ± 0.11	-
Nitrate nitrogen (g/kg)	0.024 ± 0.003	-
Calcium (g/kg)	90.3 ± 0.8	-
Magnesium (g/kg)	5.27 ± 0.09	-
Sodium (g/kg)	2.88 ± 8.4	-
Copper (mg/kg)	55.9 ± 7.28	-
Iron (mg/kg)	2,666 ± 21	-
Manganese (mg/kg)	95.5 ± 4.01	-
Boron (mg/kg)	38.1 ± 1.28	-
Zinc (mg/kg)	91 ± 5.14	-
Total coliforms (MPN/g)	> 11,000 ± 0	-
E. coli (MPN/g)	> 11,000 ± 0	-
Salmonella spp (MPN/g)	8.42 ± 0.12	-

Note: Mean ± SE, $n = 3$.

According to Garg et al. (2006b), it is preferable to pre-stabilise both digested and undigested material to reduce initial high levels of NH_3 and NH_4 which are toxic to the worms and to eliminate the volatile gases and toxic compounds formed during the digestion process. It is also necessary to lower water contents in the sludge to levels suitable for worms (Nair et al., 2006). During the pre-stabilisation period, the temperature was expected to rise above the tolerance limit of earthworms when large volumes of materials are composted.

Vermicomposting is a well established process in organic waste treatment (Singh et al., 2011). However, due to the anaerobic nature of material from the digestion process, vermicomposting is not suitable for the raw digestate. This research investigated the options for converting the digestate to a substrate suitable for the action of composting worms and the potential of worms to convert the solid digestate to vermicastings suitable for plant growth. In this study, digestate was pre-stabilised before vermicomposting.

2 Materials and methods

2.1 Anaerobic digestate

The digestate used in this experiment was obtained from the anaerobic digester at the Environmental Technology Centre (ETC), Murdoch University receiving food and vegetable waste. The slurry was dewatered by filtration using a plastic strainer to remove excess water and to produce solid digestate.

In this experiment, sawdust was used as a bulking material. The initial characteristics of the dewatered solid and sawdust are given in Table 1. The composting worm species was *Eisenia fetida*, collected from the worm farm maintained at the ETC, Murdoch University grown on kitchen wastes and cow manure. The earthworms and substrate were free from any toxic contaminants. Mature worms with clitellum were selected for the experiment.

2.2 Pre-stabilisation

The dewatered solid was mixed with sawdust at four different proportions (Table 2) (v/v basis) and placed in plastic containers for pre-stabilisation. The containers with digestate and sawdust were turned daily to help aerate the mixture. The pre-stabilisation process was continued for 15 days, when the temperature of the mix declined to below the tolerable limit for worms (< 30°C). The water content was maintained at 70 to 80% (w/w) by spraying distilled water if required. The constituents of pre-stabilised material are given in Table 3.

Table 2 Proportion of anaerobic solid and sawdust used in the trials

Experiment	*Anaerobic digestate (%)*	*Sawdust (%)*
	Treatment (with worms)	
T1	100	0
T2	90	10
T3	70	30
T4	50	50
	Control (without worms)	
C1	100	0
C2	90	10
C3	70	30
C4	50	50

Table 3 Characteristics of substrate after addition of sawdust and pre-stabilisation for 15 days

Treatment	*C1/T1*	*C2/T2*	*C3/T3*	*C4/T4*
pH (1:10 w/v)	8.38 ± 0.04	8.14 ± 0.06	8.03 ± 0.06	7.84 ± 0.08
EC (dS/m) (1:10 w/v)	1.84 ± 0.33	1.27 ± 0.15	1.08 ± 0.05	0.77 ± 0.04
Total C (g/kg)	181 ± 0.3	224 ± 0.2	270 ± 0.7	329 ± 0.3
Total N (g/kg)	9.78 ± 0.48	8.96 ± 0.37	7.88 ± 0.37	7.43 ± 0.28
C:N	18.6 ± 0.82	25.0 ± 1.25	34.2 ± 0.92	44.4 ± 2.08
NH_4 (g/kg)	1.15 ± 0.78	0.89 ± 0.03	0.39 ± 0.04	0.25 ± 0.04
NO_3 (g/kg)	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.0	0.04 ± 0.0
P (g/kg)	4.08 ± 0.11	3.67 ± 0.22	3.43 ± 0.35	2.79 ± 0.17
K (g/kg)	4.20 ± 0.43	3.69 ± 0.16	3.37 ± 0.11	2.98 ± 0.15
Ca (g/kg)	103 ± 1.05	89.2 ± 0.52	79.4 ± 0.14	60.8 ± 0.67
Mg (g/kg)	4.9 ± 0.30	4.41 ± 0.53	3.50 ± 0.43	2.84 ± 0.12
Na (g/kg)	2.73 ± 0.04	2.42 ± 0.12	2.25 ± 0.09	1.86 ± 0.04

Notes: Values are means of three replicates (mean ± SE). Treatment descriptions are given in Table 2.

2.3 Experimental design

The experiment was carried out with four waste ratios as described in Table 2. A control was maintained for each treatment without worms. Three replicates were maintained for each treatment and control.

Vermicomposting was carried out in 2 L plastic containers covered with insect screen, for 75 days. The pre-stabilised waste mixture was added at the rate of 2 L/ container referred to as vermireactors. During the experiment no extra feed material was added at any stage. Twenty adult worms with an average weight of 0.32 g were added to each vermireactor. The containers were placed in a shaded room at room temperature. The water content was maintained throughout the experiment at 70 to 80% (w/w) by spraying adequate quantities of distilled water if required.

2.4 Analysis

The worms were weighed with their gut full on 0, 15, 30, 45, 60, 75 days of the experiment. The worms were washed, dried on paper towel and weighed on a digital balance. Cocoons and juveniles were counted at the completion of the experiment to assess which treatment favoured growth and reproduction. The worms and cocoons were hand sorted and counted from the substrate under a light source.

2.4.1 Physical and chemical analysis

The samples (approximately 10 g) were collected from each container after 0, 20, 40, 75 days of the experiment to analyse various physical and chemical parameters. Day 0 refers to the day of inoculation of worms after pre-stabilisation for 15 days. The substrate was mixed thoroughly before sample collection. Care was taken to avoid removing any cocoon or worm from the vermireactors while collecting samples. The parameters measured were pH, electrical conductivity (EC), total carbon (TC), total nitrogen, ammonium, nitrate, phosphorus, potassium, calcium, magnesium and sodium. The moisture content and trace elements such as copper, zinc, manganese, iron and boron were analysed only in the initial and final samples. All the analysis was performed according to the American Public Health Association (APHA) standards.

The pH and EC were measured using HANNA pH and EC meters, respectively in 1/10 (w/v) aqueous extracts prepared with double distilled water agitated on an end-over-end mechanical shaker for 30 minutes and filtered through Whatman No. 1 filter paper. Moisture content of the sample was determined by drying the sample at 100°C for 24 hours. TC of the sample was estimated by burning 0.05 g of sample in a LECO furnace. The sample was dried, ground and sieved through a 2 mm screen prior to the analysis. Total nitrogen was determined by Kjeldahl method. The sample was digested with concentrated H_2SO_4 in the presence of H_2O_2 and mercuric sulphate as a catalyst to convert organic nitrogen into ammonium which was then determined using a spectrophotometer at 650 nm. Ammonium nitrogen was extracted at a ratio of 1/10 (w/v) with 2 M KCl (Rayment and Higginson, 1992). The samples were shaken on an end-over-end shaker for 2 hours, filtered through Whatman No. 1 filter paper, treated with Nessler's reagent and measured in a spectrophotometer at 420 nm. The nitrate content was determined by a colorimeter using cadmium reduction method in the extract prepared by shaking the sample in 1:10 distilled water for 2 hours. Total phosphorus was determined by wet acid digestion using concentrated H_2SO_4 and 4% H_2O_2, and then adding sodium molybdate, hydrazine sulphate to the digested sample. It was allowed to react for 20 minutes and then detected using a spectrophotometer at 820 nm (Allen and Jeffery, 1990). The sample was digested with concentrated H_2SO_4 and 30% H_2O_2 for K, Ca, Mg, Na, Cu, Fe, Mn, Zn analysis using an atomic absorption spectrophotometer.

2.4.2 Microbial analysis

Total bacterial count, total coliforms, *Escherichia coli* and *Salmonella* sp. were tested in the initial digestate and in the vermicastings to evaluate the microbial content. All the analysis was performed according to the Australian standards for the most probable number (MPN) technique-total coliforms (Australian standard, 5013.3), *E. coli* (Australian standard, 5013.26) and *Salmonella* sp. (Australian standard, 5013.10). The total bacterial count was tested through membrane filtration of various diluted samples and then counting the colonies on nutrient agar plates as colony forming unit (CFU)/g of sample.

2.4.3 Germination test

The maturity of compost can be assessed by a seed germination test as an indication that the final produce was mature and not phytotoxic (Raj and Antil, 2011). The sample (1:20 compost: distilled water) was extracted in warm water (60°C) by shaking for 3 hours on

an end-over end shaker (Kato et al., 2005). Ten mL of extracts from all trials were poured in Petri-dishes lined with filter paper and ten seeds of *Raphanus sativus* were placed and incubated in the dark at ambient temperature. The germination percentages and germination index (GI) with respect to control were determined after five days. GI was calculated as the percentage of seed germinated on filter paper in Petri-dishes with treatment (compost extract) multiplied by average length of roots (mm) expressed as percentage of control with distilled water (Aparna et al., 2008; Raj and Antil, 2011).

$$GI(\%) = \frac{\text{seed germination (\%)} \times \text{root length of treatment}}{\text{seed germination (\%)} \times \text{root length of control}} \times 100$$

2.4.4 Statistical analysis

Statistical analysis was performed using Statistix 8.0 data analysis software. Two-way ANOVA was used to examine the significance of the differences among treatments on various studied parameters using a repeated measures model for different sampling times. Homogenous groups were identified by pair-wise comparison using least significance difference (LSD) at $p = 0.05$.

3 Results and discussion

After mixing with different proportions of sawdust and pre-stabilisation, there was a decrease in pH, EC, N, P, K, Ca, Mg, NH_4, and Na concentrations of the digestate with increase in proportion of sawdust (0th day, Tables 4 and 5). Total C concentration and hence C:N value also increased due to the high C content (647 g/kg) of sawdust.

There was complete mortality of worms in T1 and T2 within one week after worms were added to pre-stabilised substrate. High NH_4 concentration may have played a key role in worm mortalities. The initial NH_4 concentrations were 1.15 g/kg and 0.9 g/kg in T1 and T2, respectively, even after pre-treatment for 15 days. These values were close to the lethal limit of 1 g of ammonia /kg for worms as reported by Edwards (1988). Moreover, there could be oxygen deficiency caused by poor aeration of compacted digestate. Masciandaro et al. (2000) observed that worms left the sludge when the percentage of anaerobic sludge increased. In this experiment, there was no chance for escape by worms as the containers were closed with mesh. However, in T3 and T4, the added sawdust might have improved the aeration, providing proper structure and a carbon source for the digested material, and diluted the NH_4 concentration making the conditions favourable for survival and growth of worms. Moreover, it was believed that the addition of sawdust increased the microbial nitrification (see below) as C provided food for bacteria.

The complete mortality of worms in 100% and 90% digestate was a key finding in the present study but contrasted with the results by Garg et al. (2006b) who observed the maximum growth of worms in 10% biogas plant slurry (from anaerobic digestion of cow manure with pH 8.3, TKN –0.74 g/kg and C:N –80). No NH_4 concentration was measured in the study by Garg et al. (2006b) so it is not possible to establish a definitive reason for the difference. Similar mortality to the present study was noticed by Gunadi and Edwards (2003), when vermicomposting fresh cattle manure due to anaerobic conditions that developed during the process. Whereas, in the case of 70% and 50%

anaerobic digestate, the addition of sawdust made the conditions favourable for the action of worms. Other studies (Banegas et al., 2007) also revealed that using sawdust as a bulking material for the treatment of sludges is effective as observed in this study.

Table 4 Changes in pH, EC (dS/m), total carbon (g/kg), total nitrogen (g/kg), C:N, NO_3 (mg/kg), and NH_4 (mg/kg) concentration over time during the vermicomposting experiment

	Days	*C1*	*C2*	*C3*	*T3*	*C4*	*T4*
pH	0	8.38^{a}	8.14^{c}	8.03^{def}	8.03^{def}	7.84^{gh}	7.84^{gh}
	20	8.29^{ab}	8.01^{ef}	7.91^{fg}	7.84^{gh}	7.76^{hi}	7.60^{jk}
	40	8.26^{abc}	7.84^{gh}	7.82^{gh}	7.67^{ij}	7.51^{kl}	7.43^{lm}
	75	8.16^{bcd}	7.78^{ghi}	7.77^{ghi}	7.45^{lm}	7.49^{kl}	7.35^{m}
EC	0	1.84^{a}	1.27^{g}	1.08^{h}	1.08^{h}	0.77^{i}	0.77^{i}
	20	1.62^{b}	1.44^{cd}	1.36^{ef}	1.38^{cde}	1.154^{gh}	1.19^{fgh}
	40	1.67^{ab}	1.43^{cd}	1.40^{cde}	1.23^{g}	1.52^{bc}	1.31^{fg}
	75	1.62^{b}	1.41^{cd}	1.43^{cd}	1.51^{bc}	1.23^{g}	1.32^{fg}
C	0	181^{klm}	224^{g}	270^{de}	270^{de}	329^{a}	329^{a}
	20	161^{no}	190^{jk}	219^{gh}	216^{gh}	291^{b}	290^{bc}
	40	159^{no}	182^{kl}	209^{hi}	198^{ij}	279^{cd}	265^{e}
	75	156^{o}	170^{mn}	195^{j}	177^{lm}	246^{f}	244^{f}
N	0	9.78^{b}	8.96^{cd}	7.88^{fg}	7.88^{fg}	7.43^{fgh}	7.43^{fgh}
	20	9.78^{b}	8.76^{d}	7.65^{fgh}	8.03^{ef}	7.15^{h}	7.68^{fgh}
	40	9.76^{b}	8.62^{de}	7.59^{fgh}	9.09^{cd}	7.21^{h}	8.06^{ef}
	75	10.0^{b}	9.01^{cd}	7.79^{fgh}	11.40^{a}	7.28^{gh}	9.59^{bc}
C:N	0	18.6^{hi}	25.0^{f}	34.2^{d}	34.2^{d}	44.4^{a}	44.4^{a}
	20	16.5^{ij}	21.8^{g}	28.8^{e}	26.9^{ef}	40.6^{b}	37.9^{c}
	40	16.3^{j}	21.1^{g}	27.6^{e}	21.7^{g}	38.6^{bc}	32.9^{d}
	75	15.5^{j}	18.9^{h}	25.0^{f}	15.7^{j}	33.8^{d}	25.5^{f}
NO_3	0	0.04^{k}	0.03^{k}	0.03^{k}	0.03^{k}	0.04^{k}	0.04^{k}
	20	0.21^{jk}	0.25^{jk}	0.25^{jk}	0.82^{i}	0.22^{jk}	0.46^{j}
	40	1.22^{h}	1.19^{h}	1.22^{h}	2.14^{ef}	1.04^{hi}	1.69^{g}
	75	2.95^{c}	2.45^{d}	2.40^{de}	4.36^{a}	1.99^{f}	3.78^{b}
NH_4	0	1.15^{a}	0.89^{b}	0.39^{de}	0.39^{de}	0.25^{fgh}	0.25^{fgh}
	20	0.65^{c}	0.44^{d}	0.26^{fg}	0.23^{fgh}	0.18^{hij}	0.19^{hij}
	40	0.42^{de}	0.29^{f}	0.21^{ghi}	0.15^{ijk}	0.14^{jk}	0.14^{jk}
	75	0.36^{e}	0.28^{f}	0.16^{ijk}	0.13^{jk}	0.13^{jk}	0.11^{k}

Notes: Treatment descriptions are given in Table 2.
The mean values followed by different letters are statistically different (ANOVA; LSD; $p = 0.05$).

Table 5 Changes in P, K, Ca, Mg and Na concentrations (g/kg) over time during the vermicomposting experiment

	Days	*C1*	*C2*	*C3*	*T3*	*C4*	*T4*
P	0	4.08^{ab}	3.67^{cde}	3.43^{ef}	3.43^{ef}	2.79^{gh}	2.79^{gh}
	20	4.08^{ab}	3.78^{de}	3.56^{de}	3.60^{cde}	2.80^{gh}	2.72^{gh}
	40	4.38^{a}	3.89^{cd}	3.62^{cde}	3.72^{de}	2.65^{h}	2.83^{gh}
	75	4.41^{a}	3.94^{cd}	3.72^{de}	3.98^{bc}	2.81^{gh}	3.07^{fg}
K	0	4.20^{bcd}	3.69^{fg}	3.37^{ij}	3.37^{ij}	2.98^{kl}	2.98^{kl}
	20	4.25^{abc}	3.87^{def}	3.48^{ghi}	3.66^{fgh}	2.50^{m}	2.96^{kl}
	40	4.35^{ab}	3.87^{def}	3.62^{fgh}	4.06^{de}	2.50^{m}	3.06^{jkl}
	75	4.53^{a}	3.93^{ef}	3.82^{ef}	4.22^{abc}	2.76^{lm}	3.24^{ijk}
Ca	0	102^{bcd}	89.2^{fgh}	79.4^{ijk}	79.4^{ijk}	60.8^{l}	60.8^{l}
	20	106^{bc}	89.9^{fgh}	80.7^{hij}	81.5^{hij}	62.1^{l}	67.6^{l}
	40	111^{ab}	93.9^{defg}	84.4^{ghij}	93.3^{efg}	70.7^{kl}	78.6^{jk}
	75	116^{a}	98.2^{cdef}	92.8^{efg}	103^{bcd}	87.4^{ghij}	87.6^{ghij}
Mg	0	4.91^{a}	4.41^{bc}	3.50^{def}	3.50^{def}	2.84^{g}	2.84^{g}
	20	4.75^{ab}	4.20^{c}	3.20^{fg}	3.50^{def}	3.13^{fg}	3.08^{fg}
	40	4.84^{ab}	4.22^{c}	3.34^{def}	3.71^{de}	3.24^{fg}	3.32^{ef}
	75	4.85^{a}	4.30^{c}	3.39^{def}	3.75^{d}	3.39^{def}	3.34^{def}
Na	0	2.73^{a}	2.42^{bc}	2.25^{de}	2.25^{de}	1.86^{hij}	1.86^{hij}
	20	2.52^{b}	2.26^{cde}	2.12^{ef}	1.89^{ghi}	1.81^{ijk}	1.56^{l}
	40	2.35^{cd}	2.06^{fg}	2.01^{fgh}	1.74^{jk}	1.88^{hij}	1.38^{m}
	75	2.35^{cd}	1.92^{ghi}	1.93^{ghi}	1.65^{kl}	1.76^{ijk}	1.28^{m}

Notes: Treatment descriptions are given in Table 2.
The mean values followed by different letters are statistically different (ANOVA; LSD; $p = 0.05$).

3.1 pH and EC

The pH decreased with increase in proportion of sawdust which was due to acidic nature of the sawdust. The pH decreased over time towards neutral in both treatment and control (Table 4). However, the treatments with worms (T3, T4) showed lesser pH values compared to the control on all days. In T4 with 50% sawdust and worms, pH dropped to 7.35 at the end of experiment (75 days). The additional decrease in pH was possibly due to conversion of organic matter into intermediate types of organic acids and mineralisation of nitrogen and phosphorus into nitrates and orthophosphates, respectively (Ndegwa et al., 2000). The neutralisation of alkaline pH was greater in vermireactors than the control and this was also observed by other researchers (Khwairakpam and Bhargava, 2009). The pH values were significantly decreased ($p < 0.05$) on all days except for C4, on 40th and 75th day.

In general, EC values increased at the end of incubation in both treatment and control except C1 which had lesser EC value compared to the initial solid waste (Table 4). This increase was attributed to loss of organic matter and release of different mineral salts during the mineralisation process (Kaviraj and Sharma, 2003).

3.2 Carbon and nitrogen

The addition of carbon-rich sawdust increased the total C content of the substrate, but total C in the waste decreased over time in both treatments and control. The total C decrease in the substrate over time was due to the mineralisation of organic matter (Kaviraj and Sharma, 2003). The treatments with live worms had a greater decrease compared to those without worms (Table 4). The greater decrease in T3 and T4 may be due to mucus and enzymes added by worms which builds up the microbial population and hence contributes to accelerated C losses through microbial respiration in the form of CO_2 (Suthar, 2007b).

Total nitrogen concentration remained almost the same in the treatments without worms while those with worms showed a gradual increase (Table 4) and the values were significantly higher ($p < 0.05$) on all days in T3 and T4 than C3 and C4. This was attributed to the role of worms in nitrogen mineralisation. Tripathi and Bhardwaj (2004) reported increase in nitrogen in the form of mucus, nitrogenous excretory materials, growth stimulating hormones from worms and even from decaying worm tissue after death. Nitrogen concentration increase may also be due to loss of organic carbon (Viel et al., 1987). The decrease in pH was also considered to be a factor in nitrogen increase as at lower pH, less nitrogen is lost as volatile ammonia (Hartenstein and Hartenstein, 1981). However, the initial N content in the waste and the extent of decomposition influence the final nitrogen concentration. The final product was 1.3 to 1.5 times more N-rich than the initial substrate. However, this increase was less compared to other experiments where Garg et al. (2006a) observed 4.4 to 5.8 fold increase in total N in different feed mixtures at the end of vermicomposting.

The decrease in C and increase in N, after the addition of sawdust and during the process lowered the C:N ratio. The treatment with worms showed more reduction in C:N in the final product compared to those without worms (Table 4).

There was decrease in NH_4 and increase in NO_3 concentrations throughout the experiment in both control and treatment (Table 4). The NO_3 content of 100% digestate (C1) increased from 0.04 g/kg on day 0 to 3 g/kg at the end of experiment. Treatment 3 had the highest NO_3 content among all the trials. Ammonium concentration decreased drastically from 1.15 g/kg to 0.36 g/kg in C1. On the 75th day, T4 had the lowest NH_4 content of 0.11 g/kg among all the treatments.

The observed changes in NH_4 and NO_3 are attributed to increased microbial activity and aeration of solid waste. The treatment with worms showed more than 2 fold increases in NO_3. The worms play a major role in N mineralisation and its accumulation as NO_3 (Atiyeh, 2000). The ammonia was converted into nitrite and nitrate through nitrification by micro-organisms (Bernal et al., 1998) which was probably facilitated by increased aeration of substrate by worms (Nair et al., 2006).

3.3 Other macronutrients

There was a slight increase in P concentrations in all reactors except C4 on the day 40 sampling (Table 5). The P increase is probably due to mineralisation of organic matter. It was claimed that in vermireactors phosphatases are produced in the earthworm gut and also by the action of micro-organisms (Lee, 1992). This study confirmed the hypothesis that P concentration increases by the action of worms. A higher increase of K concentration was observed in vermireactors than the control. It may be due to increased

microbial activity (Suthar, 2007a). Similar increase was observed during vermicomposting of sewage sludge (Gupta and Garg, 2008). There was also an increase in Ca and Mg concentrations except, there was lower Mg concentration in control (C1, C2, C3) compared to the initial solid waste. The concentrations of Ca and Mg were always higher in treatment than control on all sampling days. There was a decrease in Na concentration observed relative to the initial waste mixture. The decrease was higher in treatment compared to control and the values were significantly different (at $p = 0.05$). The actual process is unknown but it was possible that the decrease may be due to absorption of Na by worms, although Mg and Ca were clearly not absorbed.

3.4 Micronutrients

The concentrations of Fe, Mn, Zn, Cu, B were lower in the final product compared to the initial digestate in both treatment and control (Table 6). The dilution effect of the sawdust caused most of the changes in trace element concentration. The lower concentration in the substrate in vermireactors compared to control was attributed to accumulation of these elements in the tissues of worms as they are known to bioaccumulate micronutrients and heavy metals (Khwairakpam and Bhargava, 2009).

Table 6 Micronutrient concentrations in initial solid waste and final product

Treatment	*Mn (mg/kg)*	*Zn (mg/kg)*	*Bo (mg/kg)*	*Fe (mg/kg)*	*Cu (mg/kg)*
Initial sludge	95.52^{a}	91.01^{a}	38.13^{a}	2666^{a}	55.94^{a}
C1	93.35^{a}	90.77^{a}	24.8^{b}	2537.83^{a}	52.00^{a}
C2	67.16^{b}	78.60^{b}	21.13^{c}	2192.49^{b}	42.13^{b}
C3	63.09^{bc}	72.6^{c}	18.87^{d}	2106.183^{b}	37.96^{bc}
T3	61.74^{bcd}	62.24^{d}	16.3^{e}	1992.63^{bc}	33.32^{cd}
C4	55.49^{cd}	60.18^{d}	16.67^{ef}	1843.39^{cd}	30.64^{d}
T4	53.67^{d}	49.28^{e}	14.87^{f}	1643.96^{d}	22.73^{e}

Notes: Treatment descriptions are given in Table 2.
The mean values followed by different letters are statistically different (ANOVA; LSD; $p = 0.05$).

3.5 Microbial changes

There was a decrease in pathogenic microbial population in vermireactors compared to control (Table 7), but there was still high load of total coliforms (11×10^4 MPN/g) in C1 and C2 even after 75 days of composting. In C3 and C4, there were decreased pathogen populations compared to the initial digestate which was due to effects caused by addition of sawdust and normal composting effect. In vermireactors, however, 97% (T3) and 99.9% (T4) reduction in total coliforms was observed. Similarly, the reduction in *E. coli* after vermicomposting was 99.9% compared to 95 to 98% in the control. *Salmonella* sp. population was reduced after 75 days in all the containers except C3 where significantly higher numbers were counted. The reduction of *Salmonella sp* was more prominent in vermireactors. Reduction of microbial pathogens by vermicomposting was also observed by other researchers (Nair et al., 2006). However, there were more colonies of TBC observed in T3 and T4 compared to control which suggests that the activity of worms

controlled pathogens but favoured beneficial microbial activity. This high TBC could also be a reason for pathogen reduction.

Table 7 Microbial population (MPN/g) in the initial solid and final product (n = 3, ±SE)

Treatment	*Total coliforms*	*E. coli*	*Salmonella sp*	*TBC (CFU/g)*
Initial digestate	> 110,000	> 110,000	2 ± 0.7	ND
75th day				
C1	110,000 ± 0	5,250 ± 5,454	0.93 ± 0.81	330,000 ± 90,000
C2	110,000 ± 0	5,231 ± 5,480	1.95 ± 2.33	480,000 ± 56,000
C3	7,800 ± 4,525	1,535 ± 2,654	8.24 ± 13.6	440,000 ± 85,000
T3	81.5 ± 137.2	0.55 ± 0.32	< 0.3	790,000 ± 40,000
C4	313 ± 127	2.43 ± 0.41	0.33 ± 0.04	380,000 ± 100,000
T4	2.3 ± 0	1.61 ± 0.97	< 0.3	960,000 ± 65,000

Notes: Treatment descriptions are given in Table 2.
ND – not determined.

3.6 Germination test

Germination percentage and GI were increased in extracts from all reactors compared to the initial sludge (Table 8). In general, GI values were higher in extracts from vermireactors than control. Highest GI was in T4. The initial digestate had low germination (phytotoxic) probably due to low DO, and high ammonium concentration (Zubillaga and Lavado, 2006). The effect of composting (control), as well as the action of worms in vermireactors, reduced levels of ammonium, increased nitrates and improved aeration. GI values greater than 80% were phytotoxin free and considered as having completed maturity (Raj and Antil, 2011). This ensured the product was not phytotoxic for germination after vermicomposting.

Table 8 Germination % and GI of radish seed, in extracts of the initial and treated sludge

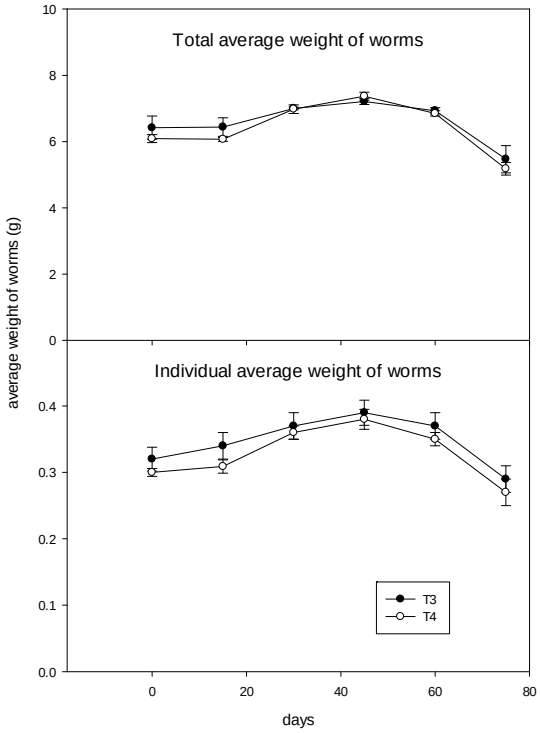
Treatment	*Germination %*	*GI*
Initial sludge	10.3^{b}	2.09^{d}
C1	79.3^{a}	65.5^{c}
C2	82.8^{a}	66.8^{c}
C3	93.1^{a}	87.5^{bc}
T3	96.6^{a}	93.3^{bc}
C4	96.6^{a}	96.7^{ab}
T4	96.6^{a}	98^{a}

Notes: Values are means of three replicates with SE. Treatment descriptions are given in Table 2.
The mean values followed by different letters are statistically different (ANOVA; LSD; p = 0.05)

3.7 Worm observations

There was a biomass gain of worms in both 70% and 50% digestate mixtures (T3 and T4). The maximum weight gain was observed on day 45 (Figure 1). Thereafter, worm weight decreased which was attributed to exhaustion of food supply (Vig et al., 2011). Yadav and Garg (2009) also observed that *Eisenia fetida* lost weight when the composition of substrate was below the worm maintenance level. Individual weight of the worms was always higher in 70% compared to 50% solid waste which may be due to a more nutrient-rich substrate. The number of adult worms from initial 20 reduced to 18.7 and 19.3 in 70% and 50% solid waste, respectively.

Figure 1 Biomass changes of worms during vermicomposting



Notes: Values are means of three replicates (±SE). Treatment descriptions are given in Table 2.

In addition to growth, the substrate also favoured breeding of worms. The production of cocoons was observed after the 5th week of vermicomposting. There were more cocoons and juveniles with 70% (15.3 cocoons and 504 juveniles) compared to 50% (8.67 cocoons and juveniles) solid waste. This implies that substrate was favourable for worm reproduction and can also be ideal for commercial worm production.

The vermicompost produced at the end of 75 days was found to be of better quality compared to compost without worms (control), which was attributed to: the action of worms that increased the rate of mineralisation of organic matter and improved aeration

of compacted digestate. It was also established that worms help to remove the harmful micro-organisms and increased the population of beneficial micro-organisms.

The present research studied the changes for 75 days. It was found that there was drastic reduction in worm biomass after 60 days which means there was exhaustion of food and the growth medium no longer supported healthy worm maintenance. Therefore, based on the measured parameters it was established that, 75 days was the maximum duration for digestate treatment.

The worms converted toxic digestate into nutrient rich safe vermicastings, in a cost-effective way which can be easily adopted for on-site treatment of digestate in developing countries. However, in this experiment, only a single type of digestate was used based on food and vegetable waste (see Table 1 for composition). The characteristics of solid digestate may vary depending on the raw material used for anaerobic digestion, which may be sewage sludge, food and vegetable waste, meat waste, garden waste, industrial waste, etc., and type of digester (Ferrero et al., 1984). For example: the proportion of ammoniacal nitrogen as a proportion of total nitrogen content varies from 12% to as high as 70% (Ferrero et al., 1984). In order to apply the vermicomposting process, the composition of the specific digestate should be known. This investigation identified the most suitable proportion of anaerobic solid to sawdust was 70:30 for providing aeration, dilution of the NH_4 concentration and for growth and reproduction worms. This proportion may not be optimal for all types of digestate as the NH_4 concentration and other characteristics of solid digestate may differ. Also, the type bulking material used may also influence the efficacy of treatment. The worm growth and reproduction are also dependent on the growth medium. The digestate properties also influence its response to bulking materials and further affect the vermicomposting. Therefore, type of feedstock used in digestion and characteristics of digestate produced after digestion may have implications for the treatment.

4 Conclusions

Anaerobic digestate of vegetable wastes by itself was not suitable for vermicomposting due to its initial low oxygen, high NH_4 and compact nature. A mixture of 70:30 (digestate: sawdust) was found to be the suitable medium for vermicomposting based on worm growth. Although the treatment without worms increased nitrification, nutrient concentration and reduced toxicity to germinating seeds, the additional beneficial changes in vermicomposting were significant. Moreover, the pathogens were reduced to safe level in vermicomposting alone. To conclude, treatment of solid digestate through vermicomposting is possible with addition of (up to 50%) sawdust to produce within 75-day safe, nutrient-rich vermicastings.

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