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RESEARCH ARTICLE

Influence of stocking density on the vermicomposting of an effluent treatment plant sludge amended with cow dung

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Abstract This paper reports the effect of earthworm population density on the vermicomposting of effluent treatment plant sludge of a bakery industry. Four waste mixtures containing 0, 10, 20, and 30 % sludge along with cow dung with five different worm population densities were established for 14 weeks under controlled moisture and temperature conditions. The results showed that average worm biomass, growth and cocoon production were lesser at higher population densities. Sexual maturity was attained in 3rd to 5th week in all waste mixtures. Worm growth was inversely related to worm population density in the waste mixture. Results also indicated that lower worm population is favorable to worm biomass production. On the other hand, mineralization and stabilization of the waste mixtures were more at higher worm populations.

Keywords *Eisenia fetida* · Population density · Cocoon production · Bakery industry sludge · Pre-composting

Introduction

Earthworms are key organisms in soil functioning and play an important role in ecosystem restoration (Jouquet et al. 2014). Use of vermicomposting to convert organic wastes into manure is well documented in literature. In this process, earthworms feed on semi-composted organic fraction of the waste and stabilize it into humus-like material. Vermicompost contains significant quantities of plant-available nutrients such as NPK and other biochemicals which are important to maintain soil health and fertility. Sustenance of the process depends on several abiotic (pH, feed substrate, substrate moisture, temperature, C/N, etc.) and biotic factors (population density of worms, presence of microbes, enzymes, etc.).

Klok (2007) reported that competition for resources like food and space may be responsible for worm population regulation during vermicomposting. On the other hand, Uvarov and Scheu (2004) reported that worm population density may affect physiological processes of individual earthworms; however, the effects of population density on physiological processes may differ between earthworm species. A bibliographic survey has indicated that average earthworm population densities in soil may range from less than one individual to several hundred individuals per square meter (Curry 1998; Lee and Plankhurst 1992). The highest earthworm densities were reported for floodplain soils, i.e., 1800–2000 individuals per square meter (Zorn et al. 2005; Lavelle and Spain 2001).

Several studies have been conducted on the vermicomposting of different wastes such as spent coffee grounds (Liu and Price 2011), sugar industry waste (Sangwan et al. 2010), plant waste (Deka et al. 2011), olive oil industry waste (Vivas et al. 2009), paper pulp industry sludge (Kaur et al. 2010), bakery industry waste (Yadav et al. 2015), activated sludge (Hait and Tare 2011), weeds (Yadav and Garg 2013), etc. But most of the studies are centered around abiotic factors such as temperature (Reinecke et al. 1992), moisture content (Hallatt et al. 1992), feed rate (Ndegwa et al. 2000), C/N ratio (Aira et al. 2006), etc. Very few studies are accessible on the effect of stocking density on

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vermicomposting of different wastes (Table 1). The available literature indicated that there is a paucity of studies on the effect of earthworm population density on vermicomposting of industrial sludge/wastes. It is important to maintain optimum worm population during vermicomposting process to have better worm growth and reproduction in a limited period. Therefore, the focus of this study was to investigate (a) the effect of earthworm population on the growth and reproduction of *Eisenia fetida* and (b) the effect of different populations on the nutrient status of vermicompost of bakery industry sludge (BIS) spiked with cow dung.

Materials and methods

Healthy non-clitellated hatchlings of *Eisenia fetida* species, each weighing 150–250 mg live biomass, were randomly picked from stock culture. Cow dung (CD) was collected from a dairy located at Hisar, India. BIS was procured from sand beds of an effluent treatment plant of a bakery industry located in Haryana, India. The procured BIS was a mixture of primary and activated sludge. The sludge was dried in sunlight for a week with periodic turnings before use in the experiments. The main physico-chemical parameters of BIS were pH 6.3 ±0.1, electrical conductivity (EC) 1.7±0.05 dS/m, total organic carbon (TOC) 366±19 g/kg; total Kjeldahl nitrogen (TKN) 21±0.3 g/kg, total available phosphorus (TAP) 8.9 ±0.21 g/kg; total potassium (TK) 3.0±0.08 g/kg; and TCa 4.3±0.07 g/kg.

Experimental setup

Four waste mixtures having different ratios of CD and BIS including one with CD only were established. Five hundred grams of each waste mixture (on dry weight basis) was taken in 2-L circular plastic containers. The compositions of waste mixtures are given below:

T_1: 100 % CD
T_2: 90 % CD + 10 % BIS
T_3: 80 % CD + 20 % BIS
T_4: 70 % CD + 30 % BIS

The composition of waste mixtures was decided based on the earlier studies conducted by the authors (Yadav and Garg 2009).

All containers were kept in the dark at room temperature (22±3 °C). The moisture content of each waste mixture was maintained at 70±10 % by adding water periodically. The feed mixtures were pre-composted for 3 weeks to eradicate volatile toxic gases if any and to make the waste mixture palatable to worms. After pre-composting, non-clitellated earthworms were introduced into five population densities (1, 2, 4, 8, and 16 worms per waste mixture). Three replicates were set for each population density. Hence, 60 vermibins were maintained in this experiment (four waste mixtures × five population densities in each feed × three replicates of each). The worms were separated from waste mixtures by hand and counted, and biomass was recorded weekly for 14 weeks. Then, all the earthworms were transferred to their respective containers. At the same, time clitellum development and cocoon production were also monitored.

The vermicomposts produced after 14 weeks were air dried at room temperature and stored in plastic containers for physico-chemical analysis. Different physico-chemical parameters of the initial waste mixtures and vermicomposts were analyzed as per standard methods. The pH and EC were determined by digital pH and EC meter using a double-distilled water suspension of each vermicompost in the ratio of 1:10 (*w*/*v*). TOC and TKN were measured using the method of

Table 1 Studies on effect of worm density on growth and reproduction of earthworms during the vermicomposting and optimum worm density

Earthworm species	Optimum population density	Feed	Duration of study	Stocking density	Reference
Lumbricus rubellus	1200 worm/m^2	Soil + Alder leaves (*Alnus glutinosa*)	4 weeks	2 to 9 worms (correspond to field densities of about 300–1350 earth-worms/m^2)	Klok 2007
Perionyx excavatus	–	Cow dung	75 days	1, 2, 3, 4, and 5 worms (correspond to field densities 0.0680, 0.1388, 0.205, 0.274, and 0.342 mg/cm^3)	Suthar 2012
Eisenia fetida	27–53 worms/kg feed	Cow dung and textile mill sludge	12 weeks	1, 2, 4, 8, and 12)	Garg et al. 2008
Eisenia fetida	0.5 kg/m^2	Sewage sludge	147 days	0–5.0 kg/m^2	Hait and Tare 2011
Eisenia andrei	8 worms/43.61 g waste (dry weight)	Pig manure	44 days	1, 2, 4, 8, 16 worms	Dominguez and Edwards 1997
Eisenia andrei	12 worms/0.5–1 L	Pig manure	60–230 days	3, 6 and 12 worms	Reeh 1992
Eisenia fetida	1.60 kg worms/m^2	Biosolids	8 weeks	0.80, 1.20, 1.60, and 2.00 kg-worms/m^2	Ndegwa et al. 2000

Nelson and Sommers (1982) and Bremner and Mulvaney (1982), respectively. TAP was analyzed using the spectrophotometric method. TK was determined after digesting the sample in diacid mixture (HNO_3 and $HClO_4$ in 9:1 *v*/*v*) using a flame photometer. C/N and C/P ratios were calculated by using the values of TOC, TKN, and TAP content.

Statistical analysis

The reported results are the average of three replicates. One-way ANOVA was used to analyze the significant differences among different waste mixtures for the studied parameters ($P < 0.05$). Tukey's *t* test was performed to identify the homogeneous type of waste mixtures for the various parameters using SPSS.

Results and discussion

Effect of population densities on growth and reproduction of worms

There was no worm mortality in any of the waste mixtures. Mean individual biomass of worms increased continuously, during the observation period, at one and two worm populations in all the waste mixtures. At other population densities, worm biomass decreased after a certain period. The reduction in biomass was more at higher worm densities. Figure 1 represents the growth of *Eisenia fetida* in T_1 at different population densities. In T_1, at 1 and 2 population densities, mean individual worm biomass increased continuously till the observation period, whereas at 4, 8, and 16 population densities, it decreased after the 7th, 8th, and 6th weeks, respectively. In T_1, mean worm biomass was 1900 ± 80 mg per worm at the population density of 1 and 841 ± 139 mg per worm at the population density of 16 (Table 2). In T_2, mean biomass was maximum (2004 ± 84 mg per worm) at the population density of 1 after the 14th week and minimum (537 ± 22 mg per worm) at the population density of 16 in the 6th week (Fig. 2). In T_3, maximum worm biomass was 1770 ± 110 mg per worm at the population density of 1 worm in the 14th week and minimum biomass was 537 ± 22 mg per worm at the population density of 16 in the 6th week (Fig. 3). Whereas in T_4, the maximum and minimum mean individual biomass were 1120 ± 70 mg per worm at population density of 1 worm on the 14th week and 600 ± 12 mg per worm at the population density of 16 worms in the 8th week, respectively (Fig. 4).

Maximum mean individual worm biomass was observed at a population density of 1 earthworm and minimum biomass was at the population density of 16 worms in all the waste mixtures. The mean individual worm biomass was significantly different ($P < 0.05$) at different population densities with respect to waste mixture (Table 2). The maximum mean individual biomass at lower population density may be due to more availability and less competition for food in these waste mixtures. The results showed that at lower population densities, earthworms gained higher biomass and vice versa. In contrast, the time taken to achieve the highest biomass was lesser at a higher population density as compared to a lower population density in all the waste mixtures. Similar observations have been reported by other workers. Suthar (2012) reported that *Perionyx excavatus* gained maximum biomass in low-stocking density trials. Dominguez and Edwards (1997) have also reported that *Eisenia andrei* gained

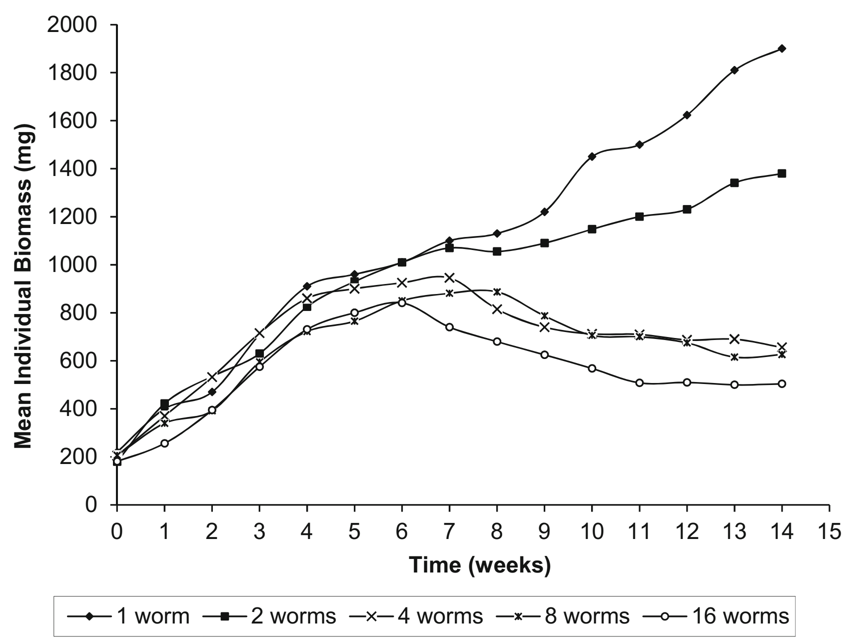

Fig. 1 Growth of *Eisenia fetida* in T_1 (100 % CD) at different population densities

Table 2 Maximum mean individual earthworm biomass achieved (mg) in different waste mixtures at different population densities (*mean* ± *SD*, *n* = 3)

Population density	T_1	T_2	T_3	T_4
1	1900 ± 80c (14th)*	2004 ± 84d (14th)*	1770 ± 110d (14th)*	1120 ± 70b (14th)*
2	1380 ± 223b (14th)*	1100 ± 57c (8th)*	1100 ± 57c (9th)*	974 ± 26b (7th)*
4	945 ± 35a (7th)*	925 ± 85bc (7th)*	890 ± 10b (7th)*	700 ± 10a (7th)*
8	887 ± 93a (8th)*	810 ± 70ab (6th)*	780 ± 11b (6th)*	654 ± 103a (8th)*
16	841 ± 139a (6th)*	700 ± 20a (6th)*	537 ± 22a (6th)*	600 ± 12a (8th)*

*Fig. in parentheses indicates the week on which maximum biomass was attained; thereafter, it declines. Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$)

maximum biomass at lower population densities during the vermicomposting of pig manure. Garg et al. (2008) reported that maximum worm biomass was gained in lesser time at the population density of 12 worms per kilogram of feed as compared to other population densities. Neuhauser et al. (1980) have studied the impact of population density on biomass growth of *Eisenia fetida* and reported that growth of worms was related to the population density. The reduction in worm biomass at higher worm densities may be due to the overpopulation of worms and non-availability of food in the waste mixtures toward the completion of the experiment. The results from the present study are in accordance with the findings of other researchers (Ndegwa et al. 2000; Hait and Tare 2011). Dominguez and Edwards (1997) have reported that, at higher population densities, earthworms grow slowly and with a lower biomass, even when the physical conditions were identical and ideal. Reinecke and Viljoen (1990) have reported that *Eisenia fetida* required 20–25 g (dry mass of cattle manure) of substrate to maintain its growth and reproduction rate for at least 48 days. Karmegam and Daniel (2009) have also reported that after achieving the maximum density in a given vermi-bed, the earthworm population tend to stabilize the density with reduced activities. These results indicated that worm population as well as feed quality have a significant effect on the growth of worms during vermicomposting.

Table 3 encapsulates the mean growth rate of *Eisenia fetida* in different waste mixtures at different population densities. The mean growth rate in waste mixtures ranged from 13.1 ± 0.14 (at population density 2 worms) to 18.09 ± 1.1 mg/worm/day (at population density 1) in T_1, 14.3 ± 0.83 (at population density 16) to 19.0 ± 0.92 mg/worm/day (at population density 1) in T_2, 10.09 ± 0.75 (at population density 16 worms) to 16.8 ± 0.15 mg/worm/day (at population density one) in T_3, and 9.52 ± 0.48 (at population density 16 worms) to 17.3 ± 0.34 mg/worm/day (at population density 1) in T_4. The results are in accordance with the findings of previous researchers. The worm growth rate for *Eisenia fetida* was found to be 14 mg/worm/day in sludge (Hartenstein and Hartenstein 1981); for *Dendrobaena veneta*, growth rate was found to be 21.3 mg/worm/day in paper sludge (Fayolle et al. 1997); for *Eisenia andrei*, growth rate was found to be 18.6 ± 0.6 mg/worm/day in food waste (Domínguez et al. 2000); for *Eisenia fetida*, growth rate was found to be 3.17–5.06 mg/worm/day in activated sludge (Hait and Tare 2011); and for *Perionyx excavatus*, growth rate was found to be 0.94–2.21 mg/worm/day in cattle dung (Suthar 2012). Slower worm growth rate at higher earthworm density, as found in this study, has also been reported for *Lumbricus rubellus* by Klok (2007).

All worms attained sexual maturity at all the population densities. The time taken for clitellum development was directly related with nutrient availability. Sexual maturity was attained in the 3–5th week in all the waste mixtures (Table 4). Neuhauser et al. (1980) reported that food availability and

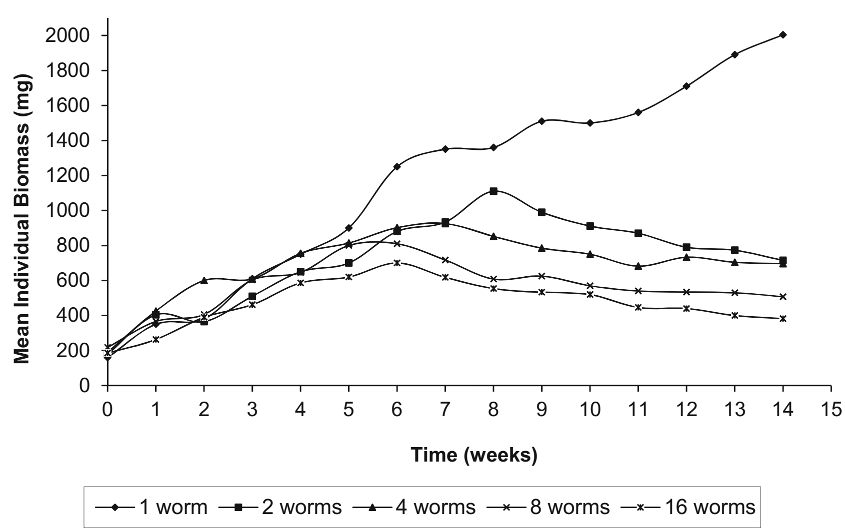

Fig. 2 Growth of *Eisenia fetida* in T_2 (90 % CD + 10 % BIS) at different population densities

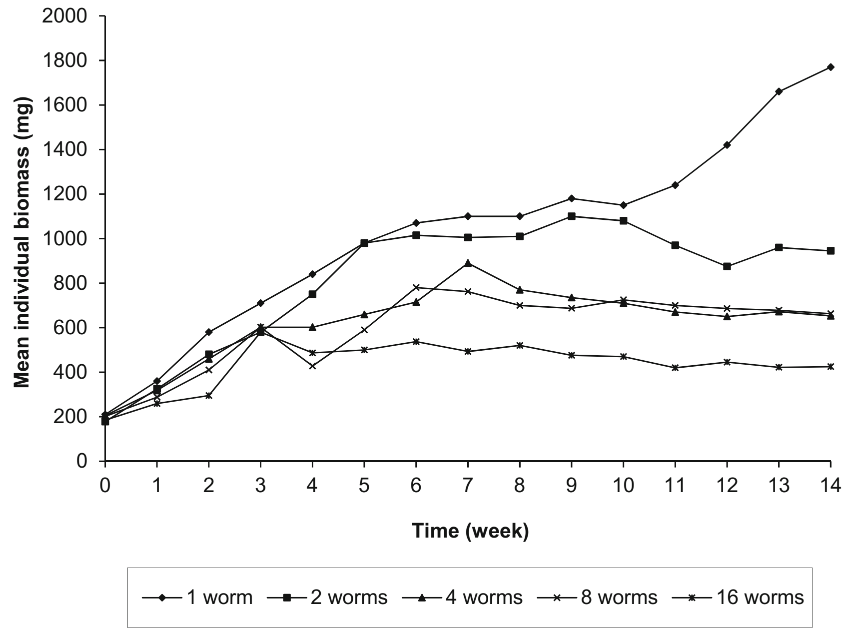

Fig. 3 Growth of *Eisenia fetida* in T_3 (80 % CD + 20 % BIS) at different population densities

population density determined the time to reach sexual maturity for earthworms. The cocoon production started between the 5th and 7th weeks in all the waste mixtures for all population densities, except at a population density of one worm. No cocoons were produced at the population density of one worm in all the waste mixtures. Although earthworms are hermaphrodite in nature, at least two worms are required for copulation which is indispensable for reproduction. That is why there was no cocoon production at singular population density.

Maximum cocoons (45 ± 4) were produced in T_1 at the population density of 16 and minimum cocoons (11.8 ± 0.8) were produced in T_2 at the population density of 2 (Table 5). Cocoon production was significantly different at different population densities in all the waste mixtures ($P < 0.05$). The time difference in the onset of cocoon production, at different population densities, may be due to the nutrient availability in waste mixtures. Cocoon production rate (cocoons/worm) decreased as the population densities increased in all the waste mixtures except in T_4 (Table 5). This may be attributed to the combined effect of higher concentration of sludge and less food availability at higher population densities (Garg et al. 2008). Hait and Tare (2011) have reported that the population density influences the worm reproduction rate because the copulation frequency is higher at low population densities and it may decrease significantly when worm density approaches the carrying capacity of the vermibin.

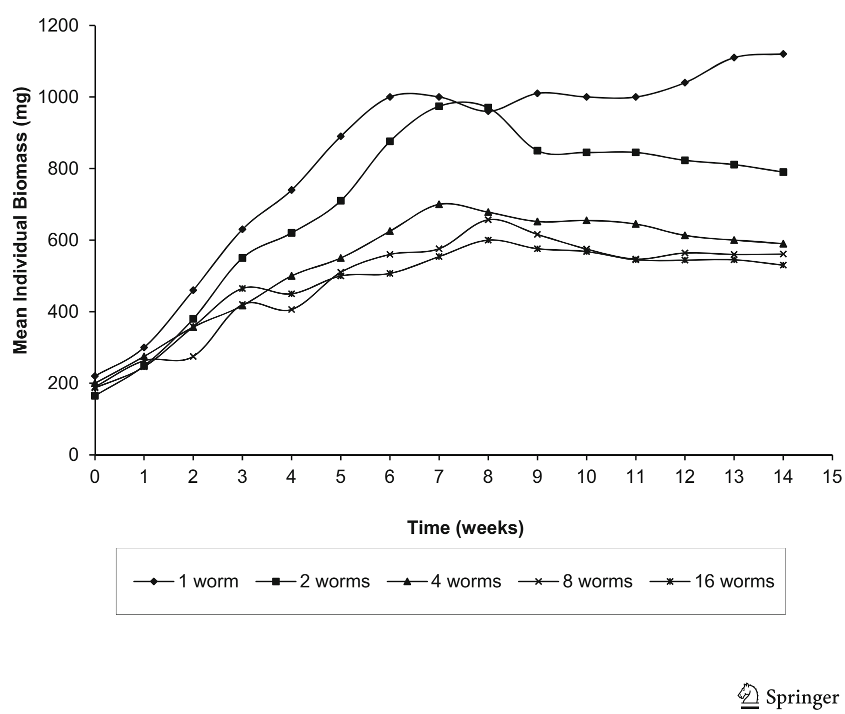

Fig. 4 Growth of *Eisenia fetida* in T_4 (70 % CD + 30 % BIS) at different population densities

Table 3 Net biomass gained (mg/worm) and mean growth rate of *Eisenia fetida* (mg/worm/day) in different waste mixtures at different population densities (*mean* ± *SD*, *n* = *3*)

Population density	T_1	T_2	T_3	T_4
Net biomass gained (mg/worm)				
1	1680 ± 120c	1884 ± 41d	1560 ± 70d	940 ± 60c
2	1200 ± 65b	920 ± 103c	922 ± 88c	809 ± 81b
4	740 ± 60a	735 ± 16b	685 ± 26b	500 ± 12a
8	681 ± 9a	592 ± 8a	578 ± 18b	476 ± 11a
16	660 ± 6a	515 ± 14a	352 ± 7a	413 ± 10a
Mean growth rate (mg/worm/day)				
1	18.09 ± 1.1c	19.0 ± 0.92d	16.8 ± 0.15b	17.3 ± 0.34a
2	13.1 ± 0.14a	17.4 ± 0.5bc	15.7 ± 0.71b	16.0 ± 0.61c
4	16.8 ± 0.2bc	16.5 ± 0.49b	15.8 ± 1.11b	12.5 ± 0.50b
8	14.07 ± 0.4a	16.5 ± 0.50b	15.9 ± 1.10b	10.4 ± 0.42a
16	15.01 ± 1.4ab	14.3 ± 0.83a	10.9 ± 0.75a	9.52 ± 0.48a

Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$)

Frederickson et al. (1997) have also reported a significant reduction in growth and reproduction of *Eisenia andrei* as population densities increased.

The hatchling production data in different waste mixtures at different population densities at the end of experiment are given in Table 6. The data showed that worm population influenced hatchling production in different vermibins. Maximum hatchlings were produced at the population density of eight worms (82.7 ± 3.7) in T_1 and minimum hatchlings were produced at the population density of two worms (12.5 ± 1.5) in T_4. Total biomass of hatchlings was maximum at the population density of eight worms (4.26 g) in T_1 and minimum was also in T_1 at the worm population density of two (0.68 g).

Effect of worm population densities on manurial values of vermicomposts

The initial physico-chemical characteristics of waste mixtures are given in Table 7. pH is one of the most frequent parameters used to characterize the vermicompost quality. Initially, all waste mixtures had alkaline pH values (8.3 to 7.7) at the outset of the process. The pH of vermicomposts was lesser than the initial pH values in all the waste mixtures. The production of CO_2 and organic acids, because of combined action of earthworms and microorganisms during decomposition of organic wastes, might result in lowering of pH of final product at the end of vermicomposting (Ndegwa et al. 2000). Reduction in pH was more at the higher worm's population densities as compared to lesser population densities. Vermicomposting process shifted the pH toward neutrality (7.5 to 7.1) in T_1 irrespective of the initial pH at all population densities. The pH values ranged from 7.1 to 6.6, 6.9 to 6.0, and 6.9 to 6.0 in T_2, T_3, and T_4, respectively (Table 8). Different worm population densities had no significant difference on pH of the vermicomposts in different waste mixtures.

Table 4 The day of start of clitellum development and cocoon production in different waste mixtures at different population densities

Population density	T_1	T_2	T_3	T_4
1	Nil (3rd)*	Nil (4th)*	Nil (3rd)*	Nil (4th)*
2	35th day (4th)*	35th day (4th)*	42th day (4th)*	35th day (4th)*
4	35th day (3rd)*	35th day (4th)*	42th day (4th)*	42th day (4th)*
8	35th day (4th)*	35th day (4th)*	35th day (4th)*	49th day (4th)*
16	42th day (4th)*	35th day (4th)*	35th day (4th)*	49th day (5th)*

*Fig. in parentheses is the week on which clitellum development was started in worms

EC of all the waste mixtures was almost the same and ranged from 1.1 to 1.3 dS/m in initial waste mixtures (Table 7). After vermicomposting, EC values were higher in final products than initial waste mixtures. The EC was maximum at highest worm population density and minimum at lowest population density in all the waste mixtures. The ECs of vermicomposts were in the range of 1.42 to 3.0 dS/m in T_1, 1.7 to 2.94 dS/m in T_2, 1.5 to 2.8 dS/m in T_3, and 1.84 to 2.84 dS/m in T_4 (Table 8). Data revealed that the EC values at different population densities were significantly different from each other ($P < 0.05$). The increase in EC may be due to loss of organic matter and release of different mineral salts in available forms such as phosphate, ammonium, potassium, etc. (Kaviraj and Sharma 2003).

TOC of vermicompost was reduced remarkably at the end of the experiment as compared to initial waste mixtures (Table 8). The data showed that TOC reduction was inversely related to the worm's population in a waste mixture. Maximum TOC loss was observed at a population density of 16 worms and minimum at a population density of 1 worm in all the waste

Table 5 Cocoons produced by *Eisenia fetida* in different waste mixtures at different population densities (*mean* ± *SD*, *n* = *3*)

Population density	T_1	T_2	T_3	T_4
Total cocoons produced				
1	Nil	Nil	Nil	Nil
2	24.3 ± 3.3a	11.8 ± 0.8a	15.2 ± 0.2a	14.9 ± 2.9a
4	20.9 ± 4.9a	18.7 ± 2.7b	24.6 ± 3.6b	35.5 ± 2.5bc
8	35.4 ± 2.0b	25 ± 3.0b	26.3 ± 1.7b	38.0 ± 5.0c
16	45 ± 4.0b	38.3 ± 3.3c	42.1 ± 1.9c	26.9 ± 4.9b
Number of cocoons produced per worm				
1	Nil	Nil	Nil	Nil
2	12.1 ± 0.15d	5.9 ± 0.2d	7.6 ± 0.1d	7.45 ± 0.35c
4	5.22 ± 0.29c	4.67 ± 0.13c	6.15 ± 0.15c	8.87 ± 0.13d
8	4.42 ± 0.09b	3.12 ± 0.12b	3.28 ± 0.07b	4.75 ± 0.13b
16	2.81 ± 0.04a	2.39 ± 0.05a	2.68 ± 0.13a	1.85 ± 0.02a

Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$)

mixtures. The reduction in TOC during the process ranged from 21.9 to 47.8 % in T_1, 19.1 to 44.9 % in T_2, 19.05 to 40 % in T_3, and 21.9 to 40.9 % in T_4 (Table 8). More reduction in TOC after vermicomposting in waste mixtures at higher population densities may be due to the presence of more earthworms in these waste mixtures. The TOC contents of vermicomposts at different population densities of 1, 2, 4, 8, and 16 worms were significantly different from each other ($P < 0.05$). This is supported by the fact that the rate of carbon reduction is almost twice in the presence of earthworms in pig manure vermicomposting than the control, revealing faster decomposition of organic matter (Aira and Dominguez 2010). Similarly, Elvira et al. (1996) have reported that earthworms modify the substrate conditions, which subsequently enhance the carbon losses from the substrates through microbial respiration in the form of carbon dioxide.

TKN content in all waste mixtures was significantly enhanced at the end of the vermicomposting period in all the population densities (Table 8). The TKN content showed a significant difference in different waste mixtures at different population densities ($P < 0.05$). The initial TKN content of the waste mixtures was in the range of 8.5 to 12.7 g/kg. It was evident from the results that TKN content increased with the increased population density in different waste mixtures. The results indicate that the maximum TKN increment (2.2- to 2.8-folds) was at the population density of 16 worms and minimum at the population density of one worm in all the waste mixtures. The TKN content of the vermicomposts ranged from 15.4 to 28.1 g/kg in T_1, 17.34 to 28.2 g/kg in T_2, 18.1 to 28.7 g/kg in T_3, and 19.3 to 29.2 g/kg in T_4 (Table 8). Tripathi and Bhardwaj (2004) have reported the addition of nitrogen in the form of mucus, nitrogenous excretory substances, growth-stimulating hormones, and enzymes from earthworms during vermicomposting. Atiyeh et al. (2000) reported that earthworms have a great impact on nitrogen transformations in manure, by enhancing nitrogen mineralization, so that mineral nitrogen was retained in the nitrate form. So due to the presence of more earthworms at higher population

Table 6 Total hatchlings produced by *Eisenia fetida* in different waste mixtures at different population densities (*mean* ± *SD*, *n* = *3*)

Population density	T_1	T_2	T_3	T_4
1	Nil	Nil	Nil	Nil
2	16.2 ± 1.2a (0.68)*	21.3 ± 0.3a (1.37)*	14.3 ± 0.4a (1.60)*	12.5 ± 1.5a (1.06)*
4	27.3 ± 0.6b (2.7)*	45.1 ± 4.1b (3.95)*	39.1 ± 0.9 (2.67)*	34.1 ± 2.1c (1.87)*
8	82.7 ± 3.7c (4.26)*	42.2 ± 0.8b (2.9)*	18.5 ± 0.5b (1.80)*	26.2 ± 2.8b (1.71)*
16	51.3 ± 5.3d (2.43)*	49.3 ± 5.3b (1.8)*	28.4 ± 1.4c (1.48)*	21.9 ± 0.1b (2.34)*

*Fig. in parentheses is the total biomass of hatchlings (g). Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$)

Table 7 Initial physico-chemical characteristics of different waste mixture (*mean* ± *SD*, *n* = *3*)

Parameter	T_1	T_2	T_3	T_4
pH	8.3 ± 0.3	8.1 ± 0.3	7.9 ± 0.02	7.7 ± 0.1
EC (dS/m)	1.1 ± 0.01	1.2 ± 0.03	1.2 ± 0.02	1.3 ± 0.03
TOC (g/kg)	510 ± 10	492 ± 15	467 ± 5.2	461 ± 13
TKN (g/kg)	8.5 ± 0.5	10.2 ± 0.19	11.5 ± 0.70	12.7 ± 0.60
TAP (g/kg)	6.4 ± 0.05	6.6 ± 0.1	6.9 ± 0.42	7.2 ± 0.6
TK (g/kg)	6.8 ± 0.3	6.5 ± 0.35	6.1 ± 0.37	5.7 ± 0.25
TCa (g/kg)	2.4 ± 0.04	2.6 ± 0.1	2.8 ± 0.07	3.1 ± 0.1
C/N ratio	60 ± 6.1	48.2 ± 2.8	40.6 ± 4.3	36 ± 1.5
C/P ratio	76.6 ± 2.3	74.5 ± 5.4	67.6 ± 4.0	64 ± 2.2
OM %	87.9 ± 4.3	84.8 ± 5.9	82.0 ± 7.1	79.4 ± 4.6

Table 8 pH, EC (dS/m), TOC (g/kg), and TKN (g/kg) in vermicomposts obtained at different population densities in waste mixtures

Population	T_1		T_2		T_3		T_4	
Density	pH	EC(dS/m)	pH	EC(dS/m)	pH	EC(dS/m)	pH	EC(dS/m)
1	7.5 ± 0.25ab	1.42 ± 0.09a	7.1 ± 0.12a	1.7 ± 0.1a	6.9 ± 0.2b	1.5 ± 0.1a	6.9 ± 0.05b	1.84 ± 0.1a
2	7.4 ± 0.25a	1.82 ± 0.06b	6.7 ± 0.20a	2.36 ± 0.04b	6.6 ± 0.55ab	1.8 ± 0.05b	6.6 ± 0.15b	2.32 ± 0.24b
4	7.3 ± 0.18a	2.56 ± 0.16c	6.7 ± 0.05a	2.6 ± 0.05b	6.6 ± 0.05ab	2.26 ± 0.14c	6.1 ± 0.13a	2.48 ± 0.08c
8	7.2 ± 0.20a	2.62 ± 0.18c	6.7 ± 0.15a	2.92 ± 0.12c	6.1 ± 0.20a	2.56 ± 0.09d	6.0 ± 0.11a	2.96 ± 0.24 cd
16	7.1 ± 0.0a	3.0 ± 0.08d	6.6 ± 0.15a	2.94 ± 0.16c	6.0 ± 0.01a	2.8 ± 0.12d	6.0 ± 0.13a	2.84 ± 0.06d
	TOC	TKN	TOC	TKN	TOC	TKN	TOC	TKN
1	398 ± 20c	15.4 ± 1.1a	398 ± 20d	17.3 ± 0.4a	378 ± 13c	18.1 ± 1.2a	360 ± 17b	19.3 ± 0.8a
2	360 ± 15b	16.9 ± 0.9a	340 ± 16c	19.5 ± 1.7ab	361 ± 18c	19.5 ± 1.1ab	341 ± 11b	21.8 ± 0.85a
4	325 ± 1b	18.1 ± 1.5ab	319 ± 5bc	21.4 ± 2.2bc	322 ± 11b	21.8 ± 0.8b	304 ± 18a	25.4 ± 1.4b
8	283 ± 6a	20.4 ± 0.7b	295 ± 7ab	24.5 ± 1.2 cd	299 ± 10ab	27.6 ± 1.6c	281 ± 7a	27.9 ± 0.9bc
16	266 ± 14a	23.8 ± 1.7c	271 ± 8a	28.2 ± 1.2d	280 ± 10a	28.7 ± 0.7c	272 ± 9a	29.2 ± 2.1c

The values of TOC and TKN were in grams per kilogram. Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$)

densities, a higher nitrogen content was added in the vermicomposts.

TAP content was also higher in vermicomposts obtained at different population densities in various waste mixtures (Table 9). TAP content increased from 6.9 to 43 % in different waste mixtures at varying population densities after vermicomposting. The data showed that an increase in TAP content was directly proportional to the worm population in the waste mixture. The increase in TAP content ranged from 10.9 to 43 % in T_1, 9.0 to 39.3 % in T_2, 7.2 to 37.6 % in T_3, and 6.9 to 36.1 % in T_4 (Table 9). The increase in TAP content at different population densities was significantly different from each other except at population densities of 8 and 16 worms ($P < 0.05$). Le Bayon and Binet (2006) studied earthworm-mediated phosphatase enhancement in soils and reported that earthworms were responsible for additional alkaline phosphatase in organic waste, produced in the worm gut and excreted through cast deposition. Yadav and Garg (2013) have reported that the increase in TAP was attributed to direct action of worm gut enzymes and indirectly by stimulation of micro flora during vermicomposting.

TK content in the vermicomposts ranged from 7.1 to 7.5 g/kg in T_1, 6.6 to 7.1 g/kg in T_2, 6.0 to 6.9 g/kg in T_3, and 6.0 to 6.9 g/kg in T_4 (Table 9). The variation in population density of earthworms in waste mixtures did not show significant changes in final TK content of the vermicomposts ($P > 0.05$). The C/N and C/P ratios of vermicomposts obtained from different waste mixtures are given in Table 9. The C/N ratios of the final

Table 9 TAP (g/kg), TK (g/kg), C/P ratio, and C/N ratio in vermicomposts obtained at different population density in different waste mixtures

Population Density	T_1		T_2		T_3		T_4	
	TAP	TK	TAP	TK	TAP	TK	TAP	TK
1	7.1 ± 0.15a	7.1 ± 0.12a	7.2 ± 0.07a	6.6 ± 0.2a	7.4 ± 0.38a	6.0 ± 0.20a	7.7 ± 0.05a	6.0 ± 0.05a
2	7.6 ± 0.05b	7.2 ± 0.09ab	7.8 ± 0.11b	6.7 ± 0.1ab	7.9 ± 0.08b	6.0 ± 0.12a	8.3 ± 0.07b	6.0 ± 0.08a
4	8.2 ± 0.05c	7.3 ± 0.14ab	8.5 ± 0.07c	6.7 ± 0.16a	8.5 ± 0.06c	6.6 ± 0.11b	9.3 ± 0.1c	6.1 ± 0.08a
8	8.9 ± 0.12d	7.4 ± 0.17ab	9.0 ± 0.08d	6.7 ± 0.1ab	9.2 ± 0.7d	6.6 ± 0.05b	9.7 ± 0.7d	6.6 ± 0.06b
16	9.2 ± 0.09e	7.5 ± 0.16b	9.2 ± 0.12d	7.1 ± 0.07b	9.5 ± 0.09d	6.9 ± 0.01b	9.8 ± 0.11d	6.9 ± 0.09c
	C/P	C: N	C: P	C: N	C: P	C: N	C: P	C: N
1	56.0 ± 3.9d	25.84 ± 0.59e	55.2 ± 2.2d	22.95 ± 0.59e	51 ± 0.91d	20.88 ± 1.97c	46.7 ± 2.5d	18.65 ± 1.72c
2	47.3 ± 1.7c	21.3 ± 0.26d	43.5 ± 1.4c	17.45 ± 0.75d	45.6 ± 1.8c	18.15 ± 2.44bc	41 ± 1.60c	15.78 ± 0.98b
4	39.6 ± 0.1b	17.95 ± 1.10c	37.5 ± 0.9b	14.9 ± 1.18c	38 ± 1.4b	14.81 ± 1.04b	32.6 ± 1.5b	11.96 ± 1.45a
8	32.9 ± 1.1a	13.8 ± 0.87b	32.7 ± 0.4a	12.04 ± 0.29b	32.5 ± 1.3a	10.83 ± 1.05a	28.9 ± 0.28a	10.07 ± 0.59a
16	27.6 ± 1.3a	11.17 ± 0.23a	29.4 ± 1.2a	9.60 ± 0.12a	29.4 ± 1.3a	9.75 ± 0.60a	27.7 ± 0.6a	9.31 ± 1.10a

The values of TAP and TK were in grams per kilogram. Mean value followed by different letters is statistically different (ANOVA; Tukey's test, $P < 0.05$)

vermicomposts obtained from different waste mixtures at different population densities were significantly different ($P < 0.05$). Initial C/N ratios of different waste mixtures were in the range of 36 to 60, and final C/N ratios ranged from 11.17 to 25.84 in T_1, 9.60 to 22.95 in T_2, 9.75 to 20.88 in 80 % T_3, and 9.31 to 18.65 in T_4. The results indicated that the worm population in a vermibin plays an important role in the rate of decomposition of organic fraction of wastes. The loss of carbon as carbon dioxide in the process of respiration and production of mucus and nitrogenous excreta enhances the level of nitrogen, which lowers the C/N ratio (Senapati et al. 1980). The results also indicated that the worm population plays an important role in the decomposition of organic wastes and mineralization. It is evident from the data that with the increase in population densities, the C/N ratio reduction was greater. This was due to greater reduction in TOC and more addition of TKN with increasing worm population.

The C/P ratio decreased at the end of the experiment in all the waste mixtures at different population densities. This indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity of organic wastes. The C/P ratios of initial waste mixtures were in the range of 64 to 76.6, and in vermicomposts, it was in the range of 27.7 to 56.0 in different waste mixtures. The C/P ratios at higher population densities were lesser than lower population densities (Table 9). The C/P ratio at a population density of one worm was insignificantly different ($P > 0.05$) with the population density of two worms, but it was significantly different with other population densities in all waste mixtures. This can be attributed to more reduction of TOC and more addition of TAP with increasing worm population in vermibins.

Conclusion

Along with physical conditions (temperature and moisture), worm population density also plays an important role in the vermicomposting process. For the adequate growth and reproduction, optimum population density should be maintained in the vermibins. The results showed that earthworm growth rate was inversely proportional to the population density. The worms at lower population density achieved higher biomass, whereas worms at higher population density achieved lower biomass. Sexual maturity and cocoon production were directly affected by the population density, i.e., the higher the number of earthworms in a bin, the higher the cocoon production. The results also showed that the physico-chemical properties of the vermicomposts are also affected by population densities. Except pH, worm densities significantly affected other physico-chemical properties (EC, TOC, TKN, TAP, etc.) of vermicomposts. The results indicate that for the vermicomposting of BIS and cow dung mixtures, a population density of 16 to 32 earthworms (*Eisenia fetida*) per kilogram of waste is optimum. For the bioconversion of BIS and cow dung into the earthworm's biomass, a lower population density seems an optimal combination, while superior quality of vermicomposts was observed in waste mixtures that contain high worm population densities.

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