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Effects of C-to-N ratio on vermicomposting of biosolids

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Abstract

The role of organic carbon and inorganic nitrogen for cell synthesis, growth, and metabolism is important in all living organisms. To provide proper nutrition for earthworms during vermicomposting, carbon and nitrogen must be present in the substrates at the correct ratio. The usual practice is to arbitrarily add either a rich nitrogenous material, or a rich carbonaceous material to the feed substrate, depending on the situation, to correct for C-to-N imbalance. In addition, the conventional determination of C-to-N ratio is not always based on the proportion of each nutrient that is available for these processes, but on their absolute content in the substrate. More so, different earthworm species are impacted differently by C-to-N ratio and feed mixture type. Therefore, pilot studies are necessary to establish optimal C-to-N ratio for a specific earthworm species and a specific feed mixture. Specifically, the focus of this study was to investigate and establish a suitable C-to-N ratio for vermicomposting of fresh biosolids (activated sewage sludge) amended with paper mulch, using *Eisenia fetida*. An optimal stocking density of 1.60 kg-worms/m^2 and an optimal feeding rate of 0.75 kg-feed/kg-worm/day (Ndegwa, P.M., Thompson, S.A., Das, K.C., 1999. Biores. Technol. 71 (1), 5–12), were used in this study. A C-to-N ratio of 25 resulted in the highest stability of the product, the best fertilizer-value of the product, and also a product with the lowest potential for environmental pollution.

Keywords: Organic-by-products; Bioconversion; Environment; Vermicomposting; Pollution; Fertilizer; Earthworms; C-to-N ratio; Biosolids

1. Introduction

Scientific investigations have established the viability of using earthworms as a treatment technique for numerous waste streams (Hand et al., 1988; Raymond et al., 1988; Harris et al., 1990; Logsdon, 1994). The action of earthworms in this process is both physical/mechanical and biochemical. The physical/mechanical processes include: substrate aeration, mixing, as well as actual grinding. The biochemical process is effected by microbial decomposition of the substrate in the intestines of the earthworms. These physical/mechanical unit processes usually represent the largest cost associated with a traditional microbial composting process. Vermicomposting, therefore, saves on all these unit operations. Hand et al. (1988) thus define vermicomposting as a low cost technology system for the processing or treatment of organic wastes.

In contrast to traditional microbial waste treatment, vermicomposting results in the bioconversion of the waste stream into two useful products: the earthworm biomass and the vermicompost. The former product can further be processed into proteins (earthworm meal) or high-grade horticultural compost (Edwards and Niederer, 1988; Fisher, 1988; Phillips, 1988; Sabine, 1988). The latter product (vermicompost/castings) is also, considered an excellent product since it is homogenous, has desirable aesthetics, has reduced levels of contaminants and tends to hold more nutrients over a longer period, without adversely impacting the environment.

The role of organic carbon and inorganic nitrogen for cell synthesis, growth and metabolism in all living organisms, is critical. For proper nutrition, carbon and nitrogen must be present in the substrate at the correct ratio. An appropriate carbon to nitrogen ratio for optimal earthworm digestion is necessary too. The conventional determination of C-to-N ratio is usually based on the absolute contents of both the C and N in the substrate and not necessarily on what proportion of each nutrient are available for these processes. Pilot studies may be needed to establish the optimal C-to-N ratio since it is not always possible to tell what proportion of each is available to the earthworms in a given feed-ration.

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Microbial decomposition is known to occur simultaneously with deliberate vermicomposting. The consortium of earthworms, the microflora living in their intestines, and those in the growth medium, enhance the decomposition process of the substrate. The intestinal mucous which consists of easily metabolizable compounds is considered to result in a priming-effect of earthworms to microbial decomposition (Albanell et al., 1988; Elvira et al., 1996; Vinceslas-Akpa and Loquet, 1997). It has been shown that some earthworms utilize microorganisms in their substrates as a food source and can digest them selectively (Edwards, 1988; Edwards and Bohlen, 1996). The efficiency of vermicomposting may, therefore, depend on number and types of microorganisms in the substrate (Hand et al., 1988). Microbial decomposition is further known to occur best when the C-to-N ratio of the substrate is approximately 25. To optimally integrate the microbial action and vermicomposting action, knowledge of C-to-N ratio requirements of both processes is vital.

The importance of C-to-N ratio with respect to population and distribution of earthworms in their natural ecology has been well reviewed by Lee (1985). Most vermicomposting studies have utilized a qualitative approach rather than a quantitative approach to addressing the issue of C-to-N ratio in the substrates. The practice has been to arbitrarily add a carbonaceous base if the material in question is too nitrogenous. Elvira et al. (1996) and Elvira et al. (1998) mixed volumetric ratios, and weight ratios, respectively, to qualitatively improve the C-to-N ratio in their studies on vermicomposting of sludges from paper mill and dairy industries, using *Eisenia andrei*. Other studies have investigated balancing the C-to-N ratio of rich nitrogenous material using rich-carbonaceous material. Albanell et al. (1988), used this approach (volume-to-volume ratio) to successfully vermicompost sheep manure using cotton industrial wastes to qualitatively balance and improve the C-to-N ratio of the feed substrate.

Although there is nothing fundamentally wrong in using qualitative techniques to adjust the substrate's C-to-N ratio, the results are very subjective and difficult to reproduce. It would be more useful to quantitatively adjust the C-to-N ratio using the properties of individual components (C-to-N ratio, bulk density, moisture contents) since this can then be used as a general guide in the formulation of a wide range of substrates for vermicomposting. Butt (1993), reported that vermicomposting of paper-mill sludge was improved by balancing the C-to-N ratio to 25, but noted that different earthworm species responded differently to the different sources of nitrogen. However, experiments were not conducted at other C-to-N ratios. Further, since the available C and N is not definite even upon balancing the C-to-N ratio, pilot studies are desirable to establish an optimal C-to-N ratio for a particular feed type and a particular earthworm species. This study investigated the C-to-N ratio requirement for the vermicomposting of biosolids, with paper mulch as the carbon base, using *Eisenia fetida*.

2. Methods

2.1. Procedure

Using the C-to-N ratio reported by Butt (1993) as a guide and the C-to-N ratios of the biosolids and paper-mulch, four different C-to-N ratios of 10, 15, 20 and 25 were investigated in this study. The C-to-N ratios of feed mixtures were prepared from the previously determined absolute values of the total organic carbon and total Kjeldahl nitrogen (TKN) of each substrate. Based on visual inspection, feed mixtures with a C-to-N ratio of greater than 25 were found to be too lean with respect to the biosolids while mixtures with a C-to-N ratio of below 10 were too biosolids rich. An optimal worm stocking density of 1.60 kg-worms/m^2 and an optimal feeding rate of 0.75 kg-feed/kg-worm/day were used in this experiment (Ndegwa et al., 1999).

Experiments were performed in worm-bins measuring 0.56 m $\times$ 0.38 m $\times$ 0.25 m (length $\times$ width $\times$ depth). This provided 0.21 m^2 of exposed top surface. Earthworms (*Eisenia fetida*, commonly known as red wigglers) were introduced into each of the respective similar worm-bins, to provide the optimal stocking density. In this case, the earthworms live-biomass loading was 0.34 kg in all four set-ups. Two replicates for each of the four C-to-N ratios were made. Each of the two replicates for every C-to-N ratio, was fed at 0.75 kg-feed/kg-worm/day. The arrangement of the units, the loading of earthworms into each unit and the feeding of each unit were done to ensure "complete randomization", as much as possible.

All the systems were fed in a single-batch with enough feedstock for the entire five weeks in which the experiment was conducted. The batch feeding was possible because the feed depth did not exceed 0.3 m and hence would not effect material heating from microbial decomposition. The experiments were conducted in an environment whose temperature ranged between 19 and 31°C. The substrate material was maintained moist at approximately 80% (w.b) by spraying/sprinkling the surface with water every two days using a spray can.

At the time of loading, the following parameters were determined for the feed: moisture content, pH, volatile solids (VS) and ash content. These analyses were either carried out immediately after the samples were obtained, or refrigerated at 4°C to minimize microbiological decomposition until analyzed.

Solid matter was determined as residue on drying at 80°C for 23 h (APHA, 1989). VS were obtained by ashing the dried sample at 550°C for 8.5 h (APHA, 1989). Determination of pH was made potentiometrically in a 1:10 suspension of the sample in de-ionized water, this is a modification of the procedure adopted from Erhart and Burian (1997). This suspension was placed on a mechanical shaker at 230 rpm for 30 m in prior to pH measurement. In the Erhart and Burian procedure, a 0.01M $CaCl_2$ solution was used instead of de-ionized water. Determinations of nutrients (N and P) were made in an independent laboratory using a Perkin-Elmer total C, N, S-analyzer. For these determinations, representative samples were dried at 80°C for 23 h (APHA, 1989) and then ground to provide a homogeneous sample. To obtain water extracts, 4 g of this homogenous sample was placed in 60 cm^3 of de-ionized water and the mixture placed on a mechanical shaker for 30 min. The mixture was then centrifuged at 4000 rpm for 10 min and the supernatant filtered through a number 40 Whatman filter paper to obtain the water extracts.

The experiments were terminated at the end of the fifth week after all the materials had been ingested by the earthworms. The worms were separated from the vermicompost and total biomass of the worms determined. Earthworm biomass growth was taken as the increase in total live earthworm biomass collected from the vermicomposted material and the bedding. Values were determined as live weight after hand sorting and removal of all extraneous material. The vermicompost was analyzed for the VS, ash content, moisture content, pH, and the nutrients (N and P) using the methods already described. Vermicompost in this work refers to a mix of the worm's castings and digested, as well as undigested feedstock.

2.2. Experiment design and analysis

Effects or responses to be investigated were product stability, worm's biomass generation, pH and nutrients; N, and P (both total and soluble components). The factor of interest was the C-to-N ratio at four levels/treatments. Each level was replicated twice. This design fits the classical one-way or single-factor experiment with multiple treatments. A one-way analysis of variance was therefore conducted on each of the responses studied. The analyses of variances within the levels investigated for all the responses studied, were conducted on a PC-based statistical software (SAS) using the GLM procedure, while Duncan's multiple range test was used for making the pair-wise comparisons. The level of significance in all cases was at $\alpha = 0.05$.

3. Results and discussion

The feedstock components' parameters (%P, %N, VS, pH) were individually determined (Table 1) and then combined on a weighted-basis to provide a single representative feedstock parameter for each of the four C-to-N ratios investigated. These weighted-parameters are presented in Table 2. Water extract parameters (%P, %N) from the individual feedstock components were also determined individually and later combined on a weighted-basis to parameterize the extract. The respective weighted-values are also presented in Table 2. The nutrient measurements were expressed on a dry matter basis. In all the worm bins, moisture was maintained at $78.9 \pm 0.9\%$ (w.b).

The percentage changes of all the parameters investigated in this experiment and the subsequent statistical analysis of variances amongst the four levels of C-to-N ratios investigated in this study are shown in Tables 3 and 4. The trends of these same parameters with respect to the levels of C-to-N ratios, are also presented in Figs. 1, 2 and 3.

The live earthworm's biomass values (see Table 3 and Fig. 1) indicated only a slight change within the duration of this experiment. An increase in live-earthworm's biomass was observed for C-to-N ratios of 10 and 15, while a decrease was observed in the other C-to-N ratios. Although there were no significant differences ($\alpha = 0.05$) in the percent change of live-earthworm's biomass among the four C-to-N ratios investigated, there was a definite trend displayed, whereby, the worm's biomass growth decreased as the C-to-N ratio of the feed increased.

Table 1
Feedstock components' parameters

Component	%N	%P	%VS	%C	pH
Biosolids	6.02	2.51	70.0	38.9	7.38
Paper-mulch	0.07	0.01	86.0	31.4	7.61
Biosolids water extract	1.69	0.21			
Paper-mulch water extract	0.04	0.00			

Table 2
Feedstock weighted parameters

Feed mix C-to-N ratio	%N	%P	%VS	pH
10	3.80	1.58	76.1	7.46
15	2.68	1.11	79.4	7.51
20	2.08	0.86	81.1	7.53
25	1.71	0.70	82.1	7.55
10 (Water extract)	1.07	0.13		
15 (Water extract)	0.76	0.10		
20 (Water extract)	0.59	0.07		
25 (Water extract)	0.49	0.06		

Table 3
Percentage changes in the nutrients and other properties after five weeks of vermicomposting[A, B]

Feed C-to-N ratio	% Changes[C]					
	Earthworm's biomass	VS	TS	N	P	pH
10	5.95[a]	−4.20[a]	−40.67[a]	−14.51[a]	13.53[a]	−28.79[a]
15	2.40[a]	−7.08[b]	−40.04[a]	−3.99[a]	22.76[a]	−31.17[a]
20	−1.35[a]	−7.61[b]	−36.93[a]	−4.21[a]	30.32[a]	−29.99[a]
25	−1.40[a]	−9.17[c]	−36.44[a]	8.44[b]	37.72[a]	−18.30[b]

[A] A plus (or no sign) sign denotes an increase while a minus sign denotes a reduction.
[B] [a,b,c]Quantities with the same letter in the same column were not significantly different at $\alpha = 0.05$.
[C] % Change $= \left[\frac{\text{Original}-\text{Final}}{\text{Original}}\right] \times 100$.

Table 4
Percentage change in soluble nutrients after five weeks of vermicomposting[A, B]

Feed C-to-N ratio	% Changes[C] N	P
10	−60.23[a]	0.34[a,b]
15	−67.10[b]	5.17[a,b]
20	−78.10[c]	15.08[a]
25	−78.54[c]	−9.12[b]

[A] A plus sign (or no sign) denotes an increase while a minus sign denotes a reduction.
[B] [a,b,c,d]Quantities with the same letter in the same column were not significantly different at $\alpha = 0.05$.
[C] % Change $= \left[\frac{\text{Original}-\text{Final}}{\text{Original}}\right] \times 100$.

Percentage changes in VS and TS after five weeks of vermicomposting are shown in Table 3 and Fig. 1. The percent VS reduction for the four C-to-N ratios were significantly different ($\alpha = 0.05$), with the highest reduction occurring in the C-to-N ratio of 25 while the lowest occurred in the C-to-N ratio of 10. In all C-to-N ratios, substantial reductions ranging between 36% and 41%, were observed in total solids (TS), however, no significant difference ($\alpha = 0.05$) was observed among the four levels. The VS decreased over the duration of these experiments. The reductions in VS increased for an increase in the C-to-N ratio, while TS reductions displayed the reverse trend. The VS reduction is normally used as an indicator of the degree of stability of the product, the same cannot be said of the TS reduction. Thus, the former parameter is a better indicator of the actual stabilization/treatment of the material in question. The highest degree of stability was achieved when the C-to-N ratio was 25.

The percent change in P and N are shown Table 3 and Fig. 2. Vermicomposting concentrated P in the solid product in all treatments. While some losses were observed in the total amounts of P, this loss was not proportional to the loss in solids. At higher C-to-N ratios an increase in concentration in P was observed. However, statistically, no significant difference ($\alpha = 0.05$) was observed among the four treatments. In general, vermicomposting decreased N concentration in the solids, except at the C-to-N ratio of 25. A significant difference ($\alpha = 0.05$) in the N content was observed

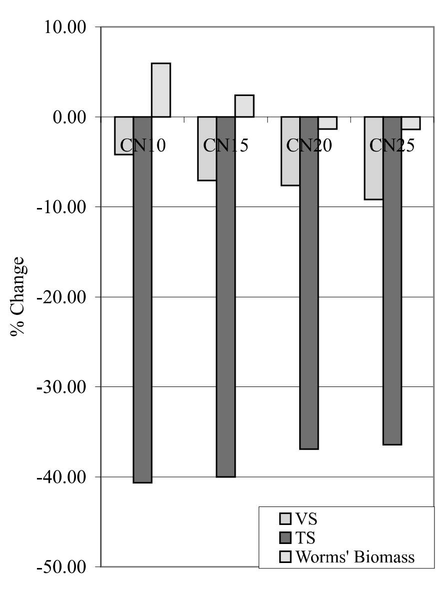

Fig. 1. Effect of C-to-N ratio on earthworm biomass, VS, and TS during the five weeks of vermicoposting.

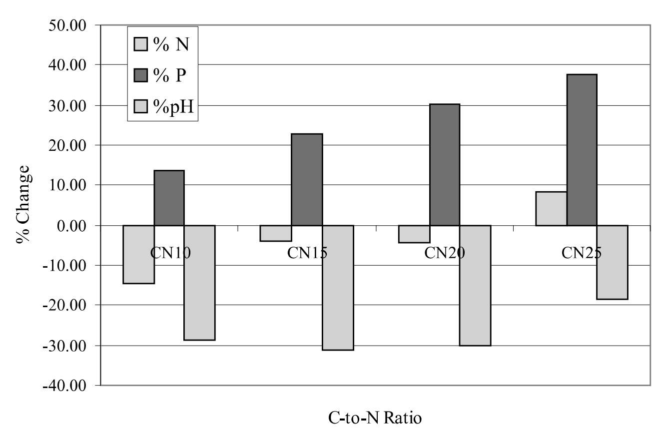

Fig. 2. Effect of C-to-N ratio on vermicompost nutrients and pH after five weeks of vermicomposting.

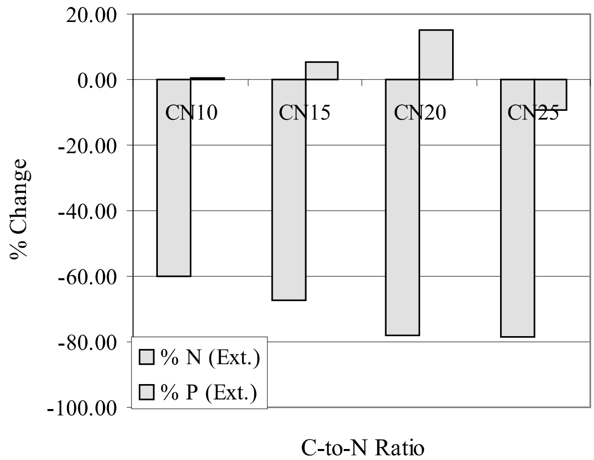

Fig. 3. Changes in soluble nutrients after five weeks of vermicomposting.

between the treatment with a C-to-N ratio of 25, and the other three treatments.

The pH of the products were lower than the pH of the feedstock in all cases (see Fig. 2 and Table 3). The final pHs of the products were 5.32, 5.17, 5.27, and 6.17, for substrate with initial C-to-N ratios of 10, 15, 20, and 25, respectively. There was generally a lowering of original pH by as much as 18–32% during the five weeks of vermicomposting. A significant difference ($\alpha = 0.05$) in pH was determined among the respective products. Statistically, the pH of the product with an initial C-to-N ratio of 25, was significantly different from the other three treatments. It is believed that the reduction in pH can be explained by the mineralization of nitrogenous compounds into (a) nitrates, (b) other NO_x compounds, and (c) other intermediate organic acidic compounds. By the end of five weeks, the most favorable pH (pH = 7.0) for the growth of worms was in the feedstock whose initial C-to-N ratio was 25.

The results of the water extracts obtained from both the feed material and the respective products (vermicompost) are shown in Table 4 and Fig. 3. A decrease in soluble P was observed only for the treatment whose initial C-to-N ratio was 25, while an increase in soluble P was observed for all other treatments. The soluble N was substantially reduced in all four treatments. This reduction increased from 60% to 79% for an increase in the initial C-to-N ratio. A reduction in either soluble N and/or soluble P reduces the potential for environmental pollution. The vermicompost whose initial C-to-N ratio was 25 was the only treatment which showed a significant reduction in both the soluble N and soluble P.

Vermicomposting fails to destroy the pathogenic organisms in the product since it is a low-temperature process. In some cases, depending upon the feed-substrate, some form of quick composting may be needed for the vermicompost to meet the EPA's PFRP (Process to Further Reduce Pathogens) guidelines for pathogen destruction for Class-A compost. These guidelines require that during composting the materials reach temperatures of (a) 40°C for at least five consecutive days and 55°C for at least three consecutive days, or alternatively, (b) temperatures of 55°C maintained for at least three consecutive days in the coolest part of compost undergoing aeration. Optimal microbial composting requires a C-to-N ratio of 20–30. Based on these experiments, feedstock with an initial C-to-N ratio of 25 would be optimal, and therefore, no C-to-N adjustment will be necessary for the composting process. Moreover, the combined action of microbes and earthworms at the mesophilic level will be enhanced since the C-to-N ratio of 25 is favorable for growth of both organisms.

4. Conclusions

The highest reduction in VS was achieved when the initial C-to-N ratio of the substrate mix was 25, and this, therefore, marked the highest degree of stability of the material achieved in this study. More nitrogen was left in the product of the substrate mix whose initial C-to-N ratio was 25. It was in this same treatment where the greatest reduction in the amount of soluble N occurred as well as the only treatment where a reduction in soluble P was observed. The near neutral pH of the products from this treatment (C-to-N ratio of 25) was also the most favorable for most purposes. The preceding parameters-combination, therefore, resulted in a product whose fertilizer value was not only higher but its potential impact on the environment was also relatively lower than that of the products from the other three treatments.

Based on the requirements of EPA's PFRP, and the need to enhance the combined decomposition of biosolids (with paper mulch as the carbon base) from the consortium of earthworms and microflora, the C-to-N ratio of 25 appears to be most effective. This latter note and the results summarized in the preceding paragraph strongly support a C-to-N ratio of 25 in the vermicomposting of biosolids amended with paper mulch.

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