The Effect of Vermicomposting Process on Organic Matter of Cattle Manure Compost and Municipal Solid Waste Compost

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Background & Aims of the Study: Considering the importance of solid waste disposal and the possibility of conversion them into rich organic fertilizers, the objectives of this study were to evaluation of effect of vermicomposting process (VP) on organic matter of cattle manure compost (CMC) and municipal solid waste compost (MSWC) by record changes of the elemental contents of C, N, H, S, and O, organic matter content and their parameter ratios that the substrates. Also, an empirical equation was developed for each substrate and vermicompost products as a function of their elemental composition and describe the stoichiometry of aerobic bioprocessing.

Material and Methods: This study is interventional. The thermal elemental analyzer was used for measurement of elemental contents and organic matter was measured by weight loss between 105°C and 550°C in a muffle furnace.

Results: Based on the results of this study, significant reduction in contents of C (69.1%, 45.6%), N (45.6%, 18.2%), H (90%, 77.5%), and S (100%, 100%) and also, significant increase in content of O (26.9%, 26.2%) was observed during VP in CMC and MSWC vermibeds, respectively. During vermicomsting of CMC and MSWC with P value of less than 0.05 between treatments, the H/C ratio was decreased from 0.13 to 0.04 and 0.07 to 0.03, the C/O ratio was increased from 0.5 to 2.22 and 0.5 to 1.25, the C/N ratio was decreased from 21.56 to 12.24 and 13.55 to 9, the C/OM ratio was decreased from 58.3 to 29.9 and 60.3 to 41.9 respectively. Also, approximate empirical formula was $C_{83}N_3O_{31}H_{133}S$, $C_{177}N_{11}O_{67}H_{143}S$, $C_{14}NO_{24}H_7$, and $C_{11}NO_{10}H_4$ for CMC, MSWC, CMC vermicomposted (CMCV), and MSWC vermicomposted (MSWCV), respectively. Developing of stoichiometry equation for each material indicated that oxygen requires for complete aerobic biodegradation of organic fraction in vermicompost products was significantly lower than substrates.

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Background

Today, due to population growth and lifestyle improvements, organic waste such as MSW and CM production has increased dramatically. On the other hand, application of chemical fertilizers in agricultural land has caused many problems such as groundwater contamination, soil degradation and loss of its fertility, air pollution, and etc (1). Therefore, the global approach has progressed to more use of organic fertilizers.

Archives of Hygiene Sciences

Volume 8, Number 2, Spring 2019

Now a day, MSW and CM after being placed under the composting process, used as organic fertilizers in agricultural lands. On the other hand, the quality of organic fertilizers or exogenous organic matters (EOMs) as an amendment is very important. Because they affect the properties of the soil and plant productivity and enter the life cycle through them (2). In the other hand, more stabilized fertilizers during long-term sequestration of organic matter (OM), provides sufficient minerals to the plants (3), and improve soil carbon content (4).

A new interest approach for improving the quality of EOMs is composting through that called vermicomposting earthworms process (VP) (3). This technology has been developed in recent years and used for stabilization of variety of organic initial materials in previous studies (5-9). Through VP, under the aerobic and mesophilic condition (6), earthworms in cooperation with the microbial communities (6) biodegraded organic wastes into a much finer, humified, and microbially active product which is rich in nutrients (10).

Compost and vermicompost products have many organic compounds that determine each of them is impossible. Therefore one of the collective analyses that used to comprise a greater or minor part of the organic matter is elemental analysis (11). The elemental analysis (commonly includes the content of C, N, H, S, and O) are useful for determination of the organic fraction of fertilizers and evaluation of their quality (12), evaluation of its biodegradability and maturity by calculation of carbon to nitrogen ratio (C/N) (11,13), determination of stoichiometry amount of oxygen required for biodegradation of organic component (11,12) and finding the correlation between total organic carbon (TOC) and the organic matter (or volatile solids) content with the aim of easier analytical measurement of TOC for each fertilizer (12).

• The Effect of Vermicomposting Process on Organic...

Bizukojc and Ledakowicz, applied elemental analysis to describe the composition of the organic fraction of MSW in order to determine an elemental formula and stoichiometry of its aerobic biodegradation (11). Komilis et al. measured the elemental content and the organic matter content of 18 organic components that are commonly found in the MSW and calculated an approximate empirical formula for the organic fraction of commingled MSW (12). Alidadi et al. determined the lignin degradation and biodegradation of organic matter under compost and vermicomposting of sewage sludge (13). Ngo et al. examined functional stoichiometry of soil microbial communities after amendment with stabilized organic matter (4). Razmjoo et al. analyzed the elemental content of compost derived from MSW to develop an approximate empirical formula for the organic fraction of the mature MSWC (14). Ngo et al. analyzed and compared the elemental composition of the three exogenous organic matter included buffalo manure, its compost, and vermicompost in presence of bamboo biochar for effect on their chemical reactivity (3). Amir et al. did some chemical analyzes, such as determining the elemental composition to demonstrate the changes in the structure of the humic acids during the CP with activated sludge (15).

Aims of the study:

To the best of the available knowledge, the aims of this investigation are to determine the effect of vermicomposting process on organic matter of cattle manure compost and municipal solid waste compost. These aims was achieved by measurement of elemental composition (CHNS-O) of the organic fraction of MSWC, CMC and their products after VP for several purposes, includes the comparison the quality, determining the levels of stabilization and maturity, approximate empirical formula, and stoichiometry describing the of aerobic biodegradation for initial materials and final fertilizers (vermicompost products).

Materials & Methods

Substrates and earthworms collection

The CMC and MSWC used in this study obtained from dairy industrial and composting plant of Isfahan, Iran, respectively. Earthworm species of Eisenia fetida (E.fetida) were collected from an academic private building that produces vermicompost.

Experimental set-up and vermicomposting techniques

This experimental study was investigated on a full scale in Isfahan, Iran. The VP was performed on two substrates includes CMC and MSWC. On the other words, two treatments were defined for this experimental, which included VP with CMC (treatment 1) and VP with MSWC (treatment 2).

Vermicomposting techniques

For the first stage, 200 kg of each substrate was sifted with 10 mm iron mesh. Then they were poured on two concrete vermibins with dimensions of 1.5 m width \times 1.5 m length \times 0.3 m depth, which was made outdoors. Then the moisture beds were maintained about 80-90% throughout sprinkling of adequate quantity of urban water. For the second stage, baskets containing E.fetida earthworms mixed with CM vermibeds were placed on the substrates so that the earthworms were brought under the baskets without mixing the basket contents. In order to get the maximum biodegradation during VP, an optimal of 1.60 kg worms/m² density was entered in vermibeds (8). The third stage relates to 6 months of VP period and its control conditions. Moreover, in order to provide suitable aeration to the earthworms and to control the temperature, the materials turning was done over at every 15th day manually. During VP, leachate was returned on beds after every time of water sprinkling to prevent its loss.

The fourth stage relates to the separating of the earthworms after the VP. For this purpose, some CM was placed in the corners of the vermibeds as the fresh food, which caused the isolation and migration of the earthworms from the vermicompost products. Then, an iron mesh of 10 mm was used to separate the residual earthworms and cocoons.

Analysis of parameters during VP

The control parameters during VP were moisture, pH, and temperature. The moisture contents were measured by gravimetric method (8). The pH was measured in 1:10 w/v waste: water extract by digital pH meter (CG, Model 824, (SCHOTT UK Ltd., UK)) (16). Moisture and pH were controlled three times a week in both the vermibins. The temperature was daily measured by a thermocouple (model Thermo, Korch novin, Iran, $\pm 0.1^{\circ}$ C) at three different locations in the vermibeds.

Sampling

In order to the elemental analysis, samples were taken from CMC, MSWC, CMCV, and MSWCV. For sampling, 500 gr of samples were taken from five points of each vermibeds and mixed, thereafter, 8 mg of the mixture was picked for analysis.

Experimental analysis

To determination of CHNS-O elements in the samples, an automated thermal elemental analyzer (ECS 4010 CHNS-O Elemental Analyzer, Costech Analytical Technologies, Inc., Italy) was used. Furnace temperature of the elemental analyzer was: 1000°C, that at this temperature, both organic and inorganic compounds are converted into elemental gases, which are separated by chromatography and detected by a thermal conductivity detector (TCD). To prepare the samples, 2-10 mg of dried and milled samples were encapsulated by aluminum foil and placed into the auto-sampler the elemental stages of analyzer. The instrument was calibrated by different standards before introducing the samples. The oxygen content in the initial materials and

Archives of Hygiene Sciences

Volume 8, Number 2, Spring 2019

vermicompost products was calculated approximatively from the Equation (1):

Oxygen %=100 - (C% + H% + N% + S% + Ash %) (1) Ash and also organic matter (OM) fractions of the initial materials and vermicompost products was determined by measurement of weight loss between 105°C, and 550°C, that combustion in 550°C done in a muffle furnace (Isuzu, Sehisakusho, Japan) for 2 h. Thus, the ash and OM contents were calculated from the Equations (2) and (3) (16):

%ASH =

 $\frac{\text{The net weight of ash at 550 °C}}{\text{The net weight of samples dried at 105 °C}} \times$

100

% OM = 100 - % ASH (3)

(2)

The increase (I-value) and reduction (R-value) percentages of measured parameters were calculated according to Equations (4) and (5): $(\%)I - value = \frac{(P2-P1)}{P2} \times 100$ (4)

$$(\%)$$
I – value = $\frac{(P2-P1)}{P2} \times 100$ (5)

Where P2 was measured parameters of vermicompost products and P1 was measured parameters of initial materials during VP.

Stoichiometry of aerobic biodegradation

The stoichiometry of organic matter in aerobic biodegradation can be described by following Equation (6):

Organic matter $+O_2$ + nutrients \rightarrow resistant organic matter

$$+CO_{2} + H_{2}O + NH_{3} + SO_{4}^{-2} + PO_{4}^{-3} + ...$$

If the solid waste organic matter is represented as CaHbOcNd, account and the composition of the resistant organic matter is represented as CwHxOyNz, biosynthesis of new cells, and production of sulphate and phosphate is not significant, then the amount of oxygen required (on a molar basis) for the aerobic stabilization of the organic fraction of initial material can be estimated by the Equation (7) (18):

$$C_{a}H_{b}O_{c}N_{d} + 0.5(n y + 2s + r - c)O_{2} \rightarrow C_{w}H_{x}O_{v}N_{z} + sCO_{2} + rH_{2}O + (d - nz)NH_{3}$$
(7)

Where r = 0.5 [b-nz-3 (d-nz)], s=a-nw, n= moles of organic matter in the output/moles of organic matter in the input. The terms CaHbOcNd and CwHxOyNz represent the empirical elemental composition of the organic material at the beginning and at the end of the process, respectively.

If the complete conversion is performed, the corresponding equation is applied according to the Equation (8):

$$C_{a}H_{b}O_{c}N_{d} + \frac{(4a+b-2c+3d)}{4}O_{2} \rightarrow$$

$$aCO_{2} + (b-3d)/2H_{2}O + dNH_{3}$$
(8)

Results

In this study, as a result of measurement of elemental contents and organic matter, during VP in both of vermibeds (CMC and MSWC), the H/C, C/N and C/OM ratios decreased and O/C ratio increased. Table 1 shows the H/C and O/C ratios, as well as C/N mass ratios of the initial materials and vermicompost products.

Also, following measurement of elemental contents, the approximate empirical formula and stoichiometry equation estimated for each substrate and vermicompost products. Table 2 shows the results of them.

| Table 1) The average elemental composition (%) and parameters ratio of the initial materials and vermicompost | | | | | |
|---|--|--|--|--|--|
| nroduets | | | | | |

| products | | | | | | | | | | |
|----------|------|------|-------|------|------|------|------|-------|-------|------|
| sample | С | H | 0 | Ν | S | H/C | O/C | C/N | OM | C/OM |
| СМС | 38.8 | 5.2 | 19.45 | 1.8 | 1.25 | 0.13 | 0.5 | 21.56 | 66.5 | 58.3 |
| MSWC | 29.8 | 2 | 14.95 | 2.2 | 0.45 | 0.07 | 0.5 | 13.55 | 49.4 | 60.3 |
| CMCV | 12 | 0.52 | 26.6 | 0.98 | 0 | 0.04 | 2.22 | 12.24 | 40.1 | 29.9 |
| MSWCV | 16.2 | 0.45 | 20.25 | 1.8 | 0 | 0.03 | 1.25 | 9 | 38.70 | 41.9 |



Volume 8, Number 2, Spring 2019

| sample | Empirical formula | stoichiometric equation |
|--------|-------------------------------|--|
| CMC | $C_{83}N_3O_{31}H_{133}S$ | $C_{83}N_3O_{31}H_{133}S+100.5O_2$ $D_2H_2O+83CO_2+3NH_3+SO_4$ |
| MSWC | $C_{177}N_{11}O_{67}H_{143}S$ | $C_{177}N_{11}O_{67}H_{143}S + 173O_2$ $SH_2O + 177CO_2 + 11NH_3 + SO_4$ |
| CMCV | $C_{14}NO_{24}H_7$ | $C_{14}NO_{24}H_7 + 3O_2 \rightarrow 2H_2O + 14CO_2 + NH_3$ |
| MSWCV | $C_{11}NO_{10}H_4$ | $C_{11}NO_{10}H_4 + 6.25O_2 \longrightarrow 0.5H_2O + 11CO_2 + NH_3$ |

Table 2) Empirical formulas and stoichiometric equations of the initial materials and vermicompost products

Discussion

Analysis of parameters during VP

The amount of the pH increased significantly from 8 to 8.9 during treatment 1 and from 7.5 to 8.2 during treatment 2 (p<0.05). The pattern of pH changes during VP in both vermibeds was similar. Thus, pH for the first time decreased and then increased that Goswami et al. reported a similar trend (5). The reduction in pH is probably due to the creation of anaerobic conditions during the period of the adaptation of the earthworms at the early stages of VP. The increases in pH are probably due to release of alkaline compounds that are soluble in water. such as ammonia (19). A change temperature during VP in the vermibeds was varied from 28-35 C and no significant difference was found between the two substrates. Also moisture content of %50-60 (suitable for earthworms (5)) was kept throughout the process.

Elemental analysis (CHNS-O)

As shown in Table 1, significant reduction in C content was observed in both the treatments (p<0.05). It might be due to mineralization and stabilization of OM through the earthworms activity and loss of carbon by microbial respiration in the form of CO₂ (19). The decrease of carbon content is the best index for total efficiency of the biodegradation process. These results are in line with other studies (7, 9, 19,20). In addition, during VP the R-value ^oC of content in treatment 1 (69.1%) was more than treatment 2 (45.6%) (p<0.05). On the other

words, CMC had the highest C content (38.8%) and its vermicompost product (CMCV) had the lowest C content (12%) among all in vessels. It was showed the faster mineralization in the CMC than MSWC (21).

As shown in Table 1, N content decreased significantly during VP in both treatments (p<0.05). It could be related to the degradation of organic nitrogen to humic acids and mineral form of nitrogen $(N-NH_4^+ \text{ and } N-NO_3^-)$ (22). Hanc et al have confirmed the increase in the mineral form of nitrogen $(N-NH_4^+)$ and $N-NO_3^-$) at the end of the VP (23). So that Dominguez and Edwards reported that during VP earthworms might mineralize organic nitrogen in the form of mucus, excreta, enzymes, and nitrogenous excrements (24). In addition, due to the pH rise during VP, volatile ammonia may be lost from vermibeds (25). Also, R-value of N content in treatment 1 (45.6%) was more than treatment 2 (18.2%) (p < 0.05). This may be explained by two reasons; 1- due to the higher pH of treatment 1, ammonia might be more volatile and 2- due to the higher organic C of treatment 1, probably nitrogen mineralization have more occurred in this vermibed.

As shown in Table 1, the oxygen content was increased during VP in both treatments (p<0.05). It evaluated by the O/C atomic ratio that was increased during VP. On the other words, C content was dominant element in initial materials (CMC and MSWC), but O content was dominant element in vermicompost products (CMCV and MSWCV). It is attributed to a relative increase of bonds containing oxygen like carboxylic and/or phenolic

Archives of Hygiene Sciences

Volume 8, Number 2, Spring 2019

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hydroxyl groups (26,27). Plaza et al. confirmed our results that shown increasing in O and O/C during humification by VP of CM alone and mixed with two-phase olive pomace (28). Also, the difference between the I-value of O content between two treatments was not significant which estimates were on average 26.4% (p>0.05). However, the I-value of the O/C atomic ratio during VP in treatment 1 (77.3%) was slightly more than treatment 2 (59.8%) (p<0.05). Fukushima et al. showed the positive correlation between increasing O/C atomic ratio and humification index (27). Thus, the results might be indicative more humification of treatment 1 than treatment 2.

As shown in Table 1, the H content gradually decreased for two treatments (p<0.05) during VP. It evaluated by the H/C atomic ratio that was decreased during VP, which these results are in line with some others (3, 28). It could be due to a decrease of aliphatic or an increase of aromatic structures (3,28) and loss of carbohydrates during VP (3). Also, the difference between the R-value of the H content between treatments was significant not (p>0.05) and on average, it was 83.5%(p>0.05). However, R-value of the H/C atomic ratio during VP in treatment 1 (67.6%) was slightly more than treatment 2 (58.6%) (p<0.05). Fukushima et al. showed the negative correlation between decreasing H/C atomic ratio and humification index (27). Thus, according to the results, it was possible to humification process of treatment 1 was more than treatment 2.

As shown in Table 1, the S content was the smallest element in all samples. It decreased in both treatments (p<0.05) during VP and the vermicompost products (CMCV and MSWCV) were free of organic-S. It probably due to the conversion of organic-S to mineral-S or release of sulfur in the form of gas (like H₂S) from substrates.

This investigation suggests that in subsequent studies to further explore the process, elemental composition changes in the earthworms' body should also be examined.

Changes in C/N ratios

C/N atomic ratio was computed by finding C and N, which were analyzed by the elemental analyzer. A decrease in C/N ratio during bioprocessing widely used to evaluate products stabilization and maturity (29). As shown in atomic ratio significantly table 1, C/N decreased during the VP in both treatments that agreed with previous reports (3,6,28). In fact, the VP caused convert solid waste into more for agronomic valuable products the application.

According to results, the main reason for the reduction in the C/N ratio was the higher rate of carbon loss compared with nitrogen loss. In addition, the changes in C/N ratio during VP depicting the R-value of 43.2% for treatment 1 that was more than treatment 2 (33.5%)(p<0.05). which it might be due to better conditions of CMC than MSWC substrates for earthworms activity.

According to previous studies, a good stabilization degree and a level of acceptable maturity was accrued in C/N ratio of < 20, while a ratio of ≤ 15 is prescribed limit for the agricultural application (29). Consequently, based on the results of this study, a high stabilization degree of organic matter and good maturity was obtained in the MSWC as an initial material and both vermicompost products. That it makes them acceptable for use as fertilizers in agriculture.

Changes in C/OM ratios

C/OM ratio was calculated by analysis organic C by the elemental analyzer and OM which were measured in a muffle furnace. The correlation between carbon and organic matter contents (or volatile solids) are still among researchers is ambiguous. Finding this ratio for different materials is useful because analysis of organic carbon by the elemental analyzer is sophisticated and expensive. Therefore, if there is a constant ratio, researchers could easily estimate the organic carbon content. Some studies have obtained different ratio for various materials. In Komilis and Tziouvaras study, they found that the ratio of six composts was to range from 0.46 to 0.69. Also, they obtained this ratio for 12 organic composts ranged from 0.48 to 0.99 (30). Also, in another research of Komilis et al. (2012), this ratio for 26 MSW components ranged from 0.40 to 0.94 (12).

As shown in Table 1, the C/OM ratio decreased during VP in materials and the R-value for this ratio between the two treatments was significantly different (p<0.05), so that the Rvalue for treatment 1 (48.7%) was more than treatment 2 (30.6%).

Empirical formulas and stoichiometry of aerobic biodegradation

As shown in Table 2, the empirical formulas of the initial materials and vermicompost products were estimated according to the average elemental composition of the organic matter.

Stoichiometry is a theoretical framework that describes the biodegradation process and indicates the quantitative relationships between the organic elements and the amount of oxygen required for the biological decomposition of the initial components in chemical reactions during bioprocessing (4,11). Stoichiometric equations for the complete oxidation of the biodegraded organic matter or the amounts of oxygen requirements are based on the initial elemental composition of the waste (11). The stoichiometric equations of initial materials and vermicompost products are presented in Table 2 that its theory used is shown in Equation (8) (materials section).

The oxygen requires varied in the range from 3 to 173 moles per 1 mole of material. It was lower in vermicompost products (CMCV and MSWCV) than initial materials (CMC and MSWC) (p<0.05). It indicates that the initial compounds have high OM biodegradable. During VP, microorganisms and earthworms use organic carbon as an electron donor and use oxygen as electron acceptor, followed by

consumption and reduction of organic carbon, the amount of oxygen required is also reduced (15).

In additionally, the highest amount of organic require was observed at the MSWCV (173 moles).

In parallel with this study, Razmjoo (14) et al. calculated empirical formulas for the organic fraction of the municipal solid waste compost that it was C 204 H 325 O 85 N 77 S.

Conclusion

In this study, the changes of elemental content (C, N, H, S, O) as an organic fraction of materials, organic matter content and their parameter ratios was investigated during the VP with E.fetida earthworms by two substrates. Based on the results, during VP, the content of C, N, H, and S were decreased and O content was increased. The H/C ratio was decreased that it probably due to decreasing of aliphatic or an increasing of aromatic structures. The C/O ratio was increased that it might be due to a relative increase of bonds containing oxygen like carboxylic and/or phenolic hydroxyl groups. The C/N ratio was decreased that it was due to stabilization and maturity during VP. Also, C/OM ratio significantly decreased during VP and its amount was different in all materials. In addition, according to the calculated chemical formulas (C₈₃N₃O₃₁H1₃₃S, C₁₇₇N₁₁O₆₇H1₄₃S, C₁₄NO₂₄H₇, and C₁₁NO₁₀H₄ for CMC, MSWC, CMCV, and MSWCV respectively), the stoichiometry equations developed for each material. The equations were indicated that oxygen requires for complete aerobic biodegradation of organic fraction in vermicompost products was significantly lower than initial organic wastes. That it represents mineralization during VP. As the results, it found that VP is a step towards achieving the objectives of sustainable agriculture, which produces valuable and highquality fertilizer.

Archives of Hygiene Sciences

• The Effect of Vermicomposting Process on Organic...

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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Archives of Hygiene Sciences

Volume 8, Number 2, Spring 2019

• The Effect of Vermicomposting Process on Organic...

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