RESEARCH ARTICLE

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Biodegradation of Lignocelluloses in Sewage Sludge Composting and Vermicomposting

Hosein Alidadi[a*], Ali Asghar Najafpoor[a], Ali Vafaee[a], Abdoalrahim Parvaresh[b], Roya Peiravi[c]

[a] Health Sciences Research Center, Faculty of Health, Mashhad University of Medical Sciences, Mashhad, Iran.
[b] Department of Environmental Health, Faculty of Public Health, Isfahan University of Medical Sciences, Iran.
[c] Department of Environmental Health, Mashhad University of Medical Sciences, Mashhad, Iran.
[*]**Correspondence** should be addressed to Dr. Hosein Alidadi; **Email:** alidadih@mums.ac.ir

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Aims of the Study: The aim of this study was to determine the amount of lignin degradation and biodegradation of organic matter and change of biomass under compost and vermicomposting of sewage sludge.

Materials & Methods: Sawdust was added to sewage sludge at 1:3 weight bases to Carbon to Nitrogen ratio of 25:1 for composting or vermicomposting. Lignin and volatile solids were determined at different periods, of 0, 10, 30, 40 and 60 days of composting or vermicomposting period to determine the biodegradation of lignocellulose to lignin. Results were expressed as mean of two replicates and the comparisons among means were made using the least significant difference test calculated ($p < 0.05$).

Results: After 60 days of experiment period, the initial lignin increased from 3.46% to 4.48% for compost and 3.46% to 5.27% for vermicompost. Biodegradation of lignocellulose was very slow in compost and vermicompost processes. Vermicomposting is a much faster process than compost to convert lignocellulose to lignin ($p < 0.05$).

Conclusions: The organic matter losses in sewage sludge composting and vermicomposting are due to the degradation of the lignin fractions. By increasing compost age, the amount of volatile solids will decrease.

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Background

The ratios of available carbon to nitrogen (C/N) are the most important nutritional factors for composting and vermicomposting process. The ideal C/N ratio is from 20 to 25. Addition of nitrogenous waste could lower an unfavorably high C/N; whereas the addition of carbonaceous waste can raise an unfavorably low C/N.

The examples of nitrogenous waste are sewage sludge, grass clipping, food waste and for the carbonaceous waste are sawdust, dry leaves, paper, and wood chips. The sawdust and woody materials are commonly used as bulking agents in sewage sludge composting or vermicomposting to raise C/N ratio (1-3).

Sewage sludge composting and vermicomposting are biological processes that convert organic matters to humic substances.

The microbial activities were essential for the decomposition and bio-oxidation of organic materials (2, 4). The bulking agents such as sawdust, straw, and most of the plant litter are the major part of biomass in nature, called lignocelluloses compounds (3, 5).

Lignocellulose is mainly consists of cellulose, hemicellulose, and lignin that take resistant to biodegradation. Lignin makes up a small percentage of sawdust. Among all naturally occurring organic compounds, lignin is probably the most recalcitrant because lignin is a complex polymer of phenylpropane units, which are cross-linked to each other with a variety of different chemical bonds.

Several properties of lignin account for its resistance to microbial attack: it is a water-insoluble, aromatic, three-dimensional polymer with a very high molecular weight (6). These recalcitrant materials have to be broken down and recycled by microorganisms to maintain the carbon cycle. White-rot fungi are the organisms able to mineralize lignin efficiently to carbon dioxide and water in compost (7).

Actinomycetes can also decompose lignin, but typically degrade less than 20 percent of the total lignin present (1, 8). Lignin degradation is primarily an aerobic process, and in an anaerobic environment, lignin can persist for very long periods (9, 10).

Because lignin is the most recalcitrant component of the plant cell wall, the higher proportion of lignin the lower the bioavailability of the substrate. The lignin fraction must first be removed from sawdust, leaving the cellulosic fraction. Thus sawdust exhibit relatively slow decomposition rate due to the high content of recalcitrant decomposable compounds such as cellulose and lignin.

The warm temperature, high moisture content, high oxygen availability, and high palatability of the sawdust to microorganisms all favor decomposition of lignin during aerobic composting (11). In addition, conditions that favor the growth of fungi, including adequate nitrogen, moisture, and temperature, all appear to be important in lignin decomposition.

The effect of temperature on lignin degradation has been studied in a few composting experiments. Horwath and Elliott (12) composted ryegrass for 45 days at a mesophilic temperature of 25 °C. Ansu *et al.* (13) studied the lignocellulose degradation by oyster mushroom using different substrates like paddy straw, non retted coir pith, and retted coir. The simultaneous activity of micro flora present in the gut of earthworms and the substrate might have intensified cellulolytic and lignolytic activity (10). The earthworms digest long chains of polysaccharides, enhancing microbial colonization. Simultaneously the structure of lignin changes, probably due to microbial oxidation and demethylation. The microbial cleavage of the aromatic rings of lignin leads to new polysaccharide and humans in the organic matter (6).

Some studies have indicated that addition of bulky agents (*e.g.*, sawdust) is important for maintaining an aerobic condition and influence of organic decomposition. The mixtures with high lignocellulose contents, such as sawdust can decrease nitrogen loss in the compost and vermicomposting processes.

Aims of the Study: The objective of this study was to determine the amount of lignin degradation and biodegradation of organic matter and change of biomass under composting and vermicomposting of sewage sludge.

Materials & Methods

Pilot Setup: This study was conducted from January to March, 2008. The sludge used in this study was obtained from the drying beds of the Wastewater Treatment Plant (located in Isfahan, Iran).

The methods of investigation were windrow composting and vermicomposting. The ratio of sludge were 3 kg of 80% moisture content mixed by 1 kg of sawdust of 5% moisture content to provide a suitable C/N ratio of 25/1. Windrow composting sizes were approximately 1.2×1.5×2.5 m^3 (height×width×length). Windrows turning were done manually every 7 to 10 days.

The vermicompost experiments were performed in plastic worm bin of 1.1×0.9×0.5 m^3 (length×width×depth). This plastic bin provides 1 m^2 of exposed top surface.

The earthworms for this study were collected from Mazandaran Province (located in the North of Iran). Eight hundreds pieces of adult *Eisenia foetida* between 0.4 to 0.5 g were placed in the vermicompost plastic bin to provide the optimal sticking density of 0.75 kg feed/kg worm per day. The biomass loading was 1.6 kg.

According to the study of Edwards and Dominguez (14), the optimum amount of substrate (72 kg) was added to the plastic bin vermicompost. The ambient and plastic bins temperatures were measured using a thermometer (Geo-Technic, Iran). The moisture content of the mixture was maintained at 60 to 70 percent throughout the vermicomposting period and the plastic bin was kept in darkness at 20 to 30 °C. The composite samples were taken from about three different points in each bin. The number of worms was manually checked and recorded on a daily basis.

Lignin analysis: For all experiments, the acetyl bromide reagent was 25% (v/v) acetyl bromide in glacial acetic acid. All samples were dried overnight at 50 °C before weighing into glass culture tubes fitted with Teflon-lined screw caps.

Weighed samples were placed in a vacuum desiccator over P_2O_5 with the caps off for at least 18 h before analyzing. The standard procedure involved removing the samples from the desiccator, adding 2.5 mL of fresh prepared acetyl bromide reagent, capping immediately, and heating at 70 °C. Then 100 µL of perchloric acid added to samples.

Heating time durations were 30 minutes. After heating, the samples were quantitatively transferred with the aid of acetic acid to 50 mL volumetric flasks that contained 10 mL of 2 M NaOH and 12 mL of acetic acid. Hydroxylamine (350 µL of 0.5 M) was added to each flask and samples were diluted to 50 mL with acetic acid.

In each sample absorption spectra were determined using an absorbance spectrophotometer (Shimadzu Corp., Japan). The absorption maximum was at 280 nm. All experiments were run in duplicate for each method (15).

Drying at 105 °C for 24 h and determining the weight loss determined the dry solid content of the samples. Volatile solids were determined by subtracting the residue of a known volume of sample after incineration at about 550 °C from the total dry solid content of the same sample.

Data analysis: All the reported results were expressed as mean of two replicates and all data were analyzed using of SPSS statistical analysis software. The comparisons among means were made using the least significant difference test calculated at p-values <0.05.

Results

The daily temperature of ambient and plastic bins varied from 8 to 15 °C and 10 to 17 °C, respectively. The moisture content in plastic bins of vermicompost varied between 70 to 75 percent and the pH ranged from 5.8 to 7.6.

A temperature range of 0 to 35 °C, a moisture range of 60 to 90 percent and pH range 5 to 9 were utilized as suitable conditions for the growth of *Eisenia fetida* (14). Thus, favorable growth conditions for the growth of earthworms, *E. fetida* were provided in this study.

The experiments of sewage sludge composting and vermicomposting processes lasted for two months. On days 0, 10, 30, 40 and 60 samples collected. The results of lignin contents in the compost and vermicompost after 60 days are summarized in Table 1. The results of volatile solids in the compost and vermicompost after 60 days are summarized in Table 2.

Table 1) Percentage of lignin content in the compost and vermicompost during the period of study

Time (day)	Compost (%)	Variation (%)	Vermicompost (%)	Variation (%)
0	3.46	0	3.46	0
10	3.58	3.35	3.62	4.42
30	3.91	8.44	5.07	28.60
40	4.23	7.56	5.16	1.74
60	4.48	5.58	5.27	2.47
Total of Variation (%)	-	24.93	-	37.23

Table 2) Percentage of volatile solids content in the compost and vermicompost during the period of study

Time (day)	Compost (%)	% of variation	Vermicompost (%)	% of variation
0	87.2	0	87.2	0
10	85.9	1.49	82.1	5.85
30	75.3	12.34	67.5	17.78
40	72.1	4.25	62.9	6.81
60	65.3	9.43	60	4.61
Total of Variation (%)	-	27.51	-	35.05

Discussion

According to Table 1, the lignin contents in the sewage sludge compost and vermicompost increased with increasing time. The lignin degradation led to production of cellulose, hemicellulose, and glucose. In the thermophilic phase of sewage sludge compost was more intense in the degradation of lignin as from 10 to 30 days, which has consistency with the observations by Hammouda and Viel (16).

At the beginning of the sewage sludge composting (days of 0 to 10), mesophilic bacteria were predominant. Once the temperature has reached more than 40 °C (days of 10 to 30), thermophilic bacteria and fungi appear in the compost piles. When the temperature reaches more than 60 °C (days of 30 to 40), the microbial activity decreases, and as the compost pile cools, mesophilic bacteria appear again. This result was supported earlier (13). In addition, during time 10 to 30 days, high increase percent observed in the vermicompost as 28.6%. After 60 days, the initial lignin (3.46%) increased to 4.48% for compost and 3.46% to 5.27% for vermicompost.

Conclusions: This study showed that biodegradation of lignocellulose was very slow in the compost and vermicompost process. This research also revealed that vermicompost was a much faster biodegradation process than the compost process in converting lignocellulose to lignin ($p < 0.05$).

The organic matter losses in compost and vermicompost are due to the degradation of the lignin fractions. Hence, in the intervals times the high losses percent volatile solids observed which due to degradation of the lignin.

The general trend of the results obtained indicates reduction in the amount of volatile solids with compost age, which indicates the increase in the biodegradation of lignin.

Footnotes

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Conflict of Interest:

The authors declare no conflict of interest.

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