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MICROBIAL COMMUNITY DYNAMICS OF COMPOST MIXTURES AFTER APPLICATION OF MICROBIOLOGICAL ADDITIVE

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Abstract

The present study aimed to analyze the dynamics of the microbial community of compost mixtures after application of a microbiological additive. Three series of compost mixtures are available with two variants each: one control and one with a microbiological additive. Quadruple sampling was performed. Main groups of aerobic microorganisms were analyzed: non-spore-forming and spore-forming bacteria, actinomycetes and molds. The total microflora of aerobic and anaerobic microorganisms was determined, and a predominance of aerobes was reported. Temperature, pH and humidity were analyzed. All variants showed an initial slight increase in microbial abundance on day 8 and a subsequent smooth steady decline as the composting process progressed. Non-sporing bacteria predominated in all mixtures. Spore-forming bacteria are least involved. Only one variant with the supplement had higher biogenicity than the control. There is no evidence that the applied supplement has a major positive effect on the microbial biota. Microbial community structures were not affected by the introduction of the microbiological additive. We consider the influence of input material and the optimal C: N ratio as the main determinant of microbial abundance.

Key words*: compost mixtures, microorganisms, microbiological additive.*

INTRODUCTION

Composting is a biochemical conversion of organic matter through the activity of microorganisms, a process well-documented across various studies (Epstein, 1997; Fontenelle et al., 2001; Cogger, 2008).

The primary agents in composting include bacteria, fungi, and actinomycetes (Sole-Mauri, 2007). Bacteria playing a main role due to their broad temperature tolerance and capacity to decompose diverse organic substrates (Maccready et al., 2013; Lopez-Gonzales et al., 2015b). The phases of the composting process, as well as its activity, are directly related to the parameters of the environment - temperature, humidity and pH (Bongochgetsaku & Ishida, 2008; Partanen et al., 2010; Franke-Whittle et al., 2014; Moreno et al., 2013).

In the composting process, four main phases can be divided, mainly on the basis of the temperature changes occurring - mesophilic (initial phase), thermophilic - decomposition phase and temperature increase up to and above 60°C, cooling phase and maturation phase - one of the most complex phases in which the synthesis of humic acids takes place (Papale et al., 2021). Microorganisms are involved in all phases. The present study examines the dynamics of microbial communities during an artificially extended mesophilic phase by maintaining suboptimal humidity. During this phase, mesophilic microrganisms, which thrive at temperatures between 25 and 40°C, predominate and initiate the composting process (Christian et al., 2009). Actinomycetes also play a vital role during this stage, producing exoenzymes that facilitate the breakdown of lignin and cellulose (Kausar et al., 2010). Microscopic fungi as well as actinomycetes are an important part of the microbial communities during the mesophilic phase of composting. The specific hyphae of fungi allow them to attack and degrade organic components that are inaccessible to bacteria. Further microscopic fungi break down lignin and cellulose more easily than bacteria and actinomycetes (Ryckeboer et al., 2003a, 2003b). Composting as a process is of serious interest to both agricultural producers and the scientific community. The relevance of composting extends beyond waste reduction, offering a dual solution to the challenges of organic waste accumulation and the necessity for soil enrichment (Waqas et al., 2023; Papale et al., 2021). However, due to the possibility of certain

problems during composting process, such as an insufficiently stable end product, the nitrogen losses, etc., solutions related to the addition of certain additives to the compost are increasingly sought (Chen at al., 2023). The addition of specific additives during the composting process can improve the characteristics of the final product both in terms of its qualities and from a sanitary point of view (Barthod et al., 2018).
The main goal of the present study is to follow the microbial community dynamics of compost mixtures after application of microbial additive.

MATERIALS AND METHODS

Within this study, three compost mixtures were prepared, each one in two varianst: one supplemented with a combination of microbiological and mineral additives, and a control. For the purpose of the study, compost mixtures were prepared with different mix of the initial waste, as well as the C:N ratio.
Variant 1 is composed of fresh weed plants: dry wheat stalks: straw: old compost starter in a proportion of 9:1:2:1, respectively. The carbon to nitrogen ratio is 30:1. Variant 2 is made up of fresh grass: onion: potato in a proportion of 20:12:5. The C:N ratio is 30:1. Variant 3 is made of fresh grass: onion: potato in a proportion of 25:30:10. The C:N ratio is 35:1.
An average sample was taken from each of the composts one day after addition, on day 8, day 19 and day 39. During the study, the main parameters related to the composting process were investigated – temperature (°C), pH and humidity (%). These parameters were investigated directly in the field inside the composters, during the sampling, by specialized electronic measuring device. Compost moisture content was intentionally kept below 30% to to assess the impact of the additives under unfavorable environmental conditions. Microbiological analyzes included determining the biogenicity (total microbial number) of each sample by counting the amounts of non-spore-forming bacteria, spore-forming bacteria, actinomycetes, microscopic fungi and total anaerobic microorganisms. Microbiological analyzes involve sterile sampling and processing within 24 hours. Microbiological analyzes were performed after preliminary homogenization of the samples, subsequent dilution and inoculation of appropriate nutrient media. To isolate the spore-forming bacteria, pre-pasteurization was performed before their inoculation. For each of the samples, the percentage participation of individual microbiological groups compared to the total microbial number is shown. The data are presented as a share in percentages for each microbiological group relative to the total microflora. The statistical analysis of microbiological results was conducted by calculating the mean and standard deviation from three replicates for each sample. The StatSoft Statistica 12 software, were used under a significance threshold of 95% to ensure the quality of the results.

RESULTS AND DISCUSSIONS

In this study, a total of 24 compost samples were collected across different intervals: one day after application of additives, and after that on days 8, 19, and 39. The microbiological results, and some additional in formention, are detailed in Table 1. The microbiological parameters quantified in colony-forming units (CFU) x 10^6 per gram of compost.
The results for the physico-chemical parameters of the investigated compost mixtures show humidity levels below the optimum. Optimum humidity at a C:N ratio of 30:1 is considered to be between 55-60% moisture (Liang et al., 2003). The presence of optimal humidity is associated with better transport of dissolved nutrients necessary to support the activity of microorganisms (Kumar et al., 2010). For the purpose of the present study, the humidity was deliberately kept below optimum values, in order to observe whether the application of additives would compensate for the lowered moisture levels. Only the supplemented sample from variant 1 showed increased levels of biogenicity compared to the control. There is no direct relationship between the acidity of the compost mixture and the total microbial number in the samples studied. The pH of all compost options, similar to other studies, are optimal for the development of the microorganisms responsible for the composting process. Sufficient aeration is provided, which further ensures the minimization of lactic acid and acetic acid (Eklind et al., 1997; Beck-Friis et al., 2003).

Table 1. Microbiological and physicochemical parameters of analyzed compost variants

	Variant	T ^{0}C	pH	Humidity, %	Total microbial number (TMN) CFUx10^6/g compost	Non-spore-forming bacteria CFUx10^6/g compost	Spore-forming bacteria CFUx10^6/g compost	*Lactobacillus* sp. CFUx10^6/g compost	Actinomycetes CFUx10^6/g compost	Micromycetes CFUx10^6/g compost	Total anaerobes CFUx10^6/g compost
One day	1+Aditive	30	7.02	25.15	89.29 ± 11.55	40.00 ± 14.33	1.92 ± 0.73	0.80 ± 0.09	42.88 ± 17.85	3.68 ± 1.62	0.0112 ± 0.01
	K1	30	6.6	26	78.41 ± 11.85	48.00 ± 22.11	1.44 ± 0.49	0.32 ± 0.09	24.00 ± 9.70	4.64 ± 0.82	0.0051 ± 0.03
	2+aditive	43	6.5	23.18	81.64 ± 11.55	67.20 ± 14.33	0.96 ± 0.73	0.64 ± 0.09	11.20 ± 17.85	1.60 ± 1.62	0.0416 ± 0.01
	K2	52	6.26	20	98.62 ± 11.85	85.12 ± 22.11	0.80 ± 0.49	0.48 ± 0.09	7.04 ± 9.70	5.12 ± 0.82	0.0601 ± 0.03
	3+Aditive	40	6.7	22.35	66.59 ± 11.55	45.76 ± 14.33	2.40 ± 0.73	0.80 ± 0.09	12.80 ± 17.85	4.80 ± 1.62	0.0316 ± 0.01
	K3	42	6.39	22.87	77.80 ± 11.85	45.76 ± 22.11	1.76 ± 0.49	0.32 ± 0.09	23.68 ± 9.70	6.24 ± 0.82	0.0380 ± 0.03
8 days	1+Aditive	35	6.12	25.06	91.26 ± 11.41	40.82 ± 15.22	2.1 ± 0.64	0.81 ± 0.08	43.74 ± 17.91	3.77 ± 1.45	0.0116 ± 0.01
	K1	30	6.7	26.12	81.02 ± 12.06	49.20 ± 22.58	1.80 ± 0.52	0.33 ± 0.09	24.93 ± 9.96	4.76 ± 0.80	0.0056 ± 0.03
	2+aditive	26	6.09	27	85.37 ± 11.41	69.72 ± 15.22	1.33 ± 0.64	0.66 ± 0.08	11.62 ± 17.91	1.99 ± 1.45	0.0424 ± 0.01
	K2	22	6.8	26	101.25 ± 12.06	87.25 ± 22.58	0.98 ± 0.52	0.49 ± 0.09	7.22 ± 9.96	5.25 ± 0.80	0.0613 ± 0.03
	3+Aditive	25	6.45	25.55	69.21 ± 11.41	46.98 ± 15.22	2.59 ± 0.64	0.81 ± 0.08	13.93 ± 17.91	4.86 ± 1.45	0.0324 ± 0.01
	K3	24	6.24	24.96	79.74 ± 12.06	47.14 ± 22.58	1.94 ± 0.52	0.32 ± 0.09	23.98 ± 9.96	6.32 ± 0.80	0.0388 ± 0.03
19 days	1+Aditive	35	6.67	25	88.33 ± 11.57	39.20 ± 14.09	1.60 ± 0.65	0.64 ± 0.09	43.20 ± 17.61	3.68 ± 1.62	0.0118 ± 0.01
	K1	32	6.74	24.88	76.81 ± 12.40	46.40 ± 22.32	1.28 ± 0.40	0.32 ± 0.09	24.48 ± 9.38	4.32 ± 0.76	0.0057 ± 0.03
	2+aditive	30	6.46	25.92	80.84 ± 11.57	65.92 ± 14.09	0.80 ± 0.65	0.48 ± 0.09	12.00 ± 17.61	1.60 ± 1.62	0.0425 ± 0.01
	K2	30	7.64	25.78	98.14 ± 12.40	84.32 ± 22.32	0.80 ± 0.40	0.32 ± 0.09	8.00 ± 9.38	4.64 ± 0.76	0.0608 ± 0.03
	3+Aditive	30	7.58	25.71	65.63 ± 11.57	44.80 ± 14.09	2.08 ± 0.65	0.48 ± 0.09	13.44 ± 17.61	4.80 ± 1.62	0.0324 ± 0.01
	K3	30	6.82	26.18	76.52 ± 12.40	44.96 ± 22.32	1.60 ± 0.40	0.16 ± 0.09	24.00 ± 9.38	5.76 ± 0.76	0.0390 ± 0.03
39 days	1+Aditive	25	6.96	29	86.91 ± 11.08	37.92 ± 13.92	1.42 ± 0.48	0.47 ± 0.09	43.13 ± 17.17	3.95 ± 1.60	0.0118 ± 0.01
	K1	25	7.02	29.38	70.70 ± 14.42	40.04 ± 23.24	1.11 ± 0.32	0.32 ± 0.09	24.49 ± 8.62	4.74 ± 0.81	0.0060 ± 0.03
	2+aditive	25	6.58	28.76	80.31 ± 11.08	64.46 ± 13.92	0.79 ± 0.48	0.47 ± 0.09	12.64 ± 17.17	1.90 ± 1.60	0.0428 ± 0.01
	K2	25	6.74	28.54	98.02 ± 14.42	82.16 ± 23.24	0.79 ± 0.32	0.32 ± 0.09	9.48 ± 8.62	5.21 ± 0.81	0.0608 ± 0.03
	3+Aditive	25	6.98	29.65	65.29 ± 11.08	43.92 ± 13.92	1.74 ± 0.48	0.32 ± 0.09	14.22 ± 17.17	5.06 ± 1.60	0.0325 ± 0.01
	K3	25	6.34	29.83	76.35 ± 14.42	44.08 ± 23.24	1.42 ± 0.32	0.16 ± 0.09	24.33 ± 8.62	6.32 ± 0.81	0.0393 ± 0.03

The results obtained show the presence of a minimal number of anaerobic microorganisms. Their amount compared to aerobic microorganisms is thousands of times lower. These data show that during the ongoing composting process we have not created anaerobic zones, accordingly there are no decay processes (Mira et al., 2003).

The initial compost mixture, and not the presence of additives, is more important for the presence of even minimal amounts of anaerobic microorganisms. For all samplings (day 1, 8, 18 and day 39) the amounts of anaerobes in mixtures with approximately similar ingredients - Samples 2 and 3 (where there is a difference only in the ratio of ingredients) show approximately similar levels of anaerobes. In the variants, on sample 1 for all samplings the levels of anaerobes compared to the other two compost mixtures were significantly lower, reaching up to 10 times lower amounts.

The starting compost mixture determines the C:N ratio, which is key to the progress of the composting process. A number of studies indicate that the main condition for a quality composting process is the correct C:N ratio (Zhu, 2007; Chang & Hsu., 2008).

Only Varinat 1 showed an increase in microbial abundance after the application of the additive. (Figure 1).

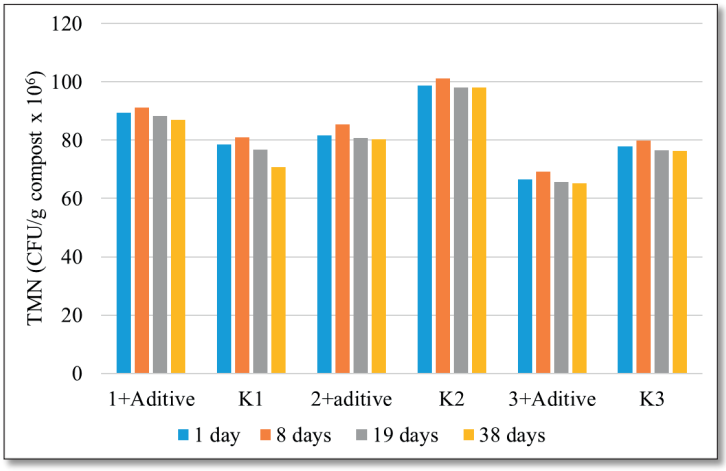

Figure 1. Total microbial number

In the remaining two variants, the controls have higher biogenicity than the samples with additives. It is clear from Figure 1 that even with the same wastes used to prepare the compost mix, their C:N ratio is of key importance. Samples 2 and 3 have the same starting waste, but in sample 2 they are in a ratio of 30:1, and in sample 3 they are in a ratio of 35:1. The sample with an optimal ratio of 30:1 (Variant 2) showed higher biogenicity.

When analyzing the data, the weak dynamics in the microbial abundance with advancing time is noticed. The amounts of microorganisms reported in individual samples are almost static. We considered this is due to the low humidity levels. In the conducted experiment, the imported additives did not lead to a reduction in the time for the onset of the thermophilic phase. We believe that in the particular experiment, the limited levels of humidity stop the initiation of the thermophilic phase, due to the reduced microbiological activity. Other scientific developments reach similar conclusions (Liang at al., 2003; Li et al., 2022).

All variants showed an initial slight increase in microbial abundance on day 8 and a subsequent smooth steady decline as the composting process progressed. In the present experiment, non-spore-forming bacteria are the dominant microbial group across all compost mixtures, a finding that aligns with observations from similar research conducted by Ryckeboer et al. (2003a; 2003b), as illustrated in Figure 2.

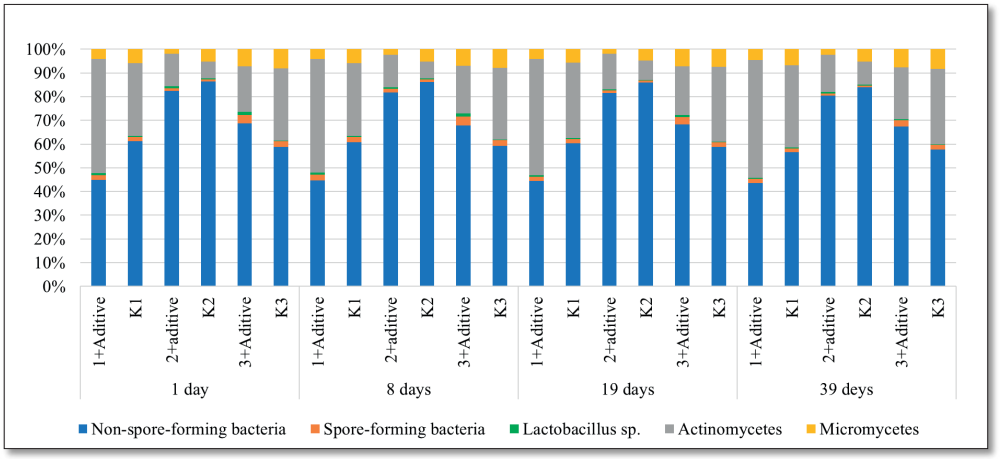

Figure 2. Percentage participation of individual microbial groups

In the present study, variant 1 with the addition of specific additives exhibited a notable increase in actinomycete populations from the onset. This observation contrasts with the findings of Beffa et al. (1996), where spore-forming bacteria were identified as the predominant group. The discrepancy between the dominance of actinomycetes in our study and the prevalence of spore-forming bacteria reported by (Beffa et al., 1996) underscores the complexity of microbial ecosystems in composting processes.

Composting proceeds through several stages, each characterized by the activity of different groups of microbes (Bertoldi et al., 1983). According to a study by Bertoldi et al. (1983) simple carbon compounds (soluble sugars, organic acids, etc.) are easily metabolized and mineralized by mesophilic and thermophilic aerobic heterotrophic and heterogeneous microflora, and natural long-chain polymers are later attacked mainly by fungi and actinomycetes. In the composting process and in the finished compost, the main share in the composition of the general microflora is occupied by non-spore-forming bacteria, followed by spore-forming bacteria, and actinomycetes and microscopic fungi are less represented (Malcheva et al., 2018). It was found that in the composting phases, with increasing temperature, the amount of actinomycetes also increases (Malcheva et al., 2018). In other research the addition of a microbial product and lavender extract to compost mixtures increased the amount of bacteria, actinomycetes and microscopic fungi, and the extract also led to the decontamination of composts by *Escherichia coli* (Malcheva et al., 2024).

This highlights the influence of environmental conditions and composting methodologies on microbial community structure. These variations show the need for further comprehensive investigations into compost microflora to better understand the interactions and contributions of different microbial groups to the composting process. In our experiment Spore-forming bacteria are least involved, with the exception of lactobacilli. The low amount of spore-forming bacteria is expected, given that these bacteria are mainly involved in the biooxidative phase of the thermophilic stage of composting (López-González, 2015b). In all variants with additives analyzed, the amounts of non-spore-forming bacteria were lower compared to the controls.

Considering that the non-spore-forming bacteria are the main microorganisms carrying out the transformation of substances in the mesophilic phase, we believe that the applied additives have a negative effect on the composting process.

In all variants, in the samples with imported additives, the amount of lactobacilli was increased compared to the controls. These data show that the introduction of the selected supplements improves the number of lactobacilli. It has been found that lactobacilli can act as promoters in the composting process, increasing the rate of the process and improving

the structure of the final product (Li et al., 2019). In addition, the lactobacilli absorb the ammonia released during the composting process, thus minimizing the unpleasant odors of the compost mass (Varma et al., 2018).

However, in this experiment, no reduction in composting time was observed as a result of an increase in lactobacilli in the samples with applied additive.

Using additives within the compost mix inhibits the development of microscopic fungi. In all studied variants, their number is smaller compared to the controls, and in Variant 2 their amount is about three times lower throughout all samplings. Fungi are one of the most important participants in the composting process. Under the influence of the enzymes released by them, such as cellulases and phosphatases, organic molecules inaccessible to other microorganisms are broken down (López-González et al., 2015a). Their reduced levels would depress the composting process, especially in the mesophilic phase and in the cooling and maturation phases.

Based on the data analyzed thus far, it appears that that the introduced additive, although it stimulates the development of lactobacilli, rather has a negative effect on the microbial community. The only group that increased in number after additives supplying was the spore-forming bacteria. However, these microorganisms are dominant during the thermophilic phase.

In this study we proved that the introduction of additives does not reduce the activation time for the onset of the thermophilic phase, accordingly we consider that the increased levels of spore-forming bacteria do not have a significant positive impact of the variants with additives.

The generated results of the share participation in % of the considered groups of microorganisms show a specific shift of the dominant community in Variant 1 with additive. In this variant, the actinomycete group is predominant on the first day. In the remaining Variants (2 and 3), regardless of the applied additive, the group of non-spore-forming bacteria dominates. In variant 1, with additive, participation (%) of actinomycetes increases with time.

We considered that in this compost mix, the imported additive has a positive impact on the composting process relative to the total amount of microorganisms. There was an increase in the total microbial number, as well as an increase in the participation (%) of the actinomycete group. Given the specificity of the starting substrate (fresh weed plants: stalks of dry wheat: straw: and starter old compost), the introduced additive enhances the quantities of actinomycetes. This leads to an increase in biogenicity compared to the control. Actinomycetes are responsible for the transformation of more complex organic components, which, with the starting mixture thus available, suggests a stable composting process (Kausar et al., 2010). For the rest of the studied variants, we have no clearly established trends for changing the percentage participation of the microbial groups as a result of the applied additives.

CONCLUSIONS

This study was conducted to assess the effects of introducing a combination of microbial and mineral additives on the dynamics of microbial communities within mesophilic compost stage. Three compost mixes were studied in two variants - with additives and control. The present study found that introduced of specific additives did not necessarily lead to an increase in total microbial abundance. Variants 2 and 3, which have the same starting materials in the mixture but with a different C:N ratio, show reduced levels of total microbial biota compared to their controls. Only Variant 1 showed an increase in microbial abundance relative to its control. When analyzing the amounts of individual groups, it was found that in all samples with additive, the amounts of non-spore-forming bacteria and fungi were lower compared to the controls. After applying additive, the amounts of lactobacilli and, moreover, the spore-forming bacteria typical of the thermophilic phase increase. According to actinomycetes group, there is no clearly established dynamics. When analyzing the percentage share of the individual groups, an increase in the participation of actinomycetes at the expense of non-spore-forming bacteria was found in Sample 1 with additive. For the rest of the studied variants, we have no clearly established trends for changing the share participation of the microbial groups as a result of the applied additive. The highest total

microbial number of the controls was Variant 2, with a mix of diverse biowastes, with low lignin levels and a C:N ratio of 30:1.

The outcomes of this research underscore the need for further in-depth investigations to identify strategies that can positively improve the microbial community in the composting process. Based on our findings, we advise against the application of microbiological additives and mineral fertilizers during the early mesophilic phase of composting. Our observations indicate that introducing these substances at this stage can slow down the composting process.

The primary goal of this study was to generate a basic database that could serve as a basis for future in-depth studies. The findings from this study provide a basic platform for conducting further detailed research on the effects of various additives on compost mixtures.

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