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DYNAMICS OF MICROBIOLOGICAL INDICATORS FOR COMPARATIVE STUDY OF COMPOST VARIANTS

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Abstract

The microbiological dynamics of different starting vegetable and animal waste was investigated. There is a 5 variants scheme of compost bunches (4 spring and 1 autumn) with different starters - two spring composts containing a combination of grape vine canes, fruit twigs and grass swath, in addition to one rabbit manure; starter: last year`s compost, two spring composts of mulberry twigs with leaves, and when the compost are turned, a different amount of clean litter and twigs is introduced; starter: soil and one autumn compost containing pepper and tomato stems and leaves, roots of tomatoes and pepper with soil, green leaves of leeks, corn and leek waste. A 6-fold microbiological analysis was started on the 7th day of the compost materials and repeated in 7-10 days during the compost reversal period. Main groups of heterotrophic microflora - ammonifying bacteria (non-spore and bacilli), actinomycetes and micromycetes are defined. The study was carried out by the method of dilution and culture of solid nutrient media with determination of cfy (colony forming units) in 1 g abs. dry substrate. The data from the microbiological analysis show differences in the course of the individual stages of composting by microbiological indicators. These differences are expressed in terms of both the total amount of microorganisms in the substrates and the dominant physiological and systematic groups of microorganisms in the microbiocenosis. The different microbiological composition of the compost materials determines a different rate of decay of the separate raw materials, which is reflected in the duration of the composting process itself.

Key words*: compost, grape vine canes, horticulture wastes, microbiological dynamics, rabbit manure.*

INTRODUCTION

Microorganisms play an important role in the composting process. They use organic matter as a source of nutrients (Rynk et al., 1992; Borken et al., 2002), as a result of the development and the activity in the formation of compost removed heat, CO2, water vapor and forming a humus (Epstein, 1997). Composting involves different types of microorganisms - bacteria, fungi and actinomycetes. They have different characteristics and functions that are vital to the process of composting (Lee, 2016). According Tiquai (2005) in addition to studies of microbial biomass, respiration rate and content of ATP, the enzyme activity is also one of the most effective methods that can be used to monitor the stability and maturity of compost.

In a typical composting process both bacteria and fungi are present (Gray et al., 1971). Earlier studies have shown that the main bacterial groups at the start of the composting process are mesophilic bacteria producing organic acids such as *Lactobacillus* spp. and *Acetobacter* spp., and later, in the thermophilic stage dominated Gram-positive bacteria such as *Bacillus* spp. and *Actinobacteria* (De Bertolli et al., 1980). According to Abu-Bakar (2015), exactly bacteria provide the fastest and most efficient composting. In his study, Partanen et al. (2010) found that bacteria from *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Deinococcus-Thermus* and from over 2000 different philotypes participated in the composting process. Fungi develop both in mesophilic and thermophilic phase of the composting, in an amount between 0.01 and 1 million per gram compost (Kowalik, 2015), as are present on the surface of the compost, when the temperature is higher. Some types of actinomycetes develop during the thermophilic phase, while others develop in

subsequent stages of maturation and ripening, by degrading resistant compounds in the final stages of humification (Lee, 2016; Adani et al., 1997; Andrews and Hirano, 1991).
Successful composting depends on a number of factors that have a direct and indirect impact on the activity of microorganisms. Tiquia et al. (2000); Fracchia et al. (2006) indicate as important factors the type of composite raw material, its nutrient composition and its physical characteristics such as bulk density, pH, moisture content, etc. According to Garcia et al. (1993) and Serra-Wittling et al. (1996), composting results in homogeneity in the composition of the product, irrespective of the starting material and the composting process itself.
Biddlestone and Gray (1985) report that the complexity of the degraded plant materials and the quality of the final product may depend on the type of biomass. In composting, the C: N ratio is considered the most important parameter as it reflects the degree of biotransformations that have occurred in compost from a chemical point of view (Saber et al., 2011). A ratio of C: N of less than 12 during the solid phase is an indicator of maturity of the compost (Bernal et al., 1998; Iglesias-Jimenez, 1993).
The purpose of the present study is to trace the dynamics of different compost variants on the quantity and quality composition of microorganisms developing in composts.

MATERIALS AND METHODS

There has been developed a 4 variants scheme of compost piles (V1, V2, V3, V4) with different row materials:
V1-Brown materials: grape vine canes and fruit twigs; Green materials: grass swath; Starter: last year compost residues.
V2-Brown materials: grape vine canes and fruit twigs; Green materials: grass swath and rabbit manure; Starter: last year compost residues.
V3-Mulberry twigs with leaves; Starter - soil. On the 7th and 13th day of building the compost pile, when it was turning, were added bedding for silkworm growing and twigs.
V4-Mulberry twigs with leaves (from a contaminated area with heavy metals); Starter - soil. On the 7th and 13th day of building the compost pile, when it was turning, were added bedding for silkworm growing and twigs from the same polluted area.
Microbiological studies include determination of non-sprouting bacteria, bacilli and micromycetes by method of selective plating and direct viable counts. They were used two solid nutrient media (meat-peptone agar for determination of non-sprouting bacteria and bacilli, and medium of Chapek-Dox for determination of micromycetes) and counting of colony forming units, recalculated to 1 g of absolute dry substrate.
The statistical processing of microbiological data includes calculation of an average of three replicates and a coefficient of variation.

RESULTS AND DISCUSSIONS

Dynamics of compost temperature and pH

For the determination of the composting phases, the temperature measurement started with the building of the compost piles and continued until their last turning (Figure 1).

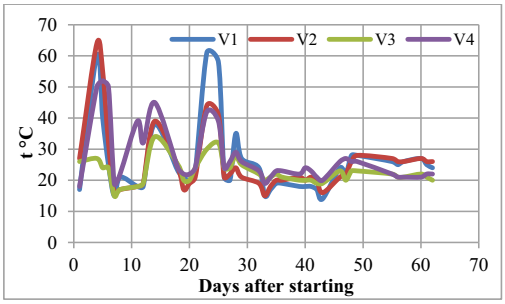

Figure 1. Dynamics of temperature in compost piles

The thermophilic phase of compost pile V1 lasts for about 25 days, and after 27 days the temperature steadily drops below 30°C. The thermophilic phase of compost pile V2 lasts for about 21 days, and after 24 days it drops permanently below 30°C. For compost pile V4 the thermophilic lasts for about 13 days, and after about 26 days, the temperature is continuously dropping below 30°C.
The compost pile V3 did not switch to a thermophilic phase. The highest measured temperature in this pile is 34°C, reached around the 13th day and, similar to V4 compost, after about 26 days, the temperature steadily drops below 30°C.

By making a comparison of the temperature at different compost piles, with the amount of material used for their building, it can be seen that from the four composting piles, with the highest temperatures and longest lasting thermophilic phase is compost pile V1, which is with the most greater mass of starting material (59,500 kg). In descending order, for this indicator are arranged compost pile V2 (56,050 kg) and compost pile V4 (32,200 kg). Compost pile V3, which has the smaller mass of the starting material (24,330 kg), did not reach the thermophilic phase.

Throughout the composting process, the pH of the compost piles was monitored and it was. between 7.8 and 8.0. During the thermophilic phase and the maturation phase, normally the medium is alkaline. Probably because of the rapid passage from mesophilic to thermophilic phases, no acidification of the medium from the microbial extraction of organic acids in the mesophilic phase was established. According to some authors, low initial pH limits microbial activity and slows temperature rise (Sundberg et al., 2004; Romanschuk et al., 2005).

Microbiological analyses

The microbiological analyses were started one week after the building of compost piles V1, V2 and V4, and on the 2nd day after the building of compost pile V3. The analyzes were repeated at each compost turn (between 7-10 days).

The results of the dynamics of the total microflora (total number of microbes in 1 g of substrate) give an idea of the degree of development, resp. settlement of the compost with microbes. This indicator is important for assessing the degree of destruction of organic wastes as far as the microorganisms carry out the mineralization of the organic compounds in them. Data from the general microflora dynamics are presented in Figure 2.

The data show a different dynamics of the total microflora in the four composting variants. It is also possible to note the different start for the presence of microorganisms in the individual variants provided by the various formulations. On the 7th day of experimentation, the highest amount of total microflora was found at V1. This amount is about 1 times higher than the same in the other variants. For this variant (V1), plant wastes have the highest microbial presence, whereas variants with a different amount of mulberry leaf and twigs (V3 and V4) have a lower microbial diversity and presence. Reducing the amount of started plant waste materials and adding the rabbit fertilizer to V2 slows the growth of microorganisms at the start of the experiment (Day 7), but in the next days of reporting, their activation is determined, most preferably on the 13th day.

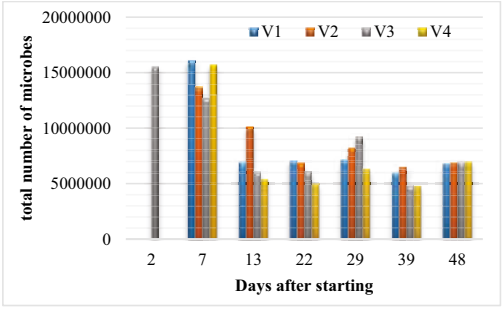

Figure 2. Dynamics of total microflora (number of microbes/g substrate)

In V2, the amount of the total microflora decreases more gradually, whereas in the other variants, the multiplication of microorganisms decreases sharply to the 13th day - 2 times at V1 and V3, and 3 times at V4, after which the breeding process gradually decreases to the end of the composting, with a slight increase in microbial growth on the 29th day after the trial. In all four composting variants it was found that on the 39th and 48th day the amount of microbes was the lowest. At the end of composting, it decreased about 2 times for variants V1, V2 and V3, and 3 times for V4 compared to the amount at the beginning of the experiment. By the seventh day of the experiment, mesophils and thermophiles are grown at V1, V2 and V4, whereas at V3 were found only mesophils, as this compost does not pass through the thermophilic phase. In the period from 22 to 29 days, temperatures rise above 60°C at V1 and above 40°C at V2 and V4, i.e. these variants pass through the thermophilic phase again. The most active is the development of microorganisms up to the 7th day of experiment. Activation of thermophiles and increase of total microflora from Day 22 to Day 29 of experimental was set-up - 1 time at V1, 1, 2 times at V2, 1, 3

times at V4. The most active is the development of mesophils in V3 during this period - the total amount of microorganisms is increased by 1, 5 times. After 29 days to 48 days, compost temperatures are below 30°C, i.e., mesophilic microorganisms develop, the composts are aged and ripened.

In the different phases of composting, different quantitative development of the studied microbial groups - non-sprouting bacteria, bacilli and micromycetes is also reported. The results for the composition of the microflora for 7 days (passage through the mesophilic and thermophilic phase) after the experimental assay in all variants and on the 2nd day (mesophilic phase) at V3 are presented in Table 1.

Table 1. Composition of the microflora 7 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V3 (2-ри ден)	13760 ± 0.262 (88.3)	1720 ± 0.291 (11.0)	100 ± 0.400 (0.6)
V1	13120 ± 0.202 (81.4)	2940 ± 0.340 (18.2)	60 ± 0.333 (0.4)
V2	11200 ± 0.179 (81.9)	2400 ± 0.300 (17.5)	80 ± 0.125 (0.6)
V3	10880 ± 0.243 (85.4)	1720 ± 0.116 (13.5)	140 ± 0.357 (1.1)
V4	13600 ± 0.074 (86.3)	2060 ± 0.243 (13.1)	90 ± 0.222 (0.6)

Mesophils and thermophiles develop in these two phases - mesophilic and thermophilic, with the highest contribution to the composition of the general microflora as non-sprouting bacteria, followed by the bacilli, which are actively involved in the initial stages of destruction of organic matter.

The microbiological analyses at the 13th day (mesophilic phase) after experimentation show a different quantitative development of the different groups of microbes (Table 2):

Table 2. Composition of the microflora 13 days after the experiment (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	5120 ± 0.195 (73.6)	820 ± 0.244 (11.8)	1020 ± 0.196 (14.7)
V2	5200 ± 0.254 (51.2)	960 ± 0.417 (9.4)	4000 ± 0.150 (39.4)
V3	4940 ± 0.202 (81.5)	880 ± 0.341 (14.5)	240 ± 0.417 (4.0)
V4	4040 ± 0.248 (74.8)	880 ± 0.455 (16.3)	480 ± 0.208 (8.9)

The lower temperature with 7°C (V1), 13°C (V2), 5°C (V3) and 18°C (V4) for 1 week is a stress factor for the development of microorganisms which limits their development and changes the role of the different groups of microorganisms in compost 1 and 3. A major share in the composition of the total microflora again occupies non-sprouting bacteria and bacilli, with the exception of composts 1 and 3, where the development of micromycetes is more active than that of the bacilli. Compost variants are in the mesophilic phase, decomposition of organic matter occurs with the development of mesophils.

The composition of the microflora 22 days after the trial of the experiment is presented on Table 3:

Table 3. Microflora composition 22 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	5600 ± 0.089 (79.1)	520 ± 0.385 (7.3)	960 ± 0.208 (13.6)
V2	4800 ± 0.208 (70.0)	500 ± 0.400 (7.3)	1560 ± 0.385 (22.7)
V3	4000 ± 0.250 (65.6)	1900 ± 0.316 (31.1)	200 ± 0.250 (3.3)
V4	3040 ± 0.164 (60.8)	1460 ± 0.342 (29.2)	500 ± 0.100 (10.0)

The composition of the total microflora follows the same trend on the 29th day of the experiment - a higher amount of ammonifiable bacteria and lower micromucites (Table 4).

Table 4. Microflora composition 29 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	6000 ± 0.200 (83.6)	920 ± 0.217 (12.8)	260 ± 0.385 (3.6)
V2	5880 ± 0.307 (71.4)	1940 ± 0.258 (23.5)	420 ± 0.238 (5.1)
V3	7920 ± 0.126 (85.5)	780 ± 0.256 (8.4)	560 ± 0.107 (6.1)
V4	5200 ± 0.192 (82.3)	520 ± 0.288 (8.2)	600 ± 0.167 (9.5)

The temperatures of the compost piles in the two last samples are close - ranging from 20°C to 26°C.

On the 39th day after experimentation, non-sprouting bacteria and bacilli dominate, with the exception of compost 2, where the amount of micromycetes is higher than that of bacilli (Table 5).

As on the 39th day and 48th day of experimentation, the temperature range suggests the development of mesophilic microorganisms.

For all compost materials, the amount of non-sprouting bacteria and bacilli is higher than that of micromycetes (Table 6).

Table 5. Composition of the microflora 39 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	5160 ± 0.194 (86.3)	800 ± 0.250 (13.4)	20 ± 0.500 (0.3)
V2	4840 ± 0.179 (74.5)	500 ± 0.200 (7.7)	1160 ± 0.086 (17.8)
V3	3960 ± 0.253 (82.2)	460 ± 0.326 (9.5)	400 ± 0.125 (8.3)
V4	3760 ± 0.266 (78.7)	760 ± 0.066 (15.9)	260 ± 0.154 (5.4)

Table 6. Microflora composition 48 days after the trial (CFU × 10^3/g compost) ± CV (%)

Variants	Non-sprouting bacteria	Bacilli	Micromycetes
V1	6020 ± 0.332 (88.3)	500 ± 0.120 (7.3)	300 ± 0.167 (4.4)
V2	5220 ± 0.096 (75.7)	1080 ± 0.093 (15.7)	600 ± 0.100 (8.7)
V3	5460 ± 0.317 (78.4)	1100 ± 0.182 (15.8)	400 ± 0.150 (5.7)
V4	5940 ± 0.168 (85.1)	820 ± 0.122 (11.7)	220 ± 0.182 (3.2)

CONCLUSIONS

The studied aerobically active composting variants pass through all phases of typical compost: mesophilic, thermophilic, aging and maturing. Only one of the composts with mulberry leaf branches (V3) does not pass into a thermophilic phase.

According to the common microflora indicator, the most active composting process starts with the highest starting material at the first variant (V1). In variants with a different amount of mulberry leaf and twigs (V3 and V4), the microbial diversity and presence is less, due to the unity of the material used. Reducing the amount of green and brown materials and adding fertilizer (V2) slows down the development of microorganisms at the beginning of the experiment, but in the next period they are activated.

Throughout the process, all the studied microbes groups have a prominent role in the composting process, with the dominant role of ammonifier bacteria - non-sprouting bacteria and bacilli. These groups of microbes, such as highly plastic, are among the most active disruptors of organic compounds in compostable materials. The lowest in the composition of the total microflora is the micromycetes.

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