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MICROBIAL AND ENZYMATIC DEGRADATION OF CARBOHYDRATES: A COMPARATIVE INVESTIGATION OF COMPOST VARIANTS

Boyka MALCHEVA[1], Milena YORDANOVA[2], Vera PETROVA[2], Pavlina NASKOVA[3], Dragomir PLAMENOV[3]

[1]University of Forestry, Faculty of Forestry, 10 Kliment Ohridski Blvd., Sofia, Bulgaria
[2]University of Forestry, Faculty of Agronomy, 10 Kliment Ohridski Blvd., Sofia, Bulgaria
[3]Technical University - Varna, Department of Plant Production, 1 Studentska Str., Varna, Bulgaria

Corresponding author email: boika.malcheva@gmail.com

Abstract

Microbial biomass carbon and enzymes, degradation carbohydrates have been investigated for the composting of various vegetable and animal wastes. A 4-variant scheme for compost piles from plant residues (vine sticks, fruit twigs, leaves, grass slopes) and rabbit fertilizer with different starters (last year's compost and soil) has been prepared. Microbial biomass and enzymes: cellulase, invertase, amylase and catalase have the highest values up to the 7th day of starting the experience. The higher momentary cellulase activity at the beginning of the experiment determines and a higher potential cellulase activity in cultivation of the variants for the first 10 days, as well as a faster rate of cellulose degradation over the entire composting period. Invertase activity values is lower than these of cellulase and amylase activities. The catalase has shown a smaller decrease at the end of the experiment compared to the beginning in comparison with the other enzymes. Microbial carbon biomass and enzyme activities have a main role in the carbohydrates degradation as an integral part of organic matter and are sensitive indicators in the composting process.

Key words*: compost, microbial biomass, enzyme activities.*

INTRODUCTION

Microbial and enzymatic degradation of carbohydrates as a major part of organic matter is essential in the preparation of organic fertilizers to improve soil fertility upon their application in soils. The composting is a controlled microbial oxidation process in which organic biodegradable waste is converted into safe and useful humus-similar products (compost), which has no phytotoxic and pathogenic properties, has been characterized by the presence of humic acids and is applied in the soil as a nourishing organic fertilizer (Weltzien, 1991; Adani et al., 1997; Epstein, 1997; Boulter et al., 2002; Lashermes et al., 2012; Li et al., 2013; Guanghui et al., 2016; Boteva and Yankova, 2017). Microbial characteristic of composting is important for optimizing the process and quality of the final product. Information on the microbial component of compost substrates can be expected to provide valuable information on the factors influencing the process, in order to support the compost variants optimization and accordingly the quality of the final product to be improved (Mondini et al. 1997; 2002; Jedidi et al., 2004; Yankova and Boteva, 2017). An extremely important part is managing the composting process, considering the factors that exert influence on it (Nedev, 2020). Ayed et al. (2007) have found a decrease in microbial biomass C and microbial biomass N in mature compost, which is probably as a result of the decreasing availability of easily degradable substrates as the municipal solid waste composting process progresses. According to these authors, the dynamics of the ratio between the two biomasses suggests a change in the composition of the microbial populations during the composting process from the prevailing bacteria and actinomycetes to the predominant micromycetes. Bouzaiane et al. (2011) concluded that the content of microbial biomass C and N and DNA during the solid waste composting process can be of great benefit for understanding the compost stability status. The basic requirement for the safe use or application of compost in agricultural land is its degree of stability, which implies a stable content of organic matter (Castaldi et al., 2004; 2008; Mondini et al., 2004). In fact, the amount of microbial biomass cause a significant role for biochemical transformations, for optimization and for the quality of the final product (Mondini et al., 2002; Jedidi et al., 2004). Microbiological activity determines the stability and maturity of the compost and it is expressed in the formation of microbial biomass by mesophilic and thermophilic bacteria, oxygen absorption and CO_2 release, as well as changes in the enzyme activity in the components (Barrena et al., 2008; Cunha-Queda et al., 2007; Jurado et al., 2014).

The evolution of total microbial biomass, Gram+ bacteria, Gram- bacteria, moulds, and enzyme activities (β-glucosidase, cellulase, protease, acid, and alkaline phosphatase) significantly depends on the type of waste (Villar, 2016). According to this author's investigation, it should be paid more attention to the ripening phase in order to optimize composting. Decreases in both the enzyme activity and the microbial community may indicate stability in the maturation of the compost. It is important to monitor microbial communities and their enzymatic activity during the time to determine if and when the compost is stable enough to be applied in the soil, or whether more time or alternative process management is required (Villar, 2016). The time required for the ripening phase is a function of the substrate and the environment and the operating conditions of the facility and it may vary from a few weeks to a year or two (Diaz et al., 2002). This lack of process control can cause environmental problems such as odors and leaching, in addition to adversely affecting the quality of the compost. Castaldi et al. (2008) propose the study of the dynamics of some enzyme activities as an appropriate indicator of stability, although they do not establish a threshold value. However, enzyme activity studies provide information on the degradation of organic matter and metabolic processes during composting, and therefore on product stability. The nature of organic substrates is also an important factor in determining the dynamics and microbial diversity during composting (Klammer et al., 2008; Ryckeboer et al., 2003; Vargas-García et al., 2010). Organic degradation is carried out by different groups of microbial populations. They develop depending on the temperature of the compost mass. Thermophilic and mesophilic groups of microorganisms have been isolated (Bernal et al., 2009). Bacteria prevail at the beginning of composting, moulds are present during the entire process, but dominate at humidity below 35%, at temperatures above 60°C their activity decreases (Shestakov et al., 2018). The decomposition of carbohydrates in compost, as well as other compounds is accomplished by a variety of microorganisms, with thermophilic forms evolving at temperatures of 50-60°C (Antonyan, 2004). Actinomycetes predominate in the process of stabilization and maturation, i.e. participate in the degradation of resistant polymers together with micromycetes. During composting, various microorganisms with cellulolytic-lignolytic activity, such as *Trichoderma viridae*, *Aspergillus niger*, *Aspergillus terreus*, *Bacillus* sp., degrade various animal and vegetable waste products, as well as farm and cattle shed waste. The compost maintains high populations with a higher percentage of Gram-negative microorganisms. All Gram-positive isolates were identified as *Bacillus* sp. (Boulter et al., 2002).

During the composting process, the starting material is transformed by a variety of biological and biochemical processes and, in which enzymes served their purpose (Gupta et al., 2015). In a study by Stutzenberger et al. (1970) the maximum cellulase activity is demonstrated at 65°C, pH 6.0. The cellulose activity of the compost is increased 10 times at logarithmic rate, while the cellulose content is reduced by 50%. According to Kubicek et al. (1998) the rate of hydrolysis of cellulose derivatives by β-glucosidase determines the induction of cellulases. Enzymes also cause an important role in the humification stage. Microorganisms capable to degrade polymers in the constituent mass of composts produce a complex of extracellular enzymes (Jurado et al., 2014). The dynamics of changes in some enzyme activities have been recognized by many authors as biological indicators of compost maturity (Bohacz and Korniłłowicz-Kowalska, 2009; Castaldi et al., 2008). Pursuant to study of Bohacz (2019) the enzymatic activity represented by the study of cellulase, protease, urease and arylsulfatase is higher during the 10 weeks of composting in compost in small amounts bird feathers and easier available lignocellulosic fraction - mainly grass, pine skin and wood sawdust than in compost containing more difficult-to-degrade lignocellulosic waste (wheat straw, wood sawdust, pine skin) and a higher content of feathers. Microbial diversity (mainly fungus) and enzymatic activity (alkaline phosphatase and β-glucosidase) cause positive effect on the mineralization of phosphorus during the production of phospho-compost (Kutu et al., 2019). Among the 19 enzymes tested in the composting process, Tiquia (2002) found that esterase, valine amino-peptidase and α-galactosidase were the most common enzymes in bird manure, whereas N-acetyl-β-glucosaminidase in pork manure. According to a study by Mondini et al. (2004) microbial carbon biomass decreases throughout the composting period (149 days), while the enzyme activity in wet fractions stabilizes between 50 (β-glucosidase, alkaline phosphatase) and 90 (arylsulfatase, acid phosphatase) days of composting. In composting plant biomass, Wei et al. (2012) have found that cellulase activity shows increasing prevalence in the later stages (24 weeks) of composting, and measured hemicellulase activities, mainly α-arabinosidase and β-galactosidase, were higher in the earlier stages (3 weeks), in response to the availability and absorption of chemically diverse biomass materials. Nakamura et al. (2004) have found that the composting materials in the composting process are dominated by the species *Cerasibacillus quisquiliarum* and *Bacillus thermoamylovorans*, which degrade gelatin and starch. They suggest that these species produce gelatinase and amylase, respectively. The increase in gelatinase is dependent on an increase in the diversity of *Cerasibacillus quisquiliarum,* whereas for *Bacillus thermoamylovorans* and amylase such trend has not been observed. The highest activity of amylase (73-129 U/g) and cellulase (75-148 U/g) has been observed in the beginning of the composting process, and maximum activities of lipase (5-10 U/g) and protease (46-72 U/g) have been established in the middle stage of the process of composting kitchen waste, dried leaves and rice bran (Fan et al., 2015). In the preparation of compost from sewage sludge and straw Niu and He (2014) found that, at the beginning of the compost, cellulase activity firstly has increased and then gradually has decreased and there is a trend to be stable. The activity of catalase is higher in the beginning of the compost and it is stable during the temperature rising, after that it quickly decreased and is maintained at a lower level. According to our previous study, the application of composts from organic waste (compost variants analyzed in this investigation) leads to activation of soil microbiological activity (Malcheva et al., 2019). Antonious (2016) establishes that the recycling of organic waste, its composting and its application leads to increased soil urease and invertase activity.

The purpose of this study is to monitor dynamically the influence of various compost variants on the accumulation of microbial carbon biomass and the activity of enzymes involved in the carbohydrates degradation as part of the organic matter of composts.

MATERIALS AND METHODS

There is a 4-variant scheme of compost piles (V1, V2, V3, V4) with different starters.

The recipes for the variants are presented in Table 1.

Microbiological studies include the determination of microbial carbon biomass by fumigation spectrophotometric method (Cai et al., 2011). The enzyme activity of the compost variants has been presented in dynamics by investigation of: cellulase, amylase, invertase by spectrophotometric method of Gradova et al. (2004) and catalase by a manganometric method (Khaziev, 1976) (instant activities). Potential cellulase activity has been presented at the beginning and end of the experiment (mesophilic phase) and, using a laboratory method according to Khaziev (1976), as in petri dish with 10 cm diameter is poured thick soil about 7 mm on which 3 strips of sterile filter paper are placed with 10/50 mm in size and cultured at 25°C, 60% maximum field moisture capacity has been maintained. The decomposed cellulose area with a standard grid shall be recorded over 10 days. Average values from the three bands are calculated.

Table 1. Recipes of compost variants

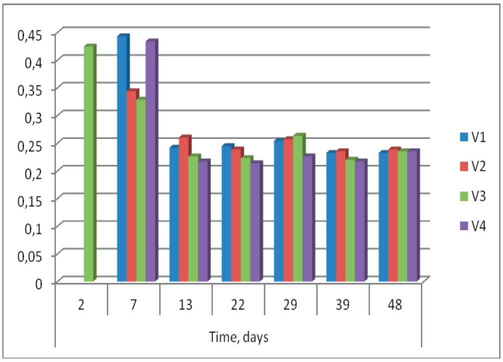
Variants	Materials	Starter
V 1	Brown materials: - Vine sticks- 26.150 kg - Fruit twigs – 10.800 kg Total brown materials: 34.950 kg Green materials: - Grass slope – 21.550 kg	Last year's compost – 3.000 kg
V 2	Brown materials: - Vine sticks – 23.450 kg - Fruit twigs – 10.500 kg Total Browns: 33.950 kg Green materials: - Grass slope – 10.000 kg - Rabbit fertilizer – 9.000 kg Total green materials: 19.000 kg	Last year's compost – 3.100 kg
V 3	Mulberry-tree twigs with leaves - 19.800 kg *The 7th day after preparing the experiment - additional insertion into the bowl, upon turning – 2.050 kg of pure litter for small bugs growing and 1.450 kg of pure twigs; the 13th day – 1.030 kg of litter and twigs.	Soil
V 4	Mulberry-tree twigs with leaves – 28.950 kg (taken from heavy metal contaminated area) *The 7th day after preparing - additional insertion into the bowl, upon turning – 3.050 kg litter for small bugs growing and twigs; the 13th day – 0.200 kg of litter and twigs taken from the same contaminated area.	Soil

Statistical processing of the data include calculating the average value of three repetitions and coefficient of variation (C.V.) by the use of Excel 2010.

RESULTS AND DISCUSSIONS

Study was started one week after the compost pile 1, 2 and 4 is prepared, and on the second day after the compost pile 3 was prepared. Analyzes were repeated upon each turn of the compost (every 7-10 days).

The results of microbial biomass carbon (MBC) dynamics provide an idea of the accumulation of organic carbon with microbial origin in compost heaps. This indicator is important for assessing the degree of degradation of compostable substrates, insofar as the mineralization of organic compounds shows (mainly carbohydrates) in them. The microbial biomass carbon dynamics data are presented in Figure 1.



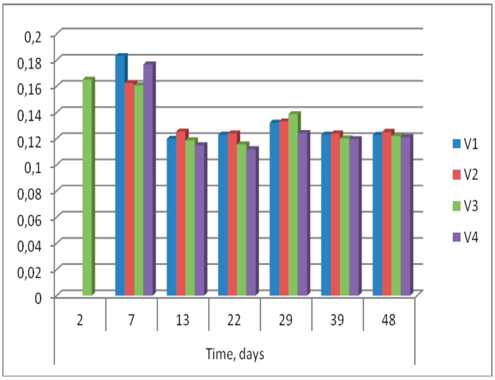
*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 1. Microbial biomass carbon (mg glucose/g soil)

The data indicate different dynamics of MBC accumulation in the four composting variants, which is determined by the differences in the compost formulations and, accordingly, by the development and activity of the microflora in them. On the 7th day of experience the highest and the amount of biomass carbon to microbial origin at V1 has been established. This amount is about 1 time higher than the same in the other variants. For this variant (V1), the brown (vine sticks, fruit twigs) and green (grass slopes) materials are in the biggest amount - a total of 56.500 kg, and the starter is last year's compost - 3 kg. According to data from our previous study (Malcheva et al., 2018), these vegetable wastes have the highest microbial presence, whereas in variants with different amounts of mulberry twigs with leaves (V3 and V4), microbial diversity and presence are lower. Reducing the amount of green and brown materials and adding rabbit fertilizer at V2 slows the development of microorganisms at the beginning of the experiment (the 7th day), but in the next reporting days their activation is most presented on the 13th day.

In V2 and V3, the amount of MBC decreased more gradually, while in the other variants (V1 and V4) the accumulation of microbial biomass sharply decreased by the 13th day - about 2 times. After that day, the biomass carbon decreases more smoothly until the end of composting, with a slight increase on the 29th day after the experiment is set. For all four composting variants, it is found that on the 39th and on the 48th days the amount of MBC reaches the lowest values. After composting it decreased about 2 times in variants V1 and V 4, and about 1.4 times in V2 and V3 compared to the amount of MBC in early experiment. The more diverse composition of vegetable waste at V1, the addition of rabbit fertilizer at V2, waste from the heavy metal contaminated area at V3, and the different amount and quality composition of the microflora in the different compost variants are relevant to this trend (Malcheva et al., 2018). Mondini et al. (2004) also establish that microbial biomass carbon decreased throughout the composting period.

According to our previously research (Malcheva et al., 2018), variants 1, 2 and 4 have passed a mesophilic and thermophilic composting phase with the development of mesophilic and thermophilic groups of microorganisms. As long as variant 3 does not go through the thermophilic phase. This fact has an effect on biomass accumulation by the 7th day of the experiment, the lowest value at V3, where only mesophilic groups of microorganisms develop, and in the other variants the activity of mesophiles and thermophiles is higher. After the 7th day, by the end of the experiment, the accumulation of MBC is close to the individual variants. Therefore, not only the amount of microorganisms is a prerequisite for their activity, and respectively for the accumulation of biomass carbon of microbial origin. Other factors also affect the temperature and humidity of the compost, the type and amount of compostable material. The same factors affect the enzyme activity of the microorganisms in the compost. Cellulase activity is presented in Figure 2.

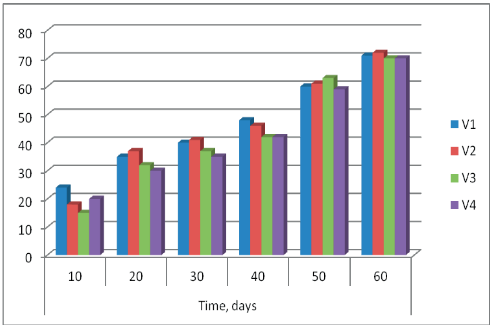


*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 2. Cellulase activity of compost materials (mg glucose/g soil)

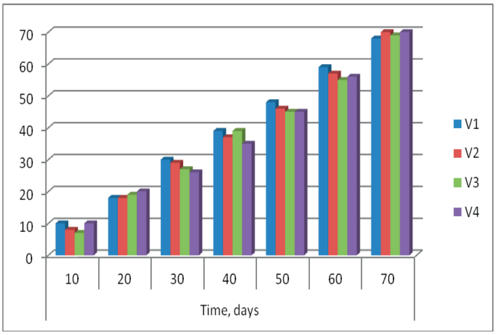
The results for cellulase activity show similar trends as for the accumulation of microbial biomass carbon - higher enzyme values by the 7th day, more sharply decrease in enzyme activity between the 7th and the 13th days, and subsequent a gradual decrease in cellulase values by the end of the experiment, with a slight increase on the 29th day. A similar trend for the highest values of cellulase at the beginning of composting was found by other authors (Niu and He, 2014; Fan et al., 2015). While in a study by Wei et al. (2012) cellulase activity shows an increasing predominance in the later stages (24 weeks) of composting, and the measured hemicellulase activities are higher in the earlier stages (3 weeks), in response to the availability and digestibility of chemically different biomass materials. Again, the enzyme activity at the beginning of the experiment was higher at V1 and V4, and lower at V2 and V3. While at the end of the experiment the cellulase values are close in all variants, the decrease from the beginning is 1.5 times for V1 and V4, and 1.3 times for V2 and V3, i.e. the reduction of cellulase activity during the composting period is less than the decrease in the accumulation of microbial biomass carbon. Despite the lower amount of accumulated microbial biomass, cellulase activity remains higher, which once again confirms the fact that the enzyme activity of microorganisms does not dependent on their amount only, but also MBC, and cellulase activity depends on the development and activity of microorganisms in compost materials. As per our research on the same compost variants (Malcheva et al., 2018), non-spore-forming bacteria and bacilli occupy a major part of the total microflora, while actinomycetes and micromycetes are developed less. Bacteria predominate at the beginning of composting, and moulds are present throughout the whole process, but dominate at humidity below 35% and temperature below 60°C (Shestakov et al., 2018).

Besides instant cellulase values, the potential cellulase activity of composting in dynamics for a period of 60-70 days has been studied. The following Figures 3 and 4 show the results for the cellulose degradation dynamics for the samples as of the 7th day (the beginning of the experiment) and the 48th day (the end of the experiment).



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 3. Degradation of cellulose (%) - the beginning of the experiment



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

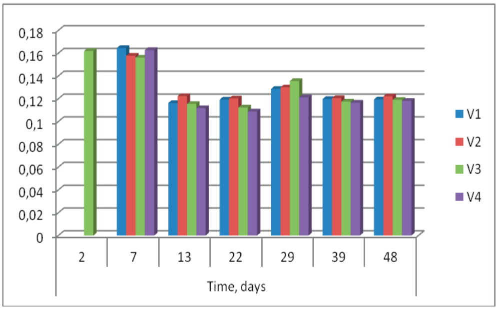
Figure 4. Degradation of cellulose (%) - the end of the experiment

As per Figure 3 and Figure 4 it has been established that higher amount of accumulated microbial biomass carbon and higher instant cellulase activity at the beginning of the experiment also lead to higher potential cellulase activity in cultivation variants as of the 7^{th} day and counting the percentage of degraded area in 10 days for a period of 60 days.

During the first 10 days a higher percentage of degraded area has been established - about 20%, increasing by 10% for the following periods and reporting for 70% of the degraded area in all variants on the 60^{th} day.

Whereas, on cultivation of samples from the day 48, the initial percentage of degraded cellulose is lower and 70% of the degraded area is established on the day 70 of cultivation, which correlates with the lower values found in the spectrophotometric determination of cellulase after the 7^{th} day up to the 48^{th} day.

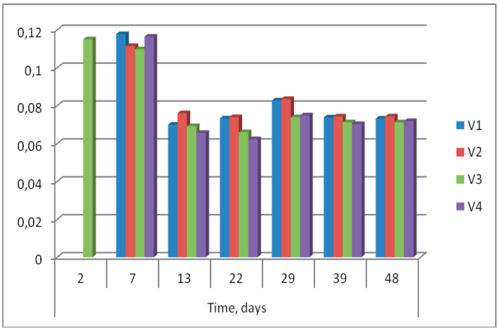
The amylase activity represents the rate of degradation of starch in the compost variants (Figure 5).



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 5. Amylase activity of compost materials (mg glucose/g soil)

The data indicate that amylase values are slightly lower than those of cellulase. The trends have been repeated - increased levels of amylase activity on the day 7, a sharp decrease from the days 7 to 13, and a subsequent more gradual decrease in enzyme activity, with a slight increase in values on the day 29. Fan et al. (2015) also establish higher amylase activity at the beginning of composting and lower at the end of the process. Again, at the beginning of the experiment, amylase activity was higher at V1 and V4, and lower at V2 and V3, and at the end of the experiment the enzyme values were close in all variants. Invertase activity has the lowest values (Figure 6).



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

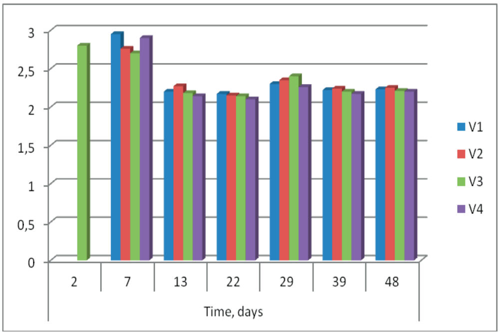
Figure 6. Invertase activity of compost materials (mg glucose/g soil)

The invertase activity is about 1.7 lower than cellulase and amylase activity. Probably the invertase activity is lower, since its producers are mainly the yeast that get killed when the compost passes through the thermophilic phase. The trends in the dynamics of invertase are the same as in previous enzymes.

Higher invertase activity was found at the beginning of the experiment and lower after the 13th to 48th day.

However, the application of compost from organic residues leads to an increase in soil invertase activity (Antonious, 2016). Cellulose, starch and sucrose are degraded by the β-glucosidase enzyme to hydrogen peroxide, which in turn is degraded to water and oxygen by the catalase enzyme.

The catalase activity of the studied compost variants is presented in Figure 7.



*Note: Average values of the indicator; C.V. up to 10% for all variants and analyzes (low dispersion)

Figure 7. Catalase activity of compostable materials (ml O_2/30 min)

The catalase activity cannot be compared quantitatively with other enzymes due to different enzyme assay methods, but it repeats the trends observed with the previous enzymes. A slight decrease in catalase activity at the end of the experiment towards to the beginning, compared to the other enzymes tested, has been established - 1.3 times for V1 and V4, and 1.2 times for V2 and V3.

Niu and He (2014) also establish a similar trend. According to the study of these authors, the activity of catalase is higher at the beginning of composting and in the period of increasing temperature is stable, then rapidly decreases and is maintained at a lower level.

The catalase values have been also influenced by the presence of catalase of vegetable origin, in addition to microbial origin.

CONCLUSIONS

Biomass carbon with microbial origin in the experienced variants of aerobic active composting has decreased at the end of the experiment compared to the beginning. The accumulation of microbial biomass is most active up to the 7th day of experience set, to a greater extent for the variant with vine sticks, fruit twigs and grass slopes (V1), as well as the variant with mulberry twigs with leaves (V4), at starter soil for both composts. The values of biomass carbon (the 7th day) are lower for the vegetable residue and rabbit fertilizer (V2) variant, as well as for the mulberry twigs with leaves, but in a smaller amount (V3), for last year's compost starter for V2 and soil for V3. MBC at the end of experiment is similar for all compost variants.

Cellulase activity of the compost variants follows the trend with the accumulation of microbial biomass - the highest enzyme values at V1 and V4, and lower at V2 and V3 at the beginning of the experiment (the 7th day), as well as close enzyme values at end of experiment (the 48th day). The higher instant cellulase activity at the beginning of the experiment also resulted in a higher potential cellulase activity in cultivation of variants during the first 10 days, as well as a faster rate of cellulose degradation over the entire composting period.

Amylase and invertase activity are lower than cellulase activity. This trend is more expressed with respect to invertase activity. And these enzymes are established at higher values at V1 and V4, and the lower at V2 and V3 initially, and similar values at the end of the experiment.

Catalase activity has followed the course of the other enzymes in the different composting phases, but in contrast, the catalase has showed a slight decrease at the end of the experiment compared to the beginning. The indicators studied - microbial biomass carbon and enzyme activities cause a significant role in the carbohydrates degradation in organics and are sensitive indicators in the composting process.

The application of the types of compost presented can support to be improved soil fertility and crop production, and to be an important step in achieving sustainable ecological farming and integrated crop production.

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