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Changes of enzymatic activity during a large-scale vermicomposting process with continuous feeding

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ABSTRACT

The presented experiment determined the enzymatic activity of eight different enzymes in three types of aged vermicomposts (from household biowaste; malt house sludge mixed with agricultural waste; grape marc). The vermicomposting was conducted in the large-scale systems of heaps with continuous feeding of the earthworms, which is applicable in practice. All vermicomposting heaps were divided into five layers of different depths, depending on the age of these layers. In all heaps, the highest number and biomass of earthworms occurred in the youngest layers, as did the highest activity of bacteria or fungi. The highest activity of all eight measured enzymes occurred in the vermicomposting process with household biowaste. The lowest activity of all enzymes showed the arylsulphatase, which did not exceed 66 μmol 4-methylumbellyferyl sulphate potassium salt. $g^{-1}.h^{-1}$. The highest enzyme activity was detected for lipase, where all values were higher than 5382 μmol 4-methylumbellyferyl-caprylate.$g^{-1}.h^{-1}$. There were marked differences between the layers in all vermicomposting heaps.

1. Introduction

Biodegradable waste is currently processed by conventional biodegradation methods such as composting and an anaerobic decomposition (Björklund et al., 1999), or a large part of biodegradable waste is incinerated (sludge, straw, plant residues) (Faaij, 2006). The less frequently used and more gentle method that can contribute to cleaner production is vermicomposting. Vermicomposting is mesophilic aerobic fermentation using epigeic earthworms which live in upper layers like *Eisenia andrei* or *Eisenia fetida* (Mc Lean and Parkinson, 1998). Organic material (ideally pre-fermented) with a C:N ratio of 25:1–30:1 serves as a food for earthworms. The ideal temperature for vermicomposting is about 22 °C, and humidity should range from 70 to 80% (Edwards et al., 2011). The earthworms usually process any organic material (Gaddie and Douglas, 1975), such as sewage sludge, animal waste, crop residues (Sinha et al., 2010), household and garden waste and some industrial waste (Atiyeh et al., 2000). Unsuitable materials for vermicomposting are the materials with high salt content or thicker layers of fresh leaves or grass, meat and dairy products (Sinha et al., 2008).

This experiment deals with vermicomposting of household waste, agricultural waste with malt house sludge and grape marc. Household waste is the waste from apartment buildings, housing areas and gardens over four seasons (Hanc et al., 2017). Agricultural waste includes some chips, straw, apples, grass or poppies and it is mixed with malt house sludge, which is difficult to dispose. This sludge remains during the barley soaking process at the bottom of the tank. Grape marc is formed after grapes crushing, draining and pressing and is the most important by-product of the wine industry. Most commonly, grape marc contains about 60%vol of stalks, peel and pulp and 40%vol of grape seeds (Flavel et al., 2005).

Enzymes are the most numerous groups of biocatalysts, with specific proteins produced inside living cells which catalyse biological reactions. The International Commission divided the enzymes into six main classes: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. The enzymes selected for this experiment were based on the evaluation of the available

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data. Selected enzymes play a part in the metabolic processes involved in the decomposition of organic matter (β-D-glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine and leucine aminopeptidase) and fall into the hydrolase class, which catalyses hydrolytic cleavage.

β-glucosidases form a very heterogeneous group of enzymes hydrolysing glucose oligosaccharides (Brenda, 2019). Acid phosphatases catalyse the hydrolysis of monoesters and phosphoric anhydrides, resulting in mineralisation of organic phosphorus (Enowashu et al., 2009). Arylsulphatase catalyses the hydrolysis of the aryl-sulphate anion by interrupting the O–S bond. Arylsulphatase contributes to the mineralisation of the organic sulphur to $S-SO_4^{2-}$ (Tabatabai and Bremner, 1970). Lipases hydrolyse acylglycerols, containing fatty acid chains with a carbon number $\geq$10, to fatty acids and glycerol. At the same time, ester bonds between glycerol and fatty acids are released (Brenda, 2019). Chitinases are an essential group of enzymes responsible for chitin degradation. They hydrolyse the glucosidic bonds of chitin, releasing the smaller nitrogen-containing organic compounds (Gooday, 1994). Cellobiohydrolase hydrolyses β-D-glucosidic bonds in cellulose and cellotetraose and releases cellobiose or glucose from the cellulose chain. It participates with endoclucanase and β-glucosidase in the decomposition of structural polymers (Mertz et al., 2007). Aminopeptidases are among exopeptidases, which are the enzymes digesting proteins during digestion. Alanine and leucine aminopeptidases release terminal N from amino acids, peptides, amides or arylamides, alanine aminopeptidase, especially from alanine or proline and leucine aminopeptidase, mainly from leucine or proline (Wickström et al., 2011).

Vermicomposting of household waste or grape marc has previously been the subject of several studies (household waste: Abbasi et al., 2015; grape marc: Domínguez et al., 2014; Gómez-Brandón et al., 2011b). However, all available studies were concerned with small-scale conditions and in the system of single feeding. Also, in some studies the enzymatic activity was determined, but only for a few enzymes (Fernández-Gómez et al. (2010a), Fernández-Gómez et al. (2010b) and Nogales et al. (2005) determined the activity of phosphatase, β-glucosidase, dehydrogenase and urease; Romero et al. (2010) determined only dehydrogenase and urease). The aim of the manuscript is to determine the enzymatic activity of eight different enzymes in three types of aged vermicomposts. The novelty of this study consists of the evaluation of changes in activity of eight enzymes between the layers, and the age of the profile in a large-scale windrow vermicomposting system with continuous feeding of earthworms *Eisenia andrei*. Newly determines the enzymatic activity of lipase, cellobiohydrolase, leucine aminopeptidase and alanine aminopeptidase in the vermicomposts. This vermicomposting took place in outdoor conditions in the system of large heaps, which is useable in practice. This system is disposing the waste at the site of its production (in the individual companies).

2. Material and methods

2.1. Experimental design

Aged vermicomposting (vermicomposting system with continuous feeding of earthworms, where the layers are gradually increasing in age) using the windrow method was set up in May 2015 at three establishments in the Czech Republic, with different biowastes, under outdoor conditions. The vermicompost with household biowaste was in Uherský Brod; the vermicompost with a mixture of malt house sludge (20%vol) and agricultural waste (80% vol) was in Veleliby u Nymburka; and the vermicompost with grape marc was in Mikulčice. The bedding layer containing earthworms (*E. andrei*), with a density about 50 earthworms per litre, was placed first. Other layers of biowaste were added every two weeks. The composition of the biodegradable material depended on the season, except the grape marc. The individual parameters of the household biowaste varied (in each layer) depending on the season. Average values for this type of biowaste are: Dry matter 32.5%; pH/H_2O 5.4 and C:N 26.7. Detailed analysis of household biowaste is mentioned in the article by Hanc et al. (2011). Average values for malt house sludge were: Dry matter 11.8%; pH/H_2O 8.3; EC: 2088 μS/cm and C:N 6. Average values for grape marc were: Dry matter 34.2%; pH/H_2O 7.6; EC: 416.5 μS/cm and C:N 21.4.

The vermicomposting heap with household biowaste was set up on an area of 5.6 × 25.5 m. Depth and the age of the layers were: V: 0–30 cm, < 3 months; IV: 30–60 cm, 3–6 months; III: 60–90 cm, 6–9 months; II: 90–120 cm, 9–12 months and I: 120–150 cm, > 12 months (Hanc et al., 2017) (Fig. 1).

The vermicomposting heap with malt house sludge was set up on an area of 6 × 10 m. Depth and the age of layers were: V: 0–20 cm, < 3 months; IV: 20–40 cm, 3–6 months; III: 40–60 cm, 6–9 months; II: 60–80 cm, 9–12 months and I: 80–100 cm, > 12 months (Fig. 1).

The vermicomposting heap with grape marc was set up on an area of 2.5 × 50 m. Depth and the age of layers were: V: 0–20 cm, < 3 months; IV: 20–40 cm, 3–6 months; III: 40–60 cm, 6–9 months; II: 60–80 cm, 9–12 months and I: 80–100 cm, > 12 months (Částkova and Hanč, 2019) (Fig. 1).

There were four samples from each layer collected at the end of the experiment. All earthworms were separated from samples, counted and weighed. The samples without earthworms were divided into three parts. The first part was dried at 35 °C to a constant weight for dry matter analysis and then ground. The second part was stored at 4 °C for pH determination and electrical conductivity (EC) analysis. The third part was frozen at −20 °C and then lyophilised for determination of microbial activity and enzymatic activity.

2.2. Agrochemical and biological analysis

The pH/H_2O and the EC were determined using a WTW pH 340 i (GeoTech, Denver, CO, USA) and WTW cond 730 (GeoTech, Denver, CO, USA), according to BSI EN 15933.

Earthworms were separated from the samples and counted manually. Afterwards, they were washed and weighed to determine biomass.

The groups of microorganisms were detected using phospholipid fatty acid (PLFA) analysis, according to Hanc et al. (2017).

The activity of β–D–glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine aminopeptidase and leucine aminopeptidase were measured in 96-well microplates. Lyophilised vermicompost (0.2 g) was extracted using 20 mL of acetate buffer (pH 5.0, $c = 50\ mmol.L^{-1}$; 1.39 g sodium acetate (CAS 127-09-3), 450 μL acetic acid (CAS 64-19-7) and 500 mL demineralised water) in an Erlenmayer flask. The mixture was homogenised using an Ultra-Turrax (IKA Labortechnik, Staufen im Breisgau, Germany) for 30 s at 8000 rpm (Štursová and Baldrian, 2011). Individual enzyme activities (Table 1) were measured in four replicates for each sample. Homogenised samples (200 μL) were pipetted into relevant wells in the microplate, and 40 μL of the substrate was then added (Table 1). The microplate was placed in a Robbins Scientific® 2000 micro hybridisation incubator (SciGene, CA, USA) at 40 °C for 5 min. The fluorescence was then measured using a Tecan Infinite® M200 (Tecan Austria GmbH, Salzburg, Austria). The microplate was placed in the incubator again for 2 h, and the fluorescence was measured again according to Baldrian (2009). Methylumbellyferol (CAS 90-33-5) (1.0, 0.1 and

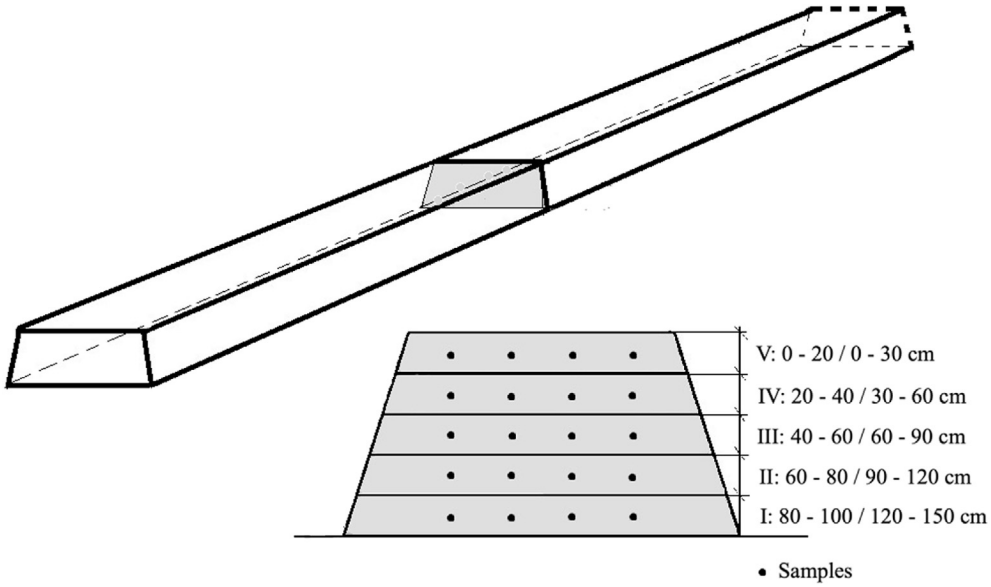

Fig. 1. Diagram of the vermicomposting heap. (The depths 0–100 cm are for the vermicomposting heap with sludge from malt house and for the heap with grape marc; 0–150 cm are for the vermicomposting heap with household biowaste.)

Table 1
Substrates used for the analysis of enzymatic activity.

Enzyme	Substrate	CAS No.	Concentration [mmol.L^{-1}]
β– D–glucosidase	4-methylumbellyferyl-β-D-glucopyranoside (MUFG)	18997-57-4	2.75
acid phosphatase	4-methylumbellyferyl-phosphate (MUFP)	3368-04-5	2.75
arylsulphatase	4-methylumbellyferyl sulphate potassium salt (MUFS)	15220-11-8	2.50
lipase	4-methylumbellyferyl-caprylate (MUFY)	20671-66-3	2.50
chitinase	4-methylumbellyferyl-N-acetylglucosaminide (MUFN)	37067-30-4	1.00
cellobiohydrolase	4-methylumbellyferyl-N-cellobiopyranoside (MUFC)	72626-61-0	2.50
alanine aminopeptidase	L-alanine-7-amido-4-methylcoumarin (AMCA)	77471-41-1	2.50
leucine aminopeptidase	L-leucine-7-amido-4-methylcoumarin (AMCL)	66447-31-2	2.50

Dissolved in dimethylsulfoxide (DMSO) (CAS 67-68-5). All chemicals are from Sigma-Aldrich s.r.o., MO, USA.

0.01 mmol.L^{-1}) and 7-aminomethyl-4-coumarin (CAS 26093-31-2) (0.1 and 0.01 mmol.L^{-1}) were used for the calibration curve.

2.3. Statistical analysis

Results were presented as the mean values of four replicates. One-way ANOVA ($p \leq 0.05$; Tukey's HSD test), including testing for normality and homogeneity of data, was performed using STATISTICA 12 software (StatSoft, Tulsa, OK, USA). Multiple regression and correlation analyses (0.05 level) were performed using IBM® SPSS Statistics 24® software (IBM, Armonk, NY, USA).

3. Results and discussion

Differences in dry matter content were found in the vermicomposting heap with household biowaste (Table 2), where the lowest value was measured in layer IV and the highest in layer III. Dry matter was the lowest in layer I in the vermicomposting heap with malt house sludge (41.4%) (Table 3) and in the vermicomposting heap with grape marc (26.68%) (Table 4). This can be caused by the rainfall and the evaporation of water (Hanc et al., 2017). The problem with evaporation is also relevant to the vermicomposting heap with malt house sludge because there was lower dry matter content in layer V layer than in layer IV, but in this vermicomposting process there was no significant difference between the layers.

The pH value was mildly alkaline. In all vermicomposting processes, the statistical differences were found. In the vermicomposting process with sludge from malt house the pH value was lower with the age of the layers, except the oldest layer, but this layer consisted of different material. In the case of the other vermicomposting heaps, the pH values were very varied and ranged

Table 2
Selected parameters of vermicomposting heap with household biowaste.

Layer	Dry matter [%]	pH/H_2O	EC [μS/cm]	Earthworms [pcs/kg]	Biomass of earthw. [g/kg]
V	40.16 ± 2.19^{a}	8.70 ± 0.13^{b}	1824 ± 139.43^{a}	125 ± 82	14.94 ± 8.93
IV	34.95 ± 1.64^{c}	8.75 ± 0.07^{b}	2041 ± 367.99^{a}	5 ± 8	0.70 ± 0.92
III	46.57 ± 1.09^{b}	7.49 ± 0.11^{a}	3640 ± 318.01^{b}	3 ± 4	0.29 ± 0.42
II	43.76 ± 0.69^{b}	8.11 ± 0.09^{c}	2076 ± 319.54^{a}	8 ± 14	0.82 ± 1.32
I	40.44 ± 0.54^{a}	7.45 ± 0.10^{a}	2317 ± 197.88^{a}	0	0

Values are the means ± SD (n = 4). Different letters (in superscript) in a column indicate significant differences (Tukey's HSD test, $P \leq 0.05$).

Table 3
Selected parameters of vermicomposting heap with mixture of malting sludge and agricultural waste.

Layer	Dry matter [%]	pH/H_2O	EC [μS/cm]	Earthworms [pcs/kg]	Biomass of earthw. [g/kg]
V	42.30 ± 4.88 ^{a}	8.82 ± 0.15 ^{b}	1098 ± 120.18 ^{a}	67.08 ± 72.15	6.72 ± 2.38
IV	46.13 ± 4.80 ^{a}	8.80 ± 0.10 ^{b}	1368 ± 132.60 ^{ab}	4.17 ± 4.19	1.28 ± 0.53
III	45.00 ± 3.02 ^{a}	8.57 ± 0.10 ^{bc}	1131 ± 254.00 ^{a}	0	0
II	43.51 ± 3.76 ^{a}	8.18 ± 0.08 ^{a}	1539 ± 256.72 ^{b}	0	0
I	41.40 ± 2.34 ^{a}	8.28 ± 0.20 ^{ac}	1483 ± 88.33 ^{ab}	0	0

Values are the means ± SD (n = 4). Different letters (in superscript) in a column indicate significant differences (Tukey's HSD test, $P \leq 0.05$).

Table 4
Selected parameters of vermicomposting heap with grape marc.

Layer	Dry matter [%]	pH/H_2O	EC [μS/cm]	Earthworms [pcs/kg]	Biomass of earthw. [g/kg]
V	41.06 ± 0.88 ^{b}	7.73 ± 0.15 ^{b}	812 ± 17.68 ^{a}	209 ± 51	32.65 ± 7.53
IV	39.20 ± 0.85 ^{ab}	8.08 ± 0.13 ^{a}	805 ± 10.00 ^{a}	18 ± 9	3.22 ± 1.80
III	38.07 ± 1.37 ^{a}	7.98 ± 0.10 ^{ab}	729 ± 31.98 ^{ab}	0	0
II	35.32 ± 1.12 ^{d}	8.08 ± 0.10 ^{a}	643 ± 34.03 ^{b}	0	0
I	26.68 ± 1.06 ^{c}	8.68 ± 0.10 ^{c}	1433 ± 131.75 ^{c}	0	0

Values are the means ± SD (n = 4). Different letters (in superscript) in a column indicate significant differences (Tukey's HSD test, $P \leq 0.05$).

from 7.45 to 8.75. It was almost the same as showed Fernández-Gómez et al. (2011) in their vermicomposting experiment with winery waste (pH 8.3), olive-mill waste and biosolids (pH 7.4) or cattle manure (pH 7.5) after six months of vermicomposting in indoor continuous-flow reactor using *Eisenia fetida*.

The electrical conductivity decreases between layer V and layer I in all heaps. The highest EC (3640 μS cm^{-1}) was measured in layer III in the vermicomposting heap with household biowaste. These layers were really varied in terms of EC values, because the composition of the layers depended on the season and the waste material from each apartment.

The highest number of earthworms (209 pcs. kg^{-1}) and the highest biomass of earthworms (32.65 g kg^{-1}) were found in the vermicomposting heap with grape marc (Table 4). The highest number and biomass of earthworms were found in the top layers where the EC was the lowest. Earthworms are sensitive to salt concentration, and the top layer also contained the highest percentage of organic matter, which serves as a food for earthworms (Gunadi et al., 2002).

In all layers, higher content of bacteria was measured than fungi. The highest ratio of bacteria/fungi was found in layer III in the heap with sludge from malt house (158.6), in which the highest activity of bacteria (61.7 μg.g^{-1}dw) was also measured in layer V (Fig. 2a). This layer was not significantly different to other layers. In contrast, the lowest ratio was found in the top layer of the vermicomposting heap with grape marc (5.4); this layer also contained the highest content of fungi (11.3 μg.g^{-1}dw; Fig. 2b). This layer was not significantly different to the other layers in the content of fungi. The vermicomposting heap with grape marc contained a concentration of fungi in all layers that was several times higher than it was in other vermicomposting heaps. Gómez-Brandón et al. (2011a) also reported nearly 100-fold higher activity of bacteria than fungi. The highest activity of these two microorganisms was in the nine-week-old layer of vermicompost from pig slurry using continuous feeding reactors and earthworms *E. fetida* (bacterial PLFAs 400 μg.g^{-1}dw and fungal PLFAs 5 μg.g^{-1}dw) (Gómez-Brandón et al., 2011a).

Activity of β-D-glucosidase showed approximately the same trend in the vermicomposting heap with household biowaste and in the heap with malt house sludge across the entire profile. The highest activity of β-D-glucosidase was measured during vermicomposting with grape marc (1642 μmol MUFG. g^{-1}. h^{-1} in layer II); the lowest activity was also measured in this vermicomposting heap (429 μmol MUFG. g^{-1}. h^{-1} in layer IV) (Fig. 3a). This variability is caused by the high proportion of seeds, which the earthworms are unable to degrade. And the highest value in this variant can be caused by the value of the EC, which was the lowest in this layer. Mondini et al. (2004) reported that, in the case of composting cotton waste and composting cotton waste mixed with some yard waste, the activity of β-glucosidase increased with the age of compost. This activity decreased after 40 d of composting, increased after 80 d of composting and decreased again after 120 d and 180 d of composting the yard waste in their experiment. Also, Fernández-Gómez et al. (2010a) measured the activity of β-glucosidase in a vermicomposting experiment with continuous-feeding *E. fetida* with liquid-paste of tomato-fruit waste. The activity increased up to 6000 μg p-nitrophenol. g^{-1}. h^{-1} (90d) and, after that, decreased until the end of the experiment (210 d). So, the activities depend on the type of biowaste. Lazcano and Domínguez (2011) were interested in the effect of vermicompost on soil fertility and plant growth. They also dealt with enzyme content in soil after application of vermicompost, manure and conventional fertilizer. The experiment showed that the Glucosidase content was statistically significantly higher in the variant with vermicompost (120,000 μg p-nitrophenol. g^{-1}. h^{-1}) than in the variant with conventional fertilizer (100,000 μg p-nitrophenol. g^{-1}. h^{-1}).

Acid phosphatase activity in the vermicomposting heap with household biowaste showed a similar trend to β-D-glucosidase activity, but in the vermicomposting process with malting sludge, the acid phosphatase activity was almost constant throughout the process (Fig. 3b). A large significant difference was found between the youngest layer (layer V) and the other layers in terms of activity of acid phosphatase in the vermicomposting heap with grape marc. The highest activity was found in the youngest layer of this heap (2035 μmol MUFP. g^{-1}. h^{-1}), and the lowest activity was in layer II of the vermicomposting heap with household biowaste (608 μmol MUFP. g^{-1}. h^{-1}) (Fig. 3b). The highest activity in the layer V in the variant with grape marc can be caused by the highest content of bacteria and also fungi. Pramanik et al. (2007) analysed acid phosphatase activities of a few biowastes after 70–85 days of vermicomposting using *E. fetida*. They tested cow dung, grass, aquatic weeds and municipal solid waste. The highest activity after 70–85 days of vermicomposting was found in vermicompost from cow dung (approximately 180,000 μg p-nitrophenol. g^{-1}. h^{-1}), and the lowest activity was measured in the variant from municipal solid waste (approximately 120,000 μg p-nitrophenol. g^{-1}. h^{-1}). Acid

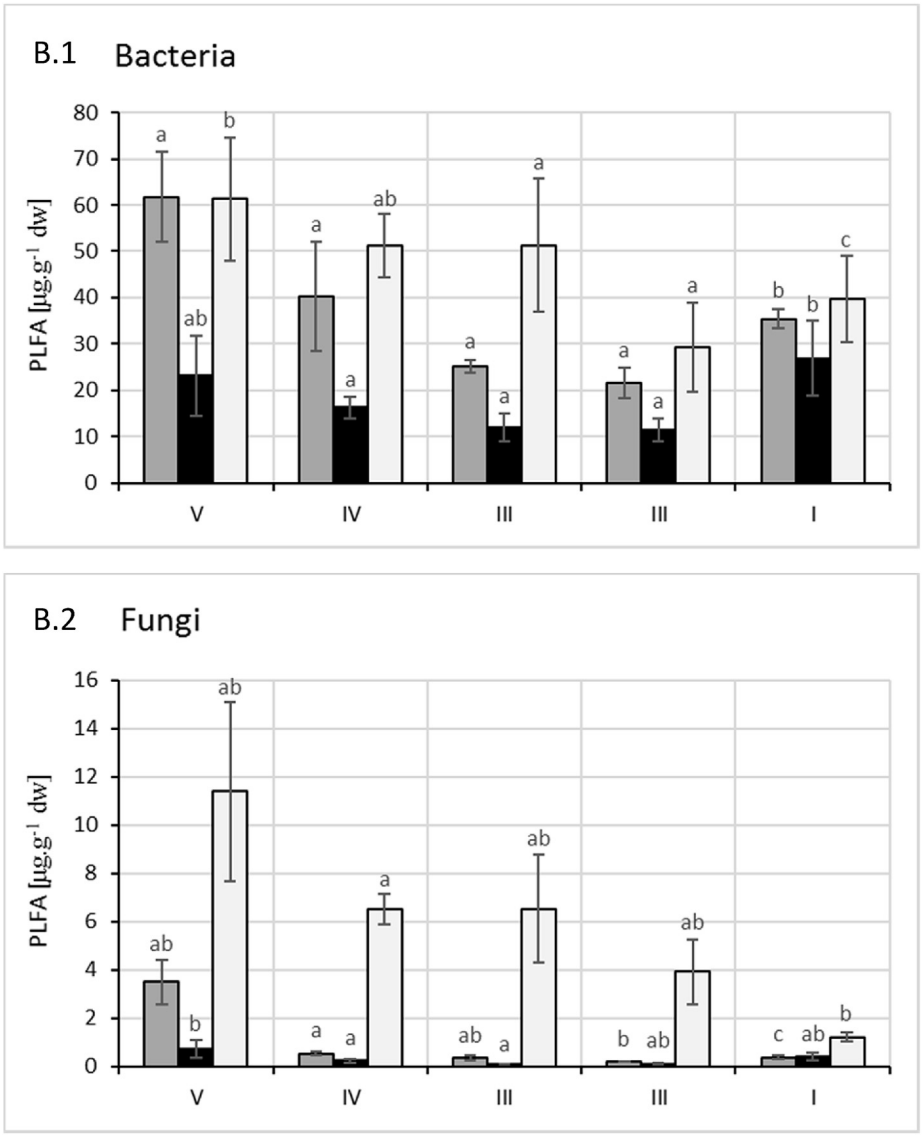

Fig. 2. Activity of bacteria (a) and fungi (v) in all layers of vermicomposting heaps. Values are the means ± SD (n = 4). Different letters indicate significant differences (Tukey's HSD test, $P \leq 0.05$). Legend: Vermicomposting heap with ▩ Household biowaste; ■ Malting sludge; □ Grape marc.

phosphatase activity reported in the experiment of Mondini et al. (2004) differed according to the type of biowaste. The experiment involved composting of organic residues (cotton waste, yard waste and a mixture of these two) in perforated plastic boxes placed outdoors under a roof for 149 days. The highest activity they measured in compost from the mixture of cotton waste and yard waste (approximately 8500 µg p-nitrophenol. g^{-1}. h^{-1}) after 40 days of composting, and the lowest activity was found at the start of experiment in compost from cotton waste (approximately 2000 µg p-nitrophenol. g^{-1}. h^{-1}). Several studies looked at the application of vermicompost to soil and its effect on enzymes in relation to soil fertility. Lazcano and Domínguez (2011) found that the vermicompost fertilized variant had a higher phosphatase content (about 160,000 µg p-nitrophenol. g^{-1}. h^{-1}) than the variant with conventional fertilizer (about 140,000 µg p-nitrophenol. g^{-1}. h^{-1}). Also, in the experiment done by Marinari et al. (2000) and Saha et al. (2008), there was a statistically significant increase in acid phosphatase content after application of vermicompost to the soil. The application of vermicompost to the soil is useful due to its effect on the transformation of organic phosphorus, which is important for the favorable environment for microbes and plant roots (Saha et al., 2008).

With regard to arylsulfatase activity in our experiment, it could be said that the trend across the profile of vermicomposting heap with household biowaste and with malting sludge is similar. However, the decline in activity of arylsulphatase was already in layer II in the vermicomposting heap with household biowaste and in the heap with malting sludge was the decline up to layer III. Arylsulphatase activity was very low throughout the process with all vermicomposting biowastes and values ranged from 13 µmol MUFS. g^{-1}. h^{-1} (layer I, grape marc) to 66 µmol MUFS. g^{-1}. h^{-1} (layer V, household biowaste) (Fig. 3c). There were no significant differences between each layer of all vermicomposting heaps. Mondini et al. (2004) also analysed the activity of arylsulphatase. This activity of arylsulphatase was many times lower than the activities of other enzymes (β-glucosidase, acid phosphatase and alkaline phosphatase), but it increased to day 120 and then decreased to day 160. Arylsulphatase activity was also measured by Růžek et al. (2015) in an experiment with green waste compost (green municipal waste, white high-moor peat, clayey soil and

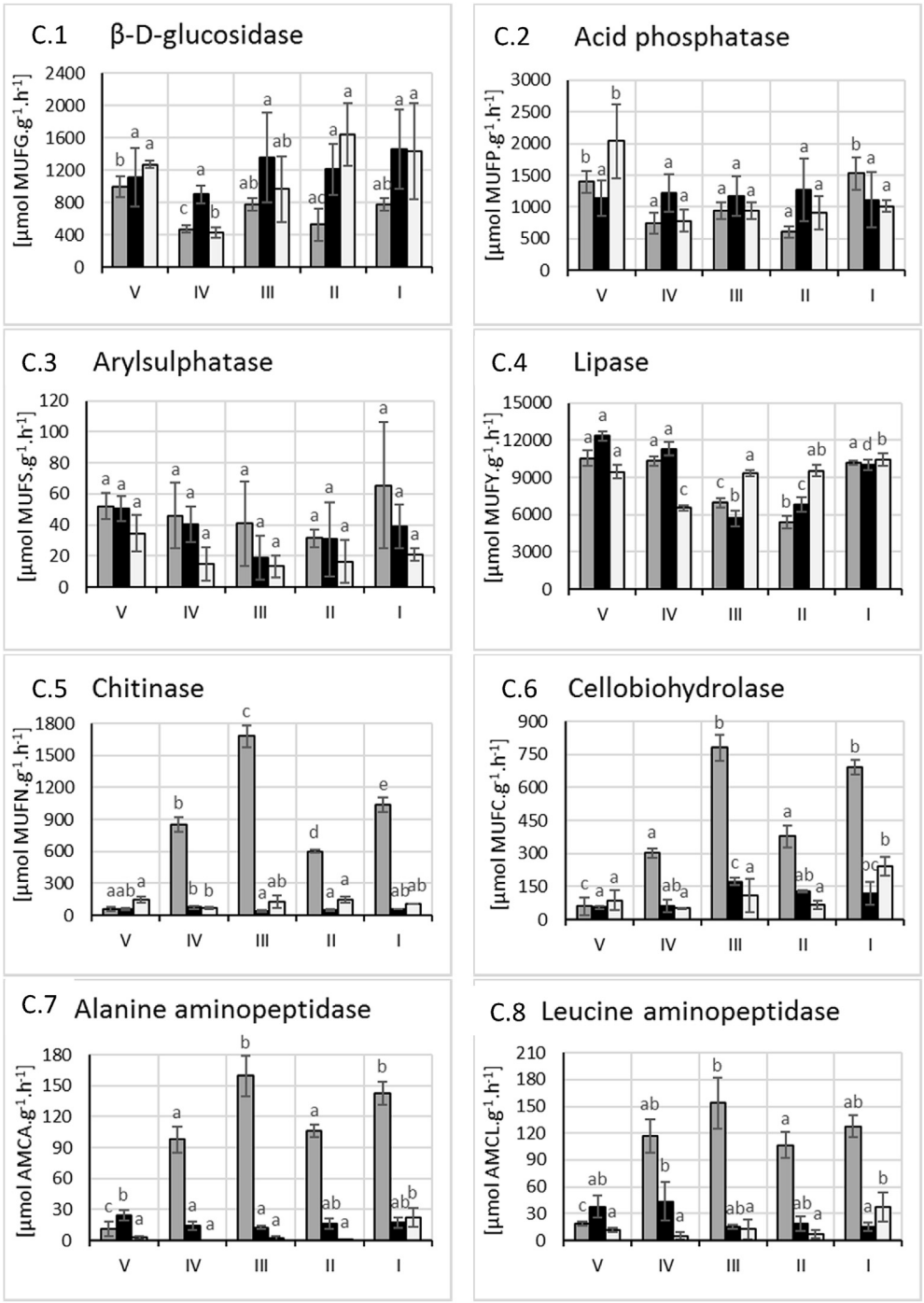

Fig. 3. Enzymatic activity in all layers of vermicomposting heaps. Values are the means ± SD (n = 4). Different letters indicate significant differences (Tukey's HSD test, $P \leq 0.05$). Legend: ▒ Household biowaste; ■ Malting sludge; □ Grape marc.

perlite). They measured the activity in the control variant (fresh compost) and three times during storage of the compost (7–14 d; 15–20 d; 21–147 d). The highest activity they measured after 15–20 days of storage (520 mg p-nitrophenol. h^{-1}. kg^{-1}) and the lowest activity they found was in the control variant (292 mg p-nitrophenol. h^{-1}. kg^{-1}). Tejada et al. (2010) investigated the effect of vermicompost from cow manure and green forage on soil properties. They found, after comparing these two variants, that vermicompost from cow dung has a better effect on soil properties due to the more labile fractions of organic matter. For arylsulfatase, the values were 14.2% higher in the variant with cow manure than in the variant with green forage.

With regard to lipase activity, the vermicomposting process with grape marc showed a trend that was the inverse of that with other vermicomposting processes. Lipase activity was very high in all vermicomposting heaps, ranging from 5382 µmol MUFY. g^{-1}. h^{-1} to 12,341 µmol MUFY. g^{-1}. h^{-1} (Fig. 3d). The lowest value was found in the vermicomposting heap with household biowaste (layer II) and the highest value was found in the heap with. malting sludge (layer V), where was also the highest pH value from every variants

and layers (8,82).

Chitinase is mainly produced by fungi, which were the most active during the vermicomposting process with grape marc, but the highest activity of this enzyme was many times higher in the vermicomposting heap with household biowaste than in others (Fig. 3e). In the case of the heap containing malting sludge, the chitinase values were very low, but there were some significant differences between the layers. The lowest values in the variant with malting sludge can be caused by the lowest values of bacteria and also fungi, which mainly produce the chitinase. Chitinase activity can be suppressed by its products such as glucose and N-acetylglucosamine (Gooday, 1994). The chitinase activity measured by Lee et al. (2016) during their composting process of a mixture of biowaste (pig and chicken manure, spent mushroom, sawdust, rice hull and addition of fertilizers contain Mo, Zn, B, Mn and Cu) decreased with the age of the compost and it ranged from 400 $\mu g\, g^{-1}$ (after 90 d of composting) to 550 $\mu g\, g^{-1}$ (at the beginning of composting).

The highest activity of cellobiohydrolase was in the vermicomposting heap with household biowaste (Fig. 3f). The level of enzymatic activity of cellobiohydrolase across the entire profile has the same trend as chitinase in the case of heap with household biowaste. The high values in household biowaste may be caused by the wide variety of constituent materials. And the highest value in layer III, can be caused by the highest value of EC.

Activity of alanine aminopeptidase was the lowest during the vermicomposting process with grape marc, where in several layers zero activity was even recorded (Fig. 3g). The highest value was measured in the vermicomposting heap with household biowaste (layer III), where was also the highest value of electrical conductivity.

With regard to leucine aminopeptidase, the highest values were 154 μmol AMCL. g^{-1}. h^{-1} for the vermicomposting heap with household biowaste (layer III), 44 μmol AMCL. g^{-1}. h^{-1} for the vermicomposting heap with malting sludge (layer IV) and only 38 μmol AMCL. g^{-1}. h^{-1} for the heap with grape marc (layer I) (Fig. 3h). The highest activity in the variant with household biowaste can be caused by the highest value of electrical conductivity in this layer (layer III).

Correlation and multiple regression analysis of the effect of microorganisms (fungi and bacteria) and earthworms on the individual enzymes were performed. Based on the dependence course (Eq. A), supplemented by the correlation analysis, it can be stated that the activity of β-D-glucosidase is significantly influenced by activity of fungi and bacteria, and also by the number of earthworms. The activity of β-D-glucosidase will be higher if more fungi and earthworms are present in vermicomposting process. In contrast, the indirect dependence was found between β-D-glucosidase and bacterial activity.

$$y = 667.063 + 122.294x_1 - 2.459x_2 + 0.39x_3$$

Eq. A: The course of β-D-glucosidase activity (y) on fungal (x_1) and bacterial (x_2) activity and on number of earthworms (x_3) in the vermicomposting process with household biowaste. R square is 44.8%.

In the cases of the vermicomposting process with malting sludge and agricultural waste and the vermicomposting process with grape marc, the activity of β-D-glucosidase also appears to be influenced by all selected biological parameters (fungal and bacterial activity and the number of earthworms) (Eq. B, Eq. C). In both processes, increased activity of β-D-glucosidase occurred when there was also increased activity of fungi and the number of earthworms. The activity of β-D-glucosidase decreases when bacterial activity increases (assuming fungal activity and the number of earthworms is constant).

$$y' = 800.219 + 2.359x_1 + 26.153x_2 - 5.05x_3$$

Eq. B: The course of the β-D-glucosidase activity (y) on fungal (x_1) and bacterial (x_2) activity and on number of earthworms (x_3) in the vermicomposting process with sludge from malt house and agricultural waste. R square is 33.3%.

$$y' = 2282.787 + 16.171x_1 - 28.747x_2 + 2.349x_3$$

Eq. C: The course of the β-D-glucosidase activity (y) on fungal (x_1) and bacterial (x_2) activity and on number of earthworms (x_3) in the vermicomposting process with grape marc. R square is 39.3%.

Based on the other dependence courses (other equations) and the correlation analysis, it is clear that the activity of acid phosphatase is positively influenced by the activity of bacteria and fungi in the vermicomposting heap with household biowaste, and by the earthworms during vermicomposting of grape marc, marked significant dependence was reported. In the case of arylsulphatase activity, very low R square indexes were found in all vermicomposting heaps. Only in the heap containing grape marc did the earthworms show statistically significant direct dependence. The activity of lipase was very highly influenced by bacterial activity during the vermicomposting process of household biowaste and by fungal activity during the process of vermicomposting of a mixture of malting sludge and agricultural waste. No statistically significant dependencies were found in the vermicomposting heap with grape marc. The activity of chitinase was negatively influenced by all parameters in the vermicomposting heap with household biowaste, but in the case of vermicomposting with malting sludge, direct dependence was found between the activity of chitinase and the number of earthworms. Activity of fungi had a significant negative effect on cellobiohydrolase activity in the case of vermicomposting of household biowaste or mixture with malting sludge. In the case of vermicomposting grape marc, only weak dependencies were found. Activity of alanine aminopeptidase was very highly influenced by the activity of fungi in all vermicomposting heaps, but only in the case of vermicomposting the mixture of malting sludge was the dependence direct. Also, the activity of leucine aminopeptidase was influenced by activity of fungi, but only during the vermicomposting of household biowaste and the mixture with malting sludge. In the case of vermicomposting of household biowaste, dependencies were found between all biological parameters and the activity of leucine aminopeptidase. The vermicomposting heap with grape marc showed no statistically significant dependencies.

4. Conclusions

The highest activity of all measured enzymes occurred in the vermicomposting process with household biowaste. The highest contents of β-D-glucosidase (994–1272 μmol MUFG. g^{-1}. h^{-1}), acid phosphatase (1139–2036 μmol MUFG. g^{-1}. h^{-1}), arylsulphatase (35–52 μmol MUFS. g^{-1}. h^{-1}) and lipase (9466–12341 μmol MUFY. g^{-1}. h^{-1}) were found in the youngest layers. On the other hand, the highest activities of chitinase (1681 μmol MUFN. g^{-1}. h^{-1}), cellobiohydrolase (782 μmol MUFC. g^{-1}. h^{-1}), alanine (160 μmol AMCA. g^{-1}. h^{-1}) and leucine aminopeptidase (154 μmol AMCL. g^{-1}. h^{-1}) were significantly higher in the vermicomposting heap with household biowaste. The enzyme values show that the most active vermicomposted material was the household biowaste, due to the heterogeneity of organic waste. Therefore, this waste appears to be most suitable for soil application. Vermicompost from domestic biowaste should best facilitate the rapid decomposition of organic

compounds into forms acceptable to plants, due to the high enzymatic activity. The least active was malt house sludge. Even so, it can be concluded that the activity of the enzymes was sufficient throughout the process and that the vermicomposting process proceeded correctly in all variants. Based on the dependence courses that were found, some enzymes are positively influenced by the number of earthworms. However, this model is only theoretical and the question of its functionality in practice should be considered. This information is in demand by people interested in vermicomposting and is useable in practice.

A well-run system of vermicomposting household biowaste, malt house sludge and grape marc in site is almost maintenance-free, low-cost and ecological, thus contributing to cleaner production.

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