Vermicomposting of distillery residues in a vertical-flow windrow system

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Abstract

Improper handling of distillery residues can have negative impacts on the environment. The present study evaluated the feasibility and processes occurring in a vertical-flow windrow vermicomposting system of distillery residues together with wheat straw. There were differences between the top and lower layers. The top and so youngest layer showed the greatest humidity and electrical conductivity among the layers. It was characterized by partially decomposed organic matter with a great amount of earthworm biomass (2.5 g kg^{-1}), which was confirmed by parameters such as C_{tot} (34%), N_{tot} (2.25%), and C/N (15.3). On the other hand, the lower layers were characterized by greater maturity, which was documented by a lesser content of microbial biomass and activity of hydrolytic enzymes, as well as a slightly alkaline pH (7.6-7.9), and lesser values for $N\text{-}NH_4^+$ (22-84 mg kg^{-1}) and dissolved organic carbon (5228-6564 mg kg^{-1}), which was indirectly proportional to the ion-exchange capacity (57-60 $mmol_+$ $100g^{-1}$). Among the examined macroelements, potassium showed the greatest content. The total contents of P and Mg increased directly with the age of the vermicomposted material, which was related to the loss of organic matter. The proportion of the available contents of P, K, and Mg constituted on average in all of the layers 11%, 64%, and 10%, respectively, of the total content. On the basis of the detected parameters, the top layer is suitable for a new windrow and for the preparation of aqueous extracts. The older layers are suitable for use as an organic fertilizer.

Keywords: Distillery residues, vermicomposting, layers, chemical and biological properties

Introduction

The increasing trend of environmental protection contributes to the growth of demand for alternative sources. The trend of recent years is the addition of bioethanol to fuels in order to reduce oil consumption. As a result, there is an increase in the number and capacity of distilleries. Biomass suitable for the production of bioethanol is divided into three groups: biomass containing simple sugars (e.g. fruit crops, sugar beet, and cane), the biomass-containing starch (e.g. cereals and potatoes), and lignocellulosic biomass (e.g. straw, energy crops, and biowaste) [1]. Distillery residues are the main waste product of ethanol production [2]. In the production of 1 L of ethanol, 15 to 20 L of distillery residues are produced [3]. The handling of distillery residues can cause environmental problems due to their seasonal production and polluting characteristics. Direct discharge of effluent into watercourses poses a serious threat to aquatic organisms. High chemical oxygen demand values and great nitrogen and phosphorus contents can lead to eutrophication of watercourses. At the same time, the coloration of fragments can reduce light transmission, which leads to inhibition of photosynthetic activity and depletion of dissolved oxygen in water [4]. Direct application of distillery residues into the soil may be problematic due to their inappropriate physico-chemical properties, especially low pH [5]. It causes inhibition of seed germination and depletion of vegetation by reducing the soil alkalinity and the availability of some nutrients, if discharged without adequate treatment [6]. Effective treatment can be achieved by using various physical, chemical, and/or biological treatment processes, either alone or in combination [7-9].

The vermicomposting of distillery residues is one of the possible solutions for handling this feedstock. Vermicomposting is an environmentally friendly technology using earthworms [10]. Compared to the feedstock and conventional compost, vermicompost contains increased and more soluble levels of major nutrients and organic matter with improved quality [11].

At this time, there do not appear to be any scientific studies on the vermicomposting of distillery residues. The aim of the study was to evaluate the feasibility of vermicomposting of distillery residues under outdoor conditions. The study sought to contribute to the understanding of the processes occurring in a vertical-flow windrow vermicomposting system.

Materials and methods

Feedstocks

The composition of the distillery residues corresponded to the just processed fruit in a grower distillery. The type of fruit was based on the growing season. The distillery processed mostly apples, pears, plums, and cherries. Distillery residues were stored in an underground tank, so a mixture with 5.7% dry matter was therefore applied. The pH value ranged in the acidic area (on average 4.9) and the electrical conductivity reached 480 μS cm^{-1}. For the experiment, dry wheat straw from compacted bales with 90% dry matter content was used. Selected agrochemical parameters of the feedstocks are shown in Table 1.

Table 1. Selected agrochemical parameters of the feedstocks used (pH and EC were determined in wet matter; other parameters in dry matter)

	Dry matter [%]	pH/H_2O	EC [mS/cm]	$C_{tot.}$ [%]	$N_{tot.}$ [%]	C/N	$P_{tot.}$ [%]	$K_{tot.}$ [%]	$Ca_{tot.}$ [%]	$Mg_{tot.}$ [%]
Distillery residues	5.7	4.9	0.48	45	2.2	20	0.25	1.61	0.75	0.17
Straw	90	7.4	1.5	46	0.6	77	0.02	0.47	0.28	0.03

Description of experiment

The experiment was set up under operating conditions at a family grower distillery in Cesov, Czech Republic (N 50°20.33638', E 15°21.89653'). The vermicompost pile occupied a ground plan 6 x 8 m. The bedding layer consisted of precomposted beef manure and grape marc with earthworms (*Eisenia andrei*), with a density of about 50 earthworms per liter, and was placed first on a flat surface. Wheat straw layers were added at half-year intervals. Distillery residues were applied on these layers every two weeks. Due to the influence of precipitation, the application of distillery residues, and the transformation of organic matter, the layers settled. After 2 years from the beginning of the experiment, samples were taken up from cross profiles. Sampling of each layer was carried out in 4 replications. The depth placement above the bedding layer and the age of each layer were as follows:

IV: 0-30 cm, 0-6 months

III: 31-60 cm, 6-12 months

II: 61-90 cm, 12-18 months

I: 91-120 cm, 18-24 months

Potential earthworms were separated, counted, weighted, and lyophilized. The resulting vermicompost sample without earthworms was divided into 3 parts and treated as required for laboratory analyses. One part of the vermicompost sample was stored at 4°C until the pH and electrical conductivity (EC) could be determined. The second part was dried at 30°C to a constant weight and ground. This was then used for analyses of the total and available contents of elements and the ion exchange capacity (IEC). The third part of the vermicompost sample was frozen at -20°C and then lyophilized for subsequent determination of the groups of microorganisms by the PLFA method and for enzyme activity.

Chemical and biological analyses

Measurements of active pH and EC were conducted on samples mixed with deionized water (1:5 w/v wet basis) using a WTW pH 340 i and Testo 240, respectively according to EN 13037 [12]. Total carbon (C_{tot}) and nitrogen (N_{tot}) were determined using the CHNS Vario MACRO cube analyzer (Elementar Analysensysteme GmbH, Germany). In this instrument, about 25 mg of the sample was burned in a catalytic furnace, and subsequently C_{tot} and N_{tot} were determined by using a thermal conductivity detector. The total contents of P, K, and Mg were determined by decomposition utilizing a wet method in a closed system with microwave heating using an Ethos 1 microwave system (MLS GmbH, Germany). The contents of ammonium nitrogen ($N\text{-}NH_4^+$), dissolved organic carbon (DOC), and the available portions of P, K, and Mg were determined in calcium chloride/DTPA (CAT) solution (0.01mol l^{-1} $CaCl_2$ and 0.002 mol l^{-1} diethylene triamine pentaacetic acid (DTPA)) at a ratio of 1:10 (w/v) according to the International BSI Standard EN 13651 [13]. The NH_4^+-N and NO_3^--N contents in the extracts were measured colorimetrically using a SKALAR SANPLUS SYSTEM®. The element concentrations were determined using inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia) with axial plasma configuration. The ion-exchange capacity (IEC) was determined conductometrically as described by Váchalová et al. [14]. Samples for the phospholipid fatty acid (PLFA) analysis were extracted in triplicate using a mixture of chloroform, methanol, and phosphate buffer

(1:2:0.8; v/v/v) according to Bligh and Dyer [15]. The extracts were analyzed by tandem gas chromatography–mass spectrometry (GC-MS; 450-GC, 240-MS Varian, Walnut Creek, CA, USA). Methylated fatty acids were identified according to their mass spectra using a mixture of chemical standards obtained from Sigma-Aldrich, Prague, Czech Republic and Matreya LLC, USA. Bacteria were determined on the basis of 17:0, 16:1 ω 9, 15:0 and 16:1 ω 7. Biomass gram positive (G+) bacteria were quantified as the sum of i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0. The gram negative (G-) bacteria were determined on the basis of 16:1ω7, 18:1ω7, cy17:0, cy19:0, 16:1ω5. 10Me-17:0, 10Me-18:0, 10Me-16:0 were used for actinobacteria and 18:2ω6,9 for fungi. The total biomass was quantified as the sum of all of the markers together with 16:0 and 18:1ω9 [16]. The labeling is typical for this research field [17]. The activities of the hydrolytic enzymes such as β-D-glucosidase, phosphatase, sulfatase, lipase, chitinase, cellobiohydrolase, alanine aminopeptidase, and leucine aminopeptidase were quantified by fluorescence detection according to Baldrian [18] and Štursová and Baldrian [19]. Briefly, the prepared suspension (0.2 g of lyophilized sample and 20 ml of 50 mmol L^{-1} acetate buffer with pH 5) was homogenized by the Ultra-Turrax instrument. Then, it was pipetted into the appropriate well in microtiter plates and the addition of the relevant substrate followed. The microtiter plates were placed in an incubator heated to 40°C for 5 minutes. Subsequently, the fluorescence of the substrate was measured using the Tecan Infinite® M200 instrument. For urease activity determination, the method according to Kandeler and Gerber [20] was applied. It is based on ammonia determination after incubation of samples with urea. Determination of nitrate reductase activity was carried out according to Kandeler [21], and it is based on nitrite determination after incubation of vermicompost with nitrate. The effect of nitrite reductase is inhibited by the addition of 2,4 - dinitrophenol.

Statistical analysis

Statistical analyses were performed using the STATISTICA 13.2 software (StatSoft, Tulsa, Oklahoma USA). A one-way ANOVA using a 95% confidence level followed by Tukey's test was performed. Spearman's correlations were explored between the agrochemical and biological parameters at the 0.05 probability levels.

Results and discussion

Agrochemical parameters

The basic agrochemical parameters are shown in Table 2.

Table 2. Basic agrochemical parameters of the layers in the large-scale vertical-flow windrow vermicomposting system. Values are the means ± SD (n=4). Different letters in a column indicate significant differences (Tukey's HSD test, $P \leq 0.05$).

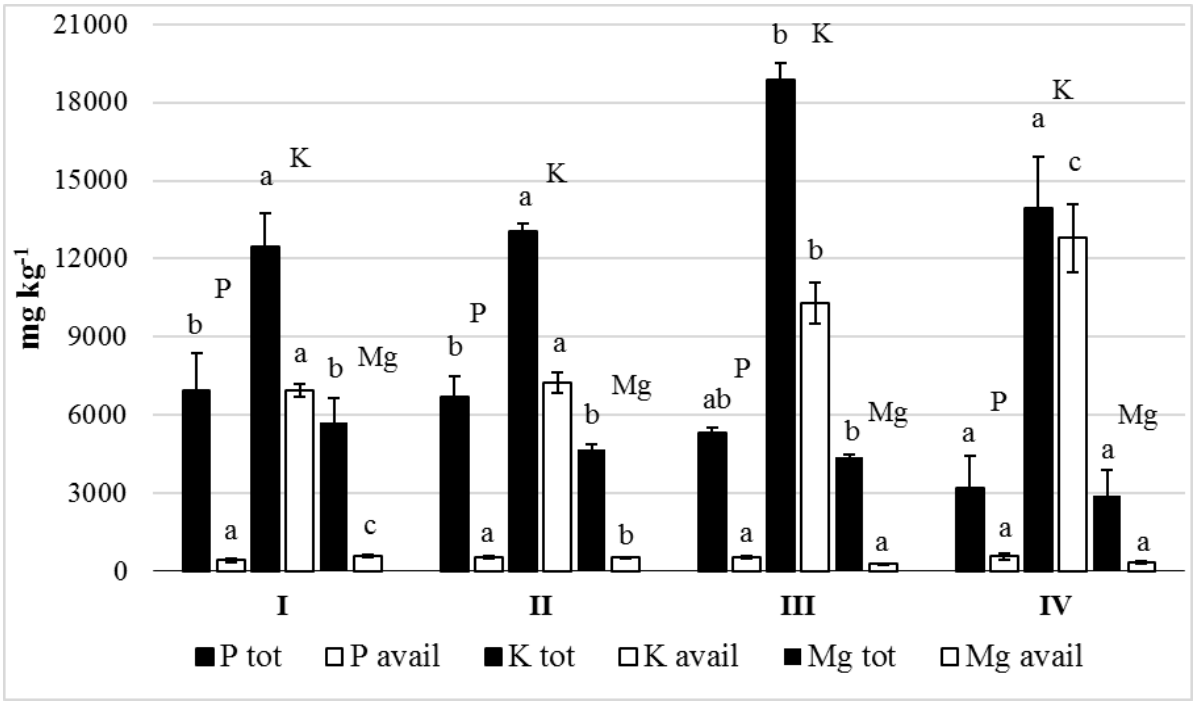
Layer	Dry matter [%]	pH/H_2O	EC [μS cm^{-1}]	C_{tot} [%]	N_{tot} [%]	C/N
IV	15.3 ± 2.42 [a]	7.41 ± 0.07 [a]	1162 ± 644 [a]	34.01 ± 5.45 [a]	2.25 ± 0.35 [a]	15.30 ± 2.80 [b]
III	25.8 ± 1.52 [b]	7.72 ± 0.04 [b]	976 ± 39 [a]	29.93 ± 1.35 [a]	2.37 ± 0.09 [a]	12.64 ± 0.76 [a]
II	25.9 ± 1.38 [b]	7.64 ± 0.10 [b]	683 ± 51 [a]	31.94 ± 3.08 [a]	2.73 ± 0.25 [a]	11.72 ± 0.55 [a]
I	28.3 ± 0.65 [b]	7.92 ± 0.09 [c]	760 ± 23 [a]	28.45 ± 2.42 [a]	2.62 ± 0.17 [a]	10.85 ± 0.24 [a]

IV: 0-30 cm, 0-6 months; III: 31-60 cm, 6-12 months; II: 61-90 cm, 12-18 months; I: 91-120 cm, 18-24 months

The dry matter increased with the age of the layers. The least dry matter content was found in the top layer. It was caused by the high humidity of the applied distillery residues (94.3%) and by exposure of the surface of the pile to weather conditions. The dry matter in the other layers was on average 1.75 times greater, with differences between the layers not statistically significant. The pH values in the vermicomposting pile ranged from 7.45 to 7.92. The greatest value was measured at the oldest layer. An increase in the pH value was observed in the direction from the youngest layer to the oldest. This can be caused by the fact that the microorganisms and earthworms which were present degraded the organic acids and partially consumed them. These values also indicate that the earthworms were able to increase the pH of the substrate from acidic values (the pH of distillery residues was 4.9) to neutral or slightly alkaline values by the gradual transformation of the organic matter. This corresponds to Singh et al. [22], who vermicomposted mixed plant residues with an initial pH of 4.3-6.9. They found a neutral pH in all treatments after 30 days of vermicomposting. The EC values in the layers did not differ significantly, nevertheless they showed a decreasing trend. A possible explanation may be the gradual leaching of salts from the layers. The total carbon content showed the greatest values in the youngest layer and showed a gradual decrease to the least value in the oldest layer. This is due to the gradual loss of volatile solids in the form of

CO_2.The amount of total nitrogen gradually increased with the age of the layers. However, the measured values did not show a statistically significant difference. Talashilkar et al. [23] attributes an increase in N_{tot} to the gradual addition of nitrogen by earthworms in the form of slime containing nitrogenous substances. The relatively low C/N value in the top layer indicates a rapid transformation of organic matter through the earthworms. The decrease in the C/N value with the age of the vermicomposted material (from 15.3 to 10.9) is consistent with the observation by Hanc et al. [24], who found a similar decrease in C/N (from 16.1 to 10.4) in a vertical-flow windrow vermicomposting system of household biowaste. Torres-Climent et al. [25] who co-composted winery-distillery wastes with animal manure, observed a decrease in the C/N from 21.9 to 13.5. Among the examined macroelements, potassium showed the greatest content (Figure 1).

Figure 1. Changes in the total and available P, K, and Mg (mg kg^{-1}) in layers I-V of the large-scale vertical-flow windrow vermicomposting system. The values are the means ± SD (n=4). Different letters above the bars within the same element denote significant differences (Tukey´s HSD test, $P<0.05$).



The total contents of P and Mg increased directly with the age of the vermicomposted material, which was related to the loss of organic matter. The average contents of P_{tot} and Mg_{tot} increased in the bottom layer 2.16-fold and 1.98-fold, respectively, compared to the top

layer. The behavior of K_{tot} was different because the bottom layer contained 10% less K_{tot} than the top layer. In the case of available contents, an increase was found in the content of Mg_{avail} (1.83-fold) and a decrease in the contents of P_{avail} (0.72-fold) and K_{avail} (0.54-fold). The proportion of the available contents of P, K, and Mg constituted on average in all of the layers 11%, 64%, and 10%, respectively, of the total content. These proportions found during the vermicomposting of distillery residues with straw were less than for grape marc vermicomposting (P = 20-42%, K = 65-79%, and Mg = 11-13%) as reported by Castkova and Hanc (2017) [26].

Parameters of maturity

In this experiment, the maturity of vermicompost was evaluated by parameters such as $N\text{-}NH_4^+$, DOC, IEC, and IEC/C_{tot} (Table 3).

Table 3. Effect of depth and age of the profile on the selected maturity indicators. Values are the means ± SD (n=4). Different letters in a column indicate significant differences (Tukey's HSD test, $P \leq 0.05$).

Layer	$N\text{-}NH_4^+$ [mg kg^{-1}]	DOC [mg kg^{-1}]	IEC [$mmol_+$ $100g^{-1}$]	IEC/C_{tot}
IV	162.05 ± 54.45 ^{c}	8799 ± 983 ^{c}	55.0 ± 3.27 ^{a}	1.64 ± 0.23 ^{a}
III	83.66 ± 21.62 ^{b}	6564 ± 379 ^{b}	60.2 ± 2.87 ^{a}	2.01 ± 0.10 ^{b}
II	21.27 ± 4.23 ^{a}	5347 ± 183 ^{a}	71.2 ± 7.37 ^{b}	2.23 ± 0.09 ^{b}
I	22.30 ± 1.92 ^{ab}	5228 ± 320 ^{a}	57.2 ± 3.20 ^{a}	2.02 ± 0.19 ^{b}

IV: 0-30 cm, 0-6 months; III: 31-60 cm, 6-12 months; II: 61-90 cm, 12-18 months; I: 91-120 cm, 18-24 months

A typical feature of immature vermicompost is greater contents of $N\text{-}NH_4^+$and DOC, and conversely lesser values for IEC and IEC/C_{tot}. This phenomenon was confirmed by values in the youngest layer IV, which was subject to the intense process of vermicomposting. With the exception of the IEC parameter, layer IV differed statistically from the other layers. A great content of $N\text{-}NH_4^+$ in organic fertilizer can have detrimental effects on the germination and root development of plants. Thompson et al. (2003) [27] set a limit value of $N\text{-}NH_4^+$ for mature compost of less than 75 mg kg^{-1}, which was met by the oldest layers I and II. DOC as the most active fraction of carbon decreased with the age of the layers. The proportion of DOC in the C_{tot} fluctuated between 1.7 to 2.6%. These two parameters

positively correlated with each other ($R = 0.55$, $p < 0.05$). The greatest value of DOC was found in the youngest layer IV (0.88%). The differences among layers IV, III, and II were significant. On the other hand, differences between the older layers II and I were non-significant, which indicates completion of the transformation processes. In these layers, 5.3 g of DOC kg^{-1} was found, which is close to 4 g kg^{-1} and below the 10 g kg^{-1} recommended by Zmora-Nahum et al. [28] and Hue and Liu [29] as a limit for mature compost. Mineralization and humification may result in a change in ion-exchange properties, which can be quantified by a parameter known as IEC. The IEC had an increasing trend from layer IV (55 $mmol_+$ /100 g^{-1}) to layer II (71 $mmol_+$ / 100 g^{-1}). This layer was the only one statistically different from the other layers. The IEC in layer I was 57 $mmol_+$ /100 g^{-1}, which could be explained by some transfer of a bedding layer consisting of precomposted beef manure and grape marc by earthworms to layer I at the beginning of the experiment. Taking into account the mineralization of organic matter the IEC/C_{tot} showed a significant difference between the youngest layer IV (1.6) and layer III (2.0). Hanc et al. (2017) [24] found that the IEC and IEC/C_{tot} during the vermicomposting of household biowaste were in the range of 52-60 $mmol_+$ / 100 g^{-1} and 2.4-3.5, respectively.

Earthworms

As is evident from Table 4, the greatest number of earthworms was found in the youngest layer (5.9 pcs/kg), and the lowest number (1.4 pcs/kg) was in the bottom layer, because this layer was already decomposed.

Table 4. Quantitative (number and biomass) and nutrient parameters of the earthworms in the windrow layers. Values are the means ± SD (n=4). Different letters in a column indicate significant differences (Tukey's HSD test, $P \leq 0.05$).

Layer	Number [in 1 kg]	E. Biomass [g kg^{-1}]	P_{tot} [mg kg^{-1}]	K_{tot} [mg kg^{-1}]	Mg_{tot} [mg kg^{-1}]
IV	5.9 ± 1.9 ^{a}	2.5 ± 0.8 ^{b}	5776 ± 855 ^{a}	3201 ± 777 ^{a}	696 ± 156 ^{a}
III	5.8 ± 2.2 ^{a}	2.6 ± 1.0 ^{b}	6904 ± 1062 ^{a}	6434 ± 1183 ^{a}	1447 ± 264 ^{a}
II	1.6 ± 1.7 ^{b}	0.5 ± 0.7 ^{a}	9036 ± 1517 ^{b}	7929 ± 2603 ^{b}	2186 ± 893 ^{b}
I	1.4 ± 0.6 ^{c}	0.4 ± 0.2 ^{a}	7226 ± 481 ^{a}	5548 ± 398 ^{a}	1514 ± 98 ^{a}

IV: 0-30 cm, 0-6 months; III: 31-60 cm, 6-12 months; II: 61-90 cm, 12-18 months; I: 91-120 cm, 18-24 months

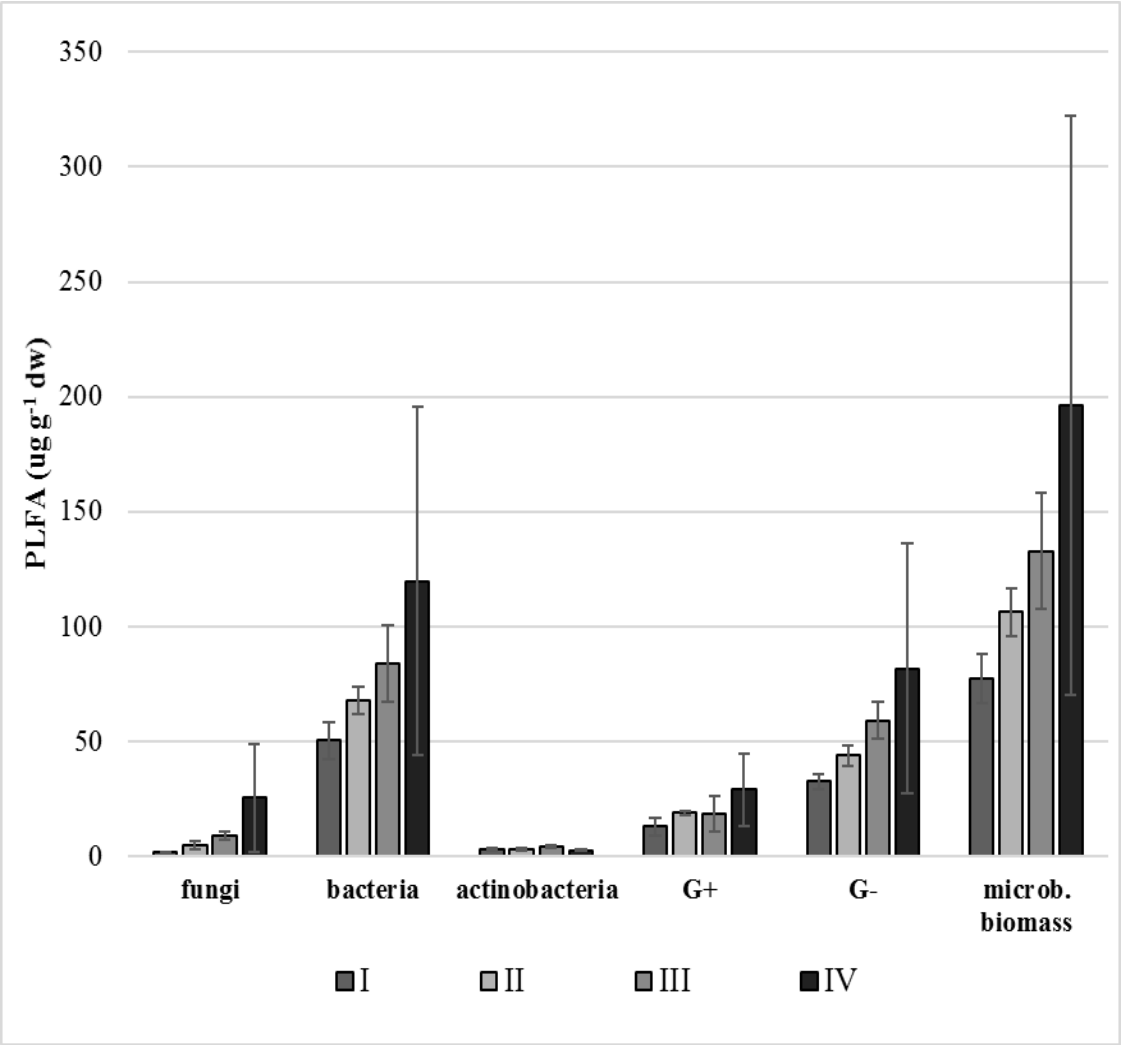
The statistically non-significant differences were found between layers III (5.8 pcs/kg) and IV (5.9 pcs/kg). The greatest biomass of earthworms was in the two top layers. The average weight of one earthworm ranged from 0.29 to 0.45 g.

The earthworms are able to accumulate nutrients from the substrate. They also contain nutrients in their urine and mucus [30]. The total contents of nutrients in earthworms is shown in Table 4. The greatest total contents of phosphorus (9036 mg/kg), potassium (7929 mg/kg), and magnesium (2186 mg/kg) were measured in layer II, which exhibited a lesser earthworm density, and which also showed statistically significant differences to the other layers. The least contents of these nutrients were found in layer IV, which was also the youngest layer. It can be stated that with the age of the layers, the contents of the observed elements in the earthworms increased. According to the Pearson`s correlation coefficient, a positive correlation was found between the total Mg content in the vermicompost and in the earthworms. This coefficient increased from layer I ($R=0.47$, $p<0.05$) to layer IV ($R=0.97$, $p<0.05$), where the greatest number of earthworms was counted. For P content, a correlation coefficient was greater than $R=0.5$, $p<0.05$ for layer I, II, and IV. Only for layer III was the Pearson`s correlation coefficient negative. In the case of K content, a negative correlation was found for all the layers except layer II, where the correlation was slightly positive ($R=0.28$, $p<0.05$). However, movement of earthworms through the profile, leakage, and the loss of organic matter must be taken into account.

Microorganisms

The total microbial biomass was very great during the whole process as it is illustrated in Figure 2. The microbial biomass decreased from 196 to 97 μg g^{-1}dw, directly proportional with the age of the layers. This is in accordance with the study by Aria et al. [31], who found that the earthworms significantly increased microbial activity in younger modules. The content of microorganisms was proportional to the presence of earthworms, which confirms that vermicomposting is the interaction of these two groups of organisms [32]. The oldest three layers showed significantly greater contents of bacteria than fungi. In layer IV, a greater content of bacteria than fungi was found, but this result was not significantly different, because this layer exhibited a great standard deviation caused by the heterogeneity of the material.

Figure 2. Changes in fungal PLFAs, bacterial PLFAs, and total microbial PLFAs biomass in the layers of the vertical-flow windrow vermicomposting of distillery residues. Values are the means ± SD (n=4).



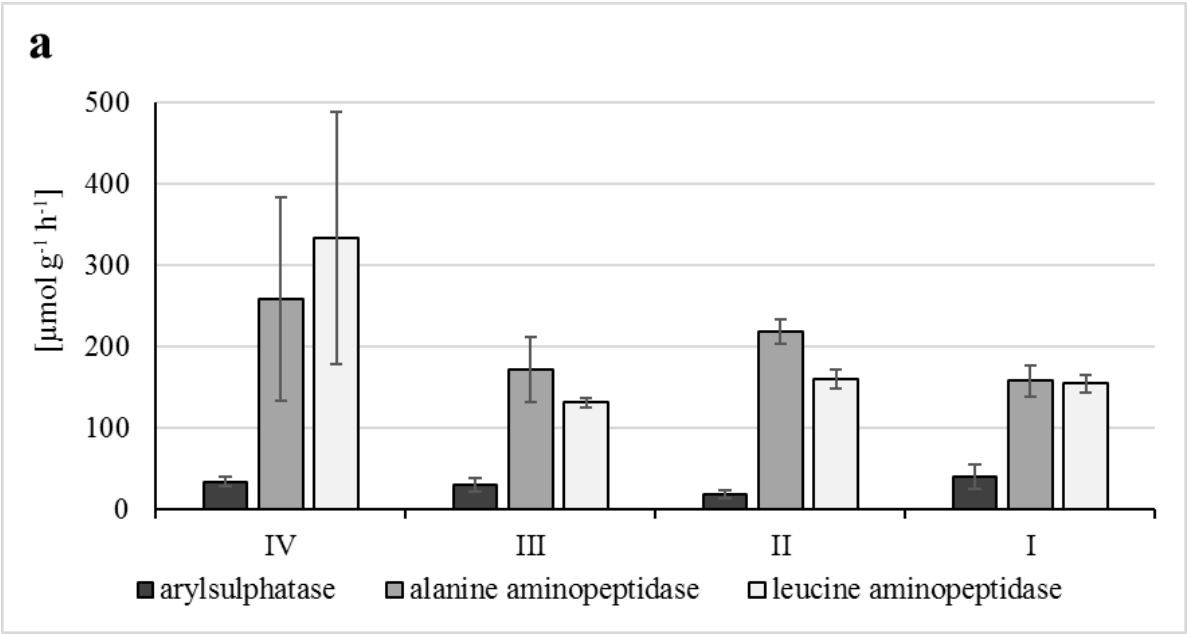
Gómez-Brandón et al. [33] vermicomposted rabbit manure for 250 days using the earthworms *Eisenia fetida*. They found the greatest total microbial activity and the greatest bacterial activity after 100 days of vermicomposting. The greatest value for fungal activity was measured in their first uptake after 50 days of vermicomposting. In the current study, the greatest bacterial/fungal ratio was found in the oldest layer I (32.5), whereas the greatest content of both microorganisms (bacterial PLFAs 119.7 μg g^{-1} dw; fungal PLFAs 25.4 μg g^{-1} dw) and the smallest ratio was observed in the youngest layer IV (4.7). The dominant microorganisms were bacteria, especially G- bacteria, then G+ bacteria, followed by fungi and finally actinobacteria, which was almost suppressed. These finding correspond with the vermicomposting of grape marc done by Castkova and Hanc [26], but in their type of

vermicompost the activities were lower. Also Fernández-Gómez et al. [34] found the same order the activity of microorganisms as we did: bacteria (43 nmol PLFA g^{-1}), G- bacteria (19 nmol PLFA.g^{-1}), G+ bacteria (18 nmol PLFA g^{-1}), fungi (8 nmol PLFA g^{-1}), and actinomycetes (4 nmol PLFA g^{-1}) after 24 weeks of vermicomposting plant waste mixed with paper-mill sludge (2:1) using *E. fetida*. In the current experiment, for most microorganisms the activity decreased during the process. Only in the case of actinobacteria was a decreasing trend with the age of the layers not observed. In this case, the greatest activity was in layer III (4.4 μg g^{-1} dw) and the least activity was in the youngest layer (2.6 μg g^{-1} dw).

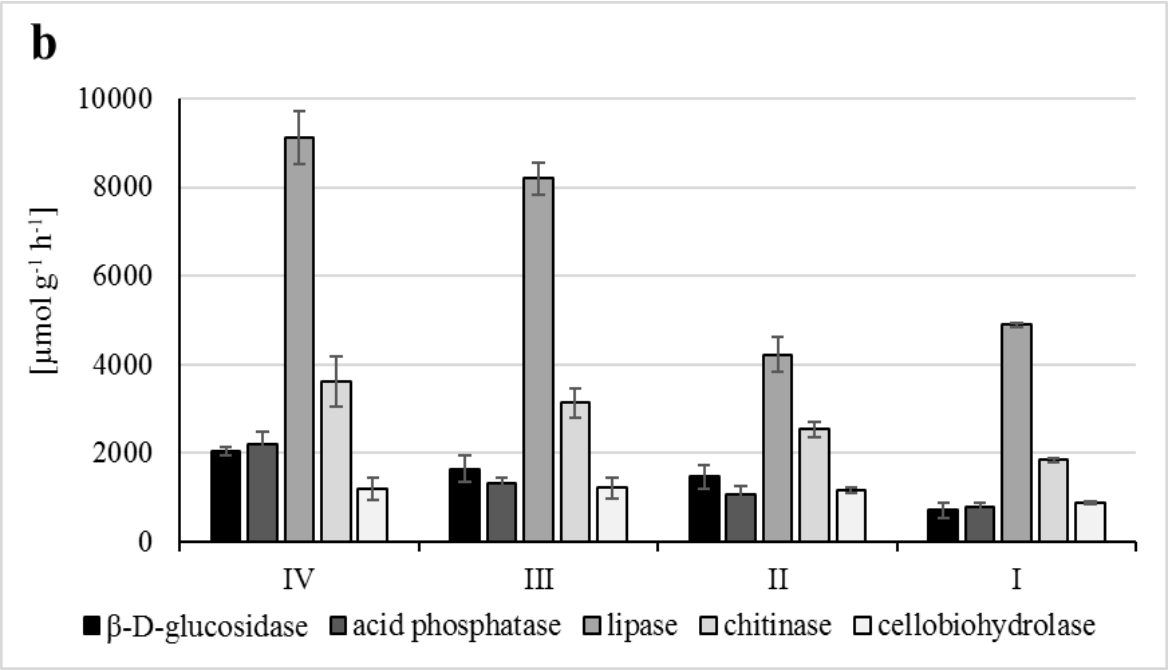
Enzyme activity

The activity of 10 enzymes is illustrated in Figure 3.

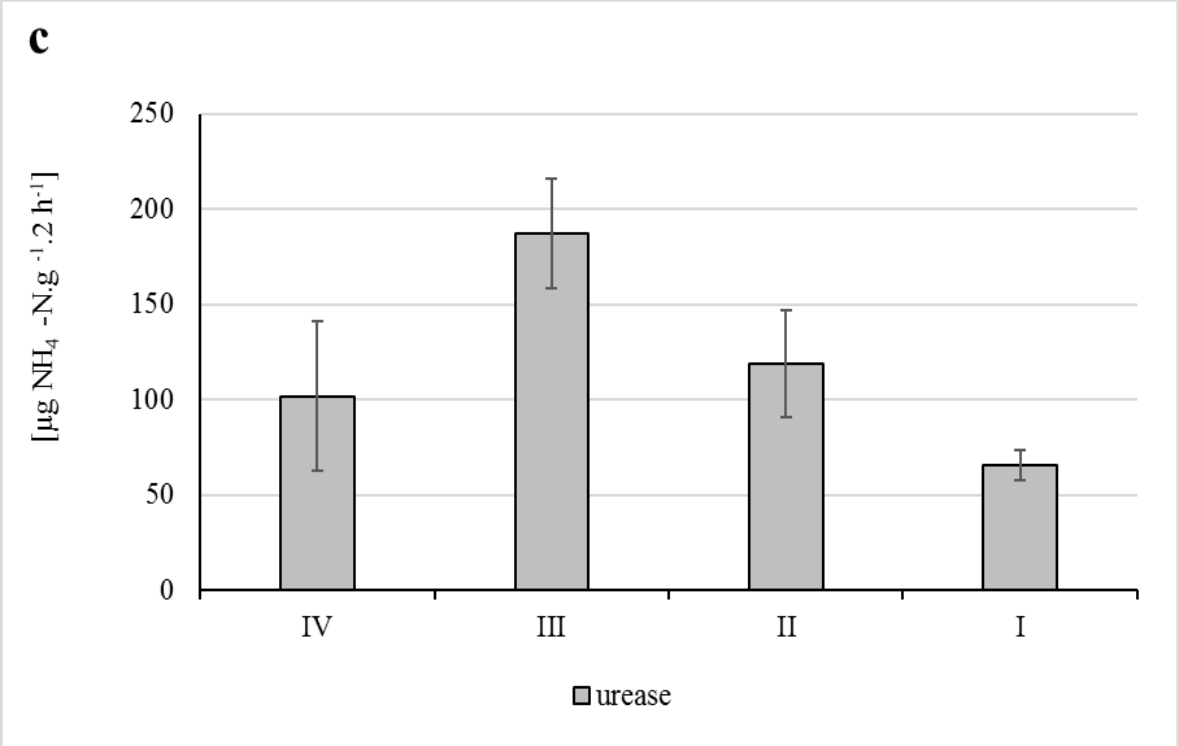
Figure 3. Enzymatic activity of arylsulfatase, alanine aminopeptidase, leucine aminopeptidase (a), β-D-glucosidase, acid phosphatase, lipase, chitinase, cellobiohydrolase (b), urease (c), and nitrate reductase (d) in all layers during vermicomposting. The values are the means ± SD (n=4).

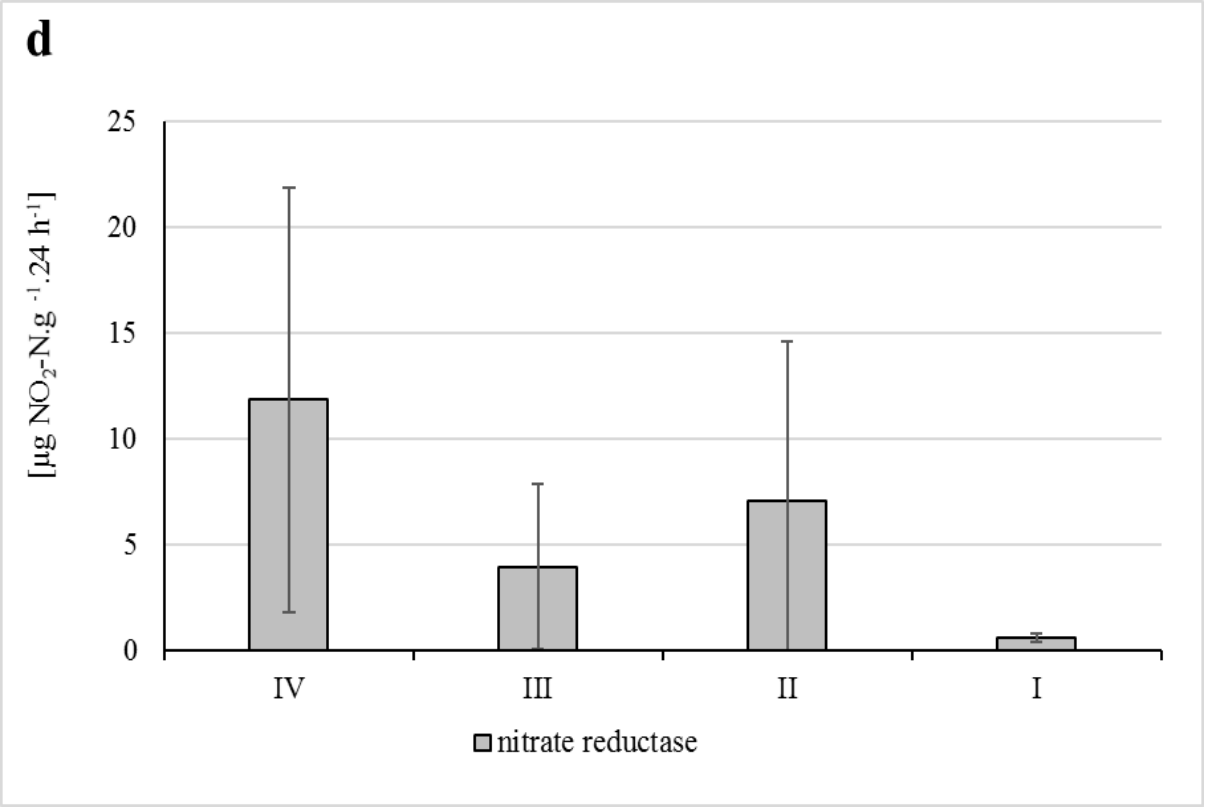


Units are μmol of specific substrate $g^{-1}.h^{-1}$. Substrates for arylsulphatase: 2.50 mmol L^{-1} 4-methylumbellyferyl sulphate potassium salt (MUFS); alanine aminopeptidase: 2.50 mmol L^{-1} L-alanine-7-amido-4-methylcoumarin (AMCA); leucine aminopeptidase: 2.50 mmol L^{-1} leucine-7-amido-4-methylcoumarin (AMCL).



Units are µmol of specific substrate $g^{-1}.h^{-1}$. Substrates for β-D-glucosidase: 2.75 mmol L^{-1} 4-methylumbellyferyl-β-D-glucopyranoside (MUFG); acid phosphatase: 2.75 mmol L^{-1} 4-methylumbellyferyl-phosphate (MUFP); lipase: 2.50 mmol L^{-1} 4-methylumbellyferyl-caprylate (MUFY);chitinase: 1.0 mmol L^{-1} 4-methylumbellyferyl-N-acetylglucosaminide (MUFN); cellobiohydrolase: 2.50 mmol L^{-1} 4-methylumbellyferyl-N-cellobiopyranoside (MUFC).





For arylsulphatase, it cannot be conclusively shown that there were statistically significant changes. This activity was very little and ranged from 39.3 µmol MUFS g^{-1} h^{-1} to 18.0 µmol MUFS g^{-1} h^{-1}. The alanine and leucine aminopeptidase showed the greatest activity in the upper layer (IV), but the least activity was measured in layer I (157.7 µmol AMCA g^{-1} h^{-1}) for the alanine aminopeptidase and in the layer III (131.2 µmol AMCL g^{-1} h^{-1}) for the leucine aminopeptidase. The greatest β-D glucosidase (2053.3 µmol MUFG g^{-1} h^{-1}), acid phosphatase (2197.6 µmol MUFP g^{-1} h^{-1}), lipase (9117.6 µmol MUFY g^{-1} h^{-1}), and chitinase (3613.7 µmol MUFN g^{-1} h^{-1}) activities were measured in the youngest layer (Figure 3b). This layer was very biologically active, with the greatest number of earthworms. On the other hand, the least activity of these enzymes except lipase was measured in the oldest layer, which was fully matured and stabilized. The greatest activity for β-D-glucosidase was measured by Fernández-Gómez et al. [35] in vermicompost from cow dung and in vermicompost from tomato-fruit waste after 12 weeks of vermicomposting. They also measured phosphatase, and its activity was multiple times greater in both vermicomposts than in our vermicompost. Nogales et al. [36] also measured these two enzymes during a laboratory study conducted with vermicomposting of grape marc for 16 weeks. The activity of these enzymes was greater

than ours, but these activities decreased with the age of the vermicompost, which was the same as in our experiment. The greatest activity of cellobiohydrolase was measured in layer III (1219.2 μmol MUFC g^{-1} h^{-1}) and the least activity was in the bottom layer (885.8 μmol MUFC g^{-1} h^{-1}). Overall, the values of the middle layers were very close due to the heterogeneity of the material. In the case of urease (Figure 3c), there was the same trend as for cellobiohydrolase. The greatest activity of urease was measured in layer III (187.1 μg NH_4-$N.g^{-1}.2h^{-1}$) and the least activity was in layer I (65.7 μg NH_4-$N.g^{-1}.2h^{-1}$). A greater urease activity (104 μg NH_4-$N.g^{-1}.h^{-1}$) was found by Romero et al. [37], who used vermicompost from grape marc to enrich the soil. Almost the same value (about 102 μg NH_4-$N.g^{-1}.h^{-1}$) was measured by Pramanik et al. [38] in their control variant of vermicompost based on cow dung after 70-85 days of vermicomposting using *E. fetida* in the process of single feeding. The activity of nitrate reductase was very low throughout the process (Figure 3d). The greatest activity was found in the youngest layer (11.8 μg NO_2-$N.g^{-1}.24h^{-1}$). The lowest value for the nitrate reductase activity was in the oldest layer (0.6 μg NO_2-$N.g^{-1}.24h^{-1}$). All the layers showed no significant differences in the activity of this enzyme, because there were great standard deviations.

Conclusion

The top and so youngest layer was characterized by partially decomposed organic matter with a great amount of earthworm biomass, which was confirmed by parameters such as humidity, C_{tot}, N_{tot}, and C/N. On the other hand, the lower layers were characterized by greater maturity which was documented by lesser contents of microbial biomass and activity of hydrolytic enzymes as well as a slightly alkaline pH, and lesser values for $N\text{-}NH_4^+$ and dissolved organic carbon, which was indirectly proportional to the ion-exchange capacity. Of the total and available nutrients studied, potassium was the greatest, followed by phosphorus and magnesium. On the basis of the detected parameters, the top layer is suitable for a new windrow and for the preparation of aqueous extracts. The older layers are suitable for use as an organic fertilizer. The results obtained could encourage companies to effectively use this valuable biowaste that is currently, unfortunately, often unnecessarily removed.

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References

[1] Muktham, R., Bhargava, S.K., Bankupalli, S., Ball, A.S.: A review on 1st and 2nd generation bioethanol production - recent progress. J. Sustain. Bioenergy Syst. 6, 72-92 (2016)

[2] Wilkie A.C., Riedesel, K.J., Owens, J.M.: Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. Biomass Bioenergy 19, 63-102 (2000)

[3] van Haandel, A.C., Catunda, P.F.C.: Profitability increase of alcohol distilleries by the rational use of byproducts. Water Sci. Technol. 29, 117-124 (1994)

[4] Mohana, S., Acharya, B. K., Madamwar, D.: Distillery spent wash: Treatment technologies and potential applications. J. Hazard. Mater. 163, 12-25 (2009)

[5] Bustamante M.A., Moral, R., Paredes C., Pérez-Espinosa, A., Moreno-Caselles, J., Pérez-Murcia, M.D..: Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. Waste Manage. 28, 372-380 (2008)

[6] Choudhary, P., Raj, A., Bharagava, R.N.: Environmental pollution and health hazards from distillery wastewater and treatment approaches to combat the environmental threats: a review. Chemosphere 194, 229-246 (2018)

[7] Wagh, M.P., Nemade, P.D.: Treatment of distillery spent wash by using chemical coagulation (CC) and electro-coagulation (EC). Am. J. Environ. Protect. 3, 159-163 (2015)

[8] Prajapati, A.K., Chaudhari, P.K.: Physicochemical treatment of distillery wastewater - a review. Chem. Eng. Commun. 202, 1098-1117 (2015)

[9] Satyawali, Y., Balakrishnan, M.: Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: a review. J. Environ. Manage. 86, 481-497 (2008)

[10] Lim, S.L., Lee, L.H., Wu, T.Y.: Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: recent overview, greenhouse gases emissions and economic analysis. J. Clean. Prod., 111, 262-278 (2016)

[11] Sinha, R.K., Agarwal, S., Chauhan, K., Valani, D.: The wonders of earthworms and its vermicompost in farm production: Charles Darwin's 'friends of farmers', with potential to replace destructive chemical fertilizers from agriculture. Agric. Sci. 1, 76-94 (2010)

[12] EN 13037 Soils improvers and growing media – Determination of pH, CEN Brussels (1999).

[13] EN 13651 Soil improvers and growing media – Extraction of calcium chloride/DTPA (CAT) soluble nutrients (2001)

[14] Váchalová, R., Borová-Batt, J., Kolář, L., Váchal, J.: Selectivity of ion exchange as a sign of soil quality. Commun. Soil Sci. Plant Anal. 45, 2673-2679 (2014)

[15] Bligh EG and Dyer WJ (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Phys. 37, 911-917 (1959)

[16] Šnajdr, J., Cajthaml, T., Valášková, V., Merhautová, V., Petránková, M., Spetz, P., Leppänen, K., Baldrian, P.: Transformation of *Quercus petraea* litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes the microbial community composition. FEMS Microbiol. Ecol. 75, 291-303 (2011)

[17] Oravecz, O., Elhottová, D., Krištůfek, V., Šustr, V., Frouz, J., Tříska, J., Márialigeti, K.: Application of ARDRA and PLFA analysis in characterizing the bacterial communities of the food, gut and excrement of saprophagous larvae of *Penthetria holosericea (Diptera: Bibionidae)*: a pilot study. Folia Microbiol. 49, 83-93 (2004)

[18] Baldrian, P.: Microbial enzyme-catalyzed processes in soils and their analysis. Plant Soil Environ. 55, 370–378 (2009)

[19] Štursová, M., Baldrian, P.: Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. Plant Soil 338, 99-110 (2011)

[20] Kandeler, E., Gerber, H.: Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fertil. Soils 6, 68-72 (1988)

[21] Kandeler, E.: Nitrate reductase activity. In: Schinner, F., Ohlinger, R., Kandeler, E., Margesin, R. (eds.) Methods in Soil Biology, pp. 176-179. Springer, Berlin, Germany (1996)

[22] Singh, N.B., Khare, A.K., Bhargava, D.S., Bhattacharya, S.: Effect of initial substrate pH on vermicomposting using *Perionyx excavatus* (Perrier, 1872). Appl. Ecol. Environ. Res. 4, 85-97 (2005)

[23] Talashilkar, S.C., Bhangarath, P.P., Mehta, V.B.: Changes in chemical properties during composting of organic residues as influenced by earthworm activities. J. Indian Soc. Soil Sci. 47, 50-53 (1999)

[24] Hanc, A., Castkova T., Kuzel, S., Cajthaml, T.: Dynamics of a vertical-flow windrow vermicomposting system. Waste Manag. Res. 35, 1121-1128 (2017)

[25] Torres-Climent, A., Gomis, P., Martín-Mata, J., Bustamante, M.A., Marhuenda-Egea, F.C., Pérez-Murcia, M.D., Pérez-Espinosa, A., Paredes, C., Moral, R.: Chemical, thermal and spectroscopic methods to assess biodegradation of winery-distillery wastes during composting. PLoS ONE 10 (2015). doi:10.1371/journal.pone.0138925

[26] Castkova, T., Hanc, A.: Change of the parameters of layers in a large-scale grape marc vermicomposting system with continuous feeding, In: Proceedings Sardinia 2017 / Sixteenth International Waste Management and Landfill Symposium, pp. 1-11. S. Margherita di Pula, Cagliary, Italy (2017)

[27] Thompson, W., Leege, P., Millner, P., Watson, M.E.: Test methods for the examination of composts and composting. The US composting council, US government printing office, Bethesda, USA (2003)

[28] Zmora-Nahum, S., Markovitch, O., Tarchitzky, J., Chen, Y.: Dissolved organic carbon (DOC) as a parameter of compost maturity. Soil Biol. Biochem. 37, 2109-2116 (2005)

[29] Hue, N.V., Liu, J.: Predicting compost stability. Compost Sci. Util. 3, 8-15 (1995)

[30] Barois, I., Lavelle, P.: Changes in respiration rate and some physicochemical properties of a tropical soil during transit through *Pontoscolex corethrurus* (glossoscolecidae, oligochaeta). Soil Biol. Biochem 18, 539-541 (1986)

[31] Aria, M., Monroy, F., Domínguez, J.: C to N strongly affects population structure of *Eisenia fetida* in vermicomposting system. Eur. J. Soil Biol. 42, 127-131 (2006)

[32] Domínguez, J., Edwards, C.A.: Relationship between composting and vermicomposting. In: Edwards, C.A., Arancon, N.Q., Sherman, R. (eds.) Vermiculture Technology, pp. 11-25. CRC Press, Taylor & Francis Group, Boca Raton (2011)

[33] Gómez-Brandón, M., Lores, M., Dominguéz, J.: Changes in chemical and microbiological properties of rabbit manure in a continuous-feeding vermicomposting systém. Bioresour. Technol. 128, 310-316 (2013)

[34] Fernández-Gómez, M.J., Díaz-Raviña, M., Romero, E., Nogales, R.: Recycling of environmentally problematic plant waste generated from greenhouse tomato crops through vermicomposting. Int. J. Environ. Sci. Technol. 10, 697-708 (2013)

[35] Fernández-Gómez, M. J., Romero, E., Nogales, R.: Feasibility of vermicomposting for vegetable greenhouse waste recycling. Bioresour. Technol. 101, 9654-9660 (2010)

[36] Nogales, R., Cifuentes, C., Benítez, E.: Vermicomposting of winery wastes: a laboratory study. J. Environ. Sci. Health B 40, 659-673 (2005)

[37] Romero, E., Fernández-Bayo, J., Díaz, J.M.C., Nogales, R.: Enzyme activity and diuron persistence in soil amended with vermicompost derived from spent grape mars treated with urea. Appl. Soil Ecol. 44, 198-204 (2010)

[38] Pramanik, P., Ghosh, G.K., Ghosal, P. K., Banik, P.: Changes in organic – C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. Bioresour. Technol. 98, 2485-2494 (2007)