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ORIGINAL PAPER

CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF LUVISOL AFTER ADDITION OF POST-FERMENTATION RESIDUE*

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Abstract

The aim of the study was to evaluate the influence of post-fermentation liquid on the chemical and microbial properties of soil as expressed by the total number of bacteria and fungi and by changes in the population of selected groups of soil microorganisms: amylolytic, cellulolytic, pectinolytic and proteolytic bacteria as well as actinobacteria. Samples obtained from the surface layer of luvisol, fertilized with post-fermentation liquid produced in an agricultural biogas plant processing pig slurry, maize silage and post-slaughter waste, were analyzed. The effect of fertilization with digestate was evaluated in a pot experiment performed on a laboratory scale. The applied fertilization affected the chemical properties of the soil, which depended on a dose of post-fermentation liquid, as well as on the duration of the experiment. The addition of post-fermentation liquid to the luvisol significantly increased the level of total nitrogen and content of organic carbon. The analysis of the fractional composition of humus compounds showed an increase in the carbon content of the humic acid fraction (C_{HA}) in the luvisol. Analyses of infrared spectra in the present study indicate a similar effect of post-fermentation liquid as in the case of humus-forming slurry. A higher concentration of post-fermentation liquid (255 kg N ha^{-1}) usually resulted in an increase in the number of individual groups of microorganisms, in particular fungi and actinobacteria, as well as the total number of bacteria.

Keywords: digestate, agricultural biogas plant, chemically and microbiologically properties of soil, soil microbiological activity.

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INTRODUCTION

Post-fermentation product (digestate) is the second product of methane fermentation after biogas. It is composed of methane bacteria, non-decomposed organic matter and mineral compounds. The process of methane fermentation leads to reduction in the organic matter content in post-fermentation liquid as a result of the removal of easily transformable carbon compounds. In recent years, the application of digestates to soil as biofertilizers has become more common. Due to its impact on a wide range of physical, chemical and biological properties of soil, a post-fermentation product can be a substitute for mineral fertilizers (Garcia-Sanchez et al. 2015, Sapp et al. 2015, Gómez-Brandón et al. 2016, Riva et al. 2016, Risberg et al. 2017). Digestate contains high density of microorganisms with wide spectrum catabolic processes (Wang et al. 2018). Conversion of nitrates into ammonium nitrogen that is more easily absorbed by plants reduces the risk of leaching into groundwater (Möller, Müller 2012). The content of other compounds in digestate, i.e. potassium, phosphorus and magnesium, is similar to the values recorded in pig slurry (Sager 2007).

According to many researchers (Cayuela et al. 2009, Alburquerque et al. 2012*b*, Chen et al. 2012, Stumpe et al. 2012), changes in the enzymatic activity of soil in combination with mineralization processes after organic matter supply are good indicators of changes in its fertility. Levels of such enzymes as dehydrogenases, phosphatases, cellulases, invertases and proteases are most often determined in order to evaluate soil's biological activity, and it is recommended to investigate several enzymes simultaneously.

The aim of the study was to evaluate the effect of digestate on the chemical and microbiological properties of a luvisol soil, expressed by changes in populations of selected groups of soil microorganisms.

MATERIAL AND METHODS

Samples obtained from the surface layer of luvisol were used in the experiment. Soil samples were subjected to air drying and then physico-chemical analyses were performed. UV-Vis spectrophotometry was used to determine the phosphorus content, and atomic absorption spectrometry was performed to measure magnesium and potassium concentrations. Organic carbon was determined by the Tiurin method. The textural composition was analyzed using the laser diffraction method (PN-R-04033: 1998). Analyses of the pH-value (in 1 mol dm^{-3} KCl, PN-ISO 10390-1997) were conducted (Table 1).

For the fertilization of soil samples, post-fermentation liquid from an agricultural biogas plant processing pig slurry, maize silage and post-slaughter waste were used. Analyses of the physical and chemical properties

Table 1

Specification of the analyzed soil

Parameter	Luvisol
pH (1mol dm^3KCl)	6.1
P (mg kg^{-1} d.m. of soil)	111.2
K (mg kg^{-1} d.m. of soil)	132.8
Mg (mg kg^{-1} d.m. of soil)	400
Organic substances (%)	1.39
Granulometric fractions (%): Sands 2.0-0.05 mm Silts: Thick 0.05-0.02 mm Fine 0.02-0.002 mm Loam <0.002 mm	 82.0 8.3 8.4
Granulometric subgroup	Loamy sand

d.m. – dry matter

of post-fermentation liquid were made in the context of its suitability as a soil improver in agriculture (Table 2). The spectrophotometric method was used for the measurement of total nitrogen (after mineralization by the Hach-Lange method), ammonium nitrogen and total phosphorus (after mineralization in sulfuric acid). Organic carbon in post-fermentation liquid

Table 2

Specification of the post-fermentation liquid used in the experiment

Parameter	Value
pH	8.37 ± 0.176
Dry mass (%)	4.11 ± 0.885
Roasting residues (%)	0.96 ± 0.045
Loss on ignition (% d.m.)	70.9 ± 5.072
Organic nitrogen (mg dm^{-3})	3975.1 ± 326.9
Ammonium nitrogen (mg dm^{-3})	65.2 ± 46.86
Total Phosphorus (mg kg^{-1})	1110.8 ± 192.3
Potassium (mg kg^{1})	2211.0 ± 78.43
Total organic carbon TOC (mg dm^{-3})	22168.7 ± 5560.8
Ca (mg dm^{-3})	586.0 ± 7.21
Mg (mg dm^{-3})	327.67 ± 64.78
Zn (mg dm^{-3})	64.98 ± 32.04
Cd (mg dm^{-3})	0.04 ± 0.006
Pb (mg dm^{-3})	0.31 ± 0.135
Cr (mg dm^{-3})	0.38 ± 0.059
Ni (mg dm^{-3})	0.27 ± 0.051
Hg (mg dm^{-3})	0.02 ± 0.003

d.m. – dry matter

was determined by the Tiurin method. Determinations of potassium and calcium were made by emission spectrometry, while the other elements were determined by0 atomic absorption spectrometry.

The influence of fertilization with post-fermentation liquid was analyzed in a laboratory scale experiment, set up in Kick-Brauckmann pots. Samples of a luvisol soil from the surface layer were mixed with appropriate doses of post-fermentation liquid (combination I: 6:0.135, combination II: 6:0.203), and then 6 kg of each sample were transferred to the pots. Three experimental combinations were used: C – the control, without nitrogen fertilization, I – samples fertilized with nitrogen dose of 170 kg N ha^{-1}, and II – soil fertilized with nitrogen in a dose exceeding the maximum dose by 150% (255 kg N ha^{-1}).

In the control and experimental samples of soils collected in the 4th (dose I) and in the 8th (dose I and II) month of the study, detailed chemical analyses were performed, such as determinations of the total organic carbon content (TOC) and total nitrogen (N_t) using a TOC and TOCN Primacs analyzer (Skalar), as well as the C/N ratio, content of organic carbon compounds extracted with 0.004 mol dm^{-3} $CaCl_2$ (EOC) and nitrogen (EN_t), extraction of humic acids by the Schnitzer method and the content of organic carbon and nitrogen in individual fractions using a TOCN Primacs analyzer from Skalar, C_{deca}, N_{deca} - carbon (nitrogen) in decalcified solutions, $C_{HA + FA}$, $N_{HA + FA}$ – sum of carbon (nitrogen) of humic and fulvic acids in extracts obtained in 0.5 mol dm^{-3} NaOH.

The carbon and nitrogen content is given in g kg^{-1} soil, while EOC, EN_t and fractional composition in mg kg^{-1} soil and as a percentage of the carbon and nitrogen pool. In addition, C_{HA} and N_{HA} were calculated from the difference of the sum of $C_{HA + FA}$ and $N_{HA + FA}$ after the precipitation of humic acids and the determination of carbon (nitrogen) of fulvic acids in a solution $C\ (N)_{HA} = C\ (N)_{HA+FA} - C\ (N)_{FA}$. The results were analyzed by ANOVA and the significance between the groups was verified by the Tukey test at $p \leq 0.05$.

Characteristics of isolated humic acids (HA) were identified based on infrared spectral analyses using a Perkin-Elmer spectrometer FTIR Spectrum BX in the wavelength range of 400-4000 cm^{-1}.

Samples for microbiological tests were collected immediately after the preparation of the pots, and later on the 60th and 150th day of the experiment. The following microbiological parameters were determined in the examined soil: the total number of bacteria and fungi, the numbers of amylolytic, cellulolytic, pectinolytic and proteolytic bacteria as well as actinobacteria. The results were expressed as colony forming units (cfu) calculated per 1 g of dried soil. Isolation of soil microorganisms was performed by placing 10 g of an analyzed soil sample in 90 cm^3 of the Ringer's solution with the following series of decimal dilutions. The inoculated media were incubated at 24°C for 4 days for bacteria and fungi, or at 28°C for 14 days for actinobacteria. Identification of bacteria belonging to particular groups was

made by coating the plates with an appropriate reagent (proteolytic bacteria – Frazier reagent, amylolytic and pectinolytic – iodine solution, cellulolytic – Congo red and NaCl solution), enabling the observation of hydrolysis zones of individual substrates and proper bacterial classification. The occurrence of illumination around the bacterial colonies indicated the bacteria secreted enzymes that were decomposing the nutrients.

The Dunn's *post-hoc* test was applied to assess the significance of differences at the significance level of $p \leq 0.05$ for the number of tested microorganisms between individual doses of fertilizer and the incubation time at 0, 60 and 150 days of testing.

RESULTS

Total organic carbon content (TOC) in the analyzed luvisol samples increased after the application of the basic dose of post-fermentation liquid compared to the TOC level of the control sample (Table 3). After 8 months since the fertilization treatment, a decrease in this parameter was observed, same as after the application of the higher dose of post-fermentation liquid. The total nitrogen content (N_t) in all combinations of the experiment increased after adding post-fermentation liquid in comparison with the control soil samples. The use of post-fermentation liquid, regardless of a dose, reduced the C/N ratio (Table 3).

The use of the digestate resulted in an increase in extractable organic carbon (EOC) in the luvisol samples (8 months) – Table 3. At the same time, the application of dose I led to a release of a slightly higher content of organic carbon soluble forms (1.11) expressed as % of organic carbon (EOC% TOC) during the 8 months of the experiment (Table 3).

The addition of post-fermentation liquid caused an increase in the content of extractable nitrogen forms in the investigated soil (Table 3). With the experiment progressed in time, post-fermentation liquid contributed to an increase in extractable nitrogen forms (in $\%N_t$) compared

Table 3

Results of organic carbon (TOC) and nitrogen (N_t) determinations, C/N ratio and content of soluble organic carbon (EOC) and extractable nitrogen (EN_t) in the luvisol

Sample	Experimental combination	TOC (g kg^{-1})	N_t (g kg^{-1})	C/N	EOC (mg kg^{-1})	EOC %TOC	EN_t (mg kg^{-1})	EN_t $\%N_t$
Luvisol	control sample	8.79_a^*	0.94_a	9.4	75.8_a	0.86_a	14.6_a	2.06
	1 dose (4 months)	9.26_a	1.11_a	8.3	71.4_a	0.77_a	115.0_b	10.3
	1 dose (8 months)	8.15_a	1.17_a	6.9	90.5_a	1.11_a	155.8_b	13.3
	1.5 dose (8 months)	8.50_a	1.14_a	7.4	72.5_a	0.85_a	135.1_b	11.7

* *a*,*b*,*c*,.. – statistically significant differences by theTukey test at $p \leq 0.05$

to the control sample, although the increase in a fertilizer dose was less effective (Table 3).

The properties of humus are a characteristic feature of different types of soil, and the composition of the fraction is modified by the type of organic material supplied to the soil. In our experiment, neither the addition of post-fermentation liquid nor the duration of the experiment modified significantly the results (Table 4). The analysis of the fractional composition of humus compounds showed an increase in the carbon content of the humic acid fraction (C_{HA}) in the luvisol as a result of the fertilization with post--fermentation liquid. Differences in the amount of humic acids resulted in changes in the C_{HA}/C_{FA} ratio, which is an indicator of the stability of soil organic matter. The application of post-fermentation liquid to a luvisol improved the stability of organic matter in the soil samples evaluated in the 8th month after fertilization (Table 4).

Table 4

Carbon content in humus fractions (mg kg^{-1}) and C_{HA}/C_{FA} ratio of the luvisol

Sample	Experimental combination	C_{deca} (mg g^{-1})	C_{deca} (%) TOC	C_{HA} (mg kg^{-1})	C_{HA} (%) TOC	C_{FA} (mg kg^{-1})	C_{FA} (%) TOC	C_{HA}/C_{FA}
Luvisol	control sample	249*	2.83	1732*	19.70	1094*	12.45	1.58
	1 dose (4 months)	248	2.68	1869	20.18	923	9.97	2.02
	1 dose (8 months)	265	3.25	2589	31.77	937	11.50	2.76
	1.5 dose (8 months)	254	2.99	2532	29.79	1140	13.41	2.22

C_{HA} – carbon in humic acids, C_{FA} – carbon in fulvic acids, C_{deca} – carbon in decalcified solutions, * values in columns do not differ statistically at $p \leq 0.05$

In soil samples, the content of nitrogen in the humic acid fraction was higher than that in fulvic acids. However, there was no positive effect of the increased dose of post-fermentation liquid on the nitrogen content in soil samples after decalcification N_{deca} (Table 5). The nitrogen content of the fulvic acid fraction (N_{FA}) increased after 8 months, but only after the application of the higher fertilization dose, in relation to both the control and the experimental dose with the basic amount of post-fermentation liquid. The N_{HA}/N_{FA} ratio increased with the duration of the experiment and with the higher dose of post-fermentation liquid (Table 5).

Figures 1 and 2 show infrared spectra (FTIR) of isolated humic acids. The spectra are a property characteristic for a given chemical compound, and the attribution of a specific position to particular functional groups within particular wavelength ranges enable the identification of these substances.

Table 5

Nitrogen content in humus fractions (mg kg^{-1}) and N_{HA}/N_{FA} ratio of the tested soils

Sample	Experimental combination	N_{deca}	N_{HA}	N_{FA}	N_{HA}/N_{FA}
Luvisol	control sample	28.8_a*	120.5_a	61.9_a	1.94
	1 dose (4 months)	121.9_b	131.0_a	57.4_a	2.28
	1 dose (8 months)	234.0_c	139.1_a	57.1_a	2.43
	1.5 dose (8 months)	172.5_b	171.7_a	65.9_a	2.61

N_{HA} – nitrogen in humic acids, N_{FA} – nitrogen in fulvic acids, N_{deca} – nitrogen in solutions after decalcification, * $a,b,c,..$ – statistically significant differences ($p \leq 0.05$) between the doses of post-fermentation liquid and dates of analyses

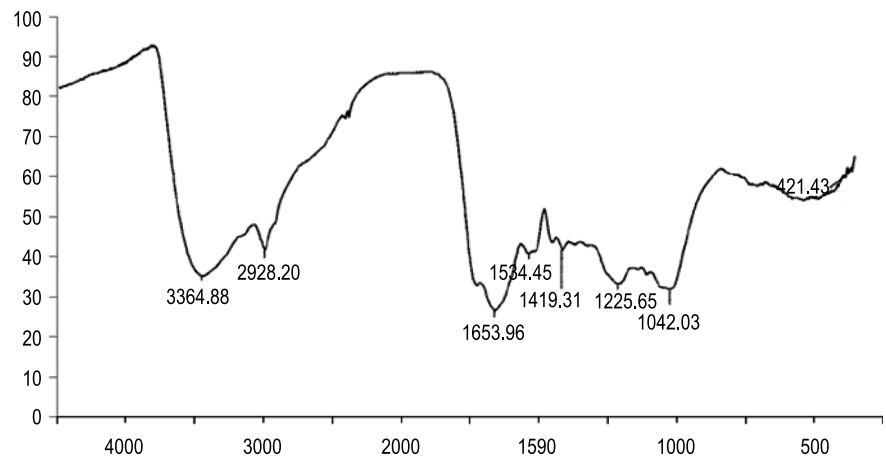

Fig. 1. FTIR spectra of humic acids isolated from the luvisol (control sample)

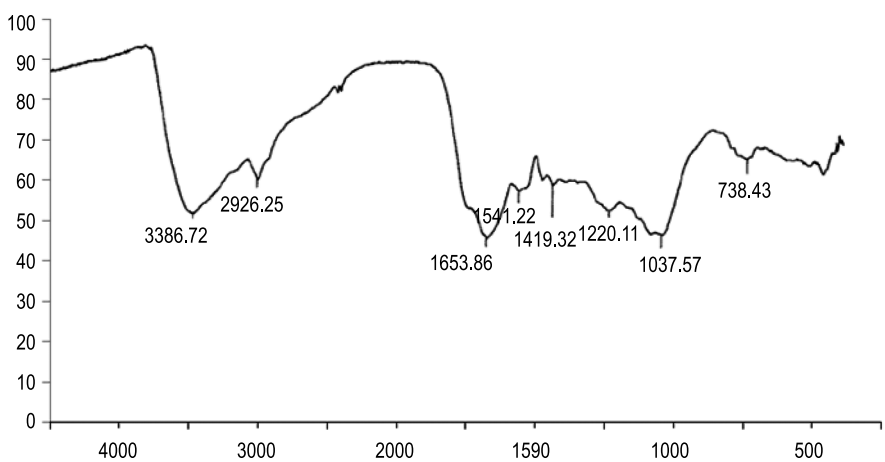

Fig. 2. FTIR spectra of humic acids isolated from the luvisol fertilized with post-fermentation liquid (dose I; 8 months)

The introduction of organic material caused the occurrence of bands with high intensity of 1030-1080 cm^{-1} in humic soil acids. The infrared spectrum analysis showed significant intensity of absorption bands occurring in the range of wave numbers 2920-2960 cm^{-1}. This band is intense for humic acids isolated from the tested soil. It is conditioned by the presence of $-CH_3$ and $=CH_2$ groups. A wide band in the range of 3100-3300 cm^{-1} was also observed in the studied soil treated with post-fermentation liquid. This band is formed due to the N-H stretching vibrations. In the range of wave numbers 1600-1660 cm^{-1}, there was a wide, intense band, associated with the occurrence of C = O stretching vibrations in peptides and tertiary amines and C = N bonds. The most distinct bands occurred in the range of wave numbers 1420-1460 cm^{-1}, 1320-1380 cm^{-1} and 1030-1080 cm^{-1}. The incorporation of organic material caused the emergence of these bands, with high intensity at 1030-1080 cm^{-1} in humic acids of the luvisol. These bands were determined by the presence of carboxylic acids, esters, lignins, and alcohols. The occurrence of the 1030-1080 cm^{-1} band indicates the presence of polysaccharides in HAs molecules (Figures 1 and 2). This is the effect of incorporating a large amount of organic matter containing proteins, carbohydrates, various forms of nitrogen and other simple organic compounds due to the addition of post-fermentation liquid to the soil.

Analyzing the soil microbiological activity, it can be concluded that the highest total number of bacteria in the luvisol samples was found directly after mixing the soil with post-fermentation liquid (Table 6). At the beginning of the experiment, the observed effect of both doses of fertilizer on the number of bacteria in the soil was statistically significant, while no significant differences were found in the subsequent stages of the experiment. Evaluation of the number of amylolytic bacteria isolated from the luvisol samples did not show any statistically significant increase as a result of the use of both doses of post-fermentation liquid. However, the number of starch-degrading bacteria was usually higher in the control samples than in the experimental ones (Table 6). In the luvisol samples, the addition of post-fermentation liquid induced a slight increase in the number of cellulolytic bacteria (Table 6). In the investigated soil, the number of proteolytic bacteria increased statistically significantly at the beginning of the experiment after the use of the higher post-fermentation liquid dose. On the other hand, there was no clear upward trend in the number of proteolytic and pectinolytic bacteria in the subsequent study periods compared to the control samples (Table 6). The determinations of the total number of fungi proved that the addition of post-fermentation liquid caused a significant increase in their number only at the onset of the experiment, and no significant effect of fermentation residue on the number of fungi isolated from the soil was found on the 60^{th} day of experiment and later (Table 6). Evaluation of changes in the number of actinobacterias in soil samples treated with post-fermentation liquid showed a general increase along with the higher dose of fertilizer, with statistically significant differences only on the 60^{th} day of incubation (Table 6).

Table 6

Changes in the total number of bacteria, amylolytic, cellulolytic, proteolytic and pectinolytic bacteria, fungi and actinomycetes in the investigated soil fertilized with post-fermentation liquid

Date of survey (days)	Control	Dose I	Dose II
The general number of bacteria (cfu kg^{-1})			
0	$1.33 \times 10^{10}{}_{c}$ *	$2.21 \times 10^{10}{}_{b}$	$3.66 \times 10^{10}{}_{a}$
60	$9.19 \times 10^{9}{}_{cd}$	$7.63 \times 10^{9}{}_{de}$	$9.87 \times 10^{9}{}_{cd}$
150	$3.21 \times 10^{9}{}_{ef}$	$1.76 \times 10^{9}{}_{f}$	$2.00 \times 10^{9}{}_{f}$
Amylolytic bacteria (cfu kg^{-1})			
0	$3.33 \times 10^{9}{}_{b}$	$3.32 \times 10^{9}{}_{a}$	$1.33 \times 10^{9}{}_{bc}$
60	$2.16 \times 10^{9}{}_{ab}$	$1.53 \times 10^{9}{}_{bc}$	$1.97 \times 10^{9}{}_{ab}$
150	$3.81 \times 10^{8}{}_{c}$	$3.53 \times 10^{8}{}_{c}$	$4.82 \times 10^{8}{}_{c}$
Cellulolytic bacteria (cfu kg^{-1})			
0	$1.44 \times 10^{9}{}_{b}$	$3.32 \times 10^{9}{}_{a}$	$1.11 \times 10^{9}{}_{bc}$
60	$4.75 \times 10^{8}{}_{cd}$	$3.65 \times 10^{8}{}_{cd}$	$3.51 \times 10^{8}{}_{d}$
150	$9.79 \times 10^{8}{}_{bcd}$	$4.96 \times 10^{8}{}_{cd}$	$1.11 \times 10^{9}{}_{bc}$
Proteolytic bacteria (cfu kg^{-1})			
0	$2.55 \times 10^{8}{}_{b}$	$3.10 \times 10^{8}{}_{b}$	$2.22 \times 10^{10}{}_{a}$
60	$9.73 \times 10^{8}{}_{b}$	$9.26 \times 10^{8}{}_{b}$	$1.70 \times 10^{9}{}_{b}$
150	$2.88 \times 10^{8}{}_{b}$	$3.86 \times 10^{8}{}_{b}$	$7.05 \times 10^{8}{}_{b}$
Pectinolytic bacteria (cfu kg^{-1})			
0	$1.89 \times 10^{9}{}_{ab}$	$2.21 \times 10^{9}{}_{a}$	$9.99 \times 10^{8}{}_{bc}$
60	$7.56 \times 10^{8}{}_{c}$	$3.27 \times 10^{8}{}_{c}$	$1.10 \times 10^{8}{}_{c}$
150	$7.61 \times 10^{8}{}_{c}$	$8.82 \times 10^{8}{}_{c}$	$1.00 \times 10^{9}{}_{bc}$
Total number of fungi (cfu kg^{-1})			
0	$3.33 \times 10^{8}{}_{b}$	$4.43 \times 10^{8}{}_{ab}$	$5.55 \times 10^{8}{}_{a}$
60	$1.30 \times 10^{8}{}_{cd}$	$1.25 \times 10^{8}{}_{cd}$	$1.97 \times 10^{8}{}_{c}$
150	$5.44 \times 10^{7}{}_{d}$	$9.92 \times 10^{7}{}_{cd}$	$1.56 \times 10^{8}{}_{cd}$
Total number of actinobacteria (cfu kg^{-1})			
0	$1.66 \times 10^{9}{}_{c}$	$1.66 \times 10^{9}{}_{c}$	$1.89 \times 10^{9}{}_{bc}$
60	$1.40 \times 10^{9}{}_{c}$	$2.29 \times 10^{9}{}_{b}$	$3.29 \times 10^{9}{}_{a}$
150	$5.27 \times 10^{8}{}_{d}$	$6.21 \times 10^{8}{}_{d}$	$6.19 \times 10^{8}{}_{d}$

* *a*,*b*,*c*,.. – statistically significant differences ($p \leq 0.05$) between the doses of post-fermentation liquid and the dates of analyses demonstrated by the Dunn's *post-hoc* test

DISCUSSION

ALBURQUERQUE et al. (2012*a*) found that the composition and stability of the digestate, dependent on the used substrates and the nature of the fermentation process, affect the dynamics of the carbon and nitrogen content

in the soil. According to Tambone et al. (2019) and Risberg et al. (2017), the liquid fraction of digestate contains much biologically stable carbone. In the present study, the addition of *post*-fermentation liquid increased the total organic carbon content (TOC) in the analyzed soil. Similar results were obtained by Ernst et al. (2008), who tested addition of fermented cattle slurry. Telesiński et al. (2017) found that the application of post-fermentation residue in three different forms (pulp, drought, granulate) resulted in an increase in the total organic carbon and total nitrogen content, which grew with an increase in the applied dose. According to Alburquerque et al. (2012*a*), the addition of post-fermentation liquid of various origins clearly showed differences in the release of carbon dioxide as a result of the mineralization of organic matter introduced with the fertilizer. The decrease in the organic carbon content noted in the 8th month of incubation was caused by the progressive mineralization of the introduced organic matter (Table 3). According to several researchers (Alburquerque et al. 2012*a*, Chen et al. 2012, Stumpe et al. 2012), the amount of mineralized organic matter in the soil may exceed the carbon pool delivered with the fertilizing dose. In the present study, the organic carbon content of the luvisol incubated in the pots for 8 months was lower than the initial value without the addition of post-fermentation liquid, indicating the possibility of soil organic matter mineralization (Table 3). The application of post-fermentation liquid, in a dose of 50 kg N ha^{-1} year^{-1}, in a four-year field experiment conducted by Odlare et al. (2008) did not cause a significant increase in the total organic carbon content in the soil (TOC), while the level of mineral nitrogen increased annually. However, Dębska (2004) stated that slurry fertilization increased the content of C_{org} and N_{org} in the soil, and that changes in the C/N ratio were caused by the higher accumulation of nitrogen than carbon. In the present study, the C/N ratio in the luvisol samples was also reduced as early as 4 months after the post-fermentation liquid application to the soil (Table 3). It needs to be highlighted that the carbon content in post-fermentation liquids is lower than in the feedstock used, like pig slurry (Tambone et al. 2013, Risberg et al. 2017).

According to Gonet and Dębska (2006), changes in the carbon content in soils induced by slurry fertilization cause changes in the content of soluble organic matter, expressed as the so-called extractable organic carbon (EOC). This trend was also observed in the our experiment. In the luvisol samples, there was an increase in the EOC content in the 8th month after the application of post-fermentation liquid to the soil in comparison with the results obtained in the 4th month (Table 3). In Dębska's research (2004), slurry fertilization also increased the content of the most mobile fraction of organic carbon (EOC). In the present study, the EOC content in the organic carbon pool decreased with the amount of post-fermentation liquid added to the soil. Also, Alburquerque et al. (2012*b*) noted a decrease in the content of water-soluble organic carbon in a field experiment, 5 months after the application of digestate.

In the present study, an increase in the dose of post-fermented liquid and the prolongation of the incubation time caused an increase in the amount of total nitrogen and its extractable forms (ENt) in the soil as compared to the control sample. Similar results were obtained by Ernst et al. (2008), who observed a slight increase in the nitrogen content after 6 weeks of soil incubation with post-fermentation liquid. In this study, in the 4th month of soil incubation, the increase in the nitrogen content for luvisol reached 0.17 percentage points (Table 3). Persistently high nitrogen content after 8 months of incubation may be the result of nitrogen binding to the biomass of microorganisms. In Dębska's study (2004), an increase in the level of nitrogen extracted with calcium chloride (EN_t) was found even 10 years after the cessation of slurry application.

The high biological activity found in the earliest analyses, induced by the increased total number of bacteria after the addition of both doses of post-fermentation liquids to soils, may indicate the immobilization of the nitrogen pool introduced with the digestate (Table 4 and 6). According to Grigatti et al. (2011) and Alburquerque et al. (2012*a*), the increased content of soluble forms of organic carbon in the soil fertilized with post-fermentation liquid may lead to the immobilization of nitrogen in biomass of microorganisms and prevention of leaching into deeper soil layers, while hindering its absorption by plants. Thomsen et al. (2013) also reported that during the fermentation process, most soluble forms of organic carbon are processed into biogas, and the digestate residue affects microbiological activity, nitrogen immobilization and mineralization processes in the soil, which affect soil fertility for a short time period. According to Odlare et al. (2008), the application of post-fermentation liquid to soil in a field experiment did not cause significant changes in the nitrogen content and available forms of phosphorus, while the content of potassium increased.

One of the indicators of the quality of humus is the ratio of the carbon content of humic acids to carbon content of fulvic acids; higher values of this ratio are characteristic for fertile soils. In the present research, the C_{HA}/C_{FA} ratio increased significantly in comparison to the control (Table 4). Similarly, Dębska (2004) noted that increased slurry applications caused the formation of unstable and easily mineralized humic acids.

The results obtained in the present study suggest that the direction of changes occurring in soil fertilized with digestate is similar to that observed in soils fertilized with liquid manure or manure. Gonet and Wegner (1993) found that pig slurry, regardless of a dose, reduces the degree of humification of isolated molecules of humic acids, similarly to changes observed in humic acids isolated from soils fertilized with farmyard manure.

Analyses of infrared spectra in the present study indicate a similar effect of post-fermentation liquid as induced by slurry in terms of humus formation (Figures 1-2). The presence of humic acids with a lower degree of humification, rich in aliphatic chains and simple aromatic compounds has been con-

firmed by Strączyńska (1993) and Dębska (2004). On the basis of IR spectra analyses of humic acids isolated from a luvisol fertilized with cattle manure, Strączyńska (1993) showed a higher share of poorly condensed aromatic nuclei in molecules of humic acids in comparison to humic acids of unfertilized soil. Based on the current results, it can be assumed that soil fertilization with post-fermentation liquid may favour the formation of humic acids with a lower degree of humification, rich in aliphatic chains and simple aromatic compounds.

Goyal et al. (2006) argued that the positive effect of organic fertilizers on the soil microbiota depends on their content of organic matter, which is a source of carbon and energy for soil microorganisms. Grigatti et al. (2011) believed that the organic matter in digestate is less susceptible to decomposition by microorganisms after its application to the soil. In turn, Terhoeven-Urselmans et al. (2009) stated that the application of liquid manure fermented in a biogas plant positively affects soil microorganisms, but probably has a less positive effect on soil's organic carbon content.

In the present study, changes in microbial activity after the use of post-fermentation liquid were observed in the soil for both doses of the soil conditioner (digestate). The higher dose of digestate resulted in a more pronounced increase in the total number of bacteria, fungi and actinobacterias. In the luvisol, the total number of bacteria on the 150th day of the experiment decreased as compared to the initial value. This situation is explained by many researchers (Grigatti et al. 2011, Alburquerque et al. 2012*a*,*b*, Stumpe et al. 2012) claiming that the addition of post-fermentation liquid introduces a high content of ammonium nitrogen into the soil. According to Barabasz (1992), the majority of soil microorganisms mainly use ammonium nitrogen. After the application of post-fermentation liquid, Yu et al. (2010) found a statistically significant increase in the total number of bacteria, fungi and actinobacterias in the rhizosphere as well as beyond it. Johansen et al. (2013) proved that the application of post-fermentation material effects a change in the soil microbial profile in comparison to non-fertilized soils, but to a lesser extent than unprocessed cattle fertilizer does. In a pot experiment, Ogbonna et al. (2018) demonstrated that the population of ammonia-oxidizing bacteria, nitrate-reducing bacteria and fungi in soil increased after 70 days since the after application of liquid digestate. In turn, Ernst et al. (2008) emphasized a decrease in the number of microorganisms after the digestate application in comparison to the treatment with slurry.

The transformation of organic matter in soil depends on the enzymatic activity of microorganisms. According to Walsh et al. (2012), the *post*-fermentation liquid stimulates the increase in the number of hydrolyzing bacteria, regardless of the type of soil, which has not been confirmed in the presented research. Alburquerque et al. (2012*a*,*b*) observed an increase in the activity of protease and β-glucosidase activity, albeit not statistically confirmed,

in soil after digestate treatment. Chen et al. (2012) found no significant differences after the use of post-fermentation liquid in the activity of β -glucosidase, cellobiohydrolase and xylan-degrading enzymes in soil, accompanied by an increasing activity of chitinase and enzymes cleaving peptide bonds. In the case of the luvisol tested in our experiment, the number of amylolytic bacteria peaked in the first dates of soil analysis after the application of post-fermentation liquid, but the number of evaluated bacteria decreased 5 months after the fertilizing treatment compared to the microbial population at the onset of the trial, without showing statistically significant differences versus the control sample. Similarly, Alburquerque et al. (2012*a*,*b*) did not observe significant differences in the amount of β-glucosidase on day 151 after the application of digestate compared to soil not fertilized and fertilized with cattle manure and mineral fertilizer. The cellulolytic activity in the luvisol in the present study was subjected to changes in individual replications, not confirming a stimulating effect of post-fermentation liquid on the discussed properties. The research performed by Chen et al. (2012) and Stumpe et al. (2012) proved the lack of an effect of post-fermentation residue on an increase of cellulase enzymes in the soil.

In summary, the influence of post-fermentation liquid as a fertilizing agent on the chemical properties of the soil studied was obvious. However, changes in the microbial activity of soil after the use of digestate varied depending on which group of microorganisms was assessed.

CONCLUSIONS

1. After the application of post-fermentation liquid, the content of organic carbon increased in the investigated soil samples, although the results obtained were dependent on the fertilizer dose and duration of the experiment.

2. The addition of post-fermentation liquid to a luvisol significantly increased the level of total nitrogen in the soil.

3. The analyses of the fractional composition of humus compounds and of the characteristics of infrared spectra of humic acids did not confirm an unequivocal influence of post-fermentation liquid on the fertility of the evaluated soil, although they indicated an increase in the stability of organic matter in the soil and the formation of humic acids.

4. Addition of the digestate to the soil resulted in an increase in the total number of bacteria, actinobacteria and, to a lesser extent, of fungi.

5. An increased dose of post-fermentation liquid generally effected an increase in the number of individual groups of investigated microorganisms, with the effect of the digestate being dependent on the time of incubation.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of interest

none

Data statement

Research data related to this article are currently deposited at the Department of Microbiology and Food Technology, University of Science and Technology in Bydgoszcz, Poland, and can be made available in any case of reasonable (science-based) interests.

REFERENCES

Alburquerque J.A., de la Fuente C., Bernal M.P. 2012*a*. *Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils*. Agr. Ecosyst. Environ., 160: 15-22. DOI: 10.1016/j.agee.2011.03.007

Alburquerque J.A., de la Fuente C., Campoy M., Carrasco L., Nájera I., Baixauli C., Caravaca F., Roldán A., Cegarra J., Bernal M.P. 2012*b*. *Agricultural use of digestate for horticultural crop production and improvement of soil properties*. Europ. J. Agronomy, 43: 119-128. DOI: 10.1016/j.eja.2012.06.001

Barabasz W. 1992. *Microbiological transformations of soil nitrogen*. II. *Biotransformation of soil nitrogen*. Post. Mikrob., 31(1): 3-33. (in Polish)

Cayuela M.L., Sinicco T., Mondini C. 2009. *Mineralization dynamics and biochemical properties during initial decomposition of plant and animal residues in soil*. Appl. Soil Ecol., 41: 118-127. DOI: 10.1016/j.apsoil.2008.10.001

Chen R., Blagodatskaya E., Senbayram M., Blagodatsky S., Myachina O., Dittert K., Kuzyakov Y. 2012. *Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities*. Biomass Bioenerg., 45: 221-229. DOI: 10.1016/j.biombioe.2012.06.014

Dębska B. 2004. *Properties of humus soil fertilized with slurry*. Rozpr. 110, ATR, Bydgoszcz. (in Polish)

Ernst G., Müller A., Göhler H., Emmerling Ch. 2008. *C and N turnover of fermented residues from biogas plants in soil in the presence of three different earthworm species* (*Lumbricus errestris*, *Aporrectodea longa*, *Aporrectodea caliginosa*). Soil Biol. Biochem., 40: 1413-1420. DOI: 10.1016/j.soilbio.2007.12.026

Garcia-Sánchez M., Garcia-Romera I., Cajthaml T., Tlustoš P., Száková J. 2015. *Changes in soil microbial community functionality and structure in a metal-polluted site: The effect of digestate and fly ash applications*. J. Environ. Manage., 162: 63-73. DOI: 10.1016/j.jenvman.2015.07.042

Gonet S.S., Dębska B. 2006. *Dissolved organic carbon and dissolved nitrogen in soil under different fertilization treatments*. Plant Soil Environ., 52(2): 55-63. www.agriculturejournals.cz/publicFiles/50518.pdf

Gonet S.S., Wegner K. 1993. *Effect of long-term fertilization with slurry, manure and NPK on the properties of humic acids*. Zesz. Probl. Post. Nauk Rol., 409: 244-249. (in Polish)

Goyal S., Sakamoto K., Inubushi K., Kamewada K. 2006. *Long-term effects of inorganic fertilization and organic amendments on soil organic matter and soil microbial properties in Andisols*. Arch. Agron. Soil Sci., 52: 617-625. DOI: 10.1080/03650340601048595

Gómez-Brandón M., Fernández-Delgado Juárez M., Zangerle M., Insam H. 2016. *Effects of digestate on soil chemical and microbiological properties: A comparative study with compost and vermicompost*. J. Hazard. Mater., 302(25): 267-274. DOI: 10.1016/j.jhazmat.2015.09.067

Grigatti M., Di Girolamo G., Chincarini R., Ciavatta C., Barbanti L. 2011. *Potential nitrogen mineralization, plant utilization efficiency and soil CO_2 emissions following the addition of anaerobic digested slurries*. Biomass Bioenerg., 35: 4619-4629. DOI: 10.1016/j.biombioe.2011.09.007

Johansen A., Carterb M. S., Jensenc E. S., Hauggard-Nielsenb H., Ambusb P. 2013. *Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO_2 and N_2O*. Appl. Soil Ecol., 63: 36-44. DOI: 10.1016/j.apsoil.2012.09.003

Möller K., Müller T. 2012. *Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review*. Eng. Life Sci., 12(3): 242-257. DOI: 10.1002/elsc.201100085

Odlare M., Pell M., Svensson K. 2008. *Changes in soil chemical and microbiological properties during 4 years of application of various organic residues*. Waste Manage., 28: 1246-1253. DOI: 10.1016/j.wasman.2007.06.005

Ogbonna C.B., Stanley H.O., Abu G.O. 2018. *Effect of liquid digestate on agricultural soil–II: microbial population dynamics*. Appl. Microbiol. Open Access, 4(1): DOI: 10.4172/2471-9315.1000145

PN-ISO 10390: 1997. *Soil quality - pH determination*. (in Polish)

PN-R-04033:1998. *Soils and mineral substances – Division into fractions and granulometric groups*. (in Polish)

Risberg K., Cederlund H., Pell M., Arthurson V., Schnürer A. 2017. *Comparative characterization of digestate versus pig slurry and cow manure – Chemical composition and effects on soil microbial activity*. Waste Manage., 61: 529-538. DOI: 10.1016/j.wasman.2016.12.016

Riva C., Orzi V., Acutis M., Boccasile G., Lonati S., Tambone F., D'Imporzano G., Adani F. 2016. *Short-term experiments in using digestate products as substitutes for mineral (N) fertilizer: agronomic performance, odours, and ammonia emission impacts*. Sci. Total Environ., 547: 206-214. DOI: 10.1016/j.scitotenv.2015.12.156

Sager M. 2007. *Trace and nutrient elements in manure, dung and compost samples in Austria*. Soil Biol. Biochem., 39: 1383-1390. DOI: 10.1016/j.soilbio.2006.12.015

Sapp M., Harrison M., Hany U., Charlton A., Thwaites R. 2015. *Comparing the effect of digestate and chemical fertilizer on soil bacteria*. Appl. Soil Ecol., 86: 1-9. DOI: 10.1016/j.apsoil.2014.10.004

Strączyńska S. 1993. *Effect of fertilization on the properties of humus sandy soil*. Zesz. Probl. Post. Nauk Rol., 411: 37-42. (in Polish)

Stumpe B., Werner S., Jung J., Heinze S., Jüschke E., Strippel C., Marschner B. 2012. *Organic carbon dynamics and enzyme activities in agricultural soils amended with biogas slurry, liquid manure and sewage sludge*. Agric Sci, 3(1): 104-113. DOI: 10.4236/as.2012.31014

Terhoeven-Urselmans T., Scheller E., Raubuch M., Ludwig B., Joergensen R. G. 2009. *CO_2 evolution and N mineralization after biogas slurry application in the field and its yield effects on spring barley*. Appl. Soil Ecol., 42: 297-302. DOI: 10.1016/j.apsoil.2009.05.012

Tambone F., Adani F., Gigliotti G., Volpe D., Fabbri C., Provenzano M.R. 2013. *Organic matter characterization during anaerobic digestion of different biomasses by means of CPMAS ^{13}C NMR spectroscopy*. Biom. Bioen., 48: 111-120. DOI: org/10.1016/j.biombioe.2012.11.006

Tambone F., Orzi V., Zilio M., Adani F. 2019. *Measuring the organic amendment properties of the liquid fraction of digestate*. Waste Manag., 88: 21-27. DOI: 10.1016/j.wasman.2019.03.024

Telesiński A., Cybulska K., Płatkowski M., Stręk M., Jarnuszewski G., Wrońska I., Mularewicz P., Kajdan T., Biczak R. Kołosowski P. 2017. *Integrated assessment of soil quality after application of the biogas fermentation residues – a laboratory experiment*. ES3 Web Conf. 22: 00176. DOI: 10.1051/e3sconf/20172200176

Thomsen I. K., Olesen J. E., Møller H. B., Sørensen P., Christensen B. T. 2013. *Carbon dynamics and retention in soil after anaerobic digestion of dairy cattle feed and faeces*. Soil Biol. Biochem., 58: 82-87. DOI 10.1016/j.soilbio.2012.11.006

Walsh J. J., Rousk J., Edwards-Jones G., Jones D. L., Williams A. P. 2012. *Fungal and bacterial growth following the application of slurry and anaerobic digestate of livestock manure to temperate pasture soils*. Biol. Fert. Soils, 48(8): 889-897. DOI: 10.1007/s00374-012-0681-6

Wang P., Wang H., Qiu Y., Ren L., Jiang B. 2018. *Microbial characteristics in anaerobic digestion process of food waste for methane production – A review*. Biores. Technol., 248: 29-36.

Yu F., Luo X., Song C., Zhang M., Shan S. 2010. *Concentrated biogas slurry enhanced soil fertility and tomato quality*. Acta Agric. Scand. Sect. B Soil Plant Sci., 60: 262-268. DOI: 10.1080/09064710902893385.