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MICROBIOLOGICAL CHARACTERISTIC OF COMPOSTS PREPARED ON THE BASIS OF SEWAGE SLUDGE SUPPLEMENTED WITH REFINED GLYCEROL

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Abstract: The paper presents microbiological characteristic of sewage sludge composted in controlled conditions together with biowastes (wheat, maize and rapeseed straw, sawdust and refined glycerol). An experiment was carried out in which the material was mixed at appropriate weight proportions and then placed in bioreactor chambers of constant air flow (4 $l \cdot min^{-1}$). The performed composting process aimed at determining the developmental dynamics of heterotrophic bacteria, molds, actinomycetes as well as bacteria from *Salmonella* genus and *Enterobacteriaceae* family.
Microbiological analyses were performed on selective substrates using Koch's plate method. Moreover, using the floatation method, the presence of live eggs of ATT (*Ascaris* spp., *Trichuris* spp., *Toxocara* spp.) intestinal parasites was assessed and levels of dehydrogenase activity were determined using 1% triphenylotetrazole chloride as a substrate. It was concluded, on the basis of the obtained research results, that the composting process reduced bacterial counts of heterotrophic bacteria, molds and the activity of dehydrogenases activity in all experimental treatments. On the other hand, no reduction was observed in quantities of actinomycetes in the composted materials whose changes in numbers were found to correlate positively most strongly with levels of dehydrogenases activity. In addition, it was found that changes in numbers of the analysed groups of microorganisms depended, primarily, on the pH value and concentrations of ammonia released from the composted materials. Furthermore, the obtained research results also revealed that the sewage sludge used in the experiment did not contain *Salmonella* spp. bacteria and live eggs of ATT intestinal parasites, and that the composting process reduced completely numbers of bacteria from the *Enterobacteriaceae* family in all compost treatments. The obtained composts fulfilled all sanitary standards complying with the requirements issued by the Minister of Agriculture and Rural Development (2008) as well as with the EU regulation (EC) No. 185/2007 from February 2007 changing EEC regulations No. 809/2003 and No. 810/2003 connected with the extension of the period of transitional requirements for composting and biogas plants as provided by the EU regulation No. 1774/2002 of the European Parliament and Council.

INTRODUCTION

At the beginning of 2008, member countries of the European Union signed a document in which they declared their will to reduce energy consumption by 20%, to increase proportions of biofuels in transport by 10% by the year 2020 and, additionally, they undertook a

decision that, by the same year, 20% of the consumed energy would derive from renewable resources. The Bill on Biocomponents and Biofuels passed by Polish Parliament provides for their considerable increase in the structure of transport fuels. The current situation on the fuel market leads to an increased interest in, among others, the first generation fuels, such as biodiesel. Biodiesel is manufactured from oils and possibly from fats. One of by-products, which develop in the course of biodiesel production process, is glycerol which, however, may cause problems with its disposal. Various attempts have already been made aiming at its appropriate management. Among the most common methods of its disposal is burning, the addition to slurry or feed, as well as soil application, where its decomposition is very slow. One of alternative methods of glycerol management is its composting with various materials.

In recent years, the problem of the organic waste management by their composting has been the object of many investigations [4, 33] with sewage sludge receiving special attention. Sewage sludge constitutes a mixture of live and dead microorganisms as well as organic and mineral components. Organic constituents may make up even 50% of the mass of dehydrated sludge and they consist mainly of carbohydrates, proteins and fats [25]. From the point of view of their sanitary properties, sewage sludges may be considered to be dangerous material as they frequently contain pathogenic bacteria, mainly *Escherichia coli, Salmonella* spp*., Shigella* spp*., Pseudomonas aeruginosa, Clostridium perfringens*. Apart from bacteria, also helminth, such as: *Ascaris* spp., *Trichuris* spp*., Toxocara* spp. can be found in sewage sludges [8, 27]. One of the methods of sewage sludges management and, simultaneously, their effective neutralisation, is composting process [1]. In the course of this process, a comprehensive stabilisation of organic substances takes place, which involves primarily their aerobic mineralisation and humification of organic matter. For the above reasons, composting is employed not only as a way of obtaining valuable organic fertilisers but, equally importantly, as a method of final stabilisation of wastes, including sludges which are unsuitable for agricultural utilisation [12, 13].

The basic role in the mineralisation process of organic matter during their composting is attributed to microorganisms. It is these microbes that transform organic substance into humus fertiliser of full value [6, 12]. According to Taiwo and Oso [29], the temperature reached during the composting process exerts an important influence on the quality of the compost obtained, as it is responsible for the destruction of pathogenic microorganisms [3], insect larvae as well as weed seeds. The temperature inside the prism is directly connected with the degree of its aeration and moisture content. Apart from the monitoring the level of temperature, the measurement of CO_2 production or the control of the succession of microorganisms, another important tool serving to check the intensity of the composting process is the determination of the activity of dehydrogenases in composts [19]. Dehydrogenases are enzymes from the group of oxidoreductases, which are found in cytoplasm and organelles of living organisms. Their task is to catalyse oxidation reaction of organic compounds, which is achieved by detachment of electrons and protons from organic compounds [5].

The objective of the performed investigations was to control the course of the composting process of sewage sludge with the addition of different structure-forming materials, including supplementation of glycerol in various doses, on the basis of the activity of dehydrogenases, counts of selected groups of microorganisms as well as quantities of the ammonia produced.

MATERIAL AND METHODS

The experiment was set up in 2009, in laboratory conditions. In the trials described, the authors used sewage sludge obtained from the left-bank Sewage Sludge Treatment Plant in Poznań, straw (wheat, maize and rapeseed) and sawdust. Their microbiological and chemical analysis is presented in Tables 1 and 2.

Table 1. The characteristics of biowastes in composts

Chamber	Components	Dry mass %	Contents	C/N initial	C/N final
K1	sewage sludge	14.70	50%	20.01	11.16
	wheat straw	90.00	30%		
	sawdust	83.77	20%		
K2	sewage sludge	14.70	50%	21.11	13.12
	wheat straw	90.00	30%		
	sawdust	83.77	20%		
K3	sewage sludge	14.70	50%	19.17	11.17
	maize straw	60.01	30%		
	sawdust	83.77	20%		
K4	sewage sludge	14.70	50%	18.10	13.13
	lupin straw	70.11	30%		
	sawdust	83.77	20%		

Table 2. The number of organisms and dehydrogenases activity in biowastes (beginning of experiment)

Groups of microorganisms	Sewage sludge	Wheat straw	Maize straw	Lupin straw	Sawdust
cfu·g^{-1} d.m. of material					
Bacteria	$1787.11 \cdot 10^6$	$10.11 \cdot 10^6$	$22.11 \cdot 10^6$	$11.00 \cdot 10^6$	$7.11 \cdot 10^6$
Molds	$158.67 \cdot 10^5$	$9.90 \cdot 10^5$	$27.89 \cdot 10^5$	$16.78 \cdot 10^5$	$929.88 \cdot 10^5$
Actinomycetes	$3353.19 \cdot 10^5$	$99.80 \cdot 10^5$	$78.80 \cdot 10^5$	$109.11 \cdot 10^5$	$429.00 \cdot 10^5$
Enterobacte--riaceae	$446.22 \cdot 10^3$	$20.45 \cdot 10^3$	0	0	0
Salmonella spp.	0	0	0	0	0
piece·kg^{-1} d.m.					
Helmints eggs (ATT)	0	-	-	-	-
µmol TPF·g^{-1} d.m. of compost·5h^{-1}					
Dehydrogenases activity	0.09	0.006	0.01	0.009	0.001

The investigations were carried out in a four-chamber bioreactor of 160 dm^3 volume each (Fig. 1) equipped with appropriate instruments, such as electronic probes for continuous registration of some process parameters (temperature, carbon dioxide, methane, ammonia and oxygen).

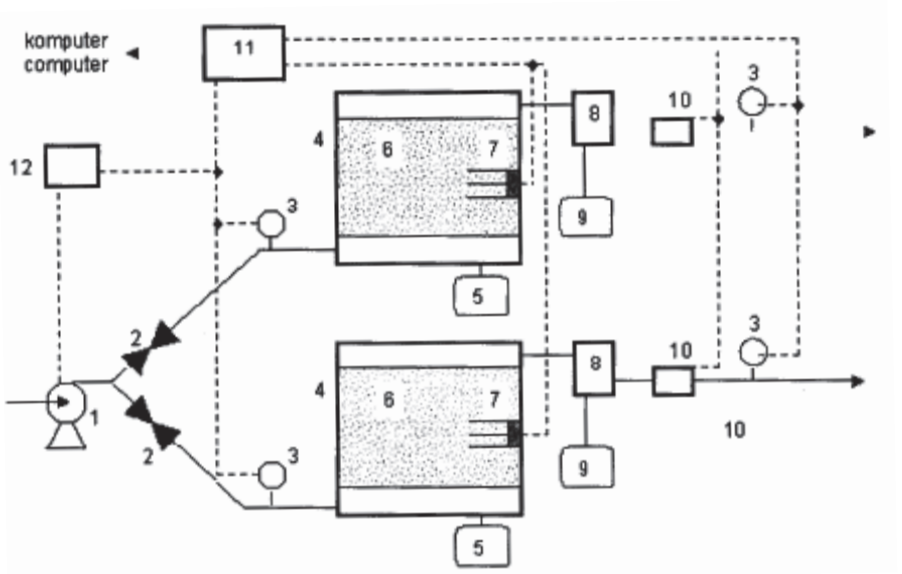

Fig. 1. Schematic diagram of the bioreactor: 1 – pump, 2 – flow regulator, 3 – flow meter, 4 – isolated chambers, 5 – drained liquides container, 6 – composted mass, 7 – sensors array, 8 – air cooling system, 9 – condensate container, 10 – multiplate gas sensor array, 11 – 32 chanel recorder, 12 – 1ir pump steering system air

The measurements of ammonia concentration were performed using MG-72 measuring heads manufactured by Alter SA, whose working accuracy was calibrated at least once a week using a model gas provided by the Messner Company for this purpose.

Materials for the experiments were mixed thoroughly in a container in weight proportions in relation to dry matter (Tab. 2). In addition, refined glycerol was added to each chamber in the following amounts: chamber K1 – 2l, K2– 3l, K3 –5l, K4 – 8l per 140 litres of the volume.

The experiment was carried out at constant oxygen flow of 4 $l \cdot min^{-1}$ in each chamber. The material in the bioreactor was composted for 872 hours (36 days), while compost samples were collected from all chambers simultaneously depending on the temperature value of the composted material.

Numbers of heterotrophic bacteria were determined on the Merck Company agar standard substrate following a 24-hour incubation at the temperature of 35°C [16]. Molds were determined during the period of 5 days at 24°C on Martin's substrate [15].

Numbers of actinomycetes were determined on a selective Pochon substrate [11] following a 7-day incubation on plates at the temperature of 26°C. *Salmonella* spp. were determined on the Merck Company XLT 4 substrate following a 18–24 hour incubation at the temperature of 37°C [17]. In order to make sure that the determined bacteria belonged to the *Salmonella* genus, Polish standard PN-Z-19000-1 was employed performing the confirming identification [20]. Merck – Chromocult® Coliform Agar substrate was used to determine bacterial counts from the *Enterobacteriaceae* family. Plates were incubated at the temperature of 37°C for 24 hours [14].

Eggs of parasites from *Ascaris* spp., *Trichuris* spp. and *Toxocara* spp. [8] genera were isolated from the sewage sludge used in the experiment with the assistance of a floating method.

Moreover, employing the spectrophotometric method, dehydrogenase activities in samples collected from the composted material were determined using 1% TTC (triphenylotetrazole chloride) as substrate following a 5-hour incubation at the temperature of 30°C and at 485 nm wavelength. The enzyme activity was expressed in μmol TPF·g^{-1}d.m. of the compost·$5h^{-1}$ [30].

All statistical analyses applied in the experiment were carried out using the Statistica 8.0 software [18].

RESULTS AND DISCUSSION

When analysing changes in heterotrophic bacteria counts (Tab. 3) in experimental compost, their highest numbers were found at the moment of trial establishment (date 1) in the compost designated as K1, which contained in its composition wheat straw and the smallest amount of glycerol – 2 l. A 24-hour composting process in controlled conditions caused an increase of temperatures in chambers by 11–23°C (Tab. 3) as well as a slight increase in pH values of the examined biowastes (Fig. 2), which, most probably, contributed to a rapid increase in bacterial counts recorded at the second date of the analyses. On this date, the highest increases in bacterial counts were observed in treatments K3 and K4, which could have been associated with the highest doses of glycerol added to them (K3–15 l, K4–20 l), which provided microorganisms with sources of carbon and energy.

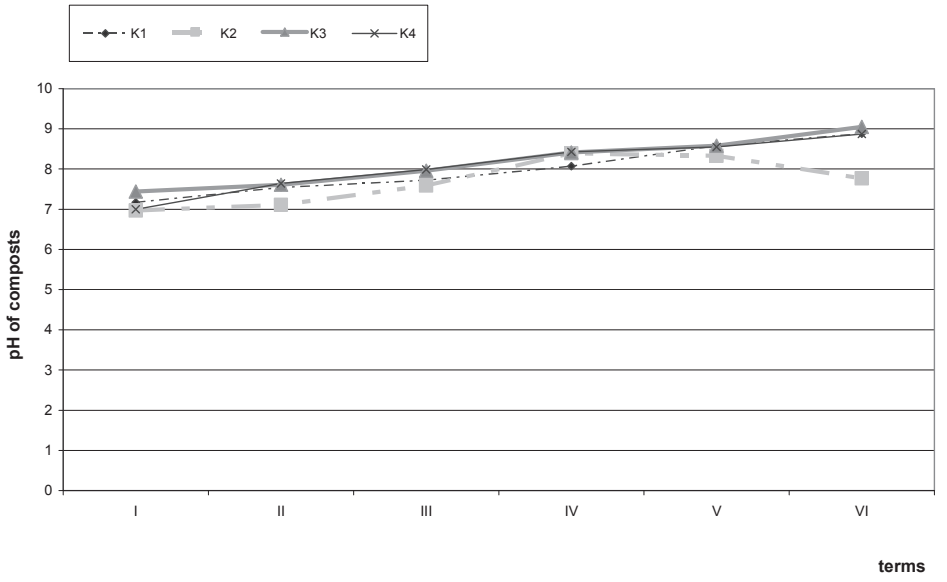

Fig. 2. The changes of pH in biowastes during composting process

Table 3. The number of microorganisms in composts (cfu·g^{-1} d.m. of material)

Kind of compost	Temperature of compost (°C)	Bacteria		Actinomycetes		Molds	
		cfu·10^5· g^{-1} d.m. of compost	Standard Deviation	cfu·10^5· g^{-1} d.m. of compost	Standard Deviation	cfu·10^3· g^{-1} d.m. of compost	Standard Deviation
I date – beginning of experiment							
K1	21	**256.70**	62.55	**127.68**	13.17	**254.77**	36.37
K2	20	**185.78**	27.13	**183.09**	3.80	**632.74**	89.00
K3	19	**124.71**	12.40	**96.59**	21.19	**224.62**	43.63
K4	20	**89.17**	26.31	**145.77**	27.65	**467.65**	98.02
II date – after 24 h							
K1	40	**3443.03**	1177.90	**726.03**	132.76	**1004.75**	77.67
K2	42	**3297.51**	735.10	**2982.67**	233.36	**1177.36**	36.77
K3	42	**5051.11**	1061.20	**2525.78**	193.69	**1794.11**	218.94
K4	31	**7046.77**	1805.47	**7777.23**	211.65	**2574.23**	442.61
III date – after 28 h							
K1	52	**10511.46**	2395.71	**698.23**	65.45	**588.11**	56.62
K2	50	**6111.70**	1318.59	**2368.98**	865.65	**344.43**	50.32
K3	42	**22272.38**	1776.00	**2301.11**	887.80	**1678.54**	160.00
K4	48	**19437.41**	6511.45	**5447.14**	969.96	**2205.30**	317.30
IV date – after 44 h (1.8 days)							
K1	65	**19.00**	1.01	**10.55**	1.13	**29.87**	4.02
K2	65	**12.88**	8.49	**60.18**	15.00	**20.00**	2.11
K3	60	**9.05**	1.50	**56.27**	4.08	**89.77**	22.48
K4	54	**34.11**	11.01	**79.99**	18.1	**120.44**	11.25
V date – after 110 h (4 days)							
K1	67	**2.34**	0.55	**73.45**	30.66	**0.96**	0.42
K2	63	**6.17**	0.98	**17.55**	28.00	**1.22**	0.16
K3	66	**1.22**	0.12	**93.68**	20.11	**1.01**	0.23
K4	65	**2.20**	0.18	**70.78**	18.33	**9.33**	4.33
VI date – after 287 h (36 days)							
K1	30	**2.00**	0.33	**199.98**	42.89	**7.88**	1.66
K2	29	**1.81**	0.22	**160.43**	67.72	**7.98**	2.34
K3	30	**34.21**	8.88	**144.70**	56.88	**13.45**	0.68
K4	31	**10.87**	3.03	**188.77**	49.44	**9.87**	1.33

On the successive date of the analyses (date III – analysis after 30h), a further increase in bacterial counts was recorded, despite the fact that the temperature in the composted biowastes continued to rise reaching the temperature of 52°C in chamber K1.

Janda and Falkowski [10] maintain that microorganisms owe their capability to grow and develop in conditions of high temperatures to specific biochemical-physiological properties of cells and, to a lesser extent, to cell morphological structure. In addition, the resistance of microorganisms to the negative influence of high temperature also depends on environmental factors (pH, substrate chemical composition).

Beginning with date IV, counts of the examined microorganisms in the composted biowastes declined dramatically together with the increase of temperatures to 54–65°C

(depending on a chamber), pH values (Fig. 2) as well as quantities of the ammonia released (Fig. 3).

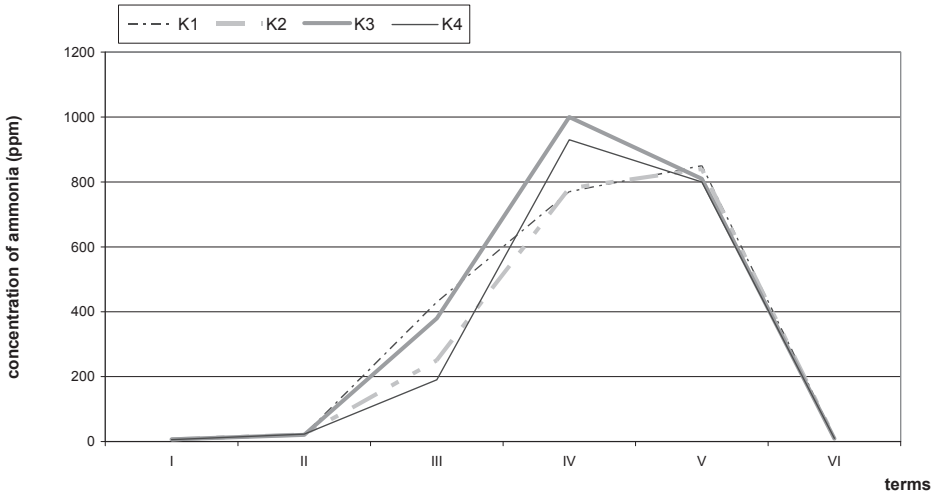

Fig. 3. The changes of concentration of ammonia in biowastes during composting process

Also Hassen *et al.* [7] reported reduced proliferation of mesophyllic bacteria together with the increase of temperature in their experiments with composted municipal wastes. According to Ishii *et al.* [9], such genera of bacteria as: *Pseudomonas*, *Achromobacter*, *Acinetobacter*, *Bacillus*, *Cellulomonas*, *Commomonas*, *Clostridium*, *Flavobacterium* and *Staphyllococcus* dominate during the mesophyllic phase of composting. On the other hand, only bacteria from the *Bacillus* genus dominated in the compost during the thermophilic phase with 70°C considered as the limiting temperature for their development [24]. Strom [26] maintains that *B. coagulans* and *B. circulans* species are dominant at the range of temperature of 49–55°C. When the temperature rises to 55–65°C – *B. brevis* and *B. stearothermophilus* species begin to dominate. After 36 days of the composting process (date VI), despite the drop of temperature to the value of 29–31°C, bacteria counts continued to remain on a low level.

When analysing the proliferation of actinomycetes in the composted biowastes (Tab. 3), their numbers were found to have increased several times following a 24-hour period of composting (date II). As in the case of bacteria, their proliferation was found the highest in treatment K4 (50% sludge + 30% rapeseed straw + 20% sawdust and 8 l glycerol). The above-mentioned phenomenon could be attributed to the inclusion of rapeseed straw in the composted biowastes because, as evident from the results of microbiological analyses shown in Table 2, in comparison with the remaining two straws, the content of actinomycetes in this treatment was the highest. In addition, the observed more intense proliferation of these microorganisms in this treatment on the second date of analyses could also have been caused by the presence of glycerol added in the amount of 8 l or by the temperature, which, in comparison with the remaining composted biowastes, reached the most optimal value (31°C) for the development of actinomycetes. During the consecutive three dates of analyses, together with the increase of individual parameters of the

composting process, i.e. temperature value (Tab. 3), pH (Fig. 2) and concentration of the released ammonia (Fig. 3), the numbers of the discussed microorganisms declined in all combinations. According to Taiwo and Oso [29], composting temperatures of the material determine the rate of many biological processes and play a key role in the succession of microorganisms understood as a change of quantitative and qualitative composition of microbial populations. Also Storm [26] reported that changes in temperature values were among the main factors conditioning the succession of microorganisms in the composting process.

However, on the basis of the performed statistical analyses (Tab. 4), it was found that in our studies changes in the total amounts of actinomycetes, similarly to the counts of heterotrophic bacteria, were not affected by temperature, but only to a limited extent, by pH values and concentrations of ammonia released from the composted materials. According to Wysocki and Lira [37], at the value of Pearson's linear correlation coefficient $0.2 \leq |\varsigma| < 0.5$ the relationship between traits was low, and at $|\varsigma| < 0.2$ – practically non-existent.

Table 4. Pearson correlation coefficient between the number of choosen groups of microorganisms (cfu·g $^{-1}$ d.m. of compost) and the value of NH_3, T and pH of compost

Kind of compost	Bacteria			Actinomycetes			Molds			*Enterobacteriaceae*		
	T	pH	NH_3	T	pH	NH_3	T	pH	NH_3	T	pH	NH_3
K1	0.09	-0.34	-0.04	-0.15	-0.38	-0.39	0.11	-0.30	0.01	0.01	0.35	0.34
K2	0.07	-0.35	-0.28	-0.01	-0.51	-0.42	-0.007	-0.37	-0.29	0.08	-0.43	-0.33
K3	-0.04	-0.28	-0.08	-0.05	-0.5	-0.40	-0.04	-0.22	-0.03	-0.05	-0.52	-0.38
K4	0.22	-0.18	-0.28	-0.06	-0.32	-0.42	0.28	-0.11	-0.20	0.18	-0.25	-0.27

Another increase of actinomycetes proliferation in our own investigations was recorded only during the last date (VI) at decreased temperature in the composted materials to the value of 29–31°C, decreased pH value and at a rapid decline of ammonia released from the biowastes. Also Wieland and Sawicka [34] reported increased numbers of actinomycetes in composted materials associated with a decrease in temperature to below 40°C. According to the above-mentioned researchers, the role of actinomycetes in the composting process is very important because these microorganisms take part in lignocellulose degradation, and act as precursors in the process of humus development. This is one of the groups of microorganisms that participate in the vitamin B12 synthesis in the composted materials as well as in antibiotic production.

Analysing the research results presented in Table 3, it was concluded that the highest numbers of molds on the day of trial establishment occurred in the composted material in chamber K2, which was composed in 30% of wheat straw as well as 50% sewage sludge, 20% sawdust and 10 l of glycerol. In addition, on the day of its establishment, the above compost was characterised by the highest C/N ratio – 21.11 (Tab. 1). The 24-hour process of composting led to a rapid increase in numbers of mould fungi, especially in K3 and K4 composts. As in the case of total bacterial counts and numbers of actinomycetes, the above phenomenon could have also been caused by the highest levels of glycerol supplementation. On the third date of the analyses (after 28 hours), together with the increase of temperature, pH and ammonia concentration released from the composted materials, the authors observed a reduction of fungi in all treatments. According to Hassen *et al.*

[7] (after Beffa *et al.*, 1996; Bollen 1993), it is not the increase of temperature during the composting process that is the main factor limiting the development of molds. The above researcher make microbiological antagonistic processes, presence of antibiotics and substrate pH changes responsible for the above phenomenon.

On the consecutive two dates of the thermophilic phase (IV and V), numbers of fungi continued to decline. Another increase in fungi numbers took place only on the VIth date of the analyses. The research results of our own studies are in agreement with data reported by Hassen *et al*. [7] as well as Ryckeboyer *et al.* [23]. The above researchers also reported declining numbers of fungi during the thermophilic phase of the composting process and their repeated increase following compost cooling. Increased numbers of fungi in the composted material together with the decline of temperature below 40°C were also reported by Wieland and Sawicka [34]. These microorganisms, apart from continued degradation of organic matter, also produce antibiotic substances used for natural disinfection of the compost. According to Błaszczyk and Fit [2], during the thermophilic phase, thermo-tolerant fungi, which grow best at temperatures lower than 50°C, are dominant.

On the basis of our own studies, it was found that on the day the experiment was established, no *Salmonella* spp. were determined in any of the analysed materials, including the sewage sludge used in the experiment (Tab. 2). Apparently, the purification processes employed in the sewage plant from which the sludge derived eliminated effectively those pathogens. Analysing the obtained research results it was found that a 24-hour composting process of biowastes (date II) increased their temperature, on average, by 11–23°C (Fig. 2) as well as pH value to more than 7 (Fig. 3). Most probably, changes of the above parameters contributed to a rapid increase in numbers of *Enterobacteriaceae* bacteria in all the experimental treatments (Fig. 4).

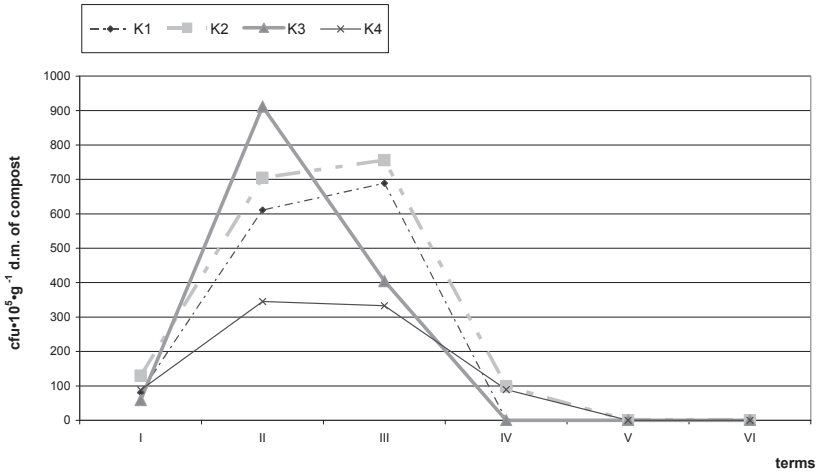

Fig. 4. The changes of *Enterobacteriaceae* number in biowastes during composting process

Zaremba and Borowski [38] maintain that optimal temperatures for bacterial growth (e.g. *Escherichia coli*) amount to 37ºC but a very good growth is also observed at temperatures ranging from 10 to 45ºC. According to the same researchers, the most optimal pH for bacterial growth is 7.2–7.4 which was also confirmed by our own investigations. On the consecutive III date of analyses, a further increase in bacterial counts from the *Enterobacteriaceae* family was recorded in treatments K1 and K2 only.

The above phenomenon is somewhat controversial because, on the above date, the temperatures in the examined treatments fluctuated from 50–52ºC, so they were by 8 to 10 degrees higher than in composts K3 and K4.

However, according to Tse-Dinh *et al.* [31] bacteria, similarly to other organisms, possess mechanisms enabling them to survive unfavourable environmental conditions, such as, for example, high temperatures. According to the above-mentioned researchers, at temperatures ranging from 30–42ºC, thermo-resistant proteins are synthesised in *Escherichia coli* cells which allow these mesophyllic bacteria to become thermo-tolerant organisms capable of developing even at the temperature of 52ºC.

Analysing the research results presented in Figure 4, it can be presumed that further increase in *Enterobacteriaceae* proliferation in combinations K1 and K2 was associated with smaller quantities of ammonia released from composts (Fig. 3). On the successive date of the analyses (IV), a total elimination of the discussed microorganisms was recorded in treatments K1 and K2. In the remaining two objects, the elimination of bacteria took place 16 hours later (date V). The above-described phenomenon was probably caused by the value of temperature, for it was observed that the elimination of *Enterobacteriaceae* took place at the temperature of 65ºC. The above observations were also corroborated by investigations carried out by Budzińska and Michalska [3]. Szejniuk, Kluczek [27] claim that temperature is the most important factor affecting pathogen survivability during the composting process. According to Epstein [6], a total elimination of *Escherichia coli* takes place at the temperature of 60ºC already after one hour. Szember [28] reports that one of the factors causing death of pathogenic microorganisms in composts is not only periodic effect of high temperature. Also a certain number of antibiotics produced, primarily, by numerous actinomycetes present in composts exert a sterilising influence. It can further be presumed that another factor, which could have reduced bacterial counts in our own investigations, was ammonia released from the composted materials, the concentrations of which, on dates IV and V, reached maximum values (Fig. 3).

Analysing changes in the levels of dehydrogenases activities in biowastes subjected to the composting process, it was found that on the day of trial establishment (date I) the levels of enzymatic activities were similar in all analysed treatments (Fig. 5). A 24-hour period of composting led to the increase of dehydrogenase activities in the compost materials in treatments K1, K3 and K4 and reduced their activities in treatment K2.

It can be presumed that differences in activities of the analysed enzymes were also associated with different concentrations of easily degradable organic matter in composted materials or with lower pH values of the compost material kept in chamber K2. A rapid decline in levels of dehydrogenase activities in all treatments was observed together with the beginning of the thermophilic phase (date III) and it continued until the termination of the experiment.

On the last date of the analyses (date VI), dehydrogenases activities remained on a similar, very low, level in all compost objects. Most probably, the observed low levels of

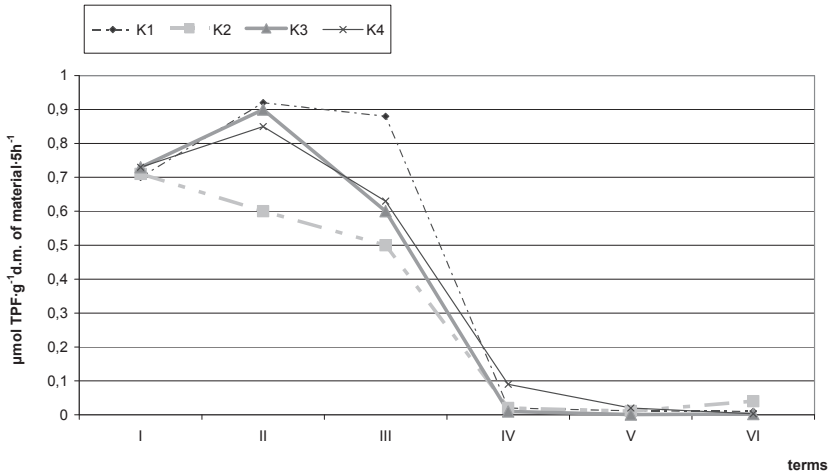

Fig. 5. The changes of dehydrogenases activity in biowastes during composting process

dehydrogenase activities towards the end of the composting process were connected with the depletion of decomposed organic matter in composts. According to Wolna-Maruwka and Sawicka [36], the highest metabolic activity of microorganisms during the composting process is observed at lower temperatures ranging from 25–45°C, and it declines rapidly during the thermophilic period only to increase again when mesophyllic conditions appear. Błaszczyk [1] reports that the highest metabolic activity of microorganisms is observed at temperatures optimal for the majority of thermophilic microorganisms, close to 60°C. This activity is attributed to several species of thermophilic fungi, which are responsible for the degradation of lignocellulose, hemicellulose and pentoses.

It is evident from the literature review [31, 35] that the activity of dehydrogenases can be positively or negatively correlated with numbers of bacteria and fungi and, consequently, be treated as an indicator of microbial activity as well as the dynamics of the composting process of biowastes. It was concluded, on the basis of the performed statistical analysis (Tab. 5), that the activity of dehydrogenases in the presented experiment was positively correlated with changes in the numbers of the majority of analysed groups of microorganisms. However, the highest positive Person's linear correlation coefficient between the above-mentioned traits in all treatments was recorded in the case of actinomycetes.

Table 5. Pearson correlation coefficient between the number of choosen groups of microorganisms (cfu·g $^{-1}$ d.m. of compost) and dehydrogenases activity (µmol TPF·g^{-1}d.m. of material·5h^{-1}) in composts

Kind of dependence	Kind of compost			
	K1	K2	K3	K4
Bacteria x dehydrogenases	0.68	0.44	0.41	0.52
Actinomycetes x dehydrogenases	0.81	0.59	0.71	0.72
Fungi x dehydrogenases	0.61	0.39	0.33	0.38
Enterobacteriaceae x dehydrogenases	-0.82	-0.90	0.73	0.63

Therefore, it can be presumed that it was this group of microorganisms that played the key role in the process of mineralisation of organic matter of composted biowastes.

CONCLUSIONS

1. No *Salmonella* spp. bacteria or eggs of intestinal parasites ATT were found in the analysed sewage sludge.
2. It was found that the slowest temperature increase in the composted material occurred in the treatment with the addition of maize straw and 5l of refined glycerol (treatment K3).
3. It was found that the composting process eliminated completely *Enterobacteriaceae* bacteria in all the experimental treatments. Therefore, all the obtained composts can be utilised agriculturally because they comply with the recommendation of the Minister of Agriculture and Rural Development (2008) as well as with the EU regulation (EC) No. 185/2007 from February 2007.
4. It was observed that the composting process helped reduce total numbers of heterotrophic bacteria and molds in all composts. On the other hand, numbers of actinomycetes remained unchanged.
5. It was determined that changes in numbers of the examined groups of microorganisms were influenced the strongest by changes in pH values followed by concentration of the released ammonia.
6. In all analysed experimental treatments, the activity of dehydrogenases exhibited the strongest positive correlation with changes in actinomycetes numbers.

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CHARAKTERYSTYKA MIKROBIOLOGICZNA KOMPOSTÓW SPORZĄDZONYCH NA BAZIE OSADU ŚCIEKOWEGO Z DODATKIEM GLICERYNY RAFINOWANEJ

Praca przedstawia charakterystykę mikrobiologiczną osadu ściekowego kompostowanego w warunkach kontrolowanych wraz z bioodpadami (słomą pszenną, kukurydzianą, łubinową, trocinami oraz gliceryną rafinowaną). Przeprowadzono doświadczenie, w którym wymieszano materiał w odpowiednim stosunku wagowym, a następnie umieszczono w komorach bioreaktora o stałym przepływie powietrza (4 $l \cdot min^{-1}$).
Przeprowadzony proces kompostowania miał na celu określenie dynamiki rozwoju bakterii heterotroficznych, grzybów pleśniowych, promieniowców oraz bakterii z rodzaju *Salmonella*, jak również z rodziny *Enterobacteriaceae*. Analizy mikrobiologiczne przeprowadzano na wybiórczych podłożach, stosując metodę płytkową Kocha. W doświadczeniu oznaczano również metodą flotacyjną obecność żywych jaj pasożytów jelitowych ATT (*Ascaris* spp., *Trichuris* spp., *Toxocara* spp.) oraz poziom aktywności dehydrogenaz, stosując jako substrat 1% chlorek trójfenylotetrazolu. Na podstawie uzyskanych wyników badań stwierdzono, że proces kompostowania przyczynił się do zmniejszenia liczebności bakterii heterotroficznych, grzybów pleśniowych oraz aktywności dehydrogenaz we wszystkich kombinacjach. Nie zaobserwowano z kolei redukcji promieniowców w kompostowanych materiałach, których zmiany liczebność najsilniej, dodatnio korelowały z poziomem aktywności dehydrogenaz. Ponadto stwierdzono, że zmiany liczebności analizowanych grup mikroorganizmów uzależnione były głównie od wartości pH oraz stężenia wydzielanego z kompostowanych materiałów amoniaku. Uzyskane rezultaty badań wykazały również, że osad ściekowy zastosowany w doświadczeniu nie zawierał bakterii *Salmonella* spp. oraz żywych jaj pasożytów jelitowych ATT, zaś proces kompostowania całkowicie zredukował liczebność bakterii z rodziny *Enterobacteriaceae* we wszystkich kombinacjach kompostowych. Uzyskane komposty spełniały więc normy sanitarne zgodne z rozporządzeniem Ministra Rolnictwa i Rozwoju Wsi (2008) oraz zgodne z przepisem Komisji (EC) Nr 185/2007 z 20 lutego 2007 r. zmieniające rozporządzenia (WE) nr 809/2003 oraz (WE) nr 810/2003 w zakresie przedłużenia okresu obowiązywania środków przejściowych dla kompostowni i wytwórni biogazu na mocy rozporządzenia (WE) nr 1774/2002 Parlamentu Europejskiego i Rady.