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IMPACT OF CRUDE GLYCEROL ON NUMBERS OF SELECTED MICROORGANISMS DURING COMPOSTING OF SEWAGE SLUDGE WITH SAWDUST AND DIFFERENT TYPES OF STRAWS*

WPŁYW DODATKU GLICERYNY SUROWEJ NA LICZEBNOŚĆ WYBRANYCH GRUP MIKROORGANIZMÓW PODCZAS KOMPOSTOWANIA OSADU ŚCIEKOWEGO Z TROCINAMI ORAZ RÓŻNYMI RODZAJAMI SŁOMY

Summary. The objective of the performed investigations was to determine the impact of the addition of various glycerol doses and different types of straws (wheat, lupine and maize straws) on some parameters of the composting process of sewage sludge. The experiment was carried out in a four-chamber isothermal bioreactor equipped in sensors for continual recording of some parameters of the composting process, e.g. temperature or degree of concentration of liberated ammonia. The performed investigations involved determination in the composted biowastes of total numbers of heterotrophic bacteria, actinomycetes, molds, Enterobacteriaceae numbers of helminth eggs (ATT). In addition, levels of dehydrogenases activity, temperature, pH and concentration of liberated ammonia in the composted material were analysed. The obtained results prove that despite changes occurring in the course of the composting process, numbers of actinomycetes and molds remained unchanged. It was found that the composting process resulted in the reduction of total bacterial counts and a complete elimination of Enterobacteriaceae. On the other hand, high level of dehydrogenases activities during the process can indicate high efficiency of organic matter mineralisation in the composted biowastes. It was concluded, on the basis of the obtained research results, that the addition of glycerol to composted sewage sludge, supplemented with such structure-forming materials as straw or sawdust, is an effective method of utilisation of these materials and provides possibilities for its further management for agricultural purposes. No indications were found to confirm that levels of glycerol doses exerted a significant impact on

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microbiological and biochemical parameters of composting. Furthermore, the final products obtained as a result of the composting process were characterised by similar C/N and NH_3 values, as well as metabolic activity of microorganisms. Therefore, it can be concluded that glycerol composting provides: an effective and environmentally-friendly way of its disposal which is of considerable importance in connection with the increasing biodiesel production and, in addition, a method for obtaining a valuable fertiliser of considerable ecological potentials.

Key words: microorganisms, dehydrogenases, wastes, compost, bioreactor, glycerol

Introduction

Use of biodiesel has grown dramatically during few years (BALAT 2005). The world total biodiesel production was around 1.8 billion liters in 2003. In Europe the most important biofuel is biodiesel. In the European Union biodiesel is the by far biggest biofuel and represents 82% of the biofuel production (BOZBAS 2008).

Producing biofuels offers an alternative to fossil fuels that can help provide solutions to many environmental problems. Using biodiesel reduces emission of carbon monoxide, sulfates, polycyclic aromatic hydrocarbons, nitrated polycyclic aromatic hydrocarbons (BALAT 2005).

In 2004, approximately 2 million tons of methyl esters were produced in the European Union and in 2010, it is estimated that 10 million tons of these compounds will be manufactured, whereas in Poland about 1.6 million tons of biodiesel are to be produced. However, all advocates of the introduction of this fuel into common use appear to forget that production of 1.6 million tons of biofuel generates many other problems both of logistic and technological nature which have to be solved. At the above-mentioned level of production, approximately 350 000 t of glycerol phase containing mainly glycerol and methanol, as well as about 16 000 t of soaps or higher fatty acids obtained from them, are to be recycled. There is no doubt that these problems can be overcome but the sooner we try to solve them, the better both for the economy and environment.

It is well known that following the process of fat transesterification, three phases are obtained: biodiesel, glycerol phase and a layer of soap separating them. Possibilities of recycling the glycerol phase and soaps will play a decisive role affecting the profitability of the biofuel production technology from rapeseed (HAYYAN et AL. 2010). The utilization of waste glycerol is becoming very important, because the amount of waste has been increasing year by year trough the increasing production of biodiesel and other oleochemicals (HUANG et AL. 2002).

On the other hand, glycerol is successfully used as carbon source for different microbial production (LEE et AL. 2001).

Improper sewage sludge management can contribute to environment degradation (BARAN and OLESZCZUK 2005) and that is why EU member states make many attempts to limit quantities of industrial and communal wastes and increasing emphasis is placed on maintaining ecological balance of the environment. One of the ways of organic waste utilisation, including various kinds of sewage sludge, can involve their use in agriculture as fertilisers. Fertiliser value of sludges is affected by the concentration of major plant nutrients (N, P, K, Mg, Ca), as well as microelements (CIEĆKO et AL. 2001, OZDEMIR et AL. 2004, VLYSSIDES et AL. 2004). However, sludges can also contain pathogenic

microorganisms whose numbers are influenced by the standard of life and health condition of the population living in a given area, kind of the employed sewage treatment plant and methods of sludge processing applied in the specific sewage treatment plant (IPEK et AL. 2001, BUDZIŃSKA et AL. 2005, SUÁREZ-ESTRELLA et AL. 2007, WOLNA-MARUWKA et AL. 2007). According to the regulation of the Ministry for the Environment (ROZPORZĄDZENIE... 2010), presence of *Salmonella* in sludge rules out agricultural utilisation of such material and, simultaneously, affects its way of further management. BUDZIŃSKA et AL. (2005) claim that *Salmonella* rods can be found in about 62% of fermented sludge samples and their survivability in this environment ranges from several weeks to even several years.

Composting appears to be one of the effective ways of sewage sludge utilisation since during this process, numbers of pathogenic microorganisms are reduced significantly or even eliminated completely and, in addition, contents of toxic and carcinogenic organic contaminations are also reduced (BARAN et AL. 2002, WOLNA-MARUWKA 2009).

BAUZA-KASZEWSKA et AL. (2010) maintain that one of the main factors affecting the appropriate course of the composting process is the value of temperature. It is one of the parameters contributing to the reduction or elimination of numbers of pathogens in the composted material. Temperature changes during composting are associated with the metabolic activity of microorganisms. It is the enhanced microorganism activity during the mesophyllic phase of the composting process that contributes, among others, to the release of large quantities of heat which can lead to the increase of temperature even up to the value of 75°C and, consequently, contribute to the hygienisation of the composted material (WOLNA-MARUWKA et AL. 2009).

Material and methods

The experiment was established in 2010 in laboratory conditions. In the described trials, the authors used sewage sludge obtained from the left-bank Sewage Sludge Treatment Plant in Poznań, straw (wheat, maize and lupine) and sawdust. Their microbiological and chemical analysis is presented in Tables 1 and 2. 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 and ammonia). 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 for this purpose a model gas provided by the Messner Company.

Materials for experiments were mixed thoroughly in a container in weight proportions in relation to dry matter (Table 2). In addition, crude glycerol was added to chamber in the following amounts: chamber K1 – control (without glycerol), K2 – 3 l of glycerol, K3 – 5 l of glycerol, K4 – 8 l of glycerol per 140 l 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 816 h (34 days), while compost samples were collected from all chambers simultaneously depending on the temperature value of the composted material. Compost samples indispensible to carry out microbiological and biochemical analyses were collected in accordance with the Polish standard PN-Z-15011-1:1998.

Table 1. Characteristics of biowastes in compost
Tabela 1. Charakterystyka bioodpadów w kompoście

Chamber Komora	Component Komponent	Dry mass Sucha masa (%)	Amount Ilość	C/N Initial Początkowe	C/N Final Końcowe
K1	Sewage sludge Osad ściekowy	14.70	42 kg w.m. (św.m.)	19.81	10.11
	Wheat straw Słoma pszenna	90.00	5 kg w.m. (św.m.)		
	Sawdust Trociny	83.77	3.20 kg w.m. (św.m.)		
	Glycerol Gliceryna	–	–		
K2	Sewage sludge Osad ściekowy	14.70	42 kg w.m. (św.m.)	20.34	12.22
	Wheat straw Słoma pszenna	90.00	5 kg w.m. (św.m.)		
	Sawdust Trociny	83.77	3.20 kg w.m. (św.m.)		
	Glycerol Gliceryna	–	3 l		
K3	Sewage sludge Osad ściekowy	14.70	42 kg w.m. (św.m.)	21.11	11.08
	Maize straw Słoma kukury-dziana	60.01	5 kg w.m. (św.m.)		
	Sawdust Trociny	83.77	3.20 kg w.m. (św.m.)		
	Glycerol Gliceryna	–	5 l		
K4	Sewage sludge Osad ściekowy	14.70	42 kg w.m. (św.m.)	21.89	12.89
	Lupine straw Słoma łubinowa	70.11	5 kg w.m. (św.m.)		
	Sawdust Trociny	83.77	3.20 kg w.m. (św.m.)		
	Glycerol Gliceryna	–	8 l		

Numbers of heterotrophic bacteria were determined on the Merck Company Agar Standard substrate following 5-day incubation at the temperature of 28°C (101621 STANDARD COUNT... 2004). Molds were determined during the period of 5 days at 24°C on Martin's substrate (MARTIN 1950).

Table 2. Number of microorganisms and dehydrogenases activity in biowastes (beginning of experiment)
Tabela 2. Liczebność mikroorganizmów oraz aktywność dehydrogenaz w bioodpadach (rozpoczęcie doświadczenia)

	Sewage sludge Osad ściekowy	Wheat straw Słoma pszenna	Maize straw Słoma kukurydziana	Lupine straw Słoma łubinowa	Sawdust Trociny
Microorganisms (cfu·g^{-1} d.m.) – Mikroorganizmy (jtk·g^{-1} s.m.)					
Bacteria Bakterie	2 011.01·10^{6}	9.09·10^{6}	48.88·10^{6}	24.60·10^{6}	11.01·10^{6}
Molds Grzyby pleśniowe	222.34·10^{5}	9.99·10^{5}	34.67·10^{5}	21.43·10^{5}	178.99·10^{5}
Actinomycetes Promieniowce	1 123.11·10^{5}	80.11·10^{5}	58.55·10^{5}	99.67·10^{5}	537.36·10^{5}
Enterobacteriaceae	889.04·10^{3}	0	0	0	0
Salmonella spp.	0	0	0	0	0
Helmints eggs (number in 1 kg d.m.) – Jaja helmintów (liczba w 1 kg s.m.)					
	0	0	0	0	0
Dehydrogenases activity (μmol TPF per 1 g of d.m. of material during 5 h) Aktywność dehydrogenaz (μmol TPF w 1 g s.m. materiału w ciągu 5 h)					
	0.08	0.008	0.001	0.007	0.001

Numbers of actinomycetes were determined on a selective Pochon substrate (KAŃSKA et AL. 2001) following a 7-day incubation of plates at the temperature of 26°C. *Salmonella* spp. were determined on the Merck Company XLT4 substrate following 18-24-hour incubation at the temperature of 37°C. In order to make sure that the determined bacteria belonged to the *Salmonella* genus, Polish standard PN-Z-19000-1: 2001 was employed performing the confirming identification. In order to determine numbers of bacteria from the Enterobacteriaceae family, a selective medium of Merck Company VRBD Agar was used (MOSSEL and CORNELISSEN 1963). Plates were incubated at the temperature of 37 ±1°C for 18-24 h. Isolated colonies were explanted onto nutrient agar (37°C for 24 h), later on agar medium with glucose (37°C for 24 h). Colonies on nutrient agar were stained using Gram's method which was followed by a rapid belt test for the identification of cytochrome oxidase presence.

Eggs of parasites from *Ascaris* spp., *Trichuris* spp. and *Toxocara* spp. (GUNDŁACH et AL. 1996) genera were isolated from the sewage sludge used in the experiment with the assistance of a floating method. Moreover, employing the spectrophotometric method, dehydrogenases activities in samples collected from the composted material were determined using 1% TTC (triphenylotetrazole chloride) as substrate following 5-hour incubation at the temperature of 30°C and at 485 nm wavelength. The enzyme activity was expressed in micromoles TPF (1,3,5-triphenyl-formasane) per 1 g of compost dry matter during 5 h (μmol·g^{-1}·5 h^{-1}) (THALMANN 1968).

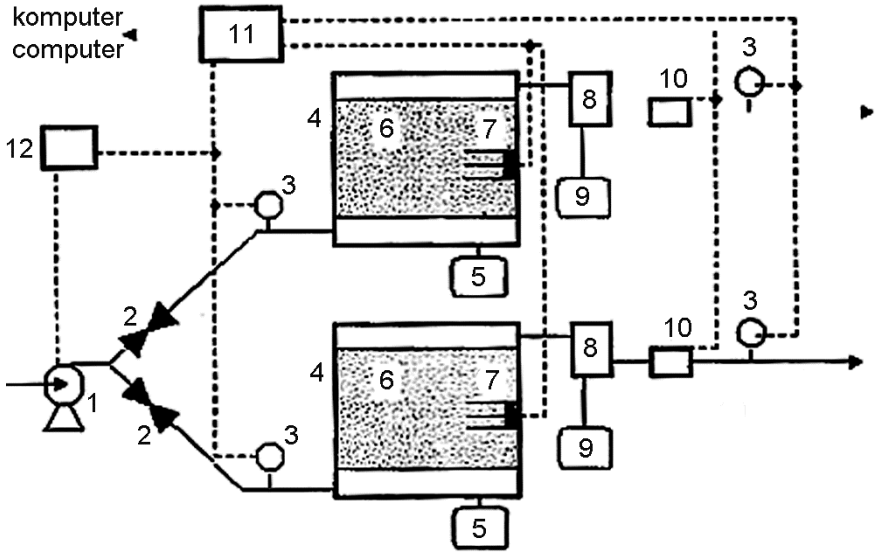

Fig. 1. Schematic diagram of the bioreactor: 1 – pump, 2 – oxygen flow regulator, 3 – flow meter, 4 – chambers, 5 – drained liquides container, 6 – composted biomass, 7 – sensors array, 8 – air cooling system, 9 – condensate container, 10 – multiplate gas sensor array, 11 – 32-chanel recorder, 12 – air pump steering system
Rys. 1. Schemat bioreaktora: 1 – pompa, 2 – regulator przepływu tlenu, 3 – przepływomierz, 4 – komory, 5 – zbiornik na odcieki, 6 – kompostowana biomasa, 7 – zespół czujników pomiarowych, 8 – system chłodzenia powietrza, 9 – zbiornik na skropliny, 10 – zespół czujników gazowych, 11 – 32-kanałowy rejestrator sygnałów pomiarowych, 12 – kontroler przepływu powietrza

All statistical analyses applied in the experiment were carried out in the Statistica 8.0 software (OTT 1984).

Results and discussion

Considering the results of microbiological analyses presented in Table 3, it was found that the highest total bacterial counts on the day of experiment establishment (date I) occurred in the material composted in chamber K1 (50% sewage sludge + 30% wheat straw + 20% sawdust). On the consecutive date of analyses (date II – after 24 h), it was probably the increase of temperature in composted materials (on average by 16--20°C) that caused quick multiplication of the discussed microorganisms in all composted biowastes. The strongest increase was observed in chamber K4 (50% sewage sludge + 30% lupine straw + 20% sawdust) to which 8 l of glycerol was introduced.

Another factor which could have contributed to microorganism development during the initial phase of the composting process could have been access to easily decomposable organic matter.

Table 3. Number of bacteria in composts (cfu·g^{-1} d.m.)
Tabela 3. Liczebność bakterii w kompostach (jtk·g^{-1} s.m.)

Kind of compost Rodzaj kompostu	Temperature of compost Temperatura kompostu (°C)	log (cfu·10^5·g^{-1} d.m.) log (jtk·10^5·g^{-1} s.m.)	log (Standard Deviation·10^5) log (Odchylenie Standardowe·10^5)
Date I – beginning of experiment – Termin I – rozpoczęcie doświadczenia			
K1	19	2.56	1.01
K2	19	2.50	1.03
K3	19	2.34	1.11
K4	18	2.47	1.02
Date II – after 24 h – Termin II – po 24 h			
K1	37	2.99	1.07
K2	35	3.36	1.72
K3	36	3.40	1.56
K4	38	3.53	1.83
Date III – after 48 h – Termin III – po 48 h			
K1	51	4.19	2.09
K2	50	4.27	2.11
K3	53	4.44	2.08
K4	53	4.28	2.01
Date IV – after 115 h (4.8 day) – Termin IV – po 115 h (4,8 dnia)			
K1	63	2.33	1.01
K2	65	2.08	1.0 6
K3	60	1.99	–0.82
K4	65	2.49	1.09
Date V – after 150 h (6 days) – Termin V – po 150 h (6 dni)			
K1	66	0.78	–0.04
K2	67	0.24	–0.72
K3	66	0.53	–0.65
K4	67	0.29	–0.72
Date VI – after 816 h (34 days) – Termin VI – po 816 h (34 dni)			
K1	29	2.14	1.01
K2	29	2.05	–0.52
K3	28	2.09	1.02
K4	31	1.95	1.01

According to MCKINLEY and VESTAL (1984), microorganisms settling organic wastes, i.e. various kinds of bacteria (e.g. *Lactobacillus*, *Leuconostoc*) are dominant during the mesophyllic phase of the composting process. They degrade easily accessible organic matter (sugars, proteins) and turn it into secondary organic compounds (primarily, organic acids) and heat. The accumulating heat increases the temperature in the composted material above that required by mesophyllic organisms. Thanks to the metabolic activity of microorganisms, the increasing temperature during the thermophilic phase results in sterilisation of the compost matter and destroys pathogenic organisms contained in it.

Therefore, beginning with the date IV of analyses, at which the temperature in the composted biowastes reached values of 60-65°C, total bacterial counts in all analysed experimental combinations decreased rapidly. PIOTROWSKA-CYPLIK et AL. (2008) maintain that temperature is one of the key factors influencing the activity of microbiological processes and, hence, the overall composting efficiency.

On the basis of our investigations, it was found that 24-hour composting process contributed to a rapid increase of temperature in all chambers of the bioreactor irrespective of the amount of the introduced glycerine dose (Fig. 2). Already after 48-hour period of composting of experimental biowastes, values of temperature corresponding to the thermophilic phase were recorded and they remained unchanged for another 102 h (until date V of analyses). Another drop in temperature was observed only during the last, date VI of analyses. According to MCKINLEY and VESTAL (1984), temperature is one of the main factors preconditioning appropriate course of the composting process. Its values, in turn, are associated with activities of microorganisms. Temperature values in the composting material, in turn, control the rate of many microbiological processes, as well as the selection and succession of microorganisms.

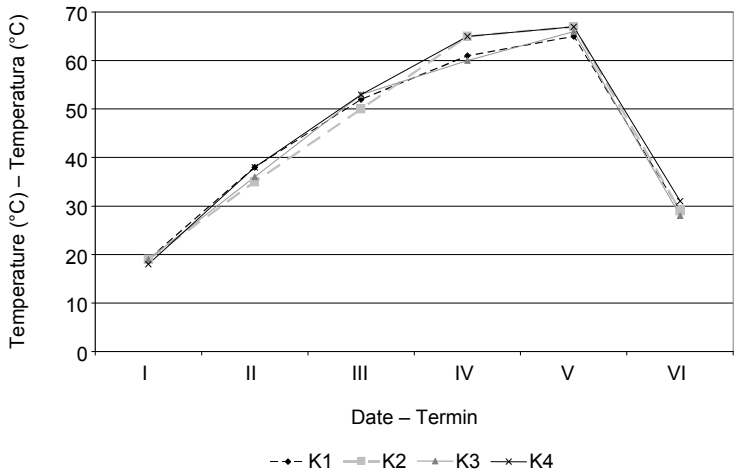

Fig. 2. Changes of the temperature in biowastes during composting process
Rys. 2. Zmiany wartości temperatury w bioodpadach podczas kompostowania

Wolna-Maruwka A., Pilarski K., Dach J., 2012. Impact of crude glycerol on numbers of selected microorganisms during composting of sewage sludge with sawdust and different types of straws. Nauka Przyr. Technol. 6, 2, #28.

The second factor, alongside temperature, affecting changes in the activity of microbiological processes, is pH of the substrate (Fig. 3) or ammonia concentration in biowaste during composting process (Fig. 4). According to the above-mentioned researchers, maintenance of the appropriate pH of the environment guarantees satisfactory environmental conditions for the development of microorganisms and, in addition, prevents losses of the compost bulk. Values of pH during the composting process continued to increase in the course of all analysed experimental combinations until date V of

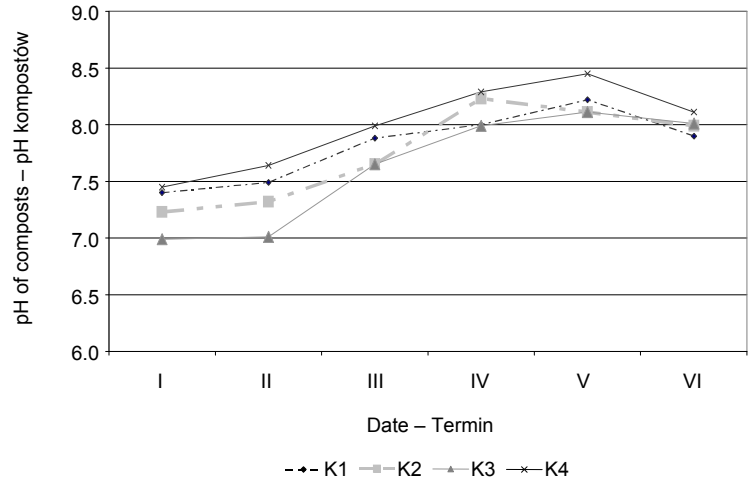

Fig. 3. Changes of pH of biowastes during composting process
Rys. 3. Zmiany wartości pH bioodpadów podczas kompostowania

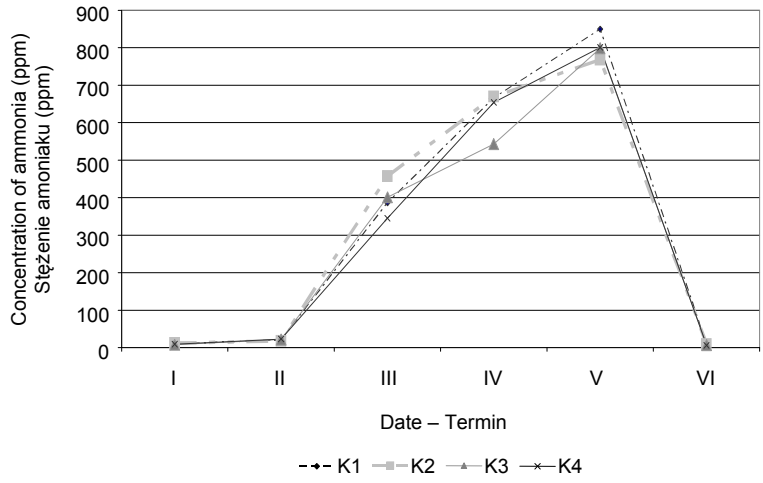

Fig. 4. Changes of concentration of ammonia in biowastes during composting process
Rys. 4. Zmiany stężenia amoniaku w bioodpadach podczas kompostowania

analyses (analysis after 6 days) when they reached their maximum (8.11-8.45) on the last date of analysis, a slight drop in pH values was recorded in all composted biowastes (Fig. 3).

However, on the basis of the performed statistical analyses presented in Table 6, it was concluded that the overall changes in pH values did not exert a significant impact on bacterial counts in consecutive dates of analyses. Furthermore, the overall analysis of changes in temperature values in the course of the performed investigations (Table 6) failed to indicate that temperature was the main factor determining counts of the considered microorganisms. It can only be presumed that the significant effect of temperature or pH values on bacterial counts referred only to a given date of analysis and not the entire period of experiment. According to WYSOCKI and LIRA (2003), at Pearson's linear correlation coefficient ranging between $0.2 \leq |\varsigma| < 0.5$, the link between traits is weak, while at $|\varsigma| < 0.2$ it does not exists practically.

HEINONEN-TANSKI et AL. (2006) reported that survivability of microorganisms in a given environment depends not only on environmental temperature and pH but also on the redox potentials, moisture content and kind of nutrients.

Another increase of proliferation of bacteria occurred not before VI date of analyses (after 34 days). Similar tendencies were reported in experiments carried out by HASSEN et AL. (2001) who, as in the case of our own studies, observed an increase in bacterial counts at the beginning of experiments followed by a decline in the rate of their multiplication caused, most probably, by temperature increase and, finally, another increase in numbers of mesophiles caused by the decline of temperature in the composted material.

It was also found that changes in numbers of actinomycetes in the composted biowastes during the experiment followed similar patterns to those of bacterial counts (Table 4).

Actinomycetes, together with bacteria proper and fungi, are considered as one of more important groups of microorganisms taking part in the mineralisation of organic matter of composted biowastes (THAMBIRAJAH et AL. 1995). Moreover, they are also ascribed a particularly important role during the cooling phase of the composted material where they are believed to contribute to further hygienisation of the material as a result of production of antibiotics. According to TUOMELA et AL. (2000), actinomycetes also include thermophilic species which, due to their enzymatic properties and resistance to temperature increases, play a vital role in the composting process.

Analysing the results of our own investigations, it was found that the inclusion of different doses of glycerol in the composted biowastes, similarly to bacteria, affected differences in numbers of actinomycetes only in the case of date II of analyses. During the consecutive dates of collection of compost samples, the amounts of the discussed microorganisms in the analysed experimental combinations were independent of the quantities of glycerol added to the analysed material. It was concluded on the basis of analysis of research results presented in Table 4 that numbers of actinomycetes in the examined compost combinations continued to increase until date III of analyses (analysis after 48 h) but their numbers declined dramatically together with the initiation of the thermophilic phase of the process only to increase again after 34 days of the composting process (date VI).

Table 4. Number of actinomycetes in composts (cfu·g^{-1} d.m.)
Tabela 4. Liczebność promieniowców w kompostach (jtk·g^{-1} s.m.)

Kind of compost Rodzaj kompostu	Temperature of compost Temperatura kompostu (°C)	log (cfu·10^5·g^{-1} d.m.) log (jtk·10^5·g^{-1} s.m.)	log (Standard Deviation·10^5) log (Odchylenie Standardowe·10^5)
Date I – beginning of experiment – Termin I – rozpoczęcie doświadczenia			
K1	19	1.87	–0.67
K2	19	2.01	1.07
K3	19	1.99	–0.66
K4	18	2.08	1.08
Date II – after 24 h – Termin II – po 24 h			
K1	37	2.78	1.11
K2	35	3.25	1.12
K3	36	2.95	1.17
K4	38	3.22	1.09
Date III – after 48 h – Termin III – po 48 h			
K1	51	3.18	1.00
K2	50	3.53	1.35
K3	53	3.35	1.09
K4	53	3.09	1.05
Date IV – after 115 h (4.8 day) – Termin IV – po 115 h (4,8 dnia)			
K1	63	1.99	0.79
K2	65	2.09	0.70
K3	60	1.83	0.62
K4	65	2.00	0.94
Date V – after 150 h (6 days) – Termin V – po 150 h (6 dni)			
K1	66	1.77	0.99
K2	67	1.53	0.90
K3	66	1.12	0.12
K4	67	1.78	0.90
Date VI – after 816 h (34 days) – Termin VI – po 816 h (34 dni)			
K1	29	2.65	1.09
K2	29	2.09	1.04
K3	28	1.95	0.46
K4	31	2.37	1.07

The performed statistical analyses revealed that, as in the case of bacteria, changes in the proliferation of actinomycetes in the composted biowastes failed to correlate with temperature changes but were found to be linked slightly with the pH value of the substrate (Table 4). It can only be presumed that changes in the counts of proper bacteria and actinomycetes in the course of the experiment were determined, to a considerable extent, by the access to organic matter.

When comparing the quantities of molds in the analysed compost samples (Table 5), it was found that on date I of analysis their greatest numbers occurred in the K4 material (50% sewage sludge + 30% lupine straw + 20% sawdust), i.e. the one that contained the highest addition of technical glycerol (8 l).

Table 5. Number of molds in composts (cfu·g^{-1} d.m.)
Tabela 5. Liczebność grzybów pleśniowych w kompostach (jtk·g^{-1} s.m.)

Kind of compost Rodzaj kompostu	Temperature of compost Temperatura kompostu (°C)	log (cfu·10^3·g^{-1} d.m.) log (jtk·10^3·g^{-1} s.m.)	log (Standard Deviation·10^3) log (Odchylenie Standardowe·10^3)
1	2	3	4
Date I – beginning of experiment – Termin I – rozpoczęcie doświadczenia			
K1	19	1.89	0.75
K2	19	2.09	1.06
K3	19	2.35	0.99
K4	18	2.27	1.11
Date II – after 24 h – Termin II – po 24 h			
K1	37	2.88	1.13
K2	35	3.00	1.09
K3	36	3.22	1.09
K4	38	2.99	1.12
Date III – after 48 h – Termin III – po 48 h			
K1	51	3.02	0.99
K2	50	2.95	1.04
K3	53	3.04	1.01
K4	53	3.12	1.04
Date IV – after 115 h (4.8 day) – Termin IV – po 115 h (4,8 dnia)			
K1	63	2.78	0.98
K2	65	2.83	1.11
K3	60	2.99	0.89
K4	65	3.60	1.12

Table 5 – cont. / Tabela 5 – cd.

1	2	3	4
Date V – after 150 h (6 days) – Termin V – po 150 h (6 dni)			
K1	66	1.82	0.56
K2	67	1.83	–0.78
K3	66	1.84	0.95
K4	67	1.66	0.46
Date VI – after 816 h (34 days) – Termin VI – po 816 h (34 dni)			
K1	29	2.00	0.66
K2	29	2.34	1.03
K3	28	2.53	1.00
K4	31	2.46	1.12

On dates II and III of analyses, fungal proliferation increased several times in all analysed experimental objects and their numbers began to decline only after the onset of the thermophilic phase of the experiment. Another increase in the numbers of molds in the composted materials was recorded only in date VI (i.e. last) of analysis. According to SAWICKA and WOLNA-MARUWKA (2008), the dominant microflora during the phases of cooling and maturing include molds and actinomycetes degrading organic polymers and producing enzymes essential for the synthesis of appropriate humus fractions.

The above results are in keeping with observations reported by RYCKEBOER et AL. (2003) who recorded declining numbers of fungi at temperatures exceeding 50°C and their fresh increase after cooling of the composted material. On the other hand, CHRONI et AL. (2009) claim that temperature increase even up to 60°C fails to eliminate completely these organisms from the composted material due to the presence of spores produced by them.

The performed sanitary examination of the sewage sludge as well as other biowastes from which the composts were made failed to identify in them bacteria from the *Salmonella* genus as well as helminth eggs (Table 2). Absence of bacteria of the *Salmonella* spp. as well as ATT eggs in the examined sewage sludge could have been associated with high effectiveness of the sludge processing method employed at the sewage treatment plant from which sludge intended for composting derived. However, the sewage sludge used in the described experiment contained high numbers of Enterobacteriaceae (Table 2). Identification of these bacteria in the applied materials appears very important because, apart from the *Salmonella* spp., this family also comprises other potentially pathogenic microorganisms such as: *Escherichia coli*, *Proteus*, *Shigella*, and *Klebsiella* which, according to BUSTAMANTE et AL. (2008), are considered as faecal contamination indicators.

When analysing changes in numbers of Enterobacteriaceae in the composted materials (Fig. 5) during the performed investigations, it was found that the applied composting process led to complete elimination of the discussed microorganisms from all composted biowastes. Beginning from date II, a strong proliferation of Enterobacteriaceae

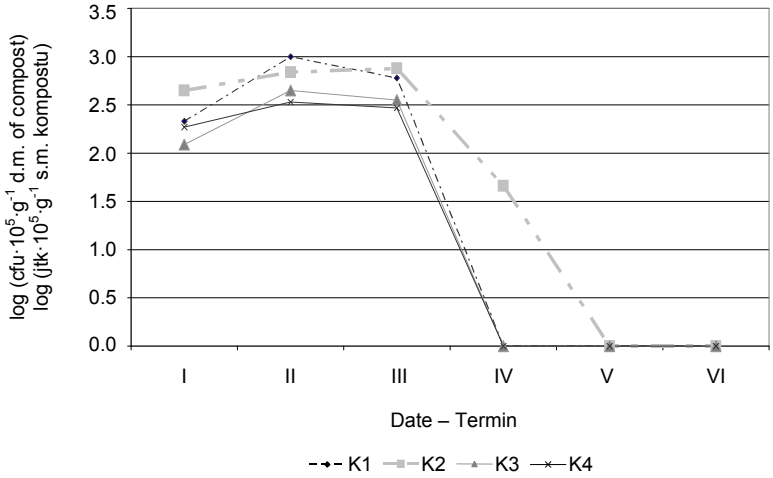

Fig. 5. Changes of Enterobacteriaceae number in biowastes during composting process
Rys. 5. Zmiany liczebności Enterobacteriaceae w bioodpadach podczas kompostowania

took place which was most probably associated with the occurrence of temperature optimal for their growth and development or access to easily decomposable organic matter during the initial period of composting.

On the basis of data presented in Figure 3, it was concluded that further increase of temperature, pH, as well as ammonia concentrations (Fig. 4) in the composted materials contributed to a rapid decline in numbers of the discussed bacteria in all experimental combinations with the exception of object K2 (50% sewage sludge + 30% wheat straw + 20% sawdust + 3 l glycerol). This phenomenon can probably be contributed to the temperature value which, at this time, attained the lowest value (40°C) in this object.

Together with further increase in temperature, pH and concentration of liberated ammonia recorded during two consecutive dates of analyses (IV and V), further reduction in numbers of the Enterobacteriaceae was observed. According to PAGANS et AL. (2006), the strongest ammonia emissions take place when the temperature increases above 45°C and pH value to about 9. In addition, ammonia liberated from the composted biowastes is one of the factors contributing to hygienisation of the composted materials. Even another decrease of temperature, pH value or concentration of the liberated ammonia failed to contribute to their fresh proliferation on the last, date VI of analyses.

However, it was observed, on the basis of the performed statistical analysis (Table 6), that the main factor affecting the elimination of the discussed bacteria in the composted biowastes was pH value, then ammonia concentration, whereas temperature contributed to this process to the least degree.

It is clear from literature review that there are three major factors contributing to pathogen elimination from composted biowastes: 1) temperature of the order of 55-70°C reached at the thermophilic phase, 2) production of phenols exhibiting antimicrobial activities and 3) the presence of microorganisms competing with pathogens for food or

Table 6. Pearson correlation coefficient between the number of chosen groups of microorganisms and physico-chemical parameters of composts
Tabela 6. Współczynnik korelacji liniowej Pearsona pomiędzy liczebnością wybranych grup mikroorganizmów a fizyczno-chemicznymi właściwościami kompostów

Kind of compost Rodzaj kompostu	Bacteria Bakterie			Actinomycetes Promieniowce			Molds Grzyby pleśniowe			Enterobacteriaceae		
	T	pH	NH_3	T	pH	NH_3	T	pH	NH_3	T	pH	NH_3
K1	0.11	0.04	0.07	–0.03	–0.17	–0.29	0.40	0.08	0.15	–0.26	–0.66	–0.50
K2	0.11	–0.19	0.13	0.02	–0.37	–0.03	0.12	–0.23	–0.03	–0.35	–0.80	–0.37
K3	0.22	–0.03	0.11	0.14	–0.21	–0.03	0.07	–0.39	–0.21	–0.19	–0.70	–0.38
K4	0.15	–0.08	–0.01	–0.09	–0.42	–0.37	0.27	–0.09	–0.004	–0.34	–0.72	–0.50

T – temperature.
T – temperatura.

producing various antibiotics that reduce survivability and growth of pathogenic microorganisms (SUÁREZ-ESTRELLA et AL. 2007). According to REINTHALER et AL. (2003), from year to year, bacteria isolated from sewage sludge, e.g. *E. coli*, are observed to develop increased resistance to a growing number of antibiotics which can contribute to difficulties associated with their elimination from composted biowastes.

Dehydrogenases belong to the group of oxidoreductases and reflect the degree of metabolic activities of microorganisms. Therefore, it seems that determination of their activity may elucidate the dynamics of the composting process.

Analysing activity changes of dehydrogenases (Fig. 6) in the course of the performed investigations, it was found that the highest mean level of activities of the exam-

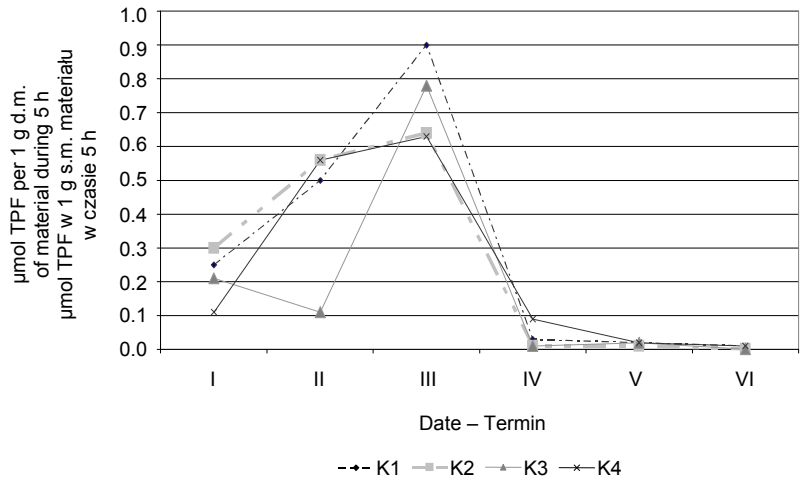

Fig. 6. Changes of dehydrogenases activity in biowastes during composting process
Rys. 6. Zmiany aktywności dehydrogenaz w bioodpadach podczas kompostowania

ined enzymes occurred in compost K1 (50% sewage sludge + 30% wheat straw + 20% sawdust). The process of composting lasting 24 h resulted in the increase of dehydrogenases activities in all composted materials with the exception of combination K3 consisting of maize straw and 5 l of glycerol. It can only be presumed that the recorded rapid decline in the activity of the above enzymes was caused by the appearance of some microbial cell metabolites exerting an inhibiting effect on the level of dehydrogenase activity.

Further temperature increase during date III of analyses contributed to a dramatic, maximal increase of enzyme activities in all composted biowastes. During the thermophilic phase, a rapid decline of dehydrogenases activities in the analysed compost combinations was observed. Moreover, this low level of dehydrogenases activities remained unchanged until the end of the experiment. The highest metabolic activity of microorganisms at temperatures ranging from 25 to 45°C was also reported by MCKINLEY and VESTAL (1984). BŁASZCZYK (2007) claims that the following groups of microorganisms: bacteria, actinomycetes and fungi are responsible for the mineralisation of composted biowastes. This opinion is corroborated by the statistical analysis presented in Table 7 from which it is evident that the highest metabolic activity during the composting process was exhibited by actinomycetes followed by bacteria and finally molds.

Table 7. Pearson correlation coefficient between the number of chosen groups of microorganisms and dehydrogenases activity in composts
Tabela 7. Współczynnik korelacji liniowej Pearsona pomiędzy liczebnością wybranych grup mikroorganizmów a aktywnością dehydrogenaz w kompostach

Kind of dependence Rodzaj zależności	Kind of compost – Rodzaj kompostu			
	K1	K2	K3	K4
Bacteria × dehydrogenases Bakterie × dehydrogenazy	0.89	0.72	0.97	0.80
Actinomycetes × dehydrogenases Promieniowce × dehydrogenazy	0.87	0.88	0.92	0.94
Molds × dehydrogenases Grzyby pleśniowe × dehydrogenazy	0.73	0.70	0.29	0.80
Enterobacteriaceae × dehydrogenases Enterobacteriaceae × dehydrogenazy	0.58	0.99	0.53	0.91

Conclusions

1. All analysed microbiological, biochemical and physical parameters from the performed experiment prove that the course of the composting process was correct.

2. Various doses of glycerol and different types of straws introduced into the composted biowastes failed to exert a significant impact on any of the analysed parameters of the composting process.

3. The applied composting process reduced total bacterial counts, although numbers of actinomycetes and molds in the composted biowastes remained unchanged.

4. Complete inactivation of Enterobacteriaceae in experimental composts confirms high effectiveness of hygienisation of composting processes which guarantees their safe utilisation for agricultural purposes.

5. Despite differences in the composition of individual components in the prepared composts, only slight differences in the reduction of Enterobacteriaceae rate were recorded.

6. The high level of dehydrogenases in the composted biowastes confirms high dynamics of the mineralisation process for which the following microorganisms were responsible: actinomycetes, heterotrophic bacteria and molds.

7. Composts obtained on the last date of analyses are characterised by a low C/N ratio, as well as a low level of dehydrogenases activity which can indicate well advanced organic matter decomposition of the examined biowastes.

8. It was found that composting of glycerol with different additives is an effective method and, at the same time, it appears to be a cheaper way of its management opening more optimistic perspectives connected with increasing biodiesel and, consequently, glycerol production.

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WPŁYW DODATKU GLICERYNY SUROWEJ NA LICZEBNOŚĆ WYBRANYCH GRUP MIKROORGANIZMÓW PODCZAS KOMPOSTOWANIA OSADU ŚCIEKOWEGO Z TROCINAMI ORAZ RÓŻNYMI RODZAJAMI SŁOMY

Streszczenie. Celem przeprowadzonych badań było określenie wpływu dodatku różnych dawek gliceryny na niektóre parametry procesu kompostowania osadu ściekowego ze słomą (pszenna, łubinową i kukurydzianą) oraz z trocinami. Doświadczenie przeprowadzono w czterokomorowym, izotermicznym bioreaktorze, wyposażonym w czujniki do stałej rejestracji niektórych parametrów procesu kompostowania, np. temperatury czy stężenia wydzielanego amoniaku. Badania dotyczyły oznaczenia w kompostowanych bioodpadach ogólnej liczby bakterii heterotroficznych, promieniowców, grzybów pleśniowych, bakterii z rodziny Enterobacteriaceae, jak również liczby jaj helmintów (ATT). Ponadto w kompostowanym materiale dokonywano analizy poziomu aktywności dehydrogenaz, temperatury, pH i stężenia wydzielanego amoniaku. Uzyskane wyniki dowodzą, że mimo zmian zachodzących w procesie kompostowania liczebność promieniowców i grzybów pleśniowych pozostała na stałym poziomie. Stwierdzono, że proces kompostowania przyczynił się do zmniejszenia ogólnej liczby bakterii i całkowitej eliminacji pałeczek należących do rodziny Enterobacteriaceae. Wysoki poziom aktywności dehydrogenaz w trakcie prowadzonych badań świadczyć może z kolei o bardzo dużej skuteczności mineralizacji materii organicznej kompostowanych bioodpadów. Na podstawie uzyskanych wyników stwierdzono, że kompostowanie gliceryny z osadem ściekowym i dodatkiem strukturotwórczych materiałów, takich jak słoma czy trociny, jest skutecznym sposobem ich utylizacji i stwarza możliwości ich dalszego zagospodarowania na cele rolnicze. Nie stwierdzono, aby wielkość dawek gliceryny wywierała istotny statystycznie wpływ na mikrobiologiczne i biochemiczne parametry kompo-

stowania. Ponadto, końcowe produkty uzyskane w wyniku powyższego procesu charakteryzowały się podobnym stosunkiem C/N, wartością NH_3, jak również aktywnością metaboliczną drobnoustrojów. Na podstawie uzyskanych wyników badań można więc wnioskować, że kompostowanie gliceryny stanowi skuteczny i przyjazny dla środowiska sposób jej zagospodarowania, co ma istotne znaczenie w związku ze zwiększeniem produkcji biodiesla, a ponadto jest sposobem na pozyskanie cennego nawozu o znacznym potencjale ekologicznym.

Słowa kluczowe: mikroorganizmy, dehydrogenazy, odpady, kompost, bioreaktor, gliceryna

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