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Regular paper

Impact of a microbial-mineral biopreparation on microbial community and deodorization of manures*

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The aim of this study was to determine the number of bacteria in poultry, cattle and swine manure in order to perform hygienization and deodorization using a microbial-mineral biopreparation. The highest number of bacteria was recorded in laying hens manure (5.1×10^{10} cfu/g). It was noted that bacteria: coliforms, *E. coli, Clostridium, Enterococcus* number was reduced (1-2 log) after the biopreparation application. The investigated odorous compound concentrations were reduced with 34–78% efficiency, depending on the type of manure and odorant. All odorous compounds were efficiently reduced only in the case of laying hen manure.

Key words: microbial contamination; manure; hygienization, deodorization

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INTRODUCTION

Intensive animal production leads to the production of large amounts of manure which may create a significant ecological hazard. Animal manure is a mixture of excreta, feed, feathers, and bedding material (Stephenson *et al*., 1990). Due to the presence of nitrogen, phosphorus, potassium, and other minerals, manure is commonly used as a fertilizer (Kuczewski & Łomotowski, 2002), however it can simultaneously be dangerous to various organisms (Gupta & Doherty, 1990; Gupta & Kelly, 1990; Gupta & Krishnamurthy, 1990). Production and processing of manure as fertilizer often results in remission of pollutants into the air, which also increases the discomfort of nearby inhabitants (Siemiński, 2008, Hayes *et al*., 2004; Persaud *et al*., 1996; Varel & Wells, 2007). In particular, formation and release of odorous compounds and pathogenic microbiota pose serious problems for animals and pollute rural locations.

The analysis of Polish agro-industry states that 79% of complaints for odor nuisance is connected with the animal production (poultry 39%, pigs 35% and cattle 5%) (Kośmider *et al*., 2002). Ventilation systems in livestock buildings and absorption/diffusion of animal manure into the soil and water are the sources of waste emission (Barowicz, 2007).

Odorous nuisance is generated by a large number of different volatile compounds derived from livestock manure, and the main odorants are: ammonia, hydrogen sulfide, thiols and volatile fatty acids (Burgess *et al*., 2001; Blaszczyk, 2007). The concentration of gaseous pollutants must be controlled and reduced, not only because of their emission into the atmosphere, but also because of the health hazard to farm animals. Odorants can cause digestion disturbances, increased sensitivity to infectious diseases, bone demineralization and anemia (Kuczewski & Łomotowski, 2002). Volatile odorous compounds can also negatively influence people working at the farms due to the allergies, chronic stress, decreased immunity, hypoxia, headaches, nausea and diarrhea (Siemiński, 2008, Nicell, 2009). Apart from these complaints, the staff of animal farms is at risk due to potential presence of pathogenic microorganisms including: *Chlamydia ornithosis*, *Bacillus anthracis, Salmonella choleraesuis var. typhi, Listeria monocytogenes, Mycoplasma spp.*, *Staphylococcus aureus*, *Streptococcus* spp., *Candida albicans, Cryptococcus neoformans*, as well as the H5N1 virus, classified in the 2nd and 3rd Groups of high risks (Dutkiewicz *et al*., 2000).

Due to the fact that the problem of air pollution from livestock production is of a high importance, numerous studies were performed concerning the use of air scrubbing (Opalinski *et al*., 2010; Opalinski *et al*., 2015), biofiltration (Tymczyna *et al*., 2004), natural antimicrobial feed additives (Varel, 2002) or multistrain probiotics (Zhang & Kim, 2014). Moreover, there is also a great need to search and develop microbiological biopreparations containing microbes naturally occurring in manure (Borowski *et al*., 2010), which will be able to remove odorous compounds and disinfect the livestock buildings (to improve the sanitary conditions).

The aim of this study was to determine the number of bacteria in poultry (laying hens, broilers, geese), cattle, swine manures and to perform hygienization and deodorization of these wastes using a microbial-mineral biopreparation.

MATERIALS AND METHODS

Manures. Poultry manure samples were collected from three different poultry farms: 1. enriched cage system (27 800 laying hens; Zgierz, Poland), 2. deep litter system (20 000 broilers; Aleksandrów Łódzki, Poland), 3. free-range system (5 000 geese; Dobra, Poland). Cattle manure samples were taken from a dairy farm (40 cows; Lisewo, Poland) and swine manure from a pig-breeding

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Abbreviations: DM, dry matter; DOM, dry organic matter; TOC, total organic carbon; TKN, total Kjeldahl nitrogen; P, total phosphorus; X, average value; S.D., standard deviation; Min, minimum value; Max, maximum value

farm with a closed cycle production system (60 sows; Wieczyn, Poland).

Sampling of manures. To perform the chemical and microbiological analysis of manures, the homogenization of samples with Bio-Gen PRO200 homogenizer was conducted. Randomly selected material (20 g) was collected from five different places. Each type of manure was mixed to obtain a homogeneous sample. Then, one part of the manure (10 g) was used for chemical analysis, while the other part (10 g) was suspended in 90 ml of sterile physiological saline (0.85% NaCl) for microbiological analysis. All analyses were performed three times using three independent replicates.

Chemical analysis. Chemical analysis of manure samples, i.e. dry matter (DM), ash, dry organic matter (DOM), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), total phosphorus (P) and pH value measurement were conducted. Dry matter, ash, dry organic matter were determined according to standard gravimetric methods (Harvey, 2000). The pH value of collected manure samples was measured using a laboratory multi-meter CP- 411 (Elmetron, Poland). Total phosphorus and total Kjeldahl nitrogen were determined by a spectrophotometric method, after mineralization of manure samples. Total Kjeldahl nitrogen content was determined using a modified Nessler method, as described by Hermanowicz *et al.* (1999), and adapted by HACH®. Total phosphorus was determined by molybdate method using also HACH DR2000 spectrophotometer. Total organic carbon was evaluated using the coulometric method (Kulomat 702Li/C, Strohlein) (Bisutti *et al.*, 2004).

Microbiological analysis. Determination of the number of microorganisms in collected manure samples was carried out before hygienization (0 hour) and after hygienization (96 hours) process performed with the investigated biopreparation. The number of microorganisms was determined using the plate method, samples were incubated 24–48 hours, depending on the isolated group of microbes: TSA with nystatin agar at temperature 28±2°C for total number of aerobic and anaerobic bacteria; BAAA, TSC and endo-LES agar were used for bacteria *Enterococcus, Clostridium* and coliforms at temperature 37±2°C; for *Escherichia coli* mFC agar and temperature 44±2°C were used. After incubation, colonies were counted and the result was expressed as colony forming units per 1 g of manure (cfu/g).

Hygienization and deodorisation of manures by biopreparation. Hygienization and deodorization process of all investigated types of manures were conducted with the use of a biopreparation. The biopreparation that was developed at Lodz University of Technology, Institute of Fermentation Technology and Microbiology (Gutarowska *et al.*, 2014), consisted of six strains of microorganisms: *Pseudomonas fluorescens* (ŁOCK 0961), *Bacillus subtilis* (ŁOCK 0962), *Bacillus megaterium* (ŁOCK 0963), *Leuconostoc mesenteroides* (ŁOCK 0964), *Enterococcus faecium* (ŁOCK 0965) and *Streptomyces rutgersensis* (ŁOCK 0967), that have been deposited in the Pure Culture Collection ŁOCK 105 ITFiM TUL. The nucleotide sequences of 16S rRNA gene of bacterial strains included in the biopreparation have been deposited with the NCBI GenBank with the numbers KJ: 919967-919972. The microbes were mixed 1:1 (v/v) and embedded on mineral carriers: perlite and bentonite (20:80 w/w). The procedure of microorganism deposition on these carriers is patent protected (Polish patent no. P393863).

Different types of manure (0.5 kg each) were tested in a laboratory set-up shown in Fig. 1. The core of that installation were two chambers with a working volume of 0.8 L each. In each experimental run, control and experimental chambers were filled with a 0.5 kg of manure. Inside the control chamber, only the examined type of manure was placed (with no addition of the biopreparation), while simultaneously in the experimental chamber, the manure surface was powdered with 50 ml of the investigated biopreparation. The chambers were tightly closed and aerated for 5 minutes (at a flow rate of 2 L/min) to provide aerobic conditions prior to sampling. Then, the aeration was turned off and hygienization and deodorization processes were continued for 96 hours. After this time, the aeration was turned on again for another sampling. Then, the exhaust air samples from each chamber were collected into Tedlar bags at the beginning and at the end of a 4 day experimental period. In order to evaluate the concentration of odorous compounds before and after the application of biopreparation, analyses were performed using gas chromatography (GC). Moreover, manure samples for microbiological analysis were collected.

Figure 1. Equipment for hygienization and deodorization of manures
1 — waste gas outlet; 2, 6 — laboratory chamber; 3 — fresh air inlet; 4 — waste gas collecting pipe; 5 — rotameter; 7 — membrane blower

Analysis of malodorous compounds. Five different odorants were analyzed: ammonia, dimethylamine, trimethylamine, isobutyric acid and hydrogen sulphide. The selection of the volatile compounds was made on the basis of a previous study (Gutarowska *et al.*, 2014). The analysis of hydrogen sulfide was performed using a Hewlett Packard gas chromatograph equipped with a Super Q 80/100 column with 3ft*1/8"glass and a flame photometric detector (FPD). Helium was the carrier gas, at a flow rate of 50 ml/min. The temperature of analysis was 210°C. Determination of dimethylamine, trimethylamine and isobutyric acid was also carried out with a Hewlett Packard chromatograph fitted with a Chromosorb 103 80/100 column (3ft*1/8" glass) and a flame ionization detector (FID). The nitrogen (50 ml/min) was used as a carrier gas, and the working temperatures were from 110°C to 240°C. Ammonia was analyzed with a Porapak N 80/100 column with parameters (3ft*1/8" SS) and a thermal conductivity detector (TDC). Helium was the carrier gas at a flow rate of 25 ml/min. The

temperature of analysis was 120°C. All analyses were performed in triplicates.

Mathematical calculations. The arithmetic mean and standard deviation for the amount of chemical components of different manures, as well as the number of microorganisms were calculated.

An ANOVA statistical analysis was performed to assess a statistically significant difference between the amount of chemical components in examined manures or number of microorganisms in the control and experimental treatment (the biopreparation application).

Decrease of the concentration of volatile odorous compounds was calculated as the reduction R [%] of a compound determined using the formula:

$$R = 100\% - \frac{C_4 \times 100\%}{C_0}$$

where C_4 is the concentration of investigated volatile odorous compound in the sample collected from the headspace of chamber after 96 hours of deodorization with the biopreparation, C_0 is the concentration of investigated volatile odorous compound in the sample collected from the chamber at the beginning of the deodorization process.

All mathematical calculations were made using Microsoft Excel and OriginPro 8.0 programmes.

RESULTS

The results showed differences in chemical composition of the examined manures (Table 1). The highest content of dry matter (712.1 g/kg) was determined in the geese manure, whereas the lowest in the cattle manure (125.0 g/kg). The difference between the content of dry matter in manures was statistically significant, except for the laying hen manure (a) and swine manure (e). The average amount of ash in the manures ranged from 16.6 g/kg (cattle manure) to 110.6 g/kg (geese manure). Lack of statistically significant difference was observed only between the laying hens (a) and broiler manures (b). Cattle and broiler manure had the highest amount of dry organic matter, 87.2% and 85.4%, respectively, while the lowest was detected in the laying hen manure (66.3%). Significant differences were observed in all types of manures, except broilers (b) and swine manure (e). The highest organic carbon concentration was determined in the geese manure (33.4%), the lowest in the laying hens (10.3%). The difference between content of total organic carbon in manures was statistically significant, except the broiler manure (b) and cattle manure (d). Nutrient, such as nitrogen, was at the highest level in the laying hens manure (7.2%), the lowest concentration was observed for cattle manure (4.5%). Lack of statistically significant differences was observed between 3 types of manure: broiler (b), cattle (d) and swine (e), the differences between the rest of manures were statistically significant. The total phosphorus amount was at the same level in all types of manure (2.3–2.7 g/kg), contrary to the pH values, where the differences were statistically significant in each manure. The highest pH value was reported for the broiler manure (pH = 8.9), whereas laying hen manure had the lowest pH value of 6.3.

The results of our experiments also indicated differences in microbiological composition of all investigated manures in relation to isolated groups of bacteria (Table 2). The highest number of almost all isolated groups of bacteria was recorded for the laying hen manure (4.0×10^7–5.0×10^{10} cfu/g), except for the total number of aerobic bacteria and *Enterococcus* sp., whose numbers were the highest in the geese manure (5.7×10^{12} cfu/g) and swine manure (1.7×10^9 cfu/g). The difference between total number of aerobic bacteria in examined manures were statistically significant, except for the laying hen (a) and broiler manures (b). The total number of anaerobic bacteria (1.2×10^5–1.3×10^{10} cfu/g) and *Enterococcus* (9.9×10^4–1.7×10^9 cfu/g) was different in each manure type, which was confirmed statistically. The number of coliforms ranged from 2.4×10^5 to 7.0×10^8 cfu/g and the differences between manures were statistically significant, except for the broiler (b) and cattle manures (d).

Table 1. Chemical characteristics of different manures

Manure	Dry matter [g/kg]	Ash [g/kg]	Dry organic matter [% DM]	Total organic carbon [%]	Total Kjeldahl Nitrogen (N_{org}+N_{NH_4}) in dry matter [%]	Total phosphorus [g/kg]	pH
Laying hens (a)	Min: 231.28 Max: 247.07 **X: 240.51** a-e S.D.: 8.23	Min: 80.21 Max: 89.63 **X: 85.86** a-b S.D.: 4.99	Min: 64.17 Max: 68.30 **X: 66.34** S.D.: 2.07	Min: 9.95 Max: 10.70 **X: 10.32** S.D.: 0.38	Min: 7.10 Max: 7.26 **X: 7.18** S.D.: 0.08	Min: 1.97 Max: 2.70 **X: 2.40** b-c-d-e S.D.: 0.38	Min: 6.28 Max: 6.30 **X: 6.29** S.D.: 0.01
Broilers (b)	Min: 545.84 Max: 553.30 **X: 549.48** S.D.: 3.73	Min: 78.48 Max: 83.51 **X: 81.64** a-b S.D.: 2.75	Min: 84.80 Max: 86.24 **X: 85.40** b-e S.D.: 0.75	Min: 25.20 Max: 26.30 **X: 25.73*** S.D.: 0.55	Min: 5.01 Max: 5.42 **X: 5.28** b-d-e S.D.: 0.24	Min: 2.01 Max: 2.41 **X: 2.27** a-c-d-e S.D.: 0.22	Min: 8.84 Max: 8.87 **X: 8.86** S.D.: 0.02
Geese (c)	Min: 709.40 Max: 712.10 **X: 711.06** S.D.: 1.45	Min: 109.30 Max: 111.90 **X: 110.63** S.D.: 1.30	Min: 75.3 Max: 76.8 **X: 75.87** S.D.: 0.81	Min: 32.90 Max: 33.70 **X: 33.37** S.D.: 0.42	Min: 6.01 Max: 6.54 **X: 6.22** S.D.: 0.28	Min: 2.08 Max: 2.51 **X: 2.31** a-b-d-e S.D.: 0.22	Min: 6.89 Max: 6.99 **X: 6.93** S.D.: 0.06
Cattle (d)	Min: 125.03 Max: 133.42 **X: 130.08** S.D.: 3.64	Min: 15.30 Max: 17.64 **X: 16.59** S.D.: 1.18	Min: 86.55 Max: 88.39 **X: 87.24** S.D.: 1.00	Min: 23.98 Max: 25.57 **X: 24.78*** S.D.: 0.80	Min: 3.99 Max: 4.87 **X: 4.48** b-d-e S.D.: 0.45	Min: 2.30 Max: 3.37 **X: 2.71** a-b-c-e S.D.: 0.58	Min: 7.49 Max: 7.50 **X: 7.49** S.D.: 0.01
Swine (e)	Min: 239.32 Max: 255.13 **X: 248.30** a-e S.D.: 6.63	Min: 38.71 Max: 52.97 **X: 45.14** S.D.:7.23	Min: 79.24 Max: 83.82 **X: 81.87** b-e S.D.: 2.36	Min: 26.54 Max: 27.98 **X: 27.17** S.D.:0.74	Min: 4.62 Max: 5.55 **X: 5.06** b-d-e S.D.: 0.47	Min: 1.98 Max: 2.68 **X: 2.37** a-b-c-d S.D.: 0.36	Min: 7.74 Max: 7.83 **X: 7.79** S.D.: 0.05

X, average value; S.D., standard deviation; Min, minimum value; Max, maximum value; a-e, b-e, etc. lack of statistically significant differences between different manures (a–e) comparison in columns

Table 2. Number of bacteria in different manures

Number of bacteria (cfu/g)	Manure				
	Laying hens (a)	Broilers (b)	Geese (c)	Cattle (d)	Swine (e)
Total number of aerobic bacteria	Min: 5.00×10^{10} Max: 5.20×10^{10} **X: 5.10×10^{10}** a-b S.D.: 1.00×10^{9}	Min: 2.25×10^{10} Max: 6.00×10^{10} **X: 3.40×10^{10}** a-b S.D.: 1.31×10^{10}	Min: 1.04×10^{12} Max: 8.50×10^{12} **X: 5.71×10^{12}** S.D.: 3.04×10^{12}	Min: 8.00×10^{5} Max: 1.09×10^{6} **X: 9.25×10^{5}** S.D.: 1.18×10^{5}	Min: 1.59×10^{10} Max: 2.60×10^{10} **X: 1.98×10^{10}** S.D.: 4.65×10^{9}
Total number of anaerobic bacteria	Min: 1.10×10^{10} Max: 1.50×10^{10} **X: 1.27×10^{10}** S.D.: 2.08×10^{9}	Min: 4.80×10^{9} Max: 1.00×10^{10} **X: 7.07×10^{9}** S.D.: 1.95×10^{9}	Min: 3.00×10^{8} Max: 6.30×10^{8} **X: 4.33×10^{8}** S.D.: 1.27×10^{8}	Min: 8.60×10^{5} Max: 2.20×10^{6} **X: 1.22×10^{6}** S.D.: 5.40×10^{5}	Min: 2.10×10^{8} Max: 3.40×10^{9} **X: 1.54×10^{9}** S.D.: 1.46×10^{9}
Enterococcus sp.	Min: 4.00×10^{8} Max: 5.00×10^{8} **X: 4.50×10^{8}** S.D.: 5.00×10^{7}	Min: 5.00×10^{7} Max: 7.00×10^{7} **X: 5.67×10^{7}** S.D.: 1.15×10^{7}	Min: 1.00×10^{6} Max: 3.00×10^{6} **X: 1.66×10^{6}** S.D.: 7.86×10^{5}	Min: 4.00×10^{4} Max: 1.56×10^{5} **X: 9.93×10^{4}** S.D.: 5.46×10^{4}	Min: 8.00×10^{8} Max: 2.89×10^{9} **X: 1.69×10^{9}** S.D.: 6.85×10^{8}
coliforms	Min: 5.60×10^{8} Max: 8.60×10^{8} **X: 7.02×10^{8}** S.D.: 1.39×10^{8}	Min: 1.20×10^{5} Max: 9.00×10^{5} **X: 4.27×10^{5}** b-d S.D.: 3.89×10^{5}	Min: 1.79×10^{8} Max: 3.90×10^{8} **X: 2.67×10^{8}** S.D.: 9.51×10^{7}	Min: 9.0×10^{4} Max: 3.8×10^{5} **X: 2.37×10^{5}** b-d S.D.: 1.45×10^{5}	Min: 7.00×10^{7} Max: 2.20×10^{8} **X: 1.19×10^{8}** S.D.: 6.43×10^{7}
Escherichia coli	Min: 3.80×10^{8} Max: 4.20×10^{8} **X: 4.00×10^{8}** S.D.: 2.00×10^{7}	Min: 6.00×10^{5} Max: 1.60×10^{6} **X: 1.03×10^{6}** S.D.: 4.30×10^{5}	Min: 4.00×10^{5} Max: 4.67×10^{5} **X: 4.34×10^{5}** c-d S.D.: 4.74×10^{4}	Min: 8.30×10^{4} Max: 4.00×10^{5} **X: 2.43×10^{5}** c-d S.D.: 1.45×10^{5}	Min: 8.00×10^{7} Max: 2.20×10^{8} **X: 1.48×10^{8}** S.D.: 5.40×10^{7}
Clostridium sp.	Min: 1.00×10^{7} Max: 6.00×10^{7} **X: 4.00×10^{7}** S.D.: 2.65×10^{7}	Min: 1.00×10^{4} Max: 1.00×10^{5} **X: 5.50×10^{4}** b-c-d S.D.: 6.36×10^{4}	Min: 3.80×10^{4} Max: 1.10×10^{5} **X: 6.87×10^{4}** b-c-d S.D.: 3.72×10^{4}	Min: 2.00×10^{4} Max: 1.00×10^{5} **X: 7.50×10^{4}** b-c-d S.D.: 3.79×10^{4}	Min: 1.00×10^{5} Max: 6.00×10^{5} **X: 3.00×10^{5}** S.D.: 2.65×10^{5}

X, average value; S.D., standard deviation; Min, minimum value; Max, maximum value; a-e, b-e, etc-lack of statistically significant difference between different manures (a–e) comparison in rows

There was no difference in the number of *Escherichia coli* (2.4×10^{5}–4.00×10^{8} cfu/g) between geese (c) and cattle manures (d), while for the other manures, the differences were statistically significant. The total number of *Clostridium* sp. (5.5×10^{4}–4.0×10^{7} cfu/g) was similar regarding to 3 manures: broiler (b), geese (c) and cattle (d), whereas only the laying hens (a) and swine manures (e) were different significantly. The application of the biopreparation for laying hen manure resulted in a reduction in the number of aerobic and anaerobic bacteria by one logarithmic unit (Figs. 2–3). There were no differences in the total number of aerobic and anaerobic bacteria with reference to the other investigated manures. Statistically significant differences were noted in the case of the number of *Enteroccocus* sp. (Fig. 4). In the case of broiler manure, the application of biopreparation reduced the number of investigated bacteria by a half of logarithmic unit. Similarly, the same observation was found in the case of geese manure but it was not statistically confirmed. The effect of hygienization was not reported in the case of laying hen, swine and cattle manures. Moreover, the number of microbes even increased after the application of the biopreparation. The highest reduction of coliform numbers, reaching two logarithmic units, was observed during broiler manure deodorization (Fig. 5). Furthermore, hygienization was also achieved during the treatment of cattle and swine manures. The number of coliforms after 4 days of geese manure deodorization dropped, but the differences between initial and final concentrations of these bacteria were statistically insignificant. The process of hygienization was also effective for *E. coli* (Fig. 6) removal in cattle and geese manure, however only in the case of cattle manure experiment, a one logarithmic unit reduction was statisti-

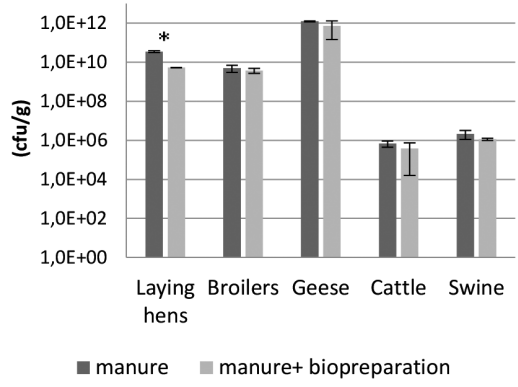

Figure 2. Total number of aerobic bacteria in different manures after hygienization
*difference statistically significant $p \le 0.05$

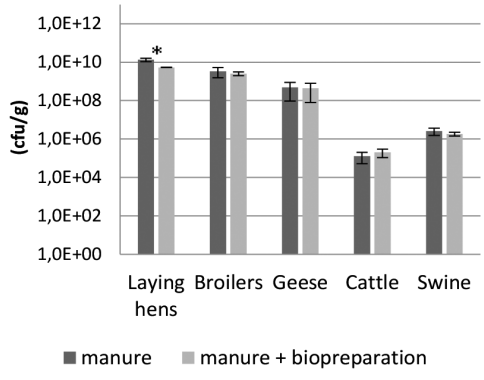

Figure 3. Total number of anaerobic bacteria in different manures after hygienization
*difference statistically significant $p \le 0.05$

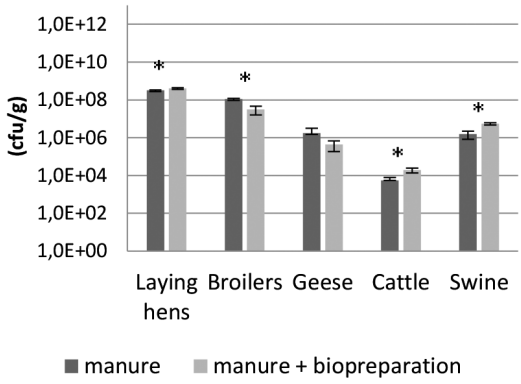

Figure 4. Number of *Enterococcus* sp. in different manures after hygienization
*difference statistically significant $p \leq 0.05$

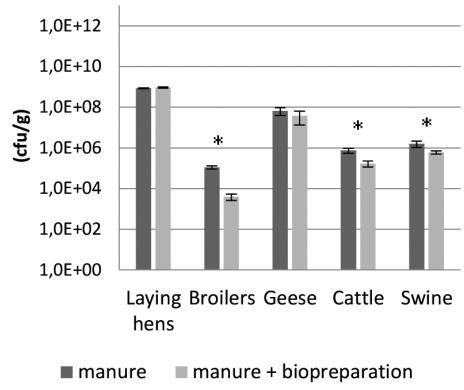

Figure 5. Number of coliforms in different manures after hygienization
*difference statistically significant $p \leq 0.05$

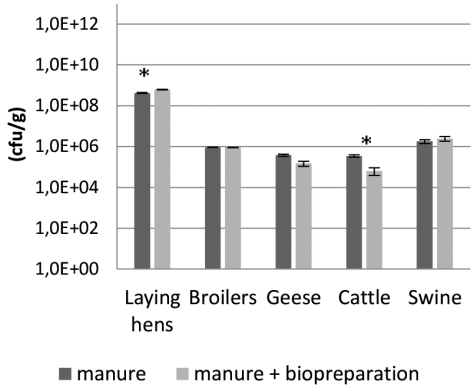

Figure 6. Number of *E. coli* in different manures after hygienization
*difference statistically significant $p \leq 0.05$

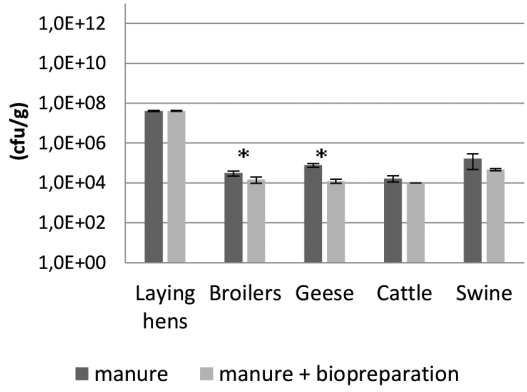

Figure 7. Number of *Clostridium* sp. in different manures after hygienization
*difference statistically significant $p \leq 0.05$

cally confirmed. A positive effect of the biopreparation application on the other tested manures, in terms of *E. coli* inactivation, was not observed. In the case of all types of examined manures, the number of *Clostridium* sp. was lowered by one logarithmic unit, but only in the case of broiler and geese manures the differences were statistically significant (Fig. 7).

Figures 8–12 show the efficiency of the biopreparation application in order to remove the investigated odorous compounds. Ammonia concentration decreased after the hygienization process with the highest effectiveness for laying hens, broiler and geese manures. Trimethylamine, isobutyric acid and dimethylamine concentrations were reduced with higher efficiency in comparison to the con-

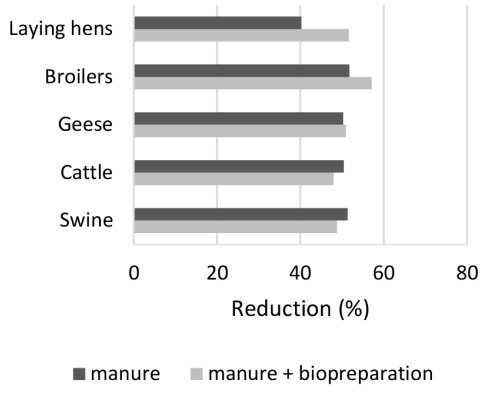

Figure 8. Ammonia reduction after 96 hours of deodorization by biopreparation

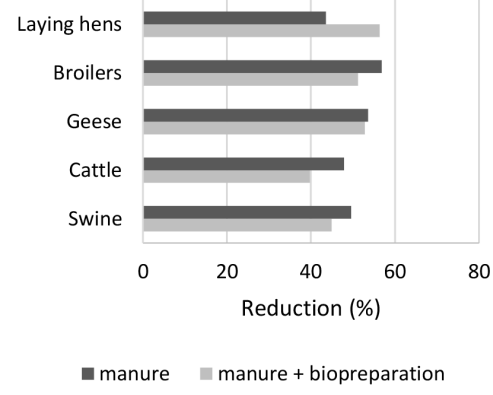

Figure 9. Dimethylamine reduction after 96 hours of deodorization by biopreparation

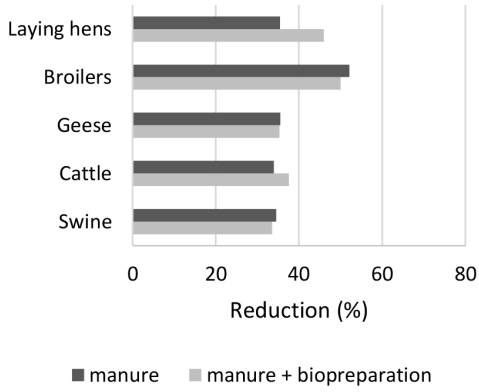

Figure 10. Trimethylamine reduction after 96 hours of deodorization by biopreparation

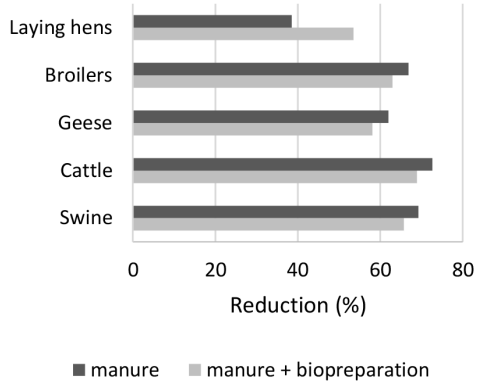

Figure 11. Isobutyric acid reduction after 96 hours of deodorization by biopreparation

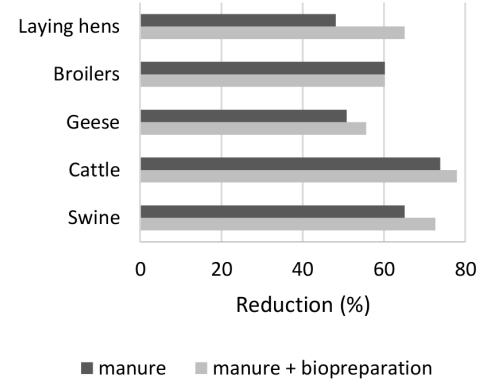

Figure 12. Hydrogen sulphide reduction after 96 hours of deodorization by biopreparation

trol treatment only in the case of laying hens manure, and the reduction level was between 45 and 56%. Furthermore, the removal of trimethylamine was observed in the cattle manure experiment (37%). It was also reported that the biopreparation was the most efficient in the removal of hydrogen sulfide. Regarding the treatment of geese, laying hens, swine and cattle manures, hydrogen sulfide was removed by 56, 65, 73 and 78%, respectively.

DISCUSSION

The dry matter (DM) content determined in case of the broiler manure was twice as high (55%) as the value obtained for laying hen or swine manures (24–25%), while for geese manure DM concentration was as high as 71%. According to Bednarek *et al.* (2010), the dry matter content in natural fertilizers such as manures, depends on the sample origin and type of manure and vary between 10 and 69% (Kwak *et al.*, 2005; Rankins *et al.*, 2002) which is in an agreement with our results.

In the present study, dry organic matter content of investigated manures was between 66% (laying hens) and 87% (cattle). Kowalczyk-Jusko (2010) and Güngör Demirci *et al.* (2004) reported similar values of that indicator, within the range of 63–80%, depending on the manure type.

The highest concentration of total Kjeldahl–nitrogen was determined in the samples of laying hen manure (7.2%) while the lowest in the case of cattle manure (4.5%). This corresponds to the results (4.5–8%) reported by Nodar *et al.* (1990), and Güngör-Demirci *et al.* (2004). However, Myszograj and Puchalska (2012) and Bednarek *et al.* (2010) noted lower values (0.4–2.8%).

The content of total organic carbon determined in the investigated manures was between 10% (laying hens) and 33% (geese), whereas Kwak *et al.* (2005) reported the concentration of this parameter in the broiler manure to be at the level of 45%, probably due to a large amount of straw and organic matter in it. Animal manure is a heterogeneous material, therefore collection of representative samples (homogenous, sampled from different places, with at least 5 replications etc.) is crucial. Thus, the chemical composition reported by different authors may differ considerably. The amount of total phosphorus in all analyzed manures was at a similar level (2.3–2.7 g/kg), which corresponds to the findings of Myszograj and Puchalska (2012). The pH value of the examined manures was between 6.3 and 8.9, depending on the type of animals. This corresponds well with the results reported by many authors. Witkowska *et al.* 2010 reported the manure pH value within the range of 4.5–6.0, determined at different weeks of broiler breeding period, while Nicholson *et al.* (2005) and Whitehead and Cotta (2001) gave the level of 6.2–8.8, depending on the types of animals (milk cows, pigs, broilers) that manure was collected from.

In the present study, microbiological analysis of manures was also performed. The total number of bacteria in the laying hen manure reached 10^{10} cfu/g, which was greater by two logarithmic units than the numbers reported by Nodar *et al.* (1992) (10^{8} cfu/g). Witkowska *et al.* (2010) determined the number of aerobic bacteria in control mulch/litter at the level of 9.5×10^{7} cfu/g, which increased to 2.3×10^{9} cfu/g after 5 weeks of broiler settlement. It was found that manures are a good environment for growth of potentially pathogenic microorganisms, including *Escherichia coli* and coliforms, *Clostridium* sp. and *Enterococcus* sp. The presence of these groups of microorganisms in poultry manure was also reported by Thurston-Enriquez *et al.* (2005). Nicholson *et al.* (2005) noticed that the most frequently occurring pathogenic microorganisms in manures (cattle, pig, sheep and broilers) were *E. coli, Salmonella* sp., *Listeria* sp., *Campylobacter* sp. Their studies have provided data on pathogen reduc-

tion during solid manure heap storage, mainly due to the increase of temperature. Hutchison *et al.* (2004) studied the zoonotic agents in fresh and stored manures (cattle, pig, poultry, sheep) for the presence of pathogens. They found the presence of *E. coli*, *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., *C. parvum* at a level between 1.0×10^1 and 4.0×10^3 cfu/g.

The biopreparation used during deodorization process in the present study was effective depending on the analyzed manure and volatile compound that was evaluated. In the case of laying hen manure, the reduction in the content of all investigated compounds i.e. ammonia, dimethylamine, trimethylamine, isobutyric acid and hydrogen sulfide was noted. The application of the biopreparation onto the broiler manure was effective only in the case of ammonia removal, whereas for geese manure, both ammonia and hydrogen sulfide were successfully removed. Moreover, only 2 odorants emitted from cattle manure (trimethylamine and hydrogen sulfide) and one from swine manure (H_2S) were reduced after application of the biopreparation. It was stated that the deodorization process carried on laying hen manure was more efficient compared to the same process performed with broilers, geese, cattle and swine manures. A high efficiency of biological deodorization has been confirmed by many authors. Mao *et al.* (2006) achieved a reduction of ammonia emission from poultry manure by 90% using a biofiltration method. Rappert and Müller (2005), Shirkot *et al.* (1994), Ghisalba *et al.* (1985) reported the same removal efficiency in the case of di- and tri- methyloamine. Parker *et al.* (2013) observed the removal of isobutyric acid content but the treatment efficiency results were lower (about 21%) than the numbers reported in the presented study. Similarly, Hirai *et al.* (2001) reached a hydrogen sulfide removal at the level of 50%, which was also lower compared to our results.

Elimination of odors from manures during animal production, with the use of mineral additives, has been studied previously. Rudzik (1998) applied kaolin and zeolite for this purpose, and the reduction in the level of ammonia and other odorants obtained in his study was about 58% and 49%, respectively. Turan *et al.* (2009) reported that expanded vermiculite had the ability to reduce the content of volatile organic compounds emitted during composting of poultry litter by about 60%. Coates *et al.* (2005), Varel and Wells (2007) applied vaccine with a bacterial strain *Geobacter* sp. NU with the iron (III) addition and a thymol additive (1.5 to 3.0 kg/m^3), for the reduction of volatile compounds emitted from pig manure slurry with good results for ammonia and volatile fatty acids elimination. Furthermore, Cai *et al.* (2007) found that topical application of zeolite to laying hen manure caused a reduction of the total odor by 51 to 67%. Ivanov (2001) used the addition of hydrated aluminum sulfate and acids: citric (at 5%), tartaric (4%) or salicylic (1.5%) in poultry houses for ammonia removal.

The conducted experiments showed that the animal wastes are different in terms of both chemical and microbiological composition. Moreover, poultry manure creates the best conditions for the growth of microorganisms and is responsible for the highest emission of volatile odorous compounds. The hygienization and deodorization process that was investigated with the use of our biopreparation demonstrated the effect of reducing the number of microorganisms in the range of 1–2 units on a logarithmic scale, whereas the concentrations of volatile compounds decreased by 46–78%, depending on the volatile compound and manure type. The best results were obtained for the laying hen manure. According to the authors of this paper, further research should be focused on the development of individual composition of the biopreparation for each type of manure. Better treatment efficiency in terms of manure hygenisation should be achieved by including in the biopreparation composition microorganisms of antagonistic activity towards microbiocenosis present in the manures.

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