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Microbial mitigation of greenhouse gas and odour emissions from poultry manure: Implications for sustainable environmental management

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Abstract: This study evaluated the effectiveness of microbial compositions (*Pseudomonas fluorescens*, *Pseudomonas putida*, *Bacillus licheniformis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Pediococcus* sp.) in reducing greenhouse gas (GHG) and odour emissions from stored poultry manure. The research consisted of two phases: (EI) a controlled 11-week column experiment with chicken manure under static chamber conditions, and (EII) a 42-week *in situ* experiment on chicken and turkey manure piles. Gas emissions (CO_2, CH_4, NH_3, H_2S, O_2) were monitored using NDIR (nondispersive infrared) and electrochemical sensors, while GC-MS analysis assessed volatile organic compounds. Results indicated that microbial treatments accelerated organic matter decomposition, increasing CO_2 and H_2S emissions compared to controls. However, they significantly reduced NH_3 (by up to 83%) and hydrogen cyanide (HCN) concentrations, demonstrating potential for odour mitigation. In *in situ* trials, turkey manure exhibited higher initial odour intensity (4.5 compared to 4.1 on a 5-point scale at 1 m distance), but both manure types stabilised to negligible levels (~1.2) after 9 weeks. Temperature and humidity strongly influenced emissions, with higher NH_3 release observed at warmer temperatures (17–19°C) and moderate humidity (64–69%). The study highlights the trade-off between microbial treatments' benefits (NH_3 reduction) and drawbacks (elevated CO_2/H_2S). Further optimisation of bacterial strains and integration with aerobic composting is recommended to balance emission control with nutrient retention. These findings contribute to sustainable manure management strategies aligned with circular economy principles.

Keywords: greenhouse gases, *Lactobacillus*, microbial treatment, odour reduction, *Pseudomonas*, sustainable agriculture

INTRODUCTION

Noticeable climate changes, progressive water pollution, and ecosystem degradation result from excessive resource consumption (Qamruzzaman, 2024). These global issues necessitate a search for more rational management approaches aligned with sustainable development, circular economy principles, and nature-based solutions (NbS) (Zyoud and Zyoud, 2025). In recent decades, highly industrialised livestock farming has expanded worldwide, with large-scale animal farms, so-called Concentrated Animal Feeding Operations (CAFOs) (Miller and Muren, 2019). According to FAO data, between 1961 and 2021 the global number of farm animals rose dramatically: chicken poultry increased by 584%, and geese by 1,181% (FAOSTAT, 2022). In 2021, there were about 30.4 bln chickens worldwide. In Poland, over 1.4 mln farms exist, most with some form of livestock production (GUS, 2020). Between 2010 and 2019, cattle herds grew by 11%, poultry by 36%, and duck and turkey flocks by over 100% (GUS, 2020). Industrial feed consumption has also increased significantly (Kupiec, 2023). Animal breeding, especially large-scale industry, is a dynamically developing sector in the world. Farmed poultry constitutes 99% of the biomass of all birds in Poland. Wild birds account for only 1% of the overall mass (own calculations). In the case of mammals, 50% of the mass is farmed mammals, the mass of humans is 35%, and the mass of wild mammals is 15%. Unfortunately, in recent years, the European Union's policy has discouraged farmers to support the Green Deal and activities related to environmental protection and climate.

Industrial animal production inevitably generates large volumes of manure, which, if improperly managed, pollutes soil, water, and air (Cattaneo *et al*., 2023; Qi *et al*., 2023). Council Directive (1991) requires proper manure storage to minimise nutrient losses and emissions (Ali *et al*., 2019). However, many farms still use outdated or inadequate infrastructure (Kupiec, 2019; Niles *et al*., 2022). Although farmers' awareness and practices have improved, environmental impacts from large-scale production remain significant, especially on air and water quality (Kupiec, Staniszewski and Kayzer, 2022).

Animal excreta are major sources of greenhouse gases (methane, nitrous oxide) and odorous compounds, e.g. swine slurry can contain over 400 volatile substances harmful to health (Pawełczyk and Muraviev, 2003). Poultry manure is particularly problematic due to its high nutrient and nitrogen content (Bayrakdar *et al*., 2017). To safely recycle poultry manure as fertiliser, biological treatment is often necessary. Technologies like ammonia stripping, zeolite adsorption, membrane separation, struvite precipitation, co-digestion, or trace element addition have been tested to mitigate emissions (Böjti *et al*., 2017; Spyridonidis, Vasiliadou and Stamatelatou, 2022).

Recent research shows promising development in using microbial biotechnologies, e.g. tailored microbial consortia for ammonia oxidation, nitrification-denitrification enhancement, or biochar-based bioreactors (Ngo *et al*., 2023; Zhang *et al*., 2024). However, large-scale implementation remains limited due to cost, process instability, and the lack of integrated solutions adapted to industrial poultry farms.

Despite these advances, knowledge gaps remain in optimising and scaling up microbial approaches for simultaneous greenhouse gas and odour mitigation in poultry manure management. There is a need for integrated, cost-effective treatment technologies that can be widely adopted by large commercial farms while meeting climate and environmental targets (Zhang *et al*., 2024).

The research discussed in this paper is part of a larger project focusing on testing a wider range of physical, chemical, and microbiological parameters of poultry manure during storage. It serves as an introduction to further experiments aimed at optimising the storage of poultry manure and other animal excrements. The research work was aimed at: 1 – diagnosis of the main problems related to the emission of selected odorous and greenhouse gases from bird droppings piles; 2 – assessment of the impact of the applied microbiological vaccines on reducing greenhouse gas and odour gas emissions, 3 – assessment of odour nuisance from bird droppings storage sites; 4 – analysis of objective and subjective weather factors on the degree of nuisance caused by bird droppings. The experiment included laboratory tests and tests in real conditions (*in situ)*.

MATERIALS AND METHODS

GENERAL INFORMATION

The experiment was carried out in two stages: EI – an indoor experiment; EII – an *in situ* experiment. Research work was carried out using chicken and turkey manure. The first stage of research was conducted between 1 March and 8 June 2022 (11 weeks). The second stage covered the period between 1 September 2022 and 6 June 2023 (42 weeks).

The research applied compositions of microorganisms used in previous own projects, which produced positive environmental effects by reducing the emission of nitrogen and phosphorus compounds from stored manure piles, as well as those used in water reclamation. Some strains were selected based on the experience of other authors and literature data.

EXPERIMENT WITH COLUMNS – STAGE I (EI)

The experiment included analyses of selected microbiological compositions, including in the form of the hard capsules, added to the manure mass. The research was carried out for chicken manure, which was obtained from large-scale animal farms in the Warmian-Masurian Province. The manure was placed in special columns with static chambers 1–4. The column experiment was conducted in unheated halls, where the temperature depended on the ambient temperature.

The research used sterile PVC containers of 120×100×116 cm. Each static chamber had a drainage column of 0.25 m of inert material: washed stone with a fraction of 16–32 mm. The columns were filled using a small hole with a diameter of 0.15 m in the upper part of the column. The drain valve was secured on the inside of the column with agrotextile mulching. In order to improve gas exchange between the internal part of the manure and the static chamber, two perforated PVC pipes of 0.70 m in height and 0.05 m in diameter were installed vertically in each column. To avoid clogging of the pipes when loading manure, the perforation pipes were wrapped with agrotextile and secured with cable ties. The upper part of the column contained a static chamber for collecting gas samples. The capacity of the chamber (space between manure and chamber walls) was approximately 0.4 m^3. After all the columns were filled, the so-called chimney with a gas intake valve attached. In each column, 0.8 Mg of manure was deposited (Fig. S1).

PREPARATION OF STATIC CHAMBERS ON MANURE PILES IN FIELD CONDITIONS – STAGE II (EII)

The research also included monitoring of emissions from manure conducted in real conditions with 5×10 m wide heaps formed directly on the ground. Two 1×1 m PVC static chambers were mounted on two heaps of chicken and turkey manure delivered from nearby large-scale farms. The volume of the chamber was the same as in the case of the column experiment (0.45×1×1 m). In order to improve gas exchange between manure and the static chamber, two perforated PVC pipes of 0.70 m in height and 0.05 m in diameter were mounted vertically in each chamber. The pipes were wrapped in agrotextile. A chimney with a gas intake valve was mounted on the top of the dome. Before placing the chamber on the heap, the manure was inoculated with the selected microbiological composition, which showed the greatest potential in stage I of the experiment. Then, the chambers were placed on the heaps (Fig. S2). One repetition was performed for each manure pile.

BACTERIAL PREPARATIONS AND THEIR APPLICATION IN COLUMNS

Liquid preparation of denitrifying bacteria

The aim was to obtain a preparation in liquid form from selected denitrifying bacteria: *Pseudomonas fluorescens*, *Pseudomonas putida*, *Bacillus licheniformis*. Incubation and passage of each of

the three bacterial strains was performed using nutrient broth based on the methodology presented by Benson (2002). Incubation was carried out at 30°C for 24 h. The preparation of the bacterial culture in a laboratory bioreactor was performed in nutrient broth based on the methodology presented by Długoński (ed.) (2021). Incubation was carried out with aeration for 48 h at a pH of 6.0–7.0. The culture was mixed using a stirrer in the bioreactor at an intensity of 80 rpm.

In the next stage, dilutions of bacterial cultures (denitrifying bacteria) were prepared and deep inoculation was performed. Representative samples were taken for microbiological tests, i.e. reflecting the condition of the entire liquid culture (Phot. S1). Deep inoculation was performed using prepared and sterile nutrient agar. The methodology was presented by Cappuccino and Welsh (2017). The next step was the preparation of the bacterial culture in a production bioreactor (with a mixer from IP System Manufacturing) using nutrient broth (Phot. S1). The methodology was based on Benson (2002) and Długoński (ed.) (2021). The characteristics of the cultures of single strains and mixtures of microorganisms are presented in Table 1.

Table 1. Characteristics of the number of single cultures and microbial mixtures after 48 h of incubation at 30°C with pH stabilisation of 6.0–7.0

Strain of bacteria	Number of microorganisms (L) (cfu·cm^{-3})	
	stage I (EI)	stage II (EII)
Liquid preparation – laboratory bioreactor		
Pseudomonas fluorescens	$2.8 \cdot 10^8$	$6.7 \cdot 10^8$
Pseudomonas putida	$1.7 \cdot 10^8$	$5.3 \cdot 10^8$
Bacillus licheniformis	$2.0 \cdot 10^8$	$2.9 \cdot 10^8$
P. fluorescens × *P. putida* × *B. licheniformis*	$1.06 \cdot 10^9$	$6.27 \cdot 10^9$
Liquid preparation – production bioreactor		
P. fluorescens × *P. putida* × *B. licheniformis*	$6.02 \cdot 10^9$	$1.31 \cdot 10^9$
Bacterial powder		
Lactobacillus fermentum × *Lactobacillus plantarum* × *Pediococcus* sp.	$3.5 \cdot 10^{10}$	$4.7 \cdot 10^{10}$

Source: own elaboration.

The production of a liquid preparation with denitrifying bacteria in stage II (EII) was similar to that in stage I (EI) of the study using the same bacterial strains and procedures.

Dried preparation from conditioning bacteria

The cultivation was carried out to obtain the biomass of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Pediococcus* sp. bacteria, which was spray-dried in the next stage. Incubation and passage of each of the three bacterial strains (*Lactobacillus fermentum*, *Lactobacillus plantarum* and *Pediococcus* sp.) was performed using MRS broth based on the methodology presented by Benson (2002). Incubation was carried out for 24 h. The preparation of a bacterial culture of *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Pediococcus* sp. in a laboratory bioreactor was performed in MRS broth based on the methodology presented by Długoński (ed.) (2021). Incubation was carried out without aeration for 48 h at a pH of 4.1–4.2.

In the next stage, dilutions of bacterial cultures were prepared and deep inoculation was performed. Representative samples were taken for microbiological tests. Deep inoculation was performed using prepared and sterile MRS agar (Cappuccino and Welsh, 2017). Plates from two successive dilutions with 30 to 300 colonies were selected for reading. The number of microorganisms (L) in 1 g of sample was calculated according to the formula:

$$L = \frac{C}{(N1 + 0.1N2)} d \tag{1}$$

where: C = sum of colonies on all plates selected for counting, $N1$ = number of plates from the first counted dilution, $N2$ = number of plates from the second counted dilution, d = dilution index corresponding to the first (lowest) counted dilution.

The research used a laboratory incubator from POL-EKO-APARATURA. The preparation of the emulsion proceeded as follows: 2 dm^3 of treated water was poured into a metal container with a volume of 10 dm^3. The water was heated using Forgast electric induction to a temperature of 60°C. After obtaining the desired temperature, trehalose was poured into the container and the substance was dissolved using an IKA mechanical stirrer. After obtaining a uniform solution, the mixture was cooled to 30°C. Maltodextrin was then added to the solution and further mixed. After dissolving the substance, bonigrass was added and mixing was continued. The prepared solution was homogenised on IKA equipment at 1500 rpm for approx. 10 min and then a bacterial culture was added to the finished solution. Everything was mixed using a mechanical mixer. After 15 min a uniform emulsion was obtained. The initial reaction of the emulsion measured with a Hach pH meter ranged from 3.8 to 4.2. Using 2 $MCa(OH)_2$, the pH of the emulsion was raised to 6.0–7.0. Aerosil was added to the obtained emulsion to reduce surface tension during spray drying.

The following substrates were used to prepare the emulsion: maltodextrin, bonigrass, trehalose. The substrates were mixed and homogenised with 5 dm^3 of bacterial culture added to the solution, and 9.62 kg of emulsion with a concentration of 33% was obtained. The emulsion was neutralised with $Ca(OH)_2$ to obtain a pH of 6.0–7.0. The obtained emulsion was introduced into a spray dryer. Drying took place at an inlet temperature of 170°C and an outlet temperature of 69°C to receive hard capsules. In order to obtain a bacterial composition with a conditioning effect, dried bacterial material containing the *Lactobacillus fermentum* strain was mixed with dried bacterial *Lactobacillus plantarum* and *Pediococcus* sp. in a ratio of 1:0.76. The composition was used in static chambers 3 and 7 (chambers with manure conditioning bacteria), and 4 (chamber with microbiological composition: denitrifiers + conditioning microorganisms). The obtained product had a homogeneous, slightly yellowish form.

In the second stage of the study (EII), the process of preparing dried strains from conditioning bacteria was similar to

the first stage of the study. The composition was used in *in situ* studies using two litters arranged on heaps in the field (actual conditions) and chambers located on them. The tested litters were inoculated with a mixture of liquid culture and dried bacteria 3 times on the dates: 1, 13 and 23 weeks of the experiment. In each of the above cases, repeatability was maintained in terms of weight and bacterial count. The characteristics of the strain cultures and mixtures of microorganisms for the dry preparation are presented in Table 1.

APPLICATION OF PREPARATIONS IN COLUMNS WITH MANURE

At the beginning of March 2022, the preparation of research columns began. Four experimental columns (0.8 Mg manure each) were prepared:

- control (column 1): untreated manure;
- denitrifying bacteria (column 2): three layers of manure, each inoculated with 13.33 dm^3 of liquid culture (*Pseudomonas fluorescens*, *putida*, *Bacillus licheniformis*; total: 40 dm^3 per column);
- conditioning bacteria (column 3): each layer received 0.82 kg of dried *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *Pediococcus* sp. (total: 2.46 kg per column);
- combined treatment (column 4): layers treated with both liquid denitrifiers (6.67 dm^3 per layer) and dried conditioning bacteria (0.41 kg per layer, totals: 40 dm^3 + 1.23 kg per column).

Liquid cultures were evenly distributed via plastic piping; dried bacteria were applied manually using a wooden beam. Columns were sealed to monitor gas emissions.

RESEARCH ON SELECTED ODOROUS AND GREENHOUSE GASES

Analyses of gases emerging in the process of storing poultry manure were conducted to assess the capacity of the proposed microbiological compositions to reduce emissions of odours burdensome to the environment and greenhouse gases contributing to climate change. Measurements of the quality of odorous and greenhouse gases (methane, ammonia, carbon dioxide, oxygen, and hydrogen sulphide) were made directly from the static chambers using sampling pipes. Measurements were performed using the Nanosens DP-28 analyser (Phot. S2). The content of CH_4 (% v/v), CO_2 (% v/v) was measured using the NDIR (nondispersive infrared) method. The content of O_2 (% v/v), NH_3 (ppm), H_2S (ppm) was measured using electrochemical sensors. Methane, ammonia and carbon dioxide concentration measurements were made in static chambers at specific time intervals. The research involved two measurements. The first measurement was made in the 3rd week from the beginning of the experiment, after the manure had stabilised in the research columns. The second measurement was performed 6 weeks after the start of the study. The tests were carried out for columns 1–4 with chicken manure. The collected gas samples were also analysed for the content of methane and its gas homologues (ethane, propane, i-butane, n-butane) and gaseous alkenes (ethylene, propylene, 1-butene).

The analysis of air samples was carried out using a Shimadzu GC-2010 Plus gas chromatograph equipped with a ZB 5ms Plus capillary column (GC-MS analyses) (Khalifa, 2018; Medeiros, 2018). Additionally, the GCMS-QP2021 SE mass detector was used. In parallel, air samples were analysed using a GCHF 18.3 gas chromatograph equipped with a TCD detector and a ShinCarbon ST 80/100 packing column (GC-TCD analysis) (Zuas and Budiman, 2016). Additionally, the share of other air components in static columns was analysed, such as nitrogen, oxygen, nitrogen fragment ion, argon, water, carbon monoxide, hydrogen cyanide, and ammonia. The analyses were performed twice, in weeks 3 and 11 of the study.

In the second stage of the experiment, the scope of work included measuring the quality of gases in two reactors (static chambers) located on two different piles of bird droppings (chamber 1 – chicken; chamber 2 – turkey) in the range of CH_4, NH_3, CO_2, and additionally O_2 and H_2S. The measurement was performed in the 3rd, 6th, 15th and 42nd week of the experiment. Additionally, analyses of the air composition and the presence of methane and its gaseous homologues (ethane, propane, i-butane, n-butane) and gaseous alkenes (ethylene, propylene, 1-butene) were performed in the 3rd and 15th week of the experiment. Gas Chromatography with Thermal Conductivity Detector (GC-MS) analyses were performed on gas samples from chambers No. 1 and 2 (chicken manure and turkey manure). Air samples for analysis were placed in a bag from which a sample was taken using a Hamilton GASTIGHT 1700 500 μL syringe.

ANALYSIS OF ODOUR NUISANCE OF MANURE PILES – STAGE II (EII)

One of the objectives of the research was to assess the effect of the microbiological composition on the occurrence and intensity of odorous gases. Hence, sensory studies were conducted on the odour nuisance of stored piles. To examine the odour nuisance of stored piles, 10 people (5 women and 5 men) aged 23–50 were selected. Each person examined the odour nuisance from the following distances: 1 m, 3 m, 6 m, 10 m, 20 m, and 40 m. From the beginning of the study, the assessment of odour nuisance was performed once a week for 1 month, and then once a month until the nuisance ceased (odorous not detectable by researchers). The assessment was performed each time from the north, south, east, and west sides of the manure. The studies were performed analogously for the two examined piles of chicken and turkey manure. A specially designed questionnaire was used for the research, with a point scale from 1 (least odour nuisance) to 5 (most odour nuisance).

Additionally, a survey of subjective perception of weather conditions was prepared to facilitate the assessment of the impact of these conditions on possible results: wind force (scale 1–5), sun exposure (scale 1–5), perceived temperature (scale 1–5), perceived humidity (scale 1–5). The results were compared with the course of weather conditions – real data: temperature (min/max), humidity (%), wind direction (N, E, S, W).

STATISTICAL ANALYSES

The following parametric statistical methods were used to interpret the results: analysis of variance (one-way ANOVA), Pearson correlation (r), Student's t-test and nonparametric – Mann–Whitney U test (Armstrong, Eperjesi and Gilmartin, 2002; Takiar, 2023; Pan *et al*., 2024).

RESULTS AND DISCUSSION

CHARACTERISTICS OF GASES EMMITED FROM POULTRY MANURE

Concentrations of substances measured in closed chambers (CH_4, NH_3, CO_2, O_2, and H_2S) are summarised in Table 2. The dominant gas across all chambers was CO_2, with the highest emissions observed in the column with denitrifiers (No. 2), where CO_2 and H_2S levels exceeded those in the control and conditioning bacteria variants. No significant statistical differences were confirmed (ANOVA $F = 1.60$, $p = 0.34$), but the observed trends indicate intensified microbial decomposition under the influence of inoculated strains. In this experiment, each treatment was tested once (single column), so there were no real replicates for each variant. Therefore, the ANOVA results should be treated only as indicative check of possible differences, and not as a rigorous test of statistical significance. The test was performed only to illustrate potential trends, not for inferential purposes.

Methane and ammonia were generally absent or detected in minimal amounts in the chambers. In the control chamber, CO_2 concentration doubled between week 3 and 11, reaching around 21% (Tab. 3). Methane stayed below 2.5% and decreased slightly over time, while HCN, initially present at about 0.7%, fell below 0.3% in both tested chambers by week 11, suggesting active microbial cyanide degradation.

Oxygen levels dropped slightly, indicating oxygen consumption due to aerobic processes inside the piles. Hydrogen sulphide (H_2S) was detected mainly in columns 2 and 4, likely as a result of intensified sulphate reduction. The effect of denitrifying bacteria on CO_2 and H_2S production suggests an overlap between nitrification–denitrification and sulphur cycles, which require further investigation using larger sample sizes.

Ni *et al.* (2012) reported NH_3 and CO_2 levels respectively 12.9–51.9 ppm and 1,755–2,295 ppm inside poultry houses. In this study, concentrations were lower due to the closed experimental setup and the lack of constant metabolic poultry emissions. The sealed conditions and microbial additions shifted the gas balance towards CO_2 dominance, which was consistent with Kreidenweis *et al.* (2021).

The targeted gas chromatography with thermal conductivity detector (GC-TCD) analysis confirmed negligible methane

Table 2. Measurement results for analysed gases in the indoor experiment (EI)

Column with static chambers	Concentration				
	CO_2	CH_4	O_2	NH_3	H_2S
	% v/v			ppm	
2nd week					
1 – control	17	0	1.2	0	2
2 – inoculated with denitrifiers	36	0	0	0	37
3 – inoculated with conditioning bacteria	17	0	3.2	0	18
4 – inoculated with a microbiological composition – denitrifying bacteria + conditioning bacteria	30	0	0	0	7
6th week					
1 – control	22	0	1.2	0	2
2 – inoculated with denitrifiers	48	0	0	0	192
3 – inoculated with conditioning bacteria	24	0	0	0	19
4 – inoculated with a microbiological composition – denitrifying bacteria + conditioning bacteria	40	0	0	0	72

Source: own study.

Table 3. The share of individual gases in the sample taken from the static chamber (EI)

Mass to charge ratio (m/z)[1)]	Compound	Chemical formula	Contents (%) in		
			3rd week chamber 1 (control)	11th week	
				chamber 1 (control)	chamber 4
28	nitrogen	N_2	61.49	46.88	54.70
32	oxygen	O_2	2.18	2.01	1.81
14	fragment ion from nitrogen	N	6.10	2.29	2.43
40	argon	Ar	2.92	1.71	1.42
16	methane	CH_4	2.25	1.68	1.55
18	water	H_2O	1.63	0.14	1.34
29	carbon monoxide	CO	0.71	0.50	0.50
27	hydrogen cyanide	HCN	0.71	0.25	0.22
44	carbon dioxide	CO_2	20.54	44.41	35.63
17	ammonia	NH_3	0.35	0.03	0.31
Sum			99.86	99.90	99.86

[1)] m/z = mass-to-charge ratio, where m is ion mass (in atomic mass units, u) and z is the number of charges on the ion.
Source: own study.

homologues. In week 3, the share of CH_4 in chamber 1 was 2.1%, dropping to 1.8% by week 11. Ammonia share fell from 0.35% to under 0.2%, while CO_2 doubled. The O_2 share decreased moderately, HCN concentrations also decreased, especially in chamber 4, supporting the hypothesis that certain strains can reduce HCN through microbial competition (Zdor, 2015).

PHYSICO-CHEMICAL PARAMETERS OF MANURE

Initial moisture content in chicken manure exceeded 50% (Tab. S1). After 11 weeks, an approximately 17% increase in moisture was observed in columns inoculated with liquid denitrifiers, compared to a 11.9% drop in the control and approx. 11.5% drop in the conditioning bacteria variant. This indicates that the liquid inoculum affects water retention and leachate dynamics. The moisture balance confirms the practical importance of application methods.

In the *in situ* piles (EII), initial humidity for turkey manure was 43.4% versus 35.4% for chicken manure (Tab. S2). During the first three months, moisture remained stable, but increased after additional inoculation in week 15, and then dropped significantly. Final moisture was 14.8% for chicken and 12.7% for turkey manure. The overall loss (20–31%) is consistent with Davam (2022) and Singh *et al.* (2018), who reported gradual drying under open conditions.

CHARACTERISATION OF ODOROUS AND GREENHOUSE GASES FROM MANURE – *IN SITU* EXPERIMENT (EII)

The *in situ* experiment (EII) analysed static piles of chicken and turkey manure under natural conditions, using repeated gas sampling and GC-MS analysis. Gas analyses in the *in situ* piles showed limited CH_4 and H_2S emissions, whereas CO_2 was detected only in turkey manure (No. 2) and showed slight fluctuations, while O_2 varied slightly. Ammonia dropped from 18 ppm to 3 ppm in chicken manure but increased over 14-fold in turkey manure by the end of the experiment (Tabs. 4–5). This highlights how substrate type and inoculation influence nitrogen turnover. The resulting effect on odour intensity over time and distance is presented in Figure 1, which shows the trend for both manure types during the *in situ* experiment.

The results of GC-MS confirmed negligible gaseous alkanes and alkenes. Methane content stayed below 2% in both manures, showing no significant anaerobic conditions, while CO and HCN shares slightly increased by week 15 in both piles, supporting the role of cyanogenic pathways during decomposition. Overall, the

Table 4. Measurement results for analysed gases in the experiment *in situ* (EII)

Column with static chambers	Contents				
	CO_2	CH_4	O_2	NH_3	H_2S
	% v/v			ppm	
Chicken manure					
3rd week	0	0	20.4	18	0
6th week	0	0	20.4	18	0
15th week	0	0	20.5	0	0
42nd week	0	0	20.8	3	0
Turkey manure					
3rd week	6.0	0	12.3	45	0
6th week	6.0	0	12.2	33	0
15th week	1.0	0	19.5	0	0
42nd week	10.0	0	10.0	472	0

Source: own study.

Table 5. The share of individual gases in the sample taken from the piles of manure – experiment *in situ* (EII)

Mass to charge ratio (m/z)[1)]	Compound	Chemical formula	Contents (%) in			
			3rd week		15th week	
			chamber 1 (chicken manure)	chamber 2 (turkey manure)	chamber 1 (chicken manure)	chamber 2 (turkey manure)
28	nitrogen	N_2	68.64	66.95	69.75	68.03
32	oxygen	O_2	20.70	19.05	21.05	19.37
14	fragment ion from nitrogen	N	4.90	5.14	3.75	3.93
40	argon	Ar	2.21	2.19	1.90	1.90
16	methane	CH_4	1.67	1.80	1.28	1.38
18	water	H_2O	0.08	0.07	0.93	0.79
29	carbon monoxide	CO	0.60	0.50	0.71	0.59
27	hydrogen cyanide	HCN	0.25	0.21	0.61	0.51
44	carbon dioxide	CO_2	0.89	3.53	0.29	2.96
17	ammonia	NH_3	0.00	0.12	0.22	0.19
Sum			**99.94**	**99.55**	**99.76**	**99.65**

[1)] As in Tab. 3.
Source: own study.

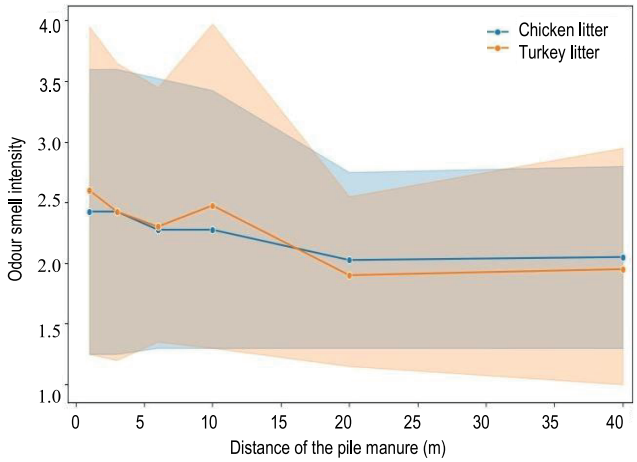

Fig. 1. Odour intensity as a function of distance from the manure pile: orange and blue shading in the line graph shows confidence intervals (95% confidence intervals) for mean odour intensity values; source: own study

results confirm that gas emissions were limited and strongly shaped by manure type and inoculated consortia, highlighting the need for further monitoring under varying environmental conditions.

ODOUR NUISANCE ANALYSIS

The odour nuisance study confirmed significant differences between chicken and turkey manure piles, with turkey manure being initially more odorous, especially at short distances (1–3 m) ($U = 320$, $p = 0.003$). Odour nuisance decreased significantly over time (ANOVA repeated measures: $F = 45.32$, $p < 0.001$), stabilising at a low annoyance level (~1.2 points) by week 19. The strongest odour intensity was recorded in the western direction, showing significant differences compared to northern and southern directions ($H(3) = 18.7$, $p < 0.001$; where H stands for the Kruskal–Wallis test statistic; Fig. 2). A clear distance effect was confirmed ($p < 0.05$ for 1–3 m) with no significant differences beyond 6 m.

Meteorological factors also influenced odour nuisance perception: lower temperatures strongly and negatively correlated with odour intensity (Tab. S3), while higher air humidity was associated with increased odour scores. Wind direction and speed showed weaker but noticeable trends (Fig. 3). The highest nuisance occurred within 1–3 m of the source (Tab. S4).

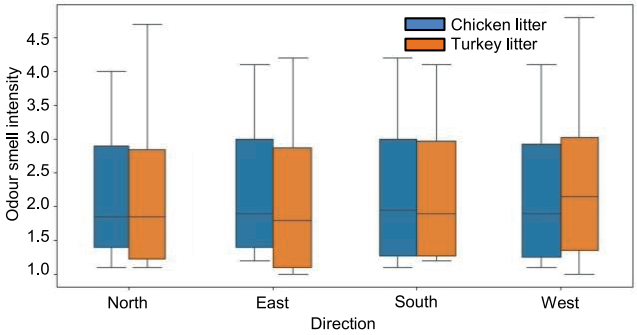

Fig. 2. Odour intensity depending on the direction of examination in relation to the litter piles: ranges indicate the distribution of median scores as a function of distance and direction; error bars represent 95% confidence intervals; source: own study

The principal component analysis (PCA) results (Fig. 4) confirmed that time was the dominant factor, clearly separating the early high-nuisance period (week 3) from the later stage (week 15). In week 3, nuisance scores, solar radiation, and air temperatures were the main drivers of odour perception, while in the later period higher O_2 and N_2 content and higher moisture played a more significant role.

Overall, the results confirm that odour nuisance can be significantly reduced by time, distance and climatic factors, and that turkey manure initially causes stronger nuisance than chicken manure under similar conditions.

POSSIBLE MECHANISMS OF REDUCED NH_3 AND HCN EMISSIONS

Microbial inoculation likely enhanced nitrification and denitrification, limiting NH_3 volatilisation by immobilising NH_4^+ and promoting NO_3^- reduction. The presence of *Pseudomonas* spp. and *Bacillus* strains is known to support these pathways (Azim, Komenane and Soudi, 2017). Hydrogen cyanide reduction could be linked to microbial competition and metabolic pathways that degrade cyanides under aerobic or low-oxygen conditions (Zdor, 2015). The *Pseudomonas* spp. and *Bacillus licheniformis* strains used in the study are known to produce urease and deaminase enzymes that accelerate the hydrolysis of organic nitrogen compounds, facilitating faster mineralisation and subsequent microbial assimilation (Azim, Komenane and Soudi, 2017). This reduces the pool of free ammonia available for volatilisation.

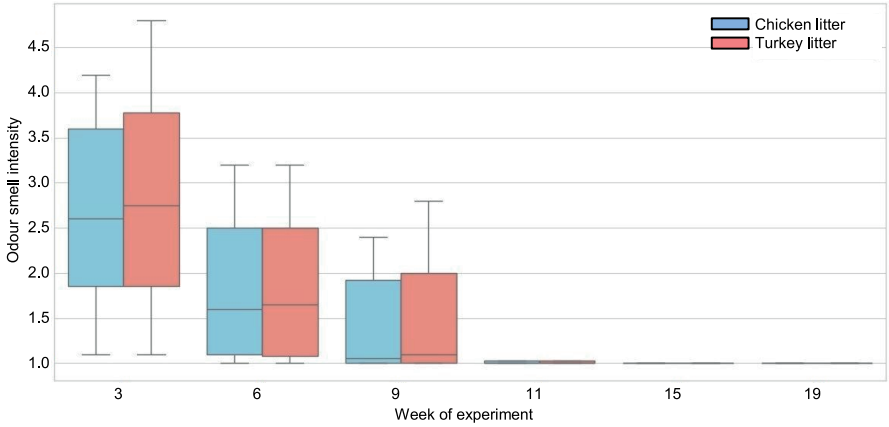

Fig. 3. Distribution of nuisance scores in individual study weeks; source: own study

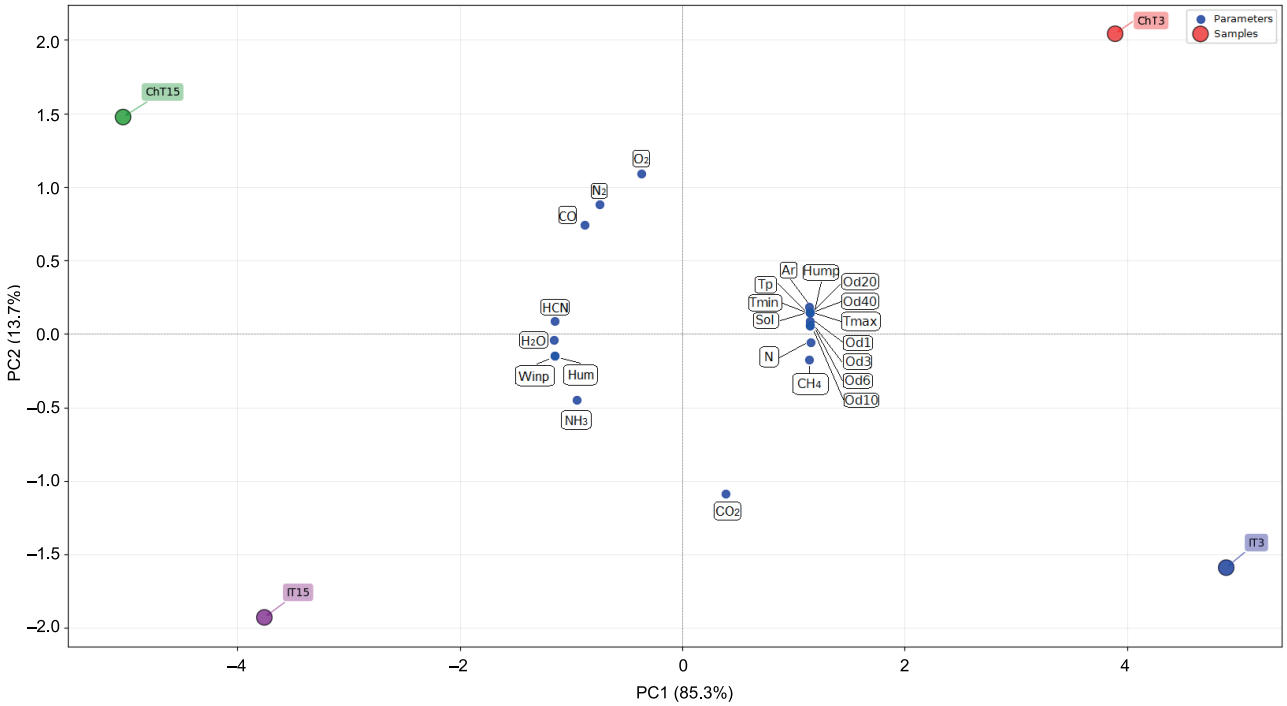

Fig. 4. Principal component analysis (PCA) results; ChT3; ChT15; IT3; IT15 = chicken manure (Ch) and turkey manure (I) in weeks 3 and 15, N_2 = nitrogen, O_2 – oxygen, N = fragment ion from nitrogen, Ar = argon, CH_4 = methane, H_2O = water, CO = carbon monoxide, HCN = hydrogen cyanide, CO_2 = carbon dioxide, NH_3 = ammonia, *Od*1–*Od*40 = nuisance depending on the distance from the pile, *Winp* – wind power (subjective value), *Sol* = insolation (subjective value), T_p = perceived temperature (subjective value), Hum_p = perceived humidity (subjective value), T_{min} = minimum temperature, T_{max} = maximum temperature, *Hum* = air humidity; source: own study

Regarding hydrogen cyanide, certain *Pseudomonas* strains (e.g., *P. fluorescens*) possess the genetic capability to degrade cyanogenic compounds via the HCN hydratase pathway under aerobic conditions (Zdor, 2015). This pathway enables the transformation of HCN into formamide or formate, which can be further metabolised by soil microorganisms. Competition for substrates and co-metabolism under mixed microbial consortia further limits the accumulation of free hydrogen cyanide in the system.

These combined processes support the observed lower emissions of both gases compared to untreated piles, aligning with the concept that bioaugmentation can mitigate gaseous emissions from manure.

CONCLUSIONS

The study confirmed that selected microbial consortia can effectively reduce ammonia and hydrogen cyanide emissions from poultry manure, although their use may increase CO_2 and H_2S emissions, highlighting the need for further optimisation. The findings demonstrate that manure type, microbiological composition, and storage conditions significantly influence greenhouse gas and odour emissions.

The research also showed that odour nuisance is the highest in the early storage period, especially for turkey manure, and strongly depends on weather conditions, particularly temperature and humidity.

Future studies should focus on refining the proportions of denitrifying and conditioning strains to balance greenhouse gas and odour mitigation and explore integrated methods, such as composting or biofiltration, to enhance sustainable manure management in line with climate protection goals.

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SUPPLEMENTARY MATERIAL

Supplementary material to this article can be found online at: https://www.jwld.pl/files/Supplementary_material_67_Kupiec.pdf.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

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