Microbial agent spraying in pig housing and slurry can potentially reduce harmful gas emissions – a preliminary study

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KEY WORDS: gas emission, microbial agents, slurry, pigs

ABSTRACT. This study aimed to evaluate the effects of spraying microbial agents in pig slurry and housing on harmful gas emissions. A total of 300, eight-week-old crossbreed ([Yorkshire × Duroc] × Landrace) growing pigs, with an average body weight of 28.2 ± 0.55 kg were used in this trial lasting 4 weeks (28 days). Experiment 1: pigs were randomly assigned to two treatments and housed in two separate rooms (150 heads/room). Slurry stored in a slurry pit, produced by growing pigs housed in one room, was sprayed with Bacillus subtilis (TRT1), while slurry from the second room was sprayed with Lactobacillus plantarum (TRT2). The results showed that L. plantarum had a better limiting effect on ammonia (NH₃), hydrogen sulphide (H₂S), and carbon dioxide (CO₂) concentrations (P = 0.01, P = 0.03 and P = 0.01 respectively) than B. subtilis. After Experiment 1, the pigs were rearranged and transferred to finishing rooms. At this point, they were subdivided and housed in 3 separate rooms consisting of 100 pigs each (Experiment 2). Subsequently, their slurry pits were sprayed with or without a mixture of microbial agents (B. subtilis and B. licheniformis) as follows: CON (no microbial agents), BSBL1 (mixed microbial agent spray 1000:1) and BSBL2 (mixed microbial agent spray 1000:2). In Experiment 2, we observed that the gases, i.e. NH₃, H₂S, total mercaptans, acetic acid, and CO₂ were strongly reduced with increasing levels of the microbial agent. Our findings clearly indicated that spraying L. plantarum in slurry exerted a greater effect on odorous gas emission compared to spraying B. subtilis. Moreover, the microbial spray mixtures provided improved positive outcomes possibly as a combined effect compared to solitary sprays.

Introduction

Atmospheric air is essential for the organism living on Earth. Despite the recognition of anthropogenic activities as the culprits of global warming and air pollutant emissions, there is a need for multi-sectoral action. Multiple reports have shown that air pollution is an enormous burden on health and is considered a major contributor to excessive mortality rate due to respiratory, cardiovascular and other diseases (Hong et al., 2019; Lelieveld et al., 2019). The intensified animal production system is accused of being the leading sector emitting the majority of atmospheric pollutants. In fact, the emitted gases cause the greenhouse effect by trapping infrared radiation and its subsequent emission in the form of reverse thermal radiation, leading to an increase in the Earth’s surface temperature (Marszalek et al., 2018). The pig industry has grown rapidly in recent years, with the intensive rearing dominating other production systems. Slurry produced during pig breeding pollutes the environment by emitting
high ammonia (NH₃) and greenhouse gases to the atmosphere (Calvet et al., 2017). In addition, the excessive use of slurry for agricultural fertilisation can lead to eutrophication of lakes and rivers, given that these heat-trapping gases are released into the Earth’s atmosphere at various stages of slurry management (Girard et al., 2009). Moreover, nutrients in manure, mainly nitrogen and phosphorous, are a significant component of pollution from agriculture to surface, ground and marine waters, damaging ecosystems through eutrophication and restricting their recreational use. Typically, slurry is defined as a liquid heterogeneous mixture of animal excreta, undigested food residues, and water used for hygienic and cleaning purposes in livestock buildings, characterised by the presence of mineral components easily assimilable to plants (Marszalek et al., 2018). Greenhouse gas (GHG) production by livestock accounts for 14.5% of total anthropogenic emissions (Twine, 2021). In particular, it has been reported that intensive pig rearing generates approx. 10% of GHG emissions from livestock, which is the second highest in this sector (Giraldi-Díaz et al., 2021); in addition, environmental issues associated with pig production concern water and air pollution (Rodhe et al., 2012). Pig slurry contains harmful substances, such as heavy metals, unpleasant odours, parasites, and pathogens that pose potential risks to the environment and public health, especially when improperly treated and applied (Sun et al., 2021). Other studies found that the majority of odour-causing substances are generated by protein degradation and if carbohydrates are limited in pig slurry during the storage period, proteins become the main source of fermentable carbon (Hwang et al., 2021). In the literature, there are various methods described to reduce gas emissions in pig slurries, including storage in hermetically sealed tanks, acidification, separation into solid and liquid fractions, anaerobic digestion and aeration (Marszalek et al., 2018). On the other hand, research has identified animal nutrition as a unique option to reduce these impacts. Initially, the use of microbial agents (probiotics) in livestock was driven by the need for alternative strategies to increase production and health of animals rather than the use of antibiotics. Probiotic supplements containing spores of *Bacillus subtilis* and *B. licheniformis* have been reported to decrease ammonia emissions by about 50%, and inconsistent results among studies are perhaps dependent on the bacterial strains used, type of feed ingredients, environmental conditions, trial duration and host age, but also the lack of sufficiently robust methodologies for determining gaseous emissions (Prenafeta-Boldú et al., 2017). There is limited research on the effects of microbial agents sprayed in to slurry materials on gas emissions in swine manure and pig houses. The present work focused on the effect of an experimental spraying of *B. subtilis* (1.0 × 10⁷ CFU/g) or *Lactobacillus plantarum* (1.0 × 10⁷ CFU/g) during the growing period (Experiment 1), or a mixture of *B. subtilis* (1.0 × 10⁹ CFU/g) and *B. licheniformis* (1.0 × 10⁹ CFU/g) during the finishing period (Experiment 2) into slurry pits as a strategy to reduce emissions of NH₃, hydrogen sulphide (H₂S), carbon dioxide (CO₂), total mercaptans (R-SH), and total acetic acid (AA) concentrations from slurry and pig house atmosphere.

Material and methods

Ethical declaration

The present study was conducted at the Gongju research unit (Dankook University). The protocol (#DK-2-2106#) for this trial was approved by the Ethics Committee of the Dankook University, Cheonan, South Korea, in accordance with the Animal Care and Use Guidelines. Microbial agents used in the study were provided by a commercial company (Powerzyme, B&B Gyeonggi-do, South Korea). Fermentation was inoculated with *B. subtilis* (1.0 × 10⁹ CFU/g) and *L. plantarum* (1.0 × 10⁷ CFU/g) in Experiment 1 (Exp. 1); and *B. subtilis* (1.0 × 10⁹ CFU/g) and *B. licheniformis* (1.0 × 10⁹ CFU/g) in Experiment 2 (Exp. 2), and incubated for 48 h. Subsequently, the material was dried at 60 °C for more than 72 h. Wood powder was used as carrier.

Experimental housing, design and sampling procedures

**Experiment 1.** Exp. 1 was strictly conducted to evaluate two microbial agents, namely *B. subtilis* (1.0 × 10⁷ CFU/g) and *L. plantarum* (1.0 × 10⁷ CFU/g) for their efficacy in reducing gas emissions. A total of 300, eight-week-old crossed ([Yorkshire × Duroc] × Landrace) healthy growing pigs, with an average body weight (BW) of 28.2 ± 0.55 kg were used in this trial for 3 weeks (21 days). Based on body weight and sex, pigs were randomly assigned to two treatment groups and housed in two separate rooms (150 pigs in each room). The pens were uniformly equipped with self-feeders and nipple drinkers to allow unlimited access to feed and water throughout the experiment. The pig room had a 0.45-m deep slurry pit under a 22.8 m² of slatted plastic floor divided equally into 4 blocks. The ambient temperature in the facilities was maintained at approximately 25 °C by a ventilation control system.
Pig slurry treatment using microbial agent spraying

system. Slurry stored in the slurry pit produced by growing pigs housed in one room was sprayed with *B. subtilis* 1.0 × 10^7 CFU/g (at an estimated dilution of 1000:5) and designated TRT1, while slurry stored in the slurry pit, produced by growing pigs housed in another room was sprayed with *L. plantarum* 1.0 × 10^7 CFU/g (at an estimated dilution of 1000:5) and designated TRT2. The slurry pits under both rooms were manually sprayed with microbial agents every morning (8:00) and evening (18:00) throughout the experiment. All pigs were fed a basal diet formulated according to the recommendations of the National Research Council (NRC, 2012) and all feed components and calculated nutritional values of the basal diet are presented in Table 1.

| Table 1. Composition of the experimental grower pig diets (as-fed basis) |
|-----------------|-----------------|
| Item            | Composition     |
| Corn            | 74.99           |
| Soybean meal (48%) | 21.31          |
| Tallow          | 1.78            |
| Dicalcium phosphate | 1.24          |
| Limestone       | 0.75            |
| Salt            | 0.20            |
| Lysine (78%)    | 0.42            |
| Methionine (99%)| 0.06            |
| Vitamin premix  | 0.12            |
| Mineral premix  | 0.10            |
| Choline (25%)   | 0.03            |
| Total           | 100.00          |
| Calculated value|                |
| crude protein, %| 16.50           |
| metabolizable energy, kcal/kg | 3300       |
| lysine, %       | 1.12            |
| methionine, %   | 0.32            |
| Ca              | 0.66            |
| P               | 0.56            |

1 provided per kilogram of complete diet: mg: vit. A (retinol) 1.3, vit. D₃ (cholecalciferol) 0.022, vit. E (tocopherol) 45, vit. K₃ (menadione) 4.2, vit. B₁₂ (riboflavin) 8.6, vit. B₉ (folic acid) 0.04; 2 provided per kilogram of complete diet: mg: Cu 15, Fe 80, Zn 56, Mn 73, I 0.3, Co 0.5, Se 0.4

Initially, fresh slurry samples were collected from the pits for analysis of odorous compounds on days 1, 7, 14, and 21. Slurry samples were collected from 4 quadrants of each room and homogenized using a slatted floor mixer (Porco, Betzenweiler, Germany), transferred to 2.6-l plastic containers, and incubated for 24 h at room temperature (25 °C) for further fermentation before analysis. Subsequently, NH₃, H₂S, R-SH, AA and CO₂ gases were determined automatically using a multi-gas monitor (Multi-RAE Lite, RAE Systems, San Jose, CA, USA) in both slurry and pig house atmosphere.

To determine gas emissions, room fans were turned off overnight (12 h), and gases were analysed the following morning directly in the room using the same apparatus.

**Experiment 2.** After Exp. 1, the pigs were regrouped and transferred to finishing rooms. At this point, they were divided and housed in 3 separate rooms with 100 pigs each, and fed a basal diet formulated according to the NRC (2012) recommendations for finishing pigs (Table 2). Their slurry pit was then sprayed with/without microbial agents mixture of *B. subtilis* (1.0 × 10^9 CFU/g) and *B. licheniformis* (1.0 × 10^9 CFU/g) as follows: CON (no microbial agents), BSBL1 (mixed microbial agents at 1000:1 dilution) and BSBL2 (mixed microbial agents at 1000:2 dilution). Slurry samples were collected on days 7, 14, 21, and 28 of the experiment. The sampling and analysis procedures of gas emissions in slurry samples and pig housing atmosphere were similar for Exp. 1 and Exp. 2.

| Table 2. Composition of the experimental finishing pig diets (as-fed basis) |
|-----------------|-----------------|
| Item            | Composition     |
| Corn            | 45.06           |
| Wheat           | 13.00           |
| Soybean meal    | 23.00           |
| Rapeseed meal   | 2.20            |
| Dried distillers’ grains with soluble, corn | 5.00 |
| Dicalcium phosphate | 1.06          |
| Limestone       | 1.00            |
| Salt            | 0.30            |
| L-lysine SO₄ (51%) | 0.24          |
| DL-methionine (50%) | 0.12      |
| L-tryptophan (10%) | 0.01          |
| L-threonine (98.5%) | 0.13          |
| Animal fat      | 5.30            |
| Molasses        | 3.20            |
| Choline (50%)   | 0.08            |
| Vitamin premix  | 0.15            |
| Mineral premix  | 0.15            |
| Calculated composition |                |
| metabolizable energy, kcal/kg | 3400        |
| lysine, %       | 0.95            |
| methionine, %   | 0.30            |
| Ca, %           | 0.76            |
| P, %            | 0.28            |

1 provided per kilogram of complete diet: IU: vit. A 10 000, vit. D₃ 2 000, vit. E 48; mg: vit. K₃ 1.5, riboflavin 6, niacin 40, D-pantothenic acid 17, biotin 0.2, folic acid 2, choline 166, vit. B₆ 2, vit. B₁₂ 28; 2 provided per kilogram of complete diet: mg: Fe (as FeSO₄·7H₂O) 90, Cu (as CuSO₄·5H₂O) 15, Zn (as ZnSO₄) 50, Mn (as MnO₂) 54, I (as KI) 0.99, Se (as Na₂SeO₃·5H₂O) 0.25

**Statistical analysis**

The obtained experimental data were statistically analysed using Student’s *t*-test in Exp. 1
and the GLM procedure of SAS version 9.0 (SAS Institute, 2002) in Exp. 2. The pig room served as the experimental unit and Duncan’s multiple range test (Exp. 2) was applied to determine the effect of microbial agent spraying in pig housing air and slurry; microbial agents were considered as a fixed variable. Data are presented as means ± standard error of the mean (SEM). The $P < 0.05$ value was adopted as statistical significance, while the $P$-value between 0.05 and 0.10 was considered a trend.

Results

Experiment 1

Table 3 shows the effect of microbial agent spraying on odorous substances in the slurry. At the beginning of the trial, spraying with *L. plantarum* in the TRT2 room significantly reduced the concentrations of odorous substances, i.e. NH$_3$, H$_2$S, and CO$_2$ compared to the TRT1 room sprayed with *B. subtilis* ($P = 0.01$, $P = 0.03$ and $P = 0.01$, respectively). At the end of week 1, 2, and 3, there was a markedly higher reduction of H$_2$S, CO$_2$, R-SH, and total AA levels in TRT2 compared to TRT1 ($P < 0.05$).

Table 4 presents the effect of microbial agent spraying on slurry odour substances in pig houses. At the beginning of the study, there was no significant difference between TRT1 and TRT2 rooms in terms of reduction of NH$_3$, H$_2$S, CO$_2$, R-SH, and total AA emissions ($P > 0.05$). By the end of week 2, the concentrations of NH$_3$, AA and CO$_2$ significantly declined in TRT2 compared to TRT1 ($P > 0.05$); however, R-SH and H$_2$S levels showed no differences between treatments. At the end of week 3, a significant reduction in AA and CO$_2$ concentrations were recorded in TRT2 compared to TRT1 ($P = 0.01$, 0.01, respectively), in contrast to NH$_3$, H$_2$S and R-SH levels, for which no significant differences were found between treatments.

**Table 3.** Effect of microbial agent spray on gas-emission in slurry

| Items, ppm | TRT1 TRT2 SEM | $P$-value |
|------------|---------------|-----------|
| Initial (day 1) | | |
| NH$_3$ | 96.21$^a$ | 88.98$^b$ | 5.20 | 0.01 |
| H$_2$S | 99.01$^a$ | 96.46$^b$ | 6.09 | 0.03 |
| R-SH | 0.00 | 0.00 | – | – |
| AA | 0.00 | 0.00 | – | – |
| CO$_2$ | 2900$^a$ | 1250$^b$ | 295 | 0.01 |
| Week-1 (day 7) | | |
| NH$_3$ | 12.27$^a$ | 9.44$^b$ | 1.56 | 0.01 |
| H$_2$S | 71.04$^a$ | 30.48$^b$ | 5.15 | 0.01 |
| R-SH | 11.00 | 3.10 | 1.64 | 0.07 |
| AA | 5.90$^a$ | 2.50$^b$ | 0.75 | 0.04 |
| CO$_2$ | 12460$^a$ | 8260$^b$ | 295 | 0.01 |
| Week-2 (day 14) | | |
| NH$_3$ | 8.13 | 5.70 | 1.14 | 0.15 |
| H$_2$S | 40.38$^a$ | 19.48$^b$ | 5.69 | 0.01 |
| R-SH | 11.40 | 9.70 | 1.63 | 0.11 |
| AA | 6.50$^a$ | 2.90$^b$ | 0.86 | 0.05 |
| CO$_2$ | 14510$^a$ | 10340$^b$ | 1329 | 0.04 |
| Week-3 (day 21) | | |
| NH$_3$ | 5.26 | 2.97 | 0.95 | 0.10 |
| H$_2$S | 20.10$^a$ | 9.68$^b$ | 1.90 | 0.01 |
| R-SH | 9.80 | 8.90 | 1.37 | 0.41 |
| AA | 5.70$^a$ | 2.10$^b$ | 0.85 | 0.03 |
| CO$_2$ | 11460$^a$ | 7400$^b$ | 1231 | 0.04 |

Table 4. Effect of microbial agent sprays on gas emissions in pig room

| Items, ppm | TRT1 TRT2 SEM | $P$-value |
|------------|---------------|-----------|
| Initial (day 1) | | |
| NH$_3$ | 3.50 | 3.25 | 0.18 | 0.67 |
| H$_2$S | 0.45 | 0.48 | 0.02 | 0.73 |
| R-SH | 5.75 | 6.00 | 0.73 | 0.78 |
| AA | 4.00 | 3.50 | 0.61 | 0.59 |
| CO$_2$ | 3350 | 3400 | 68 | 0.64 |
| Week-1 (day 7) | | |
| NH$_3$ | 4.00 | 3.00 | 0.08 | 0.28 |
| H$_2$S | 0.50 | 0.40 | 0.06 | 0.46 |
| R-SH | 6.00 | 5.50 | 0.46 | 0.55 |
| AA | 4.75$^a$ | 2.75$^b$ | 0.29 | 0.04 |
| CO$_2$ | 3500$^a$ | 3250$^b$ | 46 | 0.02 |
| Week-2 (day 14) | | |
| NH$_3$ | 4.25 | 3.75 | 0.20 | 0.21 |
| H$_2$S | 0.60 | 0.58 | 0.13 | 0.06 |
| R-SH | 6.50 | 5.00 | 0.35 | 0.10 |
| AA | 5.00$^a$ | 2.25$^b$ | 0.44 | 0.01 |
| CO$_2$ | 3600$^a$ | 3150$^b$ | 61 | <0.01 |
| Week-3 (day 21) | | |
| NH$_3$ | 4.75 | 2.50 | 0.44 | 0.35 |
| H$_2$S | 0.63 | 0.23 | 0.08 | 0.21 |
| R-SH | 6.50 | 4.75 | 0.18 | 0.11 |
| AA | 5.25$^a$ | 2.00$^b$ | 0.44 | 0.01 |
| CO$_2$ | 3700$^a$ | 3075$^b$ | 44 | 0.01 |

TRT1 = *Bacillus subtilis* [1.0 × 10$^7$ CFU/g (500 g/1000 kg dilution)], TRT2 = *Lactobacillus plantarum* [1.0 × 10$^7$ CFU/g (500 g/1000 kg dilution)]. NH$_3$ – ammonia, H$_2$S – hydrogen sulphide, R-SH – methyl mercaptans, AA – acetic acid, CO$_2$ – carbon dioxide, SEM – standard error of the mean; $^a$ – means within a row with different superscripts are significantly different at $P < 0.05$

Experiment 2

Table 5 shows the effect of spraying with probiotic mixtures on gas emissions in pig slurries.
At the end of week 1, NH$_3$ and CO$_2$ emissions were significantly reduced by increasing doses of microbial agents ($P < 0.01$ and $P = 0.01$, respectively), but there was no significant difference in H$_2$S, AA and R-SH emissions. At the end of week 2, NH$_3$, H$_2$S, R-SH and CO$_2$ emissions ($P < 0.01$, $P = 0.02$, $P < 0.01$ and $P = 0.04$, respectively) were markedly reduced by increasing doses of microbial agents, while AA levels did not differ between the treatments. At the end of week 3, R-SH, H$_2$S and AA levels were highly reduced by spraying different amounts of microbial agents ($P < 0.01$, $P = 0.01$ and $P = 0.01$, respectively) to the extent that BSBL1 and BSBL2 were free of these gases at the end of week 4. Nevertheless, no significant reduction was recorded for NH$_3$ and CO$_2$ at the same time. Interestingly, the production of NH$_3$, H$_2$S, MM, AA and CO$_2$ was significantly reduced by the increasing doses of microbial spray at the end of week 4. Promising results were also obtained for the pig room (Table 6). To illustrate this, AA and CO$_2$ concentrations were significantly lower in BSBL2 than in BSBL1 in the first week, and similarly, there was a significant reduction in NH$_3$, H$_2$S, AA, R-SH and CO$_2$ levels from week 2 to 4.

### Table 5. Effect of mixed microbial agent sprays on gas emission in slurry

| Items        | CON  | BSBL1 | BSBL2 | SEM  | $P$-value |
|--------------|------|-------|-------|------|-----------|
| Week-1 (day 7) |      |       |       |      |           |
| NH$_3$       | 19.7 | 10.3  | 9.3   | 0.8  | <0.001    |
| H$_2$S       | 5.43 | 2.30  | 0.13  | 2.70 | 0.45      |
| R-SH         | 0.0  | 0.0   | 0.0   | 0.0  | –         |
| AA           | 3.3  | 3.3   | 2.0   | 1.1  | 0.66      |
| CO$_2$       | 21133.3 | 10033.3 | 6833.3 | 1888.9 | 0.01      |
| Week-2 (day 14) |   |       |       |      |           |
| NH$_3$       | 15.3 | 7.3   | 6.0   | 0.8  | <0.001    |
| H$_2$S       | 7.23 | 3.4   | 0.80  | 0.50 | 0.02      |
| R-SH         | 9.0  | 0.0   | 0.0   | 0.0  | <0.01     |
| AA           | 4.6  | 0.0   | 4.0   | 0.0  | 0.66      |
| CO$_2$       | 46333.3 | 9466.7 | 6500.0 | 1754.7 | 0.04      |
| Week-3 (day 21) |   |       |       |      |           |
| NH$_3$       | 20.7 | 0.7   | 0.3   | 0.4  | 0.15      |
| H$_2$S       | 12.00 | 4.00  | 0.00  | 0.00 | 0.01      |
| R-SH         | 7.30 | 2.0   | 0.0   | 0.0  | <0.01     |
| AA           | 7.7  | 1.0   | 0.0   | 0.0  | <0.01     |
| CO$_2$       | 966.7 | 366.7 | 0.0   | 419.4 | 0.36      |
| Week-4 (day 28) |   |       |       |      |           |
| NH$_3$       | 23.0 | 0.0   | 0.0   | 0.0  | <0.01     |
| H$_2$S       | 8.00 | 0.0   | 0.0   | 0.0  | <0.01     |
| R-SH         | 5.56 | 0.0   | 0.0   | 0.0  | <0.01     |
| AA           | 2.0  | 0.0   | 0.0   | 0.0  | <0.01     |
| CO$_2$       | 1002.8 | 432.7 | 0.0   | 419.4 | 0.01      |

CON – normal slurry without microbial agent, BSBL1 – mixed probiotic spray (Bacillus subtilis 1.0 × 10$^9$ CFU/g and B. licheniformis 1.0 × 10$^9$ CFU/g in dilution 1000:1), BSBL2 – mixed probiotic spray (B. subtilis 1.0 × 10$^9$ CFU/g and B. licheniformis 1.0 × 10$^9$ CFU/g in dilution 1000:2); NH$_3$ – ammonia, H$_2$S – hydrogen sulphide, R-SH – methyl mercaptans, AA – acetic acid, CO$_2$ – carbon dioxide, SEM – standard error of the mean; $P < 0.05$ denotes statistical significance.

### Table 6. Effect of microbial agent sprays on gas emissions in pig room atmosphere

| Items, ppm | CON  | BSBL1 | BSBL2 | SEM  | $P$-value |
|------------|------|-------|-------|------|-----------|
| Initial (day 1) |      |       |       |      |           |
| NH$_3$     | 3.75 | 4.00  | 7.3   | 0.34 | 0.620     |
| H$_2$S     | 0.48 | 0.45  | 4.30  | 0.14 | 0.874     |
| R-SH       | 0.00 | 0.00  | 0.00  | –    | –         |
| AA         | 2.00 | 1.50  | 3.3   | 0.64 | 0.034     |
| CO$_2$     | 3400 | 3025  | 1033.3 | 117  | 0.013     |
| Week-1 (day 7) |   |       |       |      |           |
| NH$_3$     | 4.25 | 2.50  | 0.73  | 0.41 | 0.003     |
| H$_2$S     | 0.53 | 0.35  | 0.4   | 0.07 | 0.044     |
| R-SH       | 6.50 | 4.00  | 0.0   | 0.58 | 0.002     |
| AA         | 4.25 | 3.50  | 0.0   | 0.53 | 0.046     |
| CO$_2$     | 3550 | 2275  | 466.7 | 137  | 0.001     |
| Week-2 (day 14) |   |       |       |      |           |
| NH$_3$     | 4.75 | 2.50  | 0.7   | 0.18 | 0.006     |
| H$_2$S     | 0.53 | 0.35  | 0.00  | 0.02 | 0.003     |
| R-SH       | 6.75 | 3.75  | 2.0   | 0.58 | 0.004     |
| AA         | 4.50 | 3.25  | 1.0   | 0.18 | 0.017     |
| CO$_2$     | 3975 | 2650  | 566.7 | 88   | 0.001     |
| Week-3 (day 21) |   |       |       |      |           |
| NH$_3$     | 4.75 | 0.5   | 0.0   | 0.00 | 0.001     |
| H$_2$S     | 0.40 | 0.0   | 0.0   | 0.03 | <0.001    |
| R-SH       | 6.15 | 0.0   | 0.0   | 0.00 | <0.001    |
| AA         | 4.05 | 0.0   | 0.0   | 0.00 | <0.001    |
| CO$_2$     | 3750 | 1775  | 432.7 | 34   | 0.01      |

CON – normal slurry without microbial agent, BSBL1 – mixed probiotic spray (Bacillus subtilis 1.0 × 10$^9$ CFU/g and B. licheniformis 1.0 × 10$^9$ CFU/g in dilution 1000:1), BSBL2 – mixed probiotic spray (B. subtilis 1.0 × 10$^9$ CFU/g and B. licheniformis 1.0 × 10$^9$ CFU/g in dilution 1000:2); NH$_3$ – ammonia, H$_2$S – hydrogen sulphide, R-SH – methyl mercaptans, AA – acetic acid, CO$_2$ – carbon dioxide, SEM – standard error of the mean; $P < 0.05$ denotes statistical significances.

### Discussion

Microbial agents have been proposed as a suitable strategy for reducing undesirable environmental emissions from manure (Prenafeta-Boldú et al., 2017). The same author stated that Bacillus spp. (spore-forming bacteria) were best suited for this role due to their stability potential and ability to produce various hydrolytic enzymes. Studies have shown that housing, stored manure and exercise areas emit about 69–80% of total NH$_3$ in Europe (Sommer et al., 2006). Normally, NH$_3$ in livestock
facilities is mainly derived from urea. Urea in urine is relatively stable; however, when it comes in contact with urease, NH$_3$ is produced, which can subsequently be volatilised. Urease is ubiquitous in faeces, and thus contact between urea and urease readily occurs in production facilities (van Kempen, 2001). H$_2$S is a chemically unstable reducing agent, easily oxidised, and produces toxic sulphuric by-products upon combustion (Habeeb et al., 2017). It has been reported that NH$_3$ and H$_2$S emissions can pose a health risk, given their malodorous and hazardous properties, contributing to ecosystem acidification (Wu et al., 2020). Our findings in Exp. 1 showed that the TRT2 room sprayed with *L. plantarum* had significantly reduced levels of odorous substances, i.e. NH$_3$, H$_2$S, and CO$_2$ compared to the TRT1 room sprayed with *B. subtilis*. As this is a preliminary trial, there was little evidence that could explain this outcome. However, it is known that feeding a high protein diet may increase the amount of NH$_3$ and volatile organic compounds, and lower dietary crude protein concentrations decrease NH$_3$ concentrations in both fresh and stored manure (Otto et al., 2003). Previous studies focused on probiotics as feed additives in pigs and demonstrated their beneficial effects in reducing noxious gas emissions. For instance, it was reported that *Bacillus*-based probiotics potentially decreased harmful gas emissions in finishing pigs (Chen et al., 2006). Moreover, a study by Nguyen et al. (2019) reported a high reduction in gas emissions by a mixture of probiotic supplements in the diet of weaned pigs. It is normal that carbohydrates are catabolised to various products upon combustion (Habeeb et al., 2017). It has been reported that NH$_3$ and H$_2$S emissions can be correlated with the effect of microbial agents. Our preliminary results show that probiotics (microbial agents) were fed to pigs, especially *Bacillus* and *Lactobacillus* spp., they enhanced microbial fermentation in the gut.

 Planned comparisons were carried out with probiotics composed of the same microbial agent, as sprayed on the slurry in the present study, and there were some previous results that show benefits when probiotics used in pig diet lowered gas emissions; in addition, it is generally believed that *Bacillus* species are capable of hydrolysing proteins, considering that they produce a number of hydrolytic enzymes to degrade various substrates. However, most studies on probiotics were conducted in vivo, which means that we were not able to spot the mechanism of action outside the organism. Although ideal conditions of microbial activity were considered to simulate the mode of action of microbial agent in slurry, more researches are needed to further investigate the microbial activity and related factors in pig manure. Park et al. (2020) reported that fermentable carbohydrates (FC) are a promising material for reducing odour emissions from pig manure. Effective microbial products are generally utilised to reduce odour and promote fermentation in agricultural fields, and they include actinomycetes, *B. subtilis*, lactic acid bacteria, yeasts, etc. (Kim et al., 2022). The latter authors also commended the microbial agent for reducing pH, NH$_3$ concentration, and urease activity, which are part of emission factors. Kim et al. (2005) reported that the inclusion of 0.3% of a microbial agent mixture had a definite inhibitory effect on NH$_3$ and sulphide dioxide emissions. Moreover, the authors also reported that dietary probiotic supplements containing *B. subtilis* and *B. licheniformis* spores decreased ammonia emissions by approx. 50%. These differences in results between studies are possibly due to host age, environmental conditions, feed types and bacterial strains used. Meanwhile, about 50% of total sulphur is lost in the form of volatile sulphur compounds (VSCs), and common VSCs mainly include hydrogen sulphide, methyl mercaptans (R-SH) and others. It should be noted that H$_2$S is the most released VSCs, accounting for about 39–43% of emissions. Generally, NH$_3$ and VSCs are the predominant odours on the pig farm, yet they are cor-
The results of this study clearly suggested that spraying *L. plantarum* in the slurry exerted a more potent inhibitory effect on odorous gas emissions than spraying *B. subtilis*. Moreover, the mixture of microbial agents seems to have a synergetic reducing effect on gas emissions. Thanks to these findings, we can assume that the mixture of microbial agents has an collective effect on the production of noxious gases, which provides better results than solitary microbial agents. Considering the fact that these findings regarding the effect of microbial agents on gas emissions in the slurry are preliminary, our team is developing a robust methodology that will address all aspects of the problem, including optimal levels of microbial agents, mechanism of microbial action in slurry, microbial activity changes with seasons (summer and winter), exposure time, and possibly provide recommendations on the integration and application procedures to be considered among other management practices.

**Conclusions**

The results of this study clearly suggested that spraying *L. plantarum* in the slurry exerted a more potent inhibitory effect on odorous gas emissions than spraying *B. subtilis*. Moreover, the mixture of microbial agents seems to have a synergetic reducing effect on gas emissions. Thanks to these findings, we can assume that the mixture of microbial agents has an collective effect on the production of noxious gases, which provides better results than solitary microbial agents. Considering the fact that these findings regarding the effect of microbial agents on gas emissions in the slurry are preliminary, our team is developing a robust methodology that will address all aspects of the problem, including optimal levels of microbial agents, mechanism of microbial action in slurry, microbial activity changes with seasons (summer and winter), exposure time, and possibly provide recommendations on the integration and application procedures to be considered among other management practices.

**Acknowledgements**

The Department of Animal Resource & Science was supported through the Research-Focused Department Promotion & Interdisciplinary Convergence Research Projects as a part of the University Innovation Support Program for Dankook University in 2022.

**Conflict of interest**

The Authors declare that there is no conflict of interest.

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