applied sciences

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Isolation and Screening of Odor-Reducing Microbes from Swine Manure and Its Role in Reducing Ammonia Release in Combination with Surfactant Foam

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Abstract: Swine farming facilities have increased the production of malodorous gases, which negatively affects people. Hence, we developed a new feasible bio-foam technology wherein long-lasting surfactant foam, including bacteria, were sprayed on swine manure. The surfactant foam acted as a physical barrier, suppressing NH_3 release, and the aqueous-phase bacteria formed after foam breaking infiltrated in manure and degraded NH_3. In this study, we first isolated NH_3-degrading bacteria from swine manure. A bacterial consortium was prepared using the effective NH_3-degrading strains *Saccharomyces cerevisiae* NRRL Y-12632 (99.88%) (TP1), *Lactococcus lactis* subsp. *hordniae* NBRC100931^T (99.93%) (TP3), and *Lactobacillus argentoratensis* DSM 16365^T (100%) (TP5). The surfactant foam used in this study was a dry foam (foam quality 98.5–99.0% and foam density 0.025–0.026 g/cm^3), with a foam expansion of 110–112 and high foamability. Large bubbles were generated with a bubble density of 1 bubble/cm^2 and a foam lamella thickness of 0.12 mm. In a lab-scale study, foam was sprayed onto NH_3-contaminated soil or real swine manure, which reduced the NH_3 emission from the source (soil/manure) almost completely (97–100%), but NH_3 was re-emitted after foam breaking (5 h: open reactor, 7 h: closed reactor). After loading the bacteria on the foam, the initial NH_3 odor suppression was similar to that of the foam alone. However, NH_3 was effectively reduced by microbial degradation even after foam breaking. Complete odor degradation was observed after 3 days (72 h; 90–100% reduction) for the NH_3-contaminated soil, and 97.7% NH_3 in the swine manure was reduced in 24 h. Furthermore, the reagent cost for preparing stable foam was reasonable, indicating its possible field extension.

Keywords: malodor; surfactant foam; odor suppression; bio-degradation; feasibility; infiltration; surfactant; stability; surface tension; foam application; foam cover technology

Citation: Bajagain, R.; Gautam, P.; Le, T.T.N.; Dahal, R.H.; Kim, J.; Jeong, S.-W. Isolation and Screening of Odor-Reducing Microbes from Swine Manure and Its Role in Reducing Ammonia Release in Combination with Surfactant Foam. *Appl. Sci.* **2022**, *12*, 1806. https://doi.org/10.3390/app12041806

Academic Editor: Anabela Raymundo

Received: 6 December 2021
Accepted: 8 February 2022
Published: 10 February 2022

1. Introduction

Pig farming is increasing annually; pigs are majorly used for human consumption, in addition to supplying skin, fat, and other materials for use as clothing, ingredients for processed foods, cosmetics, and medicine [1–3]. A major concern of the pork industry is the odors emitted mainly from manure and from decaying feed and carcasses, which negatively affect (e.g., headaches) people living in close proximity to pig farms [4,5].

The odorous gases from manure generally contain sulfur (e.g., H_2S and mercaptans), nitrogen (e.g., amines and ammonia (NH_3)), alcohols, phenols, and volatile organic acids. These malodorous gases in swine manure adversely affect human beings, such as causing irritation to the eyes and nose, asphyxiation at high levels, nausea, headache, dizziness,

unconsciousness, and even death [6–9]. Hence, there is an urgent need to prevent malodors, which has become a serious public health concern worldwide [10].

In recent years, many innovative strategies have been made available to farmers to manage the odors emitted from livestock manure. These technologies can be categorized as physical, chemical, or biological odor control technologies [8]. Some well-established physical technologies are the adsorption, masking, and dilution of the gases emitted from facilities. The former technology, adsorption and masking, is expensive, and the latter involves the process of diluting air, wherein malodorous gases from manure are diluted by adding other gases such as camphor and indole-coumarin. These physical-based technologies are extremely useful for treating low concentrations of gases produced from manure, but appear to be ineffective for high concentrations [11,12].

Chemical methods of odor reduction include plant wet scrubbing, photocatalytic oxidation, extract spraying, combustion, and non-thermal plasma [13–15]. These chemical technologies are effective, with a removal rate greater than 90%. However, odor control by these technologies is only efficiently achieved after all odorous gases are appropriately collected from the odor source and introduced into the chemical system [16–19]. Biological methods, however, use microorganisms to remove odors. They have little or no chance of producing secondary pollution from the facilities and exhibit low energy consumption [20]. Biofiltration, bio-trickling, and bio-scrubbing are the most commonly used biological treatments in the literature. These are mostly ex situ treatments (extraction from the source and treatment using a specially designed technology) performed by designing special instruments, whereas in situ treatment (treatment on-site) for odor removal remains a challenging task for experts. The required biological time for complete reduction is relatively long, and complete removal efficiency may not be achieved at a high concentration of the gases produced from manure.

In recent years, simple surface foam spraying technology has been regarded as a promising method for physically suppressing odor and its subsequent degradation by bacteria [21]. Gautam and Mohanty [22] used surfactant foam to initially suppress volatile organic compounds (VOCs) from the source by physical processes. Park et al. (2006) used a stable bacterial foam for odor reduction in swine slurry manure. Foam, in this case, may not only act as a physical barrier but also as a biologically active intermediate that converts odorous combinations into non-odorous products. The use of surfactant foam for odor suppression has been limited in most laboratory experiments because of the unstable foam structure and the high cost of the stabilizers. Long-lasting foam is required to block the release of odor gases. Although several stabilizers have been tested to assess foam rupture, the addition of an increased amount of foam stabilizers may reduce the foamability of the surfactant [23]. Furthermore, expensive stabilizers can be an obstacle to the field application of foam technology.

Therefore, the first objective of this research was to isolate odor-reducing bacteria from swine manure, which can efficiently reduce malodors from manure. This is the first study to screen odor-degrading bacteria from real swine manure (except slurry). The second objective was to test the feasibility of bio-foam (including bacteria) application in reducing malodors from the source, which is an effective physical and biological method for odor suppression and is economically viable for subsequent field application.

2. Materials and Methods

2.1. Reagents and Materials

All chemical reagents used in this study were of analytical grade. Sodium C14-16 alpha olefin sulfonate (AOS) was used to generate foam and gelatin as a foam stabilizer. AOS was purchased from AK Precision Chemical Co., Ltd. (Seoul, Korea). Gelatin was purchased from Gelita (Berlin, Germany). NH_3 solution was purchased from Daejung (Seoul, Korea). R_2A, MRS, and Sabouraud media were obtained from Deoksan Science Co., Ltd. (Seoul, Korea). Solutions were prepared using tap water. Experimental soil was obtained from

a field near Kunsan National University (Gunsan-si, Korea), while swine manure was obtained from a swine facility located in Seocheon-gun, Chungcheongnam-do, Korea.

2.2. Preparation of Odor-Degrading Microbial Consortium (Isolation and Screening)

The ammonia-degrading bacteria were isolated from swine manure waste collected from Yongin, Korea. Ammonia-degrading bacteria were isolated using a modified culture method in Transwell plates containing R_2A, MRS, and Sabouraud media. Microorganisms were enriched in a Transwell plate containing 1 g of soil or manure and 3 mL of R_2A, MRS (for bacteria), and Sabouraud (for yeasts) medium. After culturing for 2 weeks at 28 °C, the culture was serially diluted, and 100 µL of each dilution was spread on R_2A, MRS, and Sabouraud agar plates. Colonies were selected and streaked separately on media plates until pure colonies were obtained, following which, they were sub-cultured for an odor degradation test and stored at −70 °C in media broth supplemented with 20% (*v*/*v*) glycerol.

The ability of each bacterial strain to oxidize ammonium into nitrite was determined by a colorimetric method using Griess reagent and was subsequently screened. Among several strains (52 strains) identified, 16 strains were selected depending on their nitrification ability. Among them, strains with an enhanced ability to reduce NH_3 gas were re-screened using colorimetric Gastec tubes (Gastec Inc., Tokyo, Japan), where the NH_3 concentration ranged from 10 to 1000 ppm.

Screening was performed in 250 mL Erlenmeyer flasks containing tissue papers wetted with NH_3 solution. The bacterial isolates were spiked onto NH_3 tissue. Each flask was tightly sealed and left undisturbed for degradation. Degradation experiments were conducted under a closed system at ambient room temperature (22 °C) and 30 °C. NH_3 tissue was spiked with 10 mL of bacterial solution. The NH_3 gas concentration was measured at different time intervals (0, 1, 12, 24, 36, and 48 h). Finally, three bacterial strains with enhanced NH_3 reduction abilities were selected for further experiments.

The strains were chosen on the basis of their maximum effectiveness in the removal of ammonia and the influence of surfactant foam on their growth. The 16S rRNA gene of bacteria was amplified by using PCR (Bio-Rad, Hercules, CA, USA) with forward and reverse primers 27F and 1492R, respectively, and the 18S rRNA gene of yeast was amplified by using PCR (Bio-Rad, Hercules, CA, USA) with forward and reverse primers NS1 and NS8, respectively. After sequencing, all the 16S rRNA and 18S rRNA gene sequences of phylogenetically closest neighbors were identified and retrieved from the EzBioCloud server (https://www.ezbiocloud.net/identify, accessed on 1 December 2021) and NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 1 December 2021), respectively.

The three efficient NH_3-degrading strains selected from the closed system were mixed at an equal ratio (1:1:1), and NH_3 reduction was conducted again under the open system. The NH_3 tissue was spiked with a solution of the microbial consortium (10 mL). The NH_3 gas concentration in the open flask was determined at different time intervals (0, 1, 12, 24, 36, and 48 h).

2.3. Selection of an Optimal Ratio of AOS/Gelatin Mixture for Stable Foam

Different concentration ratios of AOS and gelatin were prepared to obtain an optimal concentration ratio that could create a long-lasting foam. Various concentrations of AOS solution (0.05–0.24%) were prepared, and the foam stability was tested for each concentration. Foam stability was defined in terms of foam half-life, which is the time required to disintegrate the foam up to half of its initial volume. To determine the foam stability, a fixed volume of foaming solution (100 mL) was placed in a 1000 mL measuring mass cylinder and supplied with air at a flow rate of 1000 mL/min via a capillary tube. The air supply was terminated when the foam generated inside the cylinder reached the 1000 mL mark, and the half-life of the generated foam was determined. The optimal AOS concentration was selected based on foam stability. Gelatin, as a foam-stabilizing agent, was added to

the previously optimized AOS concentration and mixed properly until all the gelatin was dissolved. This was achieved by heating and stirring it with a magnetic stirrer at 70 °C for 1 h, following which, foam stability was tested to select an optimal combination.

2.4. Characterization of Surfactant Foam

Surfactant foam was characterized in this study by determining two different properties, namely the overall surfactant foam properties and individual bubble properties. The overall foam properties, such as foam quality, foam density, foam bubble density, and foam expansion ratio, were determined by collecting a large volume of foam in the apparatus with a specific volume/area. The foam quality, which represents the amount of air present in the total volume of the foam, was determined by collecting the foam in a 5 L jar. The foam density was calculated by weighing the foam mass with respect to the volume of the jar. Furthermore, after foam breaking, the foam quality was determined by collecting the total volume of the liquid [24]. As the liquid was collected after foam breaking, the foam expansion ratio (ratio of foam volume to liquid volume) was also similarly calculated. The individual foam property was determined using a microscope (Motic BA300, Hong Kong, China), where the foam placed in a watch glass was photographed from the microscope's lens using a mobile camera. Subsequently, the number of bubbles in a particular reference area (foam bubble density), the shape of the bubbles, lamella length and thickness, and plateau angle were determined using the reference length of the graduated watch glass.

2.5. Lab-Scale Odor Reduction Test

A lab-scale experiment was conducted to reduce the NH_3 concentration based on surfactant foam spraying technology. This study was performed in a reactor (Styrofoam box with dimensions: length, 60 cm; width, 60 cm; height, 23 cm) containing 3 kg of NH_3-contaminated soil (Figure S1), which was prepared by mixing the NH_3 solution with the soil (10 mL of NH_3-solution/kg soil). Experiments were also conducted using swine manure. For each of the applied conditions (soil and manure), three different experiments were conducted: control test (only NH_3-contaminated soil/manure, no treatment), foam alone (surfactant foam without bacteria), and bio-foam (surfactant foam with bacteria). All lab-scale experiments were performed under two different conditions: one in the open reactor and the other in the closed reactor. A closed reactor was prepared by covering the reactor's lid. In addition to the soil in the reactor, experiments with swine manure in the reactor were also conducted in an open condition, simulating the conditions of real swine facilities. The surfactant foam (using optimal concentrations of AOS and gelatin) was then sprayed inside the box containing NH_3-contaminated soil/swine manure.

A round-bottom flask with three necks was used to generate foam. The reagent solution was supplied from one of the necks of the flask, another neck was used for air, and the third was used for releasing the surfactant foam. The third neck was directed onto the soil/manure stored in the reactor (15 cm above the reactor). The surfactant foam was generated by injecting air into the flask containing the foaming solution via a capillary tube tightened with a cork [25,26]. The liquid and air flow rates were maintained at 10 and 1000 mL/min, respectively. To prepare the bio-foam, the surfactant solution was mixed with the bacterial solution in 1 (bacteria): 9 (surfactant) ratio before foam generation (the concentration of surfactant and stabilizer was constantly maintained as above). The foam quality was maintained at 99% by fixing a constant liquid and air flow rate throughout the experiment.

Sampling was performed at five different sampling points in the reactor (four corners and the center) to measure the concentrations of malodorous gases. The gas concentration was measured using a Gastec pump and Gastec tubes (Gastec, Japan) as previously described [27–33]. Gases were sampled just above the foam after being foam-sprayed. Initially, three different gases, NH_3, H_2S, and mercaptans (R-SH), were measured. The concentrations of H_2S and R-SH were considerably low in the swine manure. In addition, the background concentration of gases emitted from the contaminated soil or manure was

periodically measured during the experimental period because a small fraction of gases that were initially released before foam spraying were dispersed over the experimental area, which spread from the control box during the entire experimental period.

A cost comparison analysis was performed by comparing the current experiment with the literature related to foam application for odor control. The estimated cost was compared based on the recipes used to prepare the foaming reagent for odor reduction. The cost of the preparation of a 1 L solution was used in the calculations. The practical assumptions were similar to those in our experimental design; that is, a study reactor with 60 cm length, 60 cm breadth, and 23 cm height was used. (82,800 cm^3 volume).

3. Results and Discussion

3.1. Isolation and Screening of Odor Degrading Microorganisms

Fifty-two microbial strains were isolated from pig manure using a modified culture method in Transwell plates containing R_2A, MRS, and Sabouraud agar media. Furthermore, 16 highly efficient strains with the ability to remove ammonium–nitrogen from the first screening were molecularly identified and re-screened, as shown in Table S1. To identify these strains (TP1, TP3, and TP5), the 16S rRNA genes were sequenced and analyzed. The most similar strains were identified using the NCBI database. This study selected three strains among the 16 strains for further odor reduction study: Saccharomyces cerevisiae (TP1), Lactococcus lactis (TP3), and Lactobacillus argentoratensis (TP5). These three bacterial strains were selected based on their ability to remove NH_3 and the influence of surfactant foam on their growth. The 16S rRNA gene sequence of strains TP3, TP5, and the 18S rRNA gene sequence of strain TP1 were deposited at the GenBank database under the accession number SUB10993114 TP3 OM370997, SUB10993114 TP5 OM370998 and SUB10995932 TP1 OM417178, respectively. Phylogenetic analysis based on 16S rRNA and 18S rRNA gene sequence showed high sequence similarities with Saccharomyces cerevisiae NRRL Y-12632—TP1 (99.88%), Lactococcus lactis subsp. hordniae NBRC100931T—TP3 (99.93%), and Lactobacillus argentoratensis DSM 16365T—TP5 (100%).

As shown in Table S2, the TP1, TP3, and TP5 strains showed NH_3 removal efficiencies of 45% (1000–550 ppm), 82% (1000–180 ppm), and 85% (1000–150 ppm), respectively, under the closed system (capped jar) at ambient temperature (22 °C). The reduction efficiency of NH_3 by the three strains increased at 30 °C (still capped jar), as shown in Table S3, where the removal efficiencies of TP1, TP3, and TP5 were 49% (1000 to 510 ppm), 90% (1000 to 100 ppm), and 92% (1000 to 80 ppm), respectively.

Table 1 shows the changes in NH_3 gas in the open jar when the NH_3 solution-soaked tissue was spiked with the selected microbial consortium. Three selected strains were mixed in a 1:1:1 ratio to investigate their ability in NH_3 removal from the NH_3 source in an open jar. The microbial consortium (TP1 + TP2 + TP3) significantly reduced the initial NH_3 concentration to 3.33 ppm in 1 h after spiking 10 mL of the microbial consortium. The NH_3 concentration was reduced to 320 ppm after 10 mL of water was added to the NH_3-tissue in the control. However, the NH_3 gas concentration in the open jar increased to 70 ppm during the first experiment and 226.7 ppm after 12 h. This was because ammonia gas was continuously being generated from the NH_3 solution-soaked tissue. The NH_3 gas concentration eventually decreased to 0 ppm with the microbial consortium, while it remained at 60 ppm in the control. This study found that the microbial consortium removed all NH_3 after 48 h. Therefore, this microbial consortium was used for further foam experiments.

3.2. Optimal AOS/Gelatin Concentration for Stable Foam Generation

The optimum concentrations of AOS and the stabilizer (gelatin) were determined by measuring the half-life period of the foam. As shown in Figure S2A, the foam stability (in terms of half-life) gradually increased with increasing AOS concentration. The foam half-life increased from 10 min to 32 min when the AOS concentration was increased from

0.05 to 0.20%. However, a further increase in the AOS concentration did not increase the half-life, resulting in constant foam stability.

Table 1. Degradation of NH_3 in an open jar using the microbial consortium.

Time of Deodorization (h)	Samples	NH_3 Concentration (ppm) in the Open Jar with a Consortium of TP1:TP3:TP5 (1:1:1)		
		Concentration (ppm)	Reduction (%) in Comparison to the Control	Reduction (%) in Comparison to the Initial
0	Control	1000 (±0.0) ^{A}	-	-
	Experimental	1000 (±0.0) ^{Aa}	-	-
after bacteria spraying *	Control	320 (±34.6) ^{B}		
	Experimental	3.33 (±5.77) ^{Gd}	98.96	99.6
12	Control	226.7 (±30.6) ^{C}		
	Experimental	70 (±10.0) ^{EFb}	69.12	93
24	Control	160 (±20.0) ^{D}		
	Experimental	30 (±10.0) ^{FGc}	81.25	97
36	Control	80 (± 20.0) ^{E}		
	Experimental	3.33 (±5.77) ^{Gd}	95.83	99.7
48	Control	60 (±10.0) ^{EF}		
	Experimental	0 (±0.0) ^{Gd}	100	100

* 10 mL of the bacterial suspension (2.3×10^6–3.6×10^6 cells/1 mL) for trial/10 mL of water for control; The values are mean ± standard deviation. Different superscript lowercase letters indicate statistically significant differences only among the evaluation periods in the experiment ($p < 0.05$). Different superscript capital letters indicate statistically significant differences among the evaluation periods in the control and experiment ($p < 0.05$).

The influence of gelatin concentration (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40%) on the foam half-life was also investigated in the system by adding a predetermined concentration of AOS (i.e., 0.2%). As shown in Figure S2B, the foam stability sharply increased from 37 min to 120 min when the gelatin concentration increased from 0.05 to 0.30%. A further increase in gelatin concentration up to 0.40% did significantly increase foam stability, which remained virtually constant with a half-life of only 122 min. Therefore, 0.3% was selected as the optimal concentration of gelatin. Thus, this study selected the optimal concentration of AOS and gelatin as 0.2% and 0.3%, respectively, with a half-life of 2 h.

Gelatin is an effective foam stabilizer, and during its use, the surfactant binding ratio of the foam usually becomes smaller than normal. Refs. [34,35] identified a surfactant and stabilizer with similar compositions to our experiment (0.86 g of AOS was stabilized with 1 g of protein). A further increase in the stabilizer concentration smears out or distorts the weak bonds, such as intra- and intermolecular hydrogen connections of polymers, which further loosens (uncoils) the polymer chain. The combination of AOS and gelatin does not negatively affect biocompatibility; AOS is popular because of its superior biocompatibility and physio-chemical properties, along with its excellent foaming characteristics. Furthermore, AOS is resistant to the hardness of water and other metallic ions and is stable over a wide pH range.

3.3. Characterization of Surfactant Foam

As shown in Table 2, the foamability (foaming time to fill a 1 L vessel) of AOS was 2.4 s. The foam quality of the AOS, which indicates the ratio of gas volume to foam volume, was 98.5–99%, and foam density was 0.025–0.026 g/cm^3. The foam expansion ratio, which indicates the ratio of the foam volume to liquid volume, was 110–112. The surfactant bubbles produced in this experiment had a nonagonal shape (with nine edges) (Figure 1) with a relatively large bubble size, as indicated by the bubble density (1 bubble/cm^2). The bubble density indicates the number of bubbles present in a particular area. As shown in

Figure 1, the foam lamella thickness was 0.12 mm. In addition, the average length of the lamella corners was 0.45 cm, with an average plateau angle of 131.5°.

Table 2. Characteristics of surfactant foam generated via a foam sprinkling system.

Foam Characteristics	Unit	Value
Foamability (in 1 L cylinder)	s	2.4
Foam quality (FQ)	%	98.5–99.0
Foam stability (half-life)	h	2.0–2.1
Foam density	g/cm^3	0.025–0.026
Foam bubble density	No./cm^2	1
Foam expansion ratio	Foam/liquid vol.	110–112
Foam lamella thickness	mm	0.12
Bubble shape		Nonagon
Plateau angle	Degree (°)	131.5° (Avg.)
Liquid content in the bubble		Very less

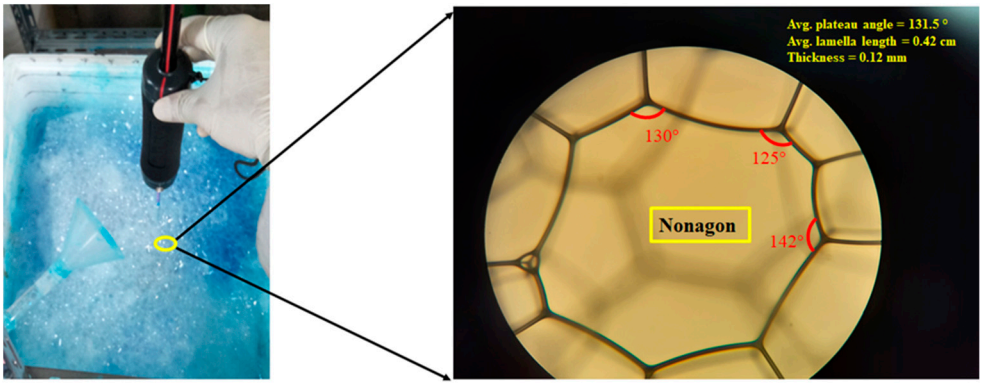

Figure 1. Characterization of a single foam bubble.

3.4. Odor Reduction Using Surface Foam-Covering

3.4.1. Open Soil Reactor System

The open soil reactor was designed to simulate manure storage in well-ventilated and open areas. As shown in Figure 2, the initial NH_3 concentration generated from the soil was 1000 ppm, which gradually reduced over time in the open soil reactor. The final residual NH_3 concentration after emission from the soil was 350 ppm ("control" in Figure 2). Spraying the surfactant foam into the reactor substantially reduced NH_3 emissions. The reduction in NH_3 concentration using surfactant foam can be classified into three stages. In stage 1, which represents the NH_3 soil covered with foam, the NH_3 concentration was reduced from 1000 ppm (initial) to approximately the background concentration in 1 h (25 ppm), which remained until 5 h. The surfactant foam lasted for approximately 5 h. The sprayed foam successfully blocked the emission of NH_3, resulting in an NH_3 reduction of 98%. In stage 2, which represents the phase after foam breaking, the emission of NH_3 restarted. The sprayed foams in the reactor gradually broke and disappeared after 5 h. After foam breaking, the NH_3 concentration at 450 ppm ("foam alone" in Figure 2) was further reduced to 325 ppm in 10 h, and was gradually reduced to 150 ppm after 72 h. This implies that the odor was emitted continuously from the soil with time; however, the addition of foam reduced the extent of emission from the soil, which lasted until the foam covered the soil. A similar result suppressing VOC emission from the source was obtained using a 40 cm-thick foam with an efficiency of 93–96% [21,22]. Here, surfactant foam acted as a good physical barrier for odor emission control. As shown in Figure 2, bio-foam (surfactant foam with bacteria) considerably reduced the NH_3 concentration even after the foam broke. Foam covering and NH_3 emission were also completely blocked in stage 1, and the NH_3 concentration was similar to the background concentration. In stage

2, after the foam broke, the NH_3 concentration increased to 375 ppm (at 5 h, "bio-foam" in Figure 2); however, this concentration was gradually reduced to 225 ppm in 10 h. The NH_3 concentration in "control" and "foam alone" at 10 h was 650 ppm and 325 ppm, respectively. The NH_3 concentration in the "bio-foam" was further reduced to 10 ppm in 72 h. These results indicate that the microorganisms in the foam infiltrated the NH_3 soil and degraded NH_3 sources.

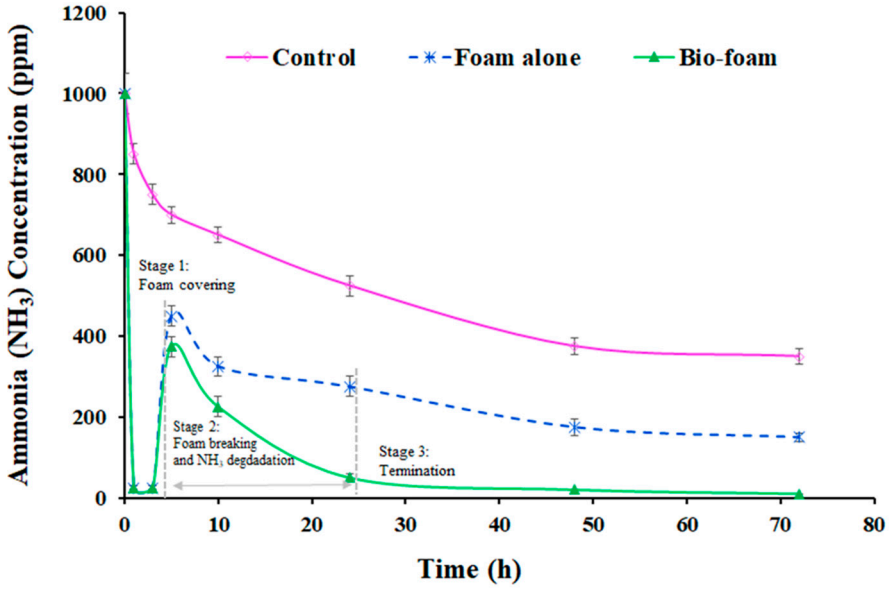

Figure 2. Change in NH_3 concentration from NH_3-contaminated soil with time in the open reactor; control (no treatment), foam alone (application of surfactant foam only), and bio-foam (surfactant foam containing bacteria).

The approximate time for the complete reduction in NH_3 from the source was about 72 h, which is low in comparison with other biological methods. Alinezhad et al. [36] required 45 days for complete NH_3 reduction. Sakuma et al. [37] achieved 100% NH_3 removal efficiency over 21 d. Huan et al. [38] achieved a 88.55% NH_3 removal efficiency after 61 days. The shorter time required during our study may be due to the addition of foam in the reactor as a bacterial transport medium. In organic-contaminated soil, the surfactant foam may have an encapsulated large surface area and can uniformly deliver the remedial agents (bacterial solution) to the NH_3 emission source [39].

3.4.2. Closed Soil Reactor System

Figure 3 shows changes in NH_3 concentration from the NH_3 soil in the closed reactor. The initial NH_3 concentration in the closed reactor remained constant at 1000 ppm for 3 h, and then decreased to 975 ppm and 900 ppm in 5 h and 10 h, respectively. The NH_3 concentration in the reactor was 625 ppm at 72 h. After spraying foam ("foam alone") in the reactor, in stage 1, the initial 1000 ppm NH_3 concentration was reduced to 30, 40, and 90 ppm in 1 h, 3 h, and 5 h, respectively, in the closed condition. In stage 2, after foam disintegrated, NH_3 concentration increased to 525 ppm in 10 h. Finally, NH_3 concentration was reduced to 325 ppm in 72 h. However, after adding bio-foam, the initial concentration was reduced to 20, 50, and 75 ppm in 1, 3, and 5 h, respectively, in stage 1 of foam application. The NH_3 concentration was increased to 350 ppm (10 h) in stage 2 after foam disintegration. The final NH_3 concentration was 30 ppm in 72 h. Thus, the bio-foam significantly reduced NH_3 concentration in the closed system.

The apparent difference in NH_3 concentration in the two different reactor systems (open and closed conditions) in all methods clearly demonstrated that the closed system prevented the release of NH_3 gas from the reactor. After the foam breakage period, the

NH_3 gas concentration gradually decreased in "control" and "foam alone" reactors up to 72 h, whereas in the "bio-foam" reactor, the concentration sharply decreased within 24–48 h, although a sudden increase in NH_3 gas was observed because of its re-emission from all of the foam sprayed reactors. The final concentrations of NH_3 gas emitted from the bio-foam reactors were similar to the background NH_3 concentration (background NH_3 near open soil reactors = 10 ppm; near closed reactors = 5 ppm). The results of this study showed that the odor-degrading, bacteria-loaded foam spraying method substantially reduced malodors from the emitting source. This implies that the sharp and pungent odor-producing ammonia can be degraded efficiently within 48 h using the bacterial-foam spraying method.

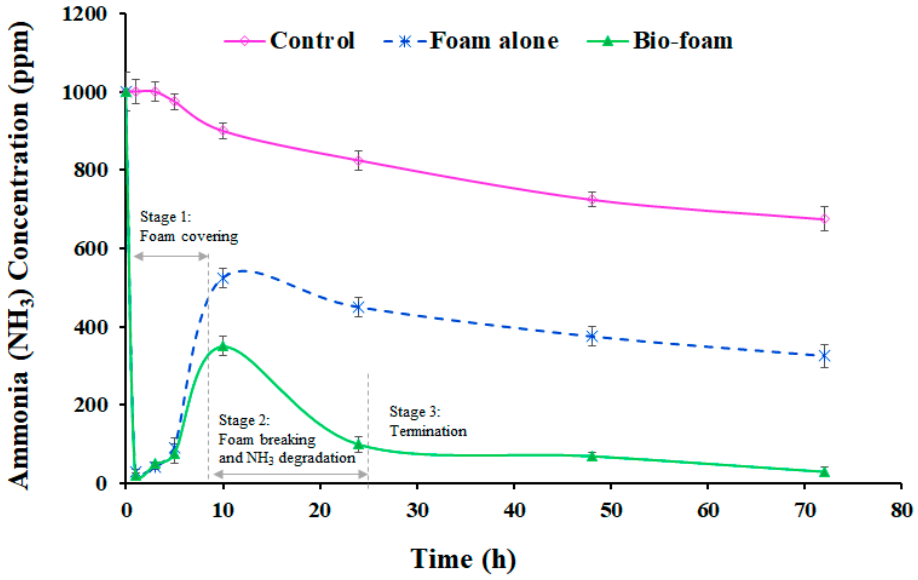

Figure 3. Change in NH3 concentration from NH_3-contaminated soil with time in the closed reactor; control (no treatment), foam alone (application of surfactant foam only), and bio-foam (surfactant foam containing bacteria).

3.4.3. Open Swine Manure Reactor

Figure 4 shows the changes in NH_3 concentration from the swine manure in the open reactor. Similar trends were observed for swine manure as those for artificially contaminated soil in all three types of treatments. In the first foam covering stage, NH_3 gas emission was completely prevented by surface foam in both "foam alone" and "bio-foam" treatments until foam was stable for approximately 6 h. The NH_3 gas concentration in the foam covering reactor was similar to its background concentration (10 ppm). NH_3 gas emission from swine manure in the control reactor (the initial NH_3 concentration; 450 ppm) remained constant until 2 h, which gradually decreased to 375 and 350 ppm at 5 and 6 h, respectively, and reached 275, 250, and 225 ppm (50% reduction) after 24, 48, and 72 h, respectively.

NH_3 gas was re-emitted in "foam alone" and "bio-foam" reactors after all foams disappeared in 6 h. The NH_3 gas concentration was 150 and 100 ppm at 6 h in "foam alone" and "bio-foam" reactors, respectively. However, the "bio-foam-treated" manure significantly reduced NH_3 emission after 12 h, which further reduced to 10 and 0 ppm after 24 and 48 h, respectively. This result indicated that microbial degradation was active even after foam breaking (after 12 h). However, the "foam-alone-treated" manure after foam breakage presented an NH_3 gas concentration of 125 ppm in 12 h, which remained at a relatively stable NH_3 concentration of 100 ppm up to 72 h. This apparent difference in NH_3 gas reduction from "foam alone" and "bio-foam" treatments was due to the effective bacterial degradation in the bacterial-foam spraying method.

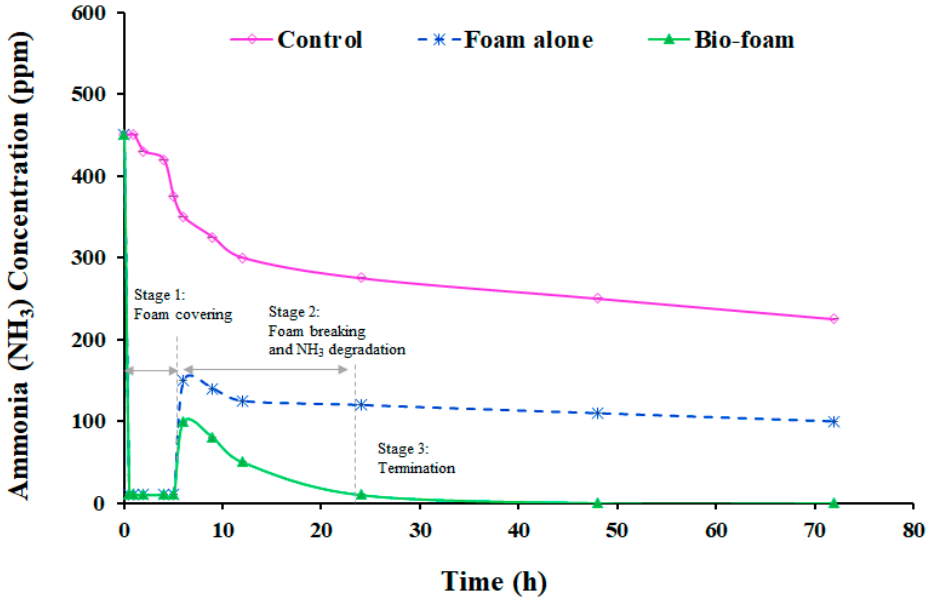

Figure 4. Change in NH_3 concentration emitted from swine manure in the open reactor: control (no treatment, only manure), foam alone (application of surfactant foam only), and bio foam (surfactant foam containing bacteria). The initial NH_3 concentration in the manure was 450 ppm.

As shown in Figures 2–4, bacteria require time to degrade odor sources because bacterial degradation is a slow process. In a case where the bacterial solution was applied individually to the swine manure, the initial NH_3 concentration would be high until bacteria became active. However, the sprayed foam reduced the NH_3 concentration to the background level during the initial period. Thus, there exists a synergetic effect between surfactant foam and odor-degrading bacteria for the complete reduction in malodorous gas emission from swine manure by individual contribution. Further studies should be conducted to completely suppress the malodors from the swine manure by applying the surfactant foam twice or thrice just before its complete breakage, which would prevent the release of odors after foam breaking.

The final residual NH_3 concentration in the reactor ranged from 0 to 30 ppm, thereby achieving a 97–100% removal efficiency within 72 h. The malodor suppression efficiency using surfactant foam and bacteria was the highest when compared with that obtained with other physical and biological methods for odor removal from manure. Chen et al. [40] used biochar as an odor suppression agent and achieved a NH_3 removal efficiency of 53% over 30 days with the repeated re-application of biochar for odor suppression. Dougherty et al. [41] achieved a maximum NH_3 removal efficiency of 80% after using the manure for approximately two months. Although other methods were found in the literature, they were either time-consuming or costly.

The cost for preparing stable foam was compared between our experimental conditions and other existing experiments in the literature. The comparison was made by assuming that the same amount of foaming reagent was required (1 L) to fill the foam completely in a 60 cm × 60 cm × 23 cm reactor. Our cost to prepare 1 L of reagent (AOS and gelatin) was approximately USD 5.7 (6788 Korean Won; KW), while the estimated cost of reagents suggested by the references was approximately USD 16.4 (KW 19,728) and USD 343.3 (KW 411,938). Our finding for preparing stable foam with AOS and gelatin may practically be feasible for field extension in terms of reagent cost.

4. Conclusions

In this study, 52 microbial strains (sequenced 16S rRNA genes) were isolated from pig manure and screened to identify NH_3-degrading microbes. Three strains were selected for odor reduction: *Saccharomyces cerevisiae* NRRL Y-12632^T (99.88%; TP1), *Lactococcus lactis*

subsp. *hordniae* NBRC 100931^T (99.93%; TP3), and *Lactiplantibacillus argentoratensis* DSM 16365^T (100%; TP5). This study found that all NH_3 was removed by the microbial consortium (1:1:1) after 48 h, while the control contained 60 ppm. This study also determined the surfactant and foam stabilizer concentrations to obtain long-lasting foam, where the optimal concentrations of AOS and gelatin were 0.2% and 0.3%, respectively, resulting in a foam half-life of 2 h.

In this study, surface foam created a physical canopy that trapped the released odor gases from the emission source. Surfactant foam cover successfully reduced the NH_3 concentration in the environment by 97–100% (100% for open reactor and 97% for closed reactor). However, as the foam broke after 6 h, NH_3 was re-emitted from the NH_3 soil and swine manure.

The bio-foam (NH_3 degrading bacteria-loaded foam) applied to the same NH_3 emission source also physically prevented the release of NH_3, and NH_3 was gradually re-emitted after foam breaking until 25 h. However, after 25 h, the degradation of NH_3 by bacteria (biodegradation) was dominant, and NH_3 degradation was completed after 72 h in NH_3 soil. In the real swine manure, the "bio-foam"-treated swine manure significantly reduced NH_3 emission even after foam breaking, which reduced to 10 and 0 ppm after 24 and 48 h, respectively.

Surface foam spraying completely prevented the emission of malodorous gases, and a significant decrease in gas concentration (90–100% efficiency) was achieved after foam breaking, within two days. In future, the results of this laboratory study can help in the possible field extension of this research.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app12041806/s1, Text S1: Measurement of soil characteristics. Text S2: Reagent and materials. Table S1: Degradation of NH_3 by different microbial strains. Table S2: Degradation of NH_3 in the capped jar (conducted at 22 °C). Table S3: Degradation of NH_3 in the capped jar (conducted at 30 °C). Figure S1: Laboratory odor reduction test; (A) experimental set-up using soil/swine manure in the polystyrene box; (B) initial sampling before foam spraying; (C) initial NH_3 concentration (pink color changed to yellow); (D) covering the manure with surfactant foam spraying technology; (E) sampling after foam spraying; and (F) NH_3 concentration after foam spraying or final sampling (no change in pink color, 0 ppm). Figure S2: Effect of (A) AOS concentration and (B) AOS + gelatin concentration on foam stability (half-life).

Author Contributions: Conceptualization, J.K. and S.-W.J.; methodology, R.B., P.G., T.T.N.L. and R.H.D.; validation, R.B., P.G. and S.-W.J.; resources, S.-W.J.; writing—original draft preparation, R.B., P.G., T.T.N.L. and R.H.D.; writing—review and editing, J.K. and S.-W.J.; visualization, R.B. and P.G.; supervision, J.K. and S.-W.J.; project administration, J.K. and S.-W.J.; funding acquisition, S.-W.J. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Korea Smart Farm R&D Foundation, funded by the Korean Ministry of Agriculture, Food and Rural Affairs, the Korean Ministry of Science and ICT, and the Korean Rural Development Administration (Grant number: 421010-03).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interests.

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