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Reducing Methane, Carbon Dioxide, and Ammonia Emissions from Stored Pig Slurry Using Bacillus-Biological Additives and Aeration

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Abstract: This study delves into the innovative application of a novel bacterial and enzyme mixture alone or combined with aeration in mitigating emissions from pig slurry storage and explores their impacts on the methane (CH_4), carbon dioxide (CO_2), and ammonia (NH_3) emissions from stored pig slurry. A dynamic chamber was used in this research to assess the efficacy of the treatments. Biological additives (HIPO-PURÍN) of specific microbial strains were tested (a mixture ofof *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis*, *Bacillus amyloliquefacien*, *and Bacillus thuringiensis*) alone and combined with an aeration system (OXI-FUCH). Controlled experiments simulated storage conditions, where emissions of ammonia, methane, and carbon dioxide were measured. By analyzing the results statistically, the treatment with HIPO-PURÍN demonstrated a significant reduction in CH_4 emissions by 67% and CO_2 emissions by 60% with the use of biological additives, which was increased to 99% and 87%, respectively, when combined with OXI-FUCH aeration, compared to untreated slurry. Ammonia emissions were substantially reduced by 90% with biological additives alone and by 76% when combined with aeration. The study was driven by the need to develop sustainable solutions for livestock waste management, particularly in reducing emissions from pig slurry. It introduces techniques that significantly lower greenhouse gases, aligning with circular economy goals and setting a new standard for sustainable agriculture. Furthermore, there is a need to validate that farmers can independently manage pig slurry using simple and effective treatments techniques with profound environmental benefits, encouraging broader adoption of climate-conscious practices.

Keywords: ammonia emissions; pig slurry; aeration; biological additives; dynamic chamber; aerobic treatment; bacillus bacteria; greenhouse gas emissions

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1. Introduction

Intensive pig farming presents a major environmental challenge in numerous countries, including Spain. The substantial presence of meat production farms in the country requires an effective management of pig slurry [1]. The Region of Murcia ranks in fourth position in Spain in terms of pig population according to the 2023 census (2,485,375 pigs) [2], only surpassed by Aragon, Cataluña, and Castilla Leon; therefore, there is a considerable need for proper waste management. This high number of pigs results in an annual production of approximately 94.7608 Hm^3 of slurries, as reported by the Ministry of Agriculture, Food, and Environment 2023.

Pig slurry, a by-product of pig farming, consists of a complex mixture of water, nutrients (including nitrogen, phosphorus, and potassium), organic matter, and microorganisms, along with some traces of heavy metals [3]. The variability in pig slurry composition across farms is significant, influenced by factors such as housing systems, diet, climate, and farm management practices [4,5]. This pig slurry is recognized for its fertilizing properties, yet it

presents considerable environmental concerns. This slurry serves as a valuable fertilizer but also poses significant environmental challenges [6].

According to the USDA Foreign Agricultural service 2023/2024 [7], the major pig-producing countries are China at 57.94 million metric tons (50% of global production), the European Union at 20.8 million metric tons (18%), and the United States at 12.39 million metric tons (11%), which generate millions of tons of pig slurry annually. Inadequate management of pig slurry can result in environmental issues such as nutrient runoff, superficial and subterranean water pollution, GHG and ammonia emissions, and soil degradation [8,9]. These challenges underscore the need for adept slurry management and creative strategies to ensure that agricultural use of pig slurry aligns with ecological preservation and public health protection.

Studies have identified three main waste streams that could be used to produce organic fertilizers: manure, sewage sludge, and food processing waste [10]. Among these three options, livestock manure represents the most significant waste stream, and pig manure is particularly associated with the highest environmental concerns regarding its safe disposal and management [11]. In Europe, the majority of produced manure is used as a fertilizer in agricultural fields without any treatment, either through spreading or grazing. The direct application to agricultural soil is among the most cost-effective choices for managing pig slurry; however, the feasibility of this alternative relies on having sufficient available agricultural surfaces and implementing sustainable application practices.

This practice of direct application of pig slurry to agricultural soil poses potential environmental risks, including nutrient imbalances, an overabundance of nutrients, and the presence of contaminants like metals [8,12]. Improper handling and application can lead to nutrient runoff, with the transfer of nitrogen from soil to surface water, often coupled with phosphorus transport, with this being the principal contributor to eutrophication [3]. Moreover, groundwater contamination is a significant concern, posing risks to drinking water sources.

Additionally, pig slurry releases greenhouse gases like methane and ammonia, contributing to climate change and air quality problems. Ammonia, nitrogen dioxide, and methane (NH_3, N_2O, and CH_4) are potent greenhouse gases [13]. These gases are primarily released during slurry storage and the composting of its solid fraction. Among them, ammonia plays a major role in acidification and eutrophication, while methane and nitrogen dioxide significantly impact climate change [3]. Unpleasant odors resulting from improper storage and spreading can affect both air quality and community well-being. The sector often grapples with neighborhood conflicts and olfactory nuisances, as documented in previous research [14]; therefore, balancing the benefits of pig slurry as a fertilizer with responsible management practices is essential to avoid negative consequences for soil quality, pathogen spread, and overall environmental health.

In recent decades, the negative environmental consequences of pig production and manure management have been controlled through stricter regulations on storage and spreading, the European Union has specific regulations regarding pig manure management through the Nitrate Directive 1991/676/EEC) [15] and the Water Framework Directive (2000/60/EC) [16]. Guidelines and regulations for mitigating the effects of livestock manure generally recommend applying nutrient limits that align closely with the actual needs of crops, with a primary focus on nitrogen.

As the intensive livestock sector faces increasing pressure to minimize the environmental impact of its operations, several methods have been evaluated as the best available technologies on the market for manure treatment, while improving the use of nutritional resources [17]. Numerous studies have been carried out to investigate the treatment of pig slurry, employing physical, chemical, and biological processes to address its environmental impact and optimize its management, such as precipitation, which involves adding chemicals to the slurry to precipitate nutrients, primarily phosphorus, into solid form. This technic is very effective in reducing phosphorus concentration, while it can be costly due to the chemical inputs, and furthermore generates additional solid waste that requires

management [18,19]. Coagulation flocculation use coagulants and flocculants to aggregate suspended particles into larger clusters that can be easily removed, and this technique is efficient at removing suspended solids and some dissolved nutrients, and enhances subsequent treatment processes. The main limitations include the use of chemical additives, which can increase costs and the environmental footprint [20]. Adsorption utilizes materials like activated carbon or biochar to adsorb contaminants from the slurry. It is effective at removing a wide range of organic and inorganic pollutants and can improve effluent quality. However, adsorbent materials can be expensive and require periodic regeneration or replacement [21]. Acidification reduces pH levels in the slurry, inhibiting microbial activity and reducing ammonia emissions. This method is advantageous in reducing odor and ammonia volatilization and can improve nutrient availability for crops. The drawbacks include the need for acid inputs and the potential for soil acidification if not managed properly [22,23]. Solid–liquid separation consists in separating the solid and liquid fractions of the slurry, often using mechanical separators. This process reduces the volume of waste needing treatment, and the solids can be composted or used as a soil amendment. However, the liquid fraction still requires further treatment [24,25]. Aerobic biological nitrogen removal uses aerobic bacteria to convert ammonia into nitrogen gas through nitrification and denitrification. It is effective for nitrogen removal and reduces odor and pathogen levels. The main limitations are the energy-intensive nature due to aeration requirements and the need for careful control of environmental conditions [26]. Anaerobic digestion involves microorganisms breaking down organic matter in the absence of oxygen, producing biogas (methane) and digestate. This method produces renewable energy (biogas), reduces pathogen levels, and stabilizes organic matter. However, it requires a high initial investment for biogas plants and necessitates the management of biogas and digestate [27,28]. Selecting an appropriate treatment method relies on several factors, including the installation and maintenance expenses, desired treatment level, and the organic matter and nutrient removal [29].

Currently, there is no definitive solution for pig slurry treatment, and treatment plants employ diverse techniques based on their preferences. Many facilities adopt a combination of the popular methods previously mentioned. However, these procedures are neither technologically practical nor economically feasible. As a result, owners of pig farming facilities must pay waste management companies for handling their manure, and larger facilities must invest in their own treatment equipment to comply with existing regulations [30].

Recently, innovated biological additives have emerged on the market, introducing additional capabilities, including the enhancement of nitrogen removal through denitrification by introducing different bacteria, which have promise in mitigating the nitrogen excess in intensive livestock areas [31]. These additives are intended to modify certain properties of the slurry by either suppressing or enhancing specific microbiological processes [32]. The benefit of this approach lies in its accessibility and cost-effectiveness. Farmers can easily implement it by following the provided guidelines and instructions.

The goal of this research was to evaluate the effect of commercially available biological additives, which are expected to improve denitrification, when applied to slurry from a fattening pig farm [31]. The application of these biological additives, particularly HIPO-PURÍN bacteria formula, introduces an innovative methodology and an original bacteria formula in this research area, highlighting innovative contributions to scientific comprehension. Additionally, this research seeks to assist in decision-making, regarding whether to use the biological additives alone or in combination with the aeration system known as OXI-FUCH, which is a newly designed aeration technology that can be installed in pig slurry storage to compare the effectiveness of the bacteria when used on the same type of slurry under consistent conditions.

The combination of HIPO-PURÍN bacteria with the aeration system OXI-FUCH is intended to reduce greenhouse gases emissions into the atmosphere. The study had two primary objectives: the initial goal was to verify the efficacy of employing a dynamic chamber in the measurement of gaseous emissions originating from slurry storage, specifically NH_3, CH_4, and CO_2, and to show the efficacy and precision of utilizing a dynamic chamber as a measurement method. By validating this methodology, the researchers aimed to determine its reliability for future emission measurements. The second objective was to analyze the gaseous emissions (NH_3, CH_4, and CO_2) throughout a slurry storage period. The researchers sought to establish its reliability for future emission measurements, in three distinct modes: in raw pig slurry, incorporating a biological additive with aeration, and without aeration, pursuing the goal of analyzing the changes in gaseous emissions throughout the storage duration. This valuable information will enhance our understanding of the factors affecting emission variations and will aid in determining the optimal periods and durations for estimating gaseous emission factors in pig manure storage, during treatment with biological additives alone or combined with an aeration system.

2. Materials and Methods

2.1. Experimental Design

The experiment was conducted at a white pig farm in Fuente Alamo, a municipality in the Murcia region of southeastern Spain. It was conducted from 18 April to 22 June 2023. The pilot study aimed to replicate the conditions of swine manure storage tanks. A portable pool with a steel and PVC structure, purchased from Bestway®, España, Spain (ref. 84265026), made in China, with circular dimensions of Ø 457 × 122 cm was used Figure 1. The slurry was divided proportionately among the experimental pools, resulting in each pool being filled up with 13 m^3 of slurry at a depth of 1 m. The chosen volume was determined to meet the requirements of the VERA protocol [33]. To implement the study on a real-world scale, we had three pools, two were treated with the same quantity of the biological additives (HIPO-PURIN), one pool with OXI-FUCH aeration techniques and the other one without, while one pool remained untreated as a control. The slurry composition and gaseous emissions were continuously tracked throughout the experiment.

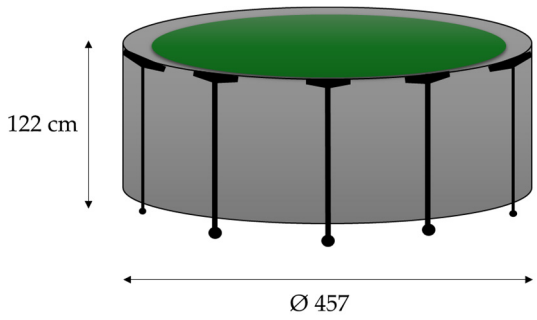

Figure 1. Bestway® Portable Pool (Ref. 84265026).

The experiment had three distinct phases. In the initial stage, no additives were introduced. During this phase, pig slurry was sampled with 3 replicates, and CO_2, CH_4, and NH_3 was measured at the same time as the replicates, in order to have initial values before starting the treatments. Subsequently, the biological additive was introduced in the other pools after the initial sampling and measurements. The concluding phase centered on greenhouse gas flux measurements, and pig slurry sampling was executed weekly. This entailed positioning the floating dynamic chamber over the pools and collecting the gas samples at T_0 = 0 min and T_{30} = 30 min post-chamber deployment, adhering to the guidelines outlined in the VERA protocol [33].

2.2. Biological Additive

The biological additive used in this experiment was obtained from the Sewervac company, Spain. Marketed under the commercial name HIPO-PURÍN, the product comprises different microorganisms, including *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis*, *Bacillus amyloliquefacien*, and *Bacillus thuringiensis*, which produce a range of enzymes such as proteases, cellulases, and lipases, the formula of HIPO-PURÍN also contains yucca extract.

Bacillus subtilis secretes proteases such as subtilisin and alkaline protease, which break down proteins into peptides and amino acids, it also produces cellulases and lipases, facilitating the hydrolysis of cellulose and lipids into simpler compounds [34]. *Bacillus megaterium* produces neutral and alkaline proteases, cellulases for cellulose degradation, and lipases for lipid hydrolysis [35]. *Bacillus licheniformis* is recognized for its alkaline protease and subtilisin activities, along with cellulases and lipases [36]. *Bacillus amyloliquefaciens* produces subtilisin and neutral proteases, cellulases, and lipases, which enable the breakdown of proteins, cellulose, and lipids [37]. *Bacillus thuringiensis*, in addition to its insecticidal properties, produces serine proteases, cellulases, and lipases that contribute to protein degradation, cellulose hydrolysis, and lipid breakdown [38].

The enzymes produced by these bacterial strains include cellulases (CMCase, avicelase, β-glucosidase, xylanase), lipases (Lip A, Lip B), amylases (α-amylase, β-amylase), and proteases (serine protease, threonine protease, cysteine protease, aspartic protease, metalloprotease). Some of the strains are facultative, allowing them to function in conditions where oxygen is limited. They can grow faster than anaerobic strains, thus avoiding the formation of sulfides and using ammonia for growth, which helps retain more nitrogen in manure and prevents its release into the atmosphere. Additionally, they stabilize pH, preventing it from rising to levels where ammonia becomes more volatile and can be released into the atmosphere.

In this study, the dosage was outlined as follows: for rehydration, a ratio of 1 kg per 100 L was recommended. The initial dose involved diluting 400 g of HIPO-PURÍN in 40 L of water, while the weekly dose entailed diluting 133.60 g of HIPO-PURÍN in 13 L of water. The cumulative dosage over a 6-week period was calculated as 2.00 kg, obtained by adding the initial and weekly doses.

For dose preparation, the product was deposited into a clean container, filled halfway with warm clean water (approximately 30 °C), stirred thoroughly, and allowed to rest for a minimum of 30 min before incorporating it to the slurry. The rehydration ratio recommended was 1 part product to at least 100 parts of water. The product is provided in bulk powder form and packaged in plastic buckets.

In the application of HIPO-PURÍN in this study, the initial dose was administered just before filling the slurry pool, in accordance with the predetermined dosage set by Sewervac technical department. The weekly or maintenance dose was applied around the study pool with a manual stirring, in order to incorporate the solution with the treated slurry. The application of the Sewervac product HIPO-PURÍN, adhered to the manufacturer's instructions and was uniformly distributed over the pig manure.

The objective of the biological additives was to allow an appropriate and easy slurry management by promoting liquefaction, achieving an effective method for controlling and reducing total nitrogen, greenhouse gases (GHG), and ammonia.

2.3. OXI-FUCH Aeration System

This research article delves into the optimization of wastewater treatment processes through the utilization of innovative technologies, biological additive alone or combined with aeration system. Focusing on the material characteristics employed, the study explored the implementation of an investment compressor and aeration modules, specifically employing microperforated upper pipes (OXI-FUCH) and weighted feeder pipes (Figure 1). The operational dynamics of the compressor, with 20 continuous hours of activity followed by 5 continuous hours of downtime, were meticulously examined. In this real-world ex-

periment, the installation of the aeration system was under the purview of the OXI-FUCH technical department.

In this study, we explored the potential of enhancing HIPO-PURÍN with an aeration system composed of linear modules of pipes, known as OXI-FUCH modules Figure 2. These modules consist of two interconnected pipes: an upper micro-perforated pipe facilitating aeration by generating two columns of microbubbles, and a lower pipe filled with sand to serve as ballast for deposition at the reservoir bottom. The microbubbles thus produced traverse the slurry from bottom to surface at a slow and uniform pace, transferring oxygen through friction. This oxygen acts as an energy source for sustaining and promoting the growth of bacteria present in HIPO-PURÍN. Our methodology involved transforming the storage tank into a miniature treatment plant by implementing these OXI-FUCH modules. The design of these modules is crucial, as it prevents the merging of air flows, thereby preventing the formation of large bubbles. This design feature promotes the efficient transfer of oxygen, with microbubbles facilitating up to 6.6 times more oxygen transfer compared to large bubbles, due to their increased contact surface area with the water. The operation of the compressor in this study was structured with a continuous operation time of 20 h, followed by a continuous off-time of 5 h.

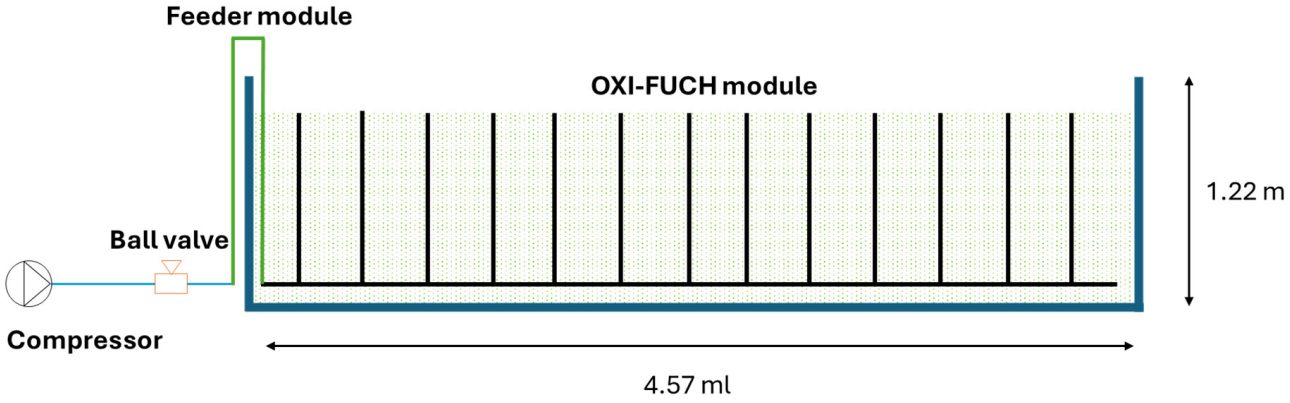

Figure 2. OXI-FUCH aeration system.

2.4. Dynamic Chamber

This research focused on the direct measurement of greenhouse gas emissions from pig slurry, utilizing a dynamic chamber as a primary methodology. The dynamic chamber, constructed from PVC as chosen in this study, was sealed to eliminate any gas exchange with the external environment. The PVC material was chosen because it is considered a non-adhesive material for the gases under investigation [33].

Complying with the VERA protocol recommendations, a specified area (0.564 m^2) of the pig slurry surface was covered, ensuring adherence to sampling standards. The chamber design included an inlet for ambient air entry and an outlet for the release of chamber air, promoting continuous gas exchange. Airflow was precisely regulated using a combination of a controlled, adjustable pump at the inlet and a suction device at the outlet. Additionally, an anemometer set to a speed of 0.2 m/s was used to monitor the flow at a speed of 0.2 m s^{-1}, as per VERA protocol guidelines [33], to monitor the airflow within the chamber. Gas sampling ports strategically positioned within the chamber facilitated representative air sampling, and the collected samples were analyzed using GASERA ONE for gas concentration measurements. The study adhered to a minimum sampling duration of 30 min per point, as recommended by the VERA protocol, considering the emission source and measurement objectives. Gaseous emissions were quantified as F (flux measured with the dynamic chamber) in kg (gas) ha^{-1} h^{-1}, calculated using the equation provided [33].

$$F = \frac{C_{out} - C_{in}}{Ab} Ai\ Vi$$

C_{out} and C_{in} represent the time-averaged gaseous concentrations of the gas (measured in kg m^{-3}) in the outlet and inlet air, respectively. The variables Ai, Vi, and Ab denote the cross-sectional area of the inlet (in m^2), the measured wind speed at the tunnel inlet (in m s^{-1}), and the source surface area covered by the tunnel canopy (in m^2).

Besides these parameters, environmental conditions such as temperature, humidity, and pressure within the chamber were routinely monitored. This thorough monitoring ensured the accurate documentation and incorporation of measurement conditions into the emission calculations.

By accurately quantifying emissions, the dynamic chamber played a pivotal role in comprehending the environmental effects of pig farming, especially pig slurry management. This understanding, in turn, can support the development of effective mitigation strategies to reduce greenhouse gas and ammonia emissions in the agricultural sector.

2.5. Gases Sampling Frequency

The aim of this study was to assess the seasonal variability in gas emissions during the storage of slurry with or without treatment. To achieve this, multiple measurements were taken, each with a duration of approximately four weeks and following a frequency of twice per week, as proposed by VERA PROTOCOL [33], conducted under varying ambient temperature conditions.

Specifically, the study focused on the measurement period of spring 2023, from 18 April to 22 June. Within each measurement period, 30 min average emission rates for each gas were computed based on air flow rates and gas concentrations obtained at 10 min intervals. Furthermore, detailed records were kept for several parameters, including manure management practices, slurry characteristics, and environmental conditions like slurry and ambient temperatures throughout the entire measurement period.

2.6. Liquid Pig Slurry Sampling and Analysis

Samples of the slurry were collected simultaneously for measurement of each of the studied gases (CH_4 and CO_2) and ammonia (NH_3). From three distinct points within the storage tank, slurry samples were collected from the surface to a depth of 20 cm, ensuring homogeneity through stirring before sampling. Subsequently, these samples were stored and transported at 4 °C to the laboratory for further examination.

All parameters were analyzed at the laboratory, except the pH and electrical conductivity (EC) that were measured in situ using HANNA multiparameter equipment with reference HI98194. The determination of Kjeldahl nitrogen (KN) content employed a modified Kjeldahl method, involving the digestion of 1 mL of pig slurry. Ammonium nitrogen (NH_4^+–N) was determined through steam distillation followed by titration with HCl 0.1 N, while total nitrogen (TN) encompassed both organic and inorganic forms, including Kjeldahl nitrogen, nitrite, and nitrates. Total phosphorus (TP) determination involved acidic hydrolysis and oxidation at 120 °C, followed by photometric analysis. Potassium (K^+) levels were measured using an atomic absorption spectrometer. Total suspended solids (TSSs) were assessed by filtering the sample through a pre-weighed standard glass-fiber filter, and the residue retained on the filter was dried and weighed using the 2440-D method (APHA–AWWA–WEF, 2012). Biochemical oxygen demand over five days (BOD_5) was determined using OXITOP WTW equipment and measured with a manometer (Darmstadt, Germany), while chemical oxygen demand (COD) was determined using photometric analysis of the chromium (III) concentration after 2 h of oxidation with potassium dichromate/sulfuric acid and silver sulfate at 148 °C (Macherey–Nagel GmbH & Co., KG, Nanocolor Test; ref. 985 028/29, Weilheim, Germany) according to German standard methods DIN 38 409-H41-1 and DIN ISO 15 705-H45 [39].

2.7. Statistical Analysis

The data were subjected to statistical analysis using one-way ANOVA, followed by Tukey's HSD post hoc test for pairwise comparisons of means (with a significance level set at $p < 0.05$). This analysis was performed using SPSS 24.0 software, to detect significant differences in greenhouse gas emissions and ammonia levels. Additionally, the parameters of pig slurry under both treatments, biological additives alone and in combination with aeration, were evaluated to draw conclusions for the study.

The statistical analysis was conducted using triple replicate sampling for both pig slurry characterization and gas measurements. The results from these replicates were averaged, and statistical analysis was applied to the mean values obtained from these triplicate experiments.

3. Results

3.1. Greenhouse Gas Emissions and Ammonia

Figures 3–5 provide a detailed overview of the carbon dioxide (CO_2), methane (CH_4), and ammonia (NH_3) emissions from both untreated and treated pig slurry samples over a period of six weeks. Notably, the treatment was applied immediately after the initial measurements, allowing for a thorough assessment of the additive's effects. These figures are instrumental in illustrating the emission patterns of the studied gases throughout the timeframe, enhancing our understanding of how the treatments impacted the slurry composition.

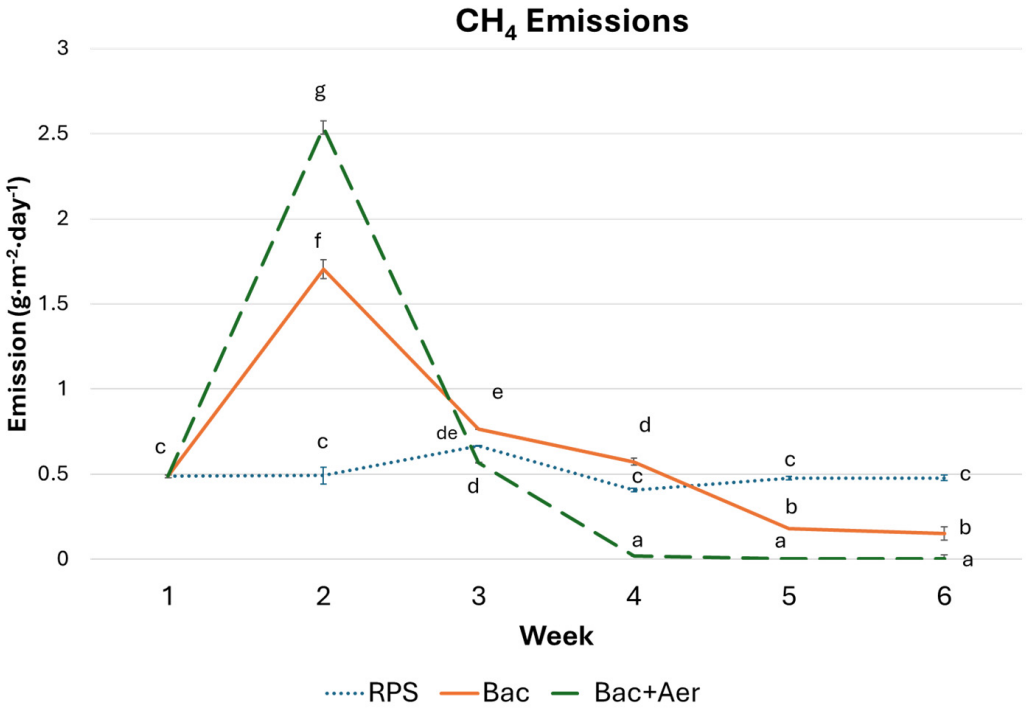

Figure 3. Methane (CH_4) progression of untreated and treated pig slurry (RPS: raw pig slurry, Bac: treated pig slurry with bacteria, Bac + Aer: treated pig slurry with bacteria and aeration). Different letters indicate significant differences between the sampling times and treatments ($p < 0.05$).

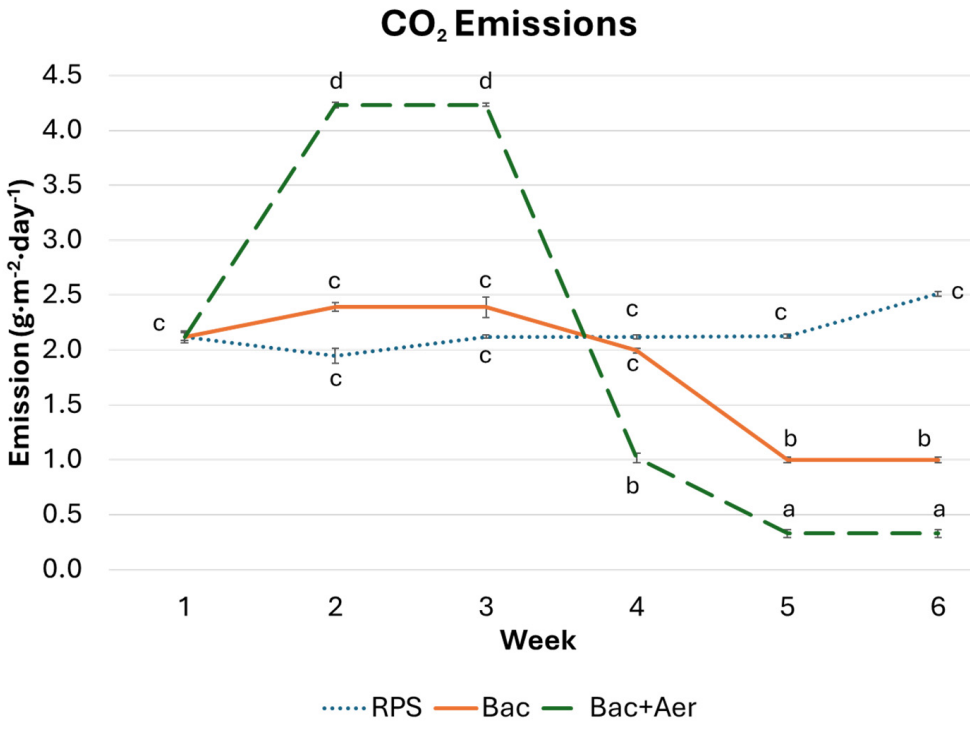

Figure 4. Carbon dioxide (CO_2) progression of untreated and treated pig slurry (RPS: raw pig slurry, Bac: treated pig slurry with bacteria, Bac + Aer: treated pig slurry with bacteria and aeration). Different letters indicate significant differences between the sampling times and treatments ($p < 0.05$).

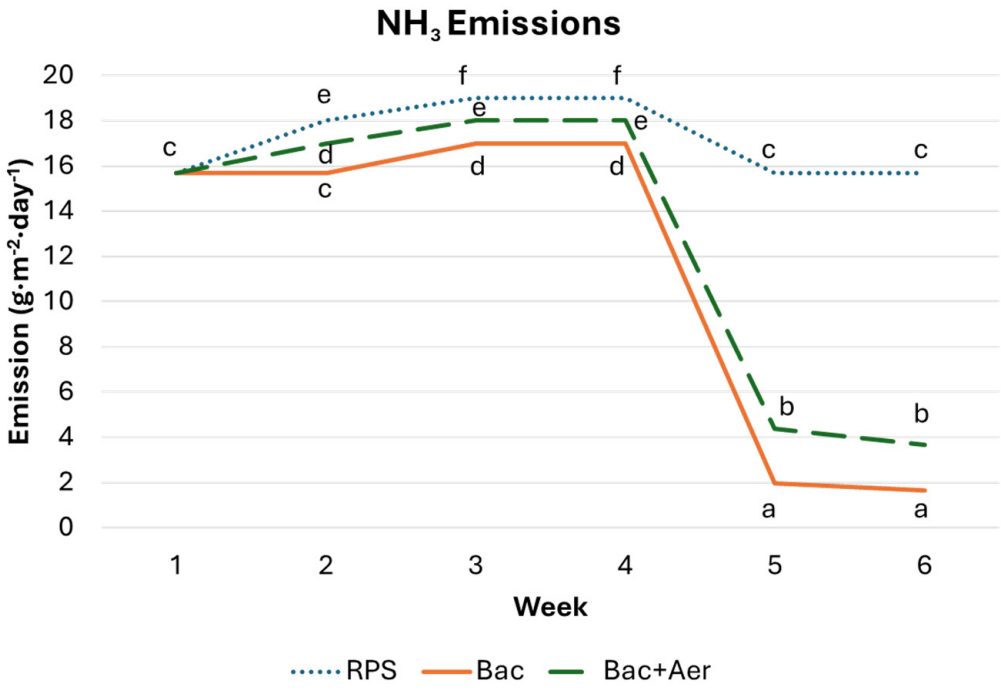

Figure 5. Ammonia (NH_3) progression of untreated and treated pig slurry.(RPS: raw pig slurry, Bac: treated pig slurry with bacteria, Bac + Aer: treated pig slurry with bacteria and aeration). Different letters indicate significant differences between the sampling times and treatments ($p < 0.05$).

3.2. Untreated and Treated Pig Slurry Analysis

Tables 1–3 present the physicochemical characteristics, macro-nutrients, and micronutrients of the pig slurry, respectively. This study investigated each parameter in both

untreated and treated pig slurry, facilitating a thorough examination of the treatment evolution and reactions during the storage of slurry. The treatment was applied subsequent to the initial slurry sampling during the first week. Significant differences ($p < 0.05$) in the concentrations of various parameters such as DBO_5, COD, total nitrogen (TN), total suspended solids (TSSs), and phosphorus were observed between the untreated pig slurry (control) and the treated slurry with bacteria alone or coupled with aeration.

Table 1. Means and standard deviation values of the physicochemical parameters' progression in untreated and treated pig slurry.

Weeks		1	2	3	4	5	6
Tª (°C)	RPS	18.05 ± 0.49 a	17.92 ± 0.59 a	21.61 ± 0.15 c	19.94 ± 0.01 b	23.1 ± 0.14 d	22.96 ± 0.06 d
	BAC	17.36 ± 0.45 a	18.12 ± 0.04 b	23.79 ± 0.07 e	18.71 ± 0.02 c	22.06 ± 0.22 d	23.42 ± 0.8 e
	BAC + AER	18.79 ± 0.11 b	17.54 ± 0.13 a	23.53 ± 0.04 d	19.89 ± 0.01 c	24.06 ± 0.04 e	24.91 ± 0.51 e
pH	RPS	7.32 ± 0.01 a	7.68 ± 0.02 b	7.71 ± 0.02 b	7.71 ± 0.02 b	7.33 ± 0.01 a	7.35 ± 0.03 a
	BAC	7.32 ± 0.03 a	7.75 ± 0.06 b	7.73 ± 0.01 b	8.00 ± 0.02 d	7.96 ± 0.04 c	7.97 ± 0.08 c
	BAC + AER	7.32 ± 0.01 a	7.3 ± 0.01 a	7.69 ± 0.03 b	8.3 ± 0.2 d	8.26 ± 0.01 d	8.46 ± 0.01 d
EC ($dS\ m^{-1}$)	RPS	22.09 ± 0.02 a	24.81 ± 0.12 c	25.74 ± 0.23 d	26.78 ± 0.01 e	23.55 ± 0.06 b	24.04 ± 0.09 b
	BAC	22.45 ± 0.56 bc	23.87 ± 0.02 c	24.56 ± 0.32 d	24.98 ± 0.1 d	19.38 ± 1.12 a	21.76 ± 1.15 b
	BAC + AER	23.54 ± 0.26 b	22.26 ± 0.67 b	22.38 ± 0.4 b	22.47 ± 0.02 b	16.16 ± 2.25 a	20.45 ± 1.63 a
TSSs ($g\ L^{-1}$)	RPS	26.57 ± 1.25 d	11.67 ± 0.15 a	22.2 ± 0.66 c	22.4 ± 2.15 c	15.53 ± 1.19 b	14.5 ± 0.86 ab
	BAC	26.97 ± 1.55 d	26.43 ± 0.35 c	17.37 ± 0.71 ab	15.5 ± 0.46 a	15.3 ± 1.35 a	19.03 ± 2.36 b
	BAC + AER	26.77 ± 1.02 d	21.23 ± 1.59 d	17.93 ± 0.57 a	25.97 ± 0.35 c	21.03 ± 0.55 b	24.07 ± 2.07 c
COD (gL^{-1})	RPS	32.5 ± 0.5 e	21 ± 0.01 c	26 ± 0.02 d	17.5 ± 0.5 b	10.9 ± 0.2 a	10.75 ± 0.12 a
	BAC	32.5 ± 0.5 e	24 ± 1.2 d	17.5 ± 0.5 c	12.5 ± 0.5 b	9.4 ± 0.01 a	10.5 ± 0.92 a
	BAC + AER	32.5 ± 0.5 e	28 ± 1.9 c	26.5 ± 1.5 bc	22 ± 1.2 a	21.3 ± 0.2 a	19.85 ± 1.68 c
BOD_5 ($g\ O_2L^{-1}$)	RPS	12 ± 0.36 c	11.68 ± 0.08 c	12.98 ± 0.03 d	9.41 ± 0.26 b	2.54 ± 0.09 a	2 ± 0.36 a
	BAC	9.94 ± 0.04 d	10.32 ± 0.54 d	7.09 ± 0.12 c	3.09 ± 0.56 b	2.01 ± 0.03 a	2.59 ± 0.23 a
	BAC + AER	11.9 ± 0.36 c	13.76 ± 0.65 e	10.62 ± 0.13 d	10.54 ± 0.14 d	6.92 ± 0.53b	3.77 ± 1,18 a

Note(s): EC: electrical conductivity, TSSs: total suspended solids; COD: chemical oxygen demand; BOD_5: biochemical oxygen demand; RPS: raw pig slurry; BAC: bacteria, BAC + AER: Bacteria with aeration. Data are the means of three replicates (SD). Different letters indicate significant differences between the treatment and the control (RPS). Significance: $p < 0.05$.

Table 2. Mean and standard deviation values of the macronutrient parameters' progression in untreated and treated pig slurry.

Weeks		1	2	3	4	5	6
N total ($g\ L^{-1}$)	RPS	2.66 ± 0.11 c	2.33 ± 0.07 c	2.33 ± 0.06 c	2.7 ± 0.21 c	1.93 ± 0.06 b	2.11 ± 0.16 b
	BAC	2.66 ± 0.14 c	2.68 ± 0.04 c	2.56 ± 0.05 c	2.29 ± 0.08 c	1.87 ± 0.12 b	1.47 ± 0.22 a
	BAC + AER	2.63 ± 0.12 c	2.65 ± 0.37 c	2.67 ± 0.04 c	2.37 ± 0.13 c	1.72 ± 0.11 b	1.52 ± 0.06 a
Na^+ ($mg\ L^{-1}$)	RPS	1504 ± 48 a	1724 ± 54.95 b	1923 ± 21.88 c	1987 ± 41 c	2060 ± 86.1 c	2324 ± 155 d
	BAC	1492 ± 40 a	1670 ± 41.11 b	1822 ± 21.06 c	2031 ± 25 d	1980 ± 64.01 d	2198 ± 122 e
	BAC + AER	1495 ± 51 a	1665 ± 48 b	1780 ± 75 bc	1979 ± 37 d	1866 ± 29 cd	2136 ± 128 e
K^+ ($mg\ L^{-1}$)	RPS	2096 ± 31 a	2451 ± 51 b	2772 ± 52 c	2975 ± 57 d	3096 ±137 d	3441 ± 210 e
	BAC	2103 ± 52 a	2297 ± 64 b	2608 ± 56 c	2966 ± 37 e	2753 ± 11 d	3173 ± 219 f
	BAC + AER	2082 ± 78 a	2319 ± 79 b	2580 ± 125 c	2925 ± 51 de	2724 ± 42 cd	3091 ± 184 e
Ca_2^+ ($mg\ L^{-1}$)	RPS	372 ± 49 bc	398 ± 11 c	430 ± 30 c	352 ± 2 bc	305 ± 11 ab	255 ± 22 a
	BAC	383 ± 41 cd	428.73 ± 16 d	348 ± 13 bc	302 ± 12 ab	240 ± 21 a	268 ± 20 a
	BAC + AER	365 ± 12 bc	411.8 ± 15 c	278 ± 43 a	296 ± 41 ab	301 ± 33 ab	299 ± 28 ab

Table 2. *Cont.*

Weeks		1	2	3	4	5	6
Mg_2^+ (mg L^{-1})	RPS	396 ± 22 c	224 ±15 a	248 ± 11 ab	272 ± 11 b	239 ± 13 ab	233 ± 1 ab
	BAC	348 ± 72 c	311 ± 11 bc	263 ± 26 ab	244 ± 6 ab	201 ± 10 a	227 ± 14 ab
	BAC + AER	341 ± 68 b	242 ±5 a	195 ± 6 a	193 ± 2 a	263 ± 19 ab	229 ± 34 a
P (mg L^{-1})	RPS	296 ± 46 b	139 ±14 ab	113 ± 6 ab	93 ±1.81 a	98 ±1.58 a	96.6 ± 2.05 a
	BAC	231 ± 97 b	70.2 ± 1.26 a	96 ±1.58 a	63.9 ± 1.58 a	64.8 ± 1.27 c	53.4 ± 1.43 c
	BAC + AER	225 ± 106 b	61.8 ± 2.04 a	82.7 ± 1.85 a	54.7 ± 0.73 c	59.4 ± 0.73 c	43.2 ± 1.59 c

Note(s): Data are the means of three replicates (SD). Different letters indicate significant differences between the treatment and the control (RPS). Significance: $p < 0.05$. RPS: raw pig slurry; BAC: bacteria, BAC + AER: Bacteria with aeration.

Table 3. Mean and standard deviation values of the micronutrient parameters' progression in untreated and treated pig slurry.

Weeks		1	2	3	4	5	6
Cu (mg L^{-1})	RPS	0.49 ± 0.05 a	0.82 ± 0.28 ab	1.45 ± 0.07 b	0.6 ± 0.15 a	1.41 ± 0.27 b	2.29 ± 0.58 c
	BAC	0.48 ± 0.05 a	0.76 ± 0.03 ab	1.36 ± 0.17 b	0.67 ± 0.19 ab	2.49 ± 0.18 c	2.44 ± 0.11 c
	BAC + AER	0.47 ± 0.07 ab	0.78 ± 0.01 b	1.49 ± 0.28 c	0.34 ± 0.06 a	1.43 ± 0.22 c	1.96 ± 0.37 d
Zn (mg L^{-1})	RPS	1.28 ± 0.06 a	2.12 ± 0.1 b	2.15 ± 0.14 b	2.3 ± 0.18 b	4.08 ± 0.67 c	5.28 ± 0.48 d
	BAC	1.39 ± 0.11 a	2.23 ± 0.09 b	2.53 ± 0.18 bc	2.98 ± 0.32 c	5.42 ± 0.48 d	5.17 ± 0.4 d
	BAC + AER	1.42 ± 0.05 a	2.45 ± 0.06 b	3.61 ± 0.31 cd	3.04 ± 0.23 bc	4.28 ± 0.64 de	4.52 ± 0.66 e
Fe (mg L^{-1})	RPS	2.8 ± 0.22 a	3.27 ± 0.28 ab	4.72 ± 0.18 de	3.69 ± 0.15 bc	4.2 ± 0.17 cd	5.46 ± 0.85 e
	BAC	3.31 ± 0.51 a	4.45 ± 0.26 c	4.7 ± 0.55 c	3.63 ± 0.28 b	5.38 ± 0.38 d	5.34 ± 0.23 d
	BAC + AER	3.36 ± 0.46 a	4.17 ± 0.11 a	6.62 ± 0.52 bc	5.53 ± 0.23 b	7.79 ± 0.76 cd	8.23 ± 0.52 d
Mn (mg L^{-1})	RPS	7.02 ± 0.61 c	4.68 ± 0.16 b	4.58 ± 0.15 b	4.21 ± 0.06 b	4.31 ± 0.54 b	4.91 ± 0.54 bc
	BAC	6.85 ± 0.57 c	5.71 ± 0.19 c	4.31 ± 0.54 b	2.75 ± 0.1 a	2.31 ± 0.06 a	2.61 ± 0.17 a
	BAC + AER	6.93 ± 1.18 d	4.43 ± 0.2 c	2.81 ± 0.15 ab	2.04 ± 0.07 a	3.41 ± 0.14 bc	1.98 ± 0.83 a

Note(s): Data are the means of three replicates (SD). Different letters indicate significant differences between the treatment and the control (RPS). Significance: $p < 0.05$. RPS: raw pig slurry; BAC: bacteria, BAC + AER: Bacteria with aeration.

4. Discussion

4.1. Methane (CH_4) Emissions

As illustrated in Figure 3, the weekly CH_4 emissions followed varied trajectories for each treatment in comparison to the control slurry. Using HIPO-PURÍN bacteria showed a notable reduction in CH_4 emission, significantly lower ($p < 0.05$) than the untreated pig slurry (0.455 g m^{-2} day^{-1} compared to 0.151 g m^{-2} day^{-1}, respectively). Combining the same bacteria with aeration resulted in improved outcomes compared to the untreated slurry (0.005 g m^{-2} day^{-1}).

The biological additives effectively reduced CH_4 emissions from the pig slurry (PS). Methane emission was observed in the control (RPS) from the third week onwards, exhibiting a significant increase relative to the first and second week, and proving to be consistent throughout the study period. The CH_4 emissions during the storage of raw pig slurry remained consistent, without showing important variations throughout the entire study duration.

After adding the biological additives, methane emissions increased in the second week, followed by a gradual decline in the subsequent weeks, until achieving a value of 0.151 g m^{-2} day^{-1}.

While microbial activity in slurry can be categorized into aerobic or anaerobic digestion, limited research has been reported on methane (CH_4) control strategies using different formulations of microbial stimulants in this field. The studies examining microbial

stimulants drew inspiration from the observed impacts of commercial biological additives in reducing GHG and ammonia a farm scale. Numerous commercial products, comprising mixtures of bacteria, enzymes, and partially decomposed organic materials are utilized for slurry treatment [40]. Wheeler [41] noted a 46% decrease in CH_4 emissions upon the addition of biological additives; Bastami [42] demonstrated a 27% reduction in CH_4 emissions from cattle slurry stored in warm conditions and a 15% reduction in cold conditions; while in other studies, the addition of microbial additives did not cause a notable decrease in CH_4 emissions [43–45].

The variability in methane (CH_4) reduction can be attributed to several factors, with temperature playing a significant role in the efficiency of biological treatment [23,46]. Bastami [44] observed an effect of CH_4 reduction, contrasting with Amon and others [43–45]. This difference could potentially be due to variations in several factors such as the dosage of effective microorganisms (EM), microbial community composition, slurry characteristics, and environmental conditions across the studies [45].

In this study, in both treatment cases, the methane emission was increased in the second week after adding the biological additives, followed by a gradual decline in the following weeks. Methane production mainly occurred between the second and third weeks during the study. The introduction of biological additives can lead to changes in the microbial population within slurry. Initially, certain bacteria that produce methane may dominate, causing an increase in emissions. However, as the microbial community adjusts, the population of methane-consuming bacteria may increase, leading to a reduction in methane emissions over time. The pH can play a crucial role in CH_4 emissions, and according to Hamood and Ishak [47,48], the optimal pH range for methane production is usually between 6.5 and 7. Within this range, the microbial communities involved in methane production thrive, leading to increased methane emissions [49]. Aeration of slurry has been shown to elevate the pH to an alkaline range in various studies [45,50,51], and this phenomenon can explain the methane reduction during the aeration of pig slurry. Furthermore, an initial peak in CH4 was observed, followed by a rapid decline in the subsequent week [51], which can be attributed to the release of trapped CH_4 in the slurry as a result of supersaturation, coupled with the establishment of aerobic conditions in the following days upon aeration. These multiple phenomena can explain the peaks in methane emissions after adding the biological additives, followed by reduction in the same gas.

The bacteria in HIPO-PURÍN produce a range of enzymes such as proteases, cellulases, and lipases that are effective in breaking down crust layers and removing organic material present in excess feed, bedding, and animal waste products. Another enzyme produced by the bacteria is urease. This enzyme breaks down urea into a form that HIPO-PURÍN bacteria can readily utilize. It has a small amount of free enzymes that start the biodegradation process. Once the bacteria are established, they handle the enzyme production. They are able to produce biosurfactants. These are naturally occurring surfactants that help with the biodegradation process and also facilitate the suspension of crust layers. HIPO-PURÍN includes a buffering agent that helps neutralize the environment in which the microorganisms are intended to act, thereby reducing the acclimatization period. Additionally, it contains one crucial final ingredient, yucca extract. This component, a natural extract from the yucca plant, consistently mitigates ammonia levels in controlled settings. This can explain the methane reduction in the treatment pig slurry by the biological additive HIPO-PURIN.

Biological additives like Bacillus bacteria and yucca extract have the potential to reduce methane emissions from stored pig slurry through multifaceted mechanisms. Bacillus species, including *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheni-formis*, *Bacillus amyloliquefaciens*, and *Bacillus thuringiensis* can disrupt methanogenic microbial pathways by outcompeting for substrates; enzymatically breaking down organic matter, by producing hydrolytic enzymes, such as cellulases (CMCase, avicelase, β-glucosidase, xylanase), lipases (Lip A, Lip B), amylases (α-amylase, β-amylase), and proteases (serine protease, threonine protease, cysteine protease, aspartic protease, metalloprotease) that help break down high molecular weight compounds [52–54]; and modulating the microbial commu-

nity to favor non-methane producing species; therefore, when applied to pig slurry, these bacteria can potentially reduce methane emissions through several mechanisms, mainly indirect anaerobic digestion enhancement by competing with methanogens for the broken down organic matter, which indirectly reduce methanogenesis by utilizing some of the available carbon sources for their own metabolism [55,56]. Yucca extract complements this by reducing foam formation, potentially trapping methane, and exhibiting antimicrobial properties that can inhibit methanogenic activity [57].

In general, aeration of the slurry resulted in decreased CH_4 levels. However, significant spikes in CH_4 emissions occurred during aeration events, especially in the second week. This trend is consistent with findings from previous studies [45,58], highlighting the limited solubility of CH_4 in water, which causes it to form small bubbles within the slurry before escaping into the atmosphere. Despite infrequent aeration, the CH_4 production persisted in the early stages, due to the existence of easily degradable organic matter, and the sporadic aeration likely failed to fully eliminate the anaerobic conditions necessary for CH_4 generation. However, with prolonged aeration, easily accessible organic matter, such as volatile fatty acids underwent aerobic degradation [59], thereby reducing the potential for CH_4 generation.

Adding the HIPO-PURÍN bacteria combined with an aeration system created an environment that was less favorable for methane production [50]. The increased oxygen levels resulting from the aeration system further supported aerobic decomposition processes, which produced less methane compared to anaerobic conditions [50–61]. Overall, this combined approach helps to mitigate methane production by creating conditions that favor alternative pathways of organic matter degradation over methanogenesis, which explain the significant methane emissions ($p < 0.05$) between using the biological additive alone and combined with aeration.

4.2. Carbon Dioxide (CO_2) Emissions

As shown in Figure 4, the weekly CO_2 emissions followed varied trajectories for each treatment, particularly notable with aeration compared to the untreated control slurry. Adding HIPO-PURÍN bacteria led to a significant reduction in CO_2 emissions, significantly lower ($p < 0.05$) than the untreated raw pig slurry (2.51 g m^{-2} day^{-1} compared to 0.998 g·m^{-2}·day^{-1}, respectively). Combining the same bacteria with aeration led to better results (0.328 g m^{-2} day^{-1}), according to our research. For the control pig slurry, CO_2 emissions remained stable throughout the entire study period, with no significant variations observed.

During the second and third weeks following the introduction of aeration combined with biological additives to the pig slurry, there were notable peaks in CO_2 emissions. This period coincided with increased bubbling and agitation of the slurry surface due to aeration, suggesting heightened microbial activity. By the fourth week, a significant decline ($p < 0.05$) in CO_2 emissions was detected, and in the fifth and sixth weeks, emissions continued to decrease steadily.

Aeration of slurry combines the effects of mixing and oxygenation, as documented in other research [62,63]. The CO_2 is produced from organic matter by microorganisms during the aerobic composting process. During the initial aeration phase, CO_2 emissions escalate quickly and there may be a spike in CO_2 emissions [64]. This is because the increased oxygen availability stimulates microbial activity, leading to accelerated decomposition of organic matter and subsequent release of CO_2. As the aerobic decomposition process continues, CO_2 emissions may remain relatively high during the initial stages of aeration [26,50,51]. This phenomenon explains the observed peaks in CO_2 emissions during the second and third weeks after initiating aeration with biological additives, the introduction of aeration could also promote the release of CO_2 trapped within the organic matter. This initial surge in CO_2 emissions signified the increased metabolic activity of aerobic microorganisms, including the Bacillus species present in the biological additive, which thrive in the presence

of oxygen. As these bacteria metabolize organic compounds, they release CO_2 as a by-product, contributing to the observed peaks in Figure 4.

However, as the aerobic decomposition process progressed and organic matter was gradually broken down, the availability of substrates for microbial metabolism decreased. Consequently, CO_2 emissions began to decline, leading to the notable decrease observed in the fourth week in Figure 4. Subsequently, during the fifth and sixth weeks, CO_2 emissions continued to decrease as the microbial population stabilized and the rate of organic matter decomposition diminished. This trend underscores the dynamic nature of microbial activity in response to changes in environmental conditions, ultimately influencing CO_2 emissions from pig slurry [26,65,66].

Overall, aeration can play a crucial role in managing CO_2 emissions from pig slurry by promoting aerobic decomposition and minimizing anaerobic processes that typically produce methane, a more potent greenhouse gas. Additionally, aeration can help improve the overall quality of the slurry by reducing odors and enhancing nutrient availability for agricultural use [67].

The reviewed studies indicated that biological additives and aeration can significantly reduce CO_2 emissions from pig manure. According to Kupper and Clemens [68,69], biological additives can reduce CO_2 emissions by 20–30%. The average CO_2% reduction in our study was 60%, which is consistent with the results (71%) reported by El bied [70].

Other studies [71,72] showed that aeration increases CO_2 emissions, because during aerobic treatment, organic matter is decomposed in the presence of oxygen, which leads to the formation of carbon dioxide, while Loyon [26] confirmed that aerobic treatment can indeed help reduce carbon dioxide emissions from pig manure over the long term when comparing the initial and final stages of pig slurry treatment with aeration after its stabilization. The overall reduction in greenhouse gas emissions was approximately 97.5%, primarily due to the complete aerobic decomposition of organic matter during the treatment process, resulting in minimal emissions post-treatment. In our study, the combination of both treatments provided a CO_2 emission reduction of up to 87%.

4.3. Ammonia (NH_3) Emissions

According to results shown in Figure 5, similar curves were observed for NH_3 during the first, third, and fourth weeks for the control and treated pig slurry. Following the initial treatment, the control and treated slurry reported significant increases ($p < 0.05$) during the three weeks previously indicated, and the values for the controlled slurry decreased in the fifth week then settled in a constant range, with the same behavior for the other treatments. NH_3 emissions significantly ($p < 0.05$) decreased when comparing control and pig slurry treated with bacteria (15.66 $mg \cdot m^{-2} \cdot day^{-1}$ compared to 1.667 $mg \cdot m^{-2} \cdot day^{-1}$, respectively). The same effects were seen when comparing the treated slurry with bacteria coupled with aeration (3.66 $mg \cdot m^{-2} \cdot day^{-1}$). In this study, we observed that NH_3 was less reduced under aerobic conditions compared to the treated slurry when only adding bacteria. The difference in the reduction in ammonia emissions between using the additives alone (90%) and combining them with aeration (76%) could be due to the specific mechanisms involved in each approach.

The average ammonia reduction in our study was 99% using only bacteria and 76% when coupled with the aeration system. Other studies [45,73] demonstrated that biological additives alone typically reduce ammonia emissions by only 10–25% and aeration alone reduces ammonia emissions by 35–50%, while El bied [70] reported that biological additives alone could reduce 77% of ammonia. Kupper [68] showed that aeration alone reduced ammonia emissions by 35–50%, while combining with biological additives could reduce ammonia emissions by 50–70%. Berg [74] confirmed that the combination of both treatments provided high reductions, with ammonia emissions reduced by up to 70%. Our findings on ammonia emissions with biological additive combined with aeration (76%) agree with the values reported by Berg and Kupper [68,74].

Once HIPO-PURÍN, previously rehydrated, is applied, it initiates a series of crucial processes. Firstly, urea is decomposed into two molecules of ammonia by the urease present in slurry and HIPO-PURÍN additive, making it available for bacterial utilization. According to Calvet [75], once urea is excreted, it undergoes a relatively rapid process that results in rapid urea saponification, leading to higher NH_3 concentrations [51], which explains the peaks during the first weeks after adding the treatment. As shown in Figure 5, there was a significant difference between the control and treated pig slurry in ammonia emissions during the second, third, and fourth weeks. To delay ammonia release until bacterial activation, the Yuca extract within the HIPO-PURÍN acts as a binding agent, allowing subsequent utilization of nitrogen and ammonia by microorganisms for growth and reproduction, with increased absorption leading to biomass conversion, which explains the small peaks in the treated slurry and the high peakks for the control.

Bacillus species are known for their potent enzymatic activity, which directly targets and metabolizes ammonia, resulting in significant reductions when added to pig slurry [76]. Bacillus bacteria can effectively reduce nitrogen and ammonia emissions from pig slurry through the nitrification–denitrification process [77]. When introduced into the slurry, Bacillus species facilitate both stages of this process and lead to reductions in the concentration of ammonia in the slurry, as ammonia is converted into less volatile nitrate forms. Subsequently, microorganisms utilize nitrogen and ammonia for growth and reproduction, with increased absorption of ammonia, leading to biomass conversion. Notably, HIPO-PURIN bacteria exhibit anaerobic functionality, even in suspension without oxygen. The establishment of an NH_3–NH_4^+ equilibrium, largely favoring non-volatile NH_4^+ due to intermittent aeration and microbial activity stabilizing pH, contributes to reduced ammonia volatilization. Furthermore, bacterial consumption of ammonia, particularly in the NH_4^+ form, significantly diminishes NH_3 emissions, highlighting the efficacy of HIPO-PURIN in mitigating atmospheric ammonia levels.

However, when combined with aeration, factors such as oxygen availability, microbial interactions, and the timing and duration of aeration may impact the efficiency of reducing ammonia [78]. Aeration may introduce conditions that alter the microbial community dynamics or affect the availability of nutrients, potentially influencing the performance of the biological additives in ammonia reduction [79,80]. This disruption could lead to reduced efficiency in lowering nitrogen and ammonia emissions, which explains the high reductions in both parameters in the treatments with and without aeration (76% and 90%, respectively). Therefore, while both strategies contributed to mitigating ammonia emissions, the observed difference could stem from the complex interplay of various factors influencing the microbial activity and ammonia metabolism within the system. Further investigation into optimizing the synergistic effects between the biological additives and aeration parameters may provide insights into improving their combined efficiency in reducing ammonia pollution from pig slurry, while also considering the additional benefit of aeration in methane (CH_4) reduction.

4.4. Compositions of the Treated and Control Raw Pig Slurry

The compositions of the slurry during the treatment process are detailed in Tables 1–3. Table 1 shows a notable contrast in electrical conductivity (EC) between the first and sixth weeks, with an increase from 22.09 μS cm^{-1} to 24.04 μS cm^{-1}, a change deemed statistically significant ($p < 0.05$) for the control slurry. Adding bacteria alone or combined with aeration reduced this significantly (21.76 and 20.45 μS cm^{-1}, respectively).

Adding biological additives led to a significant change in pH between the first and sixth weeks, attributed to microbial degradation of organic matter, leading to the formation of carbonate and ammonium [78], consequently elevating the pH value from 7.32 to 7.97 and 8.22 for bacteria alone and combined with aeration, respectively. On the other hand, aeration increased the bulk pH over the medium term, likely due to the degradation of volatile fatty acids, as previously reported [23,59,81].

The duration of storage for pig slurry has a big impact the concentration and behavior of total suspended solids (TSSs). Over time, natural settling occurs, causing heavier solid particles to separate from the liquid phase, leading to the accumulation of TSSs at the tank's bottom [82], and this phenomenon explains the significant reduction in the TSSs in raw pig slurry (from 26.57 to 14.5 g L^{-1}) between the first and sixth weeks.

Adding bacteria, the TSSs evolved from 26.97 g L^{-1} to 19.03 g L^{-1} and 24.1 g L^{-1} when combined with aeration which showed a significant effect on TSSs removal relative to the initial values.

In the context of pig slurry management, microbial cultures, particularly Bacillus bacteria strains, play a crucial role in the breakdown of organic matter. These specialized bacteria are adept at solubilizing or converting solid particles into smaller components, thereby contributing to the reduction in total suspended solids (TSSs) within slurry [83,84].

Addition of bacteria combined with aeration exhibited a small yet significant effect ($p < 0.05$) on TSSs removal. Aerobic treatment methods hold promise for reducing total suspended solids (TSSs) in pig slurry [85], but there are challenges associated with agitation during the process, which can lead to a lower removal efficiency, which happened in this study. Agitation of pig slurry can disturb settled solids, causing them to become resuspended in the liquid phase and reducing the effectiveness of TSSs removal [86].

It is noteworthy that settling is not a treatment that removes suspended solids from the liquid phase, because when the tank is cleaned or emptied, those settled solids at the bottom remain present, requiring additional steps for their removal. Therefore, settling is considered a partial solid removal technique rather than a complete one, as it only separates solids physically but does not eliminate them entirely from the system.

The correlation between COD and TSSs changes is based on the levels of inorganic and organic solids in the mixture [87]. In pig slurry, there is typically a correlation between TSSs and COD caused by the presence of both organic and inorganic suspended solids, which collectively impact the COD content [9,22]. This study validated this observation, demonstrating that a decrease in suspended solids led to significantly reduced COD concentrations across all three tanks ($p < 0.05$).

Just as COD is correlated with TSSs, BOD_5 is similarly linked to the same parameter, with BOD_5 being linked to the organic content within suspended solids. The microorganisms responsible for BOD_5 decomposition can adhere to these solids [88,89]. Consequently, effective TSSs removal can result in decreased BOD_5 levels, due to the reduction in microbial habitat and organic matter accessible for biological degradation. Notably, a significant BOD_5 reduction was noted in all three cases ($p < 0.05$), clearly showing a correlation between the BOD_5 and TSSs removal efficiency.

The findings of this study indicated a correlation between the biological additives and total nitrogen removal, because the TN had the same behavior in both treatments, where was decreased from 2.66 g L^{-1} to 1.47g L^{-1} with biological additive alone and 1.52 g L^{-1} when combined with the OXI-FUCH aeration technique.

On the one hand, Bacillus bacteria can play a role in reducing total nitrogen levels in liquid pig slurry through their ability to break down organic matter [90]. According to Shao 2003 [91], the cellulose-degrading bacteria present in pig manure, especially *Bacillus subtilis*, demonstrated superior cellulose degradation capabilities. In liquid slurry systems, organic compounds are often abundant and can contribute to elevated nitrogen levels. Bacillus bacteria, known for their proficiency in organic matter decomposition, metabolize these compounds, releasing nitrogen in forms that are more accessible for uptake or for further transformation by other microorganisms [92]. By facilitating the breakdown of organic material, Bacillus bacteria contribute to the reduction in total nitrogen content in liquid pig slurry.

On the other hand, Bacillus thuringiensis bacteria demonstrated a noteworthy capacity to reduce total nitrogen levels by 55% when introduced to pig slurry. This significant finding parallels the pioneering research that identified Bacillus thuringiensis as a potent agent in nitrogen removal and shows its effectiveness in removing total nitrogen, which can achieve

up to 82% reductions under specific conditions [93]. Similarly to the initial discovery, this study underscored bacteria's remarkable adaptability and efficacy in processing nitrogen compounds within pig slurry and revealed Bacillus thuringiensis' ability to transform nitrogen forms, resulting in a substantial decrease in total nitrogen content. In conclusion, the biological treatment yielded good results, achieving a 56% reduction in total nitrogen compared to the raw slurries.

This study demonstrates that biological additives, particularly when combined with aeration, can significantly improve the composition and quality of pig slurry by reducing key pollutants. The addition of bacteria and aeration significantly reduced electrical conductivity (EC) by 1.5% and 7.4%, respectively. Total suspended solids (TSSs) in the raw pig slurry showed a 45.4% decrease, with bacteria alone resulting in a 29.5% decrease and a 10.6% decrease when combined with aeration, affected by the absence of settling conditions. Total nitrogen (TN) was reduced by 44.7% with the addition of bacteria alone, and by 42.9% when combined with aeration.

In the final week of treatment, both treatments did not significantly affect ($p < 0.05$) Na^+, K^+, $Ca_2{}^+$, $Mg_2{}^+$, Cu, Zn, and Fe in comparison with the control slurry in the last week of treatment, while decreasing others such as P and Mn.

Bacillus bacteria are known for their ability to solubilize phosphorus, especially *Bacillus megaterium*, which means they can convert insoluble forms of phosphorus into soluble forms that are more readily available for uptake by microorganisms or plants [94]. When Bacillus bacteria are introduced to pig slurry, they may initiate processes such as phosphorus solubilization, where insoluble phosphorus compounds in the slurry are converted into soluble forms [95]. As a result, the concentration of phosphorus in the liquid portion of the slurry may decrease, as the solubilized phosphorus is utilized by the bacteria, because all bacterial species require several micronutrients, with phosphorus being particularly crucial for ATP (Adenosine Triphosphate) production. ATP conversion fuels cellular processes, with phosphorus in pig slurry typically existing in the form of phosphates ($PO_4{}^{3-}$) [96,97]. This explains the decrease in total phosphorus observed when comparing untreated pig slurry with pig slurry containing biological additives with and without aeration 96.6 mg L^{-1}, 53.4 mg L^{-1}, and 43.2 mg L^{-1}, respectively. Zhu et al. (2006) [59] investigated the impact of combining a biological additive with aeration on nutrient reduction in swine manure over a 15-day period. After just one day of aeration, all aerated treatments showed a 42% reduction in total soluble phosphorus and an increase in total insoluble phosphorus. Additionally, total Kjeldahl nitrogen decreased by about 40% in all treatments except the control.

As per the findings of Rongrong Wu [98], diverse biological approaches involving microorganisms have been identified for manganese (Mn) removal, encompassing biosorption, bioaccumulation, and biological oxidation. Therdkiattikul and Katsoyiannis [99,100] affirmed that biological oxidation employing microorganisms has emerged as a potential method for manganese removal from water sources. This observation elucidates the substantial contrast between untreated and treated pig slurry with bacteria. The former exhibited a concentration of 7 g L^{-1}, whereas the latter, treated with bacteria, displayed reduced concentrations of 2.61 g L^{-1} and 1.98 g L^{-1}, respectively.

The study results showed a significant decrease in Mn levels with biological additives. Untreated pig slurry had 4.91 g L^{-1} of Mn, while treated slurry had 2.61 g L^{-1} and 1.98 g L^{-1} without and with aeration, respectively. According to Rongrong [98], biological treatments that utilize microorganisms, such as biosorption, bioaccumulation, and biological oxidation, can remove Mn. Two other studies, by Therdkiattikul and Katsoyiannis [99,100], also supported that biological oxidation using microorganisms can used for Mn removal. Our study validated how adding biological additives to pig slurry can decrease Mn content.

Regarding cations, our findings align with those of previous studies [101], although Moral [102] reported higher concentrations of potassium (K). Several cations, including K, sodium (Na), copper (Cu), zinc (Zn), and manganese (Mn), are frequently added to diets as supplements to improve growth rates, even at concentrations surpassing physiological

requirements [103,104]. For example, pigs are estimated to excrete roughly 66% of the sodium (Na) and 59% of the potassium (K) they ingest [105]. Additionally, Clemente et al. [106] observed elevated concentrations of Cu and Zn when analyzing the separated solid fraction of pig slurries. Slurry storage could have contributed to the observed trends of increased concentrations of potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and various metals Cu, Zn, and Fe alongside a decrease in total suspended solids (TSSs) after six weeks of treatment and storage [107]. Vaporization, or evaporation, of water from slurry can concentrate the remaining solutes, including ions and metals, leading to higher concentrations in the remaining liquid phase. This concentration effect can occur as water evaporates from the surface of the slurry, leaving behind a more concentrated solution of dissolved constituents [107]. Consequently, the observed changes in ion and TSSs concentrations can be influenced by both vaporization and the biological processes previously mentioned.

5. Conclusions

This study investigated the impact of different treatment approaches, including the use of Bacillus biological additives HIPO-PURÍN and aeration using OXI-FUCH, on GHG and ammonia emissions from stored pig manure.

Methane (CH_4) emissions displayed varied trajectories across treatments, with notable reductions observed when using HIPO-PURÍN bacteria alone or combined with aeration. While initial spikes in CH_4 emissions were noted following the introduction of biological additives, subsequent weeks saw a gradual decline, indicative of microbial community adjustments favoring methane-consuming bacteria. Aeration, although generally decreasing CH_4 levels, occasionally led to spikes due to trapped CH_4 release and accelerated aerobic decomposition.

Carbon dioxide (CO_2) emissions exhibited similar trends, with significant reductions noted with HIPO-PURÍN bacteria and aeration treatments compared to untreated slurry. These reductions were attributed to enhanced aerobic decomposition facilitated by increased oxygen availability.

Ammonia (NH_3) emissions, on the other hand, saw significant decreases with the addition of biological additives alone or combined with aeration, highlighting the efficacy of HIPO-PURÍN bacteria in mitigating NH_3 volatilization.

This investigation also revealed the significant impact of biological additives and aeration on the composition of stored pig slurry. The addition of bacteria, particularly Bacillus strains, led to pH elevations and a slight decrease in electrical conductivity. Moreover, biological treatments showed promising results in reducing chemical oxygen demand (COD), biological oxygen demand (BOD_5), total nitrogen (TN), phosphorus, and manganese levels.

Overall, our findings demonstrate the multifaceted effects of biological additives and aeration on GHG from pig slurry, underscoring their potential in reducing the environmental impacts associated with slurry management.

Future studies should focus on the direct application of biological additives and implementing aeration systems in pig slurry storage ponds with larger volumes. Investigating the long-term impact and feasibility of these treatments in real-world farm environments is essential to promote sustainable livestock waste management practices.

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