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Evaluation of Laboratory-Scale Composting Reactors to Simulate Compost Barn Bedding Dynamics

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ABSTRACT

Efficient management of compost bedding is essential for sustainability and animal welfare in Compost Barns (CBs). This study evaluated laboratory-scale bioreactors as tools to simulate CB composting dynamics, focusing on temperature control, aeration, and wood shaving supplementation. Three sequential experiments were conducted. Test 1 used a 50:50 mix of shavings and compost bedding for three months. Test 2 operated 1-week cycles with 100% compost bedding without temperature control. Test 3, also lasting 1 week, applied active temperature regulation at 39°C with 100% bedding. Aeration was set at 15 mL/min in Tests 2 and 3. All experiments included initial and final measurements of pH, total solids (TS), and microbiological indicators. Results indicated that temperature control in Test 3 improved organic matter degradation and suggested enhanced pathogen reduction. These findings demonstrate that laboratory-scale bioreactors are effective for simulating CB composting and underscore the importance of temperature and aeration management. Future studies should further optimize temperature control and reactor design to enhance microbial activity and compost stabilization. To our knowledge, this is the first study to validate 3-L laboratory-scale bioreactors specifically for simulating CB bedding dynamics.

1 | Introduction

The pursuit of sustainable and efficient agriculture has driven innovations in dairy herd management (Viana et al. 2023). One notable innovation is the Compost Barn (CB) system, an alternative to conventional dairy housing that enables more effective manure management (Beaver et al. 2021). Despite its advantages, CB systems are inherently complex due to environmental and microbiological interactions, highlighting the need for further investigation. Previous studies (Oliveira et al. 2024) have demonstrated that CB systems allow for in situ composting of animal waste within the resting areas, significantly reducing the need for post-collection manure treatment. These findings underscore the relevance of understanding the composting process within CB environments and support efforts to optimize its microbiological and operational parameters.

Composting, the core biological process in CB systems, is a thermophilic microbial activity responsible for the degradation of organic matter (Xu et al. 2023). The bedding in CB systems typically consists of wood shavings, bovine manure, and urine (Peixoto et al. 2019). Manure supplies organic carbon, urine contributes nitrogen, and wood shavings regulate moisture and aeration. In addition to these functions, wood shavings play a fundamental role in absorbing excess moisture from excreta, controlling odors, and providing thermal insulation and physical

softness, which directly contribute to animal comfort. This combination supports microbial growth while minimizing odor, ultimately promoting the transformation of waste into stable, nutrient-rich compost (Silva et al. 2020, Peixoto et al. 2019). CB systems concentrate excreta in resting areas, enhancing the composting process. Composting temperatures exceeding 55°C can eliminate pathogens, improving sanitary conditions (Thomas et al. 2024). However, the efficacy of composting and animal comfort can be compromised by inadequate bedding management.

Previous studies (Andrade et al. 2022) observed spatial variability in temperature, moisture, and pH in a closed CB barn with tunnel ventilation, with some zones near feeding alleys and cooling pads exhibiting suboptimal composting conditions. Even though the carbon-to-nitrogen ratio remained outside the ideal range, biological activity was present in the inner bedding layers. Despite these limitations, cows exhibited good hygiene and locomotion scores, indicating acceptable welfare. These findings highlight the importance of adequate bedding management—including sufficient volume, regular stirring, and optimal stocking density—to maintain both composting efficiency and animal well-being.

A laboratory-scale composting system was developed in this study specifically to simulate the environmental and operational conditions of a CB system. Originally adapted from previous designs of 3-L aerobic bioreactors used to accelerate organic waste decomposition (Esteves 2023), the equipment in this work is innovatively repurposed to emulate CB-scale dynamics. This novel application includes the use of a different input material (CB, urine, and bovine manure instead of general organic waste), adjustments to aeration rates, and the strategic use of the pre-existing thermal regulation system. These adaptations enable a more realistic representation of field conditions found in CB systems.

Laboratory-scale CB models provide a controlled setting for investigating decomposition dynamics, optimizing compost management strategies, and testing key variables such as bedding materials, microbial inoculants, and airflow regimes (Kuppan et al. 2024). In addition, microbiome analysis can help elucidate the microbial community structure and its influence on compost quality (Sarwari et al. 2024). The present work advances this approach by integrating field-representative waste inputs with temperature control and daily loading, aiming to bridge the gap between small-scale experimentation and real-world CB operation.

Although there is extensive literature on laboratory-scale composting, most existing studies focus primarily on traditional organic residues, such as sewage sludge, food waste, manure, or synthetic mixtures designed to simulate biodegradable organic matter (Chen et al. 2022; Ucaroglu and Ozbek 2024; Sobieraj et al. 2023; Wyman and Salmon 2024). However, no studies have been found that simulate the compost bedding used in CB systems in controlled bioreactors. This gap highlights the originality of the present study, which stands out by mimicking, on a reduced scale, the real operating conditions of CB bedding, allowing for the controlled analysis of the physicochemical and microbiological variables involved in the process. The ability to reproduce this system in a laboratory setting, with controlled temperature, aeration, and organic load, represents a significant methodological advancement, providing an unprecedented tool to study the composting dynamics of this specific substrate. Therefore, this work introduces an innovative experimental approach to the scientific investigation of CB bedding, making a meaningful contribution to the technical and scientific understanding of the topic.

The present study aims to assemble and validate this bioreactor system, with adaptations designed to mimic the conditions of full-scale CB systems. This foundational step will support future studies focused on evaluating microbial activity and pathogen reduction, thereby contributing to the optimization of composting systems in dairy production environments.

2 | Materials and Methods

2.1 | Description of the Experimental Site

> *"This study aims to evaluate whether three laboratory-scale bioreactors can be used to simulate the CB system."*

The dairy confinement system from which samples for the CB experiment were collected is located at the José Henrique Brusque Experimental Field of Embrapa Dairy Cattle, in Coronel Pacheco, Minas Gerais, Brazil, at the geographic coordinates 21°33'26″S; 43°15'24″W. During the experiment, the CB system housed 85 Holstein dairy cows. Moisture and temperature analyses for the bioreactors were conducted at the Rumen Microbiology Laboratory at Embrapa's headquarters in Juiz de Fora, Minas Gerais.

2.2 | Experimental Design

This study aims to evaluate whether three laboratory-scale bioreactors can be used to simulate the CB system. Before the formal experiments described herein, an exploratory, non-systematic monitoring phase was conducted over several months to qualitatively assess system behavior and inform experimental planning; this stage is not included in the present analysis. Building upon insights from this preliminary phase, three follow-up experiments were designed and conducted to test different operational conditions and assess system performance.

Throughout the methodological progression of this study, each experimental test was strategically adapted based on insights gained from the preceding phase. The exploratory, non-systematic monitoring period played a critical role in guiding the design of Test 1, which served as a baseline for evaluating system behavior under moderate, unmanaged conditions. However, limitations observed in Test 1—particularly the inability of the bioreactors to sustain thermophilic temperatures beyond the initial days—prompted a methodological shift. In Test 2, the experiment was restructured into 1-week cycles, without the weekly addition of wood shavings, to isolate and verify the hypothesized temporal limits of microbial activity. This streamlined design allowed for tighter control and clearer interpretation of composting efficiency. Building on the outcomes of Test 2, Test 3 introduced active temperature control to simu-

late mesophilic conditions more precisely, enabling a targeted evaluation of microbial dynamics and hygienization potential under stable thermal environments. These sequential adjustments not only reflect an iterative and data-driven refinement process but also emphasize the study's commitment to optimizing bioreactor-based simulation of the CB system under realistic and reproducible laboratory conditions.

The first experiment (Test 1) began on January 31, 2024, and lasted for a total of three months. During this period, a mixture consisting of 50% wood shavings and 50% compost bedding was used, with proper management practices maintained throughout the process. An additional 10 g of wood shavings was added weekly. The bioreactors in Test 1 were equipped with continuous forced aeration at a flow rate of 30 mL/min. This initial experiment was conducted without external temperature regulation, relying solely on the composters' built-in heating elements to maintain internal thermal conditions. Composting temperature was continuously monitored throughout the period to evaluate thermal dynamics. The resulting temperature profile showed that the bioreactors were unable to sustain elevated temperatures throughout the entire duration. Analysis of these data suggested that the active composting phase lasted approximately 1 week. Despite the weekly addition of wood shavings, the composters frequently failed to maintain the thermal conditions required for sustained microbial activity (Nguyen et al. 2024).

To further investigate this observation, a second experiment (Test 2) was initiated on June 11, 2024, consisting of 1-week cycles without the addition of wood shavings. This test aimed to verify whether the composting process naturally declined after 1 week, thereby supporting the hypothesis of a 1-week effective composting period. The aeration rate was adjusted to 15 mL/min, and technical issues identified in Test 1 were resolved. The setup for Test 2 included 3.2 kg of compost bedding and 30 g of wood shavings distributed among the three bioreactors (Wang et al. 2021; Zhang et al. 2020).

The third experiment (Test 3), also lasting 1 week, introduced temperature control to evaluate whether this adjustment would improve bioreactor performance. In this test, only compost bedding was used, and temperature was regulated via SITRAD software, maintaining an average of 39°C. The aeration rate remained at 15 mL/min, consistent with Test 2. Mesophilic rather than thermophilic temperatures were selected to simulate realistic operational conditions in CB systems, which may not consistently reach thermophilic levels.

Although the thermophilic range (45–65°C) is generally considered optimal for organic matter degradation and pathogen inactivation, field observations in CB systems reveal temperature variability across the bedding depth (Oliveira et al. 2023; Andrade et al. 2022). The upper layers, which are more exposed to ambient air and in direct contact with the cows, often remain within the mesophilic range (30–40°C), while thermophilic conditions are mainly reached in deeper, less disturbed zones (Andrade et al. 2022; Oliveira et al. 2023). For this reason, maintaining a controlled mesophilic temperature of 39°C in Test 3 was chosen to reproduce more realistic operational conditions. Studying composting under mesophilic conditions is also relevant to evaluate microbial dynamics, organic matter stabilization, and pathogen persistence in the bedding layers most representative of the animal–environment interface.

Additionally, this setting enabled evaluation of pathogen reduction and microbial resistance under moderate thermal conditions. This experimental setup also served as a baseline for comparison with future tests, facilitating process optimization (Nguyen et al. 2024).

In both Tests 2 and 3, microbiological analyses were performed at the beginning and end of each week to assess composting efficiency in terms of organic matter degradation and pathogen reduction (Serrano et al. 2020). **Exhibit 1** summarizes the operational differences between the three experiments.

Throughout the study, bovine feces were collected weekly from the CB facility, while urine samples were obtained fresh as needed. All bioreactors were resupplied daily with feces and urine to simulate continuous loading conditions. During Test 1, the resupply procedure involved opening each bioreactor, transferring the composting material to a tray, manually mixing it with fresh feces and urine, and then returning and turning the mixture. In Tests 2 and 3, a less invasive procedure was used, in which resupply and turning were performed directly inside the bioreactors using long stainless-steel tweezers. The daily quantities of feces and urine applied to the bioreactors were calculated by scaling down the actual waste production of a CB system that, at the time of the experiment, housed 85 Holstein dairy cows and contained 612 m^3 of compost bedding. Based on literature values and local farm data, the estimated total daily excretion was 3515.6 kg of feces and 1543.6 kg of urine. These values were normalized to the volume of the bioreactors to simulate equivalent organic loading rates. As a result, each bioreactor received 19 g of feces and 8.3 g of urine per day, ensuring realistic input conditions relative to full-scale composting operations.

2.3 | Bioreactor Setup

The assembly of the bioreactors took place between November 27 and 30, 2023. The polypropylene bioreactors consist of two distinct components: an outer cylindrical housing and a reaction chamber that fits into the internal cavity of the housing, as shown in **Exhibit 2**. The cylindrical housing, measuring 40 cm in height and 20 cm in diameter, contains an electric heater and a temperature sensor on its inner wall. Two pairs of holes are located on the upper surface of the housing to accommodate two temperature sensors and the inlet and outlet air hoses. This housing provides thermal insulation and, when the internal electric heater is activated, compensates for heat loss from the reaction chamber to the external environment.

The reaction chamber itself measures 30 cm in height and 16 cm in diameter. Positioned 7 cm above the chamber's bottom is a perforated polypropylene disc with a cable gland at its center. On the chamber lid, quick-release connections for the air inlet and outlet hoses are installed, along with cable glands for temperature sensors. A hose connects the polypropylene disc cable gland to the air inlet on the chamber lid, ensuring an upward airflow within the reactor.

EXHIBIT 1 | Comparative overview of experimental setups for Tests 1, 2, and 3.

Test 1	Test 2	Test 3
No temperature control	No temperature control	Temperature controlled at 39°C
No microbiological monitoring	Microbiological monitoring conducted	Microbiological monitoring conducted
50% shavings and 50% compost bedding	Setup with 100% compost bedding	Setup with 100% compost bedding
Addition of wood shavings throughout the process	No addition of wood shavings	No addition of wood shavings

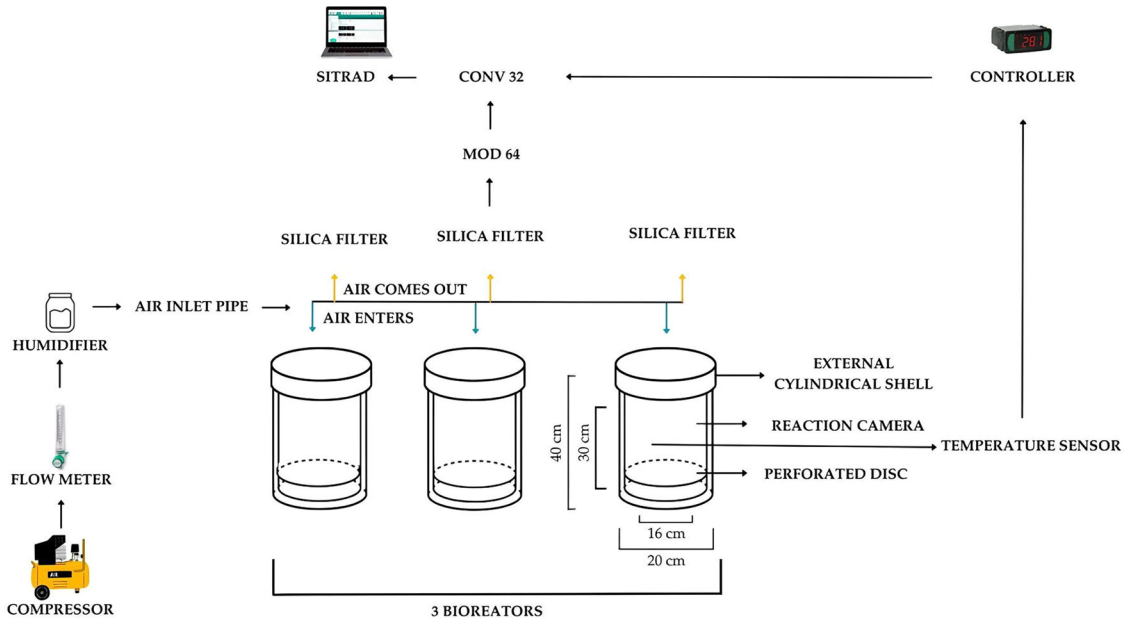

EXHIBIT 2 | Bioreactor assembly and structural components. [Color figures can be viewed at wileyonlinelibrary.com.]

Air is supplied by a compressor, with pressure regulated by external and internal controllers. The airflow is directed through the compressor's outlet valves and individual pressure valves for each bioreactor. Each bioreactor is equipped with a flow meter to monitor the amount of air supplied. After passing through the flow meter, the air is directed to a humidifier, which adds moisture to the dry air, helping to maintain adequate humidity within the bioreactors. The humidified air is then introduced into the reactors to promote forced aeration. To protect the oxygen sensor and reduce moisture content, the air passes through a container containing silica. Temperature sensors, in direct contact with the substrates inside the bioreactors, are connected to the Microsol II plus monitoring equipment to record thermal variations during the composting process.

The equipment is equipped with a differential temperature control (DTC) system that operates based on two sensors installed in each bioreactor. Sensor S1 is positioned at the mid-height of the chamber, embedded in the central region of the composting material, while sensor S2 is located at the same height but near the inner wall of the chamber. The DTC system is designed to activate an electric heater located in the cylindrical housing to reduce heat loss to the external environment. This activation occurs when the temperature difference between S1 and S2 exceeds 1.2°C. The heater remains active until the difference drops below 0.9°C, at which point it is turned off. Furthermore, the heater is not activated if the temperature measured by sensor S1 exceeds a predefined threshold. This upper limit is user-configurable and can be adjusted according to the desired composting phase. For example, during the mesophilic phase, the cutoff temperature was set to 39°C to maintain microbial activity within the optimal range and avoid overheating. All sensors are connected to individual controllers for each bioreactor, which manage the heater function and are integrated with the SITRAD software. This system enables real-time monitoring and storage of temperature data, ensuring effective thermal regulation and oversight of the composting process.

2.4 | Physicochemical Analyses

Physicochemical analyses of cattle manure, compost bedding, and wood shavings were outsourced to and performed by Engequisa Laboratórios de Análises Ambientais (Zona da Mata unit, Juiz de Fora, Minas Gerais, Brazil). The parameters evaluated included electrical conductivity (μS/cm), organic matter (mg/kg),

EXHIBIT 3 | Physicochemical characterization of composting materials.

Analysis	Cattle manure	Sawdust	Compost barn
Conductivity (μS/cm)	3983	136.9	5313
OM (mg/Kg)	104,000	60,332.2	312,000
Nitrate (mg/Kg)	196	54.1	55.6
Nitrite (mg/Kg)	45.4	—	19.5
Total nitrogen (mg/Kg)	15,171	536	6785
pH	5.89	6.96	7.91
Solids content (%)	12.3	90	34.3
Total phosphorus (mg/Kg	1072.5	0.482	672.5
TOC (mg/Kg)	214,737.6	34,995.5	96,053.1
Kjeldahl nitrogen (mg/Kg)	14,929	—	6709
Fixed solids (mg/Kg)	4000	6667	56,000
Volatile solids (mg/Kg)	104,000	993,333	312,000

Abbreviations: OM, organic matter; TOM, total organic carbon.

nitrate (mg/kg), nitrite (mg/kg), total nitrogen (mg/kg), pH, solids content (%), total phosphorus (mg/kg), total organic carbon (mg/kg), Kjeldahl nitrogen (mg/kg), fixed solids (mg/kg), and volatile solids (mg/kg). The results are presented in **Exhibit 3**. Additionally, pH and total solids (TS) were measured at the beginning (T_0) and at the end (Tf) of all experiments to provide a comprehensive analysis of process efficiency. In this context, T_0 refers to the initial compost bedding mixture introduced into the bioreactors on the first day of each test, while Tf represents the final compost material collected from all three bioreactors at the conclusion of the experiment. pH was determined using a calibrated benchtop pH meter. Samples were diluted in distilled water (1:10 w/v), homogenized, and measured after stabilization. TS were assessed gravimetrically following APHA guidelines (American Public Health Association (APHA) 2012). Approximately 10 g of each sample was dried at 105°C for 24 h, and TS (%) was calculated as the ratio of dry to wet mass (Andrade et al. 2022). Data were analyzed and visualized in R using the ggplot2 package. A bar chart was used to compare TS values between T_0 and Tf for each test. A boxplot displayed pH variation across tests and time points. These visualizations are included as Supporting Material to support the interpretation of composting dynamics.

The mass of each composting reactor was monitored by weighing the samples on an analytical balance at the beginning (Day 1) and end (Day 7) of each test. Weight data were corrected for daily substrate additions (manure and urine) to accurately assess net mass loss due to decomposition. This approach is consistent with mass balance methods applied in on-farm and laboratory-scale composting studies (Andrade et al. 2022, Poindexter et al. 2025). Statistical analysis and data visualization were performed using RStudio software. Specifically, bar plots were used to illustrate the percentage of mass loss per reactor and test, while box plots with jittered points were generated to compare the distribution of mass loss across the three experimental tests. These graphical tools allowed for a clearer interpretation of the variability and trends in composting efficiency.

2.5 | Microbiological Analyses

The microbiological analyses were conducted to verify whether the system effectively replicates CB conditions, ensuring efficient organic matter degradation, proper microbial succession, and pathogen reduction. Monitoring microbial decline at different temperature ranges allowed us to assess the effectiveness of thermal control, confirm the transition between composting phases, and determine whether the conditions promoted the expected microbial activity. Additionally, these analyses helped validate the system's ability to eliminate pathogens and optimize the composting process by identifying the most favorable operational conditions.

Microbiological analyses were conducted for Tests 2 and 3. Bedding and feces samples were collected at the beginning and end of each test week to analyze population groups and perform viable bacterial counts. Samples were diluted and inoculated on appropriate selective media, incubated under suitable conditions, and colony counts were conducted after 24 h of incubation (Serrano et al. 2020). Initial microbiological characterization included the counting and isolation of microorganisms present in the biocompost of the composters, followed by detailed biological analyses. The study was conducted at Embrapa Dairy Cattle in Juiz de Fora, Minas Gerais, where compost bedding samples were collected in sterile jars and transported aseptically in a cooler to the Rumen Microbiology Laboratory.

Microbiological analysis was performed by serial dilution in 0.9% saline solution. For this, 10 g of compost bedding was suspended in 90 mL of saline solution and agitated for 30 min, following the protocol described by Manni and Filali-Maltouf (2022), producing the initial solution. The samples were serially diluted up to eight times, and plating was conducted on Brain Heart Infusion Agar (BHI) for total colony counting. Then, 0.1 mL of each dilution was spread with a Drigalsky loop. Bacterial population differentiation was performed using selective media,

such as Mannitol Agar, Eosin Methylene Blue (EMB) Agar, and Sabouraud Agar, incubated for 24 h at 35°C.

Quantification was based on direct counting of colony-forming units (CFU), considering plates with 20–200 colonies, and the results were multiplied by the corresponding dilution factor. The microbial decay rate (MDR) was calculated using Equation (1).

$$K = \frac{1}{t}.\ln\left(\frac{N(t)}{N0}\right) \quad (1)$$

where $N(t)$ is the microbial population at time t, $N0$ is the initial microbial population (at time $t = 0$), k is the microbial decay constant, representing the decay rate, and t is the incubation or monitoring time.

2.6 | Statistical Analyses

Statistical analyses were conducted using R software to evaluate the effect of two treatments (Tests 2 and 3) on the number of microbial colonies (NC), assessed at two different time points (Initial and Final). The dependent variable NC was analyzed across three distinct microbial groups: fungi, Gram-positive bacteria (Gramp), and Gram-negative bacteria (Gramn).

Initially, assumption tests were performed to ensure the appropriateness of parametric methods. The normality of the NC variable was assessed using the Shapiro-Wilk test, while the homogeneity of variances among treatment groups was verified using Bartlett's test. Subsequently, a linear model with fixed effects was fitted using the lm() function in R, considering the factors time (Initial vs. Final), treatment (mesophilic vs. no-control), and microbial group (fungi, Gramp, and Gramn), according to the following formula: NC ~ Time + Treatment + TipoGram. This model allowed us to evaluate the individual contributions of each factor to the variation in colony counts and to detect overall trends in microbial reduction over time.

To visualize the data, exploratory graphs were created using the ggplot2 package. Boxplots with overlaid jittered points were produced to show the distribution of colony counts under each experimental condition. Line plots displaying mean ± standard error were generated with the ggline() function from the ggpubr package, faceted by microbial group to illustrate the effect of treatment over time.

Additionally, the ggbetweenstats() function from the ggstatsplot package was used to perform automated pairwise comparisons between time points. This function provided visual representations of p-values, confidence intervals, and effect sizes (rank-biserial correlation). Since the data did not meet parametric assumptions, the comparison was based on the Mann–Whitney U test. The calculated effect sizes offered estimates of the magnitude of the observed differences.

These analyses were fundamental in verifying whether the treatments resulted in a significant reduction in colony counts after the treatment period, providing statistical support for the hypothesis of microbial decay over time.

EXHIBIT 4 | Experimental setup of the laboratory bioreactor system. [Color figures can be viewed at wileyonlinelibrary.com.]

3 | Results

3.1 | Assembly of the Bioreactors

The equipment setup (Exhibit 4) consists of three 3-L bioreactors, an air compressor with external and internal pressure regulators, tubing for air and water passage (for air humidification), general pressure valves and individual pressure valves for each composter, three flow meters to regulate air intake, three humidifiers (one for each reactor), and temperature and oxygen sensors embedded in the bioreactors to monitor these parameters. This system is integrated with the SITRAD software, which records data on the operation of the bioprocess, including temperatures and respirometric data from the laboratory bioreactors. The successful setup of this equipment was a significant achievement, ensuring smooth integration and accurate monitoring of the bioprocess.

3.2 | Physicochemical Analyses

The physicochemical profiles of compost bedding, manure, and wood shavings used in the study are detailed in Exhibit 3. Parameters included electrical conductivity, organic matter, nitrogen forms, pH, moisture content, phosphorus, total organic carbon, and fixed and volatile solids. These baseline characteristics provided essential context for interpreting composting behavior and microbial activity.

3.3 | Bioreactor Operation and Composting Performance

The execution of three consecutive composting trials allowed for monitoring the evolution of system behavior and for identifying critical adjustments to improve performance. In Test 1, the bioreactors demonstrated the potential to simulate the dynamics of CB systems; however, the need for technical refinements quickly became evident. Temperature monitoring in reactors C1, C2, and C3 was essential to assess the composting progression and microbial activity (**Exhibit 5a**).

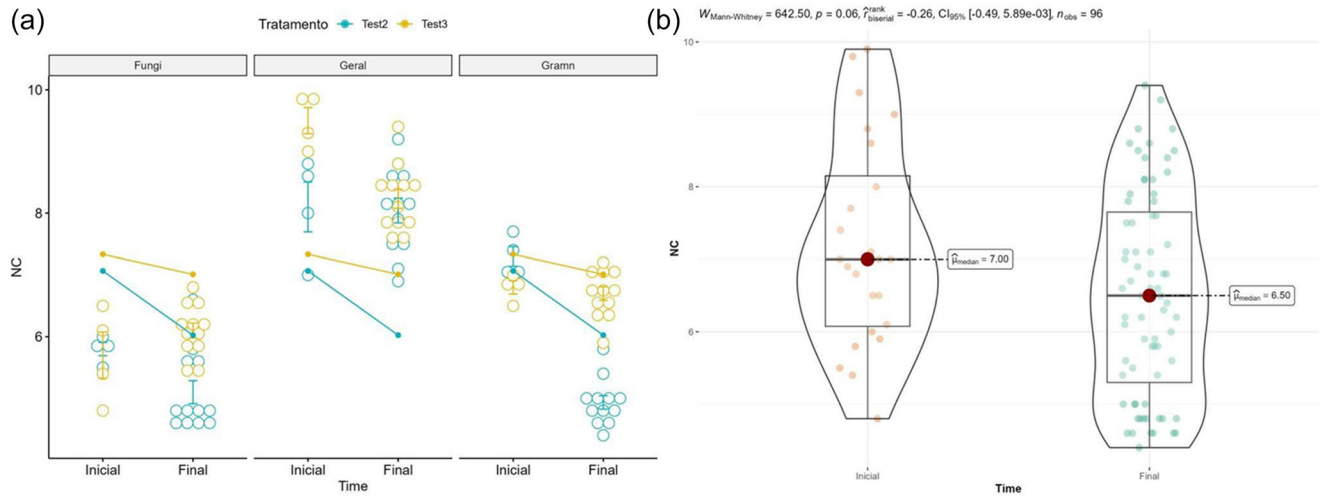

EXHIBIT 5 | Temperature monitoring under different conditions: (a) Test 1 - uncontrolled composting for 3 months; (b) Test 2 - uncontrolled composting for 1 week; and (c) Test 3 - mesophilic composting for 1 week. [Color figures can be viewed at wileyonlinelibrary.com.]

In reactor C1, temperatures reached 53.4°C in the early phase, indicating successful initiation of thermophilic composting. However, this peak was not sustained, and the system stabilized around 35°C, suggesting a transition to a phase of lower microbial activity. In C2, the temperature peaked at 49°C and then dropped to approximately 29.4°C, with brief thermophilic spikes near the end of the cycle, typically following the addition of wood shavings. These fluctuations indicated that, despite some limitations, the reactor progressed toward the compost maturation phase (Zhao et al. 2024).

In contrast, C3 displayed signs of system failure due to an assembly defect that restricted air intake, causing anaerobic conditions. This lack of oxygenation compromised microbial activity and inhibited proper decomposition, confirming that aeration is a key determinant of system performance (Dong et al. 2024). Correcting this flaw was essential for the success of the subsequent experiments.

Although the first test demonstrated that bioreactors could replicate composting dynamics, the effective composting window proved to be much shorter than that of full-scale CB systems. Even with weekly additions of structural material, the active degradation phase appeared to last only about 7 days, as evidenced by the decline in temperature and odor stabilization across all units. These findings highlight the need to adapt residence time and substrate management for lab-scale composting systems, given their limited thermal inertia and faster metabolic turnover.

With this insight, Test 2 was restructured to follow a 1-week composting cycle, and technical corrections were implemented. Aeration tubes were repaired, and wood shavings were added at the beginning of the process, ensuring better initial conditions. Real-time temperature monitoring revealed a clear rise in temperature following the introduction of fresh feces and urine, reflecting increased microbial activity stimulated by nutrient availability (Han et al. 2024, Wang et al. 2024) (Exhibit 5b). Although the thermophilic phase was short, this response was consistent with expectations for small bioreactor volumes (~1 kg per unit), where composting tends to occur more rapidly than in larger-scale systems.

Notably, bedding moisture content increased substantially during Test 2. Initial values of 51.3% rose to 66.11%, 70.21%, and 71.08% in reactors C1, C2, and C3, respectively. This rise is attributed to microbial metabolism and the release of water during organic matter breakdown, a typical phenomenon in early composting phases.

Test 3 aimed to evaluate whether controlling temperature at mesophilic levels (maximum 45°C) would improve composting consistency and pathogen inactivation (Exhibit 5c). Despite thermal regulation through the SITRAD system, the results revealed low organic degradation rates and ineffective microbial reduction. The temperature remained below the critical thermophilic threshold (>55°C), which is considered essential for efficient pathogen inactivation (Lin et al. 2022). Consequently, the process failed to achieve the sanitary targets observed in Test 2, suggesting that mesophilic composting alone is insufficient for treating organic bedding from CB systems.

3.4 | Microbial Quantification and Reduction

Microbiological analyses were conducted in Tests 2 and 3 to evaluate the effectiveness of different operational strategies in reducing microbial loads. Three target groups were assessed: Gram-positive bacteria, Gram-negative bacteria, and fungi/yeasts. CFUs ($\log_{10}$ CFU/g) were measured at the start (T_0) and end (Tf) of each 1-week composting cycle, and MDRs were calculated (**Exhibit 6**).

In Test 2, significant microbial reduction was observed, particularly among Gram-negative bacteria, whose counts decreased from 7.3 to an average of 4.9–5.3 $\log_{10}$ CFU/g. Although complete inactivation was not achieved, this represents a notable decline within a short residence time. Reductions were also recorded for fungi/yeasts (5.8 → 5.0–5.9 $\log_{10}$ CFU/g) and Gram-positive bacteria (8.6 → 7.4–8.8 $\log_{10}$ CFU/g), although to a lesser extent.

EXHIBIT 6 | Initial and final microbial counts during the composting process in Tests 2 and 3.

Microbial count ($\log_{10}$ cfu.g^{-1})					
Microorganism	Compost barn (T_0)	R1 (Tf)	R2 (Tf)	R3 (Tf)	MDR
Test 2					
Gram + bacteria	8.6	8.8	7.4	8.2	0.47
Gram − bacteria	7.3	5.3	4.9	4.6	2.37
Fungi and yeasts	5.8	5.9	5	4.7	0.6
Test 3					
Gram + bacteria	9.5	8.7	8	8	1.27
Gram − bacteria	6.8	6.4	6.7	7	0.1
Fungi and yeasts	5.7	6.1	6	6.2	−0.4

Abbreviations: MDR, average microbial decay rate; R1, bioreactor 1; R2, bioreactor 2; R3, bioreactor 3; T_0, initial time of the samples, immediately after being placed inside the bioreactors; Tf, final time of the composting process in the bioreactors after 7 days of monitoring.

The observed microbial decay supports the conclusion that active aeration, coupled with fresh substrate input, enhances pathogen reduction—even in the absence of controlled heating.

In Test 3, however, results indicated a lower efficiency of microbial inactivation. The mean MDRs were minimal, and in some instances, such as for fungi and yeasts, counts even increased over time. Gram-negative bacterial levels changed only slightly (6.8 → 6.4–7.0 $\log_{10}$ CFU/g), and Gram-positive bacteria remained elevated throughout (9.5 → 8.0–8.7 $\log_{10}$ CFU/g). These outcomes align with the thermal performance of the system, which failed to reach thermophilic temperatures necessary for effective microbial suppression (Lin et al. 2022, United States Environmental Protection Agency 1995).

The overall comparison between Tests 2 and 3 clearly demonstrates the importance of reaching thermophilic conditions to achieve meaningful reductions in microbial populations. Although mesophilic composting may support organic stabilization to some extent, it does not meet the sanitary standards required for safe reuse or land application of composted material from CB systems.

3.5 | pH and TS Dynamics

The comparison of pH and TS across the three experimental tests revealed important differences in composting performance under varying operational conditions. In Test 1, the TS content decreased from 63.1% to 50.7%, indicating moderate organic matter degradation over the three-month cycle (Wang et al. 2024, Oliveira et al. 2023). The pH increased slightly from 9.56 to 9.65, suggesting a limited shift in microbial activity, possibly due to suboptimal aeration and system failures. In Test 2, the reduction in TS was more pronounced, dropping from 51.3% to 41.4% over a 1-week period. This result reflects a more efficient composting process under improved aeration and substrate conditions. The pH in this test showed a slight decrease from 9.44 to 9.42, remaining within an alkaline range consistent with active microbial decomposition. In contrast, Test 3, which operated under controlled mesophilic temperatures (39°C), exhibited minimal reduction in TS, from 45.7% to 43.1%, and a pH increase from 8.41 to 9.16. Despite the alkaline shift, the limited reduction in solids suggests that microbial activity and degradation were insufficient under the applied thermal conditions (Wang et al. 2024, Serrano et al. 2020). Overall, while pH remained relatively stable across tests, the variation in TS and corresponding mass loss proved to be more reliable indicators of composting efficiency. These trends are further illustrated in Figures S1 and S2.

3.6 | Statistical Analyses

In Test 2, which involved a single week of composting with improved aeration and no control group, a statistically significant reduction in NC was observed. A Wilcoxon signed-rank test comparing T_0 and T_e revealed a significant decrease in Gram-negative bacterial counts ($V = 0$, $p = 0.03$), with values dropping from 7.3 to 5.3 $\log_{10}$ cfu.g^{-1}. This result indicates that, although complete pathogen elimination was not achieved, short-term operation under well-aerated conditions promoted a meaningful microbial reduction (**Exhibit 7**).

Temperature increases following the addition of fresh manure and the overall decrease in bedding moisture—key factors in microbial activity—are detailed in Exhibit 5, and help explain the improved performance of the composting process during this phase.

In Test 3, to evaluate the efficacy of mesophilic composting in reducing coliform counts (NC), a Mann–Whitney U test was performed comparing the initial (T_0) and final (T_e) microbial loads. The results showed no statistically significant reduction in NC ($W = 642.50$, $p = 0.06$), although the median decreased from 7.00 to 6.50 $\log_{10}$ cfu.g^{-1}. This suggests a tendency toward microbial reduction; however, it was not sufficient to indicate effective pathogen inactivation under mesophilic conditions alone. This interpretation is supported by the low corrected weight loss observed in Test 3 reactors, with values ranging from 0.88% to 2.93%, indicating limited organic matter degradation. In contrast, higher weight loss was recorded in Tests 1 and 2, with values up to 24.23%, suggesting that the composting conditions in Test 3 were less favorable for efficient decomposition (Lin et al. 2022). These data are presented in Exhibit 7 and Figure S3.

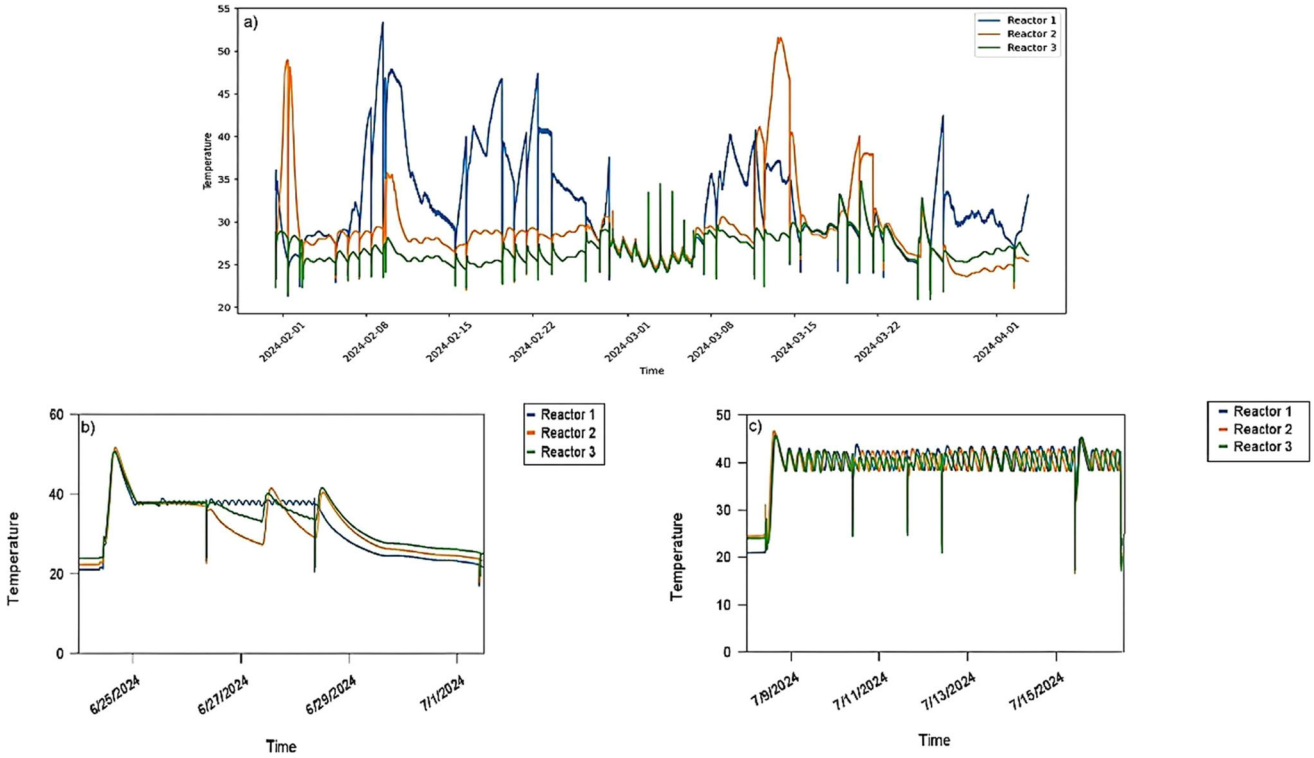

EXHIBIT 7 | Statistical analysis (Mann–Whitney *U* test) of coliform counts during Tests 2 and 3. [Color figures can be viewed at wileyonlinelibrary.com.]

4 | Discussion

Unlike previous systems, such as the one proposed by De Boer and Wiersma (2021), which were developed for general thermophilic composting without real-time control, the system implemented in this study offers a more effective approach to temperature regulation and bioprocess monitoring. By integrating temperature sensors directly into the composting substrate, the system enables continuous data transmission every 30 s to the Microsol II Plus controller. This allows precise thermal regulation via a DTC mechanism: the heater is activated when the temperature difference between the core (S1) and the reactor wall (S2) exceeds 1.2°C and deactivated once balance is restored. Additionally, a maximum temperature threshold can be set to prevent overheating. All sensor data are stored and managed using SITRAD software, ensuring automated, uninterrupted monitoring throughout the entire composting process.

This configuration enables operation under mesophilic (<39°C), thermophilic (>45°C), or uncontrolled thermal regimes, offering superior experimental control and energy efficiency. Unlike traditional models relying on manual monitoring, this system ensures precise thermal regulation, essential for optimizing microbial activity and composting efficiency.

The challenges encountered during the experiments underscore the critical role of temperature control in the composting process. The insufficient elimination of pathogens highlights the necessity of achieving and maintaining thermophilic conditions—specifically, temperatures above 55°C—to ensure both effective composting and microbial inactivation. According to guidelines from the United States Environmental Protection Agency (1995) (USEPA), for adequate pathogen control, compost must remain at a minimum of 40°C for at least 5 days, with temperatures exceeding 55°C for at least 4 h during this period. In Test 1, difficulties in reaching and sustaining thermophilic temperatures (~55°C) suggested that the composting lifespan was likely shorter than 3 months, prompting a follow-up test with a reduced duration.

In Test 2, conducted over 1 week, the shortened residence time of compost in the bioreactor system limited organic degradation, as previously observed (Qian et al. 2023). This 1-week timeframe was selected based on the thermal patterns observed in Test 1, where thermophilic conditions were evident during the first seven days but were not sustained thereafter. Similar duration of thermophilic activity—typically lasting around 1 week—has been reported in composting processes. For instance, composting protocols frequently describe an initial mesophilic phase of approximately 2–8 days, followed by a thermophilic phase that also persists por cerca de 7 a 12 dias (Ren et al. 2025, Xie et al. 2025). In a study of cattle manure composting, a thermophilic stage was observed that peaked within the first week, associated with the highest microbial activity and rapid organic matter degradation (Xu et al. 2024). These observations align with the temperature profile recorded in Test 1 of our study, justifying the decision to restructure Tests 2 and 3 into 1-week cycles, which enables a more focused evaluation of composting efficiency and system dynamics under varied operational conditions.

Based on the extended monitoring conducted during Test 1, it became evident that the active composting phase lasted approx-

imately 1 week. The temperature profile showed an initial thermophilic peak followed by a progressive decline, indicating that under bioreactor conditions, degradation occurs more rapidly than in full-scale systems due to reduced thermal inertia and smaller substrate volume. This behavior was confirmed in Test 2, where the use of a 1-week cycle effectively captured the most biologically active phase. Ongoing studies are being conducted to compare the physicochemical and microbiological evolution of full-scale CB bedding—from its initial state through the end of its operational lifespan—with the initial and final profiles obtained in the bioreactors. These complementary analyses are expected to strengthen the representativeness of the proposed model for long-term composting dynamics.

The introduction of fresh bovine feces and urine boosted microbial activity and heat generation; however, temperatures did not reach the threshold necessary for full pathogen inactivation. This was confirmed through statistical analysis: A Wilcoxon signed-rank test revealed a significant reduction in Gram-negative bacterial counts ($V = 0$, $p = 0.03$), dropping from 7.3 to 5.3 $\log_{10}$ cfu.g^{-1}. Although this indicates meaningful microbial reduction under improved aeration, complete pathogen elimination was not achieved, reinforcing the importance of thermophilic conditions for effective sanitization (Wang et al. 2024, Xu et al. 2022).

To address thermal instability, Test 3 introduced a fixed mesophilic temperature (39°C) using an automated control system (SITRAD). However, this temperature proved insufficient to reduce coliform counts effectively. A Mann–Whitney U test comparing initial and final NC values showed no statistically significant reduction ($W = 642.50$, $p = 0.06$), despite a median decrease from 7.00 to 6.50 $\log_{10}$ cfu.g^{-1}. These results suggest a trend toward microbial reduction, but not at a level indicative of effective inactivation. The outcomes of Test 3 further corroborate previous findings (Xu et al. 2024), which reported reduced pathogen inactivation during composting of cattle manure under mesophilic conditions (~39°C). In both studies, despite stable temperature regulation, microbial indicators such as coliforms were not significantly reduced, highlighting a critical limitation of mesophilic composting for sanitation purposes. In our study, the use of automated thermal control maintained consistent mesophilic temperatures, which supported organic matter transformation to a limited extent—as indicated by changes in pH and TS—but failed to achieve effective microbial elimination. These results reinforce the notion that mesophilic operation, although beneficial for thermal stability and potentially energy efficiency, does not provide sufficient thermal stress to inactivate pathogenic microorganisms. This limitation is particularly important in the context of composting cattle-derived substrates, which often carry zoonotic pathogens and thus require rigorous sanitization protocols. Exhibit 5 further illustrates that reactor temperatures remained below 45°C with minimal moisture variation, two conditions known to hinder microbial die-off (Lin et al. 2022).

Together, these findings confirm that while mesophilic conditions support microbial activity and partial degradation, they fall short for sanitation purposes. This aligns with the behavior observed in Test 3, where, despite controlled mesophilic conditions, no pathogen reduction was observed, suggesting that the present microbial community was resistant to low thermal stress. Importantly, this also reflects real-world composting variability, as many systems operate below ideal thermophilic thresholds.

In addition to temperature, aeration intensity and the incorporation of structural agents such as wood shavings played crucial roles in process efficiency. Proper aeration improved airflow, prevented compaction, and promoted microbial respiration, while structural materials like wood shavings facilitated heat distribution and microbial colonization (Guo et al. 2019, Wang et al. 2024). However, even with these interventions, the composting process exhibited a limited lifespan. The aeration rate of 1 L/h and the short processing window constrained microbial performance and organic matter degradation. Aeration rates directly influence heat retention and compost maturity, as previously reported (Han et al. 2024).

The results emphasize the importance of extending residence time and reaching thermophilic ranges (>50°C) to ensure pathogen inactivation and compost stabilization. Therefore, future trials will target these conditions and include monitoring of additional parameters such as carbon-to-nitrogen ratio (C:N), electrical conductivity, and nutrient content. These data will provide a more holistic understanding of compost maturity and microbial behavior under controlled conditions.

Finally, the successful assembly and verification of the bioreactor system represent an important step toward simulating real CB environments. Key operational improvements—such as the implementation of SITRAD software for thermal regulation—allowed for dynamic system adaptation and precise environmental control. These capabilities will be critical in upcoming experiments, which aim to test thermophilic operation, refine process parameters, and ultimately improve organic matter degradation and pathogen elimination in CB systems.

The patterns observed in pH and TS closely align with the mass loss data, collectively highlighting the differences in composting performance across the three tests. In Test 1, although affected by technical issues that compromised aeration, the extended composting period contributed to substantial mass loss (16.5%–24.2%) and a moderate TS reduction (63.1%–50.7%), suggesting ongoing, though suboptimal, microbial activity. The pH increase (from 7.12 to 9.15) further indicates progressive organic matter degradation under these conditions (Zhao et al. 2024, Wang et al. 2024).

In Test 2, the system benefited from improved aeration and the addition of fresh manure, which stimulated microbial activity and resulted in higher efficiency within a shorter time frame (Zhao et al. 2024). Notably, thermophilic temperatures (>45°C) were achieved, even if briefly, which likely enhanced organic degradation and partial pathogen reduction. This was reflected in a marked TS reduction (51.3%–41.4%), moderate mass loss (6.7%–10.3%), and a significant pH increase (6.52–9.48).

By contrast, Test 3 was conducted under controlled mesophilic conditions (~39°C). This setup led to minimal TS reduction (45.7%–43.1%), the lowest mass loss (0.9%–2.9%), and no measurable pathogen reduction. Although pH still rose significantly (5.04–9.29), likely due to ammonia release during decomposition, the limited change in solids and mass suggests that this nitroge-

nous compound degradation was not accompanied by significant lignocellulosic degradation, which is more efficiently carried out under thermophilic conditions (Zhao et al. 2024, Wang et al. 2024, Lin et al. 2022). This helps explain why such an increase in pH did not reflect in greater solids reduction or sanitization efficiency.

Additionally, the markedly low mass loss observed in Test 3 (0.9%–2.9%) reinforces the indication of limited biotransformation, as mass reduction is closely associated with the mineralization of organic matter and CO_2 release during active composting. The restricted decrease in mass suggests that microbial metabolism remained predominantly in an early-stage transformation phase, without reaching the sustained degradation rates typically associated with thermophilic activity (Zhao et al. 2024, Xu et al. 2024). This lack of substantial mass conversion further supports the conclusion that mesophilic conditions alone are insufficient to drive effective compost stabilization and sanitization (Wang et al. 2024, Lin et al. 2022).

These results highlight the influence of temperature on composting efficiency. Although thermophilic conditions (45–65°C) are known to enhance organic matter degradation and pathogen inactivation (Lin et al. 2022), several studies have reported that CB bedding often exhibits temperature stratification across its depth profile (Oliveira et al. 2023, Andrade et al. 2022, Oliveira et al. 2023). The upper layers, more exposed to ambient air and direct contact with the cows, typically remain within the mesophilic range (30–40°C), while thermophilic zones are confined to deeper, less disturbed regions. Exhibit 5 illustrates that temperatures in Test 3 remained within the mesophilic range throughout the experiment, with limited thermal stimulation of microbial activity. Consistently, Exhibit 7 shows the lowest mass loss under these operational conditions, and Exhibit 6 confirms the absence of microbiological reduction.

The limited reduction in TS and pathogen indicators under these conditions may reflect the natural constraints of microbial activity and aeration in the bedding surface layer. These observations emphasize the need for improved management strategies—such as periodic turning to mix bedding layers thoroughly, ensuring that surface material is incorporated into deeper zones, thereby promoting more uniform thermophilic conditions throughout the pack and enhancing overall compost stability.

These findings indicate that, although mesophilic operation ensures thermal stability, it is insufficient to promote substantial degradation or sanitization when applied in isolation. Aeration combined with the achievement of thermophilic temperatures is necessary to sustain microbial activity and improve composting performance.

5 | Conclusion

This study successfully assembled and validated a laboratory-scale bioreactor system capable of simulating CB bedding dynamics under controlled conditions. The experimental approach achieved its primary objectives by demonstrating that the reactors can mimic key composting processes, including organic matter degradation, thermal variation, and microbial succession.

Test 1 highlighted the limitations of long-term, unmanaged composting in small-scale systems, emphasizing the importance of optimizing operational parameters. Test 2 confirmed that 1-week cycles with active aeration promote significant microbial activity and partial pathogen reduction, while Test 3 showed that mesophilic temperature control, although thermally stable, was insufficient to achieve complete sanitization. These findings reflect both the constraints and opportunities inherent in small-scale CB simulations, particularly the impact of temperature stratification on microbial dynamics.

Overall, the results validate the use of laboratory bioreactors as viable tools for investigating CB composting under realistic operational conditions. They also underscore the critical role of temperature and aeration in composting efficiency, microbial activity, and pathogen control. Future studies should focus on achieving sustained thermophilic conditions and refining reactor design to enhance organic matter stabilization, biosafety, and representativeness of field conditions.

Complementary experiments are currently underway under controlled thermophilic conditions to further assess reactor performance, improve microbial efficiency, and optimize compost stabilization.

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Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Materials: tqem70254-sup-0001-FigureS1-S5.docx