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Effects of Rice Husk Biochar, Wood Vinegar, and a Bacillus-Lactobacillus Inoculant on Odor Intensity and Quality of Dairy Cattle Manure Compost in Cu Chi

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ABSTRACT

The mismanagement of dairy cattle manure under tropical conditions contributes to odor pollution, nutrient loss, and environmental degradation. This study evaluated the individual and combined effects of wood vinegar (pyroligneous acid), rice husk biochar, and a microbial inoculant comprising Bacillus licheniformis, Bacillus megaterium, and Lactobacillus acidophilus on odor reduction, composting kinetics, and the physicochemical and microbiological quality of dairy manure compost in Ho Chi Minh City, Vietnam. A two-phase experimental design was employed: Phase 1 screened concentration–response relationships between each amendment and odor intensity using a trained sensory panel (VDI 3882-1 scale, 0–6), and Phase 2 assessed composting performance at the optimal concentrations identified — wood vinegar at 1:40 (v/v), rice husk biochar at 5% (w/w), and microbial inoculant at 2% (v/v) — over six weeks. All amendments exhibited concentration-dependent odor reduction. The combined treatment produced the greatest improvements in organic matter content (56.11%), humic acid (17.14%), total nitrogen (19.57%), and available phosphorus expressed as P_2O_5 (11.63%), and reduced compost maturation time to four weeks compared with six weeks for the unamended control. The resulting compost met multiple quality thresholds of the Vietnamese national standard for microbial organic fertilisers (TCVN 7185:2002). These findings demonstrate that this bio-based amendment system offers a low-cost, scalable approach to sustainable dairy manure management under tropical conditions.

Key words: dairy manure; rice husk biochar; wood vinegar; microbial inoculant; odor reduction

INTRODUCTION

The intensification of dairy farming in Vietnam has generated substantial quantities of cattle manure that exceed the assimilative capacity of conventional field application methods. This imbalance exerts mounting pressure to develop environmentally sound and agronomically productive manure management strategies. Under tropical conditions with ambient temperatures of 28–32 °C and relative humidity consistently above 75%, fresh manure undergoes rapid microbial decomposition, releasing malodorous volatile compounds including ammonia (NH_3), hydrogen sulfide (H_2S), and short-chain volatile fatty acids. Such emissions not only diminish the fertiliser value of manure but also contribute to air quality deterioration and pose risks to ecological and human health.

To address these limitations, biologically based manure treatments have emerged as promising alternatives due to their environmental compatibility and potential to enhance compost quality (Zulkifli *et al*., 2019). Wood vinegar, also referred to as pyroligneous acid, is a chemically diverse byproduct obtained through the pyrolysis of lignocellulosic biomass under limited oxygen conditions. Its composition includes various bioactive compounds such as acetic acid, methanol, phenolic derivatives, aldehydes, and ketones, which collectively confer potent antimicrobial, antifungal, insecticidal, and deodorizing activities (Ouattara *et al*., 2023; Takahara *et al*., 1993). These properties have established wood vinegar as a valuable additive in agricultural and environmental applications, particularly in organic waste treatment and composting. Its efficacy in neutralizing malodorous compounds has been linked to the high concentration of volatile organic acids, which interfere with odor-generating microbial pathways (Takahara *et al*., 1993). Moreover, wood vinegar has been demonstrated to improve composting dynamics by stimulating microbial activity, promoting the proliferation of beneficial organisms, and enhancing nutrient immobilization within the compost matrix (Gama *et al*., 2024).

Rice husk biochar is a porous, carbon-rich material produced under oxygen-limited pyrolysis conditions, widely recognized for its multifunctional role in organic waste management and soil enhancement. Its distinctive physicochemical structure facilitates improved composting dynamics by serving as a physical matrix that enhances aeration, retains moisture, and provides favourable microhabitats for microbial colonisation (Vu, 2019; He *et al*., 2024). Abu *et al*. (2024) reported significant reductions in methane, nitrous oxide, and ammonia emissions when biochar was integrated into composting systems, attributing these effects to its adsorption properties and ability to regulate microbial metabolic pathways. Agronomically, biochar application has yielded favorable soil outcomes, with documented improvements in nutrient retention, cation exchange capacity, and crop productivity. Hayat *et al*. (2023) demonstrated that biochar-amended soils significantly improved rice growth indices, including plant height and grain yield, corroborating its role as a sustainable soil amendment with long-term agronomic benefits.

Bacterial consortia have been increasingly explored as inoculants to enhance composting efficiency. Among the most studied species, *Bacillus licheniformis* has been shown to significantly accelerate organic matter degradation by elevating hydrolytic enzyme activities; particularly amylase, surged up to 7.68-fold, accelerating substrate decomposition; while simultaneously raising composting temperatures to improve carbohydrate metabolism. Inoculation with this organism has also been linked to a 20% increase in humic acid content, indicating enhanced humification of the final compost product (Su *et al*., 2024; Chang *et al.*, 2023). *Bacillus megaterium* complements these effects through its broad enzymatic capacity to degrade cellulose, lignin, and other complex organic compounds commonly found in agricultural waste. Beyond direct substrate breakdown, *B. megaterium* also promotes cooperative microbial interactions within the composting community, thereby improving overall process efficiency (Kuroda *et al*., 2017). *Lactobacillus acidophilus* contributes to pH regulation by metabolizing organic acids that accumulate during early fermentation stages, thereby preventing inhibitory acidification. It has also been associated with enhanced microbial diversity, which supports the stability and resilience of the composting system (Song *et al.*, 2018; Chang *et al.*, 2023).

While the individual applications of these amendments are well documented, comprehensive studies systematically evaluate their combined effects on odor control, microbial dynamics, nutrient stabilization, and gaseous emission reduction in dairy manure composting remains are still limited, particularly under tropical climatic conditions.

The present study therefore aims to evaluate the effectiveness of, and interactions among, wood vinegar, rice husk biochar, and microbial inoculants in the integrated treatment of dairy cattle manure. Specifically, it investigates optimal dosage for odor suppression, tracks biological and physicochemical composting parameters (temperature, pH, organic matter degradation), and assesses the final product’s agronomic and microbiological quality. The findings are expected to provide practical guidance for the development of low-cost, scalable, and ecologically sound strategies for manure management in organic dairy farming systems.

MATERIALS AND METHODS

Experimental design and sample preparation

This study was conducted in two phases: (i) olfactory screening of individual bio-based amendments and (ii) composting trials conducted at the optimal amendment concentrations identified in Phase 1. All experiments were conducted under ambient conditions in Ho Chi Minh City, Vietnam (mean temperature 28–32 °C; relative humidity 75–85%), from February to October 2025. Fresh dairy cattle manure was collected from a household-scale dairy farm located in Cu Chi District, Ho Chi Minh City, where the herd size was approximately 60 animals. Samples were collected randomly in the early morning to ensure representativeness and batch consistency. Following collection, manure was stored in sealed polyethylene containers at < 5 °C and processed within 24 h.

Bio-based amendments

Wood vinegar and rice husk biochar were produced via pyrolysis at the School of Technology, Faculty of Applied Technology, Van Lang University (Vietnam). Hybrid acacia wood was pyrolyzed at 500 °C under anaerobic conditions. Dried rice husks with a particle size of less than 5 mm were pyrolyzed at 400 °C for four hours under anaerobic conditions. The principal physicochemical characteristics of wood vinegar were as follows, pH 2.83; acetic acid content 2.63%; phenol concentration 76 mg/L; furfural concentration 33 mg/L; acetone concentration 14 mg/L; and total organic acid content 24.6 mg KOH/g. The rice husk biochar was ground and sieved to obtain particles smaller than 2 mm. Its elemental composition was C 39%, O 37%, Si 29%, and K 1.4%.

The microbial inoculant used was prepared at the Home Ecological Center, Ho Chi Minh City. The product contained a 1:1:1 (v/v) mixture of three microorganisms: *Bacillus licheniformis*, *Bacillus megaterium*, and *Lactobacillus acidophilus*. Viable cell counts were quantified at the laboratory of the Home Ecological Center according to the Vietnamese national standard TCVN 4884-1:2015 (ISO 4833-1:2013), which specifies enumeration of colony-forming units (CFU) by the pour-plate technique at 30 °C. The viable cell counts of the stock inoculant were *Bacillus licheniformis*, 3.0×10^8 cfu/mL; *Bacillus megaterium*, 2.7×10^8 cfu/mL; and *Lactobacillus acidophilus*, 3.0×10^9 cfu/mL, as determined by the pour-plate method (TCVN 4884-1:2015). For Phase 1, the inoculant stock solution was serially diluted in sterile distilled water to obtain working concentrations of 0.5–4.0% (v/v). These dilutions corresponded to effective total viable cell concentrations in the range of 10^8–10^9 cfu/mL within the treatment units.

Phase 1: Odor reduction screening

Phase 1 employed a completely randomized design (CRD) to evaluate the dose–response relationship between each amendment and odor intensity. The three amendments were tested independently; no combined applications were made in this phase. Treatment levels were as follows:

- Wood vinegar: There are ten dilution ratios in distilled water - 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, and 1:90 (v/v), plus a control (non-wood vinegar).
- Rice husk biochar: There are ten incorporation rates: 1% - 3% - 5% - 7% - 9% - 11% - 13% - 15% - 17% - 19% (w/w, on a fresh manure basis), plus a control (non-rice husk biochar).
- Microbial inoculant: Using eight application concentrations: 0.5% - 1.0% - 1.5% - 2.0% - 2.5% - 3.0% - 3.5% - 4.0% (v/v), plus a control (non-microbial inoculant).

Each treatment was applied to 5.0 kg of fresh manure in a separate 10 L polystyrene foam container. Each treatment level was replicated three times; with 31 treatment levels including controls, across the three amendment types, this yielded a total of 93 experimental units. Containers were kept open at ambient temperature during the 24 h assessment window. Odor intensity was evaluated by a trained sensory panel using the 0–6 point ordinal scale defined by VDI 3882-1, where 0 denotes no detectable odor and 6 denotes an extremely strong odor. Assessments were conducted by a trained panel of three evaluators in a well-ventilated, odor-neutral room to minimize carry-over effects. Each treatment unit was evaluated at three points of time: immediately after treatment (0 hours), after 12 hours, and after 24 hours. Panelists recorded odor scores independently, and mean scores per treatment were subsequently calculated.

Phase 1 results were used to rank treatments based on mean odor scores recorded at 24 hours. The optimal concentration for each amendment was selected using dual criteria: (i) lowest average odor score and (ii) cost efficiency, with a preference for higher dilution ratios where performance differences were small. These selected concentrations were subsequently applied in Phase 2, which focused on composting performance and long-term odor control.

Phase 2: Composting with optimal amendment concentrations

Phase 2 evaluated the effectiveness of the optimal bio-based amendment concentrations, as determined in Phase 1, on the composting performance of raw dairy cattle manure. Treatments were arranged in a completely randomized design. Each composting unit comprised 5 kg of fresh dairy manure thoroughly mixed with the designated amendment(s) and placed in covered foam containers. All treatments were replicated three times, yielding a total of 18 experimental units.

The monitoring period lasted up to six weeks or until compost maturity was achieved, as assessed by sensory and physicochemical criteria in accordance with the Vietnamese technical standard TCVN 7185:2002 for microbial organic fertilizer. Compost maturity was defined by the simultaneous fulfillment of the following conditions: substantial reduction or absence of offensive odor, loose and non-sticky texture, uniform dark brown coloration, temperature stabilized near ambient (≈28–30 °C), and pH within 6.0–7.5. Temperature and pH were measured weekly using a digital instrument capable of recording temperature, humidity, and pH. Measurements were taken at the central point of each composting unit to represent core biological activity.

The optimal treatment was identified using combined technical and economic criteria, including earlier attainment of maturity compared with the control, stable temperature and pH within the defined maturation range, and marked odor reduction as indicated by low panel odor scores on the 0–6 scale, alongside lower amendment input costs (favoring higher dilution efficiency where performance was comparable). All treatments were sampled at maturity for nutrient composition and microbial analysis, and the most promising formulation was then evaluated against the key thresholds of TCVN 7185:2002.

Statistical analysis

All data were compiled and verified in Microsoft Excel 365 (Microsoft Corp., USA) and subsequently transferred to IBM SPSS Statistics v.26 (IBM Corp., Armonk, NY, USA) for statistical analysis.

RESULTS

Odor assessment at different concentrations of bio-based amendments

All three bio-based amendments exhibited clear, concentration-dependent reductions in odor intensity within 24 h ($p < 0.05$), with higher application concentrations generally yielding lower sensory panel scores (**Table 1**).

Table 1. Sensory-based odor reduction scores (mean ± sd) at 12 and 24 hours post-treatment for different concentrations of wood vinegar, rice husk biochar, and microbial inoculant (n=3)

Concentration	12h (mean ± SD)	24h (mean ± SD)
Wood vinegar		
1:5	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
1:10	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
1:20	0.67 ± 0.47 ^{ab}	0.00 ± 0.00 ^{a}
1:30	1.67 ± 0.47 ^{b}	0.00 ± 0.00 ^{a}
1:40	2.33 ± 0.47 ^{bc}	0.67 ± 0.47 ^{ab}
1:50	3.00 ± 0.00 ^{cd}	1.33 ± 0.47 ^{b}
1:60	3.67 ± 0.47 ^{de}	2.33 ± 0.47 ^{c}
1:70	4.00 ± 0.00 ^{e}	3.00 ± 0.82 ^{cd}
1:80	4.67 ± 0.47 ^{f}	4.00 ± 0.82 ^{de}
1:90	5.67 ± 0.47 ^{g}	5.00 ± 0.82 ^{e}
Rice husk biochar (%)		
19%	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
17%	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
15%	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
13%	0.67 ± 0.47 ^{ab}	0.00 ± 0.00 ^{a}
11%	1.33 ± 0.47 ^{b}	0.00 ± 0.00 ^{a}
9%	2.00 ± 0.00 ^{bc}	0.00 ± 0.00 ^{a}
7%	2.67 ± 0.47 ^{cd}	0.00 ± 0.00 ^{a}
5%	3.67 ± 0.47 ^{de}	0.33 ± 0.47 ^{ab}
3%	4.33 ± 0.94 ^{e}	1.67 ± 0.47 ^{b}
1%	4.67 ± 0.47 ^{e}	3.00 ± 0.82 ^{c}
Microbial inoculant (%)		
4%	0.67 ± 0.47 ^{a}	0.00 ± 0.00 ^{a}
3.5%	1.33 ± 0.47 ^{ab}	0.00 ± 0.00 ^{a}
3%	1.67 ± 0.94 ^{b}	0.00 ± 0.00 ^{a}
2.5%	3.00 ± 0.82 ^{c}	0.33 ± 0.47 ^{ab}
2%	3.67 ± 0.47 ^{cd}	0.67 ± 0.47 ^{b}
1.5%	4.67 ± 0.47 ^{de}	1.67 ± 0.47 ^{c}
1%	5.67 ± 0.47 ^{ef}	3.33 ± 0.47 ^{d}
0.5%	6.00 ± 0.00 ^{f}	3.67 ± 0.47 ^{d}

Notes: Values are expressed as mean ± standard deviation (SD). Different lowercase letters within the same column indicate significant differences between concentrations ($p < 0.05$).

At 12 h, wood vinegar dilutions of 1:5 and 1:10 (v/v) reduced odor intensity to undetectable levels (score = 0), whereas more dilute treatments (≥ 1:20) produced progressively higher odor scores. At 24 h, odor scores remained at zero for the 1:5 and 1:10 dilutions, whereas more dilute treatments (≥ 1:20) yielded scores above zero that increased progressively with increasing dilution ratio, consistent with a concentration-dependent pattern.

Statistical analysis indicated that wood vinegar concentration significantly affected odor scores at both 12 and 24 hours ($p < 0.001$). Lower dilution ratios (1:5–1:40) consistently yielded lower odor scores than the most dilute treatments (≥1:50), which retained noticeable odor over 24 hours. A 1:40 dilution was selected for Phase 2 based on dual criteria which were odor suppression efficacy and cost efficiency, as it produced substantial odor reduction while requiring a markedly lower input volume than the most concentrated treatments.

For rice husk biochar, odor scores at 12 h increased inversely with application concentration, such that the highest biochar rates produced the lowest odor intensities. At 24 h, higher biochar concentrations maintained minimal or undetectable odor levels, whereas lower concentrations (≤ 5% w/w) showed measurable residual odor. Biochar concentration had a statistically significant effect on odor scores at both 12 and 24 hours ($p < 0.001$). Concentrations in the higher range achieved markedly lower odor scores than the lowest tested levels ($p < 0.05$), demonstrating a clear dose–response relationship. For Phase 2, a biochar incorporation rate of 5% (w/w) was selected, as it delivered substantial odor reduction within 24 h while minimizing material requirements relative to higher-performing but more resource-intensive concentrations.

For the microbial inoculant, odor scores at 12 h decreased with increasing application concentration, with the highest tested levels yielding the lowest odor intensities. By 24 h, higher concentrations produced further reductions in odor relative to lower doses, demonstrating a positive effect of inoculant loading on odor mitigation. The effect of inoculant concentration on odor scores at 12 and 24 hours was statistically significant ($p < 0.001$). Higher inoculant concentrations produced substantially lower odor scores than the most dilute treatments ($p < 0.05$), confirming a concentration-dependent odor control effect. A concentration of 2.0% (v/v) was selected for Phase 2, as it achieved substantial odor suppression within 24 h while requiring a lower inoculant volume than the highest-performing but more resource-intensive doses.

Composting performance at optimal concentrations

The pH and temperature evolution of the composting mixtures varied among treatments (**Figure 1**). By the end of weeks 3 to 4, several amended treatments exhibited clear visual and physical indicators of compost maturity, including stabilized temperatures, complete odor abatement, and a uniform dark brown coloration consistent with advanced humification and substantial organic matter decomposition. Treatments that did not yet reach these benchmarks were maintained under observation until similar maturity signals emerged.

At the outset of composting, all raw manure mixtures exhibited initial pH values of 5.2–5.6 and temperatures of 23.9–27.2 °C; differences among treatments were not statistically significant at this stage ($p > 0.05$). By Week 2, treatment divergence had become evident. Raw manure maintained a relatively stable pH (mean 5.8 ± 0.05), whereas biochar-amended treatments attained significantly higher values (mean 6.3–6.5) relative to both the unamended control and wood-vinegar-only treatments ($p < 0.05$). Microbial inoculation alone produced only slight increases, and the differences from raw manure were not statistically significant at this stage ($p > 0.05$). Marked differences were observed from Week 3 onwards. Raw manure rose to a near-neutral value (mean 6.0 ± 0.1), while biochar-containing treatments exhibited sharp increases (mean 7.1–7.9), significantly higher than all other treatments ($F = 25.4$, $p < 0.001$).

The combined application of rice husk biochar, wood vinegar, and the microbial inoculant produced the highest pH values (up to 8.0), although these did not differ significantly from biochar-only treatments ($p > 0.05$), consistent with biochar being the primary contributor to alkalinization. At Week 4, the separation among treatments persisted. Raw manure and wood vinegar-only treatments stabilized around pH 6.2, while biochar-containing and combined treatments maintained elevated levels (pH 7.0–8.0). Biochar-containing treatments were consistently distinct from both raw manure and wood vinegar-only ($p < 0.01$). Microbial inoculation alone showed a modest but statistically significant increase (mean 6.4 ± 0.1) compared with raw manure ($p < 0.05$), yet these values remained lower than those of biochar-containing systems. By Weeks 5–6, most biochar-based and combined treatments had satisfied the physicochemical criteria for compost maturity, whereas the unamended manure remained relatively stable with only minor changes in pH and temperature. By the final composting stage, most treatments had stabilized within the pH range of 6.0–8.0, which corresponds to the range specified in TCVN 7185:2002 for microbial organic fertilizers.

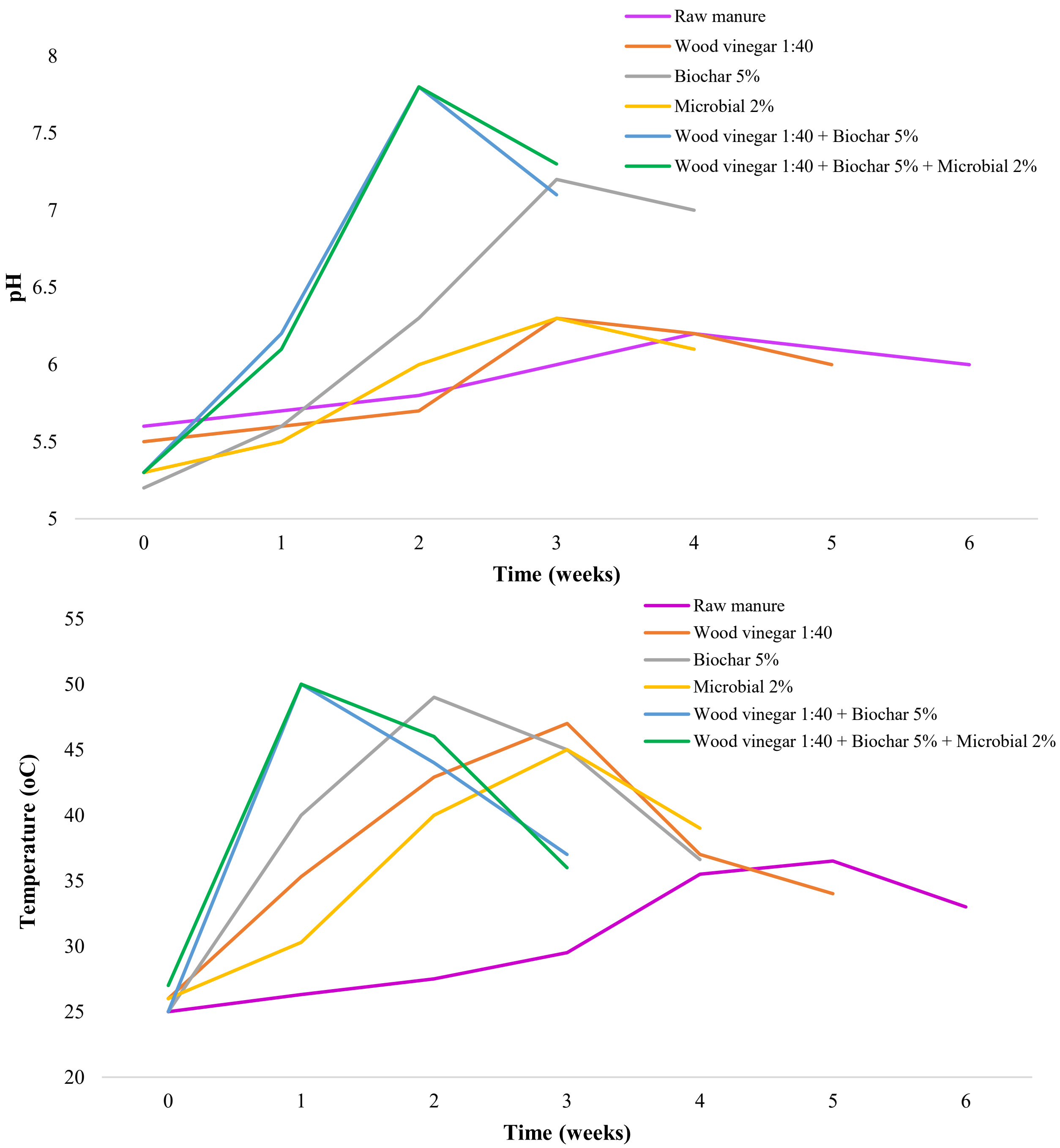

Figure 1. Temporal evolution of compost pH and temperature across various biological treatments over the composting period (n=3)

The composting temperature profiles varied significantly among treatments ($p < 0.01$), reflecting differences in microbial activity and decomposition efficiency. During the initial phase (Week 0–1), raw manure alone exhibited minimal temperature increases (mean 25–26 °C), whereas treatments amended with rice husk biochar and 1:40-diluted wood vinegar rapidly entered the thermophilic range, reaching 48–52 °C within the first week. The addition of the microbial inoculant further enhanced this effect, producing a more prolonged and stable thermophilic response compared with biochar mixed with wood vinegar alone. At the thermophilic peak (Week 2–3), biochar-containing treatments consistently achieved the highest temperatures (mean 49–50 °C), significantly exceeding raw manure (27–29 °C; $p < 0.01$). Wood vinegar-only treatments also promoted elevated temperatures (about 43–47 °C), though less pronounced than those observed with biochar-based combinations. The microbial inoculant alone contributed more to thermal stability than to peak intensity, maintaining temperatures around 40–45 °C. In the cooling phase (Week 4–5), all treatments showed declining temperatures, converging toward 36–39 °C. However, maturity defined by a combination of stabilized temperature, near-neutral pH, and absence of detectable odor was reached earlier in amended treatments. Treatment with 2% (v/v) microbial inoculant reduced the time to compost maturity to four weeks, compared with six weeks for the unamended control. Biochar-containing treatments (biochar-only and biochar + wood vinegar) also reached maturity at approximately four weeks, while raw manure required six weeks. The addition of the microbial inoculant to the biochar + wood vinegar system did not further reduce composting time (4 weeks), indicating that no additional benefit in maturation rate was obtained under these combined amendment conditions.

Table 2 summarizes the key physicochemical and microbiological quality parameters of the cattle-manure-based compost produced under the selected bio-based treatments. Humic acid content was highest in the treatment combining rice husk biochar 5%, wood vinegar 1:40, and the microbial inoculant 2%, reaching 17.14 % (± 0.008), followed by the same formulation without microbial inoculant (15.23 % ± 0.013). Treatments with biochar 5% alone (13.13 %) and microbial inoculant 2% (13.11 %) showed intermediate increases, while the wood vinegar-only treatment recorded the lowest humic acid (11.33 % ± 0.008). Fulvic acid followed a similar pattern, with the highest values (5.20 %) observed in the combined biochar + wood vinegar treatment.

Organic matter (OM) ranged from 14.12 % in the microbial inoculant 2% treatment to 56.11 % (± 0.012) in the biochar + wood vinegar + microbial inoculant formulation, which was the highest in the set. All treatments except the microbial inoculant compost met or exceeded the TCVN 7185:2002 minimum threshold of OM ≥ 22 % for microbial organic fertilizers, with biochar-containing treatments contributing the largest increases in OM content.

Total nitrogen (N) was highest in the biochar + wood vinegar + microbial inoculant treatment (19.57 % ± 0.019), followed by the biochar + wood vinegar system (16.62 % ± 0.016). The microbial inoculant treatment (7.27 % ± 0.009) and the wood vinegar-only treatment (7.28 % ± 0.007) were lowest. All treatments with biochar, including those with or without microbial inoculant, exceeded the TCVN 7185:2002 requirement of total N ≥ 2.5 %.

Available phosphorus (P_2O_5) was highest in the biochar + wood vinegar + microbial inoculant system (11.63 % ± 0.011), followed by the same formulation without microbial inoculant (9.19 % ± 0.009) and biochar-only (9.21 % ± 0.009). The microbial inoculant and wood vinegar-only treatments recorded 5.12 % and 6.90 %, respectively. All biochar-based treatments, as well as the microbial inoculant and biochar + wood vinegar + microbial inoculant treatments, met or exceeded the TCVN 7185:2002 threshold of available P_2O_5 ≥ 2.5 %.

Table 2. Post-composting physico-chemical and microbial quality under different treatments

Indicator	Unit	Control (cow manure only)	Wood vinegar 1:40	Biochar 5%	Microbial inoculant 2%	Biochar 5% + Wood vinegar 1:40	Biochar 5% + Wood vinegar 1:40 + Microbial inoculant 2%
Humic acid	% (dry wt)	8.25 ± 0.01	11.33 ± 0.008	13.13 ± 0.01	13.11 ± 0.005	15.23 ± 0.013	17.14 ± 0.008
Organic matter (OM)	% (dry wt)	20.35 ± 0.09	22.17 ± 0.012	27.11 ± 0.01	14.12 ± 0.006	49.33 ± 0.013	56.11 ± 0.012
Fulvic acid	% (dry wt)	1.87 ± 0.01	2.63 ± 0.005	3.22 ± 0.01	2.69 ± 0.004	5.14 ± 0.009	5.20 ± 0.007
Total nitrogen (N)	% (dry wt)	3.82 ± 0.01	7.28 ± 0.007	14.60 ± 0.02	7.27 ± 0.009	16.62 ± 0.016	19.57 ± 0.019
Available phosphorus (P_2O_5)	% (dry wt)	4.32 ± 0.01	6.90 ± 0.008	9.21 ± 0.009	5.12 ± 0.006	9.19 ± 0.009	11.63 ± 0.011
Boron (Bo)	ppm (dry wt)	62 ± 2	91 ± 1	144 ± 3	81 ± 2	136 ± 3	141 ± 3
Zinc (Zn)	ppm (dry wt)	58 ± 2	75 ± 1	215 ± 5	74 ± 1	201 ± 4	212 ± 4
Iron (Fe)	ppm (dry wt)	72 ± 3	63 ± 1	111 ± 2	69 ± 1	89 ± 2	88 ± 2
Manganese (Mn)	ppm (dry wt)	49 ± 2	54 ± 1	93 ± 2	62 ± 1	75 ± 2	82 ± 2
Bacillus sp.	CFU/mL	1.0×10^1	1.0×10^2	1.0×10^3	1.0×10^3	1.0×10^6	1.0×10^8
Azotobacter sp.	CFU/mL	1.0×10^2	1.0×10^2	1.0×10^3	1.0×10^3	1.0×10^3	1.0×10^3

Micronutrient levels of B, Zn, Fe and Mn were generally higher in biochar-containing treatments than in the single-amendment formulations, indicating improved retention of plant-available micronutrients under biochar-based amendments. Boron (Bo) reached 144 ppm, zinc (Zn) 215 ppm, iron (Fe) 111 ppm, and manganese (Mn) 93 ppm in the biochar-only system, with similar or slightly reduced values in the combined biochar + wood vinegar + microbial inoculant treatment. These micronutrient contents fall within typical ranges for biochar-amended composts and do not suggest phytotoxicity, provided background soil levels are considered.

Functional microbial populations were strongly influenced by treatment type. The density of *Bacillus* sp. increased by several orders of magnitude in the combined biochar + wood vinegar + microbial inoculant treatment (1.0×10^8 CFU/mL), compared with 1.0×10^2–10^3 CFU/mL in the single-amendment treatments. *Azotobacter* sp. remained relatively stable at 1.0×10^3 CFU/mL in all biochar-containing formulations, while the wood vinegar-only compost showed a lower density (1.0×10^2 CFU/mL). The density of *Bacillus* sp. in the biochar + wood vinegar + microbial inoculant treatment (1.0×10^8 CFU/mL) exceeded the minimum threshold of 10^6 CFU/g specified in TCVN 7185:2002, though direct comparison is subject to unit conversion from CFU/mL to CFU/g, which depends on compost bulk density. Overall, the post-compost quality data indicate that the combination of rice husk biochar (5% w/w), wood vinegar (1:40 v/v), and the microbial inoculant (2% v/v) yielded the greatest improvements in organic matter content, major nutrients (N and P_2O_5), humic substances, and functional microbial populations. The resulting compost product met or exceeded multiple key quality thresholds specified in TCVN 7185:2002 for microbial organic fertilizers.

DISCUSSION

The present study demonstrates that the integration of rice husk biochar, wood vinegar, and a microbial inoculant into dairy cattle manure composting produced measurable improvements in maturation rate and compost quality, with biochar emerging as the primary driver of thermophilic. Treatments incorporating biochar, either alone or in combination, consistently outperformed the control and single-amendment groups. Biochar-containing systems reached compost maturity within 4 weeks, while the unamended control required 6 weeks, and biochar-based composts showed higher organic matter (OM), humic substances, and total N/P_2O_5 content **(Table 2)**. This acceleration is consistent with the early thermophilic activation (48–52 °C in Week 1) and rapid pH stabilization (≥ 6.5–7.9 by Week 3) observed in the same treatments (**Figure 1**), suggesting that biochar promoted a more favorable physicochemical environment for microbial decomposition. The enhanced thermophilic activation observed in biochar-containing treatments is consistent with the reported capacity of biochar to improve aeration and microbial colonization through its porous architecture and high surface area (Kalina *et al.*, 2022). These properties create stable microhabitats for thermophilic and cellulolytic organisms, facilitate enzyme–substrate interactions, and reduce heat dissipation during the early composting phase (Guo *et al.*, 2020; Kalina *et al.*, 2022). The higher and more sustained temperatures in biochar-amended treatments ($\geq$ 49–50 °C in Weeks 2–3; **Figure 1**) corresponded to more rapid organic matter turnover and earlier stabilization, consistent with the lower residual odor intensities recorded in biochar-containing formulations during Phase 1 (**Table 1**; $p < 0.05$).

The rapid pH stabilization observed in biochar treatments likely contributed to enhanced microbial performance, particularly among thermophilic and cellulolytic species. The alkalinity and high cation exchange capacity of biochar are likely to have contributed to maintaining pH within the 6.0–8.0 range recommended by TCVN 7185:2002 (**Figure 1**; **Table 2**; Vu, 2019), thereby supporting conditions favorable for sustained microbial metabolic activity. This pH shift may also have selectively suppressed acid-tolerant pathogenic bacteria while favoring beneficial taxa such as *Bacillus* and *Azotobacter*, whose populations were highest in the biochar-containing treatments (**Table 2**).

The addition of wood vinegar further enhanced composting performance, particularly when combined with biochar, but had a more limited effect when applied alone. In Phase 1, wood vinegar at a 1:40 dilution reduced odor intensity to near-zero levels within 24 h (**Table 1**), likely attributable to its phenolic compounds and volatile acids, which inhibit odor-producing bacteria and neutralize malodorous molecules (Abu *et al.*, 2024; Liu *et al.*, 2023). In Phase 2, the wood-vinegar-amended composts reached maturity faster than the control but slower than biochar-based treatments, and their final OM and nutrient content (especially N and P_2O_5) were below biochar-containing formulations (**Table 2**). When applied alone, wood vinegar did not significantly improve composting efficiency or nutrient stabilization, suggesting that its primary role is supplementary and physicochemical, centered on odor and pathogen suppression rather than structural.

The microbial-based inoculant accelerated composting when used alone, reducing maturation time by one week relative to the control, and enhanced microbial densities (especially *Bacillus* sp., **Table 2**). However, when inoculant was added to the already optimized biochar + wood vinegar system, no further reduction in composting time or improvement in N/P_2O_5 retention was observed. This plateau is consistent with a threshold effect, whereby microbial activity and the prevailing environmental conditions (temperature, pH, aeration, and substrate availability) had approached near-optimal levels, thereby limiting the marginal benefit of additional inoculation (Vu, 2019; He *et al.*, 2024). The high *Bacillus* sp. density (10^8 CFU/mL) in the combined biochar + wood vinegar + microbial inoculant treatment indicates that population density per se was not the limiting factor; rather, ecological niche saturation and resource competition among microbial groups may have constrained further performance gains (Li, 2023).

The greater nutrient retention in biochar-containing treatments including higher levels of N, P_2O_5, and K_2O, indicates that biochar contributes to improved mineralization and reduced nutrient loss via volatilization and leaching. This is particularly relevant for high-moisture, protein-rich feedstocks such as dairy manure, which are susceptible to ammonia volatilization and phosphorus solubilization losses. The porous structure and high cation exchange capacity of biochar facilitate the adsorption of ammonium and phosphate ions, while its high surface area provides a stable matrix for microbial immobilization, resulting in slower nutrient turnover and more balanced nutrient release profiles (Guo *et al.*, 2020; Kalina *et al.*, 2022). The sustained populations of *Bacillus* sp. and *Azotobacter* sp. in biochar-containing treatments (**Table 2**) are consistent with the interpretation that biochar enhances microbial stability and activity throughout the composting process. The higher cell counts in biochar-amended composts, especially under favorable pH and thermophilic conditions (**Figure 1**), suggest that biochar provides both physical support (microhabitats, protection from shear stress) and chemical buffering of pH and nutrient retention necessary for maintaining a healthy microbial ecosystem throughout the composting process. In contrast, single-amendment treatments of wood vinegar alone and microbial inoculant alone exhibited more modest improvements in OM and nutrient content alongside lower microbial densities, suggesting that these amendments lack the structural and physicochemical framework required to sustain elevated microbial activity over the full composting period.

The minimal improvements observed when rice husk biochar was excluded from the treatment highlight its critical role as the core structural amendment in this system. Without biochar, composting systems exhibited delayed pH stabilization (longer acid phase, **Figure 1**), attenuated thermogenic activation (lower peak temperatures, ≈43–47 °C vs ≥49–50 °C with biochar, **Figure 1**), and slower odor reduction (**Table 1**). These findings suggest that biochar functions as a physicochemical and structural matrix that promotes microbial colonization, facilitates aeration, and adsorbs volatile organic compounds, thereby establishing a favorable microenvironment for compost maturation (Vu, 2019; He *et al*., 2024; Kalina *et al*., 2022; Li, 2023). The porous architecture and high surface area of biochar also retain moisture and support thermophilic microbial growth, both of which are critical for early compost activation. In contrast, wood vinegar and microbial inoculants, while effective in odor suppression and microbial stimulation, lack these structural attributes and perform best when integrated with biochar as the foundational amendment. This synergistic dependency has been reported in previous studies, in which biochar improved the efficacy of microbial inoculants and odor-neutralizing agents by enhancing the physicochemical conditions necessary for microbial metabolic activity (Liu *et al*., 2023; Abu *et al*., 2024).

CONCLUSION

This study demonstrates that the integrated application of rice husk biochar (5% w/w), wood vinegar (1:40 v/v), and a microbial inoculant comprising *Bacillus licheniformis*, *Bacillus megaterium*, and *Lactobacillus acidophilus* (2% v/v) significantly enhanced the composting of dairy cattle manure under tropical conditions, reducing maturation time by two weeks relative to the unamended control while improving organic matter content, humic substances, nitrogen and phosphorus retention, and functional microbial populations. Rice husk biochar emerged as the primary driver of composting performance, providing the physicochemical and structural conditions necessary for thermophilic activation and nutrient immobilization, whereas wood vinegar contributed principally to odor suppression and microbial inoculant accelerated maturation. The resulting compost met multiple quality thresholds of TCVN 7185:2002 for microbial organic fertilizers, affirming its suitability for practical application in organic dairy farming systems. The novelty of this work lies in the phased, dose–response evaluation of each amendment individually prior to their combined application under Vietnamese tropical conditions. Future research should examine the scalability of this system across diverse livestock manure types, assess long-term agronomic outcomes in receiving soils, and employ high-resolution microbiological approaches to elucidate the mechanistic basis of the observed synergistic effects.

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