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Composting domestic sewage using *Trichoderma* isolates from agricultural soils

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Abstract. *Thuy NP, Trai NN. 2025. Composting domestic sewage using* Trichoderma *isolates from agricultural soils. Biodiversitas 26: 1743-1753.* This study aimed to evaluate the efficacy of *Trichoderma* spp. in enhancing the composting process of sewage sludge from domestic treatment plants. The research focused on improving organic matter decomposition and compost quality while addressing microbial safety. Thirteen *Trichoderma* isolates were screened from agricultural soils, with *Trichoderma afroharzianum* (isolate BCS3) identified as having the highest cellulase activity. Composting trials involved six treatments: one control, five sewage sludge combinations, and a *T. afroharzianum* BCS3 bioproduct. Parameters such as temperature, moisture content, pH, and the physicochemical and microbiological properties of the final compost were monitored throughout the 30-day process. All treatments completed the composting process with characteristic thermophilic phases. Treatment 3, featuring an optimal mix of sludge and *T. afroharzianum* BCS3 bioproduct, exhibited the most significant thermophilic activity, indicating enhanced microbial activity and rapid organic matter decomposition. *Trichoderma*-treated compost showed increased organic matter, improved nitrogen and phosphorus availability, and higher concentrations of humic substances. All composts met microbial safety standards, testing negative for *E. coli* and *Salmonella*. *Trichoderma* spp., particularly *T. afroharzianum* BCS3, significantly enhanced the composting of sewage sludge by accelerating organic matter breakdown and improving nutrient profiles. This sustainable approach offers an efficient solution for sludge management, producing high-quality organic fertilizer for agricultural use.

Keywords: Bioaugmentation, compost quality, microbial activity, sewage sludge composting, *Trichoderma* spp., *T. afroharzianum*

INTRODUCTION

Wastewater treatment, while essential for public health and environmental protection, generates substantial quantities of sludge as a byproduct (Sharma et al. 2022). Effective sludge management is crucial for minimizing environmental impact and safeguarding public health (Joo et al. 2015). This material, however, is not simply waste. Sludge contains valuable resources, including essential plant nutrients like nitrogen and phosphorus, making it a potential soil amendment for agricultural purposes (Bhatt et al. 2015). Consequently, there is growing interest in developing sustainable strategies for sludge management that prioritize resource recovery and minimize disposal (Joo et al. 2015). These strategies range from minimizing sludge production through pretreatment processes to maximizing beneficial reuse options, such as the recovery of value-added products like struvite, alginate-like exopolymers, and humic substances (Gusiatin et al. 2024).

Recycling sludge in agriculture offers a potentially cost-effective and sustainable solution for waste disposal while simultaneously improving soil properties (Bhatt et al. 2015). This approach aligns with the principles of a circular economy, where waste is viewed as a resource to be reintegrated into productive systems. However, the safe application of sludge to agricultural land necessitates careful consideration of potential risks, including the presence of pathogens, heavy metals, and organic contaminants (Sharma et al. 2022; Gusiatin et al. 2024). Therefore, appropriate treatment methods are essential to mitigate these risks and ensure environmental and human health safety.

Conventional sludge disposal methods, such as landfilling and incineration, present several challenges. Landfilling can lead to leachate contamination and greenhouse gas emissions, while incineration can contribute to air pollution and require significant energy input (Joo et al. 2015). These methods are also increasingly constrained by legislative restrictions and public concerns, prompting a search for more sustainable alternatives. Composting has emerged as a promising approach for managing sludge, offering several advantages over traditional disposal methods (Manea and Bumbac 2024).

Composting is a biological process that involves controlled aerobic decomposition of organic matter. By mixing sludge with bulking agents, such as green waste or agricultural residues, an optimal carbon-to-nitrogen ratio is achieved, creating favorable conditions for microbial activity (Xu et al. 2012; Boutchich et al. 2015; Hiền et al. 2020; Manea and Bumbac 2024). This process reduces pathogens, partially degrades organic contaminants, and eliminates phytotoxic compounds, resulting in a stabilized, nutrient-rich product suitable for agricultural and landscaping applications (Mena et al. 2003). The resulting compost not only provides valuable nutrients for plant growth but also improves soil structure, water retention, and microbial activity (Sahraoui et al. 2024).

The use of microbial inoculants can further enhance the composting process. *Trichoderma* spp. a genus of beneficial fungi, has shown promise as a bio-conversion agent for composting organic waste (Cotxarrera et al. 2002; Islam et al. 2014; Komolafe et al. 2020). *Trichoderma* spp. produce a range of enzymes that accelerate the decomposition of organic matter, improve nutrient release, and suppress plant pathogens (Islam et al. 2014; Merlin et al. 2020). Several studies have demonstrated the effectiveness of *Trichoderma* spp. in improving compost quality and promoting plant growth (Islam et al. 2014; Komolafe et al. 2020). For example, Merlin et al. (2020) found that adding *Trichoderma* to sludge compost resulted in a product that met the permissible limits of good compost according to various standards. Similarly, Islam et al. (2014) observed increased plant height in vegetables grown in compost inoculated with *Trichoderma harzianum*.

Domestic sewage sludge presents a significant challenge for disposal and management, with increasing volumes generated globally. Composting offers a sustainable approach to stabilize sludge, reduce its volume, and transform it into a potentially valuable resource. This study investigates the potential of *Trichoderma* spp. to enhance the composting process of domestic sewage sludge. We hypothesize that the addition of *Trichoderma* spp. will accelerate the composting process and improve final compost quality, focusing on parameters relevant to its use as a soil amendment. This research aims to contribute to the development of sustainable sludge management strategies that promote resource recovery and minimize environmental impact, aligning with the principles of a circular economy.

MATERIALS AND METHODS

Soil samples collection

A total of twenty-one soil samples were collected from agricultural fields located in Tra Vinh Province, Vietnam. These fields were cultivated with a variety of vegetable crops, including sweet corn (*Zea mays* var. *rugosa*) (ZM), Chinese chives (*Allium tuberosum*) (AT), eggplant (*Solanum melongena*) (SM), and wax gourd (*Benincasa hispida* (BCS). Soil samples were collected from a depth of 15 cm. Five subsamples were collected randomly within each field and then thoroughly mixed to create a composite sample. Each composite sample was placed in a sterile polyethylene bag, labeled appropriately, and transported to the laboratory under cool conditions. Upon arrival, the samples were stored at 4°C until further analysis.

Isolation of *Trichoderma*

To isolate *Trichoderma* strains, 10 g aliquot of each soil sample was serially diluted in sterile 0.85% NaCl solution to obtain 10^{-4}, and 10^{-5} dilutions. Aliquots (100 µL) of each dilution were spread onto Trichoderma Selective Medium (TSM) plates supplemented with chloramphenicol (0.5 g/L) to inhibit bacterial growth. Plates were incubated at 37°C for 96 hours. Colonies exhibiting characteristics typical of *Trichoderma* spp. were subcultured onto Potato Dextrose Agar (PDA) plates for purification. Pure cultures were obtained and stored at 4°C for subsequent analysis (Mistry and Bariya 2022).

Morphological identification of *Trichoderma* isolates

Morphological characterization of the isolated *Trichoderma* strains was conducted using a two-pronged approach. For visual observation, isolates were grown on PDA plates for 3-5 days. Colony morphology, including growth rate, color, texture, and any changes in the surrounding medium, was recorded daily. For microscopic examination, slide cultures were prepared. Microscopic observations focus on the morphology of conidiophores, phialides, and conidia, including their shape, size, arrangement, and developmental stages. These morphological characteristics were used to preliminarily identify the isolated strains to the genus level (Bissett et al. 2015).

Cellulose degradation analysis

The cellulolytic activity of *Trichoderma* isolates was assessed using the plate clearance method. Czapek Dox Agar medium supplemented with 1% Carboxymethyl Cellulose (CMC) was used for this purpose. Each *Trichoderma* isolate was inoculated onto the center of a petri dish containing the CMC-amended agar and incubated at room temperature for five days. Three replicate plates were prepared for each isolate. Following incubation, the diameter of the clear zone surrounding the fungal colony was measured. This clear zone represents the area where the isolate has degraded the CMC. The diameter of the fungal colony was also measured. The difference between the total diameter (clear zone + colony diameter) and the colony diameter was calculated to determine the extent of CMC degradation. To confirm cellulose degradation, the plates were flooded with Lugol's iodine solution. The appearance of a clear halo around the fungal colony, indicating the hydrolysis of CMC, was visually confirmed. The results were used to assess the cellulolytic capacity of each *Trichoderma* isolate (Nandini et al. 2021).

Molecular identification of *Trichoderma* isolates

Molecular identification of *Trichoderma* isolates was performed by sequencing the Internal Transcribed Spacer (ITS) region of the ribosomal DNA. Mycelial biomass was obtained from pure cultures grown on PDA plates Marecik et al. (2018). Genomic DNA was extracted from the mycelial biomass using the CTAB method as described by Doohan et al. (1998). The ITS region was amplified by Polymerase Chain Reaction (PCR) using the primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al. 1990). PCR reactions were conducted in a 50 µL reaction volume containing 40 ng of template DNA, 0.2 µM of each primer, 200 µM of each dNTP, 2.5 U Taq DNA polymerase, and 1× QIAGEN PCR Buffer.

Table 1. Composition of compost treatments

Treatments	Cow manure (kg)	Sludge (kg)	Coco peat (kg)	Rice bran (kg)	Molasses (kg)	CaO powder (kg)	*Trichoderma* bioproduct (kg)
T1	60	0	40	5	1	1	0.1
T2	60	10	30	5	1	1	0.1
T3	60	20	20	5	1	1	0.1
T4	60	30	10	5	1	1	0.1
T5	60	40	0	5	1	1	0.1
T6	60	50	0	5	1	1	0.1

PCR cycling conditions included an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step at 72°C for 5 minutes was included. PCR products were separated by electrophoresis on a 2% agarose gel, stained with SafeView DNA stain, and visualized under UV transillumination. The amplified ITS region was sequenced, and the resulting sequence was submitted to the GenBank database at the National Center for Biotechnology Information (NCBI). The sequence was then compared to the NCBI database using the Basic Local Alignment Search Tool (BLASTn) algorithm to identify the closest matching sequences and determine the species identity of the isolate (Ji et al. 2020).

Bioproduct production and formulation

Trichoderma sp. was cultivated on a substrate consisting of 60% corn starch and 40% coco peat with 60% moisture content. The fungus was grown in Erlenmeyer flasks for five days. To produce the bioproduct, a mixture was formulated containing 60% rice bran, 30% corn starch, 10% additives (peptone, K_2PO_4, magnesium sulfate, ferric ammonium citrate, and potassium chloride), and a portion of the *Trichoderma* culture. The mixture was adjusted to 60% moisture content and spread onto sterilized stainless-steel trays. The trays were incubated at room temperature for seven days to allow for further fungal growth and colonization. The bioproduct was then dried at 45°C for 24 hours to reduce moisture content. The dried bioproduct was stored in sealed aluminum packages. The density of *Trichoderma* in the final bioproduct was determined by plating serial dilutions of the bioproduct onto TSM and counting the number of Colony-Forming Units (CFU).

Organic fertilizer production using *Trichoderma* sp.

Compost was produced using a mixture of cow manure, dewatered sludge from a domestic sewage treatment plant, coco peat, rice bran, molasses, Calcium Oxide (CaO) powder, and a *Trichoderma* sp. inoculum. The experiment employed a Randomized Complete Block Design (RCBD) with six treatment formulations and three replicates per treatment. Table 1 details the specific composition of each treatment. For each treatment, 60 kg of cow manure was used as a base layer. Dewatered sludge, coco peat, and rice bran were then added according to the proportions outlined in Table 1. One kilogram (1 kg) of molasses and 1 kg of CaO powder were incorporated into each mixture. Finally, a *Trichoderma* sp. inoculum, standardized at 1.6×10^8 CFU/g, was added to each compost pile at a rate of 0.1 kg per pile. The compost piles were constructed with a height of 0.4-0.6 m and a diameter of 0.7-1.0 m. The piles were covered to maintain appropriate moisture levels and incubated for 30 days to allow for microbial decomposition. Compost quality was evaluated based on a range of physical, chemical, and microbiological parameters. These included: Organic matter content, available phosphorus (P), total phosphorus (P), total nitrogen (N), total potassium (K), pH, and moisture content. Density of *Trichoderma* spp. (CFU/g dried soil), density of aerobic microorganisms (CFU/g dried soil), and colony counts of *Salmonella* spp. and *Escherichia coli* (CFU/g dried soil). Humic acid content, and fulvic acid content.

Statistical analysis

Data were analyzed using a One-Way Analysis of Variance (ANOVA) with a Randomized Complete Block Design (RCBD) to determine statistically significant differences among treatments (Panse and Sukhatme 1954). Statistical analyses were performed using the "Windostat" software. Significance was determined at the 5% level using the F-test. When significant differences were detected among treatments, Duncan's Multiple Range Test (DMRT) was used to compare means between treatment groups.

RESULTS AND DISCUSSION

Morphological identification of *Trichoderma* isolates

Thirteen fungal isolates obtained from agricultural soils were identified as belonging to the genus *Trichoderma* based on their macroscopic and microscopic features. While all isolates shared key characteristics of *Trichoderma*, significant morphological variations were observed. Isolates exhibited diverse colorations, varying from light green (ZM1, AT1, SM1, BCS1) to yellowish-green (ZM3, SM2) and dark green (AT3, BCS2, BCS3). Colony texture also varied, with ZM2 displaying compact tufts and SM3 exhibiting a looser growth pattern. Growth rates, measured as colony diameter after four days, ranged from 6.0 mm (BCS4) to 9.0 mm (AT2), with the majority of isolates demonstrating growth around 7.0 mm. Microscopic examination revealed that all isolates possessed hyaline and branched mycelium. Septate hyphae were specifically observed in isolates ZM3, BCS3, and BCS4. The primary

differentiators among the isolates were the conidiophore branching patterns and the morphology of conidia and phialides. Conidiophores were generally branched, with a verticillate branching pattern observed in isolates such as ZM2, AT2, AT3, SM3, and BCS1. However, branching frequency varied, with AT1 showing infrequent branching and AT3 and BCS2 exhibiting frequent branching. Conidia were predominantly globose and smooth-walled (ZM2, AT2, SM1, SM2, BCS1, BCS3). Notably, ZM3 produced obovoid conidia, while SM3 and BCS4 produced ellipsoidal, smooth-walled conidia. Observed phialide shapes included bottle-shaped (ZM1, ZM2, BCS1, BCS2), short and pear-shaped (AT1, SM2, BCS4), slender (SM1, BCS3), pin-shaped (ZM3), slightly inflated (AT2), and lageniform (AT3). The observed variations in colony color, conidiophore branching, and conidial and phialide morphology underscore the diversity within the isolated *Trichoderma* strains. A detailed summary of these morphological characteristics for each isolate is presented in Table 2 and Figure 1.

Cellulolytic activity of *Trichoderma* isolates

The cellulolytic activity of 13 *Trichoderma* isolates, obtained from the rhizosphere of various vegetable crops, was evaluated by measuring their ability to degrade Carboxymethyl Cellulose (CMC) on agar plates (Table 3). Variations in CMC degradation activity were observed among the isolates. Among those isolated from the rhizosphere of sweet corn (*Zea mays* var. *rugosa*), isolate ZM1 exhibited moderate cellulolytic activity, with a degradation zone diameter of 4.47±0.15 cm on agar plates.

Table 2. Morphological characteristics of *Trichoderma* isolates

Isolates code	Colony color	Colony diameter (mm)	Mycelium	Conidia	Conidiophore	Phialide
ZM1	White to light green	7	Hyaline, branched	Globose	Long, branched	Bottle-shaped
ZM2	White to light green, compact tufts	7	Hyaline, highly branched	Globose and smooth	Long, branched, verticillate	Bottle-shaped
ZM3	Yellowish to light green	6.4	Septate, branched	Obovoid	Branching, verticillate	Short, pin-shaped
AT1	White to light green	7	Hyaline, highly branched	Globose	Infrequently branched	Short and pear-shaped
AT2	White to light green	9	Hyaline, highly branched	Globose and smooth	Branching, verticillate	Slightly inflated
AT3	Yellowish to dark green	7	Hyaline, highly branched	Globose	Verticillate, frequently branching	Frequently paired, lageniform
SM1	White to light green	7.5	Hyaline, highly branched	Globose and smooth	Branched	Slender
SM2	Yellowish to light green	7	Hyaline, branched	Globose and smooth	Branched	Short and pear-shaped
SM3	White to light green, loose	8	Hyaline, branched	Ellipsoidal, smooth-walled	Branching, verticillate	Bottle-shaped
BCS1	White to light green	6.8	Hyaline, branched	Globose and smooth	Branching, verticillate	Bottle-shaped
BCS2	Yellowish to dark green	7	Hyaline, branched	Globose	Verticillate, frequently branching	Bottle-shaped
BCS3	Yellowish to dark green	7	Septate, colorless, branched	Globose and smooth	Branched	Slender
BCS4	White to light green	6	Septate, colorless, branched	Ellipsoidal, smooth-walled	Branching, verticillate	Short and pear-shaped

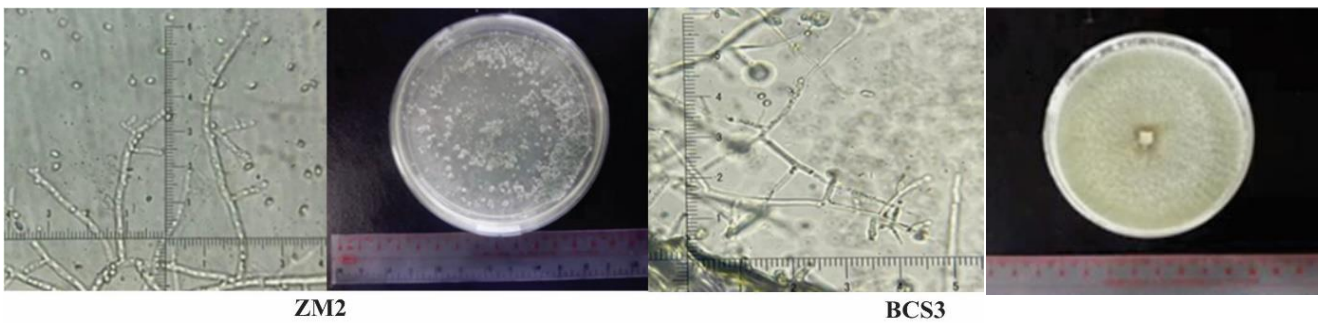

Figure 1. The morphology of colony, filament, and spores of isolated *Trichoderma* ZM2 and BCS3

Table 3. CMC degradation zone diameters of *Trichoderma* isolates

Isolates	pH of soil	Degradation zone diameter (cm)±SD
ZM1	6.8	4.47±0.15 [b]
ZM2	6.6	4.90±0.52 [a]
ZM3	6.8	6.97±0.40 [a]
AT1	6.9	6.77±0.38 a
AT2	6.8	5.63±0.91 [b]
AT3	6.8	6.27±0.67 [a]
SM1	6.5	7.00±0.40 [a]
SM2	6.5	8.40±0.46 [c]
SM3	7.2	8.07±0.51 [c]
BCS1	6.4	8.10±0.52 [b]
BCS2	6.8	7.20±0.20 [b]
BCS3	7.1	8.77±0.12 [c]
BCS4	6.8	8.57±0.31 [b]

Note: Means with different letters in the same column significantly differ at the 95% confidence level (Duncan's test)

Isolate ZM2 showed slightly higher activity with a diameter of 4.90±0.52 cm. Notably, ZM3 demonstrated significantly higher cellulolytic activity compared to ZM1 and ZM2, producing a degradation zone of 6.97±0.40 cm. Among isolates from the rhizosphere of garlic chives (*Allium tuberosum*), AT1 exhibited a degradation zone of 6.77±0.38 cm, comparable to ZM3. AT2 showed slightly lower activity (5.63±0.91 cm), while AT3 demonstrated moderate activity with a zone diameter of 6.27±0.67 cm. Isolates from the rhizosphere of eggplant (*Solanum melongena*) exhibited varying levels of cellulolytic activity. Isolate SM1 showed variability in its activity, with degradation zones ranging from 7.00±0.40 cm to 8.40±0.46 cm, suggesting potential influence of environmental factors on enzyme production. Isolate SM3 demonstrated strong cellulolytic activity with a degradation zone of 8.07±0.51 cm. Isolates from the rhizosphere of wax gourd (*Benincasa hispida*) consistently exhibited high cellulolytic potential. Isolate BCS1 displayed efficient degradation with a zone diameter of 8.10±0.52 cm. Isolate BCS2 showed a similar level of activity with a zone diameter of 7.20±0.20 cm. Isolate BCS3 exhibited the highest cellulolytic activity among all isolates, with a degradation zone of 8.77±0.12 cm. Isolate BCS4 also demonstrated high activity, producing a degradation zone of 8.57±0.31 cm.

Molecular identification

Molecular identification was conducted on isolate BCS3, selected based on its strong cellulolytic activity. The Internal Transcribed Spacer (ITS) region of the ribosomal DNA, encompassing ITS1, 5.8S rRNA, and ITS2, was amplified using primers ITS1 and ITS4. The PCR product was sequenced, and the resulting sequence was submitted to the GenBank database, where it was assigned the accession number OR856647. To determine the species identity, the obtained sequence was compared to sequences available in the GenBank database using the BLASTn algorithm. The results revealed a 99.18% sequence similarity with *Trichoderma afroharzianum*, with a 241/243 nucleotide match and a high Max score of 436 and an E-value of 0.0. These results strongly support the identification of isolated BCS3 as *T. afroharzianum*. The identification of *T. afroharzianum* strain BCS3 provides valuable insights into its potential for application in sludge composting. This molecular identification confirms the species identity and lays a strong foundation for further investigations into its biotechnological applications, particularly in the bioconversion of organic waste, such as sewage sludge, into valuable compost.

Bioproduction of *Trichoderma*

To assess the potential for bioproduction, the biomass of *T. afroharzianum* strain BCS3 was determined. The final biomass yield achieved a density of 1.6×10^8 CFU/g.

Characterization of sewage sludge

The sewage sludge used in this study was obtained from the Water Treatment Plant in Tra Vinh Province, Vietnam. Table 4 presents the physicochemical characterization of the sludge. The organic matter content of the sludge was 4.47%, with a relatively low organic carbon content of 2.03% (Table 4). Total nitrogen, phosphorus (P_2O_5), and potassium (K_2O) contents were 0.21%, 0.35%, and 0.25%, respectively, indicating moderate nutrient levels. Humic acid and fulvic acid concentrations were 0.97% and 1.40%, respectively, suggesting moderate levels of these soil organic matter fractions. Analysis of trace elements revealed the presence of calcium (0.54%), magnesium (0.13%), iron (0.99%), and aluminum (2.59%). Importantly, heavy metals such as cadmium, lead, arsenic, and mercury were not detected within the method detection limits (Table 4), indicating the sludge met the safety criteria for agricultural use. Zinc was detected at a concentration of 43.3 ppm.

Production of organic fertilizer using isolated *Trichoderma*

Temperature dynamics during composting

Temperature fluctuations were monitored throughout the 30-day composting process to assess microbial activity and organic matter decomposition. All six treatments (T1-T6) exhibited the characteristic phases of mesophilic, thermophilic, and cooling stages (Figure 2). Initially, all treatments displayed ambient temperatures of 29°C. However, by Day 5, temperatures increased significantly across all treatments, reaching a peak of 65.07°C in T3. T5 and T2 followed with peak temperatures of 63.87°C and 62.16°C, respectively. This rapid temperature rise signifies the onset of the thermophilic phase, driven by vigorous microbial activity and the breakdown of organic matter. The thermophilic phase is crucial for pathogen inactivation and the degradation of complex organic compounds. Between Days 10 and 20, temperatures in all treatments began to decline, marking the transition into the cooling phase. By Day 20, temperatures had dropped below 45°C in all treatments, indicating a decrease in microbial activity and the stabilization of the compost. At the end of the 30-day composting period, temperatures in all treatments ranged from 37.80°C (T6) to 39.33°C (T1), approaching ambient levels and signifying compost maturity. Among the treatments, T3 consistently demonstrated the most robust thermophilic performance, maintaining higher peak

temperatures for a longer duration. This finding suggests that the specific formulation of T3 was particularly effective in stimulating microbial activity and accelerating organic matter degradation.

Moisture content dynamics during composting

This study monitored moisture levels across six treatments (T1-T6) over a 30-day period to evaluate changes and their implications. At the onset of the composting process, moisture content across all treatments ranged from 58.75% (T4) to 60.83% (T1) (Figure 3). These initial levels fell within the optimal range for aerobic composting, providing suitable conditions for microbial growth and activity. During the initial 10 days, a significant increase in moisture content was observed across all treatments. By Day 5, Treatment 5 (T5) exhibited the highest moisture content (71.20%), followed by T2 (69.93%) and T4 (69.67%). This increase can be attributed to the metabolic activity of microorganisms, which generates water as a byproduct of organic matter degradation.

Between Days 10 and 20, moisture levels across all treatments stabilized, ranging between 57.53% and 60.23%. This stabilization phase suggests a balance between water evaporation, driven by heat generated during microbial activity, and the continued release of water from ongoing organic matter decomposition. For example, T3 maintained moisture levels around 59.07% by Day 20, aligning with the optimal range for sustained microbial activity. This stable phase indicates the system's ability to maintain a suitable moisture environment for efficient composting. By the final 10 days of the composting period, moisture content in all treatments decreased significantly due to water loss through evaporation. Final moisture levels ranged from 29.47% (T4) to 42.67% (T1), indicating the drying phase of the composting process. T5 and T6 retained slightly higher moisture levels compared to other treatments, potentially due to variations in aeration or differences in the composition of the composting materials.

pH dynamics during composting

The pH of the compost mixtures was monitored throughout the composting process to assess changes in acidity and alkalinity. The initial pH ranged from slightly acidic values of 4.50 (T3) to 5.28 (T1), reflecting the inherent acidity of the raw organic materials (Figure 4). Over the first 10 days, a gradual increase in pH was observed across all treatments. By Day 10, pH values ranged from 5.16 (T2) to 5.79 (T1). This rise in pH is attributed to the decomposition of organic acids and the release of ammonia from nitrogen-containing compounds within the organic matter. Between Days 15 and 25, the pH levels continued to increase as the compost matured. By Day 25, T2 and T1 exhibited near-neutral pH values of 6.50 and 6.52, respectively. This stabilization indicates a reduction in acidic intermediates and the formation of humic substances, which tend to increase soil pH. At the end of the 30-day composting process, pH levels in all treatments ranged from 5.96 (T6) to 7.26 (T2). Neutral to slightly alkaline conditions are generally preferred for most agricultural applications, as they minimize the risk of soil acidification and support optimal plant growth. The higher pH values observed in T2 and T3 suggest enhanced microbial activity and decomposition efficiency within these treatments.

Table 4. Physicochemical characteristics of sewage sludge from water treatment plant, Tra Vinh province, Vietnam

Parameters	Unit	Testing methods	Results
Organic carbon content	%	TCVN 9294:2012	2.03
Total nitrogen	%	TCVN 8557:2010	0.21
Total P_2O_5	%	TCVN 8563:2010	0.35
Total K_2O	%	TCVN 8562:2010	0.25
Total organic matter	%	TCVN 9294:2012	4.47
Humic acid	%	TCVN 8561:2010	0.97
Fulvic acid	%	TCVN 8561:2010	1.4
Iron (Fe)	%	TCVN 8562:2010	0.99
Copper (Cu)	ppm	TCVN 9286:2018	ND (MDL=5.0)
Calcium (Ca)	%	TCVN 9284:2018	0.54
Magnesium (Mg)	%	TCVN 9285:2018	0.13
Aluminum (Al)	%	TCVN 13263-14:2021	2.59
Hexavalent chromium (Cr6+)	ppm	US EPA Method 3060A + SMEWW 3500-Cr.B:2017	ND (MDL=0.90)
Arsenic (As)	ppm	US EPA Method 3051 + US EPA Method 200.7	ND (MDL=5.0)
Cadmium (Cd)	ppm	US EPA Method 3050B + US EPA Method 200.7	ND (MDL=0.7)
Lead (Pb)	ppm	US EPA Method 3050B + US EPA Method 200.7	ND (MDL=5.6)
Mercury (Hg)	ppm	US EPA Method 7473	ND (MDL=0.14)
Zinc (Zn)	ppm	US EPA Method 3050B + US EPA Method 200.7	43.3

Notes: TCVN: Vietnamese standard; ND: Not Detected; MDL: Method Detection Limit

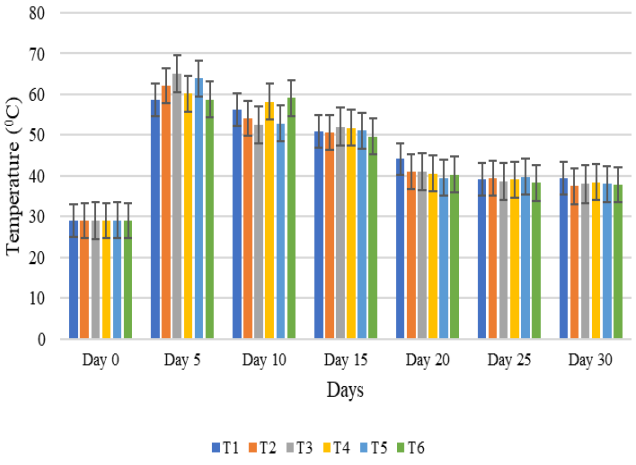

Figure 2. Temperature recorded during the 30-day composting process

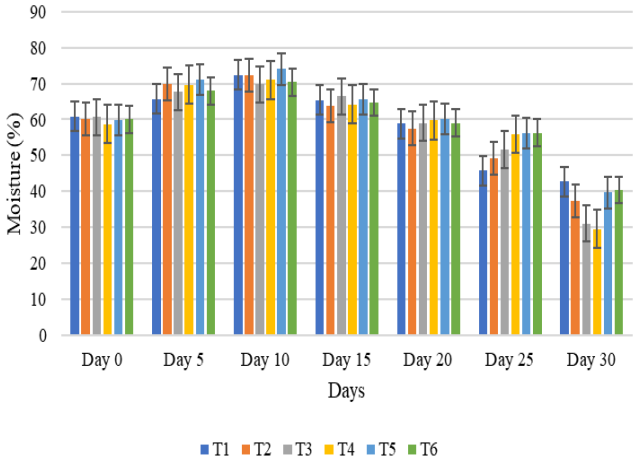

Figure 3. Moisture content recorded during the 30-day composting process

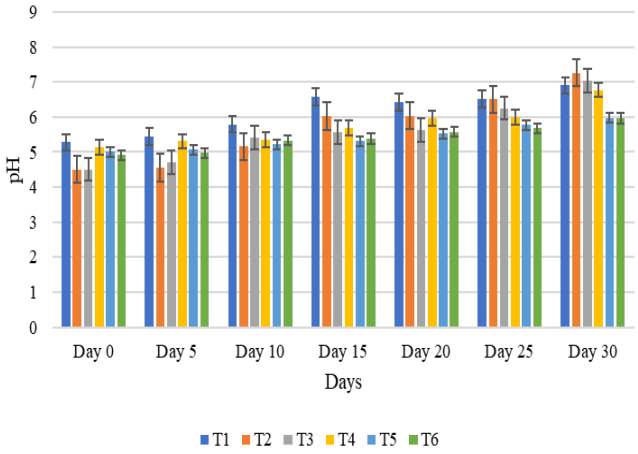

Figure 4. pH values recorded during the 30-day composting process

Chemical and microbial properties of the produced organic fertilizer

The application of *Trichoderma* spp. during the composting process significantly influenced the chemical and microbial properties of the resulting organic fertilizers. Key parameters analyzed included organic carbon content, total nitrogen (N), total phosphorus (P), total potassium (K), available phosphorus, and the C/N ratio, providing a comprehensive assessment of compost maturity and nutrient enrichment.

Chemical properties

The compost produced in Treatment 1 (T1) exhibited the highest total organic matter content (48.72±0.08%), followed by T2 (42.62±0.11%), while T4 showed the lowest (28.82±0.16%). This trend was mirrored in organic carbon content, with T1 displaying the highest level (22.14±0.31%) and T4 the lowest (13.10±0.17%). Nitrogen content varied significantly among treatments. T6 demonstrated the highest total nitrogen content (0.94±0.01%), while T1 exhibited the lowest (0.61±0.01%). Total phosphorus content also showed significant variation, with T6 exhibiting the highest levels (1.83±0.04%) and T1 the lowest (0.45±0.03%). Available phosphorus peaked in T4 (0.76±0.06%) and was minimal in T1 (0.18±0.03%). Potassium content ranged from 0.33% in T1 to 1.11% in T6, suggesting that *Trichoderma* spp. may have enhanced potassium availability within the compost. The C/N ratio, a crucial indicator of compost maturity and nutrient balance for plant growth, was highest in T1 (36.55±0.32) and lowest in T6 (14.79±0.04). This inverse relationship suggests that treatments with higher nitrogen content achieved a more balanced nutrient profile, potentially benefiting plant growth.

Humic and fulvic acid content

Humic and fulvic acids are important indicators of organic matter stability and quality within compost. Fulvic acid levels ranged from 2.52% in T4 to 4.53% in T5, with T5 demonstrating the highest fulvic acid content (4.02±0.14%). Humic acid content was highest in T6 (4.15±0.13%) and lowest in T1 (2.87±0.13%). These findings indicate that *Trichoderma* spp. may have played a role in promoting the formation of stable organic compounds during the composting process.

Microbial properties

Microbial analysis revealed varying densities of *Trichoderma* spp. across treatments. T4 exhibited the highest density (3.7×10^6 CFU/g), followed by T1 (3.2×10^6 CFU/g). T3 showed the lowest density (1.9×10^6 CFU/g). Importantly, no *E. coli* or *Salmonella* was detected in any treatment, confirming the microbial safety of the produced fertilizers for potential agricultural applications.

Trichoderma spp. are ubiquitous fungi renowned for their rapid growth, prolific sporulation, and versatile substrate colonization (Gupta et al. 2014). This study successfully isolated *Trichoderma* spp. from diverse agricultural soils, aligning with previous research (Jaisani and Pandey 2017; Anees et al. 2018; Ferreira et al. 2020). Trichoderma Specific Medium (TSM) proved effective for isolation, yielding higher fungal densities than Potato Dextrose Agar (PDA) (Attitalla et al. 2012; Alwadai et al. 2022). Morphological analysis confirmed the suitability of PDA for *Trichoderma* cultivation, as observed in previous studies (Awad et al. 2018; Mukhopadhyay and Kumar 2020; Oszust et al. 2021).

The diverse cellulolytic activity exhibited by the 13 *Trichoderma* isolates underscores their potential for biotechnological applications. Isolates from various rhizosphere sources demonstrated varying degrees of cellulolytic activity, with ZM3, AT1, SM3, and BCS3 exhibiting particularly strong activity. These findings align with previous research highlighting the cellulolytic potential of *Trichoderma* spp. (Mukhlis et al. 2013; Błaszczyk et al. 2016; Xiong et al. 2019; Carbonero-Pacheco et al. 2023). The variation in cellulolytic activity emphasizes the importance of strain selection for specific applications. Isolates with high cellulolytic activity, such as BCS3 and ZM3, hold promise for biodegradation of cellulosic waste, enzyme production, and biofertilizer development.

Table 4. Chemical and microbial properties of the produced organic fertilizers

Treat-ments	Total organic matter (%)	C-organic content	Total N (%)	Total P (%)	Total P available (%)	Total K (%)	Humic acid (%)	Fulvic acid (%)	C/N ratio	Density		
										Trichoderma (CFU/g)	*E. coli* (CFU/g)	*Salmonella* (CFU/g)
T1	48.72 ± 0.08 a	22.14 ± 0.31 a	0.61 ± 0.01 d	0.45 ± 0.03 d	0.18 ± 0.03 e	0.33 ± 0.04 e	2.87 ± 0.13 c	2.7 ± 0.08 d	36.55 ± 0.32 a	3.2×10^6	0	0
T2	42.62 ± 0.11 b	19.37 ± 0.14 b	0.76 ± 0.01 c	0.98 ± 0.08 c	0.30 ± 0.03 d	0.59 ± 0.04 c	3.43 ± 0.05 b	1.3 ± 0.10 f	25.50 ± 0.19 b	2.9×10^6	0	0
T3	34.62 ± 0.26 c	15.74 ± 0.14 c	0.74 ± 0.03 c	0.93 ± 0.05 c	0.44 ± 0.04 c	0.43 ± 0.03 d	2.71 ± 0.17 c	2.91 ± 0.05 c	21.31 ± 0.58 c	1.9×10^6	0	0
T4	28.82 ± 0.16 f	13.1 ± 0.17 f	0.86 ± 0.01 b	1.24 ± 0.09 b	0.76 ± 0.06 a	0.76 ± 0.04 b	2.74 ± 0.06 c	2.52 ± 0.04 e	15.24 ± 0.03 e	3.7×10^6	0	0
T5	32.97 ± 0.04 d	14.99 ± 0.19 d	0.84 ± 0.02 b	1.15 ± 0.10 b	0.47 ± 0.03 c	0.58 ± 0.04 c	4.02 ± 0.14 a	4.53 ± 0.10 a	17.87 ± 0.17 d	2.8×10^6	0	0
T6	30.57 ± 0.45 e	13.9 ± 0.11 e	0.94 ± 0.01 a	1.83 ± 0.04 a	0.57 ± 0.07 b	1.11 ± 0.04 a	4.15 ± 0.13 a	4.18 ± 0.08 b	14.79 ± 0.04 e	2.5×10^6	0	0
CV%	3.48	4.92	1.66	6.61	2.73	4.82	7.11	3.38	6.02			

Note: Means with different letters in the same column are not significant difference at 95% confidential level (Duncan's test)

The identification of isolate BCS3 as *Trichoderma* afroharzianum through ITS sequencing provides valuable insights into its potential for sewage sludge composting. *T. afroharzianum* is a versatile fungus with a dual role in the environment, acting as both a beneficial microorganism and a potential pathogen (Montoya et al. 2016; Inglis et al. 2020; Pfordt et al. 2020). Its adaptability and ecological versatility, as evidenced by its presence in anthropogenically polluted soils (Sherimbetov et al. 2024), make it a promising candidate for bioremediation and organic waste management. The strong cellulolytic activity of *T. afroharzianum* BCS3, coupled with its ability to induce systemic resistance in plants, promote plant growth, and antagonize phytopathogens (Marques et al. 2022), positions it as a valuable tool for sustainable waste management and compost production.

Temperature plays a crucial role in composting, driving microbial activity and organic matter decomposition (Ugak et al. 2022). All six treatments exhibited distinct temperature phases: mesophilic, thermophilic, and cooling. Treatment T3 achieved the highest peak temperature (65.07°C), indicating vigorous microbial activity and efficient organic matter decomposition. The addition of *Trichoderma* spp. significantly accelerated temperature increases in T3, demonstrating its potential as a compost activator. The thermophilic phase, characterized by rapid temperature increases and high enzymatic activity, is essential for pathogen inactivation and the degradation of complex organic compounds (Waszkielis et al. 2013; Meng et al. 2019; Cao et al. 2020). The findings of this study highlight the importance of the thermophilic phase for efficient composting and the potential of *Trichoderma* spp. to enhance this process.

Moisture content is a critical factor influencing composting success, directly impacting microbial activity and organic matter decomposition (Shen et al. 2015). All treatments maintained optimal moisture levels for aerobic composting during the initial 10 days. Between Days 10 and 20, moisture levels stabilized, indicating a dynamic equilibrium between water evaporation and release from ongoing decomposition. By the final 10 days, moisture content decreased significantly due to water loss through evaporation. Maintaining optimal moisture levels (50-60%) is crucial for efficient composting and the production of high-quality compost (Gurusamy et al. 2021). Moisture content influences nutrient transfer, porosity, temperature, oxygen uptake, and microbial activity (Pezzolla et al. 2021). A decrease in moisture content over time is indicative of decomposition and compost maturation (Jain et al. 2019).

pH is a critical parameter influencing microbial activity and compost quality. The initial pH of the compost mixtures ranged from slightly acidic to mildly acidic. Over the first 10 days, a gradual increase in pH was observed, attributed to the decomposition of organic acids and the release of ammonia. Between Days 15 and 25, pH levels continued to increase, with T2 and T1 reaching near-neutral pH values. At the end of the 30-day composting process, pH levels ranged from 5.96 to 7.26. Neutral to slightly alkaline conditions (pH 6.5-8.5) are generally preferred for most agricultural applications (Ji et al. 2023). The observed pH progression aligns with previous research (Zhang and Sun 2016; Azim et al. 2018; Hashim et al. 2022; Grgas et al. 2023). pH is an important parameter to evaluate compost maturity and stability (Ameen et al. 2016).

This study demonstrated significant variations in key chemical properties across the treatments. T1 exhibited the highest levels of organic matter and carbon, while T4 showed the lowest. Nitrogen content varied considerably, with T6 displaying the highest levels. Phosphorus content also varied significantly, with T6 showing the highest levels. Potassium content ranged across treatments, with T6 exhibiting the highest levels. The C/N ratio varied inversely with nitrogen content. Lower C/N ratios, as observed in T6, generally indicate a more balanced nutrient profile. These findings align with previous research (Hussain et al. 2015; Khater 2015; Abou Hussien et al. 2019; Lantik and Nasaruddin 2020; Kusumawati et al. 2021; Rasslan et al. 2021).

Microbial analysis of the composted material revealed variations in *Trichoderma* spp. densities across the different treatments. Treatment T4 exhibited the highest density of *Trichoderma* spp., followed by T1, while T3 showed the lowest. This variation underscores the influence of substrate composition and environmental conditions on the growth and survival of *Trichoderma* spp. during composting. Notably, T3, which experienced the most rapid initial decomposition and highest temperatures, ended up with the lowest *Trichoderma* count. This suggests that the intense thermophilic activity in T3, while potentially beneficial for overall composting, might have created a less favorable environment for *Trichoderma* in the long run. In contrast, T4, with its more moderate temperature profile and slower decomposition rate, may have provided a more conducive environment for sustained fungal growth, resulting in a higher final *Trichoderma* count. *Trichoderma* spp. play a crucial role in the composting process by secreting a diverse array of hydrolytic enzymes. These enzymes break down complex organic matter, such as cellulose, hemicellulose, and lignin, into simpler compounds, making nutrients more readily available for plant uptake. The enhanced nutrient availability and improved soil health resulting from *Trichoderma* activity contribute to the production of high-quality compost suitable for agricultural use (Ling et al. 2014; Awasthi et al. 2015; Rastogi et al. 2020). Previous studies have demonstrated the effectiveness of *Trichoderma* spp. in accelerating organic matter degradation and improving compost quality. The introduction of microbial additives, such as *Trichoderma* spp., can further enhance the composting process by increasing enzyme activity and promoting the decomposition of complex organic compounds (Barthod et al. 2018; Rastogi et al. 2020; Organo et al. 2022).

Humic and fulvic acids are crucial indicators of organic matter stability and quality within compost. Fulvic acid levels varied across treatments, with T5 exhibiting the highest content. Humic acid content was highest in T6 and lowest in T1. These findings suggest that *Trichoderma* spp. may have influenced the formation of stable organic compounds during the composting process. Humic substances play vital roles in soil health by promoting nutrient uptake, enhancing soil porosity, improving water-

holding capacity, and suppressing the activity of certain pathogens (Gerke 2018; Guo et al. 2019). The concentration of humic substances in compost is closely correlated with their maturity and overall quality (Zhou et al. 2014; Zingaretti et al. 2018). Humic substances can act as natural biostimulants, promoting plant growth and development.

In conclusion, this study confirmed that *Trichoderma* isolates, particularly *T. afroharzianum* BCS3, effectively accelerated the composting of domestic sewage sludge. The resulting compost met Vietnamese standards (QCVN 01-189:2019/BNNPTNT) for organic matter and nitrogen content, demonstrating its potential as a nutrient-rich soil amendment. While phosphorus levels required further optimization, potassium content was sufficient. Furthermore, the compost aligned with European Compost Network (ECN) guidelines, achieving desirable organic matter content and carbon-to-nitrogen ratios, indicating maturity and stability. These results underscore the efficacy of *Trichoderma*-enhanced composting for transforming sewage sludge into a safe and valuable agricultural resource. The application of selected *Trichoderma* isolates provides a sustainable approach to sludge management, generating compost that adheres to both national and international quality benchmarks.

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