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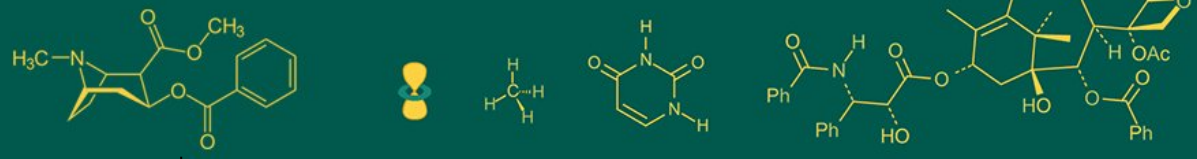

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Optimizing composting and enhancing *Agaricus bisporus* yield through thermotolerant bacterial inoculation

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Abstract
This study evaluates the impact of thermotolerant bacterial inoculation on composting processes and the cultivation of *Agaricus bisporus*. The inoculation of compost substrates with selected strains, including Bacillus subtilis and Bacillus licheniformis, enhanced organic matter decomposition and nitrogen transformation under thermophilic conditions. The treated compost achieved a faster reduction in the carbon- to-nitrogen (C/N) ratio (from 43.1 to 18.1) compared to untreated compost (from 43.1 to 23.4), indicating accelerated maturity. Ammonia emissions were reduced by 40%, and nitrogen retention was significantly improved. Additionally, the inoculated compost exhibited higher moisture content (65.32% vs. 52.09%) and electrical conductivity (431.00 mmhos/cm vs. 387.00 mmhos/cm), reflecting superior physicochemical properties .
These enhancements translated to agricultural benefits, including faster mycelial colonization (98% vs. 74%), earlier harvest readiness (18 days vs. 20 days), and higher mushroom yields (18.44 kg/q vs. 12.60 kg/q), representing a 46.35% increase. The inoculated compost also supported the production of larger fruiting bodies (10.34 g vs. 8.07 g) and more pinheads per bag (8 vs. 3). These findings underscore the efficacy of thermotolerant bacterial inoculants as a sustainable solution for integrated organic waste management and agricultural productivity enhancement.

Keywords: *Agaricus bisporus*, ammonia emissions, biological efficiency, Composting, mushroom cultivation, nitrogen transformation, thermotolerant bacteria

Introduction
Earth is the basis of agricultural production, and agricultural production is the basis of human growth. In the modern era, the ecosystem of agricultural soil suffers from a significant deterioration as a result of the unlawful path of agricultural production in terms of mismanagement of soil and wide, non -scientific use of chemical fertilizers and pesticides (Chen *et al.*, 2018) [13]. It is one of the factors that contributes to the emergence of risk sources and the lack of safety of agricultural products, which prompted the countries to search for alternative practices to exploit areas with the deteriorating soil in agriculture to optimizing productivity and ensuring food security, including the introduction of biotechnology to produce organic soil (compost) from The conversion of organic waste, which is produced in large quantities and is a long time ago a source of environmental pollution in the absence of solutions to properly manage them (Zhang *et al.*, 2017) [61]. Organic agriculture and biotechnology offer solutions to most of the issues facing agriculture and food production today, and are an important achievement that is believed to have a positive impact on the long-term sustainability of agriculture. (Wheeler, 2005) [56] The nutritional value of the vegetable product and its balanced content of biologically active components such as vitamins, mineral salts and carbohydrates is the goal that the vegetarian consumer is looking for, Mushroom cultivation is an environmentally safe bio-technique for producing a vital protein called mycoprotein (Sabri *et al.*, 2019) [46], due to its culinary, nutritional and therapeutic properties. This can only be achieved through the use of agricultural environments that provide ideal growth for mushrooms, including compost that is produced through a series of stages known as solid-state aerobic fermentation (composting), which is an effective and productive method that ensures the recycling of organic waste (crops or animal waste) through

the activity of microorganisms (bacteria, fungi and worms) and converting them into a ready medium in terms of providing the carbon and nitrogen sources necessary for the growth and stimulation of the formation of the fruiting body of the mushroom (Carrasco and Preston, 2020) [11]. The basic materials of the compost product are supplemented with some nutritional supplements such as rice bran, sugarcane, corn cobs, soybean stalks, sunflower stalks, papyrus stalks, cottonseed husks, oil palm fronds, animal manure, grains, gypsum and limestone, since they differ in their lignocellulosic composition, which are carbohydrate compounds that serve as carbon sources for the fungus growing in this organic medium (Sasaki *et al.,* 2006) [48]. On the other hand, most agricultural wastes contain low nitrogen content. Therefore, the addition of organic nitrogen or inorganic nitrogen (ammonium chloride or urea) to the substrates is necessary in order to maintain the carbon to nitrogen ratio in the organic medium, which is an important factor in the production of a profitable mushroom crop (Ruilova Cueva *et al.,* 2017) [45]. The optimum carbon to nitrogen ratio for A. *bisporus* was found to be 19/1 (Oei and Nieuwenhuijzen, 2005) [41]. Microorganisms degrade organic molecules mostly in the form of thin liquid films (biofilms) on the surface of organic particles. Bacteria are the most diverse group of organisms that compost, using a wide range of enzymes to chemically degrade a wide range of organic materials. Furthermore, bacteria have a much shorter average generation time than fungi, giving them a competitive advantage at certain stages of the composting process. If the key growth criteria of neutral pH and an ideal moisture level of 50-60% are met, most bacteria that degrade organic waste are nitrifying bacteria, which are mesothermal bacteria, generally unable to tolerate temperatures above 40 °C. These bacteria accelerate the conversion of ammonium nitrogen to oxidized nitrogen (nitrite and nitrate) and reduce the volatilization of ammonia in a process called nitrification (Wang *et al.,* 2019) [54]. Therefore, these bacteria are responsible for the majority of the initial decomposition and heat generation in the compost, which will frequently produce ammonia (NH_3) emission and volatilization during the biological decomposition of nitrogenous materials during the first three weeks of the composting period. (Chen *et al.,* 2018) [13], and thus, a large amount of nitrogen will be lost through volatilization as ammonia (NH_3) during the first thermophilic stage (>45 °C). The percentage of nitrogen lost as ammonia is estimated to be about 9.6-46% of the total initial nitrogen of raw materials during the composting of various wastes (H. Li and Yao, 2017). At the same time, the emission of ammonia not only negatively affects the environment, but also reduces the fertilizer value of the compost (Yang *et al.,* 2019). Therefore, it is very important to control the ammonia emission from fertilizers during these periods. Temperature, primary ammonium ion (NH4+) concentration, and pH are the main factors controlling the ammonia emission (Sasaki *et al.,* 2006) [48]. During composting, pH adjusting of the organic medium can help in reducing ammonia emissions. In general, the balance between ammonia and ammonium is affected by PH. Previous studies have shown that by lowering the pH, ammonia emissions can be reduced during the high temperature period by acidifying the organic medium either by adding chemicals (Yang *et al.,* 2015) [60] or by using inoculated microorganisms (Xie *et al.,* 2012) [58]. However, lowering the pH by adding chemicals to the organic medium may affect the composting process by affecting the mesophilic nitrifying bacteria that are relied upon during the early stages of the composting process. Therefore, it is necessary to rely on environmentally friendly methods, which are represented by adding thermotolerant nitrifying bacteria (TNB), which have the ability to control the pH during the high temperature period by utilizing the carbohydrate compounds of organic waste to produce organic acids at temperatures above 50 °C, thus reducing ammonia emissions (Durand *et al.,* 2017) [17]. The research results of (Li *et al.,* 2021) [35] and (Jiang *et al.,* 2016) [28] that to conserve nitrogen in the pile we can add microbial inoculants with TNB. The total nitrogen in the treatments treated with this inoculant increased by 13.01% ~ 22.10%. The results of (Curvetto *et al.,* 2002) [15] show that enriching substrates with nitrogen is important to increase the protein content of mushrooms. By raising the nitrogen content of the substrate from 0.65 to 1.3%, which would promote an increase in protein content from 17.1 to 28%. The purpose of this study is to (1) investigate the effect of inoculation on nitrogen loss from organic fertilizer prepared from palm frond waste (3) evaluate the effect of bacterial inoculation on the productivity and protein content of a fungus growing in the prepared medium.

Materials and Methods

The methodology used in this research was standardized and following the methods of

Study Area and Period

The study was conducted at one of the research stations affiliated to the Iraqi Ministry of Agriculture, Horticulture and Forestry Department, which is located in Babylon Governorate/Iraq for the period from November 2023 to May 2023. Which is 611 km southwest of Baghdad, the capital. The area is characterized by receiving a high amount of annual rainfall, high humidity, and a relatively large vegetation cover.

Microorganisms were isolated and selected based on the following criteria: (1) ability to grow on plant and animal wastes, (2) ability to grow at 50°C, and (3) ability to oxidize ammonia.

Compost reactor

The compost reactors had dimensions of 36 cm × 60 cm × 30 cm (W × L × H), and total volumes were approximately 60 L (Figure. 1). An air supplier (MA-200, Wave Point® Technology, USA) was installed at the bottom of each reactor, and the airflow rate was maintained at 1.67 L min−1. Air was continuously injected into the reactors, and the cover was perforated with the size of 10 mm diameter for air and gas ventilation. A temperature sensor (Em50, Decagon Devices, and USA) was installed at a depth of 15 cm above from the bottom of each reactor and programmed to take a measurement at every 4 h.

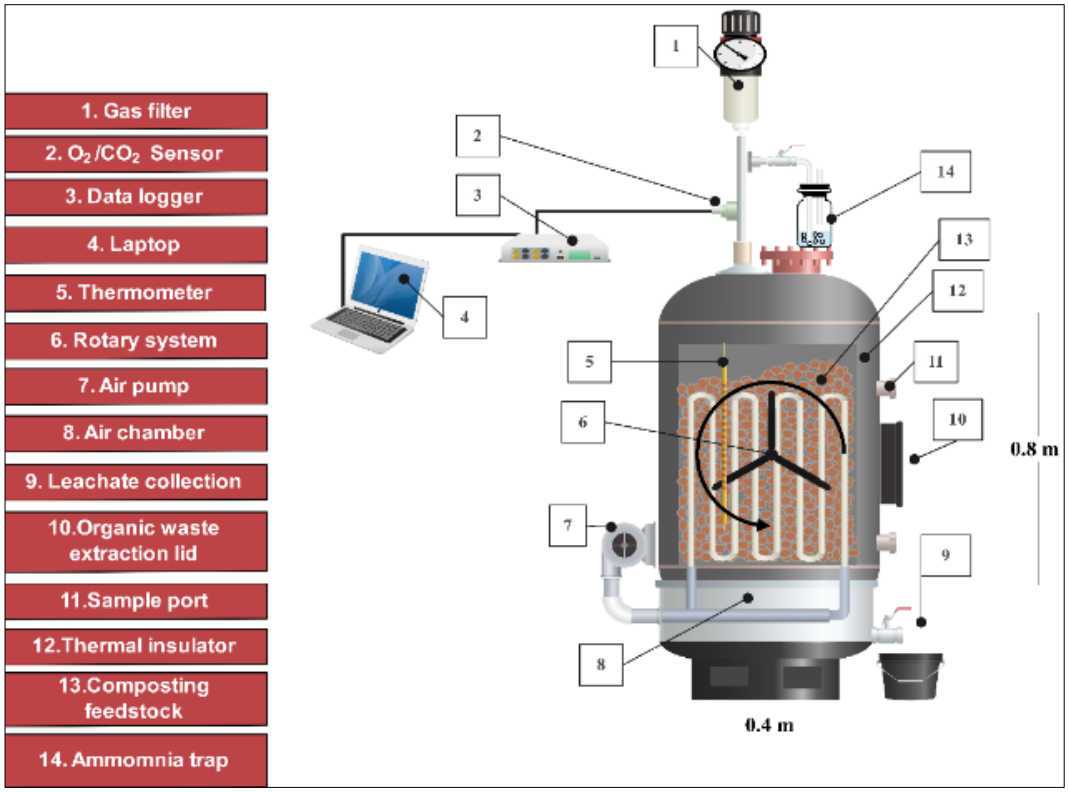

Fig 1: The schematic diagram of aerobic composting reactor

Bacterial Strains and Growth Conditions

A total of 16 bacterial strains, comprising 12 Bacillus strains (4 each of *B. licheniformis*, of *B. subtilis, B. megaterium* and 4 strains of *P. fluorescence*, were obtained from the Iraqi Microbial and Biological Resources Center, Department of Agricultural Research, Ministry of Science and Technology, Iraq (Table 1). These strains served as models to identify the most heat-tolerant strain during composting in the present study. The examined strains were spread on the surface of nutrient agar (NA) (Himedia) plate amended with cycloheximide (100 µg mL^{-1}) to prevent fungal growth and incubated at 37 °C for 24 h.

Table 1. Bacterial isolates, corresponding codes, and environmental source origins.

Species	code	Isolation Sources
B. licheniformis	*B. lich1*	Thermophilic compost piles enriched with livestock manure
	B. lich2	Soil from intensively managed farms with organic amendment.
	B. lich3	Poultry litter or dairy manure heaps.
	B. lich4	Decomposing crop residues in warm climates.
B. subtilis	*B. sub1*	Rhizosphere of wheat, maize.
	B. sub2	Phyllo sphere (leaf surface) in greenhouse grown plants.
	B. sub3	Bulk agricultural soil with moderate organic matter
	B. sub4	Soil under crop rotation systems (legumes and cereals)
B. megaterium	*B. meg1*	Manure based compost undergoing active decomposition.
	B. meg2	Orchard soil rich in decaying leaf litter and moderate to high PH.
	B. meg3	Saline soils (Arid regions)
	B. meg4	Vermicomposting
P. fluorescence	*P. flu1*	Rhizosphere of horticultural crops (tomato)
	P. flu2	Well drained soils under regular irrigation (pivot irrigated farmland).
	P. flu3	Hydroponic systems.
	P. flu4	Wetlands with abundant organic matter.

Thermal resistance testing: The thermotolerance strains of the pure bacterial cultures were determined using a modified method (Aruogu *et al.*, 2024) [7] as follows; The strains were transferred to tubes of 5 ml peptone water and incubated at 37 °C for 14 d, then heated in a Water Bath Shaker at 35,40,55,and 60°C with shaking (150 rpm) for 12 h each. After the incubation period, the cultures were plated onto nutrient agar. Bacterial isolates capable of tolerating temperatures of 50°C or higher were identified as thermophilic bacteria. The thermophilic isolates were then stored in sterile distilled water at −80°C during the entire experimental period.

Screening of xylanase producing activity bacteria

Twenty-five microliters of the inoculum prepared from each bacterial isolate under study were spread onto xylan agar plates and incubated at 30 ºC for 72 h to assess xylanase activity, which was determined by clear zones of purification and the amount of xylanase produced by each isolate was determined from the extract culture filtrate (Ammoneh *et al.*, 2014) [5].

Screening of laccase producing activity bacteria

Depending on the method described by Kuddus *et al.*, (2013) [32], bacterial isolates were cultured in 250 ml Erlenmeyer flasks containing 100 ml of nutrient broth supplemented with 0.2 mM $CuSO_4$ $7H_2O$. The cultures were incubated at 37 ºC for 48 h under agitation at (150 rpm) using a rotary shaker. Subsequently, bacterial colonies were isolated using the standard serial dilution and spread on nutrient agar medium supplemented with 0.2 mM $CuSO_4$ $7H_2O$. The plates were incubated at 37 ºC for 48 h.

To screen for laccase production, 2-3 drops of 1 mM tannic acid solution were added to the bacterial colonies. Colonies exhibiting a green coloration were identified as laccase producing isolates. Theses colonies were further sub-cultured on nutrient agar containing 0.2 mM $CuSO_4$ $7H_2O$ for isolation and reactivation of bacteria.

Collection and Preparation of the Substrate

Feedstock: Raw material samples for preparing substrates for mushroom cultivation were collected from agricultural waste from small farmers and farms affiliated to the Iraqi Ministry of Agriculture in Babil province, Iraq. (Latitude - 32.468191, longitude - 44.5501935), including date palm Residues (Phoenix dactylifera. L), which were collected during the date harvest stage on 8/2023, preceded by the collection of wheat straw residues during their harvest in April/2023, and Poultry manure. The raw materials; palm residues, wheat straw, were manually cut into 5 cm long pieces before being ground and passed through a 5 mm sieve, the chopped raw materials were soaked in clean water overnight in a 200-liter barrel for 24 h. Then, the well-soaked substrates were spread on polyethylene sheets to remove excess water, and the moisture level was adjusted to 65-70%. To make the C/N ratio of feedstock ~ 20, poultry manure and raw materials (date palm residues and wheat straw) ratio was 8:1. the pH of the feedstock mixture was maintained at 7.0 by adding gypsum (5% of total feedstock). The raw material samples and nutritional supplements were analyzed for organic carbon, pH, and nitrogen, as indicators of raw material quality. The composting experiment was conducted continuously for 35 days, for determination of physicochemical properties, precursors and humic substance, triplicate samples from each reactor were collected randomly on day 0, 7, 14, 20, 28, 30 and 35 from the surface, middle and bottom layers of the compost pile. Thereafter, samples were mixed homogenously to be ready for later tests.

Mushroom Cultivation

The pasteurized compost was inoculated with *A. bisporus* spawn at a rate of 2% (w/w) of the dry compost weight. The compost was incubated in a controlled environment at 25°C with 85-90% relative humidity to ensure optimal conditions for fungal growth. Once mycelial colonization reached 100%, a casing layer of sterilized peat moss (pH 7.5) was applied to support pinhead formation. The casing layer, approximately 2.5 cm thick, was maintained at a moisture content sufficient to form a cohesive mass when pressed, as described by (THAKUR, 2024). The growth and yield of *A. bisporus* were monitored across three flushes.

Harvesting and yield measures: Mature mushrooms were manually harvested at the wrinkly-ripe stage over three consecutive flushes. Between flushes, the bags were left unwatered for five days to allow incubation. Following Gume *et al.*, (2013) [21], the recorded yield parameters included the time (days) required for spawn running,days to the first harvest, the number of pinheads per bag (kg/q compost) on treated and untreated substrates.

Physico-chemical Analysis

Five hundred gram of compost was collected from the compost reactor in both inoculated and uninoculated treatment, and then stored at 4 ºC for subsequent evaluation of compost quality indicators and microbial efficiency:

Moisture content: Determined by oven drying compost samples at 70 ºC for 24 hours until weights constant (Guo *et al.*, 2012) [22]. The moisture content was calculated as a percentage.

PH value: Measured using a PH meter (1:10 v/w compost: water extract) according to (Dümenci *et al.*, 2023) [16].

Electrical conductivity (EC): electrical conductivity was measured in a 1:10 compost: water extract, using an EC meter (MW170, Milwaukee instruments, USA). Following the method described by (Karanja *et al.*, 2019) [30].

Total Nitrogen (TN): Total nitrogen was determined using the Kjeldahl digestion methods as described by Jackson *et al.*, (1973) [26].

Organic Carbon (OC): Organic carbon was determined according to Page, (1982) [43].

Ammonia (NH_3) Emissions

To determine the ammonia (NH_3) concentration produced during the composting process, a 2% boric acid solution (100 ml) was placed in a gas wash bottle connected to the compost reactor exhaust via a rubber tube. The bottle was sealed with a rubber stopper containing two glass tubes, allowing gas inflow and outflow. The produced gas was passed through the boric acid solution for 15 min, and the absorbed NH_3 was subsequently measured by H_2SO_4 titration. This method provided accurate NH_3 concentration measurements throughout the composting process. (Lee and Kim, 2024) [33].

Statistical analysis

Experience data was analyzed statistically by using analysis of variance (ANOVA) at significance level of (L.S.D, $P \leq 0.05$) with SPSS software (version 22.0), the least significant difference (LSD) test was performed to find significant differences between treatment means. The Graph Pad Prism software (Graph Pad Prism version 9.0.0, 2020) package was used for making graphs.

Results and Dissection

Evaluating the growth of bacterial isolates under different temperatures

By measuring colony forming units (CFUs), thermophilic bacterial growth isolated from soil is assessed at 35, 40, 55 and 60 ^{0}C as shown in Table 2. These strains' thermal preferences and adaptation mechanisms were demonstrated by their distinct temperature dependent growth patterns.

At 35 ^{0}C, pseudomonas fluorescents had the highest CFU count (224.06×10^6), whereas all strains showed moderate

development. Because of improved membrane fluidity and enzymatic control at lower temperature, this implies a preference for the lower thermophilic range. According to Akindolire *et al.,* (2022) [4], moderate temperature promote balanced lipid compositions, which provide the best possible membrane durability and permeability.

Temperature specific enzymes are activated under these circumstances, enabling effective metabolic activities with little energy consumption (Wendering and Nikoloski, 2023) [55]. These characteristics are consistent with *p. fluorescents* function in starting the decomposing of organic materials in the early stages of composting (Liu *et al.,* 2022) [36].

Bacillus species reached their optimum growth at 40 ^{0}C, with *B. licheniformis* showing the highest CFU count (423.36 $\times 10^6$). Since important enzymes including amylases, cellulases and proteases exhibit maximal activity at this temperature, it offers ideal circumstances for thermophilic bacterial development (Ajeje *et al.,* 2021) [3]. Strong thermostable enzymes and effective energy - production routes, such as glycolysis and the TCA cycle, are the main drivers of increased metabolic efficiency (Intasian *et al.,* 2021) [24]. Osmo-regulatory solutes stabilize cellular structures, and membrane with a high saturated fatty acid content guarantee stability (Yan *et al.,* 2024) [59]. The significance of thermophilic bacteria in the active composting phase is highlighted by these modifications.

Growth significantly decreased at 60 ^{0}C, and *P. fluorescens* had the lowest CFU count (4.53×10^6) because of membrane instability and enzyme denaturation. But under severe stress, *B. licheniformis* showed remarkable resilience by stabilizing cellular proteins and membranes, producing protective solvents such trehalose, and maintaining the highest CFU count (107.05×10^6) (Wang *et al.,* 2024) [53].

Table 2. Temperature-Dependent Growth of Various Thermophilic Bacteria

Strains codes	Colony forming unit ($\times 10^6$) at different temperatures			
	35°C	40°C	55°C	60°C
B. lich1	137.86	423.36	364.12	107.05
B. sub4	63.20	342.88	212.04	53.18
B. meg3	114.58	361.24	221.72	37.42
P. flu1	224.06	188.00	89.40	4.53

The Impact of Temperature on Xylanase and Laccase Activities Produced by Different Bacterial Isolates

The production and activity of xylanase and laccase by *Bacillus megaterium, Bacillus subtilis, Bacillus licheniformis, and Pseudomonas fluorescence* (Table 3), showed strain- and temperature-dependent variations, reflecting distinct metabolic adaptations and enzyme stability profiles. Xylanase, a key enzyme for degrading xylan, showed increased activity with temperature across all tested strains, which can be attributed to the general principle that enzyme-catalyzed reactions benefit from higher molecular kinetic energy at elevated temperatures, leading to enhanced enzyme-substrate collisions and catalytic efficiency (Palfey and Switzer, 2022) [62]. *B. licheniformis* displayed the highest xylanase activity at both 35°C (1.4962 U/mL) and 55°C (1.7255 U/mL), suggesting high thermostability, an adaptation to thermophilic environments (Ajeje *et al.,* 2021) [3]. In contrast, *B. megaterium* and *B. subtilis* showed moderate increases in xylanase activity with temperature (0.6174 U/mL to 0.8376 U/mL and 0.2199 U/mL to 0.2630 U/ml, respectively), suggesting these species produce enzymes that are less thermally stable but still exhibit some degree of enhanced activity at higher temperatures. *P. fluorescence* exhibited minimal xylanase activity at both temperatures (0.0566 U/mL at 35°C and 0.0914 U/mL at 55°C), possibly due to low enzyme expression or suboptimal structural configuration for xylan degradation.

Laccase, a heat liable, copper-dependent oxidase involved in lignin degradation. At 55°C, laccase activity was undetectable in *B. megaterium*, *B. subtilis*, and *P. fluorescence*, while *B. licheniformis* exhibited a significant activity decrease (from 4.116 U/mL at 35°C to 0.056 U/mL at 55°C). Laccase enzymes thermolability is responsible for dramatic reduction in activity, at higher temperatures denaturation occurs, due to disruption of their three-dimensional structure and dissociation of essential copper ions required for their catalytic activity (Jaiswal and Jaiswal, 2024). At 55°C, laccase activity disappeared emphasizes the enzyme limited thermostability and suggests that they are optimized for lower temperature functions, particularly in lignin degradation under mesophilic conditions.

Table 3: Xylanase and Laccase Enzymes Activity produced by Bacterial Strains under Different Temperatures

Strains codes	Enzyme at different temperatures			
	Xylanase		Laccase	
	35°C	55°C	35°C	55°C
B. lich1	1.4962	1.7255	4.116	0.056
B. sub4	0.2199	0.2630	0.083	0.000
B. meg3	0.6174	0.8376	2.013	0.000
P. flu1	0.0566	0.0914	0.426	0.000

Impact of Thermotolerant Bacterial Inoculation on C/N Ratio Dynamics during Organic Waste Composting

The study results (figure 2) highlight the transformative impact of thermotolerant bacterial inoculation on the carbon-to-nitrogen (C/N) ratio during organic waste composting, demonstrating enhanced microbial-driven decomposition and stabilization. The C/N ratio, a critical composting parameter, reflects the balance between carbon and nitrogen, an energy source for microbial metabolism, and essential for protein synthesis and cellular function respectively. A continuous reduction in this ratio is a sign of effective organic matter degradation and compost stabilization, ultimately indicating maturity (Bremaghani, 2024) [10].

In the initial phase (Day 0-5), untreated compost showed a slight decline in the C/N ratio, from 43.1 to 41.5, indicating limited microbial activity. On the other hand, bacteria-treated compost exhibited a sharper decline, from 43.1 to 38.2, driven by hydrolytic enzymes such as cellulases and proteases secreted by thermotolerant bacteria. These enzymes break down complex polysaccharides and proteins into simpler compounds, enhancing microbial growth, nitrogen availability, and carbon mineralization, accelerating the composting process.

In the intermediate phase (Day 5-25), untreated compost displayed C/N ratio fluctuations, peaking at 40.2 on Day 15. This temporary increase reflects an imbalance between carbon dioxide release and slower nitrogen mineralization, a common phenomenon in unamended systems (Myers, 2022; Jiménez *et al.,* 2008) [40, 29], on the other hand, bacteria-treated compost showed a permanent decline, reaching 28.1

by Day 25. Sustained enzymatic activity under thermophilic conditions enabled sufficient degradation of lignocellulosic compounds and nitrogen stabilization, key features of efficient composting (Basak *et al.*, 2025) [8].

In the final phase (Day 25-35), both treatments showed major reductions in the C/N ratio. Untreated compost dropped from 40.2 to 23.4, while treated compost decreased from 28.1 to 18.1, surpassing the maturity threshold of 20 (Kong *et al.*, 2024). The faster reduction in bacteria-treated compost is due to sustained microbial activity and enzymatic transformation of refractory organic matter into stable humic substances. Despite progress in untreated compost, its higher C/N ratio suggests a slower maturation trajectory requiring additional stabilization time. Thermotolerant bacteria, including *B. subtilis* and *B. licheniformis*, thrive under high temperatures, producing thermostable enzymes that hydrolyze lignocellulosic materials efficiently. This activity accelerates organic matter decomposition, stabilizes nitrogen within microbial biomass and humic fractions, and minimizes nitrogen losses. By reducing greenhouse gas emissions such as methane and ammonia, bacterial inoculation contributes to more sustainable composting (Cui *et al.*, 2019) [14].

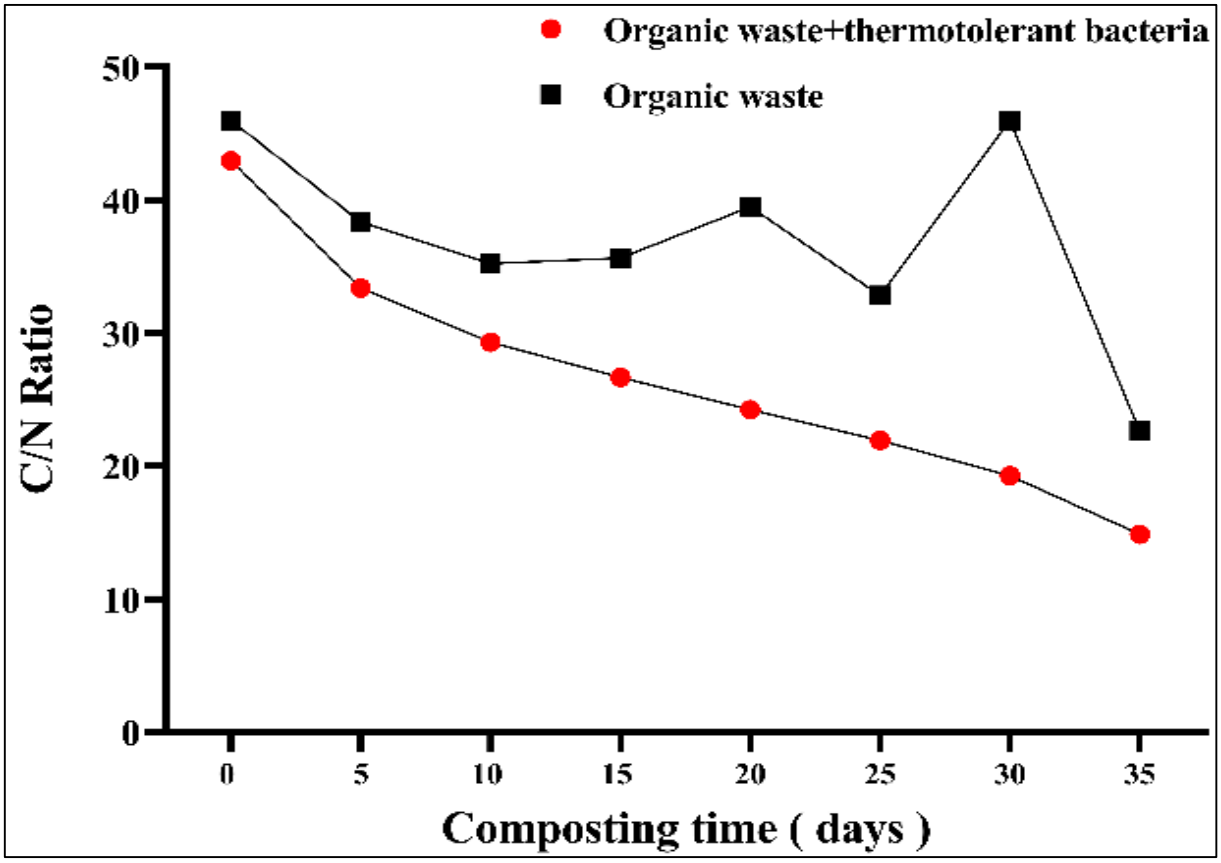

Fig 2: Comparative analysis of the carbon-to-nitrogen (C/N) ratio during the composting process of organic waste, with and without thermotolerant bacterial inoculation. The data highlight a continuous and accelerated reduction in the C/N ratio in bacteria-treated compost, indicating enhanced microbial-driven decomposition and stabilization, as compared to untreated compost. The bacteria-treated compost achieved a C/N ratio below 20 by Day 35, signifying maturity and suitability for agricultural use.

Impact of Thermotolerant Bacteria on Nitrogen Transformation during Organic Waste Composting

Figure (3) shows changes in nitrate nitrogen (NO_3^--N) concentration (g/kg) over 35 days of composting untreated organic waste and organic waste inoculated with thermotolerant bacteria.

Initial Phase (Day 0-10), NO_3^--N concentrations remained low in untreated compost, with a slight declining from ~0.4 g/kg to ~0.3 g/kg, as a result of limited microbial nitrification due to slow degradation of organic matter and ammonium-oxidizing and nitrate-forming bacteria competition. Thermophilic conditions absence is likely restricting the activity of ammonia-oxidizing bacteria (AOB), which require ammonium and oxygen (Acton, 2012). In contrast, NO_3^--N levels in bacteria-treated compost increased slightly from ~0.4 g/kg to ~0.5 g/kg, this reflect enhanced nitrification from enzymes like ureases and proteases. These enzymes hydrolyze organic nitrogen into ammonium (NH_4^+), which is oxidized to nitrate by thermophilic nitrifying bacteria (Mushtaq, 2022) [39].

During Intermediate Phase (Day 10-25), (NO_3^--N), levels in untreated compost declined to ~0.2 g/kg on Day 15, likely due to nitrogen immobilization in microbial biomass and ammonium volatilization (Whetton *et al.*, 2022) [57]. Conversely, bacteria-treated compost maintained higher nitrate levels, peaking at ~0.65 g/kg on Day 20, reflecting efficient mineralization and nitrification by thermophilic bacteria, including Nitrosomonas and Nitrobacter (Farooq *et al.*, 2022) [20].

During final Phase (Day 25-35), untreated compost recovered to ~0.4 g/kg as conditions stabilized, enabling nitrification. On the hand, Bacteria- treated compost stabilized at ~0.55 g/kg due to substrate depletion and potential denitrification in anaerobic microenvironments (Hoang *et al.*, 2022) [23].

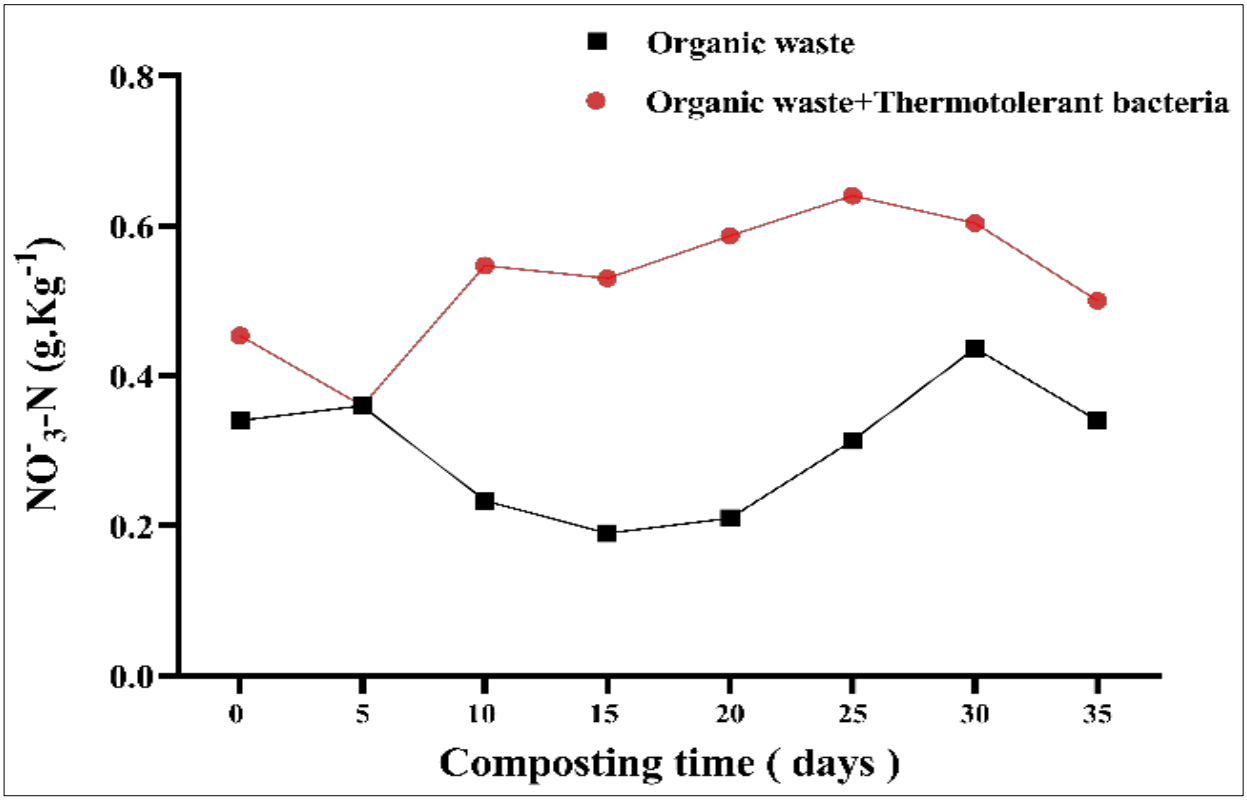

Fig 3: Comparative changes in nitrate nitrogen (NO_3^--N) concentration during 35 days of composting untreated organic waste and organic waste inoculated with thermotolerant bacteria. The bacteria-treated compost consistently exhibits higher NO_3^--N levels, reflecting enhanced nitrogen mineralization and nitrification facilitated by microbial activity under thermophilic conditions. Peaks in nitrate concentration correspond to sustained enzymatic activity and microbial transformation, demonstrating the efficiency of thermotolerant bacterial inoculation in accelerating nitrogen cycling and stabilization.

Influence of Thermotolerant Bacteria on NH_3 Emissions and Nitrogen Conversion during Composting

Ammonia emission during composting is a critical pathway for nitrogen loss, microbial degradation of nitrogen-rich macromolecules such as proteins and nucleic acids releases ammonium (NH_4^+) as an intermediate. Under thermophilic conditions, elevated temperatures and alkaline pH favor NH_4^+ volatilization as ammonia (NH_3), leading to nitrogen loss and reduced agronomic value (Hoang *et al.*, 2022) [23]. Key factors influencing NH3 volatilization include temperature, pH, and the carbon-to-nitrogen (C/N) ratio, with higher pH accelerating the conversion of $NH4^+$ to gaseous NH_3 (Amon *et al.*, 2021) [6].

The initial composting phase (day 2-5) shows a sharp rise in ammonia emissions driven by rapid degradation of nitrogen-rich substrates (Figure 4a). Peak NH_3 emissions reached 750 $mg.day^{-1}$in untreated compost but were 40% lower 450 $mg.day^{-1}$ in bacteria-treated compost. This reduction is attributed to enhanced activity of ammonia-oxidizing microbes facilitated by thermotolerant bacterial inoculation, which converts NH_4^+ to nitrate (NO_3^-) via nitrification, stabilizing nitrogen and reducing volatilization (Chamoli *et al.*, 2024) [12]. Thermotolerant bacteria also produce organic acids, stabilizing pH and mitigating NH3 loss (Liu *et al.*, 2024) [37].

After day 7, NH_3 emissions declined as nitrogenous substrates were depleted and composting transitioned to the stabilization phase. Bacteria-treated compost sustained lower emissions, reflecting efficient nitrogen transformation through microbial nitrification and ammonium oxidation.

Figure 4b shows cumulative NH_3 emissions over 35 days. Untreated compost emitted ~7000 mg, 82% higher than the ~4000 mg from bacteria-treated compost. This reduction highlights the efficacy of thermotolerant bacteria in sustaining nitrification, promoting microbial immobilization, and synthesizing stable nitrogenous compounds like humic substances, which reduce volatilization and enhance compost maturity (Mohamed *et al.*, 2024) [38].

Impact of Thermotolerant Bacterial Inoculation on Compost Properties and Mushroom Yield

Thermotolerant bacterial inoculation significantly enhanced compost quality and mushroom yield compared to the control (Table 4). Higher moisture content in the inoculated compost (65.32% vs. 52.09%) supported microbial activity and enzymatic breakdown of organic matter (Fang *et al.*, 2022), this effect stems from thermophilic bacteria enhancing porosity, water holding capacity, and producing extracellular polymeric substances (EPS) that retain moisture (Bher *et al.*, 2022). These bacteria also stabilize compost temperatures, minimizing water loss and improving structure by breaking down fibrous materials, increasing water retention and infiltration (Tie *et al.*, 2023). Neutral pH in the inoculated compost (7.00 vs. 7.24) indicates stabilization through organic acid production counteracting ammonia volatilization (Ajayan, 2021) [2]. Thermophilic bacteria influence pH by producing ammonium (NH_4^+) during nitrogen-rich organic matter breakdown, which increases pH, and releasing organic acids during lignocellulose degradation, which balances alkalinity (Shrestha *et al.*, 2017).

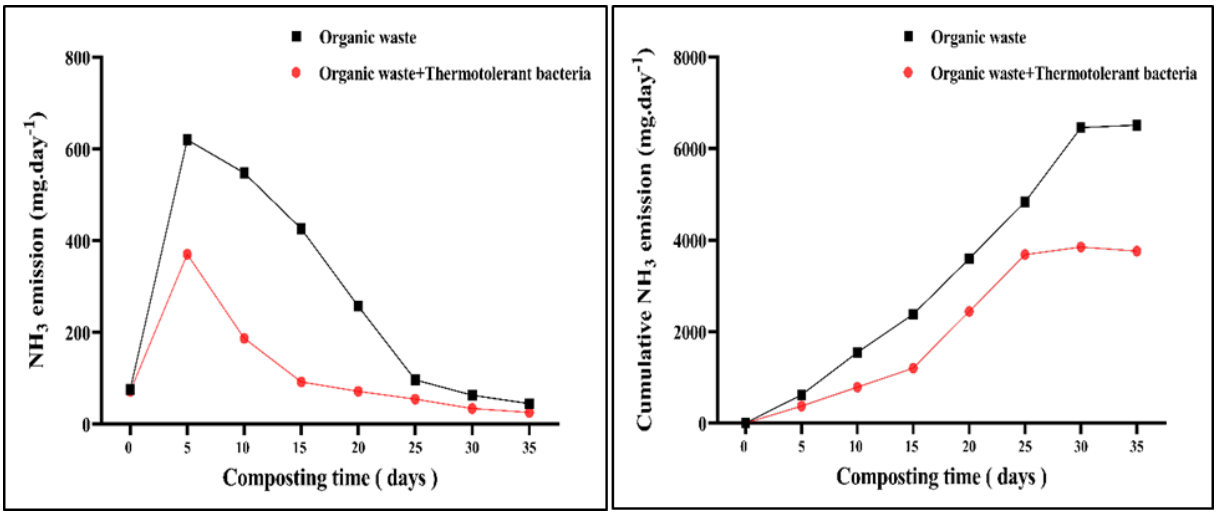

Fig 4: Impact of thermotolerant bacterial inoculation on ammonia (NH_3) emissions during composting. (A) Daily ammonia emission rates reveal a significant reduction in peak emissions for the bacterial treatment compared to the control, attributed to enhanced ammonium (NH_4^+) conversion via nitrification. (B) Cumulative ammonia emissions demonstrate the sustained nitrogen retention achieved with thermotolerant bacterial inoculation, reducing overall nitrogen losses and improving compost nitrogen efficiency.

Elevated conductivity (431.00 mmhos cm^{21} vs. 387.00 mmhos cm^{21}) reflects increased ionic concentration due to accelerated microbial mineralization of organic matter into simpler ions like nitrates (NO_3^-), phosphates (PO_4^{3-}), potassium(K^+), and calcium (Ca^{2+}), improving nutrient availability (Tiwari *et al.*, 2023).

Bacterial counts in inoculated compost were higher both before (23.00 × 10^5 CFU vs. 12.00 × 10^5 CFU) and after pasteurization (3.33 × 10^5 CFU vs. 1.34 × 10^5 CFU). Attributed to thermophilic bacteria's resilience at high temperatures. These bacteria produce heat-stable enzymes and stress-protective proteins, ensuring metabolic activity during pasteurization (Saini *et al.*, 2018). They also enhance microbial environments by outcompeting other microorganisms and improving nutrient accessibility (Iqbal *et al.*, 2023). Post-pasteurization survival is particularly important for sustaining microbial activity and supporting the subsequent fungal colonization process.

Faster mycelial colonization (98% vs. 74%) in inoculated compost is due to improved substrate quality and microbial preconditioning. Thermophilic bacteria degrade lignocellulosic materials, releasing carbohydrates and phenolics for fungal growth, while reducing microbial competition (Omoni *et al.*, 2024). Improved aeration further supports fungal proliferation. Shorter time to the first harvest (18 vs. 20 days), optimized nutrient availability and substrate conditioning by thermophilic bacteria was reflected by increased pinheads per bag (8 vs. 3), and higher fruiting body weight (10.34 g vs. 8.07 g) (Carrasco and Preston, 2020) [11]. Resulting from enhanced nutrient retention, improved compost porosity microbial activity, and moisture balance, the total yield increased (18.44 kg/q vs. 12.60 kg/q), leading to a yield Increase by 46.35% (El-Sebaaly *et al.*, 2021) [18].

Table 4. Effect of Thermotolerant Bacterial Inoculation on Compost Physical, Chemical, Microbial Properties and Mushroom Yield.

Parameter		Characteristics of compost and mushroom yield	
		Thermotolerant Bacterial Inoculation	Control
Moisture (%)		65.32	52.09
pH		7.00	7.24
Conductivity (mmhos cm^{-1})		431.00	387.00
Total dissolved solids (ppm)		211.0	170.0
Bacterial count (CFU× 10^5)	Before pasteurization	23.00	12.00
	Post pasteurization	3.33	1.34
Mycelial colonization (%)		98	74
Days taken for spawn		12	17
Days taken for I^{st} harvest		18	20
No. of mushroom pin heads/bag		8	3
Average fruiting body weight (g)		10.34	8.07
Total Mushroom yield (kg/q compost)		18.44	12.60

Conclusion

A recent strategy to optimize organic matter degradation, enhance nutrient transformation, and promote microbial efficiency by incorporation of thermotolerant bacterial inoculation into composting processes under thermophilic conditions has emerged. By accelerating the biochemical pathways responsible for nitrogen stabilization and reducing gaseous emissions, this approach addresses critical environmental challenges associated with organic waste management.

Furthermore, the inoculation synergistically improves compost maturity, fostering a biologically enriched substrate that aligns with the physiological requirements for advanced fungal cultivation systems. These findings underscore the role of thermotolerant bacterial inoculants as a cornerstone in advancing sustainable agricultural practices, offering a

scalable and eco-compatible solution for integrated waste valorization and enhanced crop productivity.

References.

1. Acton QA. Nitrogen Compounds—Advances in Research and Application: 2012 Edition. c2012.
2. Ajayan AS. Effect of thermochemical organic fertilizer on soil carbon pools, nutrient dynamics and crop productivity in ultisols. Department of Soil Science and Agricultural Chemistry, College of; 2021.
3. Ajeje SB, Hu Y, Song G, Peter SB, Afful RG, Sun F, Asadollahi MA, Amiri H, Abdulkhani A, Sun H. Thermostable cellulases/xylanases from thermophilic and hyperthermophilic microorganisms: current perspective. Front Bioeng Biotechnol. 2021;9:794304.
4. Akindolire MA, Rama H, Roopnarain A. Psychrophilic anaerobic digestion: A critical evaluation of microorganisms and enzymes to drive the process. Renew Sustain Energy Rev. 2022;161:112394.
5. Ammoneh H, Harba M, Akeed Y, Al-Halabi M, Bakri Y. Isolation and identification of local Bacillus isolates for xylanase biosynthesis. Iran J Microbiol. 2014;6(2):127.
6. Amon B, Jensen LS, Groenestein K, Sutton M. Sustainable nitrogen management for housed livestock, manure storage and manure processing. In: Reducing greenhouse gas emissions from livestock production. Burleigh Dodds Science Publishing; 2021. p. 115-160.
7. Aruogu PI, Idoko MO, Giwa HJ. Isolation, molecular characterization and screening of mesophilic and thermophilic bacteria from compost for enzymatic activity. 2024;0-28.
8. Basak B, Kumar R, Tanpure RS, Mishra A, Tripathy SK, Chakrabortty S, *et al.* Roles of engineered lignocellulolytic microbiota in bioaugmenting lignocellulose biomethanation. Renew Sustain Energy Rev. 2025;207:114913.
9. Bher A, Mayekar PC, Auras RA, Schvezov CE. Biodegradation of biodegradable polymers in mesophilic aerobic environments. Int J Mol Sci. 2022;23(20):12165.
10. Bremaghani A. Utilization of organic waste in compost fertilizer production: Implications for sustainable agriculture and nutrient management. Law Econ. 2024;18(2):86-98.
11. Carrasco J, Preston GM. Growing edible mushrooms: a conversation between bacteria and fungi. Environ Microbiol. 2020;22(3):858-72. DOI:10.1111/1462-2920.14765.
12. Chamoli A, Bhambri A, Karn SK, Raj V. Ammonia, nitrite transformations and their fixation by different biological and chemical agents. Chem Ecol. 2024;40(2):166-199.
13. Chen H, Zhang Q, Hong S, Wu J, Nie X, Lin L, Cao M. Analysis of the components of preboiled liquid of *Agaricus bisporus* and its effect on antihypertension. J Jimei Univ (Natural Sci). 2018;23(3):171-177.
14. Cui P, Liao H, Bai Y, Li X, Zhao Q, Chen Z, Yu Z, Yi Z, Zhou S. Hyperthermophilic composting reduces nitrogen loss via inhibiting ammonifiers and enhancing nitrogenous humic substance formation. Sci Total Environ. 2019;692:98-106.
15. Curvetto NR, Figlas D, Devalis R, Delmastro S. Growth and productivity of different *Pleurotus ostreatus* strains on sunflower seed hulls supplemented with N-NH4+ and/or Mn(II). Bioresour Technol. 2002;84(2):171-176. DOI:10.1016/S0960-8524(02)00013-5.
16. Dümenci NA, Temel FA, Turan NG. Role of different natural materials in reducing nitrogen loss during industrial sludge composting: Modelling and optimization. Bioresour Technol. 2023;385:129464.
17. Durand L, Planchon S, Guinebretiere M, Carlin F, Geno- FR. Genotypic and phenotypic characterization of foodborne *Geobacillus stearothermophilus*. HAL Id: hal-01591971; 2017.
18. El-Sebaaly Z, Najjar R, Sassine YN. Substrates for growing *Agaricus bisporus*. In: Mushrooms: *Agaricus bisporus*. CABI Wallingford UK; 2021. p. 66-135.
19. Fang X, Zhu Y-L, Liu J-D, Lin X-P, Sun H-Z, Tang X-H, *et al.* Effects of moisture and temperature on soil organic carbon decomposition along a vegetation restoration gradient of subtropical China. Forests. 2022;13(4):578.
20. Farooq MS, Uzair M, Maqbool Z, Fiaz S, Yousuf M, Yang SH, *et al.* Improving nitrogen use efficiency in aerobic rice based on insights into the ecophysiology of archaeal and bacterial ammonia oxidizers. Front Plant Sci. 2022;13:913204.
21. Gume B, Muleta D, Abate D. Evaluation of locally available substrates for cultivation of oyster mushroom (*Pleurotus ostreatus*) in Jimma, Ethiopia. Afr J Microbiol Res. 2013;7(20):2228-2237.
22. Guo R, Li G, Jiang T, Schuchardt F, Chen T, Zhao Y, Shen Y. Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. Bioresour Technol. 2012;112:171-178.
23. Hoang HG, Thuy BTP, Lin C, Vo D-VN, Tran HT, Bahari MB, *et al.* The nitrogen cycle and mitigation strategies for nitrogen loss during organic waste composting: A review. Chemosphere. 2022;300:134514.
24. Intasian P, Prakinee K, Phintha A, Trisrivirat D, Weeranoppanant N, Wongnate T, Chaiyen P. Enzymes, *in vivo* biocatalysis, and metabolic engineering for enabling a circular economy and sustainability. Chem Rev. 2021;121(17):10367-10451.
25. Iqbal B, Li G, Alabbosh KF, Hussain H, Khan I, Tariq M, *et al.* Advancing environmental sustainability through microbial reprogramming in growth improvement, stress alleviation, and phytoremediation. Plant Stress. 2023;100283.
26. Jackson WA, Flesher D, Hageman RH. Nitrate uptake by dark-grown corn seedlings: some characteristics of apparent induction. Plant Physiol. 1973;51(1):120-127.
27. Jaiswal N, Jaiswal P. Thermostable α-Amylases and Laccases: Paving the Way for Sustainable Industrial Applications. Processes. 2024;12(7):1341.
28. Jiang T, Ma X, Tang Q, Yang J, Li G, Schuchardt F. Combined use of nitrification inhibitor and struvite crystallization to reduce the NH3 and N2O emissions during composting. Bioresour Technol. 2016;217:210-218.
29. Jiménez EI, Silva MTB, Egea FC. Indicadores de la estabilidad y madurez del compost. Compostaje. 2008;243.
30. Karanja AW, Njeru EM, Maingi JM. Assessment of physicochemical changes during composting rice straw with chicken and donkey manure. Int J Recycling Organic Waste Agric. 2019;8:65-72.
31. Kong Y, Zhang J, Zhang X, Gao X, Yin J, Wang G, *et al.* Applicability and limitation of compost maturity evaluation indicators: A review. Chem Eng J. 2024;151386.

32. Kuddus M, Joseph B, Ramteke PW. Production of laccase from newly isolated *Pseudomonas putida* and its application in bioremediation of synthetic dyes and industrial effluents. Biocatal Agric Biotechnol. 2013;2(4):333-338.
33. Lee S-R, Kim G. Assessing Ammonia (NH_3) Emissions, Precursor Gas (SO_2, NOx) Concentrations, and Source Contributions to Atmospheric PM2.5 from a Commercial Manure Composting Facility. Appl Sci. 2024;14(23):11467.
34. Li H, Yao C. The influence of internal noise on the detection of hormonal signal with the existence of external noise in a cell system. Appl Math Comput. 2017;314:1-6.
35. Li MX, He XS, Tang J, Li X, Zhao R, Tao YQ, Wang C, Qiu ZP. Influence of moisture content on chicken manure stabilization during microbial agent-enhanced composting. Chemosphere. 2021;264(111):128549. DOI:10.1016/j.chemosphere.2020.128549.
36. Liu Q, Kong W, Cui X, Hu S, Shi Z, Wu J, *et al.* Dynamic succession of microbial compost communities and functions during **Pleurotus ostreatus** mushroom cropping on a short composting substrate. Front Microbiol. 2022;13:946777.
37. Liu Z, Cao S, He X, Liu G, Yao H, Ding S, *et al.* Effects of crayfish shell powder and bamboo-derived biochar on nitrogen conversion, bacterial community and nitrogen functional genes during pig manure composting. Bioresour Technol. 2024;402:130783.
38. Mohamed TA, Wei Z, Mohaseb M, Junqiu W, El Maghraby T, Chen X, *et al.* Performance of microbial inoculation and tricalcium phosphate on nitrogen retention and conversion: Core microorganisms and enzyme activity during kitchen waste composting. J Environ Manag. 2024;356:120601.
39. Mushtaq I. Effects of nitrogen stabilizers and crop rotation on mineral nitrogen, N cycle genes and microbial community structure and abundance in podzolic soil in boreal climate. Memorial University of Newfoundland; 2022.
40. Myers TJ. Buffering Estuarine Sediments Against Acidification. 2022.
41. Oei P, Nieuwenhuijzen B van. Small-scale mushroom cultivation. Agromisa; 2005.
42. Omoni VT, Bankole PO, Semple KT, Ojo AS, Ibeto C, Okekporo SE, *et al.* Enhanced remediation of polycyclic aromatic hydrocarbons in soil through fungal delignification strategy and organic waste amendment: a review. Indian J Microbiol. 2024:1-23.
43. Page AL. Methods of soil analysis. Part 2. Chemical and microbiological properties. 1982.
44. Palfrey B, Switzer RL. Kinetics of Enzyme Catalysis. Vol. 23. American Chemical Society; 2022.
45. Ruilova Cueva MB, Hernández A, Niño-Ruiz Z. Influence of C/N ratio on productivity and the protein contents of **Pleurotus ostreatus** grown in different residue mixtures. Rev FCA UNCUYO. 2017;49(2):331-334.
46. Sabri MA, Shafiq SA, Chechan RA. Utilization of agricultural and animal wastes in growth of novel Iraqi strains of edible mushrooms **Pleurotus ostreatus** and brown **Agaricus bisporus**. Plant Arch. 2019;19:1188-1193.
47. Saini P, Beniwal A, Kokkiligadda A, Vij S. Response and tolerance of yeast to changing environmental stress during ethanol fermentation. Process Biochem. 2018;72:1-12.
48. Sasaki H, Kitazume O, Nonaka J, Hikosaka K, Otawa K, Itoh K, *et al.* Effect of a commercial microbiological additive on beef manure compost in the composting process. Anim Sci J. 2006;77(5):545-548. DOI:10.1111/j.1740-0929.2006.00384.x.
49. Shrestha S, Fonoll X, Khanal SK, Raskin L. Biological strategies for enhanced hydrolysis of lignocellulosic biomass during anaerobic digestion: current status and future perspectives. Bioresour Technol. 2017;245:1245-1257.
50. Thakur R. Studies on utilization of paddy straw for compost preparation of button mushroom (**Agaricus bisporus**). Dr. Yashwant Singh Parmar University of Horticulture and Forestry; 2024.
51. Tie HO, Man HC, Koyama M, Syukri F, Yusoff FM, Toda T, *et al.* The effect of calcium hydroxide addition on enhancing ammonia recovery during thermophilic composting in a self-heated pilot-scale reactor. Waste Manag. 2023;166:194-202.
52. Tiwari S, Sharma B, Bisht N, Tewari L. Role of beneficial microbial gene pool in mitigating salt/nutrient stress of plants in saline soils through underground phytostimulating signalling molecules. Pedosphere. 2023;33(1):153-171.
53. Wang L, Tan Y-S, Chen K, Ntakirutimana S, Liu Z-H, Li B-Z, *et al.* Global regulator IrrE on stress tolerance: a review. Crit Rev Biotechnol. 2024:1-21.
54. Wang Y, Bi L, Liao Y, Lu D, Zhang H, Liao X, *et al.* Influence and characteristics of **Bacillus stearothermophilus** in ammonia reduction during layer manure composting. Ecotoxicol Environ Saf. 2019;180(June):80-87. DOI:10.1016/j.ecoenv.2019.04.066.
55. Wendering P, Nikoloski Z. Model-driven insights into the effects of temperature on metabolism. Biotechnol Adv. 2023;67:108203.
56. Wheeler S. Factors Influencing Agricultural Professionals' Attitudes towards Organic Agriculture and Biotechnology. ANU, Canberra; 2005.
57. Whetton RL, Harty MA, Holden NM. Communicating nitrogen loss mechanisms for improving nitrogen use efficiency management, focused on global wheat. Nitrogen. 2022;3(2):213-246.
58. Xie K, Jia X, Xu P, Huang X, Gu W, Zhang F, *et al.* Improved composting of poultry feces via supplementation with ammonia oxidizing archaea. Bioresour Technol. 2012;120:70-77.
59. Yan Y, Cao M, Ma J, Suo J, Bai X, Ge W, *et al.* Mechanisms of thermal, acid, desiccation and osmotic tolerance of *Cronobacter* spp. Crit Rev Food Sci Nutr. 2024:1-23.
60. Yang F, Li G, Shi H, Wang Y. Effects of phosphogypsum and superphosphate on compost maturity and gaseous emissions during kitchen waste composting. Waste Manag. 2015;36:70-76.
61. Zhang X, Zhao Y, Zhu L, Cui H, Jia L, Xie X, *et al.* Assessing the use of composts from multiple sources based on the characteristics of carbon mineralization in soil. Waste Manag. 2017;70:30-36.
62. Palfey B, Switzer RL. Kinetics of Enzyme Catalysis. American Chemical Society; 2022 Apr 20.