THE ABILITY OF NITRIFYING BACTERIA IN THE BIODEGRADATION OF ORGANIC WASTE AT DIFFERENT INCUBATION TIMES

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Abstract. Nitrifying bacteria play a key role in converting ammonium into nitrate, which helps eliminate foul odors and increase nitrate availability during composting. This study aimed to determine the effect of the addition of nitrifying bacterial consortium and varying incubation times on organic waste degradation, as well as to identify the nitrifying bacterial isolates involved. This research was conducted using a completely randomized design with a factorial pattern. Data were analyzed using ANOVA followed by the Tukey HSD test. Identification of nitrifying bacterial isolates was conducted based on *Bergey's Manual of Systematic Bacteriology*. The results showed that the nitrifying bacterial consortium of SA14, SA37, BA26, and BA38 could oxidize ammonium but had no significant effect on the increased nitrate content. However, the incubation time and its interaction with the bacteria significantly increased the nitrate content of the compost. The highest nitrate content was achieved at the interaction of bacterial consortium and 42 days incubation at 0.304 ppm. Phenotypic characterization identified isolates SA14 and SA37 as belonging to the genus Nitrosomonas, BA26 as a species of the genus Nitrobacter, and BA38 belonging to the genus Nitrococcus.

Keywords: ammonium, composting, nitrate, nitrifying bacteria, organic waste

A. Introduction

Organic waste produces foul odors due to the release of gases like ammonia (NH_3) and hydrogen sulfide (H_2S) [1]. Composting is an effective solution for managing organic waste because the resulting compost can be used as nutrient-rich fertilizer [2]. Composting involves the biological breakdown of biodegradable organic matter by microorganisms, which decompose complex compounds into simpler forms [3]. The use of microorganisms specifically suited to break down particular waste components is an effective strategy for optimizing the composting process.

Ammonium-containing organic waste, often produced during protein decomposition, can be degraded by nitrifying bacteria through a process called nitrification [4]. Nitrification occurs in two stages: first, ammonium is oxidized to nitrite via an intermediate compound called hydroxylamine; then, nitrite is further oxidized into nitrate [5]. Key nitrifying bacteria include *Nitrosomonas*, which oxidizes ammonium (NH_4^+) to nitrite (NO_2^-), and *Nitrobacter*, which oxidizes nitrite (NO_2^-) to nitrate (NO_3^-). Using these bacteria in composting not only accelerates the degradation process but also helps eliminate the unpleasant odors associated with organic waste.

Of the nine isolates of nitrifying bacteria that were identified from fish pond sediments: SA14 and SA37, which are ammonia-oxidizing bacteria, and BA26 and BA38, which are nitrite-oxidizing bacteria [6]. These isolates show strong potential for degrading organic waste while simultaneously removing foul odors. Importantly, the degradation of organic waste is

more efficient when these bacterial isolates are applied as a consortium rather than individually, as their complementary metabolic functions enhance the overall composting process [7].

Several factors influence the composting process, including aeration, carbon dioxide levels, moisture, temperature, carbon-to-nitrogen (C/N) ratio, pH, and particle size [8]. The duration of the composting process gives bacteria time to break down organic waste, increasing the availability of nutrients in the compost [9]. This incubation period is closely tied to the activity of nitrifying bacteria, which can be measured by the accumulation of nitrate in mature compost [10]. Nitrate is a critical indicator of compost quality, as it plays a vital role in photosynthesis, protein synthesis, respiration, plant growth, and genetic building blocks [11]. Another key indicator of composting success is the C/N ratio. Composting is most effective when the C/N ratio of the substrate is between 30 and 40%, with higher ratios leading to longer composting times.

The objectives of this study were to examine the effects of adding nitrifying bacteria on the nitrate content of compost during organic waste degradation, to assess the impact of incubation time on nitrate levels, to evaluate the combined effect of the nitrifying bacterial consortium and incubation time on nitrate accumulation, and to identify the nitrifying bacterial isolates SA14, SA37, BA26, and BA38.

B. Methods

1. Preparation of organic waste

Organic waste was taken from an integrated disposal site in Karangcegak Village, Kembaran District, Banyumas Regency. A total of 3 kg of organic waste was placed in a 10 L plastic container, which was subsequently covered gauze to allow for ventilation while preventing contamination.

2. Degradation of organic waste by bacterial consortium

A nitrifying bacteria consortium was created by mixing equal amounts of each isolate: SA14 and SA37 (ammonium-oxidizing bacteria) and BA26 and BA38 (nitrite-oxidizing bacteria). Each isolate had a population density of 10^8 CFU/mL. A total of 300 mL of this bacterial consortium, representing 10% (v/w) of the organis waste, was inoculated onto the 3 kg of organis waste using a sterile sprayer to ensure even distribution. The organic waste was then incubated for 42 days at room temperature. To maintain aeration during the incubation period, the substrate was turned over weekly.

3. Measurement of ammonium and nitrate levels

To measure ammonium and nitrate levels, 10 grams of organic waste were first pulverized using a mortar and pestle. This was then dissolved in 100 mL of distilled water in an Erlenmeyer flask and shaken at 120 rpm for 1 hour. The resulting mixture was filtered using Whatman filter paper no. 42 to separate the solid from the liquid. The ammonium content in the filtered solution was determined using the Nessler method [5]. A 1 mL sample of the solution was transferred into a test tube, and a K-Na tartrate solution was added, followed by 1-2 drops of the solution, which was then shaken. Next, 0.2 mL of Nessler reagent was added, and the mixture was allowed to stand for 10 minutes. The ammonium concentration was measured using a spectrophotometer at a wavelength of 420 nm, and the absorbance value obtained was referenced against an ammonium standard curve for quantification.

Nitrate levels were assessed using the Brucine method [5]. A 5 mL sample suspension was placed in a test tube, followed by the addition of 1 mL of NaCl and 5 mL of concentrated H_2SO_4. The mixture was thoroughly homogenized and allowed to cool. Then, 0.25 mL of Brucine-sulfanilic acid solution was added, and the test tube was gently stirred in a water bath for 20 minutes, ensuring that the temperature did not exceed 95°C. The nitrate concentration

was measured with a spectrophotometer at a wavelength of 432 nm, and the absorbance was compared to a nitrate standard curve for quantification.

4. Measurement of C/N ratio

The C/N ratio is determined by measuring the organic carbon (C) and total nitrogen (N) content in the compost. The organic carbon content is measured using the Walkley&Black method, while the total nitrogen content is determined using the Kjehdal method. The C/N ratio is then calculated using the formula:

$$\text{C/N Ratio} = \frac{\text{Organic Carbon Content}}{\text{Total Nitrogen Content}}$$

5. Measurement of the bacterial population

To assess the nitrifying bacterial populations, specific growth media were used: a Nitrosomonas agar medium for Nitrosomonas species and a selective medium for Nitrobacter species. Additionally, the total bacterial population present in the compost was quantified using Luria Bertani agar medium.

6. Measurement of temperature and pH

The temperature of the compost was monitored using a thermometer, while pH values were measured with a soil pH meter. Both temperature and pH were recorded every seven days to track changes throughout the composting process.

7. Observation of compost appearance

The compost's characteristics, including color, texture, and odor, were observed every 14 days. The evaluation of compost odor was conducted based on feedback from seven respondents, providing qualitative insights into the compost's maturation.

8. Identification of bacterial isolates

Bacterial isolates were characterized phenetically, examining their morphological, biochemical, physiological, and nutritional traits.

C. Data analysis

The data obtained were statistically analyzed using ANOVA at a 95% confidence level. The analysis showed that the treatments of incubation time and its interaction had significant effects, so further analysis was done using the Tukey HSD test with a confidence level of 95%. Identification of nitrifying bacterial isolates referred to *Bergey's Manual of Systematic Bacteriology Second Edition Volume 2* [12].

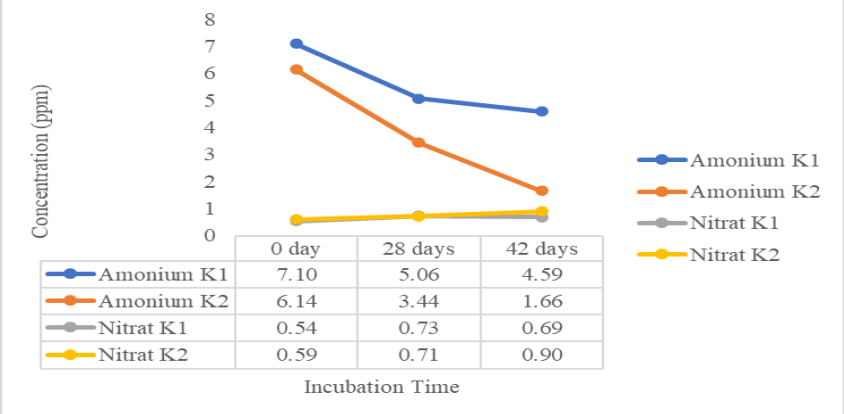
D. Results and Discussion

1. Ammonium and nitrate content of organic waste compost

The activity of nitrifying bacteria in organic matter degradation is marked by a reduction in ammonium levels and a corresponding increase in nitrate levels. In this study, the degradation of organic waste using a consortium of bacterial isolates (SA14, SA37, BA26, and BA38) led to an increase in nitrate levels in the compost. In the composting process without added nitrifying bacteria (K1), nitrate levels increased from 0.538 ppm to 0.732 ppm (36.06%) after 28 days of incubation but then dropped to 0.685 ppm (a 6.42% decrease) after 42 days. This decline in nitrate levels may be due to the incomplete nitratation process, where nitrate formation was not fully achieved. In contrast, composting with added nitrifying bacteria (K2) showed a continuous increase in nitrate levels, rising from 0.594 ppm to 0.712 ppm (19.87%) after 28 days, and then to 0.898 ppm (26.12%) after 42 days (Figure 1). This consistent rise was

due to the active role of nitrifying bacteria in converting ammonium into nitrate. The increase in nitrate levels in line with the growth in the population of nitrifying bacteria (Figure 2).

The results also showed a clear relationship between decreasing ammonium levels and rising nitrate levels in the compost. The addition of nitrifying bacteria to the composting process led to a more substantial reduction in ammonium levels compared to composting without nitrifying bacteria (Figure 1). In the composting process without nitrifying bacteria (K1), ammonium levels fell from 7.097 ppm to 5.059 ppm (28.72%) after 28 days and further dropped to 4.587 ppm (9.33%) after 42 days. However, the decrease in nitrate levels at 42 days, from 0.732 ppm to 0.685 ppm (6.42%), suggested that the nitrification process had not yet reached full completion, preventing nitrate from fully forming. In the composting process with nitrifying bacteria (K2), ammonium levels significantly decreased from 6.139 ppm to 3.437 ppm (44.01%) after 28 days, and then to 1.660 ppm (51.70%) after 42 days (Figure 1). This drop in ammonium levels was a direct result of the nitrification process, which was evidenced by the corresponding increase in nitrate levels. The rise in the population of ammonium-oxidizing bacteria further facilitated the nitrification process, enhancing the compost's nitrate content.



	0 day	28 days	42 days
Amonium K1	7.10	5.06	4.59
Amonium K2	6.14	3.44	1.66
Nitrat K1	0.54	0.73	0.69
Nitrat K2	0.59	0.71	0.90

Figure 1. Ammonium and nitrate content of compost following incubation of 0, 28, and 42 days. (K0: degradation of organic waste without nitrifying bacteria inoculation, K1: degradation of organic waste inoculated with nitrifying bacteria).

An analysis of variance (ANOVA) was conducted to evaluate the effects of adding nitrifying bacteria, the duration of incubation, and their interaction on the nitrate content of the compost. The results revealed that the addition of nitrifying bacteria did not significantly affected the increase in nitrate content ($p>0.05$) (Table 1). However, both the incubation time and the interaction between the addition of nitrifying bacteria and incubation duration had a significant impact on the increase in nitrate levels in the compost ($p<0.05$).

Table 1. The result of analysis of variance (ANOVA) on the effect of the addition of nitrifying bacteria and incubation times on the increase of nitrate content in the composting organic waste process

Source	df	Sum of Square	Mean Square	F	F Table (5%)	Sig.
K	1	0.003	0.003	1.399	4.747	0.260
T	2	0.160	0.080	34.740*	3.885	0.000
K x T	2	0.042	0.021	9.174*	3.885	0.004
Error	12	0.028	0.002			
Total	17	0.234				

The result of the Tukey HSD test showed that the addition of nitrifying bacteria and an incubation time of 42 days showed the highest average increase in compost nitrate levels in the amount of 0.304 ppm (Table 2).

Table 2. The average nitrate levels (ppm) of the organic compost following inoculation with nitrifying bacteria at different incubation times

Treatment	Mean*
K1T1	$0^{a} \pm 0$
K1T2	$0.194^{b} \pm 0.035$
K1T3	$0.147^{b} \pm 0.095$
K2T1	$0^{a} \pm 0$
K2T2	$0.118^{b} \pm 0.059$
K2T3	$0.304^{c} \pm 0.011$

Notes: K1T1: without the addition of nitrifying bacteria at incubation of 0 days, K1T2: without the addition of nitrifying bacteria at incubation of 28 days, K1T3: without the addition of nitrifying bacteria at incubation of 42 days, K2T1: with the addition of nitrifying bacteria at incubation time of 0 day, K1T2: with the addition of nitrifying bacteria and incubation of 28 days, K1T3: with the addition of nitrifying bacteria at incubation of 42 days.

*Numbers followed by the same letter were not significantly different at the 5% Tukey HSD test.

The measurement results of the bacterial population at the initial incubation of organic waste compost were 1.54 x 10^8 CFU/mL for the treatment without the addition of nitrifying bacteria (K1) and 4.30 x 10^8 CFU/mL for the treatment with the addition of nitrifying bacteria (K2) (Figure 2). The bacterial population in the K1 treatment consisted of indigenous bacteria since the organic waste was not sterilized. According to [13], all waste contains indigenous bacteria. After 28 days of incubation, the total bacterial population in the K1 treatment increased to 2.89 x 10^8 CFU/mL but subsequently decreased to 2.88 x 10^8 CFU/mL after 42 days. In contrast, the total bacterial population in the K2 treatment continued to rise, reaching 7.76 x 10^8 CFU/mL after 42 days of incubation.

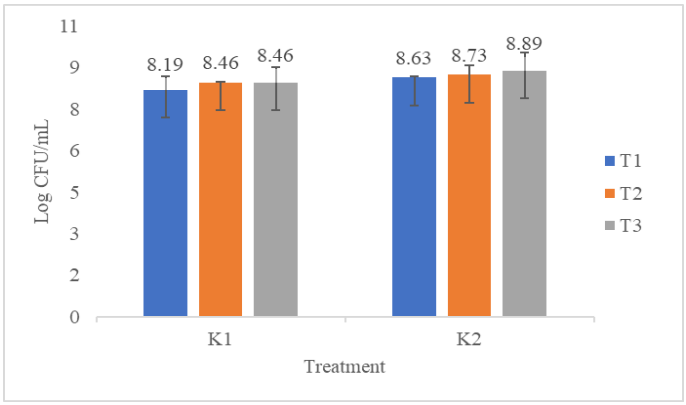


Figure 2. The total bacterial population in the organic waste compost is 0, 28, and 42 days of incubation. (K1: without the addition of nitrifying bacteria; K2: with the addition of nitrifying bacteria; T1: incubation of 0 days; T2: incubation of 28 days; T3: incubation of 42 days).

The population of ammonium-oxidizing bacteria in both the K1 and K2 treatments increased after 28 and 42 days of incubation (Figure 3). Notably, the population density of ammonium-oxidizing bacteria was higher in the K2 treatment compared to the K1 treatment. This rise in the population of ammonium-oxidizing bacteria was correlated with a decrease in ammonium levels in the compost (Figure 1). Ammonium-oxidizing bacteria contain the enzyme ammonia monooxygenase, which oxidizes ammonium into hydroxylamine [14]. Following this reaction, hydroxylamine is converted to nitrite by the enzyme hydroxylamine oxidoreductase [15].

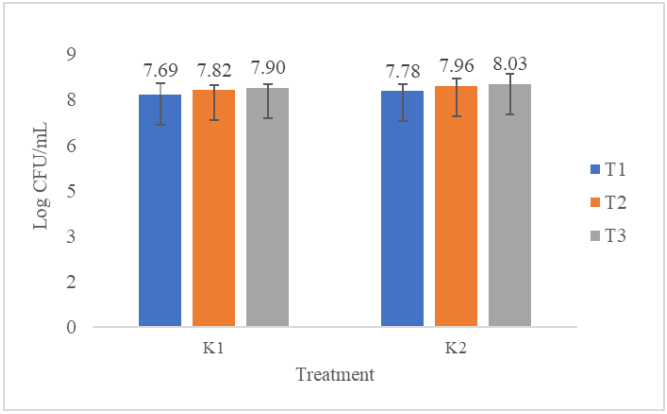


Figure 3. The population of ammonium-oxidizing bacteria at incubations of 0, 28, and 42 days. (K1: without the addition of nitrifying bacteria; K2: with the addition of nitrifying bacteria; T1: incubation of 0 days; T2: incubation of 28 days; T3: incubation of 42 days).

The population of nitrite-oxidizing bacteria showed a consistent increase in the K2 treatment throughout the 42-day incubation period, while the population in the K1 treatment decreased after 42 days (Figure 4). Initially, the nitrite-oxidizing bacterial population in the K1 treatment was 3.77 x 10^7 CFU/mL, which rise to 10.52 x 10^7 CFU/mL after 28 days. However, by the end of the 42-day incubation, this population had decreased to 6.53 x 10^7 CFU/mL. These findings indicate that the decline in the nitrite-oxidizing bacteria population in the K1 treatment correlated with a decrease in nitrate levels (Figure 1). This reduction in nitrate levels may be linked to the activity of denitrifying bacteria [16], which utilize nitrate and nitrite compounds as electron acceptors in their metabolic processes.

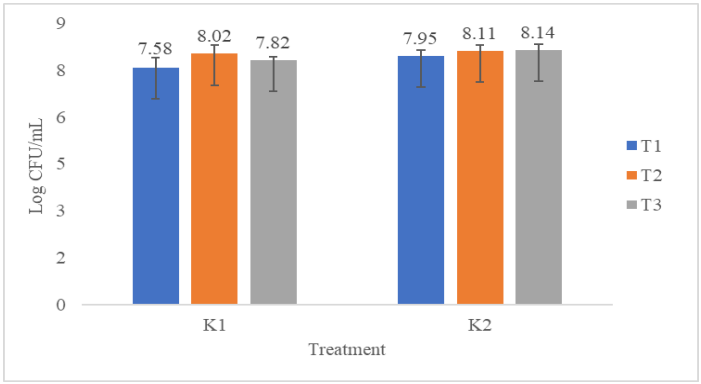


Figure 4. The population of nitrite-oxidizing bacteria following incubations of 0, 28, and 42 days. (K1: without the addition of nitrifying bacteria; K2: with the addition of nitrifying bacteria; T1: incubation of 0 day; T2: incubation of 28 days; T3: incubation of 42 days).

The initial population of nitrite-oxidizing bacteria in the K2 treatment was 8.82 x 10^7 CFU/mL, which increased to 12.87 x 10^7 CFU/mL after 28 days of incubation and further to 1.33 x 10^8 CFU/mL after 42 days. This increase in the population of nitrite-oxidizing bacteria was correlated with the rising nitrate levels in the compost, as the nitrate levels increased due to the accumulation resulting from nitrite oxidation. Nitrite-oxidizing bacteria possess the enzyme nitrite oxidoreductase, which facilitates the conversion of nitrite to nitrate [17]. Therefore, a higher concentration of nitrite-oxidizing bacteria in the compost leads to an increased nitrate content.

The C/N ratio of the compost in both the treatment without (K1) and with (K2) the addition of nitrifying bacteria decreased after 28 days of incubation but increased again after 42 days. The initial C/N ratio of the organic waste in the K1 treatment was 16.4, while it was 15.1 in the K2 treatment. The typical C/N ratio of household waste, particularly food waste, is approximately 16 [18]. The low initial C/N ratio in the organic waste is attributed to the high

levels of total nitrogen in its components, which include both organic and inorganic nitrogen (ammonium, nitrite, and nitrate). However, this total nitrogen content is not immediately available for plant uptake.

The decrease in the C/N ratio in the K1 treatment was more pronounced than in the K2 treatment. Specifically, the C/N ratio in the K1 treatment decreased by 7.22 after 28 days, while it decreased by 5.86 in the K2 treatment (Table 3). This reduction in the C/N ratio was driven by a decrease in organic carbon content as microorganisms utilized organic carbon in the compost materials as an energy source for their metabolic activities. This process resulted in the release of carbon dioxide (CO_2) into the atmosphere, leading to a continued reduction in organic carbon levels. Concurrently, ammonium produced from protein decomposition is converted to nitrate during the nitrification process, resulting in an accumulation of nitrogen in the form of nitrate [19].

After 42 days of incubation, the C/N ratio of the compost in both treatments (K1 and K2) increased. In the K1 treatment, the C/N ratio rise to 4.26, while in the K2 treatment, it increased to 0.71 (Table 3). The more significant increase in the C/N ratio in the K1 treatment can be attributed to decreased microbial activity and the death of microorganisms, which resulted in increased biomass and organic carbon content [20]. The reduction in nitrogen levels was likely due to microorganisms using nitrogen as a nutrient source to form new cells. Additionally, the denitrification process carried out by indigenous denitrifying bacteria in the compost material can also contribute to this decrease in nitrogen content, subsequently increasing the C/N ratio of the compost [21]. Furthermore, the alkaline conditions within the compost pile can influence the increase in the C/N ratio, as insufficient H^+ ions prevent the conversion of ammonia (NH_3) into ammonium (NH_4^+), resulting in the volatilization of NH_3 into nitrogen gas (N_2) in the atmosphere [22].

Table 3. C/N ratio in the composting process of organic waste by nitrifying bacteria

Treatment	Organic C Content (%)	Total N Content (%)	C/N
K1T1	21.40	1.31	16.4
K1T2	14.80	1.61	9.18
K1T3	18.11	1.35	13.44
K2T1	19.58	1.29	15.1
K2T2	16.87	1.83	9.24
K2T3	16.73	1.68	9.95

Temperature is a crucial factor influencing the rate of composting. The initial temperature of the organic waste before treatment was measured at 35.67℃. This elevated temperature was primarily due to the decomposition of carbon from easily degradable food waste [23]. By day 7, the compost temperatures increased to 37.11℃ in treatment K1 (which did not include nitrifying bacteria) and 37.89℃ in treatment K2 (which included nitrifying bacteria) (Figure 5). This observation aligns with findings by [24], indicating that compost temperatures typically rise between days 3 and 10, a sign that decomposing microorganisms are actively breaking down organic waste. Notably, the compost temperature in the K1 treatment was lower than that in the K2 treatment. This difference correlates with the higher total bacterial population observed in the K2 treatment (Figure 2). Increased bacterial activity generates more heat, leading to elevated temperatures. In contrast, the K1 treatment lacked additional activators to enhance composting, resulting in a more natural and slower decomposition process. Consequently, with fewer microorganisms present, less energy was produced, resulting in lower temperatures [25].

The observed temperature increase was not sufficient to reach thermophilic levels, primarily because the compost pile was not large enough, causing heat to dissipate quickly. This aligns with the assertion made by [26], which suggests that low temperatures below the

thermophilic range can occur when there is insufficient compost volume, leading to poor heat insulation.

From days 14 to 42, the compost temperature steadily decreased, approaching ambient temperature. On day 42, the compost temperature dropped to 30.22℃ in treatment K1 and to 30.11℃ in treatment K2 (Figure 5). The ambient temperature at this stage was 30℃. This decline in compost temperature corresponded with ongoing microbial activity as it continued to decompose the available organic matter. These results indicate that the compost had matured, as characterized by its temperature nearing ambient levels. This finding is consistent with the statement by [27] that a decrease in compost temperature close to ambient levels can signify compost maturity.

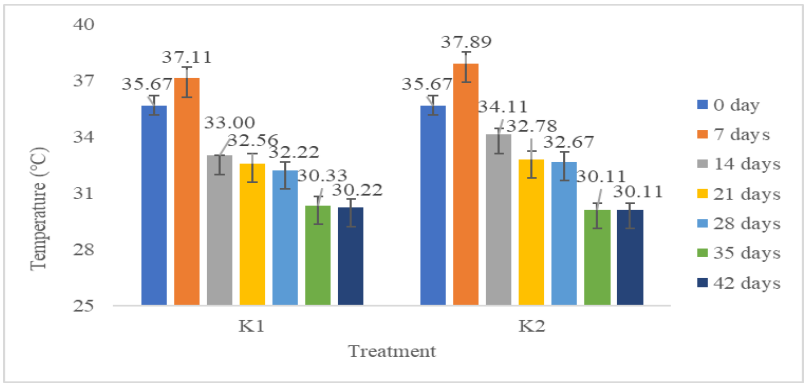


Figure 5. Weekly compost temperature of each treatment in organic waste degradation by nitrifying bacteria. (K1: without the addition of nitrifying bacteria; K2: with the addition of nitrifying bacteria).

The initial pH value of the organic waste before treatment was measured at 6.49 (Figure 6). This low pH was primarily due to the organic acids produced during the degradation of food waste [23]. Throughout the composting process in both K1 and K2 treatments, the pH values ranged from 6.49 to 7.95. This range is suitable for the activity of nitrifying bacteria, which thrive in a pH range of 6.6 to 9.7 [28]. Variations in pH levels reflect the activity of microorganisms involved in the breakdown of organic matter [29]. Specifically, an increase in pH occurs when proteins decompose into ammonia (NH_3), which then reacts with water to form ammonium hydroxide (NH_4OH), creating an alkaline environment [30]. Conversely, a decrease in pH may result from the enzymatic oxidation of inorganic compounds generated during degradation, leading to the production of H^+ cations [31].

On day 42, the pH values of the compost were 7.82 for treatment K1 and 7.78 for treatment K2. According to SNI 19-7030-2004, mature compost should have a pH ranging from 6.8 to 7.4. A pH within this neutral range is beneficial for plant nutrient absorption and can help reduce soil acidity. At neutral soil pH, plant root cell membranes are more efficient in absorbing essential nutrient ions such as nitrate, phosphate, and potassium [32].

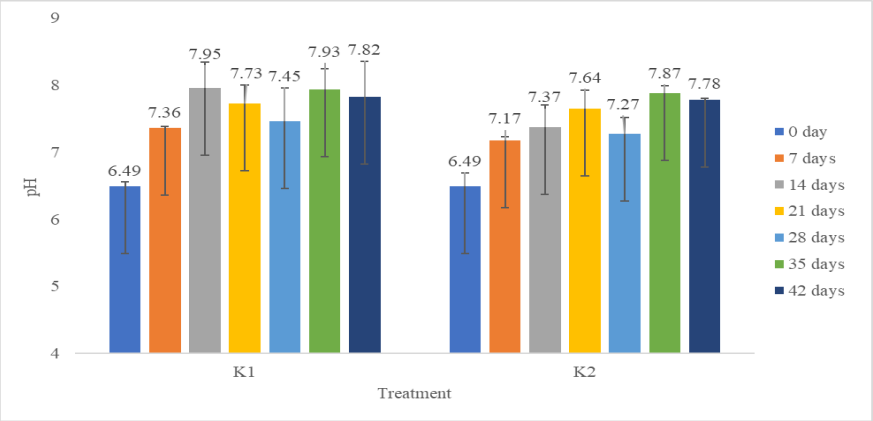


Figure 6. Weekly pH of compost of each treatment in organic waste degradation by nitrifying bacteria. (K1: without the addition of nitrifying bacteria; K2: with the addition of nitrifying bacteria).

The quality of compost can be evaluated based on its appearance, including texture, color, and odor (Table 4). At the beginning of the incubation period (day 0), the substrate had a solid, compact, and wet texture. By day 14, it began to soften and moisten. At 28 days, the texture was notably soft and moist, and by day 42, it became even more crushed, soft, and moist (Table 4, Figure 7). The presence of fibrous material in the compost indicates incomplete decomposition, which suggests that microbial activity in breaking down organic matter has decreased over time [33]. Mature compost should exhibit fewer fibers and smaller particle sizes, leading to a reduction in volume. This shrinkage is primarily due to the evaporation of water and the release of carbon dioxide (CO_2) generated during the degradation of organic matter. Ideally, high-quality compost should no longer resemble its original constituent materials [34].

The color of the compost substrate changed from brownish-green to dark brown by day 42 in both treatments K1 and K2 (Figure 7). This observation is consistent with the SNI 19-7030-2004 standard, which states that mature compost should appear blackish. Mature compost typically has a blackish-brown color that is distinctly different from its raw materials [35]. The change in color occurs as the degradation process leads to the loss of substrate pigments, resulting in a darker hue that reflects the colors of the remaining constituents [36].

Initially, from day 0 to day 14, the substrate emitted a pungent odor characteristic of organic waste. This strong smell is primarily due to the production of ammonia (NH_3) gas during the breakdown of organic matter [37]. By day 28, the odor evolved to resemble that of rotting leaves, and by day 42, it increasingly took on a soil-like aroma (Table 4). According to the SNI 19-7030-2004 standards, mature compost should have a pleasant, soil-like odor. The results indicate that the addition of a consortium of nitrifying bacteria significantly contributed to the maturation of the compost by the end of the 42-day incubation period.

(a) (b)

(c) (d)

Figure 7. Compost appearance at 0, 14, 28, and 42 days incubation times.
(a) 0 days; (b) 14 days; (c) 28 days; and (d) 42 days.

Table 4. Characteristics of organic waste compost at incubation times of 0, 14, 28, and 42 days

Incubation Time (Day)	Treatment	Characteristics of Compost		
		Texture	Color	Odor
0	K1	Compact and wet	Brownish green	Organic waste
	K2	Compact and wet	Brownish green	Organic waste
14	K1	Rather soft and moist	Brownish green	Organic waste
	K2	Rather soft and moist	Brownish green	Organic waste
28	K1	Soft and moist	Brown	Like weathered leaves
	K2	Soft and moist	Brown	Like a soil
42	K1	Soft and moist	Dark brown	Like a weathered leaves
	K2	Soft and moist	Dark brown	Like a soil

Based on phenetic characterization data, which includes morphological, biochemical, physiological, and nutritional aspects as outlined in Bergey's Manual of Systematic Bacteriology, Second Edition, isolates SA14 and SA37 have been identified as members of the genus **Nitrosomonas**. Isolate BA26 is classified as a member of the genus **Nitrobacter**, while BA38 is identified as a member of the genus **Nitrococcus**. Ammonia-oxidizing bacteria are categorized into five distinct genera: **Nitrosomonas**, which are straight rods featuring peripherally located flattened vesicles of intracytoplasmic membranes; **Nitrosococcus**, which are spherical and possess peripherally or centrally arranged stacks of intracytoplasmic membranes; **Nitrosospira**, characterized by tightly wound spirals that lack extensive intracytoplasmic membrane systems; **Nitrosovibrio,** which are curved rods that also lack extensive intracytoplasmic membranes; and **Nitrosolobus**, identified as pleomorphic lobate cells compartmentalized by intracytoplasmic membranes [38]. On the other hand, nitrite-oxidizing bacteria exhibit a diverse range of shapes, including rods, cocci, and spirilla. **Nitrobacter** cells are described as pleomorphic short rods with a polar cap of intracytoplasmic membranes. **Nitrococcus** is characterized by coccoid cells that feature tubular intracytoplasmic membranes. In contrast, cells of **Nitrospina** appear as long rods and are distinguished by the absence of flattened vesicles or tubes of intracytoplasmic membranes. Finally, the genus **Nitrospira** is recognized for its spiral shape and lack of intracytoplasmic membranes; some strains within this genus are motile and possess a single polar or subpolar flagellum [39].

Table 5. Phenetic characteristics of bacterial isolates SA14, SA37, BA26, and BA38

Characteristics	Isolate			
	SA14	SA37	BA26	BA38
Macromorphology				
Size	Small	Small	Small	Small
Form	Round	Round	Round	Round
Color	White	White	White	Cream
Edge	Flat	Flat	Flat	Flat
Elevation	Raised	Raised	Raised	Raised
Optic	Transparent	Translucent	Translucent	Translucent
Micromorphology				
Cell form	Coccus	Coccus	Coccus	Coccus
Gram	-	-	-	-
Motility	-	-	-	-
Biochemical Characters				
Catalase	+	+	+	+
Oxidase	+	+	+	+
O/F	+/+	+/+	+/+	+/+
Nitrate reduction	+	+	+	+
Citrate	+	+	+	+
Gelatin	+	+	+	+
Urease	-	-	-	-
Arginine dihydrolase	-	-	-	-
Physiological Characters				
Growth at 30℃	+	+	+	+
Growth at 35℃	+	+	+	+
Growth at 40℃	+	+	+	+
Growth at 50℃	-	-	-	-
Growth at 60℃	-	-	-	-
Growth at 70℃	-	-	-	-
Growth at pH 5	+	+	+	-
Growth at pH 6	+	+	+	+
Growth at pH 8	+	+	+	+
Growth at pH 9	+	+	+	+
Acid Form				
Glucose	+	+	+	-
Lactose	+	+	-	-
Sucrose	+	+	+	+
Fructose	+	-	+	+
Arabinose	+	+	-	+
Inositol	+	+	+	+
Raffinose	+	+	+	+
Rhamnose	-	+	-	+
Nutritional				
Glucose	+	+	+	-
Lactose	+	+	-	-
Sucrose	+	+	+	+
Fructose	+	-	+	+
Arabinose	+	+	-	+
Inositol	+	+	+	+
Raffinose	+	+	+	+
Rhamnose	-	+	-	+
Genus	Nitrosomonas	Nitrosomonas	Nitrobacter	Nitrococcus

E. Conclusion

Based on the research results, the following conclusions can be drawn:

a. The nitrifying bacterial consortium consisting of isolates SA14, SA37, BA26, and BA38 successfully oxidized ammonium during the organic waste degradation process. However, this addition did not significantly increase the nitrate content of the compost.
b. An incubation period of 42 days proved to be optimal for the organic waste degradation process, resulting in the highest compost nitrate content recorded at 0.226 ppm.
c. The combination of the nitrifying bacterial consortium (SA14, SA37, BA26, and BA38) and a 42-day incubation period yielded the best results, producing the highest compost nitrate content of 0.304 ppm.
d. Among the isolated nitrifying bacteria, SA14 and SA37 were identified as members of the genus **Nitrosomonas**, isolate BA26 was classified under the genus **Nitrobacter**, and isolate BA38 was identified as belonging to the genus **Nitrococcus**.

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G. References

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