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Study on The Use of AINA Microbial Inoculant for Aerobic Composting of Chicken Manure

Oanh Huynh Ngoc[a,b], Bang Doan Nhut[a,b], Tu Anh Nguyen Viet[a,b], Tien Pham[c], Thien Nguyen Minh[a,b,*]

[a]Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam
[b]Vietnam National University Ho Chi Minh City, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam
[c]Faculty of Medical Technology, Van Lang University, Ho Chi Minh City, Vietnam
nguyenminhthien@hcmut.edu.vn

Composting is a quick, effective, and environmentally friendly solution for treating chicken manure. Adding microorganism inoculants to composting materials makes the composting process more effective and improves product quality. This study was carried out to investigate the effect of AINA inoculant on composting of chicken manure. Four treatments (NT1, NT2, NT3, and NT4) were supplemented with AINA microbial inoculant at rates of 0 %, 0.5 %, 1 %, and 1.5 % (v/w). The results showed that the highest temperatures of NT1, NT2, NT3, and NT4 treatments were 46.7 °C, 48.3 °C, 45.5 °C, and 53.7 °C. The final composts' EC, pH, and moisture indicators had 2 - 4 mS/cm, 7.2 – 8.8, and moisture from 53 % - 56 %. The mesophilic and thermophilic bacteria densities were higher than 10^9 CFU/g and 10^5 CFU/g. The GI index of final composts in 4 treatments was 83.93, 123.64, 120.75, and 88.78 showing that the compost was mature and nontoxic to plants. The main root length and stem length indicators of NT2 and NT3 were 48.47 mm, 47.33 mm, 60.60 mm, and 56.27 mm being statistically significantly higher than with the remaining treatments ($p \geq 0,05$).

1. Introduction

Poultry farming, especially chicken, is bringing a stable income to Vietnamese farmers, especially livestock farming on an industrial scale (Lam et al., 2023). With an increase of nearly 18 % in chicken meat consumption per person by 2029 compared to 2022, expanding chicken farming is an inevitable trend (Bairagi et al., 2020). A chicken makes approximately 80-100 g of feces per day (Tanczuk et al., 2019). The accumulation of large amounts of chicken waste, including chicken manure and other waste, increases the pressure of environmental pollution (Tian et al., 2021). In Vietnam, chicken manure is commonly used as fertilizer for vegetables and fruit trees. If chicken manure has not been properly treated before fertilizing crops, using chicken manure still has many disadvantages such as causing air pollution due to odors and organic matter pollution for land, and water sources. when not handled properly, chicken manure also contains pathogens and parasites at risk to animal and human health (Manogaran et al., 2022). Rice husk is a by-product of rice processing released in large quantities into the environment each year. In Vietnam, rice husk is used as biological bedding in livestock or burned to reduce volume (Murimi and Gbedemah, 2018).

Composting is the converting process of organic wastes into organic fertilizer through the biological processes carried out by microorganisms (Nguyen et al., 2021). The composting has been extensively applied to treat agricultural and livestock wastes (Anh Hoang et al., 2021). Compost provides nutrients and changes the soil's physical and chemical properties to replace inorganic fertilizers in agricultural cultivation (Nguyen et al., 2021). The general characteristic of composting poultry manure with agricultural wastes requires a long time of about 3-6 mon. Microbial inoculation helps promote organic decomposition and metabolism and significantly shortens the composting time (Wan et al., 2020). Additional microorganisms help change the structure of the microbial community in the compost pile and provide the necessary enzymes to improve the decomposition of organic

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compounds in the compost materials (Niu and Li, 2022). This study aims to investigate the effect of microbial inoculants on the performance of chicken manure-rice husk composting and the quality of the end products.

2. Materials and methods

2.1 Composting materials

The mixture of chicken manure and rice husk used in this study was obtained from a local chicken farm (Hoc Mon town, Ho Chi Minh City, Vietnam). Microbial inoculant was supplied by AINA Joint Stock Company, Japan. TN 32 mung bean seeds were purchased from Trang Nong Co., Ltd.

2.2 Composting experimental set-up

The experiment was conducted at Department of Biotechnology laboratory of Ho Chi Minh City University of Technology. The study was arranged with 4 treatments including NT1, NT2, NT3, and NT4. Each treatment had 30 kg of chicken manure (a mixture of chicken manure and rice husk) and was supplemented with AINA microbial product with different ratios as shown in Table 1. Input materials for each treatment were mixed well and adjusted moisture by spraying water until the compost moisture was between 50 % and 60 %. The compost materials were placed into 45 L plastic bins to start the composting. The compost piles were turned once a day at 9-10 am. The composting process ended when the compost pile temperature equaled to the ambient temperature.

Table 1: Formulation of compost materials

Treatments	Materials	AINA
NT1	30 kg chicken manure – rice husk	0 % – Control
NT2	30 kg chicken manure – rice husk	0.5 %
NT3	30 kg chicken manure – rice husk	1.0 %
NT4	30 kg chicken manure – rice husk	1.5 %

2.3 Bacterial density and physical properties analysis

The central temperature, pH, electrical conductivity (EC), and moisture indicators were recorded daily during composting. The central temperature was recorded daily at 9 am using a compost thermometer (REOTEMP). The compost samples were collected daily and analyzed for moisture, pH, and Ec. Moisture content was measured by the moisture meter (A&D MX-50, Japan). For pH analysis, the compost samples were mixed with water at a 1:10 w/v ratio and vortexed by the vortex (MX-S, DLAB, China) then measured using a pH meter (HI 2211, HANNA, USA). For EC analysis, the solid samples were carried out the same way as the pH measurement method and gauged using an EC meter (EC - 98361, China).

The compost samples were collected according to the temperature of each compost pile (reached 40 °C, reached the highest, returned to 40 °C, and equaled to the ambient temperature) and analyzed for microbial parameters. The total aerobic bacteria count in the compost was checked using the 3M™ Petrifilm™ 6400 aerobic count plates. The samples were diluted decimally in sterile distilled water. Samples at successive dilutions were spread within Petrifilm plates and repeated two times for each dilution. The petrifilm plates were incubated at 30 °C for 48 h for mesophilic bacteria and at 60 °C for 48 h for thermophilic bacteria.

2.4 Effect of composts on germination index (GI) and growth indicators of mung bean

The seed germination index (GI) was a widely used indicator of compost maturity. The raw and finished compost samples were evaluated on the GI and the influence on the growth parameters of mung bean. The germination evaluation process was carried out according to the method of Zucconi et al. (1981). The compost samples were mixed with distilled water in a ratio of 1:10 w/v and shaken well at 150 rpm using the orbital shaker for 1 h. The sample solutions were centrifuged at 4,000 rpm for 10 min and filtered to obtain the compost extracts. 10 sterile mung beans were placed in a Petri dish containing sterile filter paper with 9 mL of compost extract. The control was carried out by replacing the compost extract with distilled water. The investigation was replicated three times for every treatment. The Petri dishes were incubated in the darkness for 96 h at 22 °C. The germination proportion and other indicators including root length, stem length, stem diameter, and root diameter were measured. GI was computed utilizing the Eq(1).

$$GI = \frac{Treatment\ germination\ ratio}{Control\ germination\ ratio} x \frac{Treatment\ root\ length\ mean}{Control\ root\ length\ mean} x100 \qquad (1)$$

2.5 Data statistics

Data of the growth parameters were analyzed with an ANOVA ($P \leq 0.05$) and Tukey method for posthoc analysis using R version 4.0.0 (Knezevic et al., 2007).

3. Results and discussions

3.1 Composting temperature profile

Compost center temperature was a widely used index to monitor the progress of the composting process (Hemidat et al., 2018). The ambient temperature ranged from 29 °C to 34°C throughout the incubation process. The compost pile temperature of the treatments varied from 29.5 °C to 53.7 °C with extensive changes during the composting process corresponding to the four composting phases (mesophilic, thermophilic, cooling, and maturation) (Zhang et al, 2022). The compost pile temperature increased rapidly in the first 3 - 7 d and reached the highest temperature in the 5-7 d, corresponding to 46.7 °C (NT1), 48.3 °C (NT2), 45.5 °C (NT3), and 53.7 °C (NT4) (Figure 1). During the mesophilic phase, mesophilic microorganisms digested sugars and proteins, causing rapid heat accumulation to the compost pile transition to the thermophilic phase. In the thermophilic phase, high temperatures inhibited mesophilic microorganisms' activities and constructed conditions for thermophilic bacteria to decompose proteins and other stable carbohydrates including cellulose, hemicellulose, and lignin (Nguyen et al., 2021). The thermophilic phase of all treatments (central temperature ≥ 40 °C) staying from 6 - 13 d was an essential condition to destroy harmful microorganisms in raw materials such as *Salmonella*, and *E.coli* (Lin et al., 2022). After the thermophilic phase, the temperature of the compost piles dropped rapidly due to the decline of organic matter in the raw materials, causing microbial activity to decrease (Papale et al., 2021). The composting process ended after 31 d when the temperature in the treatments equaled to the ambient temperature.

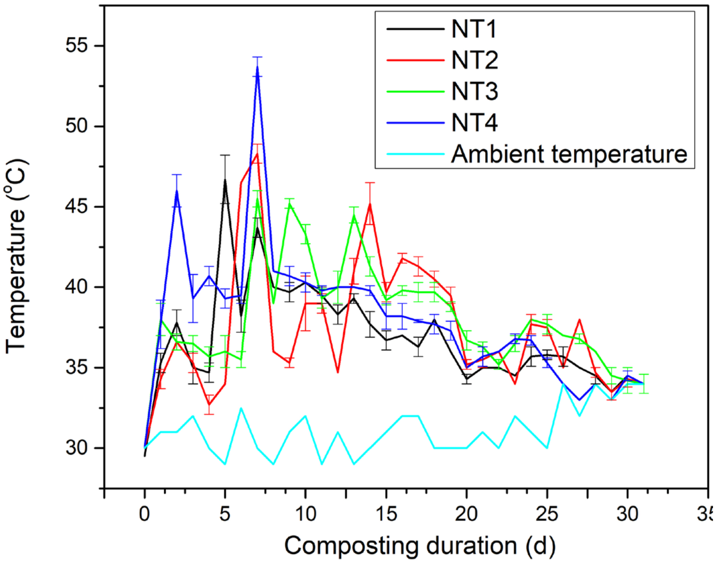

Figure 1: Changes of temperature during co-composting of chicken manure and rice husk.

Most treatments supplemented with AINA inoculant reached a higher peak temperature than the NT1 treatment. The duration of the thermophilic phase of NT2, NT3, and NT4 treatments (13, 8, and 12 d) was higher than that of NT1 treatment (6 d). NT4 treatment supplied 1.5 % v/w AINA product showed better results than other treatments with a lower rate of product addition. AINA inoculation increased the efficiency of decomposing organic compounds in compost materials. Effective microorganisms in inoculants improved the diversity of local microbes, improving the efficiency of decomposition of organic compounds and shortening composting time (Greff et al., 2022).

3.2 pH, EC, and moisture of composting

The pH of the initial stages of NT1, NT2, NT3, and NT4 treatments were 7.93, 7.87, 8.03, and 8.04. The treatments' pH decreased in 1-2 d of composting before increasing and stabilizing around 8.6 (data not shown). In the early stage, microorganisms decomposed organic compounds and released organic acids reducing the pH of the compost materials, the pH increased due to the release of NH_3 due to the decomposition of N-containing compounds in the following stage (Mengqi et al., 2023). The pH of the mature compost of NT1, NT2, NT3, and NT4 were 8.71, 8.53, 8.74, and 8.62.

The Electrical conductivity (EC) value reflected the salinity level of the compost with the mature compost value ranging from 2-4 mS/cm (Zhang et al., 2021). The lowest and highest EC values occurred in NT4 and NT2 with 1.51 mS/cm and 2.15 mS/cm. The EC value of manure increased rapidly in the first 4 d and then stabilized at the end of the composting period. The increased EC value was the release of ions from the decomposition of organic compounds (Irvan et al., 2018). The EC values of the mature composts of NT1, NT2, NT3, and NT4 were 2.68, 2.94, 2.76, and 2.55 (data not shown) that the final composts were suitable for use on crops.

Humidity impacted the growth and metabolism of microorganisms during the composting process. The appropriate moisture for composting materials was 45 - 75 % but 50 - 60 % was the optimal (Lin et al., 2022). The starting moisture of treatments NT1, NT2, NT3 and NT4 were 56.97 %, 56.46 %, 56.38 %, and 56.42 %. The moisture levels of the treatments increased and stabilized at 60 % in the next stage before decreasing at the end of the process. The final compost of the treatments had moisture levels of 52 %, 54.1 %, 54.86 %, and 54.5 % (Figure 2). The moisture levels during the composting were the appropriate humidity to enable microorganisms' activity. Water was essential for dissolving nutrients for microorganisms' physiological and metabolic activities (Ahn et al., 2008). The high humidity restricted oxygen from diffusing into the compost pile, limiting aerobic microorganisms' activity (Brito et al., 2012).

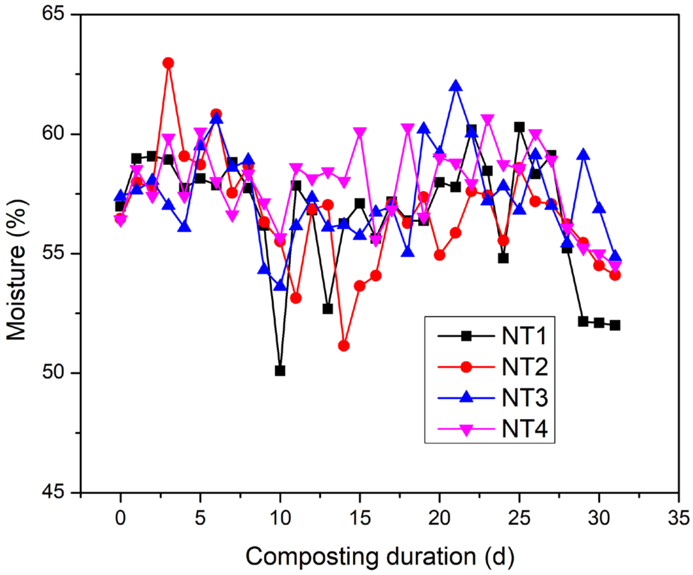

Figure 2: Changes of moisture during composting process

3.3 Microbial density

Changes in the total number of mesophilic and thermophilic bacteria in the initial and final stages were shown in Table 2. The density of mesophilic and thermophilic bacteria in the treatments supplemented with AINA product was higher than in the NT1 treatment. The addition of bacterial inoculant significantly increased the total bacterial density for the decomposition of compost materials. The density of mesophilic and thermophilic bacteria in NT4 treatment (1.5 % v/w AINA addition) reached 2.03x10^{10} CFU/g and 1.8x10^{6} CFU/g, the highest compared to the term of treatments. Adding microbial inoculant increased the bacterial density and diversified the microbial community structure increasing the speed and efficiency of the decomposition of organic compounds in raw materials (Greff et al., 2022).

Table 2: Bacterial densities of initial and end composting periods

	Mesophilic bacteria x10^9 CFU/g				Thermophilic bacteria x10^5 CFU/g			
	NT1	NT2	NT3	NT4	NT1	NT2	NT3	NT4
Initial period	0.45	0.62	0.65	2.56	3.72	4.76	18.10	3.68
End period	1.79	4.07	4.43	20.30	4.01	7.05	9.45	18.01

High microbial density corresponded to a better ability to decompose organic matter, the temperature accumulation of the compost pile to be higher and longer the duration of the thermophilic phase than that of compost piles with lower microbial density (Yasmin et al., 2022). The bacterial density of treatment NT4 was significantly higher than the other 3 treatments, so the temperature of this treatment was also higher than the different treatments. The microbial density of the 3 treatments supplemented with AINA product in the thermophilic phase was longer than the control treatment.

3.4 Effect of composts on germination index (GI) and growth indicators of mung bean

Germination index (GI) and plant growth index parameters were used to indicate the phytotoxicity and effectiveness of compost fertilizers on plants (Nguyen et al., 2020). The results of testing GI and growth indicators on mung bean were presented in Table 3. GI of NT1, NT2, NT3, and NT4 treatments was 83.93 %, 123.64 %, 120.75 %, and 88.78 % higher than 80 % that the final composts were mature and nontoxic to experiment plant (Xie et al., 2024). The GI of NT2 and NT3 treatments reached 123.64 % and 120.75 %, higher than 100 %, proving that the compost of these two treatments contained phytohormones enhancing plant growth (Dimitrijevic et al, 2024). Stem length in NT2, NT3, and NT4 treatments was 66.6 mm, 56.27 mm, and 56.57 mm significantly higher than the control and NT1 treatments with 44.27 mm and 30.94 mm ($p \le 0.05$). The main root length, root diameter, and stem diameter parameters were equivalent to the control treatment results. The addition of AINA product improved the final compost quality. The bacteria in the inoculant supplied the decomposition of organic compounds in raw materials and synthesized plant growth regulators in compost. To evaluate the potential of using microbial products was necessary to consider the parameters of the compost mixing ratio as well as the level of response to specific nutrient indicators and heavy metals (Lin et al., 2022).

Table 3: The growth parameters of mung bean

Treatments	GI (%)	Main root length (mm)	Stem length (mm)	Stem diameter (mm)	Root diameter (mm)
Control		39.20 ± 13.64^{ab}	44.27 ± 13.08^{b}	1.27 ± 0.45^{a}	2.62 ± 0.40^{b}
NT1	83.93	35.25 ± 20.40^{a}	30.94 ± 19.26^{a}	1.44 ± 0.51^{ab}	2.32 ± 0.67^{a}
NT2	123.64	48.47 ± 25.15^{b}	60.60 ± 26.00^{c}	1.55 ± 0.43^{b}	2.44 ± 0.50^{ab}
NT3	120.75	47.33 ± 29.78^{b}	56.27 ± 26.60^{c}	1.35 ± 0.52^{ab}	2.45 ± 0.61^{ab}
NT4	88.78	34.80 ± 20.6^{a}	56.57 ± 26.54^{c}	1.53 ± 0.32^{b}	2.20 ± 0.55^{a}

*Values with different letters within a column are significantly different ($p \le 0.05$).

4. Conclusions

Mixing chicken manure and rice husks has limited the decomposition of local microorganisms that are a source of environmental pollution. Applying bacterial inoculants can shorten composting time and improve the quality of mature compost. The study showed that compost maturity could be achieved after 31 d of composting chicken manure and rice husk in all treatments. The thermophilic temperature and time of all treatments supplemented with AINA preparations were higher than those of the control treatment. The treatment supplemented with 1.5 % AINA product helped the pile move to the sooner thermophilic phase (day 3) and had the highest temperature (53.7 °C) higher than other treatments. The bacterial inoculant supplement at all rates rose mesophilic and thermophilic bacterial density (20.3 x 10^9 CFU/mL and 18.01 x 10^5 CFU/mL) to improve the decomposition of material compounds. The addition of AINA product in all treatments showed that it improved the efficiency of the composting process with chicken manure and rice husk.

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