



Composting of Rice Straw–Based Materials using Aerobic Bioactivator Isolated from Rice Straw, Mahogany Bark and Cassava Peels

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Abstract

Compost is one of organic fertilizers that play an important role in maintaining soil health and supporting sustainable agriculture. Diverse aspects could be developed to increase the quality of compost. This study aims to compare the quality of compost produced by using two different bioactivators, namely aerobic bioactivator RMC (the microbial consortia isolated from composted rice straw, mahogany bark and cassava peels + additional supplement) and fermentative bioactivator (EM4 + molasses) in the composting of the mainly rice straw-based materials. Composting was conducted using a single factor completely randomized design consisting of five levels as follows: C0 (control, rice straw); C1 (rice straw + bioactivator EM4 + molasses); C2 (rice straw + bioactivator RMC + additional supplement); C3 (rice straw + cow dung + bioactivator RMC + additional supplement); C4 (leaf litter + cow dung + bioactivator RMC + additional supplement, as comparison treatment with no rice straw). Among the treatments of C0, C1 and C2, composting the same rice straw material but different bioactivators, C2 showed the highest compost quality and decomposition rate. Among the other three treatments of C2, C3 and C4 composting different materials but using the same bioactivator, C3 showed the highest compost quality, but the three treatments showed the same high decomposition rate. Based on the characteristics of the compost product, including nutrient content, the treatment C3 produced the highest quality, followed by C4 and then C2. Both bioactivators EM4 + molasses and RMC+ additional supplement tended to increase total bacteria, fungi, nitrogen-fixing bacteria and phosphate-solubilizing microbiota in the compost products compared to the control without bioactivator. A set of aerobic bioactivator RMC plus additional supplement serve as one strategy to accelerate the composting process and to enhance the compost quality.

Keywords: compost quality; fermentative bioactivator; lignocellulolytic microbiota; nitrogen-fixing bacteria; phosphate-solubilizing microbiota

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INTRODUCTION

Rice is one of the largest food commodities in the world. In 2018, worldwide rice production was estimated to be 760 million tons, yielding 760 to 1140 million tons of rice straw (FAO, 2018; Otero-Jiménez et al., 2021). The common

procedure to manage rice straw to be applied in agricultural land is by incorporating it into the soil directly, burning the biomass, and composting. Several previous studies showed that fresh rice straw incorporation in paddy fields significantly

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increased soil water content, soil organic carbon (SOC) content, total-N, available-P and available-K (Saothongnoi et al., 2014; Wang et al., 2015). However, fresh rice straw incorporation resulted in negative effects, such as increasing CH₄ emission (Bao et al., 2016), incorporating diseased rice straw enhanced pathogen numbers in the soil and increased disease severity (Zhu et al., 2014). Moreover, Liu et al. (2022) reported that using a wheat straw with a high C/N ratio impacted N deficiency due to N immobilization.

Burning rice straw is known as a cost-efficient and effective method to manage straw waste, helpful in reducing pest and disease populations that might be caused by reinfection from the pathogenic inoculum in the straw biomass, however, some previous studies reported that it caused atmospheric pollution, as well as loss of most N, 25% P, 20% K and 5 to 60% S (Dobermann and Fairhurst, 2002; Otero-Jiménez et al., 2021). The other method to manage rice straw is by converting it to become rice straw biochar or rice straw ash. Si et al. (2018) reported that the addition of rice straw biochar to farmers' fertilization treatment significantly increased rice yield in a cold waterlogged paddy field. Saothongnoi et al. (2014) reported that rice straw ash incorporation in soil resulted in no significant difference in the increase of pH, total-N, SOC, soil organic matter (SOM) and available-P compared with fresh rice straw incorporation but gave a lower increase in total C and C/N ratio and a higher increase in available-K.

Composting is a healthy method to manage a wide variety of organic waste because the composting process involves the aerobic decomposition of solid organic matter by microorganisms and passing through a thermophilic stage resulting in the sterilization effect (Finstein and Morris, 1975; Cahyani et al., 2009). Cahyani et al. (2002, 2003, 2004a, 2009) elucidated the succession of microbiota responsible for the composting process of rice straw during 145 days. They reported that the structure of microbial communities was drastically changed at the thermophilic stage, followed by the middle and curing stages. During composting, the established thermophilic phase reduced the population of pathogenic microorganisms (Asses et al., 2019). Besides reducing microbial pathogens (*Escherichia coli* and *Salmonella* sp.), thermophilic stages in the in-vessel composting of biochar-amended chicken manure and sawdust mixtures affected in reducing gaseous emissions (ammonia and CO₂)

and phytotoxicity (*Lepidium sativum* seed germination assay), and improving the quality of compost compared with the control compost products (Chung et al., 2021).

Many previous studies reported that the application of diverse composts, such as a mixture of cow manure and plant residues compost (Yang et al., 2017), unsterilized rice straw compost and microbial-fortified rice straw compost (Ng et al., 2016), a combination of rice straw compost and N fertilizer (Sharma and Dhaliwal, 2019), and a combination of rice straw compost and chemical fertilizer (Meena et al., 2016) contributed significantly to supplying plant nutrients, increasing physicochemical and microbiological soil properties, suppressing plant pathogens, improving soil fertility and health, and increasing plant growth and yield.

Besides some positive values, compost has some limitations in its utilization. The nutrient concentrations of the common compost product are not as high as those of chemical fertilizers (Che Jusoh et al., 2013; Sharma and Dhaliwal, 2019). Nutrient release is slow (Al-Bataina et al., 2016) and it takes a long time to produce mature and high-quality compost products. Applying immature compost to the soil has potential risks, since immature composts induce high microbial activity to continue aerobic decomposition that may cause self-heating, oxygen depletion, and the release of various phytotoxic compounds that often inhibit seed germination and root growth, and reduce plant production (Sullivan and Miller, 2001; Ryckeboer et al., 2003; Selim et al., 2012; Wichuk and McCartney, 2013). Alromian (2020) reported that immature compost adversely affects lettuce seed germination, plant growth and plant quality.

Important factors that can accelerate the composting process and improve compost quality are the quality of the main organic material and compost additives, the selected microbial biostarter or bioactivator, and the technology used during the composting process (Tognetti et al., 2005; Sánchez et al., 2017). Exploration and examination of microbial consortia as compost bioactivator have been reported in many previous studies, such as evaluation of the traditional bioactivator (MOL) with commercial bioactivator EM4 using Takakura Method (Wikurendra et al., 2022), the effect of exogenous cellulose-degrading bacteria as bioactivator in the co-composting of corn straw and cattle manure (Wang et al., 2022), the addition effect of rock phosphate and plant growth-promoting

rhizobacteria in the composting of corn residue (Rasslan et al., 2021), and effect of lignocellulolytic microorganisms isolated from the peel of cassava, dried rice straw and sawdust for the composting of rice straw (Cahyani et al., 2021). Various studies on compost bioactivators have been carried out, but information on the effect of microbial consortia of aerobic bioactivators isolated from several sources, such as the thermophilic stage of composting, lignocellulosic residue, and added as a set with natural bio supplement in the composting, is still limited.

This is the first study to examine the effectiveness of an aerobic bioactivator formulated from the decomposer consortia of actinobacteria, cellulolytic and lignocellulolytic bacteria, and fungi isolated from rice straw under the thermophilic stage of composting (R), the outer skin of weathered mahogany bark (M), and inner and outer peels of cassava (C) and compared with the commercial fermentative bioactivator (EM4) in the composting of the mainly rice straw-based materials. There were five compost treatments, four of which were rice straw-based materials, whereas one remaining treatment was composed of a mixture of plant litter and cow dung (1:1) that was used as a comparison treatment with no rice straw. The aerobic bioactivator was referred to as bioactivator RMC.

According to Higa and Parr (1994), effective microorganism (EM) contains selected species of microorganisms, including predominant populations of lactic acid bacteria and yeasts characterized as oxygen-tolerant-anaerobic and facultative-anaerobic microorganisms and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms in liquid culture. Thus, the composition and characteristics of microbial decomposers contained in the bioactivator RMC were completely different from those in bioactivator EM4. It is well known that bioactivator EM4 is commonly supplemented with molasses. Bioactivator RMC in the present study was supplemented with a set of rock phosphate, phosphate-solubilizing microbiota (PSM), symbiotic and non-symbiotic nitrogen-fixing bacteria (NFB), and peanut plant residue. This research provides alternative solutions for improving compost products by proposing bioactivators, supplements and compost materials adapted to the various potentials of local biodiversity and natural resources.

MATERIALS AND METHOD

Study site

The present composting study was conducted at the compost house of the Faculty of Agriculture Universitas Sebelas Maret, Central Java, Indonesia. The preparation of microbial isolates for the bioactivators (decomposer microorganisms) and the biological supplement, and for the chemical and biological analysis of the compost product were conducted at the Laboratory of Biology and Biotechnology, Department of Soil Science, Faculty of Agriculture, Universitas Sebelas Maret.

Isolation and preparation of the decomposer microorganisms, symbiotic and non-symbiotic NFB, and PSM

Decomposer microorganisms as the bioactivator RMC were isolated from three sources: 1) decomposed rice straw taken during the thermophilic stage of the rice straw composting process, 2) the outer skin of weathered mahogany bark, and 3) the inner and outer peels of cassava. The microbial isolation was performed using the spread plate method with a serial dilution series of physiological saline solution 8.5% (Gislin et al., 2018); fungal communities were isolated on potato dextrose agar (PDA) medium (Westphal et al., 2021), actinobacteria communities on starch casein agar (SCA) medium (Mohseni et al., 2013), cellulolytic bacteria, and fungi were isolated on carboxymethyl cellulose (CMC) (Kasana et al., 2008) and omelianskii media (Beukes and Pletschke, 2006), and ligninolytic bacteria and fungi were isolated on ligninolytic selection medium (Xiong et al., 2020) (Figure 1).

The NFB and PSM were isolated from the rhizosphere of peanut, maize and rice plant using yeast extract mannitol agar (YEMA) medium for symbiotic NFB (Duta et al., 2004), Jensen medium for non-symbiotic-NFB (Zebua et al., 2020) and Pikovskaya medium for PSM (Walpola and Yoon, 2013) (Figure 2). The isolation of NFB and PSM was performed using the spread plate method with a serial dilution of physiological saline solution at 8.5% (Gislin et al., 2018). Microbial consortia from each source of bioactivator RMC, symbiotic and non-symbiotic-NFB and PSM were then propagated in the liquid culture using the respective media by shaking 8 hours per day for two weeks before application. The population density of the consortium isolates of bioactivator, NFB and PSM are presented in

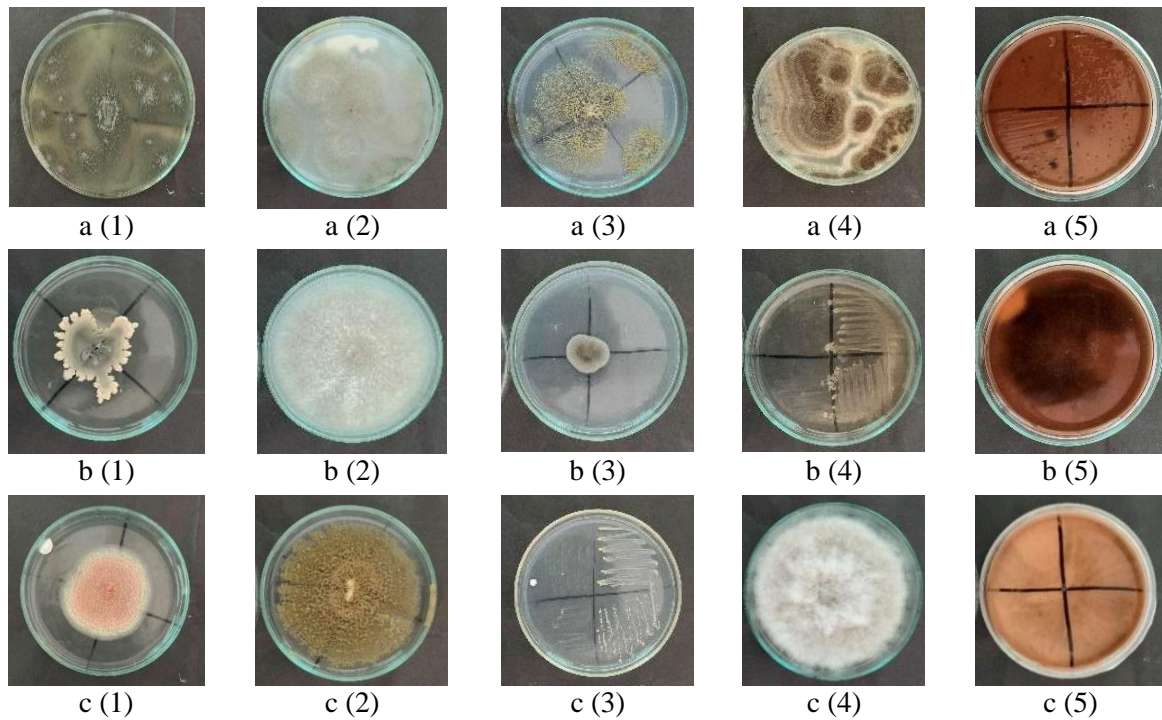


Figure 1. Representatives of the dominant isolates from each source and media used for bioactivator RMC; the source of isolates: a = Rice straw under thermophilic stage of composting; b = Mahogany bark; c = Cassava peels. The media types for isolation: 1 = PDA; 2 = Omelianskii medium; 3 = CMC; 4 = SCA; 5 = Ligninolytic selection medium

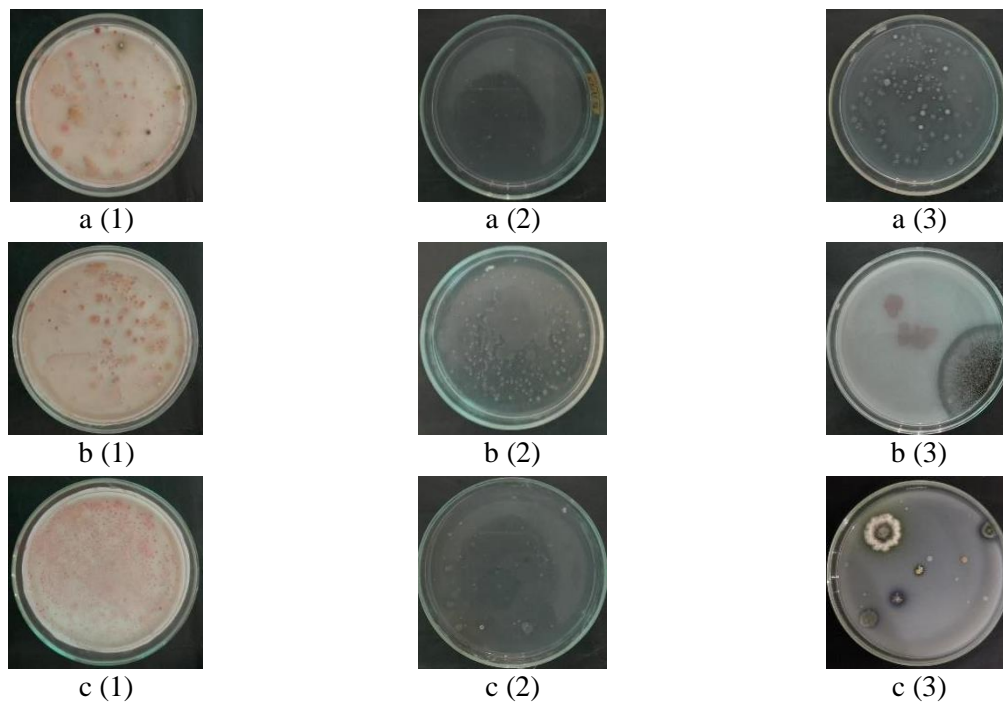


Figure 2. Representative of the dominant isolates from each source and medium for the culture of PSM and NFB to be combined in additional supplement; the source of isolates: a = Rhizosphere of paddy, b = Rhizosphere of maize, c = Rhizosphere of peanut. The media types for isolation: (1) = YEMA, (2) = Jensen, (3) = Pikovskaya

Table 1. The cultures of NFB and PSM were added at the middle stage of the composting process to ensure that the microbiota were not killed by the heat effect of the thermophilic stage.

Composting materials

The composting materials used were rice straw, cow dung, leaf litter from trees in the campus area (a mixture of river tamarind (*Leucaena leucocephala*), mahogany (*Swietenia mahagoni*) and narra tree (*Pterocarpus indicus*)), peanut plant residue, rock phosphate, commercial fermentative bioactivator of EM4 + molasses and aerobic bioactivator of RMC prepared by researchers. All plant residue materials and cow dung were dried before piling. The characteristics of compost materials are shown in Table 2.

Experimental design and procedure of composting

Composting was conducted using single factor experiment with complete randomized design (CRD) consisting of five levels of treatment: C0 (control, rice straw); C1 (rice straw + bioactivator EM4 + molasses); C2 (rice straw + bioactivator RMC + additional supplement); C3 (rice straw + cow dung with ratio 1:1 + bioactivator RMC + additional supplement); C4 (leaf litter + cow dung with ratio 1:1 + bioactivator RMC + additional supplement, as comparison treatment with no rice straw) (Table 3).

Each treatment of compost pile used 300 kg of materials with the respective composition. On the day (D) of compost set up (D0), the main

materials of each treatment were soaked or moistened and then piled layer by layer and arranged to make the compost around 1 m x 1 m x 1 m. At the first turning (D14), 300 ml of bioactivator EM4 + 300 ml molasses was applied to the compost pile C1. For the treatments C2, C3 and C4 using bioactivator RMC, 15 consortium isolates of decomposer (3 sources x 5 type media) were prepared for each consortium in a 250 ml liquid culture of the respective medium, and 10 kg peanut plant residue was applied to the compost piles. At the second turning (D28), three consortium isolates of symbiotic NFB, non-symbiotic NFB and PSM were prepared for each consortium in a 200 ml liquid culture of the respective medium and rock phosphate (75 kg ha⁻¹ or 75 kg 2,000 mg⁻¹ soil or 11.25 g per compost pile) were added to the compost piles with RMC treatment. The composting process was terminated after 77 days.

Observation and sampling during the composting process

Compost samples were taken every week, from day 0 until day 77. At each sampling time, five sub-samples were collected from a depth of about 50 cm (in the center of the compost pile) and at a depth of 30 cm and 70 cm in the middle between the center and the edge of the compost pile. In principle, the temperature of the compost pile was measured every week before sampling. To identify the detailed increasing temperature, an intensive measurement of temperature was

Table 1. Population density of consortium isolates on each liquid medium for bioactivator, PSM and NFB after two weeks of incubation by shaking 8 hours per day

Bioactivator media	Population density (x10 ¹⁷ CFU ml ⁻¹)	
	Bacteria	Bacteria
PDA	-	6.7
Omelianskii medium	236.1	8.5
CMC	70.2	10.1
SCA	25.0	5.1
Ligninolytic selection medium	31.0	1.7
NFB and PSM Culture Media	Bacteria	Fungi
YEMA	0.08	-
Jensen	0.13	-
Pikovskaya	0.18	0.05

Table 2. The characteristics of compost materials

Compost material	pH	Moisture content (%)	Organic-C (%)	Total-N (%)	C/N ratio
Rice straw	8.54	1.02	43.0	1.06	40.57
Cow dung	6.72	1.04	46.4	1.12	41.43
Leaf litter	6.62	1.13	28.6	0.42	68.10
Peanut plant residue	8.45	13.80	37.0	1.83	20.22

Table 3. Treatment design of composting

Treatment	Composting treatment		
	Compost main material	Bioactivator	Additional ingredient
C0	Rice straw	No bioactivator	-
C1	Rice straw	Bioactivator EM4	Molasses
C2	Rice straw	Bioactivator RMC	PR + RP + NFB + PSM
C3	Rice straw + cow dung (1:1)	Bioactivator RMC	PR + RP + NFB + PSM
C4	Leaf litter + cow dung (1:1), as comparison treatment with no rice straw	Bioactivator RMC	PR + RP + NFB + PSM

Note: PR = Peanut residue; RP = Rock phosphate; NFB = Nitrogen-fixing bacteria; PSM = Phosphate-solubilizing microorganisms

conducted every day for seven days after setting up the composting (D1-7), after the first turning (D22-28) and second turning (D43-49). On the day of turning, the temperature was observed twice, just before turning and 3 to 4 hours after turning. Samples taken on D77 were also used for population density analysis of PSM and NFB using the spread plate method with serial dilutions on Pikovskaya, YEMA and Jensen media. Chemical analyses of compost, namely pH H₂O (Fahrudin et al., 2021), total organic-C (Soon and Abboud, 1991), total N (Bremner and Mulvaney, 1982), C/N ratio and total P (Dinh et al., 2014) were conducted on D0 and D77.

Data analysis

Data obtained in the present study were analyzed using a one-way analysis of variance (ANOVA), followed by a Duncan's multiple range test (DMRT) at 5%. The relationship among all of the parameters was tested by Pearson's correlation analysis.

RESULTS AND DISCUSSION

Temperature and pH during the composting process

The fluctuation of the temperature during the composting process reflected the dynamic activity of the involved decomposer microorganisms. Temperature patterns are presented in Figure 3 and 4. Figure 3 shows the temperature of the compost pile that was obtained from the average temperature at five positions of the taken subsamples at each compost pile. Figure 4 presents the temperature of the center of each compost pile during the composting process. The temperature at the center of the composting pile was higher than that of the compost pile throughout the composting process.

On D4, the compost of piles of C0, C1, C2 and C3 showed the first peak temperature at the level 60 to 62 °C, whereas the compost piles C4 showed a lower peak temperature at 55 °C

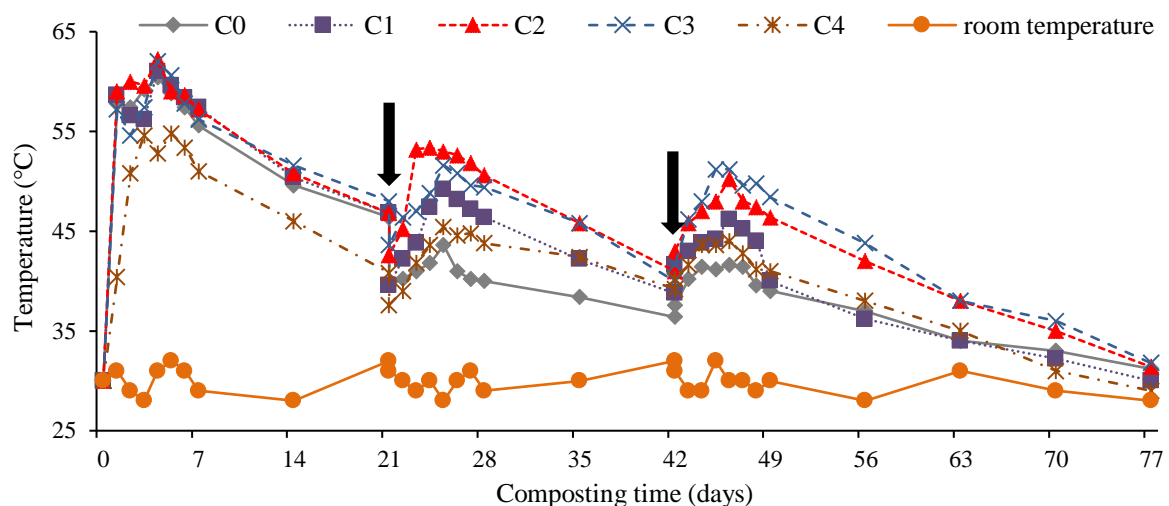


Figure 3. Temperature of compost pile during composting process; C0 = Control, rice straw; C1 = Rice straw + bioactivator EM4 + molasses; C2 = Rice straw + bioactivator RMC + additional supplement; C3 = Rice straw + cow dung + bioactivator RMC + additional supplement; C4 = Leaf litter + cow dung + bioactivator RMC + additional supplement, as comparison treatment with no rice straw. Arrows indicate the turning time of compost piles

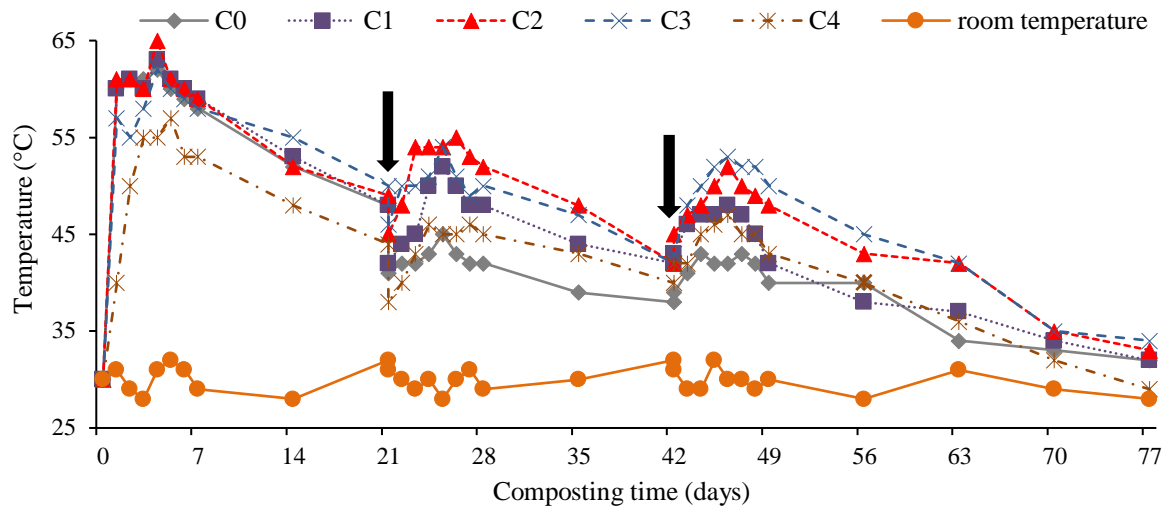


Figure 4. Temperature of the center of compost pile during the composting process; C0 = Control, rice straw; C1 = Rice straw + bioactivator EM4 + molasses; C2 = Rice straw + bioactivator RMC + additional supplement; C3 = Rice straw + cow dung + bioactivator RMC + additional supplement; C4 = Leaf litter + cow dung + bioactivator RMC + additional supplement, as comparison treatment with no rice straw. Arrows indicate the turning time of compost piles

(Figure 3). The first peak temperature of the center of compost piles was achieved at the level 62 to 65 °C for C0, C1, C2 and C3, whereas C4 showed the lower peak temperature of the center (55 °C) on D4 (Figure 4). It is indicated that the highest increase of temperature due to oxidative enzymatic decomposition in composting occurred in the center of each compost pile, whereas the closer to the outer side of the compost pile, the temperature was getting lower. After achieving the peak, the temperature of all compost piles decreased to 46 to 48 °C for C0 to C3 and 41 °C for C4 before the first turning (D21). It is important to note that this first peak temperature occurred naturally before the addition of the bioactivator.

At the first turning, the bioactivator was applied to the compost piles, namely bioactivator EM4 + molasses for C1 and bioactivator RMC for C2, C3 and C4, whereas C0 was without bioactivator. After 3 to 4 hours of the first turning, the temperature of compost piles decreased 3 to 7 °C to the level around 38 to 44 °C (Figure 3). Then, the temperature of compost piles gradually increased to the second peak on D25 at the level around 44 to 53 °C (Figure 3). The second peak indicated that the effect of the bioactivator was involved. Among rice straw-based materials of composting, the bioactivator RMC resulted in a higher effect on temperature compared with bioactivator EM4 + molasses as shown that the second peaks of C2 and C3 (53 °C and 52 °C),

which were higher than the temperature of C1 (49 °C) and C0 (44 °C) (Figure 3). The compost pile of C4, consisting of leaf litter and cow dung, showed the second peak at 45 °C.

The second turning was conducted on D42. The temperature of compost piles at the minute before the second turning was around 36 to 41 °C. At this second turning, the cultures of the beneficial microbiota of NFB and PSM as the supplement in the set of bioactivator RMC were inoculated to the compost pile C2, C3, and C4. Just three hours after the second turning, the temperature of all compost piles showed a slight increase at the range of 38 to 43 °C. On D46, the third peak temperature of all compost piles occurred as follows: C0 (41 °C), C1 (46 °C), C2 (50 °C), C3 (51 °C) and C4 (44 °C) (Figure 3). Meanwhile, the third peak temperature of the center of each compost pile was as follows: C0 (42 °C), C1 (48 °C), C2 (52 °C), C3 (53 °C) and C4 (47 °C) (Figure 4). These results indicated that by the addition of the beneficial microbial cultures of NFB and PSM at the second turning, the treatments C2 and C3 yielded the higher temperature of compost piles and the higher temperature of the center at the range 50 to 53 °C compared to C4 which ranged around 44 to 47 °C. It is important to note that leaf litter as the mixture materials with cow dung (1:1) for the treatment C4 showed a very slow decomposition, since until D46 the original form of the leaf litter was still visible. This slow decomposed leaf litter allowed

to generate space that facilitated the smooth aeration in the compost pile C4, which yielded a lower temperature level compared to C2 and C3. The composting C1 (rice straw material with bioactivator EM4 + molasses) showed a slight higher temperature level at the third peak (46 to 48 °C) compared to C4. After the third peak, the temperature of all compost piles gradually decreased until the level near room temperature (around 29 to 32 °C) at the end of composting (D77).

The changes in pH during composting of five compost piles are presented in Figure 5. On the day of the composting setting up, the pH values of the mixture materials of each compost pile were as follows: C0 (7.6); C1 (7.6); C2 (7.6); C3 (7.0) and C4 (6.8). After one day of composting, the pH of all compost piles increased sharply to around 9.17 to 9.40, then decreased to around 8.2 to 8.5 on D21 (just before turning). After the addition of the bioactivator at the first turning (D21), the pH values of all compost piles increased slightly on D28 and D35 and then gradually decreased to neutral (around 7.3 to 7.6) at the end of composting (D77).

The range of peak temperature of the compost pile C0 to C3 was ≥ 60 °C, revealing the present composting process with rice straw-based materials with a total biomass of 300 kg per compost pile (in dimension 1 m x 1 m x 1 m) reaching the high-temperature level, which leads to a sterilization/sanitation effect for reducing pathogenic microorganism (Cahyani et al., 2002,

2003, 2004a, 2004b, 2009). As reported by Asses et al. (2019) that the thermophilic stage with a temperature exceeding 65 °C during 20 days allows pathogens reduction as indicated by the reducing number of *Fecal coliforms*, *Streptococci* and *E. coli* and the disappearance of *Salmonella* at the end of the composting process. Elorrieta et al. (2003) also supported the effect of the thermophilic stage that all phytopathogenic bacteria (*Erwinia carotovora* subsp. *carotovora*, *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *syringae*) disappeared in less than 60 hours of composting, these bacteria showed low resistance to high temperatures (50, 60 and 70 °C). The other study reported the peak temperature was reached at 60 °C in the composting wheat straw with the dimensions of 10 m x 1.5 m x 1 m (Zhang et al., 2016). However, the composting process of a mixture of rice straw, goat manure and green waste with a total biomass of 20 kg was reported to reach the highest temperature at 56.2 °C on D10 (Jusoh et al., 2013). Thus, the material composition, the total biomass and the dimension size of composting determine the level and duration of peak temperature of the compost pile during composting.

The effect of bioactivator inoculation on the dynamic of compost temperature was also reported by some previous studies. Xu et al. (2019) reported that the inoculation of *Bacillus licheniformis* (TA65), *Aspergillus nidulans* (GXU-1) and *Aspergillus oryzae* (GXU-11) in

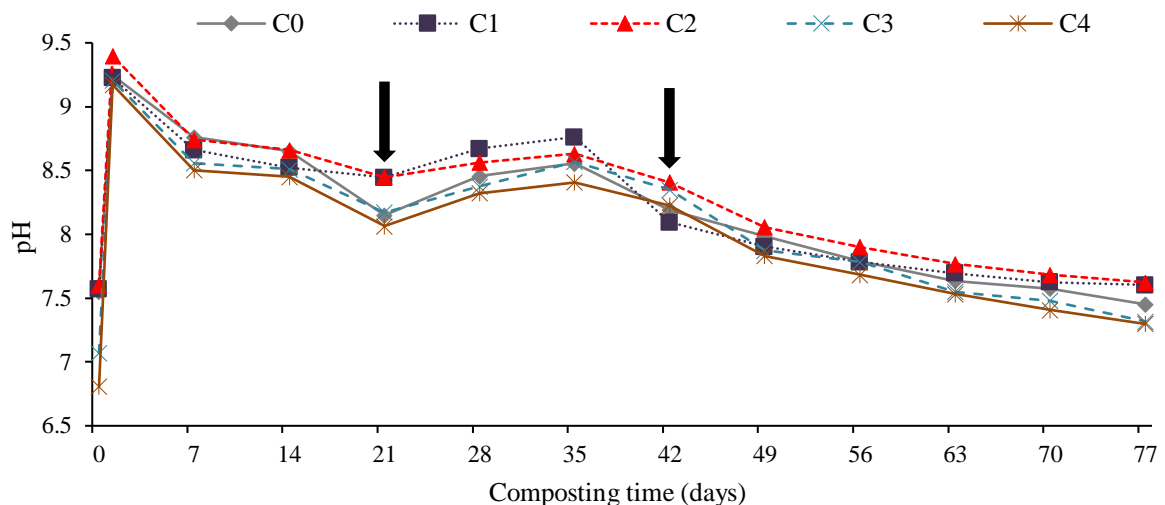


Figure 5. pH during the composting process; C0 = Control, rice straw; C1 = Rice straw + bioactivator EM4 + molasses; C2 = Rice straw + bioactivator RMC + additional supplement; C3 = Rice straw + cow dung + bioactivator RMC + additional supplement; C4 = Leaf litter + cow dung + bioactivator RMC + additional supplement, as comparison treatment with no rice straw. Arrows indicate the turning time of compost piles

twice applications (D0 and D9) as bioactivator for the composting sugarcane and dairy manure resulted the peak temperature at 55.9 °C on D10. The other studies concluded that the inoculation of a combination of *Acinetobacter pittii*, *Bacillus subtilis* subsp. *stercoris* and *Bacillus altitudinis* for composting pig manure and corn stalk resulted in the highest temperature at 67.3 °C on D7 (Li et al., 2019). By comparing with the previous studies, it was revealed that the bioactivation RMC applied in the present study extended the thermophilic stage and reached the second peak (D25) at a level as high as the first peak temperature (D7 to D10) of other composting processes from different materials and inoculants.

The pH values of compost piles in the present study at the level of 8.6 to 8.8 (D7) were also observed in the previous study using the same main materials of rice straw, which reached the pH value of 8.5 (Cahyani et al., 2002). However, Cahyani et al. (2002) did not observe the pH values between D0 to D7 during the composting process. Thus, the highest pH values at the beginning of composting (D1) at the level 9.2 to 9.4 found in the present study could not be compared with those in the previous study. Using different compost materials, Mao et al. (2018) reported that in the composting process, the main materials of pig manure and sawdust (5:1) reached the highest pH values at around 8.5 to 9.0 (D1) and then decreased to around 7.8 to 8.3 (D7). The highest temperature and pH values that were commonly found during the first week of composting were due to the intensive activity of microbial metabolic degradation of organic matter and the ammonification process (Tripetchkul et al., 2012), which resulted in the emission of CO₂, NH₃ and CH₄ (Mao et al., 2018).

Based on the temperature and pH values, the compost pile of C2 showed the highest temperature and pH values compared to compost pile of C0 and C1. On the other hand, the other compost pile with different composition of C3 showed a similar pattern and level of temperature with C2, but showed a similar pattern and level of pH level with C4. The compost pile of C4 exhibited a similar pattern of temperature and pH values, but always at the lowest temperature level compared to the other compost piles. Mao et al. (2018) disclosed that composting of a mixture of pig manure, sawdust, bamboo biochar and a high-temperature aerobic microorganism powder yielded a higher peak temperature at 68.5 °C and pH 7.5 compared with

composting of a mixture of pig manure, sawdust, bamboo biochar and another effective facultative microorganism powder, which had peak temperature 66.8 °C and pH values 7.5. The other composting process using rice straw + goat manure + green waste as the main materials with EM4 resulted in a higher peak temperature of 58.2 °C on D10 compared with the composting of the same materials without EM4 which had a peak temperature of 56 °C on D11 (Che Jusoh et al., 2013). Composting of fresh cattle manure and dry corn straw using exogenous cellulose-degrading bacteria (ECDB) (*B. subtilis* WF-8, *B. licheniformis* WF-11, *B. cereus* WS-1 and *Streptomyces nogalater* WF-10) showed a higher peak temperature at 62 °C on D10 and a lower pH of 8.31 at the end of composting compared with composting without bioactivator, which showed a lower peak temperature at 58 °C on D11 and pH of 8.40 at the end of composting (Wang et al., 2022). Thus, the results of the present study and the previous studies (Che Jusoh et al., 2013; Mao et al., 2018; Wang et al., 2022) indicated that composting with aerobic bioactivator had a higher peak temperature compared with anaerobic or facultative bioactivator.

Decomposition rate and nutritional status of compost products

Based on the ANOVA, it was revealed that the treatments affected significantly toward the contents of organic matter, total-C, total-N, total-P and C/N ratio of the compost products (Table 4). After 77 days of composting, the compost product of the treatment of C0 showed the highest value of organic matter, total-C and C/N ratio, indicating the lowest decomposition rate. Meanwhile, the compost product of C4 showed the lowest content of organic matter, total-C and C/N ratio, indicating the highest decomposition rate. The nutritional status of compost products was observed from the total-N and total-P. The compost treatments of C3 and C4 demonstrated the highest total-N contents at the values of 1.66% and 1.67% with the same notation of DMRT followed by C2 (1.61%) (Table 4). For the total-P, the compost treatment of C3 showed the highest value of total-P (1.90%), followed by C4 (1.67%) and C2 (0.94%). The three compost treatments of C2, C3 and C4 showed the same DMRT notation for the C/N ratio at the values of 20, 21 and 19, respectively (Table 4), which were lower compared to C0 (30) and C1 (25).

Among the treatments with the same main materials of rice straw (C0, C1, C2), it was shown

that the highest decrease of organic matter and total-C was indicated by C2, followed by C1 and then C0. The decrease of organic matter and total-C by C2 was 18.2% higher, compared to C0, whereas by C1 was 5.6% higher, compared to C0 (Table 4). The compost treatment of C2 showed a higher total-N, compared with C1, but the same level of total-P with C1. The two treatments of C1 and C2 had significantly higher nutrition status than that of C0, as indicated that C2 and C1 showed an increase in the total-N by 20.1% and 13.4%, and the increase in the total-P by 18.99% and 17.72% compared to C0. Among the treatments of C0, C1 and C2, the highest decomposition rate as indicated by the lowest C/N ratio was obtained by C2 (20) and then followed by C1 (25) and C0 (30).

Based on the data of organic matter, total-C, total-N, total-P and C/N ratio, it was clearly shown that the three treatments of compost of C2, C3 and C4 in the present study, which comprised different compositions of compost materials but used the same bioactivator RMC + additional supplement, indicated higher quality and higher decomposition rate compared with C1 and C0. In comparison among the three compost treatments of C2, C3 and C4, although all the treatments have the same level of decomposition rate, C3 and C4 showed a higher nutritional status of total-N and total-P compared with C2. The differences in the composition of the main materials were considered causing the difference in compost quality. El-Haddad et al. (2014) reported that composting of a mixture of rice straw supplemented and cattle dung with fungal inoculant showed a higher decomposition rate or lower C/N ratio at 17 compared with composting of rice straw which only had a C/N ratio of 66. Abdel-Rahman et al. (2016)

found that the addition of a mixture of *B. licheniformis* 1-1v and *B. sonorensis* 7-1v in the composting of rice straw and cattle dung resulted in a higher decomposition rate and lower C/N ratio at 14.9 on D80 compared with the composting with the same material without the isolates, which have C/N ratio 16.9 on D80. It is important to note that after 77 days of the composting process, with the value of the C/N ratio of C0 at level 30, indicated that the compost was still unmaturing.

Based on characteristic results on the measurement of organic matter, total-C, total-N, total-P, and C/N ratio, it can be explained that the treatment of C2 showed a higher decomposition rate and the nutritional status of N and P compared with C1. The C0 showed the lowest decomposition rate and nutritional status compared with all other compost treatments. According to the Decree of the Minister of Agriculture of the Republic of Indonesia Number 261/KPTS/SR.310/M/4/2019, the minimum technical requirements of the solid organic fertilizer for organic-C, C/N ratio and total nutrient of $N + P_2O + K_2O$ are $> 15\%$, ≤ 25 , $> 2\%$, respectively.

In the previous study, using rice straw (300 kg) with the addition of ammonium sulfate ($(NH_4)_2SO_4$) at the rate of 10 kg mg^{-1} of air-dried rice straw, Cahyani et al. (2002) reported that the changes of organic matter, total-C, total-N and C/N ratio during 145 days of composting were from 87.2 to 74.2%, 42.1 to 37.9%, 0.75 to 1.74%, 56 to 22, respectively. Although, the composting process in the present study showed the lower total-N at the level 1.66 to 1.67% compared to the previous compost product at level 1.74%, it should be considered that the period of composting in the present study was 77 days or

Table 4. Chemical properties of compost products after 77 days of composting

Treatment	Chemical properties				
	OM (%)	Total-C (%)	Total-N (%)	C/N ratio	Total-P (%)
C0	69.40±1.19 ^d	40.25±0.69 ^d	1.34±0.02 ^a	30±0.09 ^c	0.79±0.02 ^a
C1	65.52±1.76 ^c	38.00±1.02 ^c	1.52±0.04 ^b	25±0.07 ^b	0.93±0.02 ^b
C2	56.76±1.34 ^{ab}	32.92±0.78 ^{ab}	1.61±0.01 ^c	20±0.03 ^a	0.94±0.03 ^b
C3	58.93±1.32 ^b	34.18±0.77 ^b	1.66±0.03 ^d	21±0.06 ^a	1.90±0.06 ^d
C4	55.81±2.72 ^a	32.37±1.58 ^a	1.67±0.03 ^d	19±0.08 ^a	1.67±0.03 ^c
ANOVA	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}	0.00 ^{**}

Note: OM = Organic matter; C0 = Control, rice straw; C1 = Rice straw + bioactivator EM4 + molasses; C2 = Rice straw + bioactivator RMC + additional supplement; C3 = Rice straw + cow dung + bioactivator RMC + additional supplement; C4 = Leaf litter + cow dung + bioactivator RMC + additional supplement, as comparison treatment with no rice straw. Mean±standard deviation (STD) followed by the same notation in the same column shows results that are not significantly different at 5% DMRT level. ** = $p < 0.01$; * = $0.01 < p < 0.05$; ns = $p > 0.05$

shorter than the 145 days in previous composting process (Cahyani et al., 2002).

Similar to the treatment of the present study, Gaind (2014) also conducted the composting process of several compositions with rice straw as the main material (total of biomass 10 kg) for four months. On the treatment of rice straw only, Gaind (2014) reported, the changes of organic matter, total-C, total- N and C/N ratio were from 63.44%, 32.83%, 0.42%, 78.16 to 56.0%, 29.04%, 0.88%, 33, respectively, which indicated the lower organic matter, total-C and total-N, but higher C/N ratio compared with those in the present study of C0 composting treatment. Gaind (2014) also identified the characteristics of the end compost product of the other treatment of T6 with the composition of rice straw and cattle manure with fungal inoculation (three potential phytate mineralizing fungi, including *Aspergillus niger* ITCC 6719, *Aspergillus flavus* ITCC 6720 and *Trichoderma harzianum* ITCC 6721) as follows: organic matter content (52%), total-C (27%), C/N ratio (18.88) and total-N (1.43%). The compost product of the treatment of T6 (Gaind, 2014) indicated a lower organic matter content, total-C, total-N and C/N ratio compared to the compost product of the treatment C3 in the present study. Zhang and Sun (2017) conducted the composting with two treatments using the same materials, a mixture of green waste (fallen leaves and branch cutting) and cow dung, for 30 days with a different total biomass of 20 kg (T4) dan 35 kg (T7) resulted in total-N at 3.17% and 4.02% as well as total-P 0.5% and 0.46%. The results reported by Zhang and Sun (2017) indicated the higher total-N and the lower total-P of the compost products compared with the compost treatment C4 in the present study.

The decomposition process during the composting with all treatments in the present study clearly explained that enhancing the decomposition rate as indicated by reducing the C/N ratio value occurred along with the reduction of organic matter and total-C with the increase in nutritional status of total-N and total-P as revealed by the result of Pearson's correlation analysis that C/N ratio showed positive and significant correlation with organic matter and total-C ($r = 0.955^{**}$; $p < 0.01$ and $r = 0.955^{**}$; $p < 0.01$), but showed negative and significant correlation with total-N ($r = -0.972^{**}$; $p < 0.01$) and total-P ($r = -0.657^{**}$; $p < 0.01$).

Population density of total bacteria and fungi, PSM and NFB of compost products

The ANOVA and DMRT revealed that the treatment of composting (composition of compost materials and application of bioactivator) gave no significant effect on the population density of total bacteria and fungi, PSM and NFB of the end compost products. However, Table 5 presents that the total common bacteria (grown on nutrient agar/NA medium) and fungi (grown on PDA medium) on the compost treatments with bioactivator RMC (C2, C3 and C4) tended to show higher population density than compost treatment with bioactivator EM4 (C1) and compost control (C0). The compost treatments of C1 and C2 that consisted of the same of rice straw materials but used different bioactivator indicated that C2 tended to show the higher population density of total bacteria (NA medium), total fungi (PDA medium), beneficial specific bacteria of non-symbiotic NFB (Jensen medium), and phosphate-solubilizing bacteria and fungi (Pikovskaya medium), but tended to show the lower population density symbiotic NFB (YEMA medium) compared to C1.

Table 5. Population density of total bacteria and fungi, NFB and PSM of compost products

Treatment	Population density (Log10 CFU g ⁻¹)					
	NA Bacteria	PDA Fungi	YEMA NFB	Jensen NFB	Pikovskaya	
					Bacteria	Fungi
C0	9.48±1.08	9.20±1.04	8.83±1.05	8.43±1.29	9.06±1.13	2.60±3.58
C1	9.64±1.16	9.45±1.07	9.37±1.19	8.61±1.18	9.43±1.07	4.67±4.29
C2	9.98±1.30	9.54±1.20	9.12±1.22	8.87±1.30	9.48±1.17	6.64±3.80
C3	9.83±1.21	9.46±1.21	9.25±1.20	8.85±1.27	9.71±1.22	6.67±3.81
C4	9.75±1.13	9.49±1.11	9.31±1.23	8.82±1.25	9.58±1.18	6.46±3.68
ANOVA	ns	ns	ns	ns	ns	ns

Note: C0 = Control, rice straw; C1 = Rice straw + bioactivator EM4 + molasses; C2 = Rice straw + bioactivator RMC + additional supplement; C3 = Rice straw + cow dung + bioactivator RMC + additional supplement; C4 = Leaf litter + cow dung + bioactivator RMC + additional supplement, as comparison treatment with no rice straw. ** = $p < 0.01$; * = $0.01 < p < 0.05$; ns = $p > 0.05$

In the present study, based on the comparison among three compost treatments with the same bioactivator but different compost materials (C2, C3 and C4), the treatment of C2 tended to show the highest population density of total common bacteria and fungi and non-symbiotic NFB, whereas C4 had the tendency to show the highest population density of the symbiotic NFB and C3 tended to show the highest population density of phosphate-solubilizing bacteria (PSB) and phosphate-solubilizing fungi (PSF). The present findings indicated that both compost materials and the bioactivator + supplement added in each treatment potentially affected the population density of total bacteria, fungi, symbiotic and non-symbiotic NFB and PSM.

Comparative studies in composting that used aerobic bioactivator can be seen from the studies reported by Awasthi et al. (2014) and Chang and Yang (2009). Awasthi et al. (2014) reported that composting 14 kg of multiple sludge waste (vegetable waste, food waste, garden waste and office waste) with fungal inoculation (*Trichoderma viride* MTCC 793, *A. niger* MTCC 1344 and *A. flavus* MTCC 1425) for 35 days with an intensity of turning once a week resulted in total aerobic bacteria, filamentous eumycetes, total aerobic cellulolytic bacteria, total aerobic cellulolytic fungi as follow: 8.5×10^{-6} ; 6×10^{-6} ; 4.5×10^{-6} ; 5×10^{-6} and 6×10^{-6} CFU g^{-1} , respectively. Compared with the results reported by Awasthi et al. (2014), all of the compost treatments of the present study (C0, C1, C2, C3 and C4) showed a higher total bacteria per gram compost product (grown on NA medium). On the other hand, Chang and Yang (2009) reported the compost mixture of chicken waste (15%), Chinese herbal residue (15%), sawdust (35%), tea residue (10%), paper pulp (10%) and mixture of food proceeding sludge and waste from poultry and livestock slaughterhouse (15%) during with inoculation *Aspergillus fumigatus* O4 showed total mesophilic fungi, thermotolerant fungi, mesophilic phosphate-solubilizing fungi and thermotolerant phosphate-solubilizing fungi at the amount of 1.7×10^6 , 7.4×10^5 , 1.4×10^6 and 6.5×10^5 CFU g^{-1} , respectively. Thus, the population density of total PSF found by Chang and Yang (2009) was lower than those in all compost treatments in this present study (C0, C1, C2, C3 and C4).

There was limited information about the effect of bioactivator EM4 in composting related to the observation of total microorganisms in compost products. The only study was conducted by

Wahyuni et al. (2010), which explained that the composting of vegetable waste by the addition of EM4 showed a higher total PSB at the level of 42 CFU ml^{-1} compared with the control treatment (without EM4) at the level of 30 CFU ml^{-1} of extract compost.

Comparative studies which observed the microbial population density in the compost product without bioactivator treatment were conducted using the results of the studies by Pepe et al. (2013) and Asses et al. (2019). Pepe et al. (2013) reported that the composting of a mixture of pomace with the kernel (65%), liquid sewage sludge from industrial processing of vegetables (potatoes and carrots) (22%) and borland from the distillation of molasses (13%) with a total biomass of 1,200 kg for 113 days yielded the population density of aerobic bacteria (8.1-8.2 log CFU g^{-1}) and aerobic N-fixing bacteria (3.4-3.7 log CFU g^{-1}). Compared with the population density of bacteria reported by Pepe et al. (2013), it could be explained that total bacteria and NFB obtained in the present composting process (C0, C1, C2, C3 and C4) had a higher level of the population density. Using compost materials from local manufacturers of broiler farming and slaughtering, sewage sludge, cardboard, agricultural waste and wood dust, Asses et al. (2019) reported that total fungi at an amount of 6 log₁₀ CFU g^{-1} were observed in the compost product after 90 days composting, and this result was lower compared to the total fungi observed in the compost treatments of the present study (C0, C1, C2, C3 and C4).

Although ANOVA and DMRT indicated no significant effect of the treatments, Pearson's correlation analysis showed a positive and significant correlation between total bacteria with symbiotic NFB ($r = 0.940^{**}$; $p < 0.01$), non-symbiotic NFB ($r = 0.941^{**}$; $p < 0.01$) and PSB ($r = 0.941^{**}$; $p < 0.01$) as well as the positive and significant correlation between total fungi and PSF ($r = 0.959^{**}$; $p < 0.01$). These findings indicated that increasing growth of total bacteria was simultaneously followed by the increasing growth of specific beneficial bacteria of NFB and PSB as well as the increasing total fungi was followed by the increasing specific beneficial fungi of PSF.

CONCLUSIONS

Bioactivator RMC + additional supplement showed the higher effect compared to the bioactivator EM4 + molasses in accelerating

composting process and increasing the quality of compost product as indicated in a comparison of the composting using the same materials of rice straw (C0, C1 and C2). The treatment of C2 showed the lowest C/N ratio and the highest nutrient content. On the other hand, applying the same bioactivator RMC + additional supplement but using different compositions of compost materials (C2, C3 and C4) showed that the three treatments indicated the same high level of decomposition rate, but the highest quality was obtained by C3 (rice straw + cow dung). Among all the five treatments, based on the characteristics of compost product, including the nutrient contents of N and P, the compost product of C3 showed the highest quality. Future study is needed to elucidate the functional mechanism mediated by each isolate in the consortia of bioactivator RMC in the composting process.

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