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Microbial quality and macronutrient of vermicompost produced by *Eisenia fetida* in dairy wastewater solids

EULIS TANTI MARLINA[1,♥], YULI ASTUTI HIDAYATI[1], ELLIN HARLIA[1], DEDEN ZAMZAM BADRUZZAMAN[1], NAOMI MEYNADHEA[2], NUR ARISTAWIDYA RAHAYU[2]
[1]Microbiology and Waste Management Laboratory, Department of Livestock Product Technology, Faculty of Animal Husbandry, Universitas Padjadjaran. Jl. Ir. Soekarno Km. 21, Sumedang 45363, West Java, Indonesia. Tel.: +62-22-7798241, ♥email: eulis.tanti@unpad.ac.id
[2]Program of Animal Husbandry, Department of Livestock Product Technology, Faculty of Animal Husbandry, Universitas Padjadjaran. Jl. Ir. Soekarno Km. 21, Sumedang 45363, West Java, Indonesia

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Abstract. *Marlina ET, Hidayati YA, Harlia E, Badruzzaman DZ, Meynadhea N, Rahayu NA. 2024. Microbial quality and macronutrient of vermicompost produced by* Eisenia fetida *in dairy wastewater solids. Biodiversitas 25: 2729-2737.* Dairy Wastewater Solids (DWS) contain enough nutrients for the growth of earthworms and utilize the nutritional requirements of livestock waste for growth. The growth of earthworms affects the quality of vermicompost or castings that are useful as organic fertilizers. This research aimed to determine the growth of *Eisenia fertida* using DWS as a growth medium, and its effect on the quality of vermicompost produced. The organic material decomposed in DWS by indigenous microorganisms and earthworms can convert DWS into organic fertilizer through composting. Rice straw was added as a carbon source to obtain the ideal C/N ratio for the growth of decomposing microorganisms. Good growth of decomposer microorganisms can increase the availability of nutrients in DWS as a source of plant nutrition. *E. fetida* was used in vermicomposting for 14 days after the initial decomposition phase had passed. This research used the experimental method of Completely Randomized Design (CRD) with three stocking density treatments (T1: 6.6 g/L; T2: 13.3 g/L; T3: 20.0 g/L) and six replications. The results showed that higher stocking density promoted an increase in the biomass weight of earthworms. Various indigenous microorganisms act as decomposers and work with earthworms to convert organic matter into inorganic matter as a plant nutrient. The stocking density treatment had no significant effect on vermicompost production, total nitrogen content, and P_2O_5 vermicompost but had a significant effect on K_2O, Ca, Mg, population of Nitrogen-Fixing Bacteria (NFB) and Phosphate-Solubilizing Bacteria (PSB). Vermicomposting, DWS, and rice straw mixture produced vermicompost with high functional microbial and macronutrient concentrations.

Keywords: Dairy wastewater solid, earthworm, *Eisenia fetida*, macronutrient, microbial quality, vermicompost

INTRODUCTION

The milk processing industry is one of the food manufacturing sectors that contributes industrial waste in the form of sludge originating from wastewater treatment. Increasing processed milk production affects expanding the volume of wastewater produced. An average of 2.5-3.0 L of wastewater is made from 1 L in the dairy industry process (Ritambhara et al. 2019). If the amount of waste water is not handled correctly, it causes environmental pollution. Dairy wastewater solids has characteristics that can burden the environment (Marlina et al. 2019; Tabelini et al. 2023). Handling dairy industry wastewater generally uses a series of processes combining physics, chemistry, and biology. The purpose of this process is to reduce various dissolved organic materials and other organic compounds with the help of anaerobic bacteria; then, filtration helps absorb various remaining chemicals and separates water from solids. Solids sourced from this treatment are known as Dairy Wastewater Solids (DWS). DWS contains sufficient organic material as a source of nutrition for indigenous microorganisms. Several types of bacteria and yeast are found in DWS (Al-Wasify et al. 2017).

The nitrogen (N) content in milk sludge is relatively high, which plays a good role in the growth of decomposer microorganisms that break down organic materials into inorganic compounds for plants. However, the C/N ratio of DWS is still low for optimal microorganism metabolism, so a mixture of other materials is needed as a carbon source (Zhao et al. 2020). Rice straw is an agricultural waste with a fairly high carbon content, so it acts as a mixture of milk sludge to obtain a C/N ratio suitable for the growth of indigenous microorganisms in DWS so that it can be converted into organic fertilizer. The process of converting DWS into organic fertilizer includes initial decomposition stage and continues with vermicomposting. Each stage of the process cannot be separated from the role of Indigenous microbes as decomposers and earthworms as detritivores, which break down organic material into simple compounds that are available as plant nutrients (Hidayati et al. 2021; Gibb et al. 2022). Vermicomposting is a composting process that involves earthworms as detritivores, which continue the bioconversion process carried out first by microorganisms so that the composting process runs faster (FAO 2020; Kholiya and Poudel 2021). Pre-composting or initial decomposition is a term used in vermicomposting that means the initial active phase of composting when the pile temperature exceeds 55°C (131°F) for a minimum of three days. If these conditions are met, parasites, pathogens, and weed seeds are destroyed, and heat energy is removed from the raw material mixture (Marlina et al. 2020; Hawari et al. 2022). The microbes that play a dominant role in the initial decomposition process are bacteria. Bacteria act as decomposing microbes that first degrade organic materials, synthesize nitrogen compounds, dissolve phosphates, and have better diversity than other microbes (Biyada et al. 2021).

Vermicomposting is a synergistic process between microorganisms as decomposers and earthworms as detritivores. The earthworm *Eisenia fetida* has been widely used as a detritivore in vermicomposting organic materials (Jaikishun et al. 2018; Ramnarain et al. 2018; Raza et al. 2019; Bin Dohaish 2020; Shafique et al. 2023). Simple organic materials produced by decomposers during initial decomposition are then converted by earthworm *E. fetida* into nutrients plants need. Nitrogen (N), P_2O_5, K_2O, Mg, and Ca are needed for plant growth. Vermicompost is an organic fertilizer rich in nutrients and several functional microbes, including Nitrogen Fixer Bacteria (NFB) and Phosphate Solubilizing Bacteria (PSB), which have a positive impact on soil fertility (Dume et al. 2023; Raimondo et al. 2023; Yakkou et al. 2024). One of the quality standards for organic fertilizer is regulated in Government Regulations through Minister of Agriculture Regulation No. 01 of 2019, one of which states the requirements for a total $N+P_2O_5+K_2O$ content in solid organic fertilizer to be at least 2 (Department of Agriculture of the Republic of Indonesia 2019). This research aimed to study microbes and nutrient quality in organic fertilizer produced from DWS through the detritivore activity of earthworms.

MATERIALS AND METHODS

Pre-composting

The materials prepared for the pre-composting process included Dairy Wastewater Solids (DWS) and rice straw. Then, calculate the mass of the compost mixture with a C/N ratio of 25. Mix the DWS and rice straw according to the calculated level, put it in a compost bag with a volume of 50 L for the aerobic fermentation process, and incubate for 7 days aerobically.

Medium preparation

According to the labels stated on the packaging of each media, Nutrient Agar (NA) media, Potato Dextrose Agar (PDA) media, Jensen medium, and Pikovskaya medium were used for total bacterial growth, fungal growth, NFB growth, and PSB growth, respectively. Dissolve each medium in distilled water to 1 L, pour it into a Schott bottle, and heat it on a hot plate stirrer. Sterilization was carried out using an autoclave to a temperature of 121°C for 15 minutes with a pressure of 1 atm. Pour 10-15 mL of each medium (cool) into the petri dish.

Total bacterial population count

Take a 10-g sample from the compost bag and grind it in a mortar. Then, transfer it to an Erlenmeyer flask, add 90 mL of NaCl solution, and homogenize the mixture. Transfer 1 mL of the suspension into 9 mL NaCl solution using a 1,000 µL micropipette and homogenized using a vortex. Then, as previously done 1 mL of the 10^{-2} dilution to the10^{-3} dilution. Repeat this process to get dilutions until 10^{-10}. Take 1 mL from the 10^{-10} dilution using a 1,000 µL micropipette for bacterial inoculation. After this, 1 mL from the 10^{-10} dilution was placed in a petri dish, and then poured nutrient agar media 1 mL of 10^{-10} dilution was placed in a petri dish. Then nutrient agar media was added, and the medium was mixed well and allowed to solidify. Once the medium solidifies, incubate it at 37°C for 24 hours. Bacteria colonies grown on media were calculated using the following formula (Sukmawati and Fahrizal 2018):

$$\frac{\text{CFU}}{\text{g}} = \sum colony \times \frac{1}{\text{Dillution Factor}}$$

Where:
N : Number of bacterial colonies per gram (CFU/g)
∑ Colony : Number of bacterial colonies on the plate (30-300)

Vermicomposting

After the seven-day pre-composting process, *Eisenia fetida* were planted in media with a stocking density as per treatment (T1: 6.6 g/L; T2: 13.3 g/L; T3: 20.0 g/L). The vermicomposting process lasts 14 days with regular inspection and aeration. Harvesting was done using manual methods. The material containing the worms was dumped in a pile on a flat surface above a light source because the worms move quickly to avoid the light; they can be easily identified, picked, and collected in a container. After separating the earthworms and growth medium, each was weighed.

Macronutrient analysis

The total nitrogen (N) content of vermicompost was determined using the Kjeldahl method (FAO 2021). In this method, the sample was digested with sulfuric acid to convert all the nitrogen into ammonium sulfate, which was then titrated with a standard solution of sodium hydroxide to determine the amount of nitrogen present. The amount of P_2O_5 was determined using the wet destruction method (BSN 2018), which involves the sample being digested with a mixture of nitric and perchloric acid. The resulting solution was heated until dry and then dissolved in water to determine the amount of P_2O_5 present. The amount of K_2O was analyzed using the flame photometer method (Wiyantoko et al. 2021). In this method, the sample was introduced into a flame, which excites the atoms and causes them to emit light. The light intensity was then measured to determine the amount of K_2O present. The levels of Calcium (Ca) and Magnesium (Mg) were measured using the wet oxidation method (Kim et al. 2021). In this method, the sample was digested with a mixture of nitric and perchloric acid, and the resulting solution was then analyzed using an Atomic Absorption Spectrophotometer (AAS) to determine the levels of Ca and Mg present.

Nitrogen-fixing and phosphate-solubilizing analysis of bacteria

Nitrogen-Fixing Bacterial (NFB) and Phosphates-Solubilizing Bacterial (PSB) populations were enumerated using the total plate count method with serial dilutions, carried out up to 6 x dilution (10^{-6}). NFB was grown on the Jensens Medium and PSB on the Pikovskaya medium (Arsita et al. 2020). The results of the 6x dilution series were taken aseptically to be transferred to a petri dish and planted in Jensen medium using the pour plate method. After incubation for 24 hours at room temperature, observations were made to determine nitrogen-fixing activity, which was indicated by a color change from yellowish-white to blue around the colony growth. The growing colonies were observed for macroscopic characteristics: shape, color, form, elevation, surface, edges, and microscopic observations, namely cell shape and gram staining (Zhang et al. 2022).

Data analysis

ANOVA and Tukey's test were used to analyze microbial populations in the initial decomposition process, the increase in earthworm biomass, verbed shrinkage, N-fixing Bacteria, and P-solubilizing bacteria populations in vermicomposting with SPSS 26 (2022).

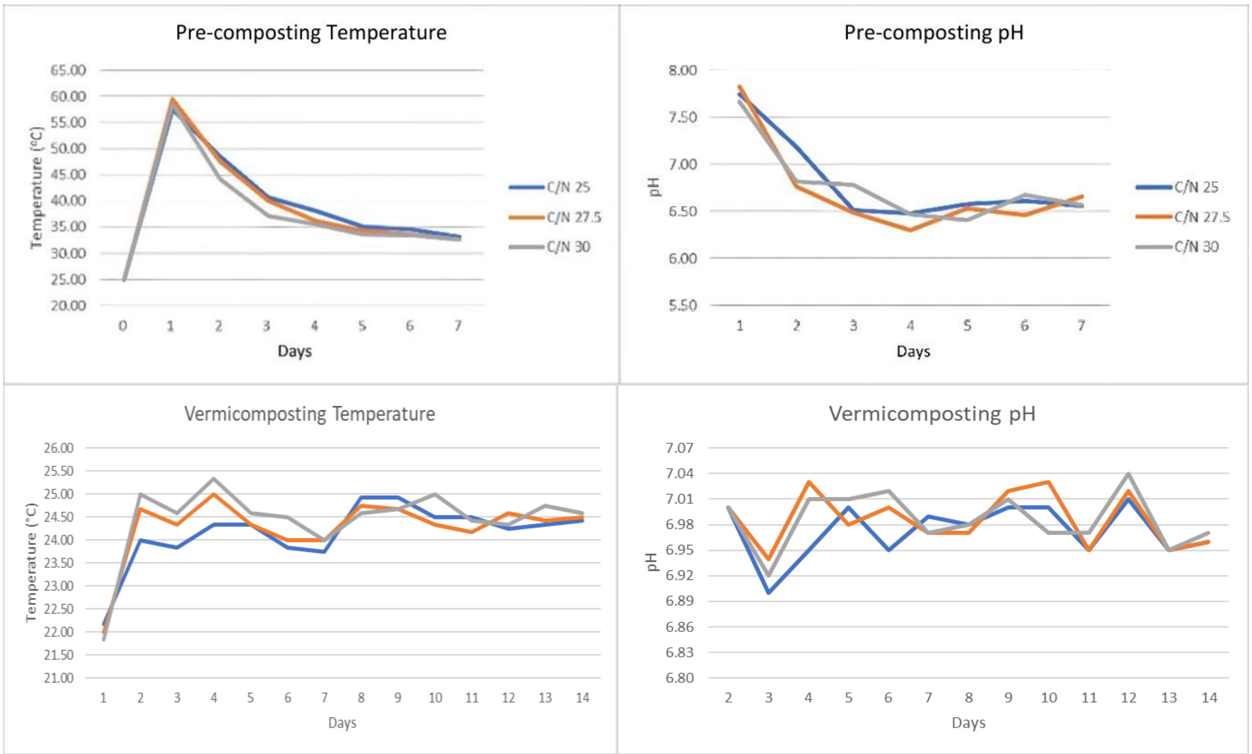
RESULTS AND DISCUSSION

Pre-composting and vermicomposting temperature

The temperature during composting reflects the ongoing activity of microorganisms. It increases on day 1 of pre-composting and gradually decreases until the 7th day of incubation (Figure 1).

During the pre-composting process, microbial growth progresses through several phases: mesophilic, thermophilic, and back to mesophilic again. The mesophilic phase (20-45°C) lasts very short, namely a few moments after the substrate is stacked and put into a compost bag when the decomposing bacteria start to work. The thermophilic phase (45-65°C) lasts several days, but gradually, the temperature decreases until it reaches the mesophilic phase.

A gradual combination of microbial activities led to an increase in temperature values. This combination of activities went through specific routes: degradation of organic materials can increase temperature, and microorganisms active in a specific temperature range participate in composting, causing a gradual temperature rise. The temperature of each phase in the pre-composting process describes different decomposer activities. Some decomposers that play a role in composting include fungi, firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, and Chloroflexi (Aguilar-Paredes et al. 2023). Furthermore, when the temperature reaches room temperature, it is the right time to plant earthworms for the following bioconversion process. The temperature during the vermicomposting process did not experience temperature fluctuations like in the pre-composting process (Figure 1). Earthworms are very sensitive to low temperatures (<12°C) and high temperatures (>30°C) (Viljoen and Reinecke 1992; Jesna and Livingstone 2023).


Pre-composting Temperature

Pre-composting pH

Vermicomposting Temperature

Vermicomposting pH

Figure 1. Temperature and pH during pre-composting and vermicomposting

High temperatures in the composting process were closely related to controlling pathogenic bacteria in waste. An effective composting process usually involves a thermophilic phase, where the temperature in the compost pile can reach between 55°C and 70°C. At this temperature, various pathogens, including pathogenic bacteria, viruses, fungi, and parasites, die, or their numbers decrease significantly (Gurtler et al. 2018; Hamzah et al. 2020). In the context of waste processing, mainly domestic and agricultural waste that may contain pathogens, composting with proper temperature control is a crucial step to ensure that the final product is safe for use as fertilizer.

Total bacterial and fungal Population on pre-composting

The C/N ratio is the primary source of nutrition for bacterial growth and a constituent that influences bacterial activity. The optimal C/N ratio at the beginning of the decomposition process to be effective in decomposing organic materials or substrates was around C/N 25-30. In this ratio range, bacteria reach the best conditions to maintain the population and carry out their activities in synthesizing proteins that form single cells (Azis et al. 2023). The total population of bacteria and fungi during the pre-composting process is presented in Figure 2.

During the thermophilic phase, total bacteria and fungi reached their highest population, and the C/N ratio influenced the population. A C/N ratio 25 produced the highest total bacterial and fungi population in the thermophilic phase, i.e., days 1-3 (Figure 2). However, in the mesophilic phase (days 4-7), total bacteria and fungi were relatively the same in the C/N ratio. During composting, microbes break down organic compounds to obtain energy for metabolism and obtain nutrients to maintain their growth. However, C and N were the most important: C was used as an energy source, while N was used to build cell structures.

Generally, the bacteria in DWS and rice straw mixed are bio decomposers which can break down lignocellulosic components, including cellulose, hemicellulose, and lignin, which are polymers into simple monomers (Xu et al. 2021). Ritambhara et al. (2019) stated that bacteria in DWS include *Bacillus cereus, Bacillus subtilis, Enterobacter*, and *Streptococcus faecalis*. Bacteria obtain energy through metabolic processes to reduce complex organic compounds in the form of carbohydrates and proteins into simpler compounds. Furthermore, through this metabolic process, the element Carbon (C) is produced, which is used by bacteria for growth, and Nitrogen (N), which is used to construct bacterial cells (Tortosa et al. 2021). Bacteria experience growth through several phases, including a lag phase, namely metabolic activity that occurs without cell division or a preparation phase for division. The exponential log phase is where cell division occurs very quickly. The stationary phase balances the number of bacteria that grow in the stationary phase and the number of bacteria that die. The decline phase is the death phase due to the bacteria starting to lack nutrition, known as toxic metabolism (Papale et al. 2021).

Adding rice straw to DWS creates an optimal C/N ratio for microorganism growth during decomposition. DWS has a low C/N ratio of 15.20, while microorganisms require a ratio between 25 and 30 for their activities (Rastogi et al. 2020). Therefore, it is necessary to add carbon source materials to achieve the ideal C/N ratio for microorganism growth in the decomposition process.

Increase earthworm biomass and vermibed shrinkage

During the 14th day vermicomposting process, earthworm biomass increased, and vermin shrinkage decreased (Table 1). Based on this table, it can be seen that the increase in biomass of *Eisenia fetida* earthworm produced by treatment T1 was significantly different from treatment T_2 and treatment T_3. The average increase in biomass of *Eisenia fetida* earthworms in T_1 treatment (26.33%) was higher, followed by T_2 (9.17%). Finally, T_3 (0.50%), which indicated low stocking density (6.6 g/L), produced the highest increase in earthworm biomass. This outcome is believed to be due to the low stocking density of earthworms, which impacts the competition for food. A small population leads to abundant food availability, ensuring that earthworms receive the appropriate proportion of food and sufficient nutrition (Putri et al. 2020).

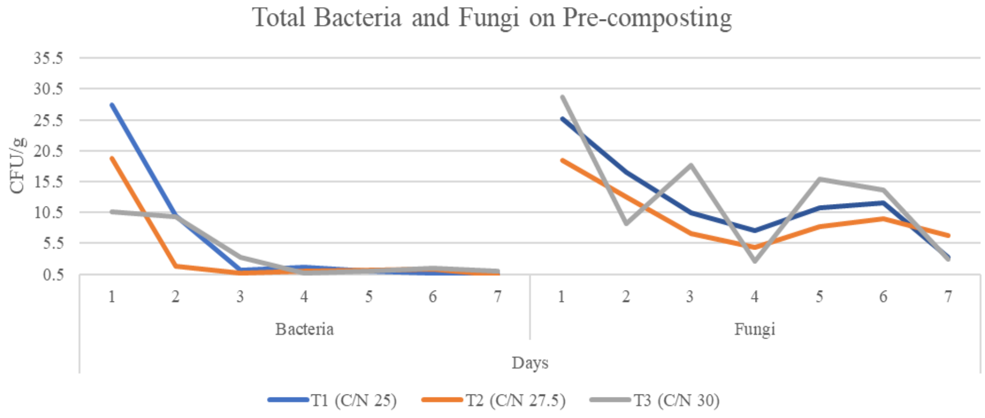


Figure 2. Total bacteria and fungi on pre-composting

Table 1. Average of increased earthworm biomass and vermibed shrinkage

Stocking densities	Increase earthworm biomass (%)	Vermibed shrinkage (%)
T1	26.33^{c}	27.33^{a}
T2	9.17^{ab}	31.00^{a}
T3	0.50^{a}	28.83^{a}

Note: T1: 6.6 g/L; T2: 13.3 g/L; T3: 20.0 g/L. ^{a,b} Means with the same letter were not significantly different at 0.05

Table 2. Average of nitrogen-fixing bacteria and phosphate-solubilizing bacteria in vermicompost

Stocking densities (g/L)	NFB (x10^{7} CFU/g)	PSB (x10^{7} CFU/g)
T1	5.5 ± 1.85^{a}	4.4 ± 1.42^{a}
T2	8.2 ± 1.39^{b}	8.4 ± 3.19^{b}
T3	8.7 ± 1.32^{b}	8.5 ± 2.22^{b}

Note: T1: 6.6 g/L; T2: 13.3 g/L; T3: 20.0 g/L. ^{a,b} Means with the same letter were not significantly different at 0.05

The highest increase in biomass during vermicomposting was obtained from the lowest stocking density treatment (T_1). In the treatment (T_3) with the highest stocking capacity, the observed increase in earthworm biomass was much less compared to other treatments. This is due to the denser population of earthworms in the treatment, which has increased competition for nutrients and food. This high level of competition could have hindered the growth and development of earthworms, which resulted in the observed minimal increase in biomass. Therefore, it can be concluded that the density of earthworms is significant in their growth and development and that overcrowding could harm their overall health and well-being. The density of earthworms in each area plays an essensial role in their ability to absorb oxygen and food. Therefore, at the high stocking density, the limited space available for the earthworms to move causes them to compete fiercely for dissolved oxygen. High stocking density hampers their ability to absorb oxygen, negatively impacting their health and well-being (Devi et al. 2023). Therefore, it is essential to maintain optimal stocking densities to promote the growth and development of earthworms.

Vermicomposting is a process that utilizes earthworms to break down organic matter into nutrient-rich compost. During this process, a vermibed was used as a habitat for the earthworms to live in and is typically made up of a mixture of organic materials such as DWS and rice straw. As the earthworms consume the organic matter in the vermibed, their biomass increases, and the vermibed experiences shrinkage. The organic material is converted into earthworm cell mass, which maintains the earthworms' bodies and supports their growth. Interestingly, a recent study has shown that the stocking density of earthworms in the vermibed does not impact vermicompost production. When the amount of worms in an environment becomes too high and surpasses the environment's capacity to support them, the amount of vermicompost produced per worm may decrease due to increased competition for food and space. In such a situation, adding more worms without sufficient organic material does not significantly increase vermicompost production (Kauser and Khwairakpam 2022). The vermicompost produced is a mixture of organic compounds from the DWS and rice straw-broken down in the worm's digestive tract and excreted as worm feces (casting). The vermibed itself does not go through the degradation process in the worm's digestive tract and is used as a stable habitat for the earthworms. Overall, vermicomposting is an efficient and sustainable way of producing nutrient-rich compost, and understanding the role of the vermibed in this process is crucial for its successful implementation.

Nitrogen-fixing and phosphate-solubilizing bacteria in vermicompost

The impact of vermicomposting earthworm stocking density on populations of N-Fixing Bacteria (NFB) and Phosphate-Solubilizing Bacteria (PSB) is presented in Table 2. The addition of earthworm stocking density to the vermicomposting substrate resulted from a mixture of DWS and rice straw, significantly affecting the N-fixing bacteria population. In their digestive system, earthworms provide an ideal environment for nitrogen-fixing bacteria and phosphate-solubilizing bacteria to reproduce. The digestive conditions of earthworms are rich in microorganisms and enzymes that support the biological activity of these bacteria. As earthworms move and excrete feces (vermicast), they distribute the bacteria throughout the compost pile. The earthworm's activity increases the bacterial population throughout the vermicompost pile. This reason can explain why the more significant the worm population per unit area of the vermibed, the higher the content of nitrogen-fixing bacteria and phosphate-solubilizing bacteria in the vermicompost (Wang et al. 2022).

The data presented in Table 2 highlight an exciting finding: a correlation between the stocking density of earthworms and the population of P-solubilizing bacteria. The population of these beneficial bacteria ranged from 4.4×10^7 CFU/g to 8.5×10^7 CFU/g, depending on the density of earthworms present. This study demonstrates that increasing earthworm stocking density in a vermicomposting substrate made with a mixture of DWS and rice straw has a significant impact. The stocking density of earthworms on vermicomposting substrate resulting from a mixture of DWS and rice straw significantly affected the population of phosphate-solubilizing bacteria ($F<0.05$). Results showed that there was a significant difference ($P<0.05$) in the population of phosphate-solubilizing bacteria in the earthworm stocking density of 6.6 g/L (T1) compared to other treatments. Increasing the stocking density significantly increased Nitrogen-Fixing Bacteria (NFB) and Phosphate-Solubilizing Bacteria (PSB) populations.

Functional bacteria in organic fertilizer provide positive value for plant growth. Free-living microorganisms carry out non-symbiotic biological nitrogen fixation. Enterobacteriaceae, *Bacillus*, *Azotobacter*, *Azosprilium*, and *Herbasprilium* have been proven capable of N_2 fixation (James et al. 1997; Thomas et al. 2021; Ikhwani et al. 2021). *Azotobacter* populations are influenced by fertilization.

Furthermore, N-fixing bacteria found in organic/biological fertilizers can reduce the dose of nitrogen fertilizer in rice plants by up to 25% (Ruiz et al. 2020). Plants and most microbes cannot fix nitrogen into compounds needed in their cells and generally get nitrogen from ammonium and nitrate compounds in the soil. *Rhizobium* is an N-fixing bacteria that have an essential role in fixing free N_2 from the air so that it becomes a nitrogen compound that is beneficial for plant growth (Fahde et al. 2023; Rafique et al. 2024)

Three NFB bacterial isolates and two PSB isolates were found in the vermicompost, as shown in Table 3. The primary function of Phosphate-Solubilizing Bacteria (PSB) in organic fertilizer is to solubilize insoluble forms of phosphorus in organic matter or rock phosphate. PSB produces organic acids and enzymes, such as phosphatases, which break down complex organic phosphorus compounds or mineralize insoluble inorganic phosphate and are readily available for plant uptake (Souza et al. 2023). Several genera of bacteria are known to include phosphate-solubilizing species, such as *Bacillus, Pseudomonas,* and *Rhizobium* (Janati et al. 2023).

Nitrogen-fixing bacteria, such as *Rhizobium, Azotobacter*, and *Azospirillum* genera, possess several microscopic characteristics that enable them to perform the crucial biological process of nitrogen fixation. The genus NFB is generally made up of rod-shaped (bacilli) bacteria that can exist singly or in chains. These bacteria are classified as Gram-negative, meaning they have a thin peptidoglycan layer sandwiched between an inner cytoplasmic membrane and an outer membrane. The outer membrane contains Lipopolysaccharides (LPS), which are responsible for pathogenicity and interaction with host plants. *Rhizobium* species often have flagella, which helps them move around in liquid environments and colonize plant roots. Colonies can appear smooth, round, or irregularly shaped on suitable growth media (Hadija et al. 2021).

The identification results of bacteria growing on Jensen's media showed the presence of three different colonies, while two colonies were found on Pikovskaya media. All three colonies identified on Jensen's media exhibited the characteristics of nitrogen-fixing bacteria (N1, N2, and N3). Additionally, two colonies were identified as phosphate-solubilizing bacteria (P1 and P2) (Table 3). Phosphate-Solubilizing Bacteria (PSB) are a group of microorganisms that can solubilize insoluble forms of phosphorus in the soil and are readily available for plant uptake. Depending on their genus and species, these bacteria can have different shapes, such as rod-shaped (bacilli), spherical (cocci), and filamentous forms. Also, their cell wall structures can be Gram-positive or Gram-negative, depending on their genus and species. Moreover, many phosphate-solubilizing bacteria are motile, which means they possess one or more flagella to move through liquid environments or across solid surfaces (Khan et al. 2022).

Phosphate-solubilizing bacteria are decomposer bacteria that consume simple carbon compounds. Through this process, bacteria convert energy in soil organic matter into valuable form for other soil organisms in the food chain. These bacteria can break down soil pollutants and retain nutrients in their cells. Therefore, phosphate compounds such as Al-phosphate, Fe-phosphate, and occluded-phosphate in acid soil, while in alkaline soil, phosphate generally compounds as Ca-phosphate. This binding causes the phosphate fertilization to be applied inefficiently. It is usually assumed that occluded phosphate is not plant-available, so it must be given in high doses (Schubert et al. 2020; Timofeeva et al. 2022).

Macronutrients in vermicompost

The quality of organic fertilizer is determined by the content of macronutrients, namely elements needed by plants in large quantities. The macronutrient content of vermicompost from a mixture of Dairy Wastewater Solids (DWS) and rice straw by *Esenia fetida* with different stocking densities did not produce different N and P_2O_5 contents (P>0.05), but different K_2O, Ca, and Mg contents at different stocking densities (Table 4). Plants require a range of critical macronutrients to support their growth and development. Among these macronutrients are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), which play essential roles in various plant functions such as photosynthesis, protein synthesis, and energy transfer. These macronutrients are crucial for plants to thrive and produce healthy crops (Jose 2023; Niazi and Monib 2023). Requirements for macronutrient content are regulated in the Regulation of the Minister of Agriculture of the Republic of Indonesia No. 01/2019. These macronutrients are in the form of total N, P_2O_5, K_2O, Ca, and Mg.

The phosphorus pentoxide (P_2O_5) content in vermicompost was directly proportional to the Nitrogen (N) content. If the level of nitrogen content in vermicompost is high, it leads to a higher P_2O_5 content in the vermicompost. Vermicompost is a solid organic fertilizer that contains macronutrients needed by plants. Macronutrients result from the synergistic breakdown of organic material between microorganisms and earthworms. According to Minister of Agriculture Regulation No. 1 of 2019 concerning Minimum Technical Requirements for the Quality of Solid Organic Fertilizer with macronutrients resulting from the sum of the macronutrient percentages ($N + P_2O5 + K_2O$) of a minimum of 2%, the nutrient content obtained from this research meets the requirements for organic fertilizer, as shown in Table 4.

Table 3. Macroscopic and microscopic characteristics of functional bacteria in vermicompost.

Functional bacteria	Isolates	Macroscopic	Microscopic
NFB	N1	white, circular	Bacilli, Gram (-), motile
	N2	white, irregular	Bacilli, Gram (-), motile
	N3	white, rhizoid	Bacilli, Gram (-), motile
PSB	P1	yellowish, circular	Coccus, Gram (+), non-motile
	P2	white, irregular	Bacilli, Gram (-), motile

Note: NFB: Nitrogen-Fixing Bacteria; PSB: Phosphate-Solubilizing Bacteria

Table 4. Macronutrients in vermicompost

Stocking densities	**N**	**P_2O_5**	**K_2O**	**Summation of $N+P_2O_5+K_2O^*$**	**Ca**	**Mg**
				-------- % ---------		
T1	1.37 ± 0.12^a	0.22 ± 0.02^a	1.43 ± 0.06^a	3.02	5051.37 ± 212.91^a	2232.15 ± 178.19^a
T2	1.36 ± 0.21^a	0.22 ± 0.01^a	1.57 ± 0.04^b	3.15	9158 ± 3597.78^a	4238.95 ± 1465.35^{ab}
T3	1.36 ± 0.13^a	0.23 ± 0.03^a	1.63 ± 0.02^b	3.22	4920.38 ± 701.23^a	2426.23 ± 514.34^b

Note: T1: 6.6 g/L; T2: 13.3 g/L; T3: 20.0 g/L. ^{a,b}: Means with the same letter were not significantly different at 0.05, *Referring to the requirements in the Minister of Agriculture Regulation 01/2019 (at least 2)

In vermicomposting, microorganisms and earthworms complement each other in breaking down organic material and improving the quality of the final result. Therefore, combining their activities produces compost rich in nutrients, has a better structure, and is more beneficial for plant growth. Microorganisms, such as bacteria and fungi, start the decomposition process by breaking down organic material from a mixture of DWS and rice straw into smaller parts. They digest cellulose, lignin, and other organic components, producing organic matter more easily digested by earthworms (Lou et al. 2022). Earthworms, especially types such as *Eisenia fetida* (red worms), consume organic material that microorganisms have partially decomposed. As earthworms digest organic material, they break it down further in their digestive system. This process produces vermicast (worm droppings) rich in nutrients and microorganisms.

Earthworm excreta, consisting of mucoprotein, ammonia, urea, and possibly uric acid and allantoin, contributes to a significant amount of nitrogen in the soil (Lang et al. 2023). The research revealed that the nitrogen content in vermicompost was not influenced by stocking capacity; however, it did affect the K_2O, Calcium, and Magnesium content. In previous studies, it was stated that the factors that influence the nutrient content of vermicompost are the nutritional balance (C/N ratio) of the vermibed, vermiculture techniques, and composting conditions (Ndegwa et al. 2000; Devi et al. 2023). In vermicomposting, converting organic matter into phosphorus pentoxide (P_2O_5) and K_2O involves several biological and chemical processes. Initially, organic matter undergoes microbial decomposition facilitated by microorganisms present in the vermicompost. These microorganisms break down complex organic compounds into simpler forms, releasing phosphorus compounds. Earthworms play crucial role in vermicomposting by ingesting organic material and breaking it down further in their digestive system. During digestion, earthworms release enzymes that break down organic matter, including phosphorus-containing compounds. As earthworms digest organic matter, it undergoes further microbial mineralization within the worm's gut. Therefore, microorganisms in the earthworm's digestive tract continue to break down organic compounds, releasing phosphorus in various forms (Vos et al. 2022; Hu et al. 2024).

Dairy Solid Wastewater (DWS) treatment involves aerobic fermentation and microbial and earthworm activity to create high-quality organic fertilizer and biomass. The organic material in DWS provides the necessary nutrients for the growth of microbes and earthworms and converts them into nutrients required for plant nutrition. This research provides a solution for managing waste in the milk processing industry, thereby reducing its environmental impact.

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