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Effect of Effective Microorganisms in Fermentation of Rice Husk and Anchovy Head Using Lab-scale Treatment

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ABSTRACT

The rice husk and anchovy filet industry is one of the world's most successful industries and generates many biowastes with valuable bioproducts. Combining effective microorganisms (EM) with anchovy head (AH) and rice husk (RH) will produce a beneficial fertilizer and make nutrients more valuable to the plant. Thus, this study aims to determine the most effective anchovy head and rice husk treatment ratio for agricultural applications to reduce waste disposal from anchovy heads and rice husks worldwide, which may cause environmental problems. In this study, rice husk and anchovy head were fermented at five different ratios of treatment weight for 25 days: Treatment A with 100% AH, treatment B with 100% RH, treatment C with 50% AH:50% RH, treatment D with 70% AH:30% RH, and treatment E with 30% AH:70% RH. The pH, temperature, and number of colonies of every treatment were measured every 0, 5, 10, 15, 20, and 25 days. This study shows that treatment D with a ratio of 70% AH: 30% RH is the most suitable based on pH range within 6–8.5, consistently increasing temperature and the highest number of colonies to be applied to plants. The ratio of rice husk and anchovy head of treatment D in this study can be used further by other researchers to discover their potential, especially for the agricultural industry.

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INTRODUCTION

Rice is the principal carbohydrate source in Malaysia, with a total paddy cropping area of about 0.70 million ha, and generates significant waste, including rice husk,

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straw, and bran (Firdaus et al., 2020). It is also a standard waste product in rice countries such as China, India, Bangladesh, Cambodia, Vietnam, and Southeast Asia. About 20%–33% of the paddy weight comprises rice husk, and 1,000 kg of paddy rice produces 200 kg of the husk (Pode, 2016). Rice husk is a cellulose-based fiber and contains approximately 40% cellulose, 30% lignin group, and 20% silica (Chindaprasirt et al., 2007). The high silica (Si) in rice husk makes it valuable in the agricultural industry because it can increase the soil's moisture content (Ramli et al., 2023). Furthermore, it showed that *Brassica oleracea* var. *alboglabra* L. (Chinese kale) uptake nitrogen (N) and increases growth by rice husk addition to palm oil-based compost (Ramli et al., 2023). The same is valid with anchovy heads. Paone et al. (2021) stated that the anchovy filet industry produces a large amount of biowaste, such as fish heads, bones, and tails, which can extract many potentially valuable bioproducts. Therefore, it is necessary to evaluate the nutritional makeup of fish waste to establish whether it can offer plant nutrients like nitrogen, a combination of nitrogen and phosphorus, or enrich a compost. According to Unggang et al. (2023), fish-based fertilizers typically contain macronutrients with recorded high elements of nitrogen (N), potassium (K), and phosphorus (P).

However, fish industry by-products such as heads, viscera, skin, bones, and scales (Paone et al., 2021) were previously considered waste and a cause of environmental damage. However, when both industries grow, the waste will pollute the ecosystem since there will be dumping of abandoned waste that does not benefit the earth. The rice husk and anchovy head waste must be treated and converted into fertilizer to avoid this problem.

Effective microorganisms (EM) are microbial inoculants containing numerous microorganism species that can stimulate plant growth and improve soil fertility (Talaat, 2019). Combining EM with organic waste will produce a beneficial fertilizer because it can make nutrients more beneficial to plants and increase their life expectancy. After all, many beneficial microorganisms in EM solution, such as lactic acid bacteria and yeast, will help improve plant production—biofertilizers from organic waste benefit nutrition management through their considerable potential to improve the efficiency of nutrient usage. Compared to ordinary fertilizers, the nutrients are released very slowly because they are bound to the nano-dimensional adsorbents (Zulfiqar et al., 2019). Plants receive air nitrogen directly from microbes in the biofertilizers (Karki, 2020). In addition, the product produced is eco-friendly, cost-effective, and can increase crop yield by 20%–30%. Furthermore, the most important is that it can help to reduce the waste produced by the food and fish industries. It is also more efficient for agricultural purposes. When biofertilizers are made from organic waste, waste is reduced, while nutrients in organic waste will boost plant product development and bring advantages to customers and the globe.

Utilizing agricultural by-products, like anchovy heads and rice husk, has enormous potential for value-added product creation and sustainable resource management. However, applying lab-scale treatment techniques to ferment these by-products effectively is still difficult. Furthermore, the utilization of these by-products is restricted due to the lack of a systematic strategy to improve the fermentation process, resulting in waste and lost chances for value development. In order to improve the procedure and maximize resource usage, it is necessary to look at how EM affects the fermentation of rice husks and anchovy heads at a lab-scale level. Therefore, the first objective of this study was to evaluate the effect of EM on the fermentation of rice husk and anchovy head by monitoring key parameters, including pH, temperature, and microbial activity. The second objective was to determine the most effective anchovy head and rice husk treatment ratio to be used as biofertilizers for agricultural applications.

MATERIALS AND METHODS

Effective Microorganism Activated Solution (EMAS) Preparation

The authentic EM, which contains yeast, lactic acid, and phototrophic bacteria, was obtained from EMRO Malaysia Sdn. Bhd. (Malaysia). The EM solution was required to produce an Effective Microorganism Activated Solution (EMAS) in the fermentation sample. The concentrated EM solution diluted with chlorine-free water and molasses (EMRO Malaysia Sdn. Bhd., Malaysia), which serves as a food supply for the microbe, was added to activate the process further. One part of the EM microbial inoculants and one part of the molasses were combined with 20 parts of chlorine-free water to activate the EM and kept that solution for three to five days in an airtight ferment container (Jusoh et al., 2013).

Fermentation Treatment Process

The sample materials were weighed and mixed into a different closed container (500 ml). The fermentation containers were labeled treatments A to E and their control with a different ratio (Table 1). Each treatment was done in triplicates. Then, water was added until the moisture content of each fermentation mixture reached 60% (wet basis). The fermentation containers were then stored in dark conditions to prevent excessive heat loss and to preserve moisture. The moisture content was maintained at 50%–60% throughout the twenty-five days of the fermentation period by adding water.

Table 1
Different ratios of sample anchovy head (AH) and rice husk (RH) supplemented with effective microorganisms (EM)

Treatment	% Ratio (w/v)	% EM
A	100% AH	5
B	100% RH	5
C	50% AH: 50% RH	5
D	70% AH: 30% RH	5
E	30% AH: 70% RH	5
Control A	100% AH	-
Control B	100% RH	-
Control C	50% AH: 50% RH	-
Control D	70% AH: 30% RH	-
Control E	30% AH: 70% RH	-

The mixtures were turned every five days to maintain porosity. The pH and temperature were measured and recorded.

A 5-ml EM solution was added to a given mixture for treatments A, B, C, D, and E. An amount of 350 ml of sterile distilled water was added to each fermenting mixture. The samples were mixed well. All the samples were placed in a dark place at room temperature for 25 days.

Measurement of pH and Temperature

A pH meter (No. H1 8915 ATC, Hanna Instruments Sdn. Bhd., Malaysia) and a digital thermometer (RS PRO RS40, Wired Digital Thermometer, Malaysia) were used to record pH and temperature of samples at 0, 5, 10, 15, 20 and 25 days. A graph was plotted to observe the ratio treatment.

Measurement of the Total Number of Bacteria

For a total number of bacterial analyses, a sample was serially diluted by adding 1 ml of the sample to 9 ml of distilled water in a test tube. Each dilution sample was distributed on a nutrient agar plate and incubated for 24 hr. After 24 hr, the number of colonies on each agar plate was determined. The number of bacteria in each treatment was calculated using the following formula (Sieuwerts et al., 2008):

Total number of bacteria [Colony Forming Unit (CFL)/ml] =

$$\frac{\text{Number of colonies} \times \text{total dilution factor}}{\text{Volume of culture plate (ml)}}$$

RESULTS

Changes of pH

According to Figure 1, all treatments except for treatments B and E are within the range of pH 6.00–7.00, while for treatment E, the pH range is 5.00–7.00. At the same time, for treatment B, the pH drastically dropped from pH 5.35 on day 0 to 4.31 on day 25.

Changes of Temperature

Figure 2 depicts the temperature profiles of five fermentation treatments (treatment A – treatment E). Immediately after fermentation begins, all treatments show an increase in temperature. On day 5, the temperature for treatment rose to 25.60°C from 23.23°C for treatment A, 25.23°C from 23.83°C for treatment B, 25.40°C from 24.00°C for treatment C, 25.70°C from 23.30°C for treatment D, and 25.53°C from 23.30°C for treatment E. Treatment D reached the highest values of 25.70°C on day 25 compared to other treatments. All treatments with EM have a temperature higher than the control treatment except for day 0 treatments D and E. However, all treatments increased in temperature along 25 days of fermentation.

Total Number of Bacteria

The number of colonies of each fermentation process of making biofertilizer from rice husk and anchovy head based on treatment ratio is shown in Figure 3. It shows that the total number of bacteria proves the existence of microorganisms sourced from rice husks and anchovy heads. The highest number of bacterial colonies was in treatment D, with a total bacterial presence of 37.87 × 10^5 CFU/ml, followed by treatment A, 35.3 × 10^5 CFU/ml. For treatments A and D, the

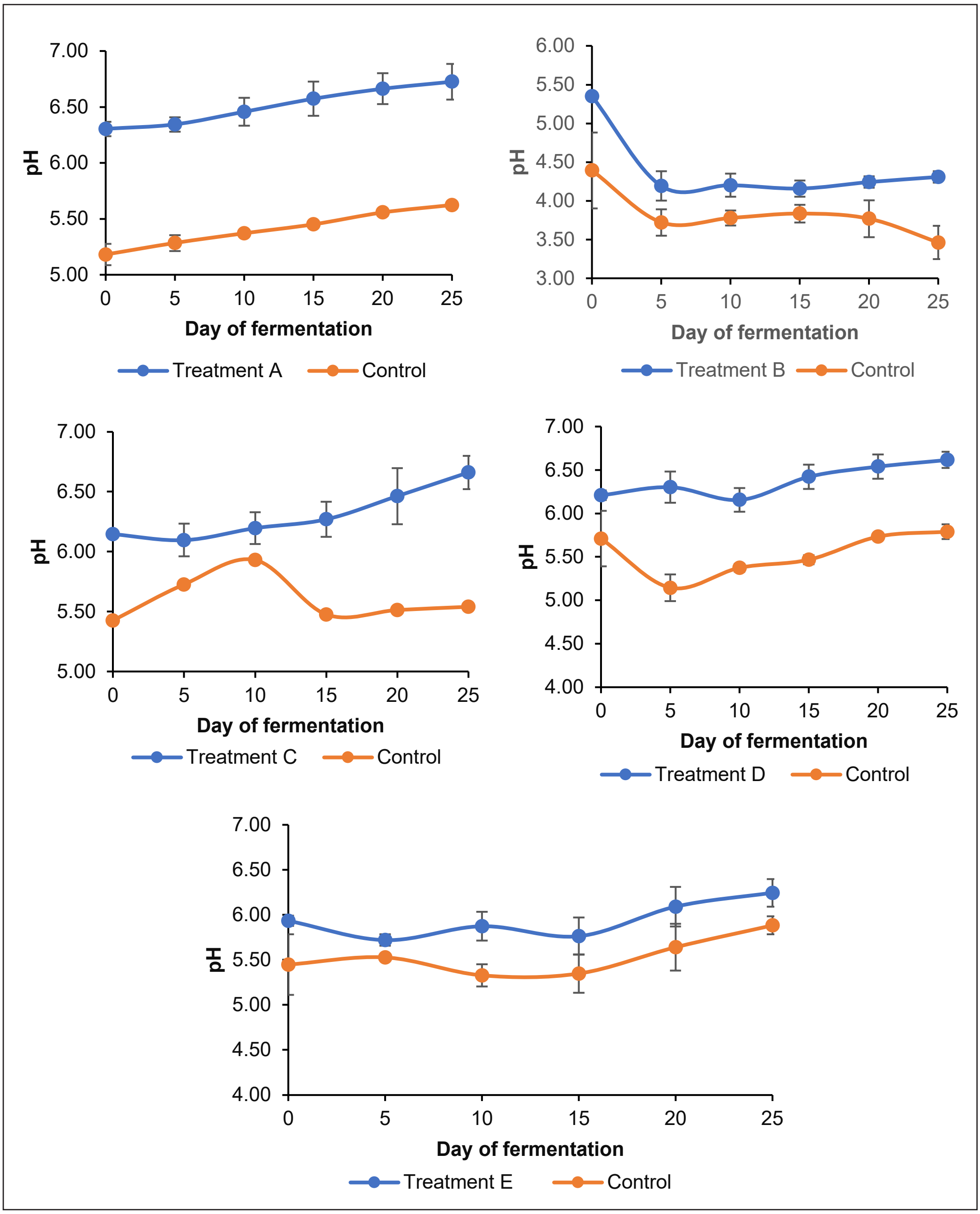

Figure 1. The pH of treatments A-E with it controls during the fermentation process
Note. Treatment A = 100% AH; Treatment B = 100% RH; Treatment C = 50% AH : 50% RH; Treatment D = 70% AH: 30% RH; Treatment E = 30% AH: 70% RH. Error bars = Standard deviation

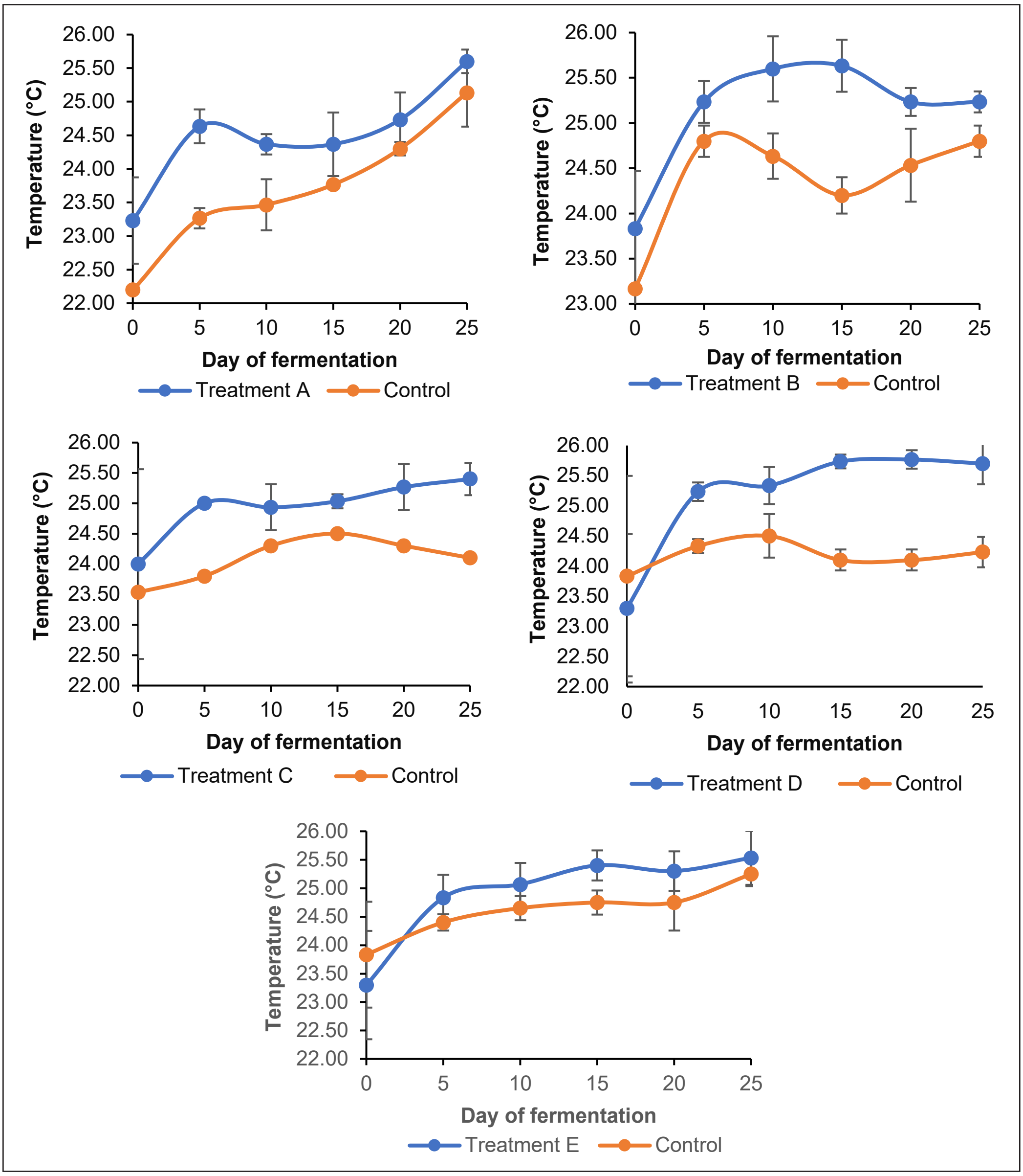

Figure 2. The temperature of treatments A-E with it controls during the fermentation process
Note. Treatment A = 100% AH; Treatment B = 100% RH; Treatment C = 50% AH : 50% RH; Treatment D = 70% AH: 30% RH; Treatment E = 30% AH: 70% RH. Error bars = Standard deviation

number of colonies increased until day 15 and started to drop on day 20. The graph trend for all treatments showed that the number of colonies decreased at the end of fermentation.

DISCUSSION

pH and Temperature During Fermentation

Based on the result, microbial activity of treatment with EM is higher than control

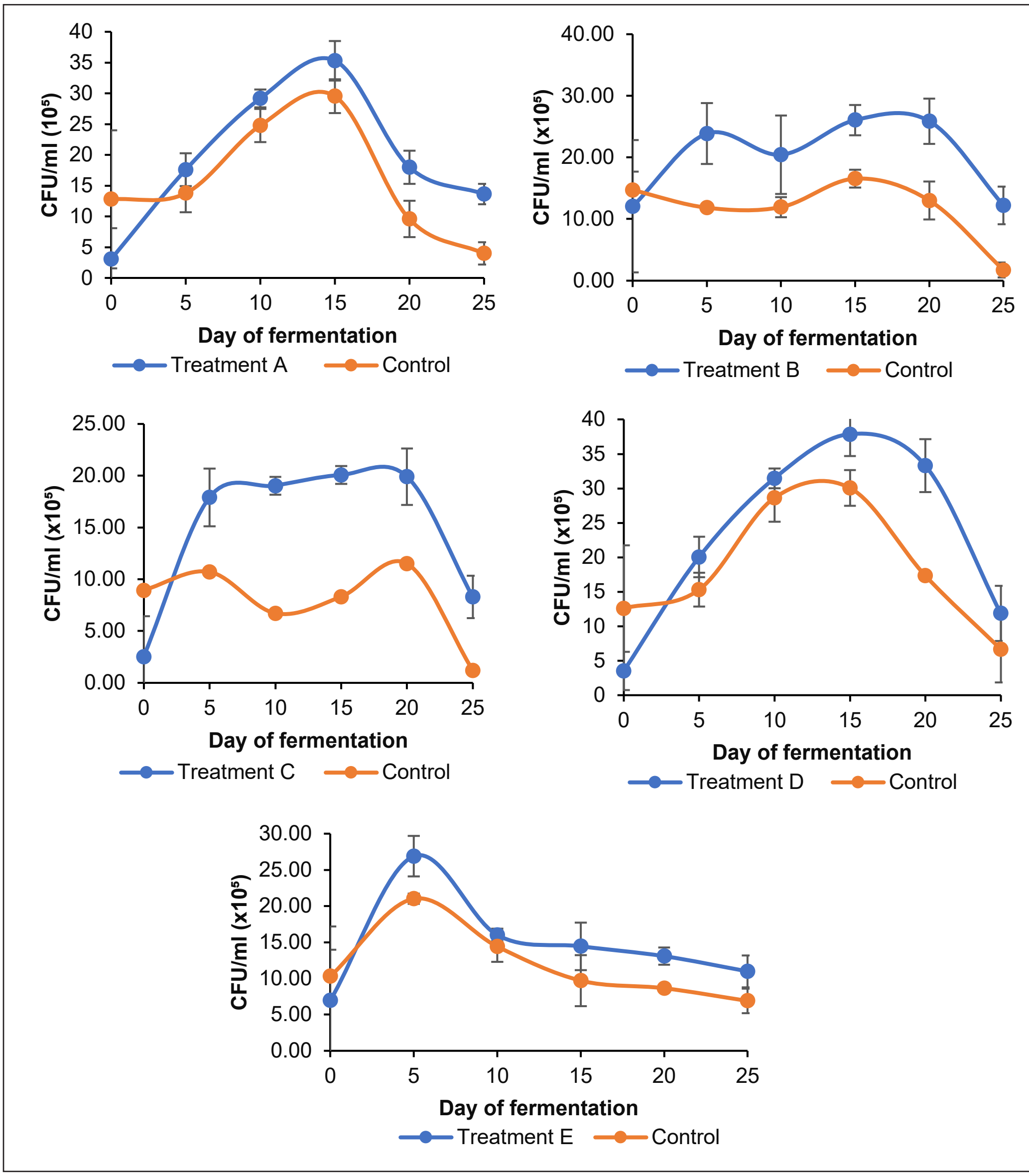

Figure 3. The total number of bacteria (CFU/ml) of treatments A-E with its controls during the fermentation process

Note. Treatment A = 100% AH; Treatment B = 100% RH; Treatment C = 50% AH : 50% RH; Treatment D = 70% AH: 30% RH; Treatment E = 30% AH: 70% RH. Error bars = Standard deviation. CFU = Colony Forming Unit

treatment, resulting in higher pH and temperature at the end of fermentation. It shows that EM plays a role in increasing microbial activity during the fermentation process. Changes in pH indicate that incubation works well. Based on the result obtained, the pH of all treatments was increasing, except for treatment B. Based on a previous study, this indicates a good quality compost within the suggested range of pH 6–8.5 (Jusoh et al., 2013; Romero-Perdomo et al., 2015). This range is optimal for promoting microbial growth and activity.

Heat is released during the fermentation treatment process. According to Kornievskaya et al. (2020), microorganisms that decompose organic matter produce energy from heat, carbon dioxide, and water vapor, causing temperature rises. The heat generated by the population of microorganisms' respiration and decomposition of sugar, starch, and protein also contributes to the increase in temperature during the fermentation process (Jusoh et al., 2013). The rising temperature is a good indicator of microbial activity in the fermentation process, as higher temperatures indicate more microbial activity. Even though no microbial tests were performed on the sample, based on the high temperature achieved, it is reasonable to conclude that the fermentation treatment with EM has higher microbial activity than the control treatment without EM. A similar study by Molina-Favero et al. (2008) found that temperatures between 29 and 32ºC produce biomass, auxins, and nitrous oxide in bacteria belonging to the genus *Azospirillum* during the fermentation process.

Total Microbial Colonies During Fermentation

As the day of fermentation increases, then the number of colonies increases. The increased colony count could be attributed to adequate microbial nutrient intake in EMAS, allowing microbes to reproduce actively during fermentation. The fact that there were more colonies in the EM treatment than in control indicates that EM affected the growth of many microbial colonies because they still had access to sufficient energy from molasses for growth.

Microorganisms derived from organic waste materials can produce fertilizer in the same way organic solid waste, which contains a high concentration of nutrients like N, P, K, and other organic materials (Bamdad et al., 2022). Some microorganisms, such as plant growth-promoting rhizobacteria (PGPR), turned into biofertilizers because they release phytohormones, particularly cytokinins (Wong et al., 2015). The biological functions of phytohormones, such as auxins, cytokinins, and gibberellins, are useful in contributing to the observed physiological traits and crop yield of plants. Furthermore, using microorganisms derived from organic waste can improve soil structure and quality. However, with the addition of EM to organic waste materials, the number of microorganisms is greater than that of organic waste alone. As a result, using EM will improve the efficiency of organic waste fermentation and shorten the time required to convert organic waste to biofertilizer. However, several environmental factors, such as temperature, pH, the presence of oxygen, and the availability of food for these microbes, impact microbial growth, leading to unstable growth and a decline in the number of bacteria (Febria & Rahayu, 2021).

Identification of Best Treatments Ratio

The pH is gradually increasing for treatment A, as is the temperature. However, the number of colonies formed increased until day 15 and decreased until day 25. The pH of treatment B is only higher on day 0, but it tends to decrease to around 4.0–5.0. However, when the temperature and number of colonies are analyzed, the graph shows that the number of colonies decreased when the temperature was high. The number of colonies increased when the temperature was slightly reduced. In treatment C, the pH and temperature rise concerning the fermentation day. However, the trend of the number of colonies may be unstable because the number of colonies increased for a few days, then decreased, and this cycle continued. In treatment D, the pH and temperature rise with the fermentation day. The trend of graphs of colony number for treatment D is nearly identical to treatment A's. When day 20 arrived, the number of colonies decreased. The pH differences in treatment E were not particularly noticeable, and the differences from the beginning to the end of fermentation were also minor. However, the temperature rises during the fermentation process. Nonetheless, when the number of colonies was examined, the number of colonies of treatment E decreased.

So, after considering the pH, temperature, and number of colonies, treatments A and D are suitable for biofertilizers. Treatment D, on the other hand, has the best treatment ratio. It is because, when comparing the number of colonies, both treatments begin to decline after day 15. The number of colonies, however, varies significantly between days 15 and 20. The number of colonies in treatment A decreases from 35.3×10^5 to 18×10^5 CFU/ml. On the other hand, the number of colonies in treatment D has been reduced from 37.87×10^5 to 29.23×10^5 CFU/ml. The number of colonies in treatment A is higher than in treatment D. According to Stoffella and Kahn (2001), longer fermentation times result in fewer microbes and less substrate for growth. Based on that statement and the results obtained, it can be concluded that treatment D with a ratio of 70% AH:30% RH can be applied to the plant around day 15 of fermentation because the microbial activity is active at that time. If microbial activity declines, sugar energy sources, such as molasses used in this study, must be added to allow microbes to redevelop.

Comparison of AH and RH Volume Ratio

Another factor can be investigated to determine the best treatment ratio. This study concludes that anchovy head and rice husk volume influence microbial activity during fermentation based on the volume ratio of anchovy head and rice husk. When the volume of AH is high, microbial activity rises, resulting in the highest number of colonies. It occurs in treatments A and D. However, when the volume of the rice husk is greater than the volume of the anchovy head, the treatment becomes unstable because the number of colonies fluctuates based on the trend of the number of colonies in treatments B and E. It could happen because both anchovy head and rice husk contain nutrients. Although no nutrient content analysis was performed on the sample in this study, based on the findings, it can be assumed that the nutrient content of the anchovy head and rice husk influenced microbial activity during the fermentation process. Yusof et al. (2022) supported that anchovy heads have high nutritional quality such as carbohydrates, protein, fat, calcium, iron, fiber, zinc, vitamins A, B12, D, E, DHA, EPA, and trans-fat. Meanwhile, rice husk has a lot of silicon and potassium, which are nutrients that can be utilized by indigenous microorganisms and can be used to improve soil quality (Milla et al., 2013).

CONCLUSION

All fermentation parameters revealed in this study show a similar pattern for both treatments, with and without EM. The measured parameters show that the decomposition of AH and RH occurs in both treatments. The increasing pH, temperature, and number of colonies indicate that organic matter decomposition occurs over 25 days. All treatments are suitable to be used on plants. Nevertheless, treatment D is the best treatment ratio, with a 70% AH:30% RH. Even though treatments A and D have the same graph pattern, there is a difference in the number of colonies between the treatments, which indicates that treatment D is the best treatment ratio. The amount of anchovy head and rice husk also affected microbial activity during fermentation. Using EM in fermentation increases microbial activity, which will shorten the time for organic waste to become biofertilizers. Plants benefit from EM as well because it increases crop yield. Finally, the ratio of rice husk to anchovy head in treatment D in this study should be studied further by other researchers to discover its potential, particularly for the agricultural industry.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported herein.

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