Assessment of bacteria and fungi in food waste compost using hybrid dehydrated food waste associated with Effective Microorganisms (EM)

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Abstract. Food waste is a significant global issue, leading to soil contamination and greenhouse gas emissions. To address this problem and reduce reliance on chemical fertilizers, composting food waste using Effective Microorganisms (EM) and dehydrated techniques has been explored. This study aims to comprehensively investigate the composting process at different stages using EM-related dehydrated food waste. Bacteria and fungi colonies were measured during early, premature, and mature stages of composting in two systems. The results showed varying trends in bacteria and fungi populations, with mesophilic bacteria dominating the early stage and thermophilic bacteria increasing in System 2 at the mature stage. Fungi colony counts decreased over time in both systems. Correlation analysis indicated a negative correlation between mesophilic bacteria and fungi with pH and temperature, while thermophilic bacteria and fungi in System 2 showed a positive correlation. Dehydrated food waste was found to enhance bacterial and fungal growth, promoting efficient composting under specific pH and temperature conditions. These findings highlight the potential of using dehydrated food waste and EM in sustainable waste management practices and

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agricultural applications, fostering a more resilient and eco-friendly future.

1 Introduction

Food waste is a significant global issue that fuels issues with soil contamination and greenhouse gas emissions. All of these wastes have the potential to end up in landfills, where, if they are not properly disposed of, they may decay and generate methane, a potent greenhouse gas that hastens climate change. Additionally, the disposal of food waste resulted in a large loss of nutrients that could have been employed in agriculture. Chemical fertilizer is now the main source of agriculture because it provided a low-cost and effective source of nourishment. These fertilizers can have both immediate and long-term harmful consequences on the ecosystem, including the buildup of heavy metals in soils and the eutrophication of neighbouring rivers. Additionally, these chemical fertilizers do not improve the soil's characteristics, such as its structure and capacity for water infiltration, which is crucial for reducing soil deterioration in agricultural practices.

As a result, food waste can be used in agriculture as a fertilizer to replace the chemical fertilizers that are typically employed in the sector and as a disposal alternative. Composting is the best option in this case for keeping food waste out of landfills and converting it into a useful resource. Traditional composting's disadvantage is that it takes a while to mature because the breakdown of organic material solely depends on microorganism presence in the system which is highly dependent on the environmental factors. The process can be sped up using a variety of techniques and technologies, including Takakura, Bokashi and dehydrated techniques [1]. Effective microorganisms (EM) are used in these methods as an expediting agent. Leachate is toxic to EM, which contains many of the bacteria and fungi needed for composting [2]. As a result, dehydrated food waste is required, especially for composting so that can be applied to soil application and planting [3]. Composting confers numerous benefits, such as being replete with essential nutrients necessary for plant growth and harbouring microorganisms that facilitate the composting process [4]. The inclusion of wet food waste in the composting process has the potential to amplify the generation of leachate, consequently exerting an influence on microbial activity [1]. The desiccated food waste has been recognized for its capacity to enhance the levels of macro-nutrients, namely nitrogen (N), phosphorus (P), and potassium (K), as well as stimulate microbial activities, as documented by Xin et al., (2021) [5]. Nevertheless, the discourse surrounding the impact of this approach on the process of composting is notably scarce. Furthermore, a lot of studies concentrate on compost quality during the maturation stage. The early and premature phase, which is equally crucial to determining the viability of the compost, has rarely been examined. The goal of this study is to better understand composting at every stage of composting using EM-related dehydrated food waste. To determine the stability of the compost, it is necessary to measure the temperature, fungus, and bacteria that are present during the composting of food waste [6].

2 Materials and Method

2.1 Preparation of Effective Microorganisms (EM)

EM is widely utilized in various fields such as organic farming, waste management, soil improvement, and other environmental applications. The diverse group of microorganisms in EM had a positive impact by working together, preventing harmful pathogens, making the soil more fertile, and enhancing the overall well-being of the ecosystem. The primary components used in the preparation of EM include molasses, washed-rice water, peels from oranges and pineapples, and tempeh. The orange, pineapple peels, and tempeh were diced into smaller pieces before being combined with the solution. Subsequently, the solution was meticulously prepared. A solution was prepared by combining 1 litre of washed-rice water with 75 grams of molasses in a small bottle. The molasses served as a nourishing food for the microorganisms, helping them to thrive and multiply. Subsequently, the bottles were gently covered with a permeable cloth or lid, which enabled the exchange of gases. The bottles are also kept in a room that is warm and dark, with an ideal temperature ranging from 25°C to 35°C. The temperature range provided the best conditions for the growth of microorganisms and the process of fermentation. In addition to that, the mixtures were left to ferment for around 7 to 10 days. A pleasant yet tangy odour of the fermented mixture suggests that the EM is prepared for utilization. Following the completion of the fermentation process, the mixture underwent a filtration step to remove any remaining impurities or solid particles. The EM solutions were carefully moved into a sterile and sealed container to be kept for later use. The EM was stored in a cool and dark environment, to ensure that the microorganisms remained alive and usable for future purposes.

2.2 Composting experiments

The raw food waste was obtained from a nearby cafeteria in the College of Engineering Studies, UiTM, Shah Alam. This comprised peels from fruits and vegetables, and other scraps from plant-based materials found in the kitchen. In this research, meat, dairy products, oily foods, and cooked leftovers were refrained. These items have the potential to attract pests, impede the composting process, and generate unpleasant odours. Therefore, the food waste was cut and broken down into smaller pieces to speed up the composting process. This also helps the bacteria break down the food waste more effectively by providing them with a larger surface area to work on. Figure 1 shows the framework of the composting experiment. Briefly, in each system, a total of 3 kilograms of food waste and 2 kilograms of sawdust were utilized. In the case of system 1, a mini composter was utilized to dehydrate and mix the raw food waste with sawdust. Following a period of eight hours in the mini composter, the mixture was relocated into a container, where it was then given time to undergo the composting process. The 10% by volume of EM was included in the container and mixed evenly. The second system, known as manual food composting, involved placing all food waste into a container. Subsequently, sawdust and effective microorganisms (EM) were introduced before the initiation of the composting process. Regular monitoring is necessary for all composting systems to maintain the ideal level of moisture, which should be damp but not excessively wet. In addition, it was necessary to periodically turn or mix the materials to ensure proper aeration and facilitate the process of decomposition. The temperature was recorded accordingly using a thermometer stick.

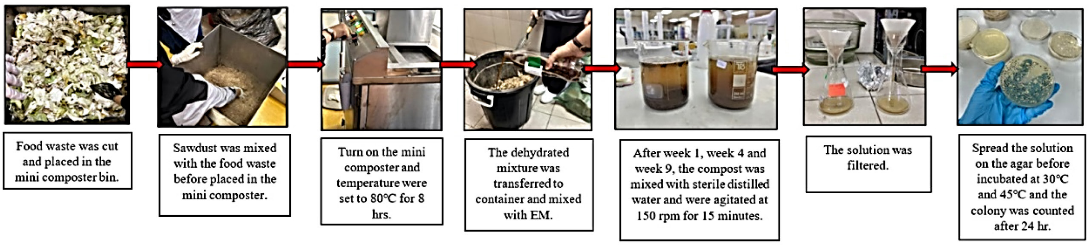

Fig. 1. The framework of composting experiment and colony determination

2.3 Determination of bacteria, fungi, and pH in compost

The sampling procedure encompassed three distinct weeks of observation during the food composting process, specifically denoted as week 1 (early stage), week 4 (premature stage), and week 9 (mature stage) for both system 1 and system 2. The temperature measurements for each respective system during the week were duly recorded. A quantity of 100 g of the compost was procured weekly, and subsequently subjected to dilution with 100 ml of sterilized distilled water. Following 15 minutes at 150 rpm, the solution underwent a process of filtration to eliminate any residual impurities or solid particulates present within the solution. This filtration procedure involved the utilization of a sieve with a mesh size of 63 μm [7]. Each filtrate was recorded for pH. For determining the colony of bacteria and fungi, nutrient agar (HiMedia) and potato dextrose agar (HiMedia) were prepared on Petri dishes. A serial dilution was performed utilizing Phosphate-Buffered Saline (PBS, OXOID) as the diluent. A volume of 100 uL of suspension was carefully dispensed onto the agar surface and subsequently evenly distributed using an L-spreader in a sterile manner. The Petri dishes were hermetically sealed using parafilm and subsequently positioned within an incubation apparatus. The incubator's temperature was set at 30°C for the mesophilic condition, while for the thermophilic condition, the temperature was maintained at 45°C. The specimens were subjected to an incubation period of 48 hours, or precisely two days. During the specified time frame, bacteria and fungi exhibited growth and subsequently developed discernible colonies on the surface of the agar medium.

2.4 Statistical analysis

The determination of the correlation between composting processes involving bacteria, fungi, and temperature, specifically in the context of mesophilic and thermophilic conditions, can be achieved through the application of statistical analysis. This analysis entails the utilization of Pearson's correlation coefficient to examine the relationship between the colony-forming units (CFU) and temperature data. The data collected were subjected to analysis utilizing Microsoft Excel version 365. A bar chart was generated to visually represent the observed trend, while Pearson analysis was employed to ascertain the correlation. Based on the concept of perfect correlation, a correlation coefficient of approximately 1 signifies the presence of a perfect relationship. Conversely, a correlation coefficient falling below 0.5 or exceeding 0.5 suggests the existence of a strong relationship. In contrast, a correlation coefficient ranging from 0 to 0.3 indicates a weak relationship, with the positive or negative sign denoting the direction of the variables involved. A correlation coefficient of 0 signifies the absence of any correlation between two variables, whereas a correlation coefficient of 0.5 denotes a moderate level of correlation [8].

3 Materials and Method

3.1 Bacteria and Fungi

Fig.2 (A) shows the result of bacteria and fungi colonies that exist during the composting of food waste in different systems. This subtopic explains the trends of the colony based on the system at every stage of composting. At the early stage of composting, systems 1 and 2 have high mesophilic bacteria compared to thermophilic bacteria. Generally, the number of bacteria colonies at the early and premature stages was stable but greatly changed at the mature stage. The mesophilic bacteria in both systems were sustained until the premature stage and reduced by 0.8-fold at the mature stage. These declines may be due to the natural progression of the composting process, where mesophilic bacteria begin to decrease as the compost matures. This study aligns with Hamzah et al. [9] which the number of mesophilic and thermophilic bacteria colonies was reduced at its reached mature stage.

For thermophilic bacteria, similar trends were observed as mesophilic bacteria in system 1 which also reduced by 0.8-fold but in system 2, the thermophilic bacteria increased by 1.3-fold. This could be attributed to the rapid increase of specific bacterial species that are well-adapted to the next phases of composting. It also explains that the dehydrated raw food waste contains less moisture content which reduces the microorganism content [10]. Moreover, the temperature recorded throughout the composting stage shows that the temperature in System 2 is slightly higher compared to System 1 (Table 1). This explains the ability of the thermophilic bacteria to present in system 2 is more favourable.

For the fungi colony in system 1, the number of mesophilic fungi was reduced significantly at the premature stage by 0.7-fold as shown in Fig.2 (B). While steady reduction trends were observed in system 2, which was 0.7-fold from the early stages to the mature stage. The trends for thermophilic fungi in system 1 were reduced by 0.5-fold, while the fungal colony was reduced by 0.8-fold at the premature stage before increasing by 1.3-fold at the mature stage. Compared to bacteria, the fungi colony was changed significantly at every stage of composting. The decrease in fungi counts could be influenced by changes in environmental conditions, such as temperature or availability of organic matter, which may not be favourable for fungi growth during the composting process. Since the temperature recorded during the composting process was between 28°C to 34°C, therefore the presence of thermophilic bacteria and fungi was also inhibited.

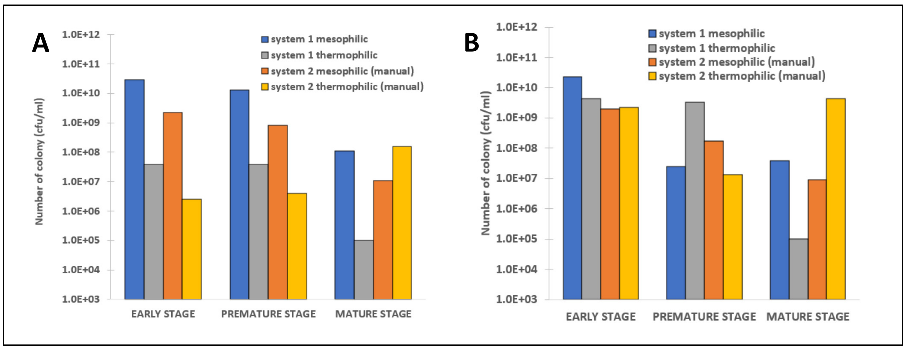

Fig. 2. The trend of (A) bacteria and (B) fungi existing in the compost at three different stages for system 1 and system 2

Briefly, in early food waste composting, mesophilic bacteria outnumber thermophilic bacteria. Mesophilic bacteria are better at using a variety of organic substrates found in food waste, explaining this phenomenon. Food waste starts composting in a fresh, degradable state. Mesophilic bacteria thrive in this environment because resources are abundant thus increasing the population. The first week of food waste composting may have had a low temperature, causing thermophilic bacteria to lag. Thus, thermophilic bacteria may decrease from the early to mature stage. Ryckeboer et al. [11] found that in the early stages of composting, substrates maintain the same temperature as the environment. Therefore, the mesophilic fungi and bacteria can degrade fresh organic waste around 20 °C to 40 °C.

Fig.2 shows that food waste composting bacteria and fungi are unstable, resulting in a population reduction. Bacteria-fungi competition may explain the phenomena. The ratio between mesophilic bacteria and fungi shows that bacteria are more favourable to the conditions compared to fungi. However, at the thermophilic condition, the fungi are higher indicating the tolerance of fungi to high temperatures. Chinakwe et al. [12] found that most microbial species cannot withstand thermophilic composting conditions. This was observed in both systems suggesting, dehydrated raw food waste does not influence the ratio of bacteria and fungi in the compost system. Instead, the dynamic between bacteria and fungi might benefit the composting, especially in stagnant temperature composting.

3.2 Correlation analysis between bacteria and fungi with pH and temperature

The study further measured the correlation between the number of bacteria and fungi colonies concerning pH and temperature to understand the composting process.

Table 1 shows the recorded value for pH and temperature during composting stages. The pH value for both systems was in acidic conditions due to the EM solution. As the compost turns into the maturity stage, the pH increases to neutral. This might be due to the dynamic changes in microbial activities. For temperature, the range was between 28℃ to 31℃ for system 1 while in system 2 was in the range of 32℃ to 34℃. No dramatic temperature changes for both composting might be due to the food waste used in this study which is only fruit and vegetables. Organic food waste such as vegetables can sustain the temperature during composting [13]. Moreover, Alves et al. [14] studied organic waste proportions in composting and found that temperature change did not occur as indicated in the literature. The temperature did not follow the traditional pattern of 40°C in the first stage, 65-70°C in the thermophilic phase, and a progressive decline in the final maturation stage. Composting residue proportions may affect this temperature deviation.

Table 1. The table shows the pH and temperature values recorded throughout the composting process for System 1 and System 2

Composting stage	**pH**		**Temperature (℃)**	
	System 1	**System 2**	**System 1**	**System 2**
Early stage	4.28	4.47	28	32
Premature stage	6.18	6.43	30	32
Mature stage	6.72	7.23	31	34

Fig. 3 shows Pearson's correlation analysis which left-sides indicate negative correlation and positive correlation on right-sides. A strong negative correlation was observed for mesophilic bacteria and fungi in both systems while a positive correlation for thermophilic bacteria and fungi in system 2 with respect to pH and temperature. However, the strength of the correlation between thermophilic fungi and pH was low at 0.2758 which indicates the thermophilic fungi have less influence as the pH changed.

From the figure, it can be concluded that pH and temperature have a reverse correlation to mesophilic bacteria and fungi which higher pH and temperature reduce the abundance of mesophilic bacteria in both systems. However, thermophilic bacteria and fungi in system 2 are higher as the temperature and pH were increased. By using dehydrated food waste, the number of bacteria and fungi in both conditions can be enhanced at room temperature and acidic pH. Meanwhile, the manual system shows that mesophilic bacteria and fungi prefer room temperature and acidic pH while thermophilic fungi and bacteria require high temperature and neutral pH. Pot et al. [15] discovered that the compost microbiota changes during optimizing treatments, including during development. Microbial diversity increases throughout maturation, suggesting that composting pH and temperature might affect mesophilic and thermophilic bacteria and fungi.

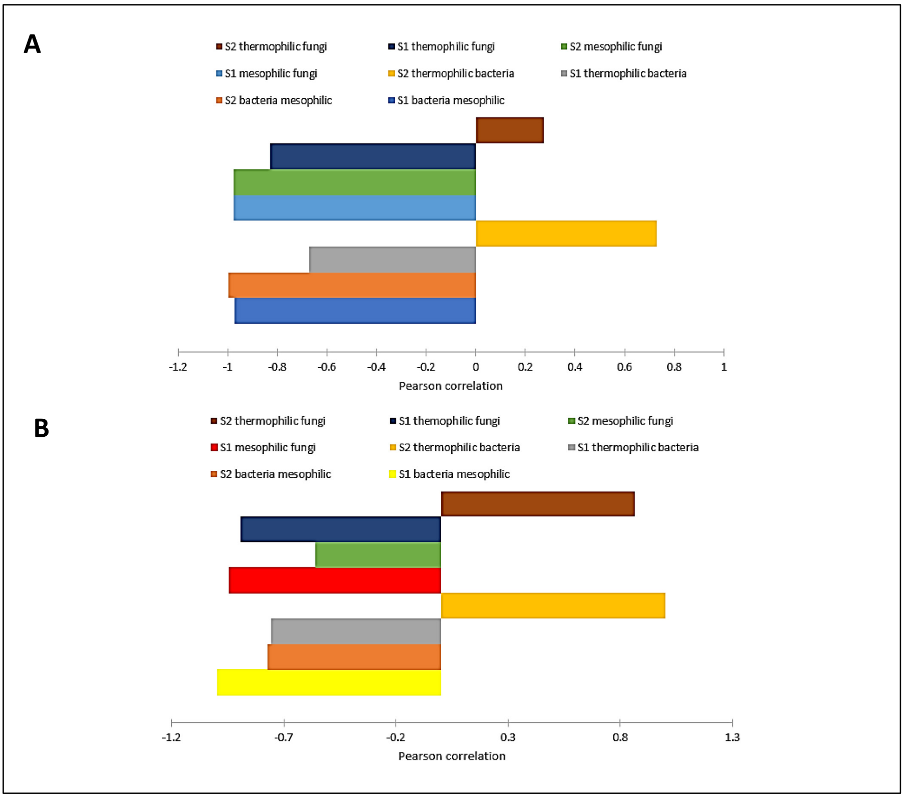

Fig. 3. The Pearson correlation analysis of bacteria and fungi concerning (A) pH and (B) temperature

4 Conclusion

In conclusion, food waste contributes to soil contamination, greenhouse gas emissions, and nutrient loss in agriculture, posing a significant environmental challenge. The main source of nutrition in agriculture, chemical fertilizers, have negative effects on the ecosystem. Composting food waste is a viable solution to this problem, as it can replace chemical fertilizers and divert waste from landfills. Effective Microorganisms (EM) can speed up the decomposition process, but hazardous discharge necessitates the use of dehydrated food refuse. Nonetheless, the impact of this strategy on decomposition phases is primarily unexplored. This study investigated decomposition at various phases using EM-related dehydrated food waste and measured the temperature, bacteria, and fungi to determine the compost's stability. Both systems produced compost with varying levels of mesophilic and thermophilic bacteria and fungi, as demonstrated by the results. As compost matured, System 1 demonstrated a decrease in bacterial and fungal colonies, whereas System 2 demonstrated an increase in thermophilic bacteria and fungi. The interaction between bacteria and fungi may play an important role in decomposition and contribute to the observed dynamic changes. The correlation analysis between bacteria, fungi, pH, and temperature yielded insightful information. The study found a strong negative correlation between mesophilic bacteria and fungi and pH and temperature, indicating that higher pH and temperature reduce mesophilic microorganism abundance. Thermophilic bacteria and fungi in System 2 exhibited a positive correlation with pH and temperature, indicating that they prospered under conditions of high temperature and neutral pH.

The use of dehydrated food refuse promoted bacterial and fungal growth in both systems, resulting in efficient composting at room temperature and acidic pH for mesophilic microorganisms and high temperature and neutral pH for thermophilic microorganisms. These results support the viability of using dehydrated food waste to optimize composting processes and emphasize the significance of understanding the microbial dynamics at various composting stages. This study contributes important knowledge to the fields of decomposition and sustainable waste management practices. It highlights the importance of contemplating decomposition as a multi-stage procedure, as opposed to focusing solely on the quality of the ultimate product. The findings can aid in the development of efficient decomposition techniques that utilize the benefits of EM and dehydrated food refuse. Implementing these strategies can not only reduce the environmental impact of food waste but also improve the sustainability of agriculture by providing nutrient-rich compost as an alternative to chemical fertilizers. This study illuminates the potential of using dehydrated food waste and EM in decomposition processes, paving the way for more comprehensive and effective waste management strategies in agriculture and beyond. Based on these findings, additional research on various decomposition materials and conditions can promote sustainable practices to address global food waste and environmental challenges. Planet Earth's future can be made more sustainable and resilient if we employ the power of decomposition. Future studies may include the identifications and dynamics of bacteria and fungi during composting to further evaluate beneficial microorganisms for well-stabilized compost.

Acknowledgement

The authors would like to thank the Universiti Teknologi MARA for Geran Komuniti CE-SIR grant (600-RMC/CE-SIR 5/3 (009/2023)) and College of Engineering,UiTM for supporting this research.

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