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Composting of Oil Palm Empty Fruit Bunches by Microbial Inoculant

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Abstract. Composting is a natural process that occurs as a result of microbial succession and marks the degradation and stabilization of organic matter in waste. The use of microbial additives during composting is believed to be highly efficient, as it increases the production of various enzymes, resulting in a faster rate of waste degradation. The process converts waste into a humus-like substance that can enhance the physical, chemical, and biological properties of soil. In this study, to assess the contribution of microorganisms to the composting process, we selected the thermophilic bacteria degrading *Bacillus subtilis*-ReK$_1$Hs-Cr$_1$ as the inoculant. The analysis of temperature, pH, moisture, microbial population, compost maturity, seed germination test, and C:N ratio were all monitored during the composting process. Physical changes in the compost, such as color, texture, and smell, were also observed. The findings showed that the inoculation of this thermophile with the addition of chicken manure was able to raise the temperature up to 67.3^{o}C, which may have resulted in the elimination of pathogens, which is above the recommended temperature level of 55^{o}C for 3 consecutive days. There was an increase in the microbial population (4.6 x10^5 -2.9 x10^7) at the start of the experimental works and the temperature decreased as the composting process proceeded because of the maturity phase. The pH; 7–9 and moisture content; 51–63 % of compost materials were in the range of optimum composting conditions. The weight of the compost materials in all three treatments, except for the control, decreased by 47% to 55%. The color of the compost turned to dark brown, the obnoxious odor was eliminated, and the texture changed to lose and easy pulverize at the final stage of composting. High germination index (GI) was obtained from the test, indicating that the final product is free from phytotoxic substances and thus suitable as a soil conditioner/fertilizer. Therefore, *B. subtilis* ReK$_1$Hs-Cr1 is the best candidate as an inoculant added with chicken manure to degrade OPEFB on a large scale using windrow technology.

Keywords: *Bacillus subtilis*-ReK1Hs-Cr1; Composting; Germination index; Oil palm empty fruit bunch; Thermophilic strain

1. Introduction

Oil palm production is a major agricultural industry in Malaysia. As a result, there has been an increase in the amount of waste generated by the oil palm industry, especially empty fruit bunches (EFB). Solid wastes from oil palm plantations, such as trunks, fronds, and oil palm empty fruit bunches (OEEFB), are a source of concern for the environment. As a result, composting is preferred and environmentally sound, whereby organic waste is

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reduced to organic fertilizer and soil conditioners through biological processes (Shukor *et al.*, 2018; Pan, Dam, and Sen, 2012). In composting, the higher-plant material breaks down under the influence of aerobic thermophilic microorganisms present in the waste to a material rich in organic nutrients. Oil Palm Empty Fruit Bunch (OPEFB) fiber is a lignocellulosic waste from palm oil mills that contains 12% lignin and 75% cellulose (Hermansyah *et al.*, 2019). Lignocellulose components are the main building block of plant cell walls where the cellulose fibrils are embedded in an amorphous matrix of lignin and hemicellulose (Zoghlami and Paës, 2019). Lignin is a structural polymer found in vascular plants, which plays essential functions in their survival. It provides rigidity to the plant and binds plant cells together, imparting resistance to impact, bending, and compression. However, cellulose is a highly regular polymer of any hydro-D-glucopyranase unit linked with ß (1-4) linkage, and this molecule can be easily metabolized by various cellulolytic microbes. Bacteria play a significant role in the humification processes associated with soil and compost. The enzymatic ability to cleave alkyl-aryl ether bonds enables bacteria to degrade oligomeric and monomeric aromatic compounds released during lignocellulose degradation. Composting has been used as a system for the disposal of organic waste, to produce organic fertilizer, and to improve soil biological fertility. Zulkepli and Tuah (2008) reported thermophilic bacteria had the potential to enhance the biocomposting of OPEFB which can reduce the weight of OPEFB and cause lignocellulose loss in 7 days of degradation. He also reported that the maximum temperature achieved during the composting of OPEFB by lignocellulose-degrading bacteria was only 45^{o}C which did not meet the minimum requirement of 55^{o}C for three consecutive days sanitized compost material as suggested by Jones and Martin (2003). The four most commonly used composting methods in the industry are open static piles, turning windrows and piles, aerated static piles, and in-vessel systems. Among these options, an in-vessel composting system has many advantages because it requires less space and provides better control over agitation, aeration, and mixing of the compost materials. Typically, two phases are engaged in in-vessel composting systems: a high-rate phase in the vessel composter and a curing phase in an outside composting pile. In this study, a locally isolated thermophilic lignocellulose-degrading bacteria from a hot spring (*Bacillus subtilis* ReK$_1$Hs-Cr$_1$) (Zulkepli and Tuah, 2008) were investigated for its capability to enhance composting of shredded OPEFB by using an in-vessel composting system. Non-inoculant and inoculant bin-type composting of OPEFB were compared by monitoring parameters which include temperature, microbial population, pH, C: N ratio, and Germination Index during the 90-day period.

2. Materials and Methods

2.1. Organism maintenance and preparation

Bacteria used in this work was the lignocellulose-degrading thermophilic strain Bacillus subtilis-ReK$_1$Hs-Cr$_1$ obtained from a hot spring in Ranau, Sabah (Zulkepli and Tuah, 2008). This strain has been selected due to its capability to sustain high temperatures, thus helping in elevating the temperature of compost materials and ensuring the elimination of both animal and plant pathogens during the composting process. The organism was maintained on Nutrient Agar (2 g/L yeast extract, 2 g/L meat extract, 5 g/L peptone, 4 g/L NaCl, 10 g/L glucose, and 12 g/L agar in a liter) at 4°C (Zahari *et al.*, 2021a). Subcultures were made weekly and cultivated at 40°C. To achieve successful and rapid composting of shredded OPEFB, inoculation of bacteria was needed. The sterilized raw materials were inoculated with 10% broth (Zahari *et al.*, 2022) inoculum (10^7 CFU/mL) of Bacillus subtilis-ReK$_1$Hs-Cr$_1$.

2.2. Composting process

Lab-scale in-vessel composting was carried out in accordance with Figure 1. Black bins with a capacity of 69 L and a height of 0.65 m (65 cm) were prepared. Approximately 10 holes with diameters of 1.5 to 2.0 cm were drilled at the bottom of each bin to collect leachate and similar holes were drilled on the sides of the bins for aeration purposes. The composting process involved four treatments, each with two duplicates, as shown in Table 1.

Table 1 Various treatments carried out in this study.

Treatment	Description
(K) - Control	: Shredded Empty fruit bunches (EFB) only
(R1)	: EFB + chicken manure
(R2)	: EFB + inoculant (*B. subtilis*-ReK$_1$Hs-Cr$_1$)
(R3)	: EFB + chicken manure + inoculant (*B. subtilis*-ReK$_1$Hs-Cr$_1$)

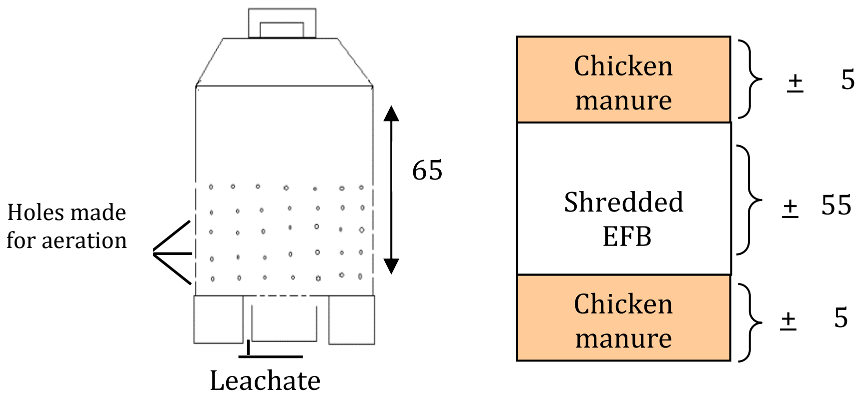


Figure 1 In-vessel composting (a) (bin-type) and (b) arrangement of EFB and chicken manure in the bin.

2.3. Sampling and analysis

The reduction weight of the compost along with color and texture, was observed at the initial and the end of the composting process. The temperature of the compost was recorded daily at the surface, middle, and bottom of the layer by using a digital temperature probe. pH value was analyzed by using a pH meter (1: 10 waters: extract). Moisture content was analyzed by drying 10 g of the sample at 120^{0}C until a constant weight was obtained. In maintaining the moisture content of all treatments, inoculants were added according to the weight of the compost materials, which is 5 mL for every kilogram of waste in the compost weekly. For biomass concentration, the microbial population (CFU/mL) was tested every 15 days. The Spread Plate Technique was used to obtain the CFU/mL after 24 h at 40°C (Taliesin and John, 2021). The inoculant was added on a weekly basis. Leachate was collected and added back into the compost material to control the moisture content. The carbon and nitrogen contents were analyzed using CNHS/O analyzer 2000 and Inductively Coupled Plasma (ICPOES, Perkin Elmer, USA). All samples were done in triplicates to obtain accurate data. The seed germination index (GI) was carried out to determine compost maturity and stability. 10 g of green bean seed ore were placed on filter paper soaked in 5 mL of compost extract. The petri dish was placed in a dark room at room temperature. For control, 1 g of seed was soaked in distilled water. The experiment was conducted in triplicates and incubated for 3 days. After incubation, the root length and seed germination index (GI) was calculated using the equation by Fetouh and Hassan (2014) below:

$$GI\ (\%) = \frac{seed\ germination\ in\ treatment\ (\%)\ x\ root\ length\ in\ treatment}{seed\ germination\ in\ control\ (\%)\ x\ root\ length\ in\ control}\ x\ 10$$

3. Results and Discussion

3.1. Physiochemical changes during the co-composting process

The changes in temperature during the 90-day composting process of different treatments are shown in Figure 2 (a). There are four stages included in this process which are mesophilic, thermophilic, cooling, and maturity compost. The duration of each stage depends on the initial composition of the mixture in a treatment bin. In the mesophilic phase, a variety of bacteria, actinomycetes, and fungi quickly break down the C-rich substrates at moderate temperatures, usually between 15 and 40 °C. Heat is produced by this aerobic metabolism. As time passed, the temperature rose rapidly to more than > 45 °C (Thermophilic phase) in less than 15 hours during the initial stages. The results show that the temperature was increased in all treatments studied, describing a peak of microbial activity. For Treatment 1 (R1), (R2), and (R3), the maximum temperature reached was 58.8°C, 56.8°C and 67.3 °C on day four, respectively. The thermophilic phase of Treatment 3 (R3) was maintained for 14 days, while for other treatments, the thermophilic phase was maintained for only 8 days. While for the control (K), the thermophilic phase only lasted 4 days, and the maximum temperature only reached 50.2°C. This shows that the addition of an inoculant of bacterial strain and chicken manure has a positive effect on the composting, especially during the thermophilic phase, that help in elevating the temperature of compost materials during composting process. The high temperature achieved during the composting process ensured the elimination of both animal and plant pathogens. Only three to four days at a temperature of 55 °C was sufficient for eliminating the pathogens (Zhao, Ta, and Wang, 2017). Kim *et al.* (2019) reported that pathogens can be killed at 55 °C. Additionally, almost all pathogens are eliminated in a few days making the compost product suitable for agricultural use. Besides, a rise in temperature during composting are the consequence of the breaking down of the readily available organic matter and nitrogen compounds by microorganism (Raut *et al.*, 2008). As time increase, the organic matter becomes more stabilized, thus slower the microbial activity and decomposition rate. A steady temperature was achieved at the last stages of composting, indicating that less degradation (cooling phase) process took place. The final maturing phase is characterized by lower temperatures below 25°C and reduced oxygen uptake rates of aerobic microorganisms. During this stage, degradation of the more refractory organic compounds continues, and soil meso and macrofauna enter.

Figure 2(b) illustrates the weekly pH changes of the composting process for the different treatments over the 90-day period. The initial pH values of all treatments of the composting process were nearly neutral, which were 7.6 for (R1), 7.5 for (R2), 7.5 for (R3), and 7.7 for Control (K). During the composting process, the pH values for all treatments increased until week 3. For Treatment 1, the pH of the compost material gradually increased to 8.7 at week three from the initial pH of 7.5. The pH value at week three was observed to be the highest pH value, 8.7, throughout the composting process. Then, the pH value slightly decreased from week four to week six. This trend has been observed in all treatments. The pH rise was due to the organic acid volatilization and microbial decomposition (mineralization) that release ammonia from the organic nitrogen sources (Umsakul, Dissara, and Srimuang, 2010). pH for (R1) has been decreased to 7.1 at week 11 then the value raised again to 8.8 in the final stage of the composting process. A similar trend was observed in (R2), (R3), and Control (K). At the final stage of the composting process, the results obtained for (R1), (R2), and (R3) and Control (K) were 8.8, 8.1, 8.5, and 8.1 respectively.

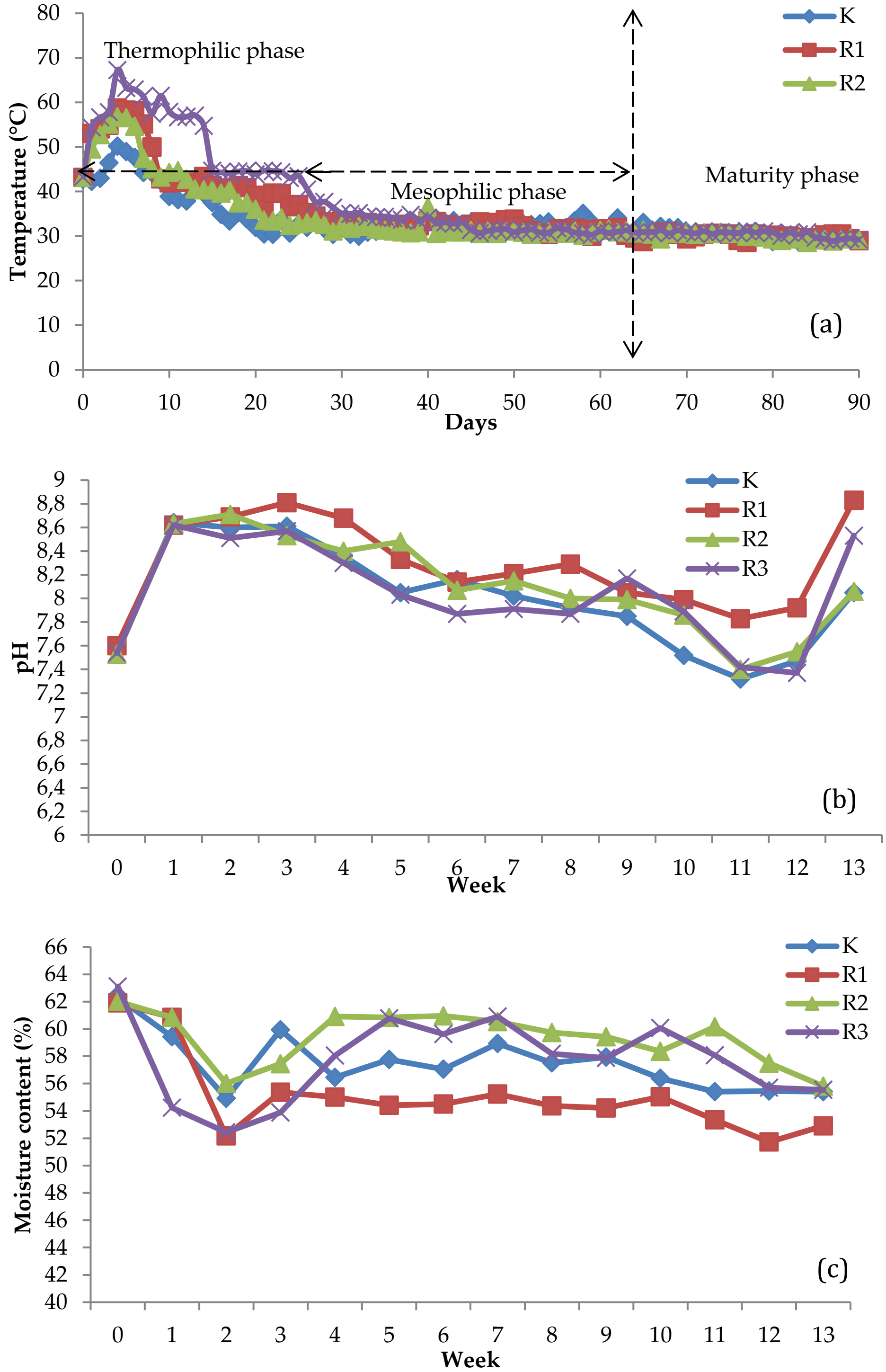


Figure 2 Profile of physiochemical elements that use different treatment conditions. (a) Temperature changes during composting. (b) pH changes during different waste substrates. (c) Changes in moisture content during composting

Previous research found that microbial activity increased the likelihood of achieving a suitable pH range of 5.5–9.0, while the composting process is most effective at pH values between 6.5 and 8.0 (Pan, Dam, and Sen, 2012). The pH of all treatments was alkaline and stable due to ammonia generation, which could improve the composting process by inhibiting pathogenic fungi that prefer acidic conditions (Saidi *et al.*, 2008).

Figure 2 (c) shows the weekly moisture content of the composting process of different treatments during the 90-day period. Moisture appears to be a significant component controlling microbial activity (Anastasi, Varese, and Marchisio, 2005), as low moisture content hinders the growth of helpful microbes (Tiquia, 2010), whereas excessive moisture might cause anaerobic conditions, resulting in the creation of unpleasant odors and poisonous volatile compounds (Saidi *et al.*, 2008). The moisture content of Treatment 1 (R1), Treatment 2 (R2), Treatment 3 (R3), and Control (K) was shown as 51 %, 56 %, 52 %, and 55 %, respectively. The moisture content for all treatments was increased when the temperature of the compost was decreased. This high moisture level in all treatments resulted in a cooling effect. This is due to the gaseous exchange by reducing diffusion and consequently constraining oxygen uptake by microbes, thus resulting in lower microbial activity (Zahari *et al.*, 2021b). The average moisture content for Treatment 1 (R1) was 59.5 % throughout the composting process. The moisture content was maintained in the range of 52 % to 62 % for the Control (K) throughout the composting process. The moisture content for Control (K) decreased from 62 % at the begining to 55% during the final stage of the composting process. For (R2), the moisture content obtained in the initial stage was 58 %. The moisture content slightly increased to 58 % at week 1. The moisture content showed a gradual decrease at week two to 44 % even though inoculants were added to all bins as usual and increased again to the optimal range (61%). At week three, there was a slight increase and decrease in moisture content of (R2) until week 11. At week 11, the moisture content noted an obvious decrease to 48 % and continued to decrease to 47 % in week 12. The decrease in moisture content may be caused by the decrease in microbial activity in compost, which has a direct effect on a high content of organic matter (Kazami *et al.*, 2016). Generally, the moisture content for all treatments showed a decreasing trend till the end of the composting process. The addition of inoculants into the compost materials is adequate to maintain the moisture content of the treatments. Occasionally, leachate from the compost was collected and added to the compost materials to maintain the moisture content. A comparative study in Table 2 showed the performance of various compost processes using different microbial inoculants within the present work.

Table 2 Summary of the previous studies by different microbial inoculants used for composting of EFB

Compost feedstock	Inoculant Used	Duration (days)	Maximum Temperature Recorded (⁰C)	Descriptions	References
EFB	*Bacillus lincheniformis* – NAS_3 dHs-Cr_1	90	63.6	The final ratio of C:N R3 is low compared to other treatments 14:1, which indicates that the compost is mature and stable. The weight reduction of compost R3 was the highest among the treatments, which reduced by 53.1%.	(Tiong, 2011)
EFB	*Trichoderma*	30	24	C:N ratio stabilized, increased nitrogen (N), phosphorus (P), and potassium (K) were found in compost, enhanced soil micronutrient, plant growth performance, and crop yield production	Siddiquee, Shafawati, and Naher (2017)
EFB	Consortium	-	45	The addition of specialized microbes to EFB-POME co-compost may help to reduce the magnitude of waste biomass generated from palm oil mills and provide crop fertilizers.	(Krishnan *et al.*, 2017)
EFB	Lactobacillus and actinomycetes	64	52	The inoculation of the microbial additive into EFB compost can improve the efficiency of EFB decomposition	(Lim, Chua, and Lee, (2015)
EFB	*Bacillus subtilis*-ReK_1Hs-Cr_1.	60	67.3	High germination index obtained from the test indicated that the final product is free from phytotoxic substances and thus suitable as a soil conditioner/fertilizer.	Present work

3.2. Bacterial count

The microbial community of different treatments was studied during the composting process. Bacteria and fungi that constitute the microbial community structure of the composting environment are present and active during the composting process. Different bacteria and fungi can have a beneficial or detrimental impact on the entire composting process. Their diversity also hints at the composting mechanisms at work. From Figure 3, which shows the microbial population of different treatments during the 90-day composting process, the microbial population in the compost material of all treatments was almost the same for (R1), (R2), (R3), and Control (K), which are $7.2\times10^6 – 2.8\times10^7$, $5.0\times10^5 – 2.9\times10^7$, $4.6\times10^5 – 2.9\times10^7$ and $1.5\times10^6 – 2.9\times10^7$ $CFUmL^{-1}$, respectively. This is because the compost materials from all treatments were from the same source. The population of microorganisms is slightly decreased for all treatments from day 1 until day 15 because the high temperature during the thermophilic phase of composting (> 45 °C) killed the pathogens as well as other microorganisms in the compost. The microbial population, therefore, continued decreasing until the end of the composting process. This is because the microbial cannot survive without nutrients and oxygen (Riedel *et al.*, 2013). While for the control, the microbial population decreased in week 45, and the compost materials were in the mesophilic phase. In this phase, it is most suitable for most of the microorganisms to grow and reproduce. Thus, the population of microorganisms in the compost increased again.

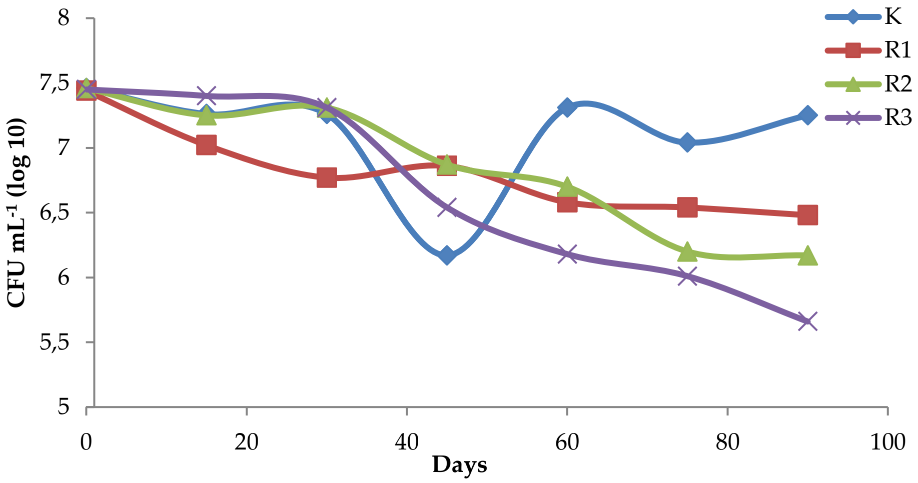


Figure 3 Profile of microbial population according to the respective composting treatment.

3.3. Compost stability and maturity

In this framework, examining the maturity of the product was necessary to complete its characterization and identify any potential negative impacts on the soil. The germination test is one of the tests to determine the compost's stability and maturity. According to Tiquia (2010), the germination index (GI), which includes the measurements of relative seed germination and relative root elongation, has been used to assess the toxicity of the compos. It has been observed that poor germination indices are typically achieved at the outset of the bio-stabilizing process as a result of the rapid onset of biological activity and the generation of germination-inhibiting substances such as alcohols, phenolic compounds and organic acids (Kazemi *et al.*, 2016). From our finding (Figure 4), it was shown the germination of green beans of different ages of compost material. It was observed that after day 3, all seeds were germinated with an extensive and healthy root system. However, on day 15 of the composting process, the GI of green beans was very low for all the treatments. The GI for (R1), (R2), (R3), and Control (K), were 54%, 60 %, 59 %, and 66 %, respectively. These low GI values point to the presence of phytotoxic substances for germination and growth, as suggested by Banegas *et al.* (2007). As the composting process went on, the GI value for all treatments increased significantly to 97 %, 97 %, 97%, and 96 %, respectively, at day 30. According to Tiquia (2010), a GI value of 80 % has been employed as an indicator for the removal of phytotoxicity in composts. From this study, although the GI value for (R2) and (R3) dropped at day 45 and continued to increase till the end of the composting process (day 90), the GI values for all treatments obtained are still considered matured. The GI value, which dropped at day 45, may also be caused by the complete turning of the entire compost material a few days before day 45 and also due to the release of toxic concentrations of ammonia again in the compost. It is suggested that the compost turning frequency influenced the composting rate, the time necessary to reach full maturity, and the elimination of phytotoxicity from spent litter during composting (Tiquia, 2010). The obtained GI values showed a trend of decreasing phytotoxicity with composting time, as suggested by Gigliotti *et al.* (2005). The GI values for all treatments increased till the end of the composting process, which were 103 % for (R1) and (R2), 108 % for (R3), and 102 % for Control (K). The high GI value obtained shows that the compost materials were in the maturity stage and safe to be used.

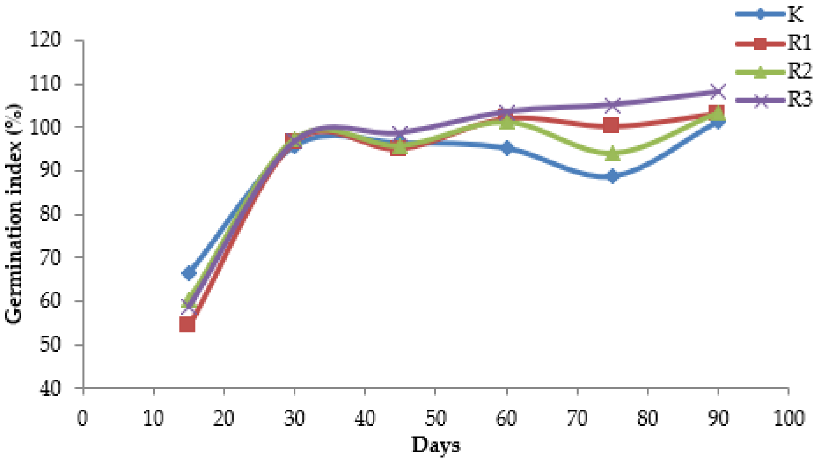
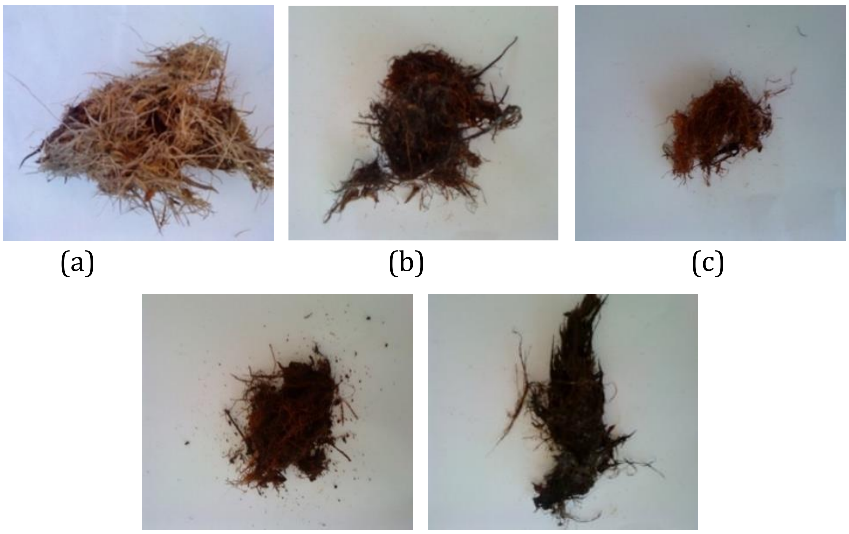


Figure 4 Germination Index of all treatments of shredded OPEFB composting during the 90-day period.

3.4. Weight reduction of the compost material

The initial weight of the compost materials in all treatments during the composting process was 76.7 kg, 60.3 kg, 76.6 kg, and 59.8 kg for (R1), (R2), and (R3) and Control, respectively. The weight of the compost material in Treatment 3 showed a significant decrease in weight to 33.3 kg, which is a 57 % reduction compared to the initial weight. The weight of the compost materials of (R1), (R2), and Control (K) has been reduced by 54 %, 55%, and 34 %, respectively. The weight reduction of the compost materials in all treatments was associated with the evaporation and leaching of moisture (Jain, Daga, and Kalamdhah, 2019).



(a) (b) (c)

(d) (e)

Figure 5 The condition of compost materials at the beginning and final stage of the composting process. (a) OPEFB before composting, (b) Control (K) after composting; (c) Treatment 1 (R1) after composting; (d) Treatment 2 (R2) after composting, and (e) Treatment 3 (R3) after composting

The sample taken to test the pH, moisture content, colony forming units, carbon to nitrogen ratio, and as well as tested compost maturity through seed germination tests from time to time has also contributed to the reduction in weight of compost materials in all treatments. Figure 5 (a)- (e) shows the condition of compost materials at the beginning and final stages of the composting process. During the composting process, gradual changes in the texture and other characteristics of the shredded OPEFB were observed after 90 days. The changes in color, odor, and texture for (R1), (R2), (R3), and Control (K) were the same. The color of the compost materials changed from filemot at the beginning to dark brown humus-like in the final stage of the composting process. This result is comparable to a study by Pan, Dam, and Sen (2012), which reported a black-colored humus-like substance developed after 120 days of decomposition of common organic wastes inoculated with three bacteria, *Bacillus subtilis* B1U/1, *B. subtilis* D3L/1, and *Pseudomonas* sp. RAT/5. The compost material produced an obnoxious smell at the initial stage. As the composting process proceeds, the compost becomes fragrant humus at the final stage of the experiment. Lastly, the texture of the compost materials changes from conglomerate become loose and easily pulverized at the end of the composting process.

4. Conclusions and Recommendations

Composting is the most widely used technology for the biological stabilization of organic solid waste, owing to its perceived ease of operation in comparison to anaerobic procedures. However, the proper evolution of degrading processes must be continuously monitored, as standard criteria for compost quality evaluation usually provide insufficient or incorrect information, which can be damaging to process operation and performance. The selection of the most appropriate raw material is critical for successful composting, as it contributes to the process's operation and performance. This study highlights that thermophilic strains *Bacillus subtilis*-ReK_1Hs-Cr_1 were able to raise the temperature up to 63.7^{o}C, which may have resulted in the elimination of pathogens. A subsequent decrease in pile temperature at the end of composting correlates well with other characteristics of a compost used to evaluate its stability or maturity in a compost. The pH, 7–9, and moisture content; 51–63 % of compost materials were in the range of optimum composting conditions. The weight of the compost materials in all three treatments excluding control was reduced by approximately 47–55 %. The color of the compost was turned to dark brown; the obnoxious odor was eliminated, and the texture changed to lose and easily pulverize at the final stage of composting. The high germination index obtained from the test indicated that the final product is free from phytotoxic substances and thus suitable as a soil conditioner/fertilizer. Some suggestions can be made to improve the efficiency of composting. The performance of composting can be further enhanced by improving the turning/tilting process, such as by using a mechanical tilting mechanism. Furthermore, the compost's quality as a bio-fertilizer must be confirmed through detailed analysis of other physicochemical and microbiological parameters, as well as studies on larger-scale processes.

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