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Biodegradation of Chicken Feather Waste with *Bacillus subtilis* in Vermicomposting

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ABSTRACT

Poultry waste (chicken manure [CD] and chicken feathers [CF]), and agricultural waste, including mushroom media residue (MMR) and banana trunks (BT), were utilised in this vermicomposting with different proportions (15 ratios). This study primarily introduced earthworms (*Eudrilus eugeniae*) and *Bacillus subtilis* (3.9×10^{-5} CFU/mL) as degradation agents. Both agents were added on day 14 into bins, mixed thoroughly, and the subsequent substrate was then retained for the 60-day composting process. T9 treatments demonstrated enhanced biodegradability of chicken feather waste, achieving a biodegradation rate (Kb%) exceeding 80%. The earthworm population increased by 62%, and there was a 53% weight gain in earthworm reproduction at day 60 for the ratio of 6:2:1:1. The maximum pH value was recorded in T1 (7.11±0.06). In contrast, the maximum EC value (ms/cm) was recorded at 3.5±0.40 in T4. The vermicompost contains a nitrogen compound (N) range (1.9%–4.4%), a potassium content (K_2O) between 7.81%–22.9%, and phosphorus (P_2O_5) within 0.91%–3.03%. The investigation revealed that T11 had the highest dehydrogenase enzyme (DHA) activity, whereas T13 showed a greater catalase activity, and T3 exhibited the maximum keratinase enzyme activity. DHA activity showed a correlation with K_2O (r^2=0.507) and also demonstrated a correlation with P_2O_5. Correlation studies have found that enzyme activity and physico-chemical properties influence the vermicomposting process of chicken feathers.

Keywords: *Bacillus subtilis*, biodegradation, chicken feathers, enzyme activity, *Eudrilus eugeniae*

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INTRODUCTION

In order to convert agricultural waste into valuable products, the manipulation of microbes has emerged as a strategy that is environmentally sustainable and cost-effective, and preserves the validity of structure and product efficacy (Daroit & Brandelli, 2014). Utilising earthworms for composting diminishes organic waste, stabilises organic resources, and enhances plant biomass output. Earthworms facilitate the degradation of organic waste by feeding, fragmentation, aeration, turnover, and dispersion, in conjunction with enzymatic digestion aided by bacteria. Thus, vermicomposting is a more effective and safer method for managing agricultural waste compared to traditional composting, which results in nutrient depletion, prolonged processing times, and inferior compost quality.

Keratin has the potential to be utilised in a variety of applications in the agricultural and industrial sectors, such as animal feed, adhesives, organic fertilisers, leather processing chemicals, cosmetics, pharmaceuticals, and biomedical materials (Mengistu et al., 2023), however most chicken feathers are still burned or thrown in the trash (Alahyaribeik & Ullah, 2020). Its keratin may be utilised as a valuable bioresource to produce high-value metabolites and products with additional value. In Malaysia, an estimated 17.745 million tonnes of waste is contributed by chicken poultry (Zayadi, 2021). This keratinase waste is primarily composed of chicken feathers, which are disposed of through land-filling, burning, and open dumping. The gradual degradation of chicken feathers results in their prolonged accumulation of keratinase waste in the environment due to their slow degradation process. Besides being insoluble in water, keratin protein has been shown in the literature to be best enhanced for degradation by heat treatment and somewhat acidic pH conditions (Fagbemi et al., 2020). Concerns about the keratin waste from chicken poultry, an alternative approach by using microbes or maybe further with specific microbial keratinases that are able to catalyse the biodegradation of keratin by using the vermicomposting method.

Bacillus spp. are abundant producers of lignocellulose-degrading enzymes, such as xylanases and cellulases. Nonetheless, an extract from *Bacillus subtilis* exhibited significant cellulase activity in cellulose disintegration, thereby facilitating cellulose degradation (Siu-Rodas et al., 2017). Furthermore, identifying and screening microorganisms capable of degrading feathers, including bacteria and fungi, has been documented (Elhoul et al., 2016; Mini et al., 2017; Zhang et al., 2016). Previous research indicated that two keratinolytic strains, *Bacillus licheniformis* BBE11-1 (Liu et al., 2014) and *Stenotrophomonas maltophilia* BBE11-1 (Fang et al., 2013), can hydrolyse feathers; however, their growth and enzyme production conditions differ significantly, rendering them unsuitable for the degradation of bulky quantities of feathers. Peng et al. (2019) reported that keratinase alone does not hydrolyse feathers. Limited research has investigated the degradation of keratin derived from chicken feathers. A study indicated that *Bacillus* spp. C4 destroys merely 75% of a 5% (w/v) chicken feather suspension over 8 days, employing a laborious and inefficient

method (Patinvoh et al., 2016). Consequently, using earthworms (vermicomposting) and *Bacillus* sp. as agents for the degradation of chicken feathers aims to enhance degradation efficiency and facilitate the conversion into compost products. A suggestion indicated that *Bacillus* sp. may facilitate the degradation of organic materials by generating enzymes that stimulate microbial activities (Peng et al., 2019).

Production of several enzymes, such as dehydrogenase and catalases, can be utilised to determine the maturity and effectiveness of the vermicomposting process. The presence of microbial composition will produce enzymes to decompose the initial substrate. Barrena et al. (2008) indicated that dehydrogenase serves as a useful enzyme for reflecting the biological activity of composting. Simultaneously, catalase activity was assessed to evaluate the breakdown of hydrogen peroxide during degradation. The elevated catalase activity signifies the efficient breakdown of organic materials during degradation. This study examines how changes in enzyme activity during the vermicomposting process are closely related to the presence of physicochemical properties and nutrients produced, as shown in Table 3, using correlation tests.

MATERIALS AND METHODS

Compost Materials and Environmental Site

The substrate comprises mixtures of chicken feathers (CF), mushroom media residue (MMR), banana trunk (BT), and chicken dung (CD). CD and CF have been collected from the poultry farm and the chicken processing centre in the Northern region of Malaysia. CF were dried to ease cutting and placed into smaller pieces using scissors. All substrates were soaked for 14 days using tap water by changing water every day until the electrical conductivity (EC) of the substrate mixtures reached below 2.0 ms/cm (Ahmad, 2020). Pre-composting was adjusted for about 14 days, with a temperature at the environmental site of around 22–35°C, daylight and a moisture content of 55% – 80%, and substrate pH between 7 and 7.6 before composting to keep the earthworms healthy. All substrates were mixed in different (dry volume) amounts of organic matter into bins with proportion ratios (Table 1).

Composting System and Treatment

The composting method was performed with slight modifications based on different proportions of chicken waste. All mixtures were homogenised to obtain 15 different proportions (Table 1). All ratios were made in five replicates. Earthworms were obtained from the Compost Worm Breeding Centre and the Vermicompost Producer, Plant Protection and Plant Quarantine Division, Department of Agriculture Malaysia, Tambun Tulang, Perlis. The earthworms were undergoing an adaptation and breeding using the proportion of 3:2:1 for 2 weeks before being placed in the experimental bins. All treatments were received 10 healthy *E. eugeniae* placed in the experimental bins.

Five replicates of bins were set up with the addition of earthworms, and *B. substilis* was purchased from the website of the American Type Culture Collection (ATCC). The suitability of added agents for vermicomposting immediately after pre-composting of substrate, the EC value reaches below 2 ms/cm approximately for 14 days (Ahmad, 2020). 1 ml of *B. subtilis* (3.9×10^{-5} CFU/mL) was inoculated into each treatment. Treatment without *B. subtilis* was used as a control; These bins were kept undisturbed for 60 days and sprinkled with water daily to keep them moist.

Compost Preparation

The composting process was carried out using only slight modifications to the proportions of chicken waste in bins located at the composting house at Universiti Teknologi MARA (Malaysia). On day 14, the inoculation of the composting agents, *E. eugeniae* and *B. subtilis*, started and was recorded as day 0 of organic matter degradation (initial). Five samples from each replicate were collected from each bin at a depth of 15 cm at 60 days. Approximately 100 g of vermicompost was collected from each bin to determine the enzyme activity and physico-chemical properties. Compost samples were kept under -4°C in the dark storage for a maximum of 4 days before sample preparation for compost determination to reduce the risk of microbial growth. Determining enzyme activity assays were done immediately after the sample was prepared. Sample preparation has potential effects on the enzymes' activity during storage.

Table 1
Different proportions (dry volume) of organic matter (OM) waste and MMR used as the main raw material

Treatment	Degradation agents	Organic matter (OM) waste and proportions			
		MMR	CD	CF	BT
T1	*Bacillus subtilis*	6	3	null	1
T2	*Bacillus subtilis*	6	null	3	1
T3	*Bacillus subtilis*	6	1.5	1.5	1
T4	*Bacillus subtilis*	6	2	1	1
T5	*Bacillus subtilis*	6	1	2	1
T6	*Bacillus subtilis* + *Eudrilus eugeniae*	6	3	null	1
T7	*Bacillus subtilis* + *Eudrilus eugeniae*	6	null	3	1
T8	*Bacillus subtilis* + *Eudrilus eugeniae*	6	1.5	1.5	1
T9	*Bacillus subtilis* + *Eudrilus eugeniae*	6	2	1	1
T10	*Bacillus subtilis* + *Eudrilus eugeniae*	6	1	2	1
T11	*Eudrilus eugeniae*	6	3	null	1
T12	*Eudrilus eugeniae*	6	null	3	1
T13	*Eudrilus eugeniae*	6	1.5	1.5	1
T14	*Eudrilus eugeniae*	6	2	1	1
T15	*Eudrilus eugeniae*	6	1	2	1

Earthworm Population and Reproduction

Population and reproduction of earthworms were determined individually by self-count, and weight was measured using a digital scale. The vermicompost produced has been sieved using a fine 4 mm mesh to separate the compost material from the earthworms. The population and reproduction of earthworms were quantified based on the treatment and replicates, as shown by the formula provided in Equations 1 and 2:

Population: After number of *Eudrilus euginea* - Before number of *Eudrilus eugenia* [1]

Reproduction: After weight of *Eudrilus euginea* - Before weight of *Eudrilus eugenia* [2]

Biodegradable Rate (%)

Organic matter (OM) waste was weighed for all the treatments and replicates at day 0 and day 60 of the vermicomposting period using a weight scale to measure the organic matter conversion during vermicomposting. The biodegradation rate was measured with a slight modification to determine the degradation rate (Manyuchi et al., 2013; Manyuchi et al., 2014).

$$\text{Biodegradation rate (\%)} = 100 \times (B\text{-}A)/B \quad [3]$$

Where, B is the dry weight of the organic matter before degradation, and A is the dry weight of the organic matter after degradation.

Enzyme Activity Assays

The activity of three microbial enzymes (dehydrogenase, catalase and keratinase) was determined on days 0 and 60. For measuring dehydrogenase (DHA), the triphenyl tetrazolium chloride (TTC) method was used based on He et al. (2013), with a slightly modified method and dehydrogenase activity was determined by using the triphenyl tetrazolium chloride method according to the procedure described by Barrena et al. (2008). Values of DHA are expressed as mg of triphenyl formazan (TPF) released g dry matter-1 h-1 and are presented.

Dehydrogenase activity was measured using a calibration curve of dehydrogenase activity with different TTC concentrations as recommended in (Pourakbar et al., 2020). In order to measure the dehydrogenase enzyme activity, a calibration curve was developed using 3 ml of the 3% v/w TTC substrate and 5 ml of methanol as an extractor. Afterwards, the TTC solution was vortexed and filtered through the sample. A UV-visible

spectrophotometer revealed the solution at 485 nm, chloride (TTC). The absorbance of six different concentrations of TTC has been inserted into the standard curve scatter graph. Then, the calibration curve was developed in line according to the standard curve graph's linear equation, with ($r2$= 0.9918), with the equation of a straight line being y = 0.0022x+0.0622. Therefore, the vermicompost sample from each treatment was measured using the procedure given.

Keratinase activity was used on a total of 5 g of the compost sample, as described in the procedure by Kamarudin et al. (2017). Keratinase activity was defined as the amount of enzyme causing a 0.01 absorbance increase between the sample and control at 595 nm under the given conditions. The unit of keratinase was in (µg) in every 5 g of the compost. The keratinase standard curve was created using a concentration in which 5 µg represents 1% of keratin azure. A scatter plot's points are strongly correlated with a linear regression line according to the standard curve graph's linear equation, which ($r2$=0.9901), with the equation of a straight-line being y = 3.18x +0.0349. The value of the keratinase enzyme activities (x) was determined by inserting the absorbance into the formula (Equation 4).

x = Absorbance- Intercept slope [4]

Characterising the Physical and Chemical Properties of Biodegradation

pH value and the electrical conductivity (EC) of the substrate were measured at day-0 and day-60 using Hana Instruments, HI98107. The Oakton EC100 portable conductivity meter (EC) was used to determine the electrical conductivity in the compost by inserting the rod into the compost. All the treatments and replicates were sent to the Global Testing and Consultancy for Rubber (G-TACR) for the measurement of the chemical properties such as nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O).

Data Analysis

The enzyme activities of compost samples were carried out in five replicates and presented as mean ± standard deviation. SPSS 16.0 software was used to perform a correlation analysis of enzyme activities and physico-chemical properties, as well as the biodegradation rate (Kb)% between the composting agents.

RESULTS AND DISCUSSIONS

In the present study, the changes in composting pH were recorded at day 0 and day 60. pH value of the biodegradation process was recorded during pre-composting (initial stage), 7.2–7.5. The peak of pH sharply reduces the value and indicates an acidic pH value when the composting process begins. However, all treatments were indicated in the optimum range of pH value after completing the biodegradation activity and in accordance with the

range of 6.5–7.5 of composting produced, excluding T7, T9 and T12. The three treatments (T7, T9 and T12) indicate that the presence of organic waste leads to an increase in acidic content in the bins, which is a potential danger to the survival of earthworms (Katiyar et al., 2017). However, the dramatic changes in pH recorded during day 60 of composting might be related to the biodegradation of organic materials by a composting agent.

Results also indicate that T7 and T9 were degraded with the combination of both agents, while T12 was found in the earthworm degradation agent only. This finding slightly contradicts the previous finding. The biological oxidation of organic substances during this period may facilitate the movement of hydrogen from the organic substrate to the inorganic acceptor (Aghayani et al., 2018). Hydrogen is an acid cation; particularly, acid cations influence the media or soil pH.

The pH values recorded during the biodegradation process, comprising varying quantities of substrate and either *B. subtilis* or earthworm as the degradation agent, remained within the slightly acidic range of 6.0–7.11. Furthermore, these tests indicated a lower pH of 6.13 in T7, despite the presence of both composting agents. However, this discovery was not parallel with the consistent occurrence of organisms in degradation. Hazardous material waste may contain compounds that are less hazardous or nontoxic. A greater number of earthworms significantly improves the aeration of the compost, hence preventing a decline in pH, whereas aerobic conditions affect ammonium utilisation and pH increase. Furthermore, biochar and earthworms can improve soil structure and boost microbial population and activity (Zhang et al., 2021).

The EC values in all treatments were observed to be under the acceptable limit of 4 ms/cm on the final day of the biodegradation process (day 60). The lowest average EC was obtained in T11 and T6. Meanwhile, the highest was in T1 and T4 (3.5 ms/cm), respectively, at day 60. Electrical conductivity of less than 3.5 µs/cm has been suggested as the optimal value for compost use as an agricultural fertiliser (Chen et al., 2014). In the present study, the changes of EC values between the pre-composting range value of 0.1–0.2 ms/cm in bins were initially recorded, followed by determination on day 60. However, the final EC at day 60, respectively, from T1, T2, T3, T4, and T5, ranged from 3.1 to 3.5 ms/cm, indicating high EC values.

EC reflects the total ion concentration in the compost material, i.e., the concentration of soluble salts. In pre-composting, chicken wastes, mushroom media residue (MMR), banana trunk, and chicken dung mixture were flushed using a volume of water that may eliminate the toxic effect, or soluble ions in the compost material. During composting, the increase in EC may also be due to the biodegradation of organic matter. As small organic molecules decompose and the pH increases, total ions decrease, decreasing EC (Abid & Sayadi, 2006; Geng et al., 2010).

Earthworm Population and *Bacillus subtilis* Population Count

The earthworm population was observed to increase in numbers (population) during the entire biodegradation of chicken feathers at 60 days. The absence of mortality of earthworms during the process demonstrated that a proportion of all treatments provides an optimal environment for earthworms to survive. Treatment T11 showed an 85% increase in the population and a 64% weight gain. This study indicates that the number of earthworms on day 60 for the biodegradation of chicken feathers was not significant in T6 and T9. In contrast, earthworm weight gain reached its highest in T11 but was also not significantly different from T6, T8, T11, T13, T14, and T15. In addition, this study showed a greater number of earthworms and earthworm weight gain in vermicomposting for the proportion ratio without chicken feather (6:3:0:1) and T9 (6:2:1:1). Treatment T7 (6:0:3:1) resulted in a low number of earthworm population, similar to T12 when inoculated with *B. subtilis*. The appropriateness of waste proportion for vermicomposting is evaluated based on the suitability of growth and reproduction of earthworms in compost, which contributes to the nutrient composition of the final product, namely vermicompost.

Li et al. (2023) hypothesised that phosphate-solubilising *Bacillus subtilis* (PSB) may exhibit significant adaptability to the MMR in an aerobic composting environment and improve phosphorus availability through enhanced synergy. Duan et al. (2020) investigated the effects of *B. subtilis* inoculum addition. They observed a subsequent decrease in the C/N ratio resulting from the synthesis of humic compounds in symbiotic association between plant growth-promoting bacteria (PSB) and indigenous microorganisms. Throughout the vermicomposting process, all bins' organic matter (OM) progressively declined until day 60 due to substrate utilisation as energy for microbial metabolism.

In the compost process, earthworm castings (vermicast) offer numerous advantages, including the acceleration of composting, suppression of pathogens, elimination of contaminants, and increased compost quality (Zhang & Sun, 2015). Vermicast promotes microbial growth and activity due to its high cation exchange capacity, crucial for neutralising pH during composting. However, limited knowledge refers to the influence of vermicast from agricultural waste. The population of *B. subtilis* in all treatments within the bins increased on day 60, indicating the compatibility of the degradation agents throughout the biodegradation process.

Biodegradable Rate (Kb)%

The biodegradation rate of vermicompost (Kb)% in a 15-proportion ranged from 66% to 81% (Table 2). The biodegradation rate in T6 and T9 indicated that the maximum (Kb)%, at 81%, was indicated by the action of the earthworm (*E. eugeniae*) and the *B. subtilis* agent during composting (Table 2). In T6, a portion of organic matter was without chicken feathers, but T9 showed a ratio of chicken dung that was double the amount of chicken

Table 2
Earthworm population (number of earthworms), reproduction (earthworm weight gain), Bacillus subtilis *(CFU/mL), and biodegradable rate (Kb)% of chicken feather at day 60*

Treatment	Earthworm (*Eudrilus eugeniae*)		*Bacillus subtilis*	Biodegradable rate (Kb)%
	Number of earthworms	Reproduction (weight gain)	Population X10^6	
T1	n.a	n.a	1.05X10^6	72^{cd}
T2	n.a	n.a	1.98 X10^6	68^{f}
T3	n.a	n.a	1.75 X10^6	71de
T4	n.a	n.a	5.3 X10^6	77^{b}
T5	n.a	n.a	10.5 X10^6	73^{cd}
T6	**79^{a}**	**58^{a}**	1.35X10^6	81^{a}
T7	9.4^{d}	3.9^{bc}	3.98 X10^6	70de
T8	42.9^{bc}	**27^{abd}**	1.75 X10^6	70de
T9	**60.9^{ab}**	**53^{a}**	22.3 X10^6	81^{a}
T10	21.9^{cd}	8^{bc}	5.5 X10^6	76^{b}
T11	**85^{a}**	**64^{a}**	n.a	72de
T12	11^{d}	-15^{c}	n.a	76^{bc}
T13	47^{b}	**33^{ab}**	n.a	66^{f}
T14	**62^{ab}**	**43^{ab}**	n.a	73^{cd}
T15	51^{b}	**25^{abc}**	n.a	73^{cd}

Note. According to Tukey's analysis, the mean value within a column, followed by the same letter, is not significantly different at the $p< 0.05$ level, n.a = not available

feathers, resulting in a biodegradation rate of 81%. In T6, it is not substantial due to the proportion without CF.

All treatments achieved a biodegradation rate exceeding 60% within the 60-day composting procedure. The study involved vermicomposting assessments that were not influenced by the increase in material temperature during composting, since the earthworms (*E. eugeniae*) and *B. subtilis*, as agents, would be affected by temperature elevation and produce heat. While most composting models related heat production to the biodegradation of organic matter or oxygen levels, vermicomposting, in conjunction with bacteria in the compost pile, is more critical for stabilising agents under composting conditions. Composting without *Eudrilus eugeniae* or *B. subtilis* is less successful, as shown by a biodegradation rate below 80%, indicating significant variation when both agents are present. The biodegradable rate was markedly greater in the treatment that combined the degradation agent (*Eudrilus eugeniae*) with *B. subtilis*, followed by the treatment with *B. subtilis* alone.

The dynamics in the biodegradation process were discussed when comparing the population (number of earthworms) and reproduction (weight gain) under different

proportions, revealing significant differences in the *E. eugeniae* population, which contributes to the biodegradation rate compared to the treatments by *B. subtilis* and *E. eugeniae*, respectively. These results show that *E. eugeniae* fed on the substrate mixture other than chicken feather, while adding *B. subtilis* increased the degradation of the chicken feather. Therefore, the effects of proportion without CF in substrate ratios also affected the *B. subtilis* population as well as its microbial metabolic activities.

Dehydrogenase Activity (DHA Activity)

Research has shown that enzyme-catalysed oxidation of simple carbon substrates is the initial step in the biodegradation of organic materials (Pourakbar et al., 2020). At day 60, a maximum dehydrogenase activity was recorded (38.0±1.01) U/mL in T11. The increase in dehydrogenase activity indicated greater availability of complex compounds, which enhanced microbial activity. The DHA activity, however, has been recorded at lower levels in T8 (8.84±0.36) U/mL and T15 (11.7±0.91) U/mL. Treatments T8 was the treatment with both degradation agents, while T15 was the treatment with the earthworm only.

The final biodegradation at day 60 was considerably faster in mature compost, regardless of the proportion of substrate ($p<0.05$). Data were significantly different in the treatment of single degradation agents, *Bacillus* only (19.23±4.31) U/mL introduced, followed by the treatment of earthworm and *Bacillus* (23.02±8.3) U/mL; meanwhile, DHA activity was higher using the earthworm agent only (27.1±9.3) U/mL.

Catalase Activity

The relative catalase activity was enhanced until day 60 of the biodegradation of chicken feathers. High catalase activities indicate the effective biodegradation of organic matter in compost. This study's finding of high catalase resulted in 18.6±0.66 U/mL in T13 and lower in T10 (8.33±2.34) U/mL. However, analysis of correlation in Table 3 showed no significant correlation at the 0.05 level with $r^2= 0.100$ between DHA activity and catalase enzyme activity.

Keratinase Activity

Keratinase activity observed at 60 days showed that T3 indicated the highest keratinase activity (0.15±0.20) U/mL, followed by T6 (0.11±0.10) U/mL. Keratinase activity recorded in this study is slightly different and quantifies keratinase activity higher in treatment with *B. subtilis*, followed by treatment using both agents.

In general, keratinase was able to degrade feathers. He et al. (2018) investigated the enzymes involved in the hydrolysis of various feathers by a particular type of *B. subtilis* and subsequently identified four enzymes related to keratin hydrolysis. Keratin-degrading

microorganisms in this study were derived from chicken feathers, and *Bacillus* was added to the treatment. Based on previous research, the bacteria present are reflected in the optimum pH and temperature of the keratinase activity of these microorganisms. However, the correlation analysis in Table 2 showed no significance at the 0.05 level with other enzymes and physico-chemical properties.

Physico-chemical Properties

In this study, a nitrogen compound in the 15 proportion ratios presented varied between 1.87% and 4.46%. In contrast to this study, treatment without a nitrogen source (ratio 6:0:3:1) recorded higher N% compared to the proportion with chicken dung (T12), while organic matter biodegradable recorded higher in proportion with chicken dung. However, another source of N added, such as chicken dung, in this study showed an opposite effect for N% and Kb% results. In addition, N was determined to be higher in proportion, even without chicken dung. Based on the substrate used in this study, all substrates can be considered as low nitrogen content material (Kumla et al., 2020).

The phosphorus content varied between 0.35% and 3.04% P_2O_5, and the potassium content varied between 7.81% and 22.9% K_2O_5. However, this research slightly different compared with result obtained by Phukan et al. (2013) indicated the total K_2O content varied between 0.69%, 0.73%, and 0.65%, while the P_2O_5 content varied between 1.1%, 1.51%, and 0.82% using slurry method (non-enriched and enriched) and conventional method, for 3.04% P_2O_5 nutrient content was shown in T7 vermicomposting bins, where the proportion without chicken dung recorded the highest 22.9% K_2O_5 nutrient content at T8.

However, this study's findings were concurrent with Bhat et al. (2013) findings, which contained approximately 2.47% P_2O_5 and 2.37% K_2O for both nutrient contents. Based on the trial, the higher potassium content in this research resulted from BT being added as a substrate in each bin. Additionally, Soobhany et al. (2015) also reported a higher nutrient content determined in vermicomposting than in conventional composting processes.

Many researchers concluded that vermicomposting is a recommended solution for the degradation of agricultural waste. In addition, a type of organic waste utilised as substrate in this vermicomposting affects the nutrient content of the finished products. Meanwhile, bacilli in this study affected the degradation of chicken feathers. The nutrient content in several proportions indicated the suitability of the vermicomposting method to convert a bulk of raw chicken feather waste into biofertiliser, enabling more effective agricultural waste management. Soil bacteria, earthworms, and community structure are closely related to waste degradation. The difference in the proportion of substrate also affected the diversity of the population and the nutrient content after degradation occurred. However, this finding shows that the degradation of all substrates into biofertiliser could contribute to soil quality and crop productivity.

Table 3
Correlation between enzyme activity and physico-chemical properties at day 60 of the biodegradation of chicken feather

	Dehydrogenase	**Keratinase**	**Catalase**	**N**	**P_2O_2**	**K_2O**	**pH**	**EC**
Dehydrogenase	1	r^2=0.017 p=0.897	r^2=0.100 p=0.445	r^2=0.207 p=0.112	r^2=0.108 p=0.410	**r^2=-0.504**** p<0.001	r^2=-0.227 P=0.081	r^2=-0.350* P=0.006
Keratinase		1	r^2=0.16 p=0.903	r^2=-0.015 P=0.907	r^2=-0.035 p=0.791	r^2=-0.046 p=0.726	r^2=0.194 p=0.137	r^2=-0.007 p=0.96
Catalase			1	r^2=-0.083 p=0.527	r^2=-0.220 p=0.092	r^2=-0.006 p=0.962	r^2=0.151 p=0.249	r^2=-0.193 p=0.139
N_2				1	**r^2=0.570**** P<0.001	r^2=0.205 P=1.117	**r^2=-0.716**** P<0.001	r^2=-0.017 P=0.899
P_2O_2					1	r^2=0.333** P=0.009	**r^2=-0.550**** P<0.001	r^2=-0.076 P=0.563
K_2O						1	r^2=-0.144 P=2.72	r^2=-0.079 P=0.547
pH							1	r^2=0.308* P=0.017

Note. *Correlation is significant at the 0.05 level (2-tailed)

CONCLUSION

The presence of degradation agents, such as earthworms and *Bacillus*, along with various substrates in composting, contributes to an efficient composting process based on proportion ratios. The biodegradation of chicken feathers was most effectively demonstrated at a ratio 6:2:1:1 in the presence of both earthworms and *Bacillus* degradation agents. The inoculation of *Bacillus* influenced the bacterial population succession by changing the physico-chemical parameters, particularly pH, during the biodegradation process. The nitrogen compounds (N) ranged from 1.9% to 4.4%, while the potassium (K_2O) level ranged from 7.81% to 22.9%, and the phosphorus amount varied from 0.91% to 3.03% P_2O_5 in the compost produced in this study, all of which are considered to be acceptable. The addition of chicken dung affected the N% level in chicken feather degradation. Besides, this work utilised MMR and BT derived from agricultural waste as substrates, contributing to P_2O_5 and K_2O. The optimal biodegradation observed was in the ratio of 6:2:1:1, yielding N (3%), P_2O_5 (1%), and K_2O (17%) nutrient content. Thus, this study suggested that an 80% biodegradation rate using vermicomposting might be considered effective for chicken-feather degradation with *Bacillus*. This study also recommends the addition of *B. subtilis* into chicken feather vermicomposting, when combined with mushroom media residue (MMR), might accelerate the breakdown process and enhance biomass assimilation. A specific keratinolytic microorganism or, preferably, a specific microbial keratinase could be recommended to catalyse the degradation of chicken feathers. For future application in agriculture, an abundance of chicken feathers can be utilised as a value-added ingredient in the biofertiliser industry. Overall, further studies to test compost effectiveness in various soil types or crop requirements could improve soil health and fertility. Furthermore, it will bring about innovative waste management possibilities while improving our understanding of the crop production cycle.

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