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## Changes in aerobic fermentation and microbial community structure in food waste derived from different dietary regimes

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**Abstract:** This study aimed to analyze the relationship between food components and food waste aerobic fermentation efficiency. Different food wastes were designed to be reflective of different dietary regimes, including formulated (R1), high oil/fat and salt (R2), high oil/fat and sugar (R3), and vegetarian (R4) diets, after which the physicochemical properties, enzyme activity, and structural characteristics of food waste microbial communities were examined to explore the potential mechanisms of food waste degradation under different dietary regimes. The main results of this study demonstrated that the physicochemical properties and hydrolase activity of different food waste were significantly different. The species richness in R2 and R3 food waste was higher than that of R1 and R4, whereas the community diversity of R1 and R4 food waste was higher than that of R2 and R3. At the genus level, the dominant bacteria in the four food waste types were *Bacillus*, *Thermoactinomyces*, *Paenibacillus*, and *Cohnella*.

**Keywords:** food waste; aerobic fermentation; dietary regimes; physicochemical properties; enzyme activity; microbial community

## 1. Introduction

Food waste (FW) is an important part of municipal solid waste (MSW) and includes food residues and kitchen waste. FW is mainly derived from residues in restaurants and cafeterias, as well as waste from food processing, including perishable organic wastes such as leftovers, vegetables, and peels (Thyberg and Tonjes, 2016). Relevant research indicates that approximately 1.3 billion tons of food waste are generated globally each year and the amount of food waste is expected to increase in the next 25 years (Chen et al., 2017; Petracchini et al., 2017). Food waste is a critical component of environmental sustainability and can be a valuable resource if properly processed (Conrad et al., 2018). Various treatment and disposal strategies have been developed to utilize and recycle the current abundance of food waste. For example, hydrogen, methane, lactic acid, and other substances have been obtained from food waste anaerobic digestion (Li et al., 2019). Similarly, aerobic composting has been shown to significantly decrease the weight and volume of food waste, which can ultimately be transformed into highly fertile and stable humus (Bernal et al., 2009). However, regardless of whether aerobic composting or wet or dry anaerobic fermentation are used to process food waste, the treatment effectiveness is largely dependent on the waste components. For instance, the outcomes of treating food waste via dry anaerobic and wet anaerobic processes can vary depending on the solid contents

of the waste material. Li et al. (2019) reported differences in CH<sub>4</sub> gas production during the anaerobic digestion of different food waste components. Qureshi et al. (2017) also reported differences in pectinase and lipase activity during the solid fermentation of different fruit waste. Moreover, Wang et al. (2017) revealed differences in microbial community structure and composting effects during aerobic composting, which were attributed to differences in food waste feeding ratios. Different regions are affected by varying climates and religious beliefs, thereby affecting the dietary habits of people. For instance, the vast majority of Europeans and Americans follow so-called "western diets," which are mainly characterized by higher intakes of sugar, salt, and fat (Conrad et al., 2018). Moreover, people who follow this diet typically exhibit an increased risk of certain diseases such as hypertension and diabetes, and it is estimated that 1.12 billion people worldwide will be obese by 2030 (Kastorini et al., 2011; Brigham et al., 2015). In India, approximately 35% of the population follows a strictly vegetarian diet, which has been passed down from generation to generation. Vegetarian diets are generally thought to promote health. Compared with carnivores, vegetarians have lower plasma cholesterol and lower risk of hypertension and obesity (Key et al., 2006). However, regardless of eating habits (i.e., food composition), it is still unclear whether generated food waste is easier or more difficult to process, and very few research has focused on determining the effect of eating habits on food waste treatment and management. Food waste composed of various components has its own unique problems during treatment. High oil, salt, and fat food waste has been long known to cause substantial problems during transportation and disposal, whereas vegetarian food

waste contains large amounts of cellulose and hemicellulose and therefore it cannot be easily degraded during composting and fermentation. Therefore, it is important to consider the eating habits of the population when designing food waste treatment strategies.

The biochemical degradation of food waste is composed of a series of complex biochemical reactions (Bernal et al., 2009). During the fermentation process, environmental factors and microbial abundance, diversity, and community composition of food waste are known to change significantly. Physicochemical properties are the main factors influencing the degradation of food waste. Generally, as temperature rises during the composting process, organic matter and moisture contents are significantly reduced, whereas pH gradually increases with the decomposition of nitrogenous organic matter such as protein (Bernal et al., 2009; Wang et al., 2019). A large number of studies have assessed the composting process by monitoring changes in physicochemical properties and hydrolytic enzyme activity. Chang and Hsu (2008) sought to optimize composting conditions by exploring the dynamic changes in physicochemical properties during the composting process. Awasthi et al. (2018) studied the biological mechanisms of the composting process and found that hydrolytic enzyme activity increased and then decreased during aerobic composting, which was consistent with the findings of previous studies (Awasthi et al., 2017; Qureshi et al., 2017).

Microbial agents play an important role in the aerobic fermentation of food waste. Zhang et al. (2014) added lignocellulosic decomposers at the beginning of composting,

which greatly improved the waste degradation rate. Onwosi et al. (2017) reported that the use of microbial agents can shorten the composting process and increase the density of dominant bacteria. The biomass of indigenous microorganisms is small during the early stages of composting, and thus it takes some time for these organisms to reproduce (Xi et al., 2015). Therefore, it is necessary to add a microbial compound agent to food waste prior to aerobic fermentation. Artificial addition of high-efficiency microbial compound agents can adjust the structure of the microflora and improve microbial activity, thus improving composting efficiency, shortening the fermentation cycle, and improving the quality of composting products (Li et al., 2020). Bacterial activity is closely related to the composting process, and the changes in the microbial community structure of food waste can be determined via sequencing of bacterial 16S rRNA genes. Numerous studies have demonstrated that the microbial community during aerobic composting changes in stages with temperature (Wang et al., 2017; Nakasaki et al., 2019). However, the effect of food waste composition associated with different eating habits on microbial community structures remains unclear.

Therefore, this study sought to characterize the effect of food waste composition derived from different eating habits on aerobic fermentation and microbial community structure. In this study, food waste was generated to be representative of different eating habits, including formulated (R1), high oil/fat and salt (R2), high oil/fat and sugar (R3), and vegetarian (R4) diets to identify dynamic changes in bacterial community structure and biochemical degradation mechanisms. The analysis and discussion of the correlation of physicochemical indicators, hydrolytic enzyme activity, and microbial

community changes can directly reflect the characteristics of the aerobic fermentation of food waste and the succession of microbial communities. Based on these results, the biochemical degradation of food waste under four different dietary regimes was characterized, with special attention to the ability of current fermentation strategies to improve the efficiency of food waste aerobic fermentation.

## 2. Materials and Methods

### 2.1 Materials and experimental setup

The food waste was designed to be representative of formulated diet, high oil/fat and salt, high oil/fat and sugar, and vegetarian diets, which were based on different well-characterized dietary proportion structures (Food and Agriculture Organization of the United Nations, 2016). Different ingredients were procured, cooked, and ground, after which a microbial compound agent was added. This added EM solid microbial agent (containing *Bacillus*, *Actinomycetes*, *Yeast*, *Lactobacillus*, and other microorganisms) was obtained from China Agricultural University. The properties and proportions of the feedstocks are summarized in Table 1.

The aerobic fermentation reactor was outfitted with a custom-designed thermostat for peritoneal fluid with four cuboid iron boxes inside measuring 15 cm long × 8 cm wide × 15 cm high (Fig. 1). In order to create a good aerobic state and facilitate airflow, an aeration head (1.8 L h<sup>-1</sup>) was installed at the bottom of the reactor so that oxygen could be introduced from bottom to top, which was combined with artificial stirring twice a day at fixed time intervals. Afterward, the different food waste compositions and microbial agents were fully mixed in the reactor at a 10:1 ratio to initiate aerobic

fermentation.

## **2.2 Sample collection**

The waste heap was turned and sampled regularly to ensure that the food waste remained in an aerobic fermentation state. Prior to the experiment, the food waste was allowed to ferment in natural ventilation conditions, and each sample was made of a mixture of parallel samples randomly collected from different cross-sections and depths of each reactor on days 0, 8, 13, 18, 23 and 28. The samples were then divided into three parts; one of the parts was dried in an oven at 30 °C and then used to determine the physicochemical properties of food waste. Another part was made into a crude enzyme solution to measure hydrolase activity at 24 hours. The last part was stored in a -20 °C freezer to characterize microbial community structures via 16S rRNA sequencing.

## **2.3 Analytical methods**

### **2.3.1 Chemical and enzyme analysis**

The following physicochemical properties and enzyme activity were measured during the aerobic fermentation process using the methods described above (Zhang et al., 2011; Loh and Ting, 2016): pH, temperature (T), moisture content (MC), total nitrogen content (TN), organic matter content (OMC), carbon-nitrogen ratio (C/N), and protease, amylase, lipase, and cellulase activity. All samples were analyzed in triplicate. Samples were oven-dried at 105 °C to determine moisture content (MB90 moisture meter, America). The oven-dried samples were further heated at 550 °C for 5 h for the determination of OMC. Total organic carbon content (TOC) was calculated by dividing



OMC by 1.8 (Tweib et al., 2014). TN was determined via Kjeldahl nitrogen determination. C/N was determined as the ratio of TN to TOC. pH was determined using a pH meter (pH700, Eutech, China). The temperature was measured using a five-point method, whereby thermometers were inserted around the feedstock and at the center point. Protease activity was determined using the Folin's phenol reagent method, amylase activity was measured using the starch-iodine colorimetric method, lipase activity was analyzed with the alkali colorimetric method, and cellulase activity was determined with the 3, 5-dinitrosalicylic acid method.

### **2.3.2 Microbial community analysis**

To determine the effect of different food waste compositions on microbial community structure during aerobic fermentation, five samples were periodically collected from each reactor, sample A on day 8, sample B on day 13, sample C on day 18, sample D on day 23, sample E on day 28. A1-E1 were designated as R1 (formulated diet), A2-E2 as R2 (high oil/fat and salt diet), A3-E3 as R3 (high oil/fat and sugar diet), and A4-E4 as R4 (vegetarian diet). After DNA extraction, the V4 region of the bacterial 16S rRNA genes was amplified using primers 515F (5'- GTG CCA GCM GCC GCG GTA A -3') and 806R (5'- GGA CTA CHV GGG TWT CTA AT -3'). Afterward, the amplification products were purified and submitted for sequencing (Ion S5TMXL platform; Novogene, Beijing, China). The sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using the UPARSE pipeline (version 7.0.1001). OTUs were classified taxonomically using the SILVA database (version 132). The tag sequence with the highest abundance was selected as a

representative sequence within each cluster. Between-groups Venn analysis was performed with the R programming language (version 3.4.1) to identify unique and common OTUs (Edgar et al., 2013). The alpha diversity of each sample was determined as with the “ACE,” “Chao1,” “Shannon,” and “Simpson” diversity indexes. Rarefaction curves were calculated and plotted with the R programming language to compare the bacterial OTU diversity between different samples (Zhang et al., 2019). The relationship between flora and environmental factors were analyzed with distance-based redundancy analysis (dbRDA) and Spearman correlation analysis. Taxonomic composition analysis was used to characterize microbial community structures.

All sequences derived from this study have been deposited in the National Center for Biotechnology Information Sequence Read Archive under the accession number SRP250398.

## **2.4 Statistical analysis**

Analysis of Variance (ANOVA) was conducted using the SPSS 22.0 statistical software package. Repeated measures ANOVA was used to identify significant differences between the replicate trial results. All results were deemed statistically significant when P-values were  $< 0.05$ .

## **3. Results and discussion**

### **3.1 Physicochemical properties**

Physicochemical properties are important factors affecting the aerobic fermentation process of food waste. Therefore, in this study, the time-dependent changes in

temperature, moisture content, pH, total nitrogen content, and organic matter content of different food waste were measured.

Temperature (T) is an important factor that affects the aerobic fermentation rate and microbial physiological activity of food waste. Excessively high temperatures can lead to widespread microbial death and serious effects on the hydrolase system. Conversely, if the temperature is too low, the growth and metabolic capacity of microorganisms will be inhibited, thus affecting the food waste fermentation process (Liu et al., 2017). Fig. 2a illustrates the time-dependent temperature changes during the aerobic fermentation process of the four food waste groups. From day 0 to day 8, all groups exhibited the same increasing temperature trend, whereby the temperature increased from 29.10 °C to the maximum value of 44.77 °C. In the early stage of the reaction, the growth and metabolism of microorganisms were vigorous and therefore the organic matter was consumed and utilized by microorganisms and a large amount of heat was released (Xi et al., 2015). After the temperature reached 44.5 °C, a stable state was maintained. At this time, the temperature of each reactor had no significant change, and the floating range was between 42.5 °C and 44.5 °C. No significant differences were observed between R2 and R3 on days 23 and 28 ( $P = 0.608$ ;  $P = 0.256$ ), which may indicate that the composition of R2 and R3 had no significant effect on temperature during the late fermentation period. Between days 18 and 28, the temperature of R1 was always the highest. This may be because compared with R2, R3, and R4, R1 contained more proteinaceous organic matter, which was more readily degraded by microorganisms, thus producing more heat to maintain the temperature of the system.

During the composting process, the moisture content (MC) not only affects the metabolism of microorganisms but also determines the food waste aerobic fermentation efficiency. When the moisture content is too high (i.e., > 70%), it is difficult to maintain the air circulation inside the reactor, and food waste will also tend to produce acid decay gas due to anaerobic fermentation. In contrast, excessively low moisture contents inhibit microbial activity. Fig. 2b illustrates the time-dependent moisture content changes during the aerobic fermentation process of the four food waste groups. Between days 0 and 13, the moisture content of all four experimental groups exhibited a rapidly decreasing trend from 57.40%, 44.27%, 53.69%, and 59.27% to the lowest values of 24.43%, 20.33%, 20.87% and 26.73%, respectively. This was likely because the internal material temperature continued to rise, as well as ventilation and artificial agitation, and therefore the water evaporation rate was greater than the amount of water that was generated by the microbial decomposition of organic matter. On day 13, the moisture content of R2 and R3 was 20.33% and 20.87%, respectively, which was significantly lower than that of the other two experimental groups ( $P < 0.05$ ) given that their moisture content was affected by the fat and oil content in the system. Relevant studies have shown that fat and oil can inhibit the composting process (Chang and Hsu, 2008; Maliki and Lai., 2011). This could be because the metabolic enzyme activity of microorganisms is affected by high fat/oil content and therefore relatively less water is produced. On day 13 of fermentation, R2 and R3 exhibited visible lumps, and the moisture content of R1 and R4 also decreased to approximately 25%. When the water content is too low (i.e., < 15%), microbial activity comes to a halt (Ahn et al., 2008).

Therefore, after day 13, small amounts of distilled water (75 mL) were repeatedly added to each of the four reaction chambers to improve the moisture content and ensure a smooth food waste aerobic fermentation process. At the same time, bacterial metabolism became active, and microbial oxidative decomposition of organic matter also produced substantial amounts of water. During the fermentation process, the moisture content of R4 was significantly higher than that of the other groups ( $P < 0.05$ ), this was attributed to the higher water content of vegetables, which comprised a large proportion of R4.

pH is another key factor that affects the aerobic fermentation efficiency of food waste and is therefore a significant indicator to monitor during food waste fermentation. The pH variations of the food waste with different properties were relatively consistent within 28 days. It can be observed from the figure that the pH gradually increased, and the initial pH was generally between 3.90 and 3.96 (Fig. 2c). When  $\text{pH} < 6.0$ , acidophilic microorganism activity was strong, which could easily degrade readily fermentable organic materials in the system into small organic matter molecules. This generated organic acids and released substantial amounts of heat, which made it difficult for the pH of the food waste to rise. With the rise in temperature and the continuation of the aerobic fermentation process, organic acid gradually volatilized and organic nitrogen in the materials gradually decomposed under the action of microorganisms, which entered into the system in the form of ammonia nitrogen. Consequently, pH values gradually increased (Zhang et al., 2017). The pH of R4 was the highest during the composting process, whereas the pH of R2 and R3 rose relatively

slowly and exhibited no significant differences ( $P = 1.000$ ). This was because R4 was mainly composed of vegetables, which was consistent with a relevant study that found composting vegetable waste maintained high pH values (Liu et al., 2017; Ghinea and Leahu., 2020). Moreover, R2 and R3 contained higher fat and oil, which led to the production of fatty acids in the system. Chang and Hsu (2008) concluded that the formation of fatty acids after the hydrolysis of fat was the key mechanism of system acidification. Therefore, in the process of aerobic fermentation, changes in pH were the result of mutual transformation between different substances.

Nitrogen is an important nutrient element in the aerobic fermentation process of food waste. Fig. 2d shows the time-dependent changes in the total nitrogen content of food waste. Between days 0 and 13, the TN contents of R1, R2, and R3 were higher than that of R4, this was because R4 contained a minimal protein content. There were no significant differences in the TN changes between R2 and R3 during the early and late stages of fermentation ( $P = 0.771$ ;  $P = 0.158$ ), which may be caused by the similar composition of R2 and R3. In the process of fermentation, nitrogen-containing organic matter such as protein was decomposed via microbial activity, and a large amount of ammonium nitrogen was produced, which made the total nitrogen content begin to rise in the initial stage of the reaction (Wang et al., 2016). With the gradual volatilization of ammonium nitrogen from the material, the pH began to rise, and protease activity began to increase, while the total nitrogen content in the material decreased accordingly (Zhang et al., 2017). On day 18, the TN of the four groups (R1- R4) was 0.98%, 0.62%, 0.81%, and 0.77%, respectively (Fig. 2d). Among them, the total nitrogen of R1, R2,

and R4 showed an increasing trend. This was because during the later stages of aerobic fermentation, the mineralization and decomposition of a large number of organic substances led a reduction in dry matter, and a decrease in nitrogen loss results in relatively high TN concentrations, thus leading to a gradual increase in TN content (Bernal et al., 2009). These results were consistent with the observations of previous studies (Tweib et al., 2014; Ghinea and Leahu, 2020). In contrast, the TN content in R3 exhibited a decreasing trend. This TN decrease in R3 may be related to the changes in the microbial community structure. Relevant studies identified *Paenibacillus* as a nitrogen-fixing bacterium during the composting process, and decreases in their abundance may result in TN decreases (Attar, 2018; Zhang et al., 2019).

*Paenibacillus* abundance in R3 exhibited a decreasing trend in the late stage of fermentation, and was lower than that of the other three groups (Fig. 5b). Therefore, the TN content of R3 exhibited a decreasing trend on day 18. Except for the 13th day, the TN content of R1 was the highest. Notably, the protein content of R1 was higher than that of the other groups, and its organic matter degradation rate was also the fastest, which resulted in an overall decrease in carbon to nitrogen ratio coupled with an increased total nitrogen content (Bernal et al., 2009). It can be said that the change in TN resulted from the concentration effect caused by ammonia volatilization and organic matter mineralization. Based on the total nitrogen results, the formulated diet (R1) food waste nitrogen content was the largest, which indirectly indicated that R1 waste contained more nutrients to sustain microorganism growth and reproduction.

Organic matter content (OMC) is an important indicator of compost maturity, and

its dynamic changes can directly reflect the aerobic fermentation process (Wang et al., 2019). During the entire fermentation process, the organic matter content of the four experimental groups declined slowly, from 80.40%, 79.18%, 79.20%, and 77.71% to 71.20%, 70.60%, 71.18%, and 72.66%, respectively (Fig. 2e). This may be due to the carrier of the compound microbial agent that was used (i.e., mainly wheat straw), which had a complex structure and more insoluble cellulose, which is difficult to be degraded by microorganisms and therefore the organic matter degradation of food waste was not enough to cause the organic matter in the waste heap to change (Wang et al., 2016). A microbial agent containing rice bran was added to citrus peel compost, after which the organic matter decline rate was only 10% after 35 days of municipal solid waste composting (Wang et al., 2019). Another study used cornstalk as a bulking agent in co-composting sewage sludge and municipal solid waste, which resulted in approximately 8.5% decreases in organic matter after composting (Zhang et al., 2017). During early fermentation stages, the water and nutrient content of the reactor remained sufficient as the system temperature increased, and the growth and metabolism of thermophilic microorganisms were vigorous. Easily degradable organic matter was consumed and decomposed first and thus the organic matter content began to decline and hydrolase activity began to increase. The organic matter content of R1 decreased the fastest (i.e., by 9.20%), followed by that of R2 and R3, which decreased by 8.57% and 8.01%. This was likely because the fat content of these materials was more difficult to degrade than protein and sugar. Compared with R1, R2, and R3, there were statistically significant differences in the OMC changes of R4 in the early and late



fermentation periods ( $P < 0.05$ ). The organic matter content in vegetarian waste declined the least. This was because these waste contained more cellulose, which is one of the most difficult food waste compounds to degrade (Zhang et al., 2014). From an organic matter degradation standpoint, the formulated diet (R1) food waste was the most favorable. Therefore, it is reasonable to speculate that the composting process of the formulated diet food waste was relatively faster than that of the other dietary regimes.

The C/N ratio is an important indicator of food waste compost maturity. Fig. 2f shows the time-dependent changes in the C/N ratio of food waste. From days 0 to 28, the C/N ratio of the four food waste groups changed from 35.78, 43.19, 43.60, and 98.39 to 31.74, 53.25, 56.80, and 44.85, respectively. Related studies have found that the C/N ratio in the composting process of food waste generally decreases from an initial value of approximately 35 to below 25 (Wang et al., 2016; Wang et al., 2017). In contrast, the C/N ratio in this study was generally high, which may be caused by differences in the detection methods. The aforementioned studies used a CNS elemental analyzer to determine C/N ratios, whereas the present study determined C/N ratios by calculating the ratio of TN to TOC. TN content values in the present study were low because the total nitrogen determined by the Kjeldahl method does not account for the nitrate value, and therefore the C/N ratio of the present study was higher (Zhang et al., 2011). The C/N ratio of R1 decreased from the beginning to the end of the experiment during the aerobic fermentation process, whereas the C/N ratio of R2 and R3 increased significantly. This indicated that the aerobic fermentation process of R2 and R3 was

affected by its components, which led to pronounced C/N ratio changes. This may have been because the microbial community structure of high oil/fat, high sugar, and high salt food waste was different from that of the formulated diet food waste. High C/N ratios are closely related to changes in microbial community structure, which was supported by the microbial community composition data determined herein (Fig. 5a). The *Thermoactinomyces* and *Bacillus* abundance of R2 and R3 was higher than that of R1. Zhang et al. (2020) found that *Thermoactinomyces*, *Bacillus*, and *Pseudomonas* maintained high bacterial abundance in compost with high C/N ratios. The C/N ratio of R4 showed a significant decrease compared to the initial stage of aerobic fermentation, which may be due to the relatively low total nitrogen content and higher organic matter content of R4 during aerobic fermentation.

### 3.2 Hydrolase activity

Hydrolase activity is an important indicator for food waste aerobic fermentation monitoring. Changes in substrate hydrolase activity during different fermentation stages can reveal the process of mineralization and decomposition of organic matter in food waste from a microbial structure standpoint (Awasthi et al., 2017). During the early stage of fermentation, the abundance of fermentable materials (i.e., substrates) and the large number of reproducing microorganisms translates to a large concentration of hydrolytic enzymes that could degrade organic substances, which could effectively degrade complex macromolecular organic matters in food waste. During the post-fermentation period, the microorganisms were restricted by the internal environmental factors of the system, their metabolic activities began to decelerate, and

the activity of substrate degrading enzymes began to decline, which indicated that the whole aerobic fermentation process was coming to an end.

Variations in hydrolase (protease, amylase, lipase, cellulase) activity were examined to identify microbial dynamics for various organic waste degradation processes. In this study, the activity of protease, amylase, and lipase increased first and then decreased, whereas the activity of cellulase remained at a very low level. Moreover, significant differences were observed in the variation of the activity of the four hydrolases examined herein between different food waste compositions ( $P < 0.05$ ), which indicated that the enzyme activity characteristics of each food composition were significantly different, which was closely linked to the different microbial flora characteristics associated with food compositions.

Fig. 3a illustrates the time-dependent protease activity changes during the aerobic fermentation of food waste. The protease activity of the four food waste groups increased at first and then decreased. The protease activity of the R4 reached a maximum value of 70.05 U on day 13. After 18 days, the enzyme activity of R1, R2, and R3 reached their maximum values of 116.70, 100.06, and 104.67 U, respectively. However, during this period, there were no significant differences in the protease activity of R2 and R3 ( $P = 0.459$ ;  $P = 0.053$ ), which may be because the salt and sugar contents in the food waste examined herein did not significantly affect microbial protease secretion. A high protease activity indicates that the microorganisms consume and degrade the protein organics quickly. During the early stage of fermentation, with the increase of material temperature, the content of protein organic matter in the reactor

was sufficient, and the microbial metabolism and growth capacity was strong, thus secreting a large number of protease substances. Due to the high protein content in R1, R2, and R3, the protease activity in the system was higher than the protease activity in R4. In the middle and late stages of fermentation, the metabolic activity of microorganisms gradually decreased, and the protease activity of the four experimental groups also decreased after reaching a peak.

Fig. 3b illustrates the time-dependent amylase activity changes during the aerobic fermentation of food waste. The amylase activity of the four groups increased at first and then decreased. The amylase activity of R1 and R3 reached its maximum value on the 8th day at 733.30 and 666.67 U, respectively. On day 13, the amylase activity of R2 and R4 reached its maximum value at 400.00 and 533.33 U, respectively. During aerobic fermentation, starch and saccharides were first consumed and decomposed by microorganisms when the materials contained a substantial proportion of rice, which could provide sufficient energy for amylase-secreting microorganisms. Therefore, amylase activity in the four food waste groups remained at a high level. Moreover, oil and salt are known to inhibit the fermentation process, which in turn can affect the hydrolase activity (Chang and Hsu, 2008; Chen et al., 2008; Maliki and Lai., 2011). Therefore, the amylase activity of R1, R3, and R4 food waste was higher than R2. Additionally, there were significant differences in the amylase activities of R2 and R3 during the fermentation period, which may be due to the high salt content in R2 affecting the amylase activities in the system. These results indicated that the effect of sugar on amylase activity is less than that of salt.

Fig. 3c illustrates the time-dependent changes in food waste lipase activity. During aerobic fermentation, there was no significant difference in lipase activity between R2 and R3 ( $P = 0.730$ ). This could be due to the similar oil composition in R2 and R3. During the fermentation process, the lipase activity of the four food waste groups increased at first and then decreased. Due to the low fatty acid content in the materials, fewer nutrients were required to induce lipase secretion from microorganisms. Therefore, the lipase activity in the four experimental groups remained low. The lipase activity of R2, R3, and R4 reached the maximum values of 4.13, 6.03, and 1.55 U on day 13, respectively, whereas the lipase activity of R1 reached its maximum value of 4.10 U on day 18. Oil and fat are known to be more difficult to decompose than carbohydrates and proteins (Chang and Hsu, 2008). The starch and protein organic contents of R1 were more abundant than in the other treatments. Such organics were used first in the process of organic degradation, whereas small amounts of fatty substances that were initially difficult to degrade were consumed and decomposed later, and therefore the maximum lipase activity of R1 manifested later than in the other three experimental groups.

Fig. 3d illustrates the time-dependent changes in food waste cellulase activity. Notably, the changes in cellulase activity in the four experimental groups remained largely constant throughout the entire process, and the enzyme activity remained low. The maximum cellulase activity of the R1, R2, R3, and R4 groups was 0.01, 0.01, 0.0089, and 0.130 U, respectively. This was because the cellulase was prone to denature and inactivate under strongly acidic conditions, and the optimal pH for cellulase

produced by common fungi ranges from neutral to slightly alkaline. Moreover, the cellulose degradation process is much more complex than that of other organic materials such as sugars and proteins (Awasthi et al., 2017). The changes in cellulase activity were consistent with the changes in pH value of food waste in the four groups (Fig. 2c). On day 23, the pH value of R1 and R4 increased slightly and, at the same time, cellulase activity also showed a slowly increasing trend (Fig. 3d). On the other hand, the composite microbial agent carrier used in the experiment was mainly wheat straw, which contains substantial amounts of refractory cellulose, and therefore the cellulase activity of the four food waste groups remained low.

Overall, the amylase and protease activity of R1 was higher than that of other treatments due to its primary protein and starch composition. From R2 and R3 hydrolase activity results, the influence of salt and sugar on amylase activity was significant, but exerted no significant effects on protease activity. Moreover, the cellulase activity of R4 was higher than that of the other three treatments.

### **3.3 Microbial community structure**

#### **3.3.1 OTUs clustering analysis**

The total number of OTUs and the number of shared OTUs among the four food waste groups (R1, R2, R3, and R4) and OTU changes within each group can be easily observed from the Venn diagram (Fig. 4). Notably, it can be observed that 6,446 OTUs were commonly shared between R1, R2, R3, and R4, which accounted for a significant proportion of the total OTUs. Given that the system was small and the temperature and

pH changes did not vary considerably throughout the entire reaction, the overall community changes remained relatively consistent. These results indicated that the number of OTUs in the aerobic fermentation process of the four food waste groups was similar. However, the number of unique OTUs in the R3 group was the largest (i.e., 21). The independent OTUs of R3 decreased sharply compared with the other three groups, indicating that the material properties in R3 had a great influence on community composition (Fig. 4c). All of these observations suggest that the microbial diversity in the R3 group was relatively higher than that in the R1, R2, and R4 groups. According to the lipase activity results (Section 3.2), the effect of salt on lipase activity was greater than that of sugar, whereas the OTU cluster analysis results demonstrated that the effect of sugar on community composition was greater than that of salt. Some studies have reported that both sugar and salt can affect microbial activity. For instance, Li et al. (2019) investigated the effect of salt content on the anaerobic fermentation of food waste and reported that high salt content could inhibit the metabolic activity of microorganisms. Moreover, Medeiros et al. (2006) found that fungi abundance would increase in response to high sugar content. Therefore, both salt and sugar contents have different effects on the physiological and biochemical indexes of microorganisms. The adverse effect of salt on physicochemical properties such as water content and pH is more significant than that of sugar (Li et al., 2019). Therefore, different food waste components in the waste disposal process are worth further discussion. R1 exhibited the highest number of shared OTUs, showing that this diet had the least effects on food waste-associated community changes.

### 3.3.2 Microbial community diversity

To investigate the diversity of microbial communities during the aerobic fermentation of different food waste compositions, the alpha diversity analysis index (Shannon, Simpson, Chao1, ACE) of different samples at a 97% consistency threshold was statistically analyzed. The Chao1 and ACE indices can be used to calculate community richness while the Shannon and Simpson indices are usually used to reflect the diversity of microbial communities. The Chao1 of R2 and R3 were 258.73 and 272.21, respectively, and the ACE indices of R2 and R3 were 269.69 and 281.42, respectively (Table 2), which were all higher than those of the R1 and R4 groups. In contrast, the Shannon and Simpson indices of the R1 and R4 groups were higher than those of R2 and R3. All of these data indicated that the species richness of R2 and R3 was higher than that of R1 and R4, whereas the community diversity of R1 and R4 was higher than that of R2 and R3. Generally, more astringent environmental conditions lead to a simpler community structure (Vasconcelos et al., 2016). After the elimination of various adverse environmental factors, only microorganisms with a strong tolerance can survive. In these cases, the growth of a single species is typically more vigorous, thus resulting in a simpler whole-community diversity. Kitamura et al. (2015) found that a food waste community contained a large number of *Firmicutes*, which inhibited the growth of other bacteria, thereby reducing community diversity. Despite oxygen limitations, Partanen et al. (2010) reported high temperatures during the high-temperature composting stage, which was coupled with a low species richness, where *Actinomycetes* and *Thermoactinomyces* were predominant.



### 3.3.3 Taxonomic composition analysis

Fig. 5 illustrates the genus-level distribution of the top 30 microbial communities in the four food waste groups. The dominant bacteria in the food waste aerobic fermentation process included *Bacillus*, *Thermoactinomyces*, *Paenibacillus*, *Cohnella*, and others. All of these are known to secrete hydrolase during the fermentation process, thereby effectively promoting the decomposition and transformation of food waste organic matter (Ke et al., 2010; Rai et al., 2010; Wushke et al., 2013; Msarah et al., 2020). The abundance of *Bacillus* was the highest in all the dietary groups examined (i.e., 37.60, 48.24, 40.55, and 40.08%, respectively) (Fig. 5a). Upon further analysis of the *Bacillus* classification level, it was found that the main *Bacillus* species of the four food waste groups was *Bacillus coagulans*, with an abundance of more than 20%. Related studies have shown that *Bacillus coagulans* is highly acid-resistant and can decompose sugars to produce L-lactic acid at 45~50 °C. Moreover, it can also secrete large amounts of protease and amylase during the fermentation process to promote the decomposition and transformation of macromolecular organic matter (Msarah et al., 2020). The relative abundance of *Thermoactinomyces* in the four food waste groups was 18.69, 34.45, 27.56, and 23.83%, respectively. These organisms exhibit heat resistance and are known to survive in harsh environments, metabolizing at 45 °C and producing a variety of heat-resistant enzymes, such as amylase, serine, protease, and lipase (Ke et al., 2010). The presence of these bacteria can effectively promote the aerobic fermentation process of food waste, which was consistent with the aforementioned results that determined that the enzyme activity remained high during the aerobic fermentation of

food waste. Additionally, the relative abundance of *Paenibacillus* in the R1 group was 11.73%, which was higher than that of the R2 and R3 groups. *Paenibacillus* is able to secrete more proteases (Rai et al., 2010), which is consistent with the previously reported high protease activity in food waste from formulated diets. At the genus level, there were a large number of *Bacillus* and *Thermoactinomyces* in R2 and R3, which accounted for more than 70% of the total bacterial flora, this was more than in R1 and R4, indicating that these bacteria may have a certain preference for oil/fat, salt, sugar, and other food waste. Among these classifications, *Thermoactinomyces* are known to be heat-resistant and quite suitable for survival in adverse environmental conditions, which might have inhibited the growth of other bacteria, thereby decreasing community diversity (Ke et al., 2010; Kitamura et al., 2015). Specifically, the relative abundance of *Cohnella* in R4 was the highest, at 4.91%. This bacterium played an important role in degrading cellulosic substances, which increased cellulase activity during the later stage of fermentation (Wushke et al., 2013).

Fig. 5b illustrates the intra-group abundance of species at the genus level during the aerobic fermentation of different food waste. Although the food waste components were different, four types of bacteria (*Bacillus*, *Thermoactinomyces*, *Paenibacillus*, and *Cohnella*) occupied a large proportion in almost every fermentation period. These bacteria also showed a tendency to increase first and then decrease during the fermentation process. Such results indirectly revealed the dynamic biochemical degradation process of food waste, which was consistent with the above-mentioned changes in hydrolase activity.

### 3.3.4 Environmental factors correlation analysis

Distance-based redundancy analysis (dbRDA) is typically used to reflect the relationship between the quantitative characteristics and environmental factors of different sample species compositions (Warton et al., 2012). In this study, dbRDA was used to reveal the relationship between microbial community structure composition and environmental factors during food waste aerobic fermentation. dbRDA1 and dbRDA2 explained 48.90% and 29.72% of the total variation, respectively (Fig. 6a). The length of the arrow line represents the correlation between pH, T, M (moisture content), and community distribution, and the angle between the arrow line and the sorting axis reflects the correlation between environmental factors and the sequencing axis. Longer lengths indicate a stronger correlation. When the angle between environmental factors is smaller, this demonstrates a positive correlation between the two. It can be observed that pH was negatively correlated with T, M ( $P < 0.05$ ), and there is a significant correlation between T, pH, M and community distribution (Fig. 6a). Such results were consistent with a previous study (Zhang et al., 2011; Awasthi et al., 2018; Sun et al., 2020). Moreover, there was a significant positive correlation between pH and community distribution in R1 and R4, indicating that R1 and R4 microbial communities were more sensitive to pH variation than R2 and R3. Moreover, R2 and R3 were also insensitive to T and M due to their characteristically high oil/fat and salt/sugar content. Unlike pH, the influence of T on different food waste community changes was not significant. Given that the experimental temperature was largely maintained at approximately 45 °C, the dominant bacteria of different food waste did not change

significantly.

Spearman rank correlation is mainly used to demonstrate the relationship between environmental factors and the abundance of microbial species during aerobic fermentation. Correlation and significance P values between pairs were obtained to explore the relationship between environmental factors and species. There was a certain correlation between three environmental factors (T, pH, and M) at the genus level for a total of 35 bacterial species (Fig. 6b). Moreover, temperature had a positive correlation with a total of 28 bacterial species, whereas pH and moisture content had a negative correlation with most bacteria. *Brevibacillus* had a significant positive correlation with temperature, which was consistent with literature that reported that *Brevibacillus* has the ability to degrade cellulose at high temperatures (Maeda et al., 2011). The four bacteria (*Treponema*, *Truepera*, *Bellilinea*, and *Azotobacter*) exhibited a significant inverse relationship with pH. Among them, *Bellilinea* is known to be able to use a variety of carbohydrates as a carbon source in acidic and anaerobic environments (Yamada et al., 2007). *Alcaligenes* had a significant positive correlation with pH and moisture content ( $P < 0.05$ ), which was consistent with the fact that *Alcaligenes* cannot easily survive in low pH and high salinity environments (Kim et al., 2019)

### 3.4 Evaluation and suggestions

This study characterized the biochemical degradation of food waste under four different dietary regimes. The formulated diet food waste exhibited a rapid decline in organic matter and a high hydrolytic enzyme activity, whereas the high oil/fat and salt food waste exhibited low pH, and its high salt content affected hydrolase activity during

aerobic fermentation. Compared with salt, the negative impact of sugar on physicochemical properties was much smaller. Therefore, future studies should focus on the removal of oil/fat and salt from food waste during the composting process.

Vegetarian waste had relatively stable physicochemical properties and enzyme activity during aerobic fermentation. However, the negative effects of high water content and the slow degradation of organic matter should be considered in vegetarian waste composting processes.

Current laboratory-scale study results suggest that improving diet quality was an important factor that affected food waste aerobic composting. Therefore, diet quality should be improved by avoiding high oil/fat, high sugar, and high salt diets. At the same time, the above-described information can help individuals and organizations to implement in-situ food waste treatment as much as possible. For instance, oil and salt in waste should be pre-filtered or removed during the aerobic fermentation process of household food waste to facilitate food waste transportation and centralized treatment.

#### 4. Conclusions

In the process of aerobic fermentation, the physicochemical properties and hydrolase activity of different food waste were found to be significantly different. The dominant bacteria of the four food waste were *Bacillus*, *Thermoactinomyces*, *Paenibacillus*, and *Cohnella*. The species richness of high oil/fat and salt and high oil/fat and sugar food waste was higher than formulated and vegetarian diet waste, whereas the community diversity of formulated and vegetarian diet food waste were

higher than that of high oil/fat and salt and high oil/fat and sugar waste. Taken together, these results are conducive to improving the fermentation efficiency of food waste compost.

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**Figure captions:**

Fig. 1 Food waste aerobic fermentation reactor

Fig.2. Time-dependent changes in physicochemical properties (T, MC, pH, TN, OMC, C/N) during the aerobic fermentation of the four food waste groups examined herein.

Fig.3. Changes in hydrolytic enzyme (protease, amylase, lipase, and cellulase) activity over time during the aerobic fermentation of the four food waste groups examined herein.

Fig.4. (a) OTU changes within the R1 group. (b) OTU changes within the R2 group. (c) OTU changes within the R3 group. (d) OTU changes within the R4 group. (e) Total number of OTUs and number of shared OTUs between the four food waste groups.

Fig. 5. Relative inter-group (a) and intra-group (b) species abundance at the genus level during the aerobic fermentation of different food waste (samples A1, B1, C1, D1, E1 correspond to the R1 group; samples A2, B2, C2, D2, E2 correspond to the R2 group; samples A3, B3, C3, D3, E3 correspond to the R3 group; samples A4, B4, C4, D4, E4 correspond to the R4 group).

Fig. 6 (a) dbRDA analysis of the relationship between distances based on flora and environmental factors. (b) Spearman correlation analysis of the relationship between specific genera and environmental factors.

Table 1 Properties and proportions of the feedstocks

	Formulated diet	High oil/fat and salt	High oil/fat and sugar	Vegetarian diet
Rice	363 g	363 g	363 g	363 g
Lean meat	100 g	0 g	0 g	0 g
Fat meat	0 g	100 g	100 g	0 g
Cabbage	250 g	250 g	250 g	250 g
Celery	0 g	0 g	0 g	100 g
Spice powder	0.35 g	0.35 g	0.35 g	0.35 g
Salt	3 g	12 g	3 g	3 g
Sugar	3 g	3 g	12 g	3 g
Water	150 mL	150 mL	150 mL	150 mL
Oil	3 mL	6 mL	6 mL	3 mL
Microbial agent	80 g	80 g	80 g	80 g

Table 2 Alpha diversity indexes of each group

Groups	Community diversity		Community richness	
	Shannon	Simpson	Chao1	ACE
R1	4.303	0.884	224.619	229.625
R2	3.879	0.859	258.734	269.689
R3	3.819	0.811	272.214	281.424
R4	4.351	0.902	251.246	248.896

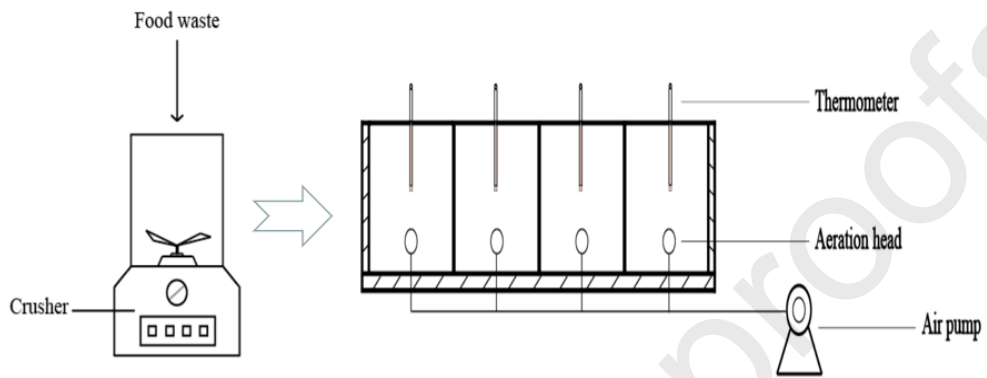


Fig. 1 Food waste aerobic fermentation reactor



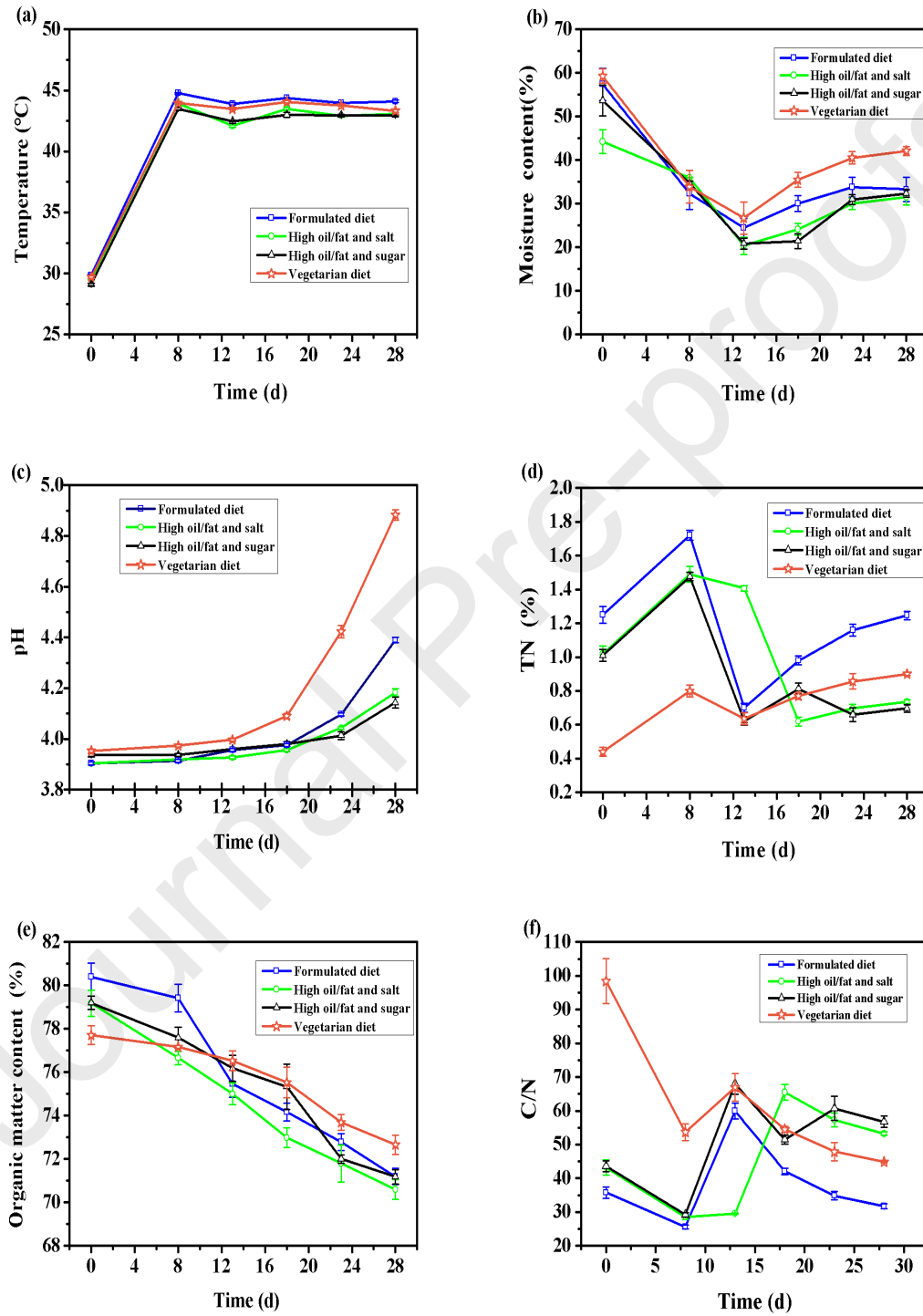


Fig.2. Time-dependent changes in physicochemical properties (T, MC, pH, TN, OMC,

C/N) during the aerobic fermentation of the four food waste groups examined herein.

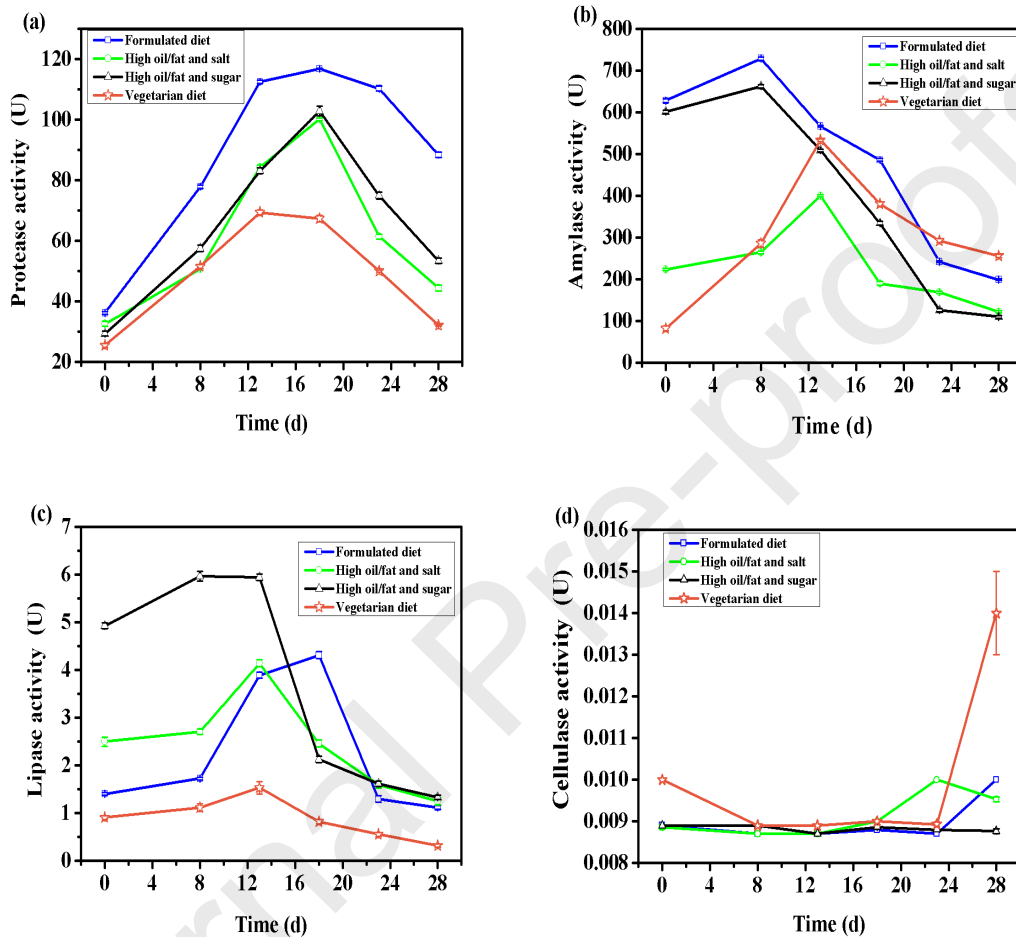


Fig.3. Changes in hydrolytic enzyme (protease, amylase, lipase, and cellulase) activity over time during the aerobic fermentation of the four food waste groups examined herein.

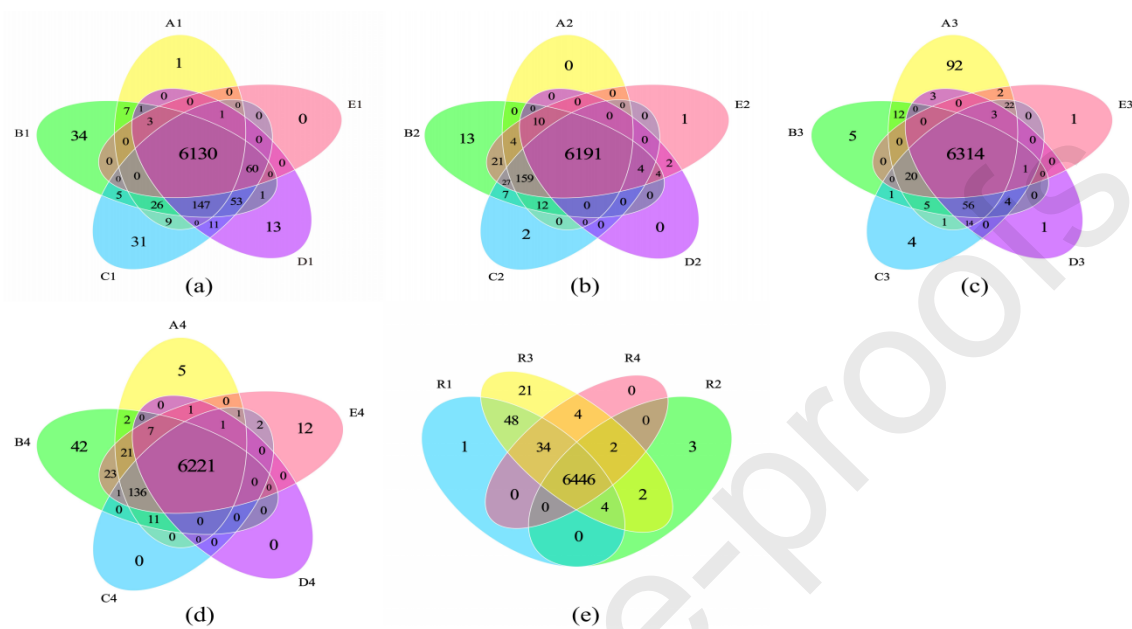


Fig.4. (a) OTU changes within the R1 group. (b) OTU changes within the R2 group. (c) OTU changes within the R3 group. (d) OTU changes within the R4 group. (e) Total number of OTUs and number of shared OTUs between the four food waste groups.

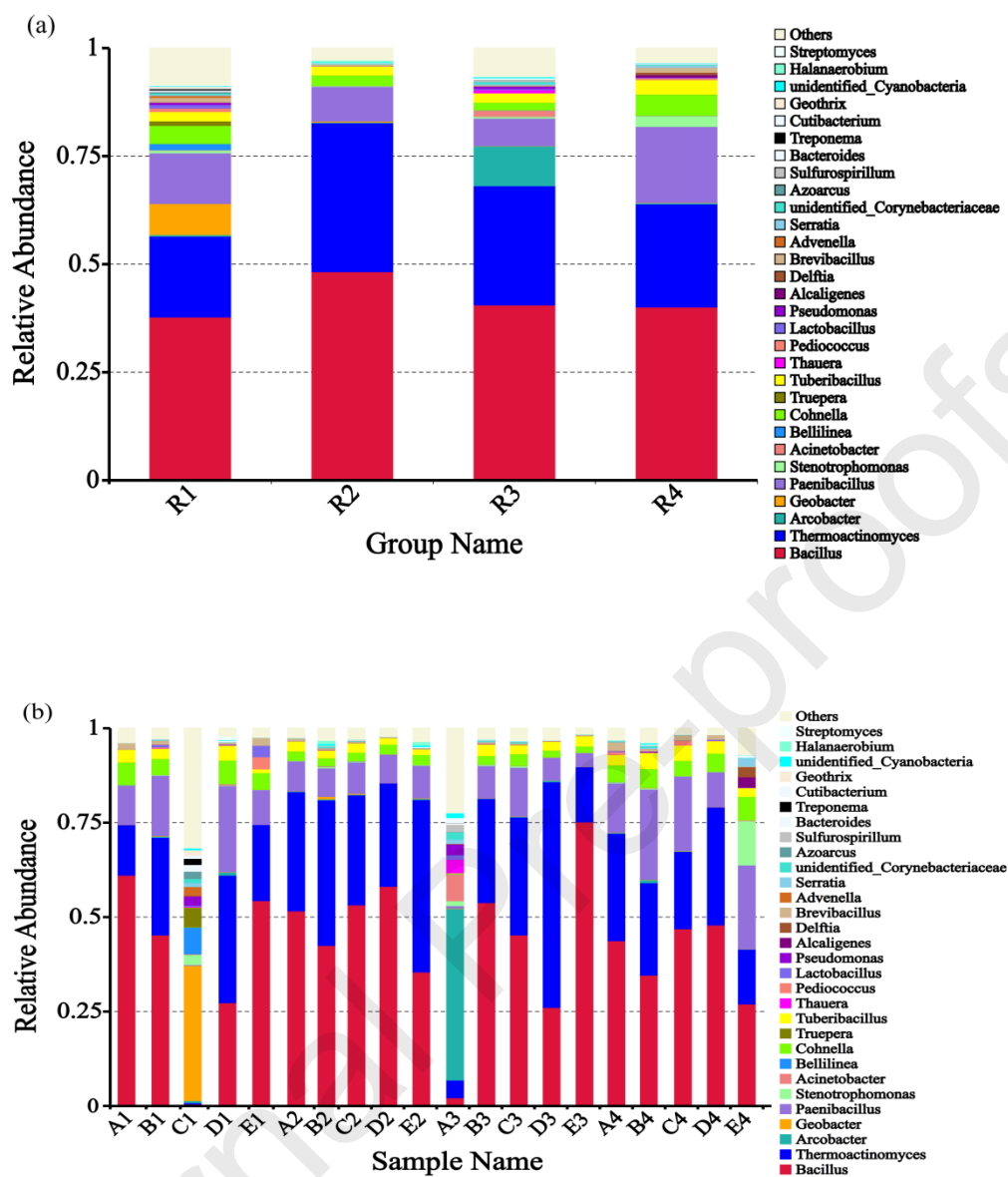


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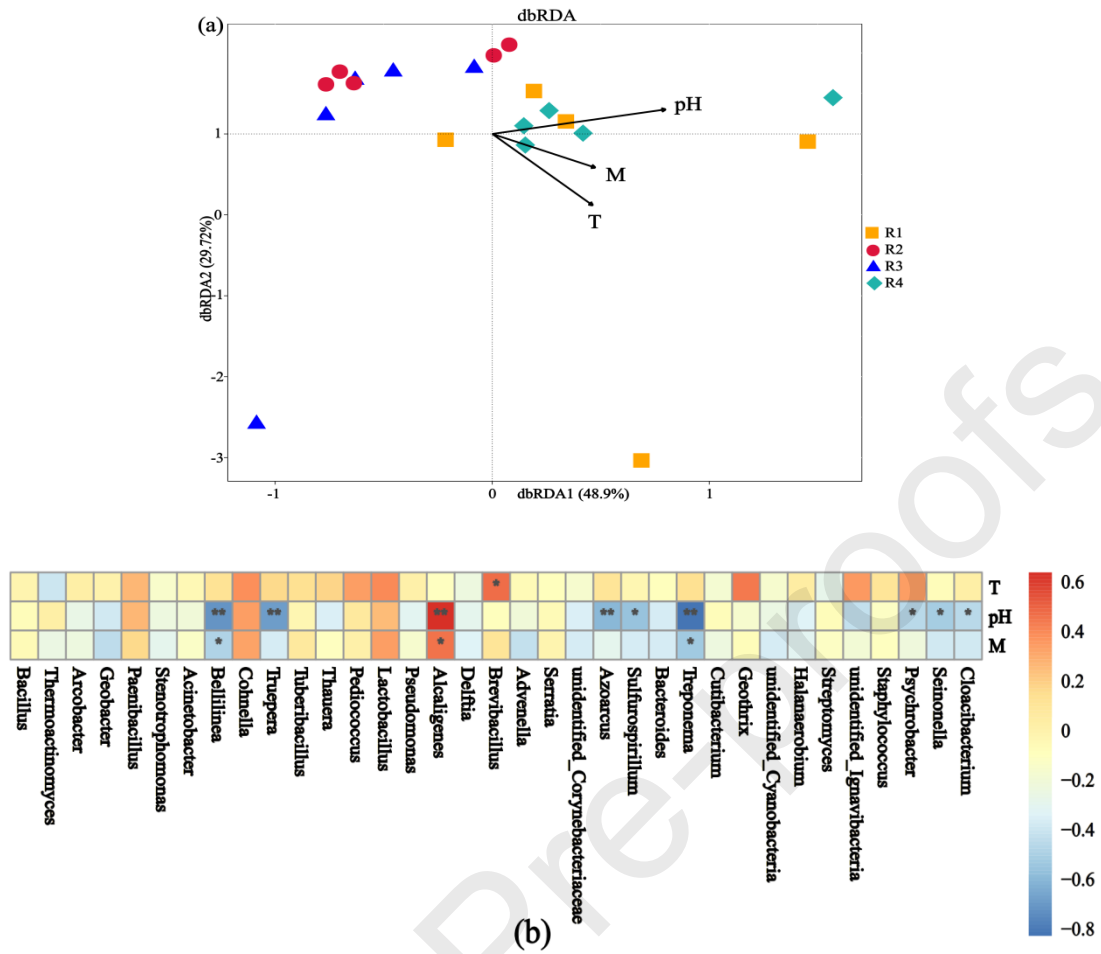


Fig. 6 (a) dbRDA analysis of the relationship between distances based on flora and environmental factors. (b) Spearman correlation analysis of the relationship between specific genera and environmental factors.

## **CRedit Authorship Contribution Statement**

**Yanzeng Li:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology,

Writing - original draft, Writing - review & editing.

**Zhou Chen:** Data curation, Formal analysis, Investigation, Methodology, Writing - review & editing.

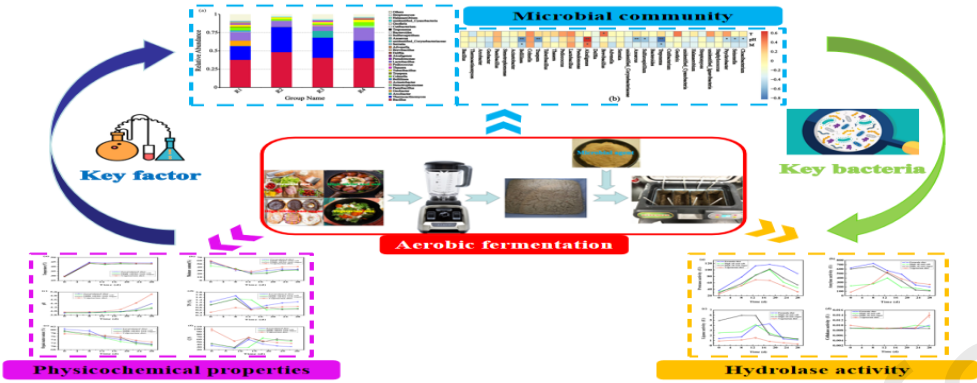
**Yanyan Peng:** Formal analysis, Investigation, Methodology.

**Kaiming Zheng:** Data curation, Formal analysis.

**Chengsong Ye:** Data curation, Methodology.

**Kun Wan:** Formal analysis, Methodology.

**Shenghua Zhang\*:** Funding acquisition, Writing - review & editing, Supervision, Review.



**Highlights:**

- Biochemical degradation mechanism of different food waste was proposed.
- The physicochemical properties of different food waste were significantly different.
- The change trend of hydrolase activity in different food waste was consistent.
- *Bacillus* et al. were the dominant degraders in the food waste aerobic fermentation.