Received: 26 October 2022 | Revised: 16 November 2022 | Accepted: 22 November 2022

DOI: 10.1002/elsc.202200067

Engineering in Life Sciences

RESEARCH ARTICLE

Rapid in-situ aerobic biodegradation of high salt and oily food waste employing constructed synthetic microbiome

Song Xu[1,2] | **Lidan Tao**[1,2] | **Jingjing Wang**[1,2] | **Xiaoxia Zhang**[1,2] | **Zhiyong Huang**[1,2]

[1]Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China

[2]National Technology Innovation Center of Synthetic Biology, Tianjin, China

Correspondence
Zhiyong Huang, Applied Microbial Ecology Engineering Lab, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, No. 32, West 7th Road, Tianjin Airport Economic Area, Tianjin 300308, People's Republic of China.
Email: huang_zy@tib.cas.cn

Funding information
National Key Research and Development Program of China, Grant/Award Number: 2018YFA0901200; Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project, Grant/Award Number: TSBICIP-PTJS-001; General Program from the National Natural Science Foundation of China, Grant/Award Number: 41977200

Abstract

The high salt content of food waste (FW) severely limits microbial physiological activity and reduces its biodegradability. In this study, a salt-tolerant thermophilic bacterial agent that consists of four different substrate degradation functional strains was evaluated for efficient high salt and oily FW in solid-state aerobic biodegradation disposers. The phy-chemical properties, enzyme activities, microbial community structure, and function during the biodegradation process were evaluated under high salt (5%) stress. The results showed that the agent promoted the degradation rate, increased the matrix temperature, decreased the moisture content (MC), and enhanced enzyme activities without putrid smell. High-throughput sequencing indicated community structure succession between different groups and the positive contribution of the inoculated functional strains. During the FW biodegradation process, the *Bacillus* sp. inoculated was the dominant genus in the agent group. Furthermore, CCA further confirmed the positive effects of the four inoculated strains on high salt and oily FW aerobic biodegradation. Functional prediction and metabolite results both confirmed that the agent was more efficient in carbon, amino acid, and lipid metabolism, which demonstrated that the synthetic microbial consortium holds a potential advantage for efficiency and subsequent resource utilization for organic fertilizer.

KEYWORDS
aerobic biodegradation, food waste, microbial community, salt tolerance, thermophilic bacteria

1 | INTRODUCTION

In recent years, the contradiction between the acceleration of the urbanization process and the difficulty of disposal of municipal solid wastes (MSWs) has become increasingly coincidental [1], especially food waste (FW), which is an essential part of MSWs. According to the data from the National Bureau of Statistics, the Chinese urban permanent population was 848.43 million in 2019, and the urbanization rate of permanent residents reached 60.6% [2]. Compared with developed countries, the main reason Chinese traditional FW biodegradation is its high salt and oily characteristics and the lack of source reduction and classification systems. The "garbage siege" has put

Abbreviation: FW, food waste.

tremendous hazardous impact on people and pressure on the urban management work of many local governments. Implementing source reduction and resource disposal of FW will not only help reduce the volume of MSWs, to alleviate the waste of nowhere to landfill, it can also greatly improve the composition of FW. This greatly reduces the generation of landfill leachate and malodorous gas and effectively reduces the hazardous risk of dioxins produced by the incineration of domestic waste, which will play a role in solving the problem of MSW disposal. Therefore, it is imperative to reduce the quantity and resource disposal of FW on the spot. The use of carbon dioxide, water, ash, and a small amount of residues as organic fertilizers is an important way to realize the elimination of FW, which should be produced and eliminated [3]. The most prominent problem is that a large amount of water content, high salt [4], and oily FW enter the municipal domestic waste collection and transportation system, which is an important component of MSWs, including food residues and kitchen waste [5]. Compared with other countries, especially traditional Chinese cooking and people's lifestyles, it has led to remarkable characteristics of high salt and oily contents in the FW.

At present, in addition to FW anaerobic biodegradation, the aerobic treatment of FW has been widely recognized because it can significantly reduce large quantities of FW to produce organic fertilizer raw materials. In addition, the in situ disposal of FW has been widely promoted since FW easily produces a severe odor in the process of collection and transportation [6]. However, due to the limitation of the treatment site area, method and technology, the pretreatment of this in situ treatment mode is only a simple backlog of FW and then directly into the bioenhanced processor. Therefore, the salt and oil contents gradually accumulate with the increasing addition of FW, which will lead to high salt and oil contents. High salt and oil concentrations severely inhibit the physiological activity of degrading microorganisms, so their ability to degrade FW also decreases [4, 7]. The high concentration of Na^+ in FW causes an increase in the osmotic pressure of the aerobic degradation environment, which will affect the activity of degradation microorganisms and interfere with their metabolism [7]. The oil/fat concentration of FW in China is approximately 1%, and a high content of oil/fat will hinder the access of oxygen required for microbial metabolism. Therefore, it affects the growth and metabolism of microorganisms and negatively affects the rate at which microorganisms decompose organic matter and the quality [8].

Most of the research on application scenarios of salt-tolerant bacteria focuses on sewage treatment [9], soil remediation [10], plant growth promoting bacteria [11], etc. In the research of FW biodegradation, most is about the fermentation of anaerobic bacteria and related metabolites [1, 3]. To our knowledge, there are few studies on FW-degrading microbial agents with wide salt-tolerant ability, in particular, the in situ aerobic solid-state biodegradation process under conditions where high salt and oil/fat exist. Previous studies on the related strains were about the enhancement of their functions, but there were few studies on their performance under the pressure of salt and oil resistant environments. Therefore, in this research, we constructed a bacterial agent composed of salt-tolerant thermophilic degrading strains for starch, oil, protein, and cellulose. In addition, the performance, microbial community structure evolution, and metabolites of the agent in FW biodegradation were studied under high salt and oil/fat stress. This research aims to provide a novel agent for the aerobic process of high-salt and oily FW in situ biodegradation.

Practical Application

- The high salt content of food waste (FW) severely limits microbial physiological activity and reduces its biodegradability. In this study, a salt-tolerant thermophilic bacterial agent that consists of four different substrate degradation functional strains was evaluated for efficient high salt and oily FW in solid-state aerobic biodegradation disposers. The research demonstrated that the synthetic microbial consortium holds a potential advantage for FW efficiency and subsequent resource utilization for organic fertilizer under high salt and oily stress.

2 | MATERIALS AND METHODS

2.1 | Functional strain screening, salt tolerance, and identification

The soil samples were taken from the FW trash bins of Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. Since the FW trash is often scattered on the ground, the sampling depth is approximately 0–10 cm. Each of the samples was streaked on various appropriate selective medium plates (Starch, protein, oil, and cellulose degradation medium), and the degradation ability was compared and then incubated at 50°C and 180 rpm for 24 h. After incubation, different morphological colonies were isolated, purified, and stored in 25% glycerin at −80°C for further studies. All the selected strains

were inoculated into various corresponding selective liquid media and cultured at 50°C and 180 rpm, and the inoculation volume was 10%. At this time, the salinity of the medium was adjusted to 1%, 5%, 10%, 15%, and 20%, the initial pH was 7, and samples were taken on an ultraclean bench 24 h after inoculation to test the change in OD_{600}. All samples were analyzed in triplicate. Potential functional strain DNA was extracted using a QIAGEN DNA extraction kit, and 16S rRNA gene sequences were amplified and identified using the National Centre for Biotechnology Information BLAST service (http://www.ncbi.nlm.nih.gov/BLAST) for each isolate.

2.2 | Construction of microbial agent and bulking carrier

FW is mainly composed of four components: starch, protein, oil, and cellulose. Four different functional complementary strains with the highest degradation efficiency and the best resistance to salinity abilities were selected and formed into a high-efficiency bacterial agent after the plate confrontation test. The initial input rate at 1:1:1:1 was tested as the agent group, and each strain was diluted to 10^8 CFU/ml. To avoid FW cohesive behavior, adjust the C/N ratio and maintain ventilation, wheat bran and sawdust were added to serve the purpose of the bulking agent. The biodegradation agent was composed of wheat bran (1 kg), sawdust pieces (0.5 kg, <2 cm), and 250 ml of mixed functional agent. The control treatment was composed of wheat bran (1 kg), sawdust (0.5 kg), and 250 ml of distilled water.

2.3 | FW disposer

The household in situ FW biodegradation equipment was manufactured by Hainan GT Environmental Technology Development Co., Ltd. (Hainan, China). The effective capacity of the entire FW disposer is 10 L, and the length, width, and height dimensions are 39 × 45 × 40 cm. After the FW disposer lid was closed, the stirring mode was 10 min mixing and 20 min halting rotation. An aeration air inlet was provided at the side of the FW biodegradation disposer. When the stirring shaft rotated, the air in the reaction chamber was driven to circulate evenly. There is a plastic thermal insulation jacket designed to keep the process temperature (Figure 1).

2.4 | Experimental design

The FW was modified to be representative of high salt and oil/fat based on 2016FAO (Food and Agriculture Organization of the United Nations) dietary proportion structures. Rice 564 g, fat meat 156 g, cabbage 388.5 g, spice powder 0.54 g, salt 125 g, sugar 4.8 g, and oil 100 ml, add up to a total of approximately 2500 g with distilled water. Different ingredients were procured, cooked, ground, and then added to the FW disposer [12]. The high salt and oily FW experiment using functional agent was conducted over 72 h, and a control without the agent was also conducted under the same conditions. The residual matrix from different layers inside the equipment was collected, and each sample was formed every 8 h to determine the physical-chemical properties, enzyme activities, and microbial community and function.

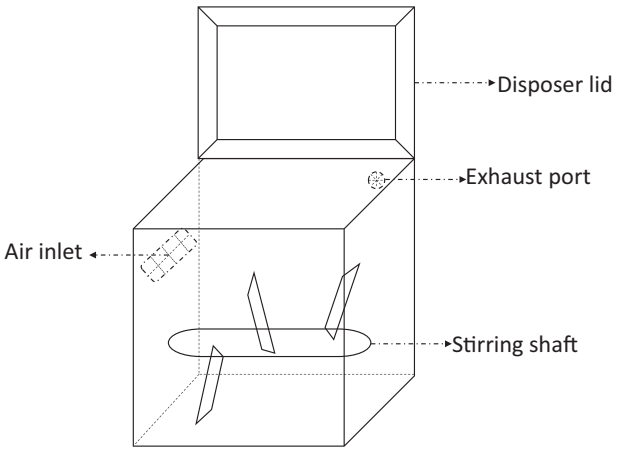

FIGURE 1 Schematic diagram of food waste (FW) aerobic digestion disposer

2.5 | Total mass reduction (TMR) and dry matter degradation (DMD) rate

$$\text{TMR rate } (\%) = \frac{C - (A - B)}{C} \times 100\%$$

$$\text{DMD degradation rate } (\%) = \frac{[C - (A - B)] \times (1 - MCT)}{D} \times 100\%$$

where A is the total weight of the initial degradation system, including the agent and disposer, and the total weight is directly weighed with a weighing balance. B: The remaining weight of the residue matrix and disposer at hours 0, 8, 16 … and 72; the remaining weight is directly weighed with a weighing balance. C: the initial total weight of all materials added. D: The initial total dry weight total weight of materials added. MCT: the moisture content (MC) of the residue matrix at hours 0, 8, 16 … and 72. All data were weighed in triplicate.

2.6 | Physical-chemical and enzyme activities analysis

The cabin matrix temperatures in the FW biodegradation equipment with agent and control groups, denoted as Agent CT and Control CT, respectively, were measured by a mercurial thermometer at hours 0, 8, 16 …, and 72. Room ambient temperature (Agent RT and Control RT) was recorded at the same time. The temperature increase (Agent ΔT and Control ΔT) at the same time as the agent and control was the increase in the temperature of the matrix, that is.

$$\Delta T(^{\circ}C) = CT - RT)$$

The electronic conductivity (EC) and pH were determined using a pH meter and an EC meter, respectively (W/V = 1:10). The samples were oven-dried at 105°C ending with a constant weight to determine the MC. The wet bulk density (BD) in the matrix was determined following previous methods [13]. All samples were analyzed in triplicate.

$$\text{MC } (\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%$$

$$\text{BD } (\text{kg/L}) = \frac{\text{Mass}}{\text{Volume}}$$

The total carbon content (TOC) and total nitrogen content (TN) in the matrix were determined and analyzed following standard methods [14]. Protease activity, amylase activity, lipase activity, and cellulose activity were measured by using commercial chemical assay kits (Beijing Solarbio Technology Co., Ltd.). All samples were analyzed in triplicate.

2.7 | DNA extraction and 16S rRNA sequencing

In this study, all FW degradation matrix samples were extracted using a Power Max Soil DNA Isolation Kit (MO Bio Laboratories, Solana Beach, CA). Samples (0.25 g) were taken, and the subsequent steps were carried out according to the instructions of the kit. The extracted genome was identified by electrophoresis, and the content and quality of the genome were determined by a Nanodrop 2000. The V4 hypervariable region of 16S rRNA was detected by the Illumina MiSeq sequencing platform. In the present study, the bacterial universal primer sequences used were F515 5′-GTG CCA GCM GCC GCG GTA A -3′ and R806 5′- GGA CTA CHV GGG TWT CTA AT -3′.

2.8 | Detection of degradation metabolites

The detection of degradation metabolites was analyzed following the methods described in a previous paper. Briefly, the samples were freeze-dried by a vacuum freeze-dryer (Scientz-100F). The freeze-dried sample was crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz. Dissolve 100 mg of lyophilized powder with 1.2 ml 70% methanol solution, vortex 30 s every 30 min six times in total and place the sample in a refrigerator at 4°C overnight. Following centrifugation at 12,000 rpm for 10 min, the extracts were filtered (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China, http://www.anpel.com.cn/) before UPLC–MS/MS analysis. Then, the sample extracts were analyzed using a UPLC–ESI–MS/MS system (UPLC, SHIMADZU Nexera X2, www.shimadzu.com.cn/; MS, Applied Biosystems 6500 Q TRAP, www.appliedbiosystems.com.cn/). The effluent was alternatively connected to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS.

2.9 | Bioinformatics and statistical analysis

Each sample yielded at least 40,000 reads, and basic data analysis was performed by QIIME 2 (version v.2019-07) software, according to the relevant process. The sequences were aligned against the Silva database (version: http://www.arb-silva.de/). Denoise and cluster high-quality sequences, divide ASVs (features), and obtain their species classification according to the sequence composition of features. The alpha diversity, including Chao 1, ACE, Simpson diversity, and Shannon diversity, was subjected to statistical analysis using QIIME 2. Data were analyzed using multivariate correlational and ordination methods in the R statistical environment (R Core Team, 2019) using the R package vegan. PICRUSt2 was used to analyze the functional characteristics of the microbial community.

All data in this study were analyzed in triplicate and expressed as the mean ± standard deviation using Excel 13.0. One-way analysis of variance (ANOVA) with least-significant difference (LSD) at a 95% confidence level was used to test for statistically significant differences. The 16S rRNA gene sequences in this study were deposited in the National Center for Biotechnology Information (NCBI)

Sequence Read Archive (SRA) under accession number PRJNA739490.

3 | RESULTS AND DISCUSSION

3.1 | Strain screening, salt resistance ability, and agent construction

There are 25, 16, 6, and 11 isolations can grow on starch, protein, oil, and cellulose elective medium plates respectively. Then, two strains with maximum degradation effects (transparent circle diameter) on protein, starch oil, and cellulose were selected to test salinity tolerance. Salinity is an important environmental factor that affects the growth and metabolism of microorganisms during FW degradation. To compensate for the effects of microbial degradation on high-salt FW and expand the application range of FW biodegradation, the salt tolerance of strains was studied to construct the bacterial consortium. From the perspective of salinity tolerance, the starch-degrading strain *Bacillus subtilis* D-7 has better salinity tolerance; when the salinity is 150 grams per liter, there is still obvious sign of growth with an OD_{600} higher than 0.60. Protein-degrading strain Bacillus *sp*. PT-4 showed a wider salinity tolerance ability than PT-8. The oil-degrading strain *Bacillus lichenifomis* Y-1 has better salinity tolerance than Y-4. The cellulose-degrading strain *Brevibacillus borstelensis* CL-1 has better salinity tolerance than CG-3 (Figure S1). Finally, the bacterial agent was constructed and constructed to evaluate the stability and degradation capacity based on the proper division of labor and functional compartmentalization strategies, which contained four different substrate degradation functional strains, that is, *Bacillus subtilis* D-7 and *Bacillus pumilus* PT-4, *Bacillus lichenifomis* Y-1, and *Brevibacillus borstelensis* CL-1.

3.2 | The total mass reduction (TMR) and dry matter degradation (DMD) rate changes

During the high salt and oily FW biodegradation process, the TMR is one of the most important indicators. Therefore, the TMR was selected as the indicator to detect the degradation rate of the newly constructed bacterial agent. Figure 2A shows that the novel agent FW biodegradation system had a relatively higher TMR than that with no inoculated functional strains. At the initial 8 h, the TMR difference between the two systems was slight, but from 8 to 72 h, the TMR difference between these two systems became increasingly larger. In the end, the TMR of the novel bacterial agent was 45.1%, while the TMR of the blank control group was 36.2%. Figure 2B shows that the DMD rate of the agent was approximately 81.8%, while that of the control was only 54.6%. Most importantly, the constructed agent biodegradation systems ran steadily, while the control group sustained release exuded an unpleasant sour smell during the entire FW biodegradation process. It is well known that the smell of distributed and on-site FW biodegradation equipment, which is always close to residential areas or workplaces, is the most worthy of consideration in actual operation. Therefore, the novel bacterial agent we constructed is particularly suitable for application with this equipment and is easier to accept by people living nearby.

3.3 | The phy-chemical properties changes

Temperature is one of the most important factors affecting FW aerobic biodegradation. Appropriate temperatures can more effectively maintain the physiological activity of microorganisms, thereby improving their degradation effects [15]. Figure 2C shows that the room temperature (RT) of the control group was always higher than that of the agent group due to the seasonal ambient temperature. The compartment matrix temperatures of the agent fluctuated between approximately 28.3°C and 49.3°C, which were higher than those of the control, which fluctuated between approximately 27.3°C and 40.3°C, especially after 8 h of the degradation experiment. The temperature in the agent compartment matrix was maintained at approximately 49°C, started from 16 h and lasted until 48 h. In contrast, in the blank control group, the compartment matrix temperature was maintained at approximately 38°C, lasting from 8–48 h. The cabin matrix temperature of the blank control group could not continue to increase, and the temperature also dropped sharply to 28.5°C in the later descending process stage. In addition, the curve of agent ΔT was higher than that of control ΔT in the matrixes. In the entire degradation stage after 8 h of high-oily and high-salt FW, the temperature of the agent treatment group was significantly higher than that of the control group ($p < 0.01$). Figure 2C shows that the novel agent group temperature increase range was significantly higher than that of the control, which suggested that more heat was released by the novel thermophilic agent metabolic activities. In addition, this also implies that the FW has basically been degraded completely. When high salt and oily FW are added, the microorganisms in the material can undergo respiration and metabolism by virtue of suitable environmental conditions and sufficient substrates in the degradation equipment, releasing energy at the same time to increase the material temperature and accelerate the

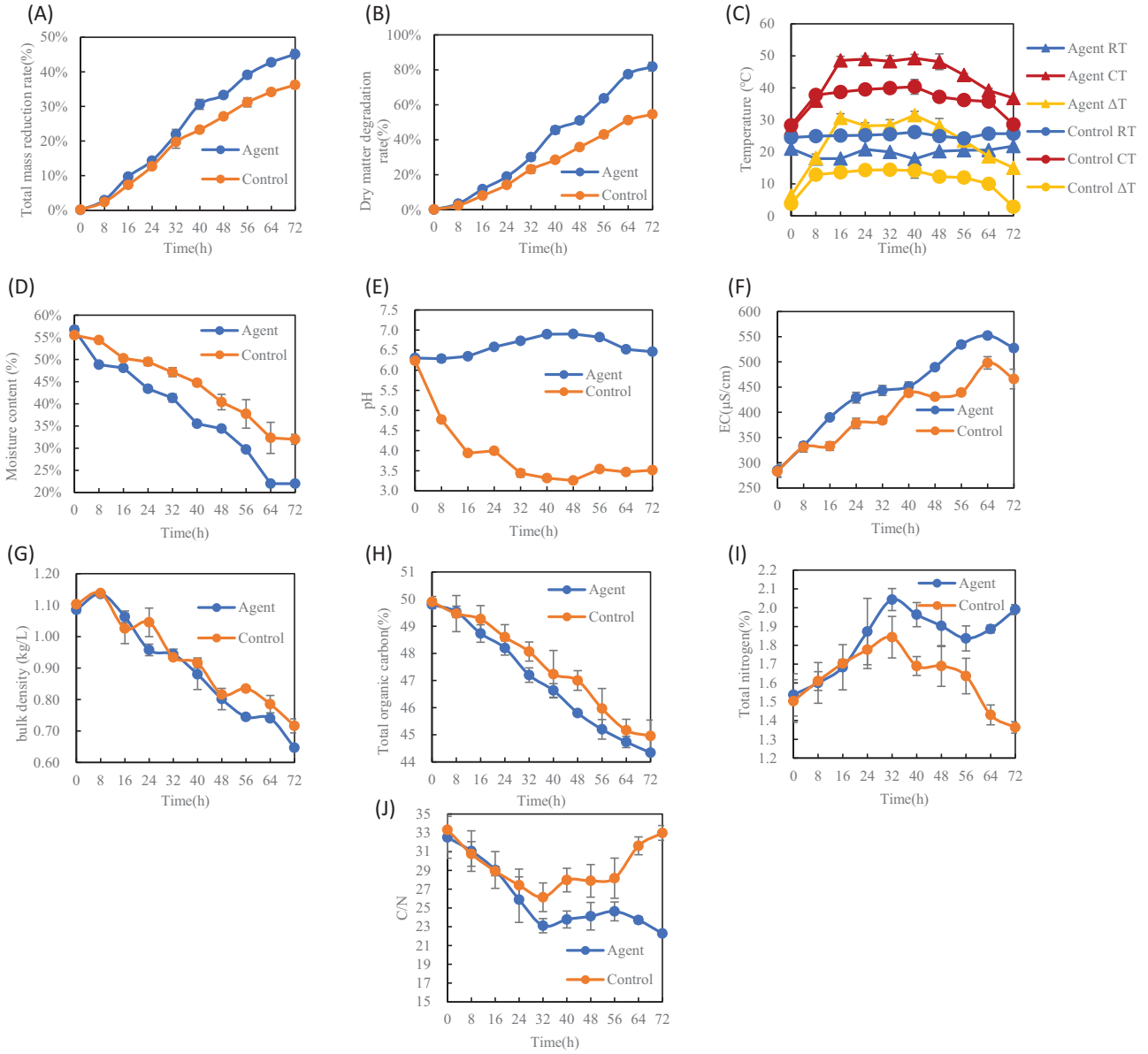

FIGURE 2 The degradation and dynamics of the physicochemical properties changed during the FW aerobic biodegradation process between the agent and control groups

degradation process. In the process of FW biodegradation, the content of easily degradable organic matter decreases rapidly with increasing material temperature, which also indicates that temperature changes can characterize the degradation process of organic matter in materials to a certain extent.

Moreover, MC, EC, pH, and wet BD are other significant indicators that affect the FW biodegradation efficiency. Figure 2D indicates that the matrix MC after the high salt and oily FW was added in both treatments showed downward trends, from 55% at the beginning of the experiment to approximately 22%–32% at the end. The matrix MC of the control was approximately 10% higher than that of the agent group at the end. Generally, an environment with neutral pH can better maintain the physiological and biochemical activities of microorganisms [16]. Figure 2E shows that the pH variations of the FW between different groups were significantly different within 72 h. The pH of the agent group was maintained at approximately 6.5–7.0 during the high salt and oily FW biodegradation process, whereas the pH of the control group sharply dropped to approximately 3.5 within 32 h, which might be because of acidophilic microorganism activity and the large amount of organic acids produced. This reflected that there was an acidification process of FW without an inoculation agent specifically under conditions where high salt and oil/fat existed. The EC of the two treatment groups gradually increased along with the degradation process of FW, which

is consistent with previous research results (Figure 2F). BD showed a gradual decreasing trend during FW degradation except for the initial thermophilic phase because of the water generated when the FW rapidly degraded (Figure 2G). This trend might be attributed to the FW volume reduction and MC loss. Although the BD in agent and control had almost the same consistent downtrend, the BD in agent was significantly lower than control due to the higher MC in control.

As shown in Figure 2H, the TOCs of the agent and control groups were significantly reduced from 49.80% and 49.90% to 44.34% and 45.30%, respectively ($p < 0.05$). The TOC reduction rate is small, which might be due to the bran and sawdust carrier of the novel constructed agent and shorter degradation time. The carrier has a complex structure and lignocellulose, which makes it difficult for microorganisms to degrade in a short period of time [17]. Nevertheless, this study still proved that the novel bacterial agent had an excellent degradation effect, whereas the control group had a significantly lower TOC reduction rate ($p < 0.05$) [12]. Moreover, the TN of the agent group was significantly increased after FW biodegradation ($p < 0.05$), while the TN change of the control group was not obvious (Figure 2I). This might be because a large amount of nitrogenous organic matter (protein) is mineralized and degraded by microorganisms, which leads to a decrease in dry matter and a gradual increase in TN during the FW biodegradation process [18]. Considering the changes in the community structure, previous studies proved that most *Bacillus* are nitrogen-fixing bacteria that are more abundant in the agent group [19]. In addition, C/N is another important indicator of FW biodegradation maturity (Figure 2J). After FW biodegradation, the C/N ratios of the agent and control groups changed from 33.31 and 31.17 to 34.06 and 21.96, respectively. The postbiodegradation C/N ratio of the agent group confirmed the indicator for FW biodegradation maturity with a significant decrease ($p < 0.05$), relevant studies have found that the C/N ratio generally decreases from approximately 35 to below 25 during the FW composting process [17]. However, the C/N ratio of the control group increased, but it was not statistically significant, which may be due to the lower TN and organic matter degradation rate.

3.4 | The enzyme activities changes

Most organic substances in nature can be degraded by microorganisms if the degradation time is not considered. Microbial extracellular enzymes can depolymerize high molecular weight organic matter [16, 20]. Extracellular enzyme activities play very important roles during the FW aerobic biodegradation process. Therefore, to understand the main different FW organic substance degradation precedences, protease, amylase, lipase, and cellulase activities were examined during the FW aerobic biodegradation process between the agent and control groups (Figure 3).

Starch, as an important part of FW, is a valuable raw organic material for the production of high-value end products, such as organic fertilizer [14]. Amylase is a general term for enzymes that hydrolyze starch and glycogen, which can hydrolyze starch into reducing sugars such as glucose. Figure 3A illustrates that from the beginning of the FW added to 40 h, the amylase activity of the agent group was always maintained at a high state, reaching the maximum value of 1690 U at 8 h. During the FW aerobic degradation process, foods with high starch content, such as rice, generally easily and first decomposed and consumed by microorganisms that can secrete amylase so that they can secrete a large amount of amylase. However, the amylase activity of the control group was significantly lower than that of the agent group ($p < 0.01$) throughout the biodegradation process of FW, which might be because high salt and oily are known to inhibit normal microorganism growth and reproduction, eventually affecting the activities of the enzyme [21].

Because chickens, ducks, fish, eggs, and other foods contain high protein, the protein content in FW is relatively high. Studying the changes in protease activity will help to understand the protein conversion in the entire FW biodegradation process. Overall, the protease activity of the agent and control groups first increased and then decreased, and both reached maximum activity at 16 h, 719 and 940 U, respectively, as shown in Figure 3B. During the initial stage of the FW degradation process, the protein organic matter content is very sufficient, so microorganisms can proliferate in large numbers, and a large amount of proteases are produced, which also leads to a gradual increase in the temperature of the system at the same time. The protease activity of the agent group was significantly higher than that of the control group ($p < 0.01$), which may be because the salt and oily contents affected microbial protease function or secretion. Moreover, the protease activity of the two treatment groups significantly decreased to a relatively low activity plateau, which may indicate that the protein content in the system was basically completely degraded after 32 h.

Traditional Chinese food has a high oil/fat content after being processed by frying, roasting, etc., indicating that it has a great influence on lipase activity during FW biodegradation. After adding the high salt and oily FW, the lipase activity of the agent group continued to increase. At 24 h, the lipase activity reached a maximum of 625.4 U, whereas the lipase activity of the control group reached its maximum value of 266.3 U at 36 h, and then gradually decreased. Similarly, the lipase activity of the control group

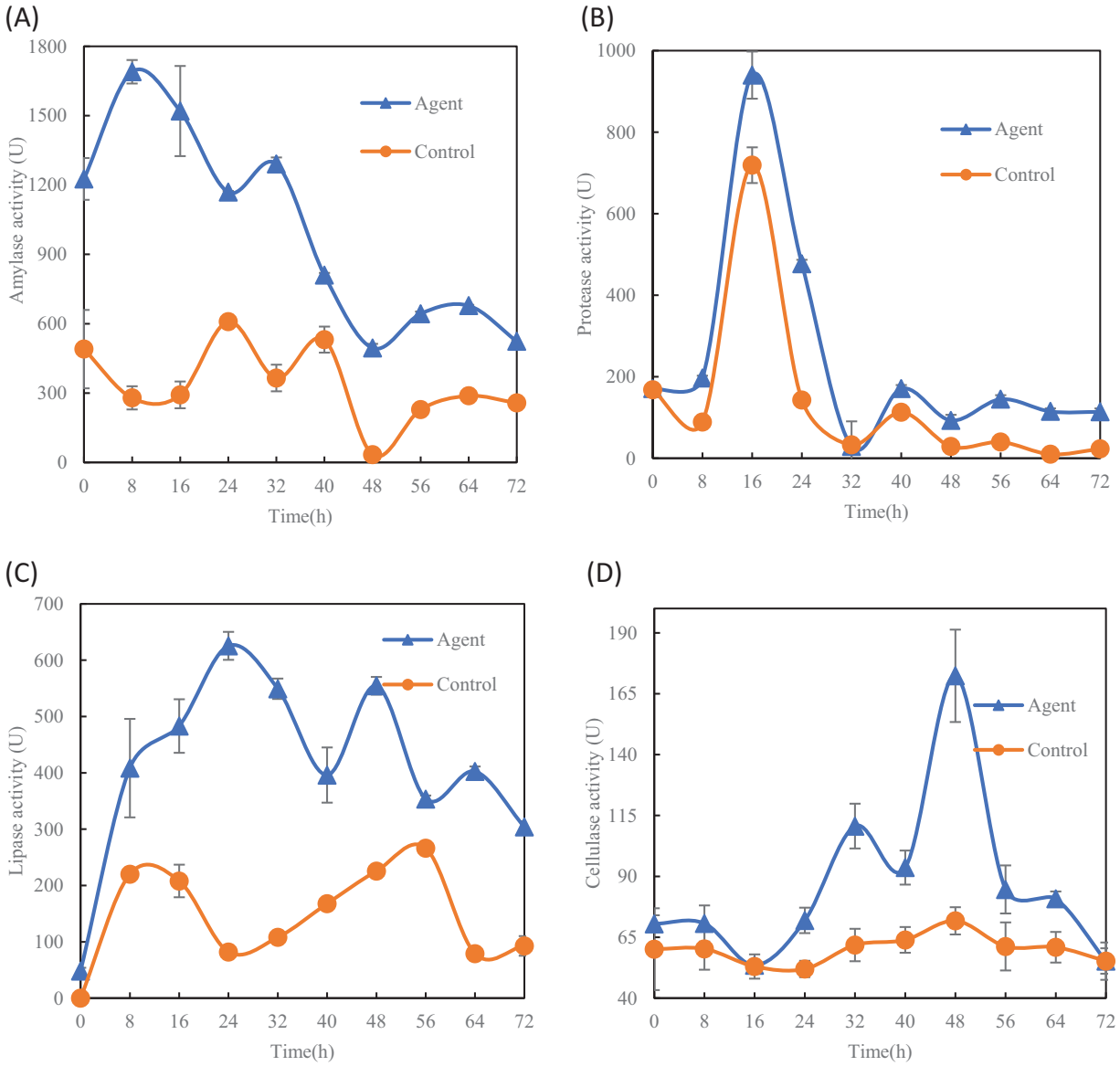

FIGURE 3 The enzyme activities changed during the FW aerobic biodegradation process between the agent and control groups

was still significantly lower than that of the agent group (Figure 3C).

Cellulase is a complex enzyme system composed of the C1 enzyme, Cx enzyme and β-glucosidase, bacteria, fungi, animals, etc., can produce cellulase. Figure 3D illustrates the cellulase activity changes during high salt and oily FW biodegradation. The cellulase activity of the agent group was also low in the initial state, reaching the highest value of 172.4 U at 48 h. Obviously, compared with the other three enzyme activities, the cellulase activity remained low throughout the entire FW biodegradation process, especially in the control group. This is because cellulase is easily denatured and inactivated under strong acid conditions. In addition, the degradation process of cellulose is much more complicated than that of other organic substances (such as sugar and protein).

Overall, during the whole FW biodegradation process, the activity of various enzymes in the agent group was higher than that of the control group, which may be because the characteristics of high salt and high oily inhibited the indigenous microbial activity and processing ability. In the initial stage of FW degradation, sufficient water and nutrient content (i.e., substrates) provided material energy for the vigorous mass growth and metabolism of thermophilic microorganisms. Similarly, microorganisms secrete a large number of hydrolyzable enzymes, which can effectively degrade the complex macromolecular organic matter in FW. With the continuous degradation of the substrate, the microorganisms are subject to various constraints within the system's internal environmental factors, their metabolic activity begins to slow down, and the enzyme activity begins to decrease, indicating that the

entire degradation has stabilized and ended. Most notably, through our research, we found that the maximum activities of amylase, protease, lipase, and cellulase occurred at 8, 16, 24, and 48 h, respectively. These results confirm the selectivity of the novel constructed FW biodegrading thermophilic agent for the utilization of different substrates, and degradation is carried out in sequence according to the degree of difficulty of substrate degradation.

3.5 | Microbial community characteristics

3.5.1 | Community structure compositions

A total of 2,024,723 pairs of reads were obtained by sequencing 20 samples. After quality control and splicing of double-ended reads, a total of 1,929,602 clean reads were generated. Overall, these reads were assigned to 14 phyla, 25 classes, 59 orders, 91 families, 162 genera, and 231 species.

Figure 4A illustrates the relative abundance of dominant bacteria changes during the FW biodegradation process between different treatment groups at the phylum level. Overall, the dominant bacteria in the high salt and oily FW aerobic biodegradation process were mainly Firmicutes, Proteobacteria, and Cyanobacteria, accounting for more than 95%. In the initial stage of FW aerobic degradation (0–8 h), the dominant bacteria were Proteobacteria and Cyanobacteria. However, Firmicutes became the dominant bacteria followed by Proteobacteria in the agent group, while Proteobacteria became the dominant bacteria followed by Firmicutes in the control group at 16 h. The relative abundance of Firmicutes in the agent group was higher than 90% from 24 to 72 h. In contrast, Proteobacteria was the dominant bacteria in the control group from 0 to 40 h, and then the relative abundance of Proteobacteria and Firmicutes became almost the same.

Figure 4B shows the top twenty bacterial species-level distributions of these two groups. The dominant species were *uncultured_bacterium_f_Mitochondria* and *uncultured_bacterium_o_Chloroplast* at 0 h. Then, the dominant genera in the agent group were *Bacillus subtilis* and *Oceanobacillus caeni* from 16 to 72 h, and their total relative abundance accounted for more than 90%. The dominant genus *Bacillus* in the agent group was the thermophilic bacterium we added. Previous studies have shown that *Bacillus* has environmental stress resistance ability and can effectively promote organic matter degradation [22]). It is worth noting that the relative abundance of *Oceanobacillus caeni*, which was not added to the degradation process, also gradually increased. Some related studies proved that *Oceanobacillus* can survive in a high-salt environment and play an important role in carbon and nitrogen metabolism [23]. In contrast, the dominant species of the control group were *uncultured_bacterium_g_Klebsiella, Pediococcus acidilactici*, *Pantoea ananatis*, and *Lactobacillus crispatus*. *Pediococcus acidilactici* was one of the most abundant species, and the relative abundance gradually increased. Shipeng Zhou et al. also found that *Pediococcus* is one of the donminat genera during FW biodegradation and is an indigenous microbial consortium in FW [24]. *Lactobacillus* often exists during the FW natural corruption process [25]. The most worthy of attention is the presence of *Escherichia coli* during the degradation of FW in the control group. Some types of *E. coli* can cause symptoms such as diarrhea and hemorrhagic colitis. Overall, there were significant differences in the microbial community structure change trends between the agent and control groups (Figure S2).

3.5.2 | Community alpha diversity

The rarefaction curve indicated variation in ASV (amplicon sequence variants/features) density within all the samples, while the sequence coverage sufficiently captured the bacterial community diversity (Table 1). To study the bacterial community alpha diversity during the high salt and oily FW biodegradation process between the agent and control groups, the alpha diversity analysis indices of different samples (ACE, Chao1, Shannon, and Simpson) were calculated. The ACE and Chao 1 indices can reflect community richness, and the Simpson and Shannon indices can reflect community evenness and diversity in the samples.

From Table 1, the Chao1 and ACE indices decreased during the initial 0–16 h and then increased afterwards in the control group, while they decreased during the initial 0–24 h and then increased intensely in the agent group. At the end stage of the degradation of FW, the Chao1 and ACE index of the agent group was significantly higher than that of the control group, demonstrating that the novel agent may have higher resistance to higher salt and oil/fat than the FW indigenous microorganisms. According to the Shannon and Simpson indices, there was a slight drop and then little fluctuation trend in the control group. In the agent group, the Shannon and Simpson index decreased from 0 to 16 h and then increased sharply at 16–24 h. However, the Shannon and Simpson index decreased again from 48–56 h with a slight increase at the end stage. Overall, the Shannon and Simpson indices of the control group were visibly higher than those of the agent group during the FW biodegradation process. Generally, only bacteria that have a strong tolerance can survive under high salt and oil/fat conditions in the study. Additionally, the inoculation of the novel constructed thermophilic agent

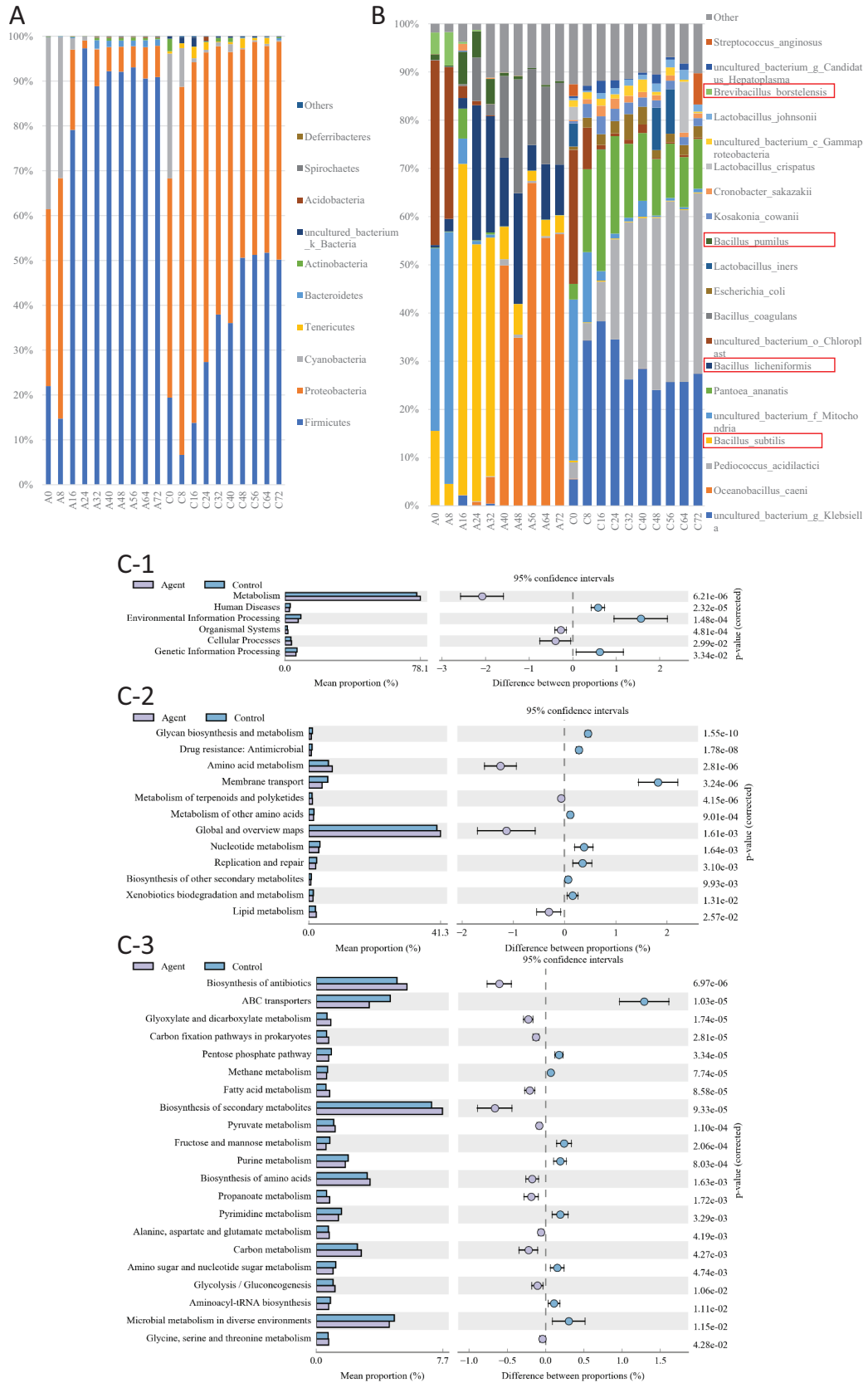

FIGURE 4 The relative abundance of dominant bacteria in samples between agent and control groups at (A) phyla level (top 10), (B) species level (top 20, the red wire frame represents the functional strain added), and the variation in microbial community function profiles analyzed by PICRUSt2 based on the KEGG pathway (C-1: level 1, C-2: level 2, C-3: level 3)

TABLE 1 The change trends of microbial community alpha diversity indices at different times between the agent and control groups

Sample ID	ACE	Chao1	Simpson	Shannon	Coverage
A0	127.00	127.0	0.722	2.565	1
A8	127.00	127.0	0.654	2.354	1
A16	115.00	115.0	0.591	2.374	1
A24	53.00	53.0	0.676	2.188	1
A32	154.00	154.0	0.734	3.017	1
A40	147.00	147.0	0.708	2.850	1
A48	149.00	149.0	0.779	3.066	1
A56	147.00	147.0	0.535	2.197	1
A64	150.00	150.0	0.662	2.778	1
A72	152.00	152.0	0.654	2.702	1
C0	171.22	171.0	0.836	4.009	1
C8	145.18	145.0	0.825	3.550	1
C16	95.00	95.0	0.786	3.310	1
C24	101.00	101.0	0.802	3.292	1
C32	104.45	104.5	0.805	3.263	1
C40	108.15	108.0	0.806	3.316	1
C48	97.28	97.0	0.802	3.308	1
C56	88.19	88.0	0.780	3.024	1
C64	92.24	92.0	0.793	3.138	1
C72	96.00	96.0	0.779	3.154	1

Note: A: Agent group, C: Control group, 0, 8, 16, 24…: 0, 8, 16, 24…hours.

(*Bacillus*) also resulted in a simpler community structure. These results are consistent with previous research findings showing that the proliferation of *Bacillus* will inhibit other bacterial growth, but the specific functional vitality of the entire microbial community structure is more powerful [26].

3.5.3 | Function prediction

Microbial community function was predicted by PICRUSt based on the KEGG pathway (Figure 4C). The KEGG pathway results illustrated that the pathways were mainly involved in metabolism, human diseases, environmental information processing, organismal systems, cellular processes, and genetic information processing. Specifically, the agent group metabolism was significantly higher than that of the control group ($p < 0.05$). A total of 27 metabolic functions were predicted in all samples with the most enrichment in global and overview maps, amino acid metabolism, membrane transport, nucleotide metabolism, replication and repair, and lipid metabolism. In the agent group, the abundance of amino acid metabolism and lipid metabolism were significantly higher than those in the control group ($p < 0.05$). López-González et al. [14] proved that amino acid metabolism can be used as an important indicator of compost maturity because amino acids are important carbon sources and energy sources for microbial reproduction and metabolism [27]. Importantly, the prediction functions related to human diseases, such as infectious diseases: bacterial, immune disease, etc., were at higher levels in the control group than in the agent group. The addition of thermophilic bacteria can effectively inhibit the growth and reproduction of pathogens by significantly increasing the substrate temperature, which is conducive to minimizing human health risks in FW biodegradation. The level 3 KEGG ortholog function prediction results showed that the relative abundance of biosynthesis of secondary metabolites, biosynthesis of antibiotics, biosynthesis of amino acids, carbon metabolism, fatty acid metabolism, carbon fixation pathways in prokaryotes, glyoxylate and dicarboxylate metabolism, pyruvate metabolism, propanoate metabolism, glycolysis/glucocorticosis, glycine, serine and threonine metabolism, and alanine, aspartate and glutamate metabolism transport and catabolism in the agent group were significantly higher than those in the control group. Notably, the sequences related to fatty acid and carbon metabolism were significantly higher, indicating that the agent group had an excellent effect on the lipid and carbon degradation of high salt and oily FW. In addition, secondary metabolite biosynthesis, transport, and catabolism were proven during the FW biodegradation process [28]. With easily degradable substances (such

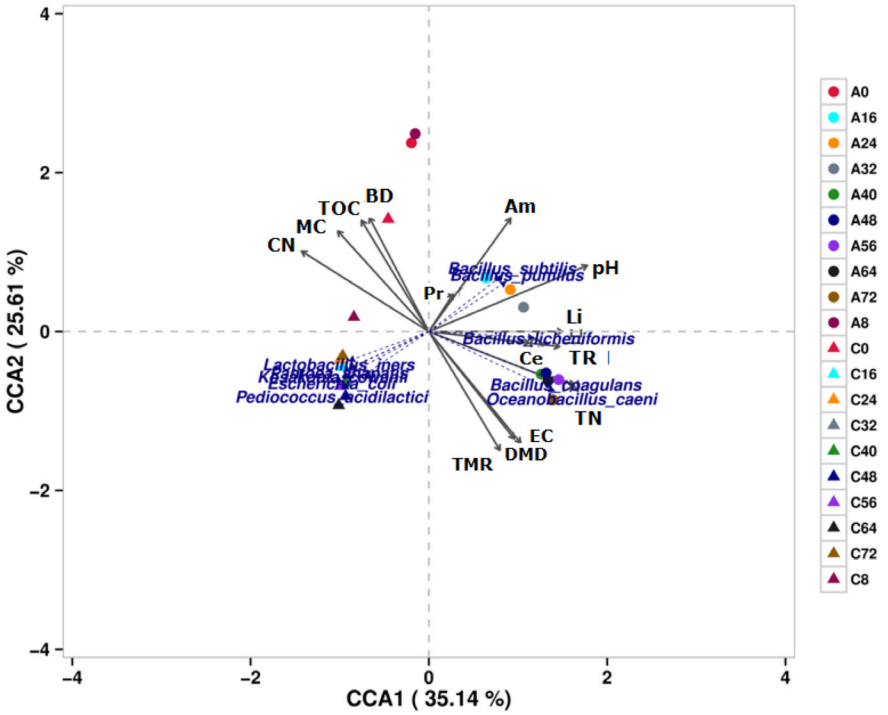

FIGURE 5 CCA analysis of the relationship among degradation, related phy-chemical factors, enzyme activity, and microbial community structure composition (at species)

as starch and protein), the inoculation of bacterial agents does not significantly improve their degradation function or effectiveness. However, the more complex substances that are difficult to degrade (such as oil/fat and cellulose), the bacterial agents can significantly improve the degradation effect.

3.5.4 | Correlation analysis

CCA is typically used to reflect the relationship among the total mass reduction rate (TMR), dry matter degradation rate (DMD), physicochemical factors, enzyme activity, and community structure of different groups at the species level. CCA1 and CCA2 explained 35.14% and 25.61% of the total variation, respectively (Figure 5). First, the CCA results showed that there was a significant correlation between the TMR, DMD, temperature increase (TR), MC, pH, EC, BD, total organic carbon (TOC), total nitrogen (TN), C/N, lipase activity (Li), cellulose activity (Ce), protease activity (Pr), amylase activity (Am) and microbial community distribution. MC was negatively correlated with TMR, DMD, EC, and TR. Such results were consistent with a previous study [29]. Generally, there were positive correlations between TMR, DMD, EC, TR, pH, enzyme activities, and most bacterial community distributions in the agent group, indicating that agent group microbial communities had higher degradation efficiency, enzyme activity, and temperature increasing ability under high salt and oil/fat FW stress. Notably, amylase activity, protease activity, lipase activity, and cellulase activity were positively correlated with *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus lichenifomis*, and *Bacillus coagulans*. Finally, CCA proved that the microbial community structure and function of the two treatment groups were different. The above correlation analysis results indicated that the environmental indicators, enzyme activity and the microbial community are closely related to each other and can be used to indirectly characterize microbial metabolism, thereby revealing the mechanism of biodegradation.

3.6 | Biodegradation metabolites

Operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (AB Sciex), a total of 3256 metabolites were obtained by analyzing these two groups of postbiodegradation samples herein. Through reliability model orthogonal projections to latent structures-discriminant analysis (OPLS-DA), we can filter out metabolites that are not related to categorical variables and analyze nonorthogonal variables and orthogonal variables separately to obtain more reliable metabolite differences. Therefore, 2402 differential metabolites were obtained between the agent and control groups, of which 1078 were upregulated and 1324 were downregulated when adding the novel agent, especially the higher production of various amino acids in the agent group. The complex metabolic reactions and their regulation in organisms

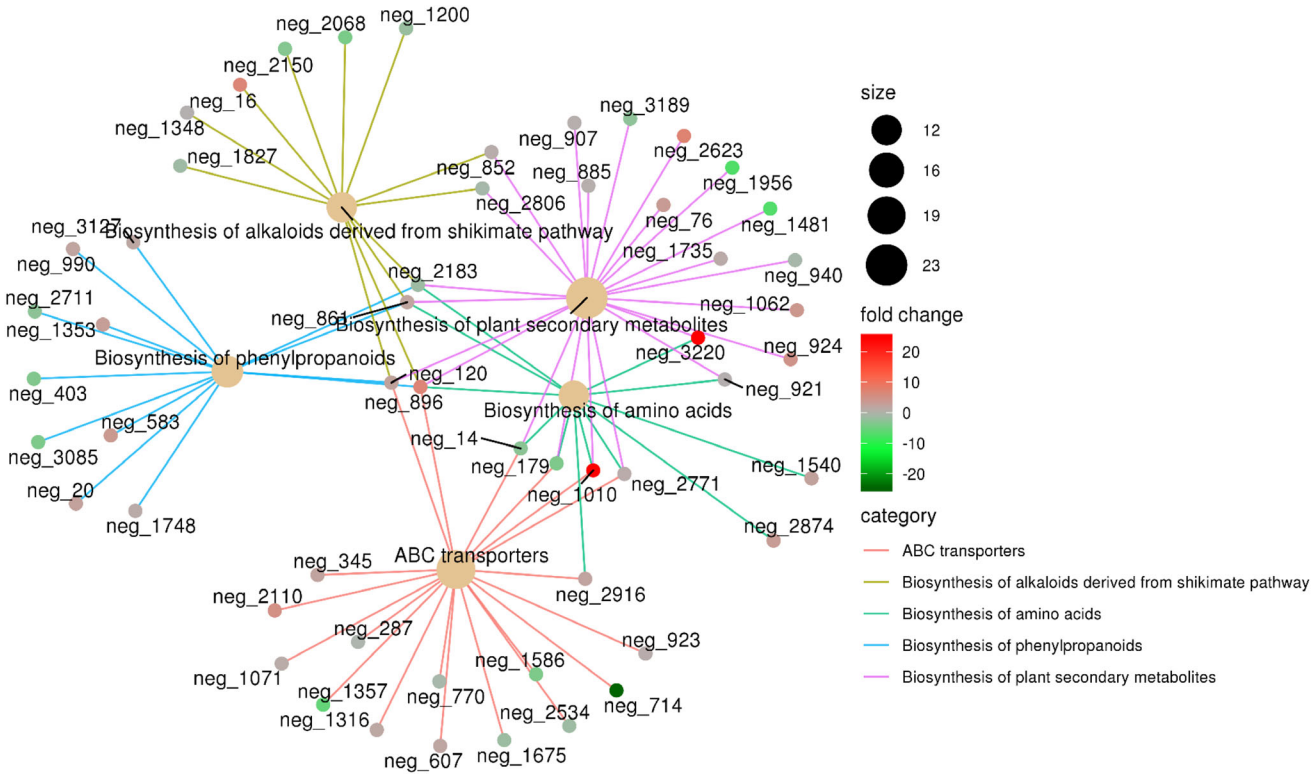

FIGURE 6 The control versus agent metabolite KEGG pathway enrichment network diagram

do not proceed independently. Different genes and proteins often mutually influence and regulate, eventually leading to systemic changes in metabolites. A total of 244 differential metabolites could be annotated using the KEGG database (Figure 6). ABC transporters, biosynthesis of phenylpropanoids, biosynthesis of alkaloids derived from the shikimate pathway, biosynthesis of amino acids, biosynthesis of plant secondary metabolites, and biosynthesis of plant hormones were the top five metabolic pathways with the greatest differences. It should be noted that amino acids [30], plant secondary metabolites [31], and plant hormones [32] play pivotal roles in the growth of crops. In addition, ABC transporters are involved in the transport of plant secondary metabolites [33]. The biodegradation metabolites were consistent with the changes in the novel agent group community structure and function. These metabolites indicated their advantages as raw materials for subsequent organic fertilizer production.

4 | CONCLUSIONS

In this study, the salt-tolerant thermophilic bacterial agent showed higher biodegradation efficiency and enzyme activities for dealing with high salt and oily FW. During the FW biodegradation process, the microbial community structure was found to be significantly different between the agent and control groups, and *Bacillus* sp. inoculated was the dominant genus in the agent group. In particular, we also conducted comparative studies on the degradation metabolites postdegradation in different treatment groups. Taken together, this research provides a novel microbial agent and process strategy that is conducive to achieving more efficient high salt and oily FW biodegradation and resource utilization in the future.

AUTHOR CONTRIBUTIONS

Song Xu: Investigation, conceptualization, formal analysis, writing – original draft, writing – review and editing. Lidan Tao: Methodology. Jingjing Wang: Formal analysis. Xiaoxia Zhang: Data curation. Zhiyong Huang: Funding acquisition, supervision.

ACKNOWLEDGEMENT

This work was financially supported by the National Key Research and Development Program of China (Grant Nos. 2018YFA0901200), Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project (Grant Nos. TSBICIP-PTJS-001), and the General Program from the National Natural Science Foundation of China (Grant Nos. 41977200).

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Curry N, Pillay P. Biogas prediction and design of a food waste to energy system for the urban environment. *Renew Energy*. 2012;41:200-209.
2. NBS. *China Statistical Year Book*. China Statistics Press; 2020.
3. Bernal MP, Alburquerque JA, Moral R. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour Technol*. 2009;100:5444-5453.
4. Liu N, Wang Q, Jiang J, Zhang H. Effects of salt and oil concentrations on volatile fatty acid generation in food waste fermentation. *Renew Energy*. 2018;113:1523-1528.
5. Zhang D-Q, He P-J, Jin T-F, Shao L-M. Biodrying of municipal solid waste with high water content by aeration procedures regulation and inoculation. *Bioresour Technol*. 2008;99:8796-8802.
6. Ke X, Sun JC, Liu C, et al. Fed-in-situ biological reduction treatment of food waste via high-temperature-resistant oil degrading microbial consortium. *Bioresour Technol*. 2021;340(11):125635.
7. Li RP, Ge YJ, Wang KS, Li XJ, Pang YZ. Characteristics and anaerobic digestion performances of kitchen wastes. *Renew Energy Res*. 2010;28:76-80.
8. Nakasaki K, Nagasaki K, Ariga O. Degradation of fats during thermophilic composting of organic waste. *Waste Manag Res*. 2004;22:276-282.
9. Guo J, Zhou J, Wang D, et al. Biocalalyst effects of immobilized anthraquinone on the anaerobic reduction of azo dyes by the salt-tolerant bacteria. *Water Res*. 2007;41(2):426-432.
10. Dagar JC, Yadav RK, Dar SR, et al. Liquorice (Glycyrrhiza glabra): a potential salt-tolerant, highly remunerative medicinal crop for remediation of alkali soils. *Curr Sci*. 2015;108(9):1683-1688.
11. Chookietwattana K, Maneewan K. Selection of efficient salt-tolerant bacteria containing ACC deaminase for promotion of tomato growth under salinity stress. *Soil Environ*. 2012;31(1):30-36.
12. Li Y, Chen Z, Peng Y, Zheng K, Zhang S. Changes in aerobic fermentation and microbial community structure in food waste derived from different dietary regimes. *Bioresour Technol*. 2020;317:123948.
13. Jain MS, Paul S, Kalamdhad AS. Kinetics and physics during composting of various organic wastes: statistical approach to interpret compost application feasibility. *J Clean Prod*. 2020;255:120324.
14. López-González JA, Suárez-Estrella F, Vargas-García MC, López MJ, Jurado MM, Moreno J. Dynamics of bacterial microbiota during lignocellulosic waste composting: studies upon its structure, functionality and biodiversity. *Bioresour Technol*. 2015;175:406-416.
15. Ling L, Wang S, Guo X, Zhao T, Zhang B. Succession and diversity of microorganisms and their association with physicochemical properties during green waste thermophilic composting. *Waste Manag*. 2018;73:101-112.
16. Awasthi MK, Wong J, Kumar S, et al. Biodegradation of food waste using microbial cultures producing thermostable α-amylase and cellulase under different pH and temperature. *Bioresour Technol*. 2017;248:160-170.
17. Wang X, Zhang W, Gu J, Gao H, Qin Q. Effects of different bulking agents on the maturity, enzymatic activity, and microbial community functional diversity of kitchen waste compost. *Environ Technol*. 2016;37:2555-2563.
18. Ghinea C, Leahu A. Monitoring of fruit and vegetable waste composting process: relationship between microorganisms and physico-chemical parameters. *Processes*. 2020;8:302.
19. Zhang W, Cheng L, Wang Y. *Grid-Connected Power Converters with Synthetic Inertia for Grid Frequency Stabilization*. Conference paper, Springer International Publishing; 2019:297-303.
20. Hiraga K, Miyamoto K, Maeda Y, et al. A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*. 2016;351:1196-1199.
21. Maliki AD, Lai KM. Design and application of a precomposting test step to determine the effect of high fat food wastes on an industrial scale in-vessel composting system. *Int Biodeterior Biodegr*. 2011;65:906-911.
22. Msarah MJ, Ibrahim I, Hamid AA, Aqma WS. Optimization and production of alpha amylase from thermophilic Bacillus spp. and its application in food waste biodegradation. *Heliyon*. 2020;6:e04183.
23. Didari M, Spröer C, Makhdoumi A, Sánchez-Porro C, Ventosa A. Oceanobacillus longus sp. nov., a moderately halophilic bacterium isolated from a salt lake. *Int J Syst Evol Microbiol*. 2016;66:10.
24. Zhou SP, Zhou HY, Xia SN, Ying JM, Zheng YG. Efficient biodegradation of food waste through improving the microbial community compositions by newly isolated Bacillus strains. *Bioresour Technol*. 2021;321:124451.
25. Jeon D, Chung K, Shin J, Min Park C, Gu Shin S, Mo Kim Y. Reducing food waste in residential complexes using a pilot-scale on-site system. *Bioresour Technol*. 2020;311:123497.
26. Kim M, Abdulazeez M, Haroun BM, Nakhla G, Keleman M. Microbial communities in codigestion of food wastes and wastewater biosolids. *Bioresour Technol*. 2019;289:121580.
27. López-González JA, López MJ, Vargas-García MC, Suárez-Estrella F, Jurado M, Moreno J. Tracking organic matter and microbiota dynamics during the stages of lignocellulosic waste composting. *Bioresour Technol*. 2013;146:574-584.
28. Wei H, Wang L, Hassan M, Xie B. Succession of the functional microbial communities and the metabolic functions in maize straw composting process. *Bioresour Technol*. 2018;256:333-341.
29. Sun L, Han X, Li J, et al. Microbial community and its association with physicochemical factors during compost bedding for dairy cows. *Front Microbiol*. 2020;11:254.
30. Dan L, Tq B, Cl B, et al. Insights into thermal hydrolyzed sludge liquor – Identification of plant-growth-promoting compounds. *J Hazard Mater*. 2021;403:123650.
31. Nina T, Manuel L. The evolution of functional in plant secondary metabolites. *Int J Plant Sci*. 2003;10:1086.

32. Umehara M, Hanada A, Yoshida S, et al. Inhibition of shoot branching by new terpenoid plant hormones. *Nature*. 2008;455:195-200.
33. Yazaki K. ABC transporters involved in the transport of plant secondary metabolites. *FEBS Lett*. 2006;580:4.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Xu S, Tao L, Wang J, Zhang X, Huang Z. Rapid in-situ aerobic biodegradation of high salt and oily food waste employing constructed synthetic microbiome. *Eng Life Sci*. 2024;24:e2200067.
https://doi.org/10.1002/elsc.202200067