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Research article

# A novel approach to enhance high optically active L-lactate production from food waste by landfill leachate

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ABSTRACT

The recycling of food waste (FW) through anaerobic fermentation into lactic acid (LA), with two isomers L-LA and D-LA, aligns with the principles of a bio-based circular economy. However, FW fermentation is often limited by competing pathways, acidification inhibition, and trace metals deficiency. This study investigates the introduction of landfill leachate, containing buffering agents (ammonia) and trace metals, into FW fermentation. Various dosages of landfill leachate, ranging from 90 (LN-90) to 450 mg/L (LN-450) based on inclusive ammonia calculation, were employed. Results showed that LA production peaked at 43.65 ± 0.57 g COD/L in LN-180 on day 6, with a high optical activity of L-LA at 92.40 ± 1.15 %. Fermentation pathway analysis revealed that landfill leachate amendment enhances hydrolysis (as evidenced by increased activity of amylase, α-glucosidase, and protease) and glycolysis (resulting in enhanced utilization of carbohydrates and glucose). The inclusive ammonia in leachate plays a crucial role as a buffer, maintaining optimal pH conditions (5–7), thereby reducing volatile fatty acid production and thus intensifying LA orientations. The increased activity of L-lactate dehydrogenase (L-LA generation) and decreased NAD-independent lactate dehydrogenase (LA consumption) in properly dosed leachate further explained the high accumulation of L-LA. Dominance of lactic acid bacteria, including *Streptococcus*, *Enterococcus*, *Klebsiella*, *Bifidobacterium*, *Bavariicoccus*, and *Lacticaseibacillus,* accounted for 91.08% (LN-90), while inhibitory effects were observed in LN-450 (4.45%). Functional gene analysis further supported the enhanced glycolysis, L-lactate dehydrogenase, and nitrogen assimilation. Finally, a network analysis indicates a beneficial effect on the genus *Enterococcus* and Klebsiella by landfill leachate addition. This study demonstrates the efficiency of utilizing landfill leachate to enhance LA recycling from FW fermentation, aligning with the concept of circular economy by transforming waste into valuable resources.

## 1. Introduction

The United Nations declares that nearly one-third of food is annually lost or wasted globally, which resulted in more than 1.3 billion tons of food waste (FW), accounting for 8–10% of global greenhouse gas emissions (Pradhan et al., 2021; Xu et al., 2023; Li et al., 2024). FW is prone to rotting, breeding of pathogens, and releasing odorous gases when treated inappropriately, posing public and environmental health concerns (Zhang et al., 2020b). Considering the rich organics within FW, the upgrading of FW into valuable products through anaerobic fermentation with mixed cultures has attracted wide attention, which simultaneously achieves safe disposal and economic benefits (Lee et al., 2022).

Among the fermentation product spectrum, lactic acid (LA) is one of

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the most important platform molecules and possesses a widespread application in the pharmaceutical, food, textile, and chemical industries (Shahab et al., 2020; Askarniya et al., 2023). LA is also the precursor of biodegradable plastic polylactic acid (PLA), making the industrialized application of such a process more promising (Sun et al., 2022; Ali et al., 2023). Nevertheless, strong microbial competition with lactic acid bacteria (LAB) normally occurred during anaerobic fermentation with mixed cultures (Holtzapple et al., 2022; Kumar et al., 2022). LA is easily degraded to acetic acid, which is further consumed by methanogens (Cheng et al., 2020). The active LA could also be converted into propionic acid, butyric acid, valeric acid, and caproic acid when coexisting with the genus of *Clostridium*, *Veillonella*, and *Veillonella* (Shahab et al., 2020). In addition, fungi *Saccharomyces cerevisiae* also compete for carbohydrates for ethanol production with LAB (Senne de Oliveira Lino et al., 2021). These competitive pathways finally declined the LA yield from FW fermentation. Thus, reliable alternative methods to reduce unexpected substrate consumption for obligate lactate production in an environmentally sound are worth pursuing.

Treating waste by waste is an effective way for the circular economy. Among the waste, landfill leachate contains large amounts of dissolved organic matter, ammonia, salts, and other refractory/toxic compounds, potentially threatening the safety of the environment and ecosystems (Wu et al., 2022; Qian et al., 2024). Landfill leachate can be treated by biological treatments (e.g., activated sludge), chemical treatments (e.g., Fenton process and precipitation), and physical treatments (e.g., adsorption and membrane processes), in which a tradeoff is typically required between the efficiency and cost (Teng et al., 2021; Hassan et al., 2023). Interestingly, large works have proved that landfill leachate is also a promising substrate to co-digest with FW for methane production (Liu et al., 2022, 2023; Peng et al., 2022; Zou et al., 2023), which is economically favorable. It should be noted that heavy metals and toxic organic compounds easily form chelates within landfill leachate (Lei et al., 2019). These chelates are more accessible for uptake into methanogens and thus restrain the methanogenesis process (Gao et al., 2021; Song et al., 2023), which is potentially beneficial to the accumulation of carboxylic acids (including LA). However, little is known about how landfill leachate addition impacts the LA fermentation pathway during FW fermentation.

Importantly, LA is a chiral platform molecule, which includes L-lactic acid (L-LA) and D-lactic acid (D-LA) (Li et al., 2016). The commercial value of promising biodegradable plastic PLA is strongly determined by the optical activity (OA) of L-LA (Rajeshkumar et al., 2021). Especially, the activity of D-lactate dehydrogenase (D-LDH) is more vulnerable to chemical inhibition than L-lactate dehydrogenase (L-LDH). Li et al. (2021) reported that the addition of salt (a content of landfill leachate) at 30 g NaCl/L could facilitate optically pure L-LA production. Zhang et al. (2020b) also reported that ammonia at 300 mg/L (another content of landfill leachate) enriched the genus *Streptococcus*, *Lactococcus*, *Enterococcus*, and *Corynebacterium*, which were related to L-LA production, finally increasing the OA of L-LA (OA-L) by five-folds. The other components within landfill leachate might also affect the chiral LA generation during FW fermentation. However, the effects of complicated landfill leachate on the composition of the microbial community (especially LAB), key genes related to LA, such as L-LDH and D-LDH, and OA-L, remained unknown.

To explore the effectiveness of landfill leachate amendment on valuable chiral L-LA production from FW, this study proposes a novel approach to improve L-LA production from FW fermentation using landfill leachate. The LA production and OA-L were first compared with different dosages of landfill leachate. Analysis of the fermentation pathways including solubilization, hydrolysis, and acidification provided insight into the regulation of landfill leachate on FW fermentation. The guidance of landfill leachate on the microbial community and functional genes related to LA production were also investigated. Finally, relationships among landfill leachate addition, LA, metabolites, enzyme activity, and key microorganisms were investigated by network analysis. This study provides a novel way to recycle FW for valuable chiral L-LA production.

## 2. Material and methods

### 2.1. Characteristics of FW, leachate, and inoculums

FW was collected from a college canteen in Shanghai, China. The main content of the FW was rice, which is rich in carbohydrates and protein. A blender was employed to mill the FW into a slurry state before being used as substrate. The main characteristics of the slurry FW were as follows: pH at 7.2 ± 0.1, moisture content at 54.09 ± 0.8%, and volatile suspended solid (VSS) at 45.73 ± 0.96%. Leachate was obtained from a landfill in Shanghai, China. The main characteristics of the leachate were as follows: pH at 9.0 ± 0.1, COD at 640.2 ± 21.0 mg/L, and $NH_4^+$-N at 479.3 ± 12.3 mg/L. Waste activated sludge (WAS) was initially inoculated to provide fermenters, which was an excellent inoculum for L-LA production during FW fermentation according to our previous study (Li et al., 2015, 2021; Xue et al., 2018; Zhang et al., 2020b). The WAS was taken from a secondary sedimentation tank of a wastewater treatment plant (WWTP) with an $A^2/O$ process in Shanghai, China. The concentrated WAS was obtained after a 24-h sediment with discarding the supernatant that was then filtered with a 1mm × 1 mm sieve to remove impurities. The main characteristics of the concentrated WAS were as follows: pH at 6.5 ± 0.1, moisture content at 92.5 ± 0.7%, and VSS at 5.78 ± 0.49%.

### 2.2. FW fermentation with different dosages of landfill leachate

The fermentation reactors (working volume at 500 mL) were set at an initial concentration of substrate at 60 g COD/L. The ratio of FW and WAS was set at 18:1 based on the total suspended solid (TSS) calculation according to a preliminary study (Table S1). To avoid free ammonia nitrogen inhibition (Zhang et al., 2020a, 2020b), the inclusive ammonia addition was strongly emphasized at a proper concentration (<500 mg/L) in final reactors. After the mixing of FW with WAS, leachate was added to the reactors by calculating desired ammonia concentrations at 90 mg/L (LN-90) using 93.9 mL of leachate, 180 mg/L (LN-180) using 187.8 mL of leachate, 270 mg/L (LN-270) using 281.7 mL of leachate, 360 mg/L (LN-360) using 375.5 mL of leachate, and 450 mg/L (LN-450) using 469.4 mL of leachate, in which the blank was run without leachate addition. As suggested in our previous study regarding the optimal fermentative conditions (Zhang et al., 2017), the temperature was controlled by a water bath at 35 °C. Mechanical stirring at 100 rpm was employed to ensure the homogeneousness of the mixture. The pH was adjusted to 9.0 with 4M NaOH or 1M HCL three times a day. All reactors run for 9 days. 30 mL of the substrate was taken out and centrifuged daily at 10,000 G for 15 min. The supernatants were filtered by a 0.45 μm filter membrane and placed in a 10 mL brown glass vial at 4 °C for the subsequent determination of total lactic acid (T-LA), L-LA, D-LA, ammonia, and volatile fatty acids (VFA).

### 2.3. Effect of leachate on the solubilization, hydrolysis, and acidification processes

Solubilization and hydrolysis are normally related to the conversion of dissolved organics within FW, which was plotted by three-dimensional excitation and emission matrix fluorescence (3DEEM) in this study. The determination of carbohydrates and protein was used to reflect the solubilization process of FW. In addition, critical hydrolytic enzymes, e.g. amylase, a-glucosidase, and protease, were determined in the first three days of FW fermentation, which were responsible for the hydrolysis of starch, carbohydrates, and proteins (Luo et al., 2020). To further reveal the effect of ammonia on acidification, 5 g/L glucose was used as a model substrate to ferment, in which WAS was used as inoculum under different concentrations of ammonia at 0 mg/L, 90 mg/L,

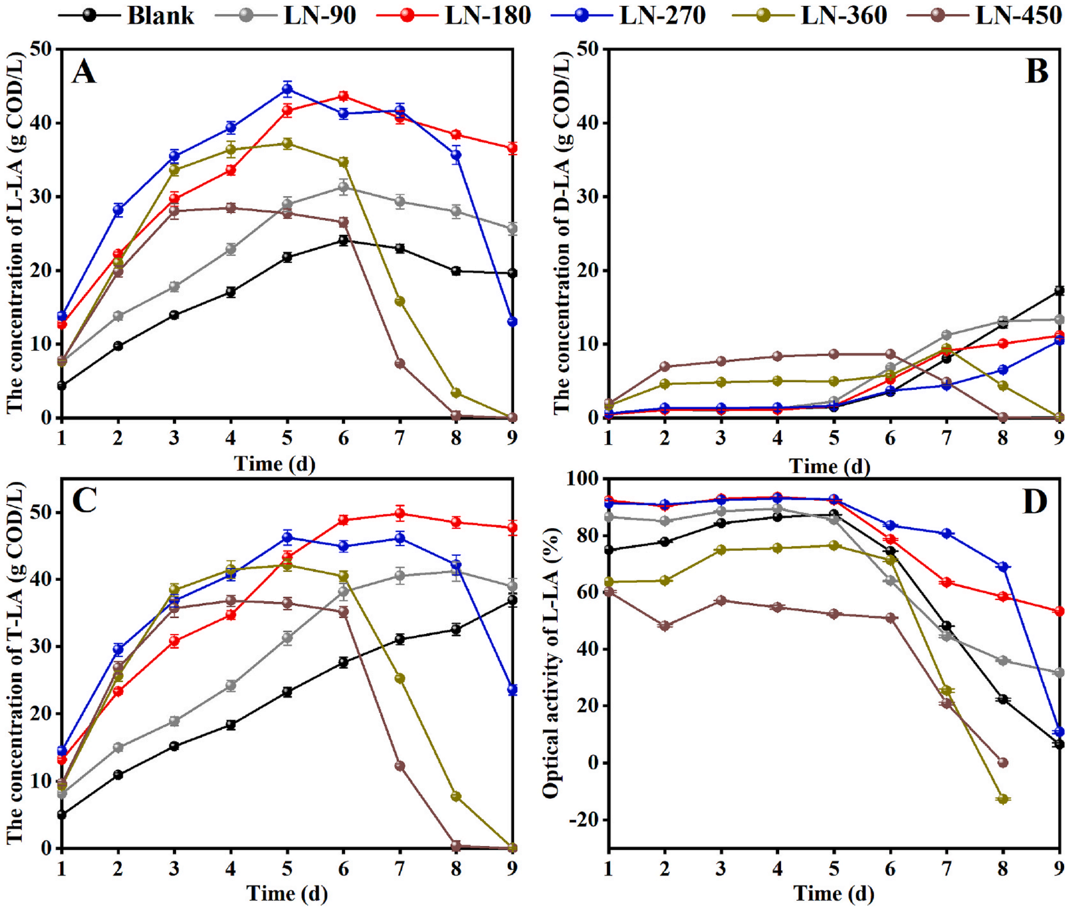

**Fig. 1.** Impact of leachate on the production of L-LA (A), D-LA (B), T-LA (C), and OA-L (D).

180 mg/L, 270 mg/L, 360 mg/L, and 450 mg/L, respectively. The fermentation parameters were as same as in part 2.2.

### *2.4. Effect of landfill leachate on the activity of L-LDH, D-LDH, and NAD-independent lactate dehydrogenase (iLDH)*

L-LDH, D-LDH, and iLDH were responsible for the production of L-LA, D-LA, and the consumption of LA to pyruvate, respectively (Li et al., 2015). On day 5, 25 mL of fermentation broth was fetched from the reactors. The supernatant was removed by freezing centrifugation at 10, 000 G for 10 min at 4 °C. The precipitate after centrifugation was retained and fixed in 20 mL with 0.05 M Tris-HCL (pH 7) shaken well, and then frozen and centrifuged again, repeating the above operation three times. The clean precipitate with microorganisms was fixed in 15 mL of the Tris solution and ultrasonicated in an ice water bath for 20 min (2 s of ultrasonication and 2 s of pause). Finally, the broken cell debris was removed by centrifuging at 4 °C and 10,000 G for 10 min, and the supernatant (cellular extracts) was taken and stored at 4 °C for the subsequent determination of enzyme activity.

The activity of n-LDH was determined using an enzyme labeling method. 40 μL of 300 mM sodium succinate, 40 μL of 24 mM sodium pyruvate, 40 μL of 18 mM fructose 1,6-bisphosphate (FDP), 40 μL of 4.2 mM reduced coenzyme I (NADH), and 80 μL of cellular extracts were added to the enzyme-labeled plate. Kinetics were carried out at 340 nm wavelength and 27 °C using an enzyme labeler. Before determination of the activity of L-LDH, the cell extracts were immersed in water at 50 °C for 3 min to inactivate D-LDH, and then the L-LDH activity was kinetically analyzed according to the n-LDH assay. The D-LDH activity was calculated according to *Eq (1)*:

$$\text{D-LDH} = \text{n-LDH} - \text{L-LDH} \quad (1)$$

The activity of i-LDH was also determined using an enzyme labeling method. 50 μL of 80 mM DL-lactic acid 50 μL, 50 μL of 3 mM oxidative coenzyme I (NAD$^+$), 50 μL of 50 mM Tris-HCL (pH 7), and 50 μL of cell extracts were added to the enzyme labeling plate. Kinetics were carried out at 570 nm wavelength and 27 °C using an enzyme labeler.

### *2.5. Microbial community and predictive functional profiling using the KEGG database*

Samples were withdrawn from reactors on day 6 under different dosages of landfill and the blank for microbial analysis by Personal Biotechnology Co. Ltd. (Shanghai). The genomic DNA from triplicate samples was pooled to minimize variation and amplified using the primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) in the V3-V4 region. Double-end (Paired-end) sequencing was adopted by using the Illumina Miseq platform. All raw sequences were deposited in the SRA database with reference PRJNA1110027 under the accession numbers: SRR29031887, SRR29031886, SRR29031885, SRR29031884, SRR29031883, SRR29031882.

### *2.6. The correlation network analysis*

The correlation network analysis among landfill leachate addition, T-LA, L-LA, D-LA, metabolites (carbohydrates, proteins, ammonia, nitrate, acetate, propionate, butyrate, valerate, and pH), enzyme activity (amylase, α-glucosidase, protease, L-LDH, D-LDH, and i-LDH), and top 15 microorganisms at the genus level was performed. The Spearman's coefficients were calculated, in which $R > 0.8$ and $p < 0.05$ were retained. Gephi (version 0.9.7) was adopted for visualization. In

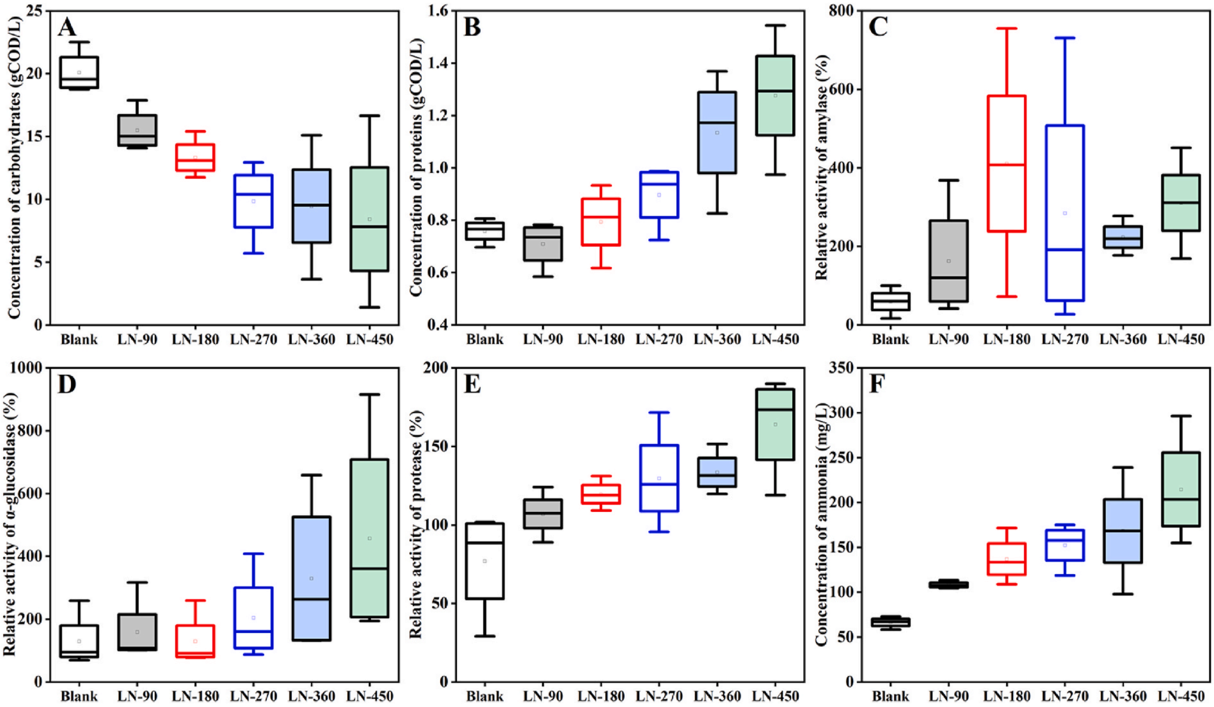

**Fig. 2.** Effect of leachate addition on the concentration of carbohydrate (A), protein (B), the relative activity of amylase (C), α-glucosidase (D), and protease (E), and the concentration of ammonia (F) in the first three days.

addition, the Mantel test investigated deep relationships using R (version 4.3.1).

### 2.7. Analytical methods

Chiral L-LA and D-LA were determined on an HPLC (LC-2030, Shimadzu) equipped with an Astec CLC-D column (5 μm, 15 cm × 4.6 mm). The wavelength of UV detection was 254 nm and the mobile phase was 5 mM $CuSO_4$ (1.0 mL/min). VFA, including acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, and n-valeric acid, was determined on a gas chromatograph (GC-2030, Shimadzu) equipped with a flame ionization detector and an INERTCAP column (30 m × 0.25 μm × 0.25 mm). The details of dissolved organic matter and humic acid were determined by 3DEEM (HITACHI, F-4700). VSS, TSS, $NH_4^+$-N, protein, carbohydrates, COD, pH, the activities of hydrolase (amylase, protease, α-glucosidase), and intracellular enzymes (L-LDH, D-LDH, i-LDH) were measured as described previously (Li et al., 2015, 2021; Xue et al., 2018; Zhang et al., 2020b).

### 2.8. Calculations

$$\text{T-LA} = [\text{L}] + [\text{D}] \quad (2)$$

$$\text{OA-L}\ (\%) = ([\text{L}] - [\text{D}]) / ([\text{L}] + [\text{D}]) \quad (3)$$

where [L] and [D] are the concentrations (g COD/L) of L-LA and D-LA, respectively.

### 2.9. Statistical analysis

All fermentation tests were performed in triplicate and results were expressed as mean ± standard deviation. Analysis of variance was used to test significant differences, with $p < 0.05$ being considered statistically significant.

## 3. Results and discussion

### 3.1. Landfill leachate drives FW fermentation into L-LA production with high OA

The maximum production of L-LA in the blank was 24.08 ± 0.69 g COD/L on day 6 and then kept stable, which was similar to our previous study (Li et al., 2021). Interestingly, after amendment with leachate, the highest production of L-LA was 31.30 ± 1.08 g COD/L (LN-90, day 6), 43.65 ± 0.57 g COD/L (LN-180, day 6), 44.60 ± 1.09 g COD/L (LN-270, day 5), 37.20 ± 0.74 g COD/L (LN-360, day 5), and 28.03 ± 1.03 g COD/L (LN-450, day 3) (Fig. 1A), respectively, representing a relatively high level a LA concentration (Song et al., 2022). It should be noted that L-LA was quickly consumed in the LN-270, LN-360, and LN-450. Different from L-LA generation, the production of D-LA was highest in the blank (17.28 ± 0.50 g COD/L, day 9) (Fig. 1B). This indicated that leachate possibly inhibited the D-LA fermentation pathway, thus intensifying L-LA generation. Due to the dominance of L-LA among chiral isomers, a similar trend to that of L-LA was observed in the production of T-LA (Fig. 1C). The average OA-L in the first 5 days was 82.23 ± 5.53 % (blank), 87.09 ± 1.91 % (LN-90), 92.40 ± 1.15 % (LN-180), 92.20 ± 0.94 % (LN-270), 70.98 ± 6.47 % (LN-360), and 54.55 ± 4.57 % (LN-450) (Fig. 1D), respectively. The declined OA-L after day 5 is related to the decreased concentration of L-LA (Fig. 1A) or the increased concentration of D-LA (Fig. 1B) as calculated by *Eq (3)*. To take the L-LA maximum production, OA-L, and stability of L-LA into account, the LN-180 was recommended as the preferable amendment.

Currently, the co-fermentation of FW and leachate mainly focuses on anaerobic digestion for methane production (Liu et al., 2023). Liu et al. (2022) reported that the addition of leachate provided $NH_4^+$ to avoid acidification inhibition and trace metals (Fe, Co, Mo, and Ni) for microbial growth, in which methane productivity reached 5.87 ± 0.45 L/(L*d). Zou et al. (2023) achieved a 94.4% improvement in specific methanogenic activity after leachate participation. Nevertheless, it is also believed that excess addition of leachate easily leads to the formation of chelates, restraining the methanogenesis process (Gao et al.,

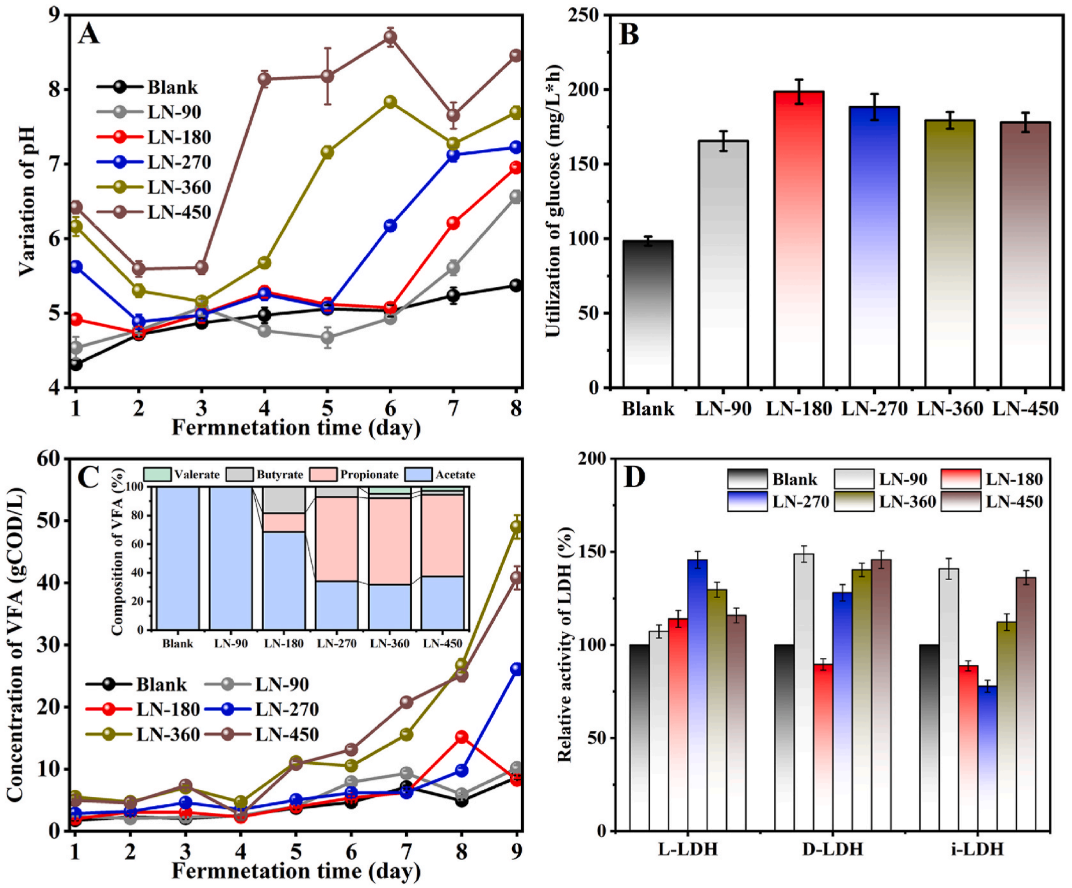

**Fig. 3.** Effect of leachate addition on the pH (A), glucose utilization (B), the production and composition of VFA (C), and the activity of L-LDH, D-LDH, and i-LDH (D).

2021; Song et al., 2023). From the point of view of the authors, this is the first study to investigate L-LA production from FW fermentation by the regulation of leachate. Compared to methane, value-added L-LA is a preferable product (Henard et al., 2016). LA is also a versatile platform molecule and easily be converted into acetate, propionate, butyrate, valerate, and even caproate (Shahab et al., 2020). In addition, this study adopted leachate to steer FW fermentation into L-LA with high OA (44.60 ± 1.09 g COD/L, 92.83 ± 0.03%), making it more promising as the precursor of the biodegradable plastics PLA (Rajeshkumar et al., 2021).

### 3.2. *Effect of landfill leachate addition on the solubilization and hydrolysis processes*

During the anaerobic fermentation of FW, the solid organics solubilized to liquid nutrient substances such as carbohydrates and protein (Wu et al., 2024). Normally, the determined concentration of the soluble organics was a balanced value between the solubilization and utilization, in which the first three days were considered as dominated by the solubilization process (Li et al., 2021). Similarly, carbohydrates were the dominant substrate compared to protein during FW fermentation, typically in China (Luo et al., 2021). During this period, the average concentration of carbohydrates in the blank (20.09 ± 0.68 g/L) is higher than that in the LN-90 (15.49 ± 0.58 g/L, $p > 0.05$), LN-180 (13.33 ± 0.48 g/L, $p < 0.05$), LN-270 (9.85 ± 0.35 g/L, $p < 0.05$), LN-360 (9.45 ± 0.39 g/L, $p < 0.05$), and LN-450 (8.42 ± 0.27 g/L, $p > 0.05$) (Fig. 2A, time curves in Fig. S1). This indicates that the addition of leachate might weaken the solubilization or enhance the utilization of carbohydrates. The addition of landfill leachate was also advantageous to the accumulation of protein especially in LN-270, LN-360, and LN-450 (Fig. 2B, time curves in Fig. S2). The concentration of protein in the FW fermentation was relatively low (<1.6 g COD/L) compared to the carbohydrates (Fig. 2A), potentially contributing less during the subsequent LA generation.

Nevertheless, solubilized carbohydrates and proteins are macromolecular substances, that can not be directly utilized by functional microorganisms. Amylase, α-glucosidase, and protease are crucial hydrolases, that could convert macromolecular polysaccharides and proteins into micromolecular monosaccharides and amino acids (Li et al., 2021). Amylase is typically for the hydrolysis of starch, in which the average relative activity was increased by 174.7% (LN-90, $p > 0.05$), 594.4% (LN-180, $p > 0.05$), 381.6% (LN-270, $p > 0.05$), 277.9% (LN-360, $p < 0.05$), 425.2% (LN-450, $p < 0.05$), respectively, compared to the blank (Fig. 2C, time curves in Fig. S3). This indicated that the landfill leachate amendment is beneficial to the hydrolysis of carbohydrates, which could be further supported by the enhanced activity of α-glucosidase (Fig. 2D, time curves in Fig. S4). Besides, improved protease activity was also observed with landfill leachate addition (Fig. 2E, time curves in Fig. S5).

Importantly, the inclusive ammonia in the landfill leachate also was reported to improve the solubilization and hydrolysis (Zhang et al., 2020b; Zou et al., 2023). In this study, the average concentration of ammonia was 66.24 ± 2.45 mg/L (Blank), 108.05 ± 4.35 mg/L (LN-90), 136.80 ± 4.92 mg/L (LN-180), 152.26 ± 6.18 mg/L (LN-270), 168.17 ± 6.18 mg/L (LN-360), and 214.49 ± 7.41 mg/L (LN-450) (Fig. 2F, time curves in Fig. S6). Interestingly, a part of ammonia was converted into nitrate, which also observed in our previous ammonia amendment fermentation system (Zhang et al., 2020b). This is possibly related to the operation of intermittent alkaline supplements, which introduced part of dissolved oxygen to react with ammonia. The average of nitrate was

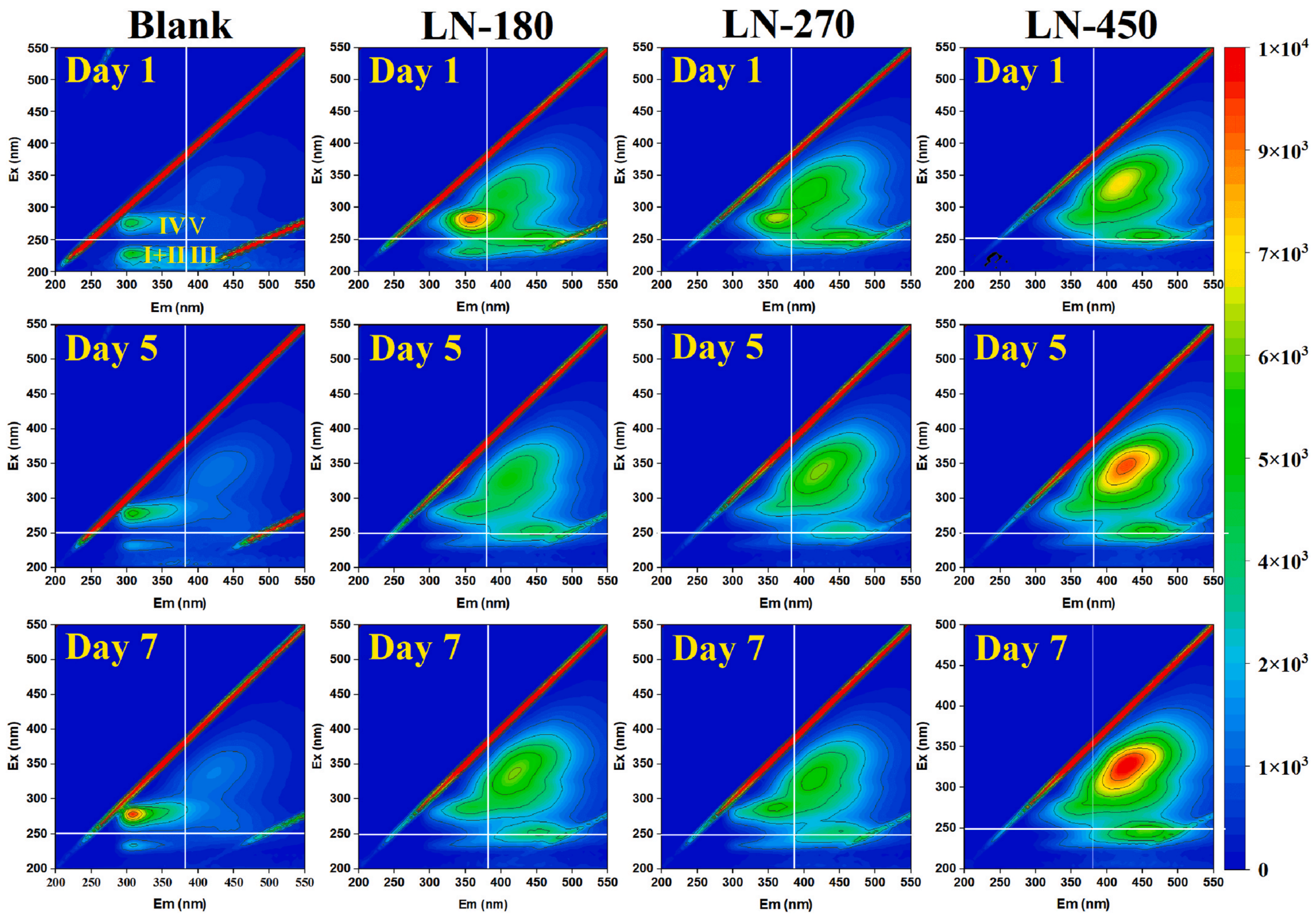

**Fig. 4.** 3DEEM mapping of fermentation broth in the blank, LN-180, LN-270, and LN-450 on day 1, day 5, and day 7.

linear to the initial ammonia addition as follows: LN-450 > LN-360 > LN-270 > LN-180 > LN-90 > Blnak (Fig. S7). To consider the sum of ammonia and nitrate, the content of nitrogen surpasses the theoretical value in the Blank, LN-90, and LN-180, which might be related to the release from FW or disruption of WAS. However, they are lower than the theoretical value in the LN-270, LN-360, and LN-450, suggesting a higher nitrogen utilization. In high dosages of landfill leachate, microorganisms possibly secreted more extracellular polymeric substances to resist the adverse conditions, requiring nitrogen for protein synthesis (Nouha et al., 2018).

### *3.3. Regulation of acidification process and the activity of LDH by landfill leachate*

Carbohydrates are the dominant substrate during FW fermentation in this study (Fig. 2A). Subsequently, carbohydrates are hydrolyzed to sugar and metabolized to generate carboxylic acids including VFA and LA (Wu et al., 2020; Song et al., 2024), resulting in a decrease of pH in the fermentation broth. As shown in Fig. 3A, in the first three days, the average pH was 4.63 ± 0.23 (Blank), 4.79 ± 0.22 (LN-90), 4.88 ± 0.11 (LN-180), 5.16 ± 0.32 (LN-270), 5.54 ± 0.44 (LN-360), and 5.87 ± 0.39 (LN-450), suggesting a higher pH with landfill leachate amendment. It has been reported that the inclusive ammonia in the landfill leachate played a crucial role as a buffer to regulate fermentative pH (Lv et al., 2021), avoiding the reversely undissociated carboxylic acids inhibition under low pH (Xu et al., 2022). Compared to the blank with a long-duration acidification process (5.37 ± 0.06 on day 8), the fermentative pH with landfill leachate addition quickly rose to 6.56 ± 0.09 on day 8 (LN-90), 6.95 ± 0.04 on day 8 (LN-180), 7.12 ± 0.09 on day 7 (LN-270), 7.83 ± 0.07 on day 6 (LN-360), and 8.14 ± 0.11 on day 4 (LN-450) (Fig. 3A). This indicated that the participate of landfill leachate could efficiently accelerate the fermentation process, while the production of LA relied on proper landfill leachate optimization.

During acidification, the glucose is first metabolized to the crucial intermediate, pyruvic acid, well-known as the glycolysis process (Li et al., 2021). In the fermentation of pure glucose, the utilization rate of glucose was 98.39 ± 3.02 mg/(L*h) in the blank, which was significantly ($p < 0.05$) increased to 165.57 ± 6.61 mg/(L*h) in the LN-90, 198.66 ± 8.14 mg/(L*h) in the LN-180, 188.38 ± 8.71 mg/(L*h) in the LN-270, 179.38 ± 5.60 mg/(L*h) in the LN-360, and 178.04 ± 6.51 mg/(L*h) in the LN-540 (Fig. 3B). This indicated an enhanced glycolysis process with landfill leachate amendment, possibly related to the inclusive ammonia and trace metals supplement to enhance the metabolic activity (Ye et al., 2018a; Zhang et al., 2020b). The intermediate pyruvic acid is further converted into competitive VFA with LA (Li et al., 2015). The VFA concentration was lower than 13.5 g COD/L in the first 6 days in all reactors. The final VFA concentration on day 9 in the blank, LN-90, and LN-180 was similar at around 8–10 g COD/L, while it increased to 26.11 ± 0.94 (LN-270), 49.04 ± 1.88 (LN-360), and 40.81 ± 1.86 (LN-450). The high concentration of VFA in the LN-270, LN-360, and LN-450 was in line with the quick consumption of LA from day 6 to day 9 (Fig. 1A). In addition, the main content of VFA is acetate in the blank, LN-90, and LN-180 (>69%), while propionate accounted for around 57–60% in the LN-270, LN-360, and LN-450 (Fig. 3C). Zou et al. (2023) also reported a propionate-dominated VFA during co-digestion of FW with landfill leachate for methane production. This might be related to the acetate consumption due to acetoclastic methanogenesis (Pan et al., 2021). Unfortunately, the biogas was not collected in this study, which requires investigation for further evaluation.

The relative activity of L-LDH, the crucial enzyme for L-LA generation (Li et al., 2015), was increased by 7.29 ± 0.03% (LN-90), 14.06 ± 0.05% (LN-180), 45.74 ± 0.05% (LN-270), 29.70 ± 0.04% (LN-360), and 15.91 ± 0.04% (LN-450) compared to the blank (Fig. 3D). This is in line with the high production of L-LA in this study (Fig. 1A). Nevertheless, the enhanced relative activity of D-LDH contradicted (except LN-180) to the decreased D-LA production (Fig. 1B). This is possibly related to the unbalanced reactions between D-LA generation and consumption. In addition, the low activity of i-LDH, the crucial enzyme for L-LA consumption (Li et al., 2015), was relatively low in the LN-180 and LN-270. This indicates a low LA consumption rate and explains its higher accumulation of LA (Fig. 1C).

### *3.4. Potential effect of other inclusions in the landfill leachate on LA generation*

It should be noted that ammonia is an important basis for the calculation of landfill leachate addition to run the FW fermentation under different landfill leachate amendments. The variation of ammonia was determined and analyzed in section 3.2. While the other inclusions within landfill leachate such as metals and refractory organic matter also potentially play crucial roles during the LA-related pathways.

For the metal inclusions, the trace metals in landfill leachate accounted for around 15–421 mg/L of Fe, 0.04–12.8 mg/L of Co, 2.26 mg/L of Mo, and 0.19–13.4 mg/L of Ni, which are key stimulators for the growth of fermenters (Lv et al., 2021). For example, our previous finding has revealed that the participation of Fe could enhance LA production, and the process of Fe(II)→Fe(III) was strongly related to the isomerization of L-LA to D-LA (Li et al., 2017). Metal Na and K are also typical characterizations of landfill leachate, resulting in a high salinity. We also proved that high salinity at 30 g/L of NaCl could inhibit the generation of D-LA, achieving an optical pure L-LA production (Li et al., 2021). For the hazardous heavy inclusions, we found that a proper addition of copper is also beneficial to LA production during FW fermentation (Ye et al., 2018b).

Besides metal inclusions, refractory organic matters including humic/fulvic-like components are also a typical component of landfill leachate (Priyanka & Saravanakumar, 2023). 3D EEM is extensively used for the characterization of dissolved organic matter during organic waste fermentation (Li et al., 2020). As suggested by He et al. (2013), the 3DEEM spectra could be divided into five regions, in which regions I, II, and IV are protein-like fluorescence regions derived from protein-like and phenolic compounds while regions III and V belong to the fulvic- and humic-like fluorescence. As shown in Fig. 4, the spectra density in regions I, II, and IV was relatively low, indicating a low concentration of protein compounds. This is in line with the determination of protein titration in Fig. 2B. Interestingly, the humic-like fluorescence in region V increased with the addition of landfill leachate on day 1, which further increased with the progress of the fermentation process till day 7. It has been reported that landfill leachate was characterized as rich in humic substances (Gu et al., 2019). Importantly, humic acids can enhance the hydrolysis (α-glucosidase and protease) and electron transfer, thus leading to increased performance of anaerobic digestion (Zou et al., 2023). In this study, the inclusive humic acids in landfill leachate might also play a role during optical L-LA generation from FW.

More importantly, heavy metals and toxic organic compounds easily combine and form chelates (Lei et al., 2019), which might restrain the methanogenesis process (Gao et al., 2021; Song et al., 2023), thus being beneficial to the accumulation of LA. In addition, ammonia, metals, and refractory organic matter might also play synergistic effects on FW fermentation for LA production, requiring further investigations.

**Table 1**
α-diversity indices of microbial samples under different landfill leachate addition during FW fermentation (Higher Chao1, Shannon, Simpson indices indicate higher richness and diversity).

| | Good's coverage | Chao1 | Shannon | Simpson |
|---|---|---|---|---|
| Blank | 0.99 | 659.61 | 5.08 | 0.84 |
| LN-90 | 0.99 | 418.54 | 4.40 | 0.80 |
| LN-180 | 0.99 | 489.77 | 4.87 | 0.82 |
| LN-270 | 0.99 | 463.17 | 4.70 | 0.81 |
| LN-360 | 0.99 | 615.63 | 4.06 | 0.79 |
| LN-450 | 0.99 | 898.25 | 5.65 | 0.94 |

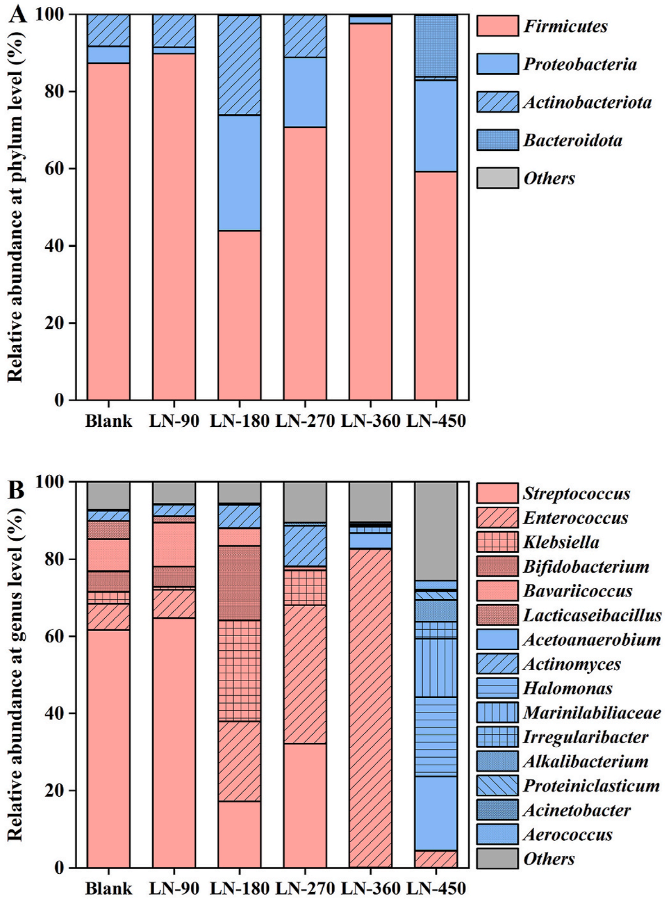

**Fig. 5.** Microbial community at the phylum level (A) and genus level (B) under different dosages of landfill leachate on day 6 (the red legends in genus level belong to LAB). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### *3.5. Effects of landfill leachate dosage on the microbial community structure*

The α-diversity indices are listed in Table 1. The Good's coverage was 0.99 in all reactors, indicating convincing sequencing results. The Chao1 index analysis found that the richness declined in the LN-90, LN-180, LN-270, and LN-360 while increased in LN-450. Similar trends were found in the microbial diversity indices (Shannon and Simpson). It is thus speculated that the proper addition of landfill leachate is possibly beneficial to some special microorganism enrichment, thus leading to decreased richness and diversity.

The production of LA from FW fermentation is highly dependent on the microbial community (Xue et al., 2018). At the phylum level, the main microflora were *Firmicutes*, *Proteobacteria*, *Actinobacteriota*, and *Bacteroidota* (Fig. 5A), which commonly existed in WAS and played a

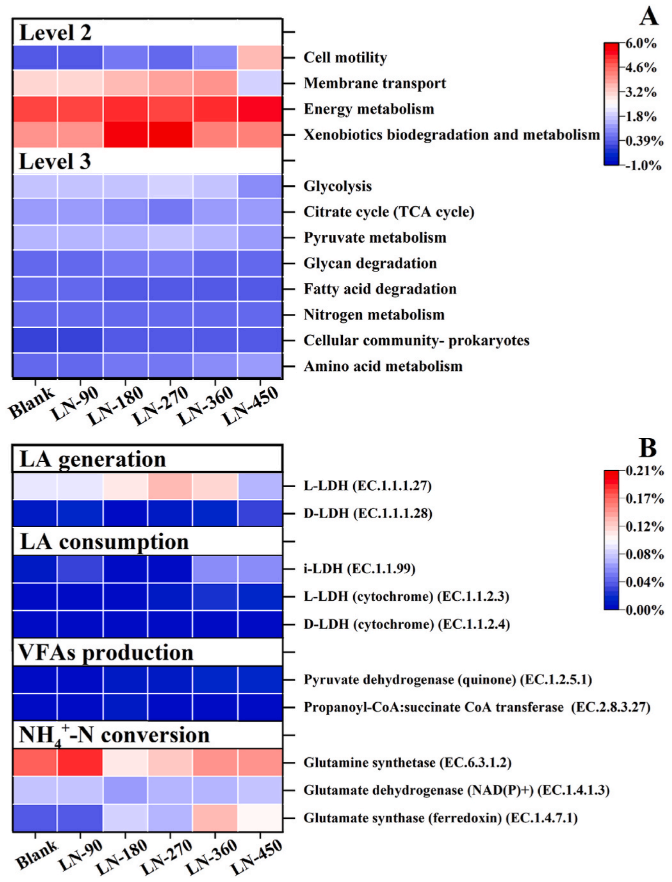

**Fig. 6.** Relative activity of functional pathways at KEGG level 2 and level 3 (A) and metabolic genes (LA generation, LA consumption, VFA production, and $NH_4^+$-N conversion) based on the KEGG database under different dosages of landfill leachate.

crucial role in the degradation of complex organics (Ye et al., 2018b; Li et al., 2021). The relative abundance of *Firmicutes* declined, while *Proteobacteria* and *Actinobacteriota* increased in the LN-180 and LN-270 compared to the blank. Considering the optimal LA production in such dosage of landfill leachate (Fig. 1), *Proteobacteria* and *Actinobacteriota* might play a key role here. At the genus level, LAB including *Streptococcus*, *Enterococcus*, *Klebsiella*, *Bifidobacterium*, *Bavariicoccus*, and *Lacticaseibacillus* (Feng et al., 2017; Zhang et al., 2020b; Bian et al., 2024) accounted for 89.80% (Blank), 91.08% (LN-90), 88.00% (LN-180), 78.11% (LN-270), and 82.75% (LN-360) while decreased to only 4.45% (LN-450) (Fig. 5B). The low abundance of LAB in LN-450 is probably related to the high addition of landfill leachate, which introduces more toxic substances, reaching the inhibition threshold point for LAB. Genus *Acetoanaerobium*, responsible for acetic acid production (Sangavai and Chellapandi, 2019), was enriched in the LN-450, possibly related to the low concentration of LA in high dosages of landfill leachate. In addition, the genus *Halomonas* and *Marinilabiliaceae*, the salt-resistant microflora (Williamson et al., 2016; Huang et al., 2024), were enriched to 20.55% and 15.18% in the LN-450, respectively.

### 3.6. *Alteration of functional genes related to LA metabolism by leachate steering*

To better elucidate the functions of the microbial community amended by different landfill leachates, functional pathways at KEGG level 2 and level 3 were predicted by the PICRUSt based on the 16 S rRNA gene sequencing data (Ye et al., 2018b; Zou et al., 2019). As shown in Fig. 6A, the KEGG level 2 pathways including cell motility, membrane transport, energy metabolism, and xenobiotics biodegradation and metabolism were enhanced by landfill leachate addition, especially in the LN-180 and LN-270. Zhang et al. (2020b) have reported that the inclusive ammonia in the leachate was able to increase the relative activity of cell motility and membrane transport, which is consistent with this study, finally facilitating the production of LA. The enhanced energy metabolism involves the generation of energy (ATP), which might also drive the fermentation to the LA direction. The increased activity of xenobiotics biodegradation and metabolism could enhance the degradation of exogenous toxins (Singleton, 2004), thus favoring organic matter degradation and maintaining stable fermentation processes for LA. Regarding the KEGG level 3 pathways, the glycolysis pathway was enhanced by landfill leachate addition, which was in line with Fig. 3B. The enhanced fatty acid degradation pathway by landfill leachate addition could also explain its higher accumulation of VFA in Fig. 3C. In addition, the activity of nitrogen metabolism and amino acid metabolism was also increased in LN-270, which possibly contributed the ammonia accumulation in Fig. 2F, further impacting the LA metabolism.

The specific gene abundance of LA generation, LA consumption, VFA production, and $NH_4^+$-N conversion was exhibited in Fig. 6B. The relative abundance of L-LDH increased by 4.62% (LN-90), 29.17% (LN-180), 46.18% (LN-270), 32.92% (LN-360), which was in line with the enzyme activity in Fig. 3D. However, the decreased activity of L-LDH enzyme contradicted with the gene abundance in LN-450, which requires further investigation, as well as D-LDH. A similar trend was observed between the activity of the i-LDH enzyme and the gene abundance, further indicating a low LA consumption in LN-180 and LN-270. The total relative abundance of pyruvate dehydrogenase (EC.1.2.5.1) and propanoyl-CoA: succinate CoA transferase (EC.2.8.3.27), responsible for the acetic acid and propionic acid production, was much lower than the LDH abundance. This is consistent with the dominant LA production in this study. The function of gene glutamate synthase (ferredoxin) (EC.1.4.7.1), encoding for nitrogen assimilation, was enhanced by landfill leachate addition. In our previous study, an enhanced glutamate synthase was also observed in an ammonia amendment fermentation system along with an increased LA production (Zhang et al., 2020b). This further indicates that inclusive ammonia might play a key role during the LA fermentation from FW in this study.

### 3.7. *Networks in landfill leachate amendment fermentation system*

Networks among landfill leachate addition, T-LA, L-LA, D-LA, metabolites, enzyme activity, and the top 15 microorganisms at the genus level were constructed. The topological metrics are listed as follows: edge at 194, node at 32, average degree at 12.13, average weighted degree at 10.93, diameter at 6, average path length at 1.99, density at 0.39, and average clustering coefficient at 0.74, suggesting a successful construction of networks (Fig. 7A).

Landfill leachate addition was significantly negatively correlated with D-LA ($r = -1.00$, $p < 0.05$), suggesting an adverse effect of landfill leachate on D-LA generation. This is in line with the decreased production of D-LA with landfill leachate addition in Fig. 1B. In addition, positive relationships were observed between landfill leachate and α-glucosidase ($r = 0.94$, $p < 0.05$) and protease ($r = 1.00$, $p < 0.05$). This indicates that landfill leachate could improve the activity of hydrolase, which also follows the finding in Fi.g. 2D and 2E. Landfill leachate addition was also significantly negatively correlated with LAB *Streptococcus* ($r = -0.89$, $p < 0.05$), *Bavariicoccus* ($r = -0.94$, $p < 0.05$), and *Lacticaseibacillus* ($r = -0.97$, $p < 0.05$), while it was beneficial to the enrichment of LAB *Enterococcus* and *Klebsiella* (Fig. 5B). The enriched key LAB *Klebsiella* was significantly positively correlated with acetogenesis *Actinomyces* ($r = 0.89$, $p < 0.05$). However, the specific relationship between *Klebsiella* and *Actinomyces* requires pure culture experiments to validate. These relationships were further exhibited by the mental test (Fig. 7B).

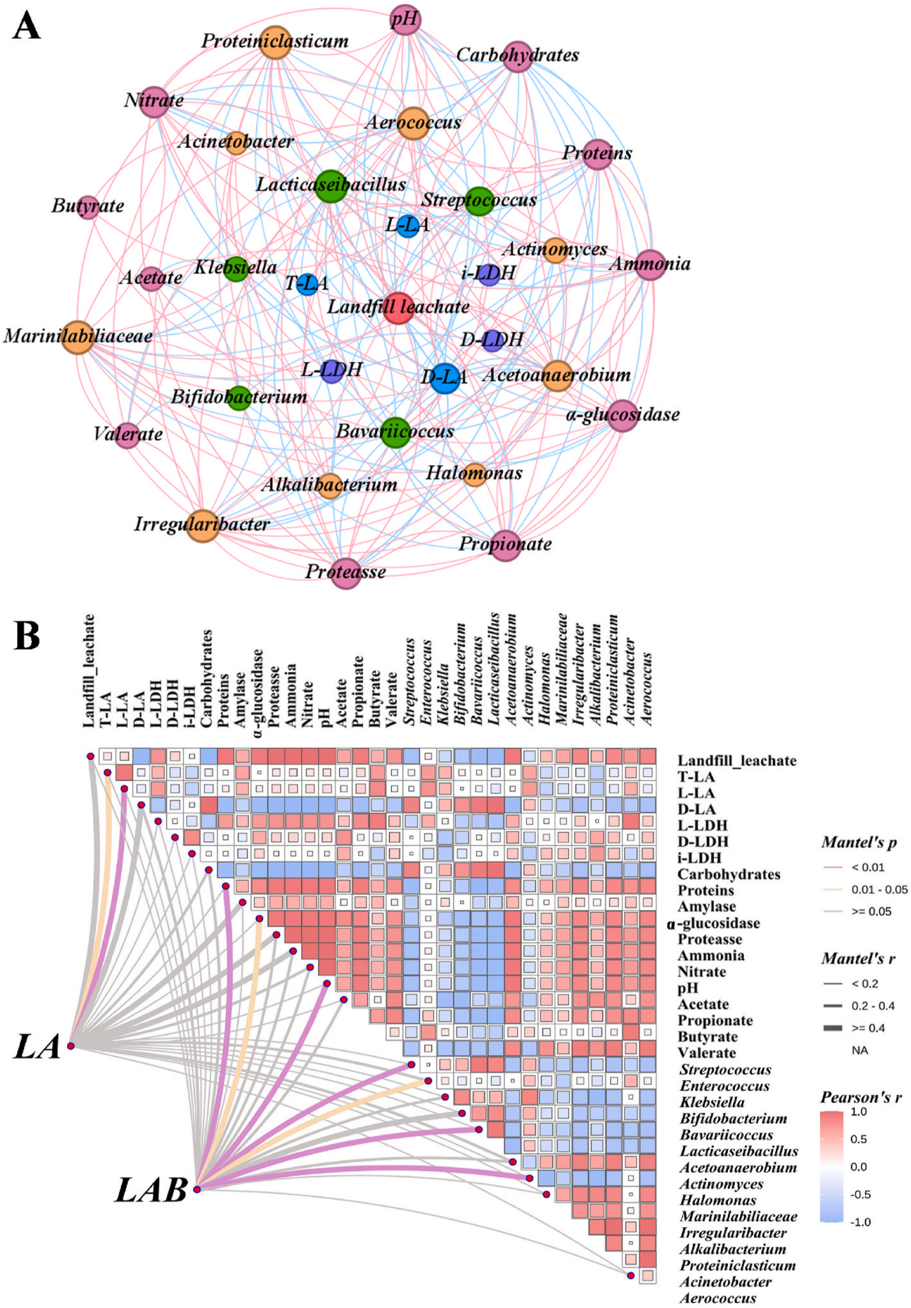

**Fig. 7.** Network analysis among landfill leachate addition, T-LA, L-LA, D-LA, metabolites, enzyme activity, and top 15 microorganisms at the genus level (A). The size of the nodes is proportional to the degree of the node. Red lines indicate positive interactions and blue lines indicate negative interactions. Mantel test of landfill leachate addition, T-LA, L-LA, D-LA, metabolites, enzyme activity, and top 15 microorganisms at the genus level (B). The color gradient indicates the size of the Spearman correlation coefficient ($p < 0.05$). The edge width corresponds to the size of the distance correlation and the edge color denotes the statistical significance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### 3.8. *Prospect and implication*

The recycling of FW with value-added products to replace the low-economy methane biogas is urgently chasing. Before methanogenesis, LA is generated during the acidification process during FW fermentation (Li et al., 2015). Among them, LA is widely employed in the pharmaceutical, food, textile, and chemical industries (Shahab et al., 2020; Askarniya et al., 2023), especially as the precursor of biodegradable plastic PLA (Sun et al., 2022; Ali et al., 2023). The recovery of LA from FW follows the concept of a circular economy. The expected LA production is around 4 million tons by 2025 with a market price of \$1300–1600/ton. The payback period of the LA recycling from FW is six years and the return on investment is around 20%. Currently, 90% of lactic acid is produced by microbial fermentation (Song et al., 2022). In China, the government encourages such green, economical, and promising technology based on the policies of a zero-waste city.

However, FW fermentation is normally limited by VFA inhibition, trace metals deficiency, and poor digestate dewaterability. Landfill leachate has been proven to be a promising co-substrate with FW (Lv et al., 2021). The landfill leachate can provide ammonia as a buffer for stable pH to overcome VFA inhibition and trace metals to stimulate the activity of functional microorganisms (Zhang et al., 2020b; Lv et al., 2021). In addition, large amounts of divalent cations ($Ca^{2+}$ and $Mg^{2+}$) in landfill leachate were introduced to the fermentation system, which can

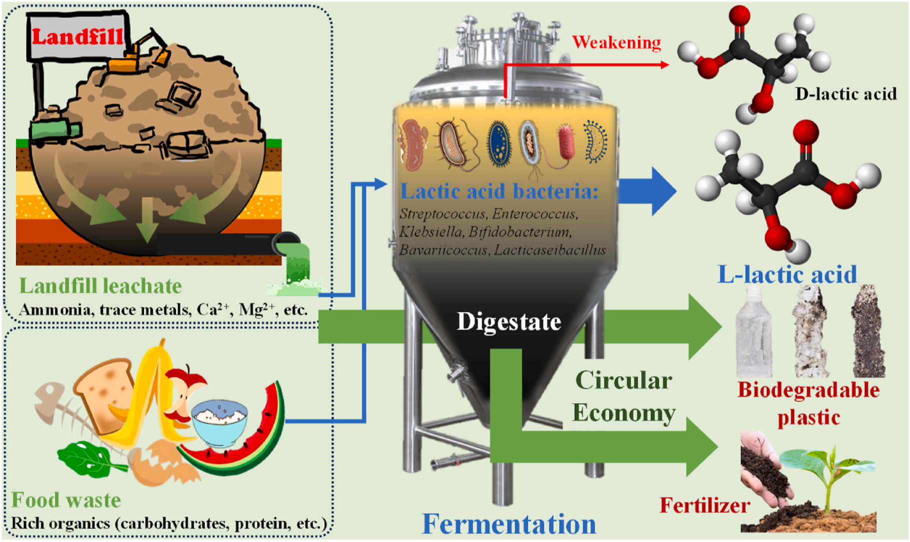

**Fig. 8.** Schematic of FW fermentation for LA production under a circular economy concept by the amendment of landfill leachate.

also improve the dewatering of the digestate (Lv et al., 2021), thus simplifying the subsequent separation process. Such amendment is environmentally sustainable and consistent with the concept of treating waste with waste. Finally, LA could be recovered from the separated fermentation broth by solid phase extraction, solvent extraction, reactive extraction, membrane extraction, in situ removal with anion exchange resin, electro-electrodialysis, chromatography, precipitation, molecular distillation as summarized by Song et al. (2022). Currently, research has successfully reported the generation of LA from organic fermentation. Future work should focus more on the separation and purification process, and the recycling of digestate composting as fertilizer to exploit the value-added product from FW to further support the circular economy (Fig. 8).

## 4. Conclusion

Varying levels of LA production were observed across different treatments. LA production reached 24.08 ± 0.69 g COD/L in the control sample (Blank, day 6), while supplementation with landfill leachate (LN-90, LN-180, LN-270, LN-360) led to increased LA production, peaking at 44.60 ± 1.09 g COD/L (LN-270, day 5). However, the highest dose of landfill leachate (LN-450) resulted in a decrease in LA production, yielding 28.03 ± 1.03 g COD/L (LN-450, day 3). Analysis of the optical activity of L-lactic acid (OA-L) revealed similar trends, with the highest OA-L observed in the LN-180 treatment (92.40 ± 1.15 %), and a decrease in the LN-270 (92.20 ± 0.94 %), LN-360 (70.98 ± 6.47 %), and LN-450 (54.55 ± 4.57 %).

Fermentation pathway analysis indicated that landfill leachate amendment is beneficial for hydrolysis and glycolysis, and plays a crucial role as a buffer to maintain optimal pH conditions (5–7), reducing volatile fatty acid production and thus intensifying LA orientations. The observed increase in L-LDH activity and decrease in LA consumption further explained the high accumulation of L-LA in the presence of landfill leachate. Analysis of the microbial community composition revealed significant shifts, with LAB including *Streptococcus*, *Enterococcus*, *Klebsiella*, *Bifidobacterium*, *Bavariicoccus*, and *Lacticaseibacillus* dominating in most treatments. However, the LN-450 treatment exhibited a drastic decrease in LAB abundance (4.45%), potentially contributing to the observed decrease in LA production. Functional gene analysis further supported the enhanced glycolysis, L-lactate dehydrogenase, and nitrogen assimilation (related to ammonia) in the presence of landfill leachate. A network analysis explored potential relationships among landfill leachate addition, LA production, metabolites, enzyme activities, and key microorganisms, providing valuable insights into the complex dynamics of FW fermentation in the presence of landfill leachate.

## CRediT authorship contribution statement

**Wenjuan Zhang:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Jiaxin Shi:** Writing – original draft, Visualization, Validation, Methodology, Investigation. **Yue Li:** Validation, Investigation. **Yonghong Ma:** Visualization, Methodology, Investigation. **Aisha Khan Khanzada:** Writing – review & editing. **Hussein E. Al-Hazmi:** Writing – review & editing. **Xianbao Xu:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization. **Xiang Li:** Writing – review & editing, Conceptualization. **Gamal Kamel Hassan:** Writing – review & editing. **Gang Xue:** Resources, Conceptualization. **Jacek Makinia:** Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.122497.

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