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# The Effect of pH on the Production and Composition of Short- and Medium-Chain Fatty Acids from Food Waste in a Leachate Bed Reactor at Room Temperature

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**Abstract:** This study evaluated the hydrolysis and acidogenesis of food waste at different operating pHs (uncontrolled, 5.5, 6.5, 7.5, 8.5) in a leachate bed reactor (LBR) at room temperature. LBR operation at pH 6.5–8.5 resulted in a hydrolysis yield of 718–729 g SCOD/kg $VS_{added}$, which was statistically ($p \leq 0.05$) higher than that obtained at pH 5.5 (577 g SCOD/kg $VS_{added}$) and the uncontrolled pH (462 g SCOD/kg $VS_{added}$). The hydrolysis rate at pH 6.5 was the highest amongst all the pH values. Stabilization at pH at 6.5 also resulted in a high fatty acid (FA) yield of 643 g $COD_{FA}$/kg $VS_{added}$. Butyrate was the main FA at the pH of 5.5–6.5, while acetate was the main FA at the pH of 7.5–8.5. At the uncontrolled pH, lactate production was the highest, indicating a shift in the microbial community from fatty-acid-producing bacteria to lactate-producing bacteria. The compositions of medium-chain fatty acids, such as caproate, were the highest at pH of 5.5.

**Keywords:** food waste fermentation; leachate bed reactor; pH; short-chain fatty acids; medium-chain fatty acids

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## 1. Introduction

Over 2 billion tons of municipal solid waste (MSW) was generated globally in 2016. At the current rate of generation, MSW is anticipated to further increase by 69% to reach 3.40 billion tons by 2050 [1,2]. A large portion of MSW is food waste, constituting up to 45% of MSW [1,2]. The main components of food waste are fruits and vegetables, which are disposed in large quantities by local markets and grocery stores. The bulk of the food waste in many developing and developed countries is disposed of through landfilling, resulting in adverse health and environmental effects including greenhouse gas (GHG) emissions, the contamination of subsurface environments and loss of habitats [3,4]. Therefore, sustainable approaches for managing food waste are being intensively researched. The conversion of food waste to fatty acids via the acidogenic fermentation process is an emerging biotechnology that combines the sustainable management of food waste with resource recovery.

Acidogenic fermentation is carried out by a consortium of bacteria (mixed microbial culture) under anaerobic conditions to produce different fatty acids (FAs) from heterogenous waste such as food waste through multi-step concurrent biochemical reactions. Fatty acids with 2–5 carbon atoms (e.g., acetate, propionate, butyrate, valerate) are categorized as short-chain fatty acids (SCFAs), and those with 6–8 carbon atoms (e.g., caproate, hexanoate, etc.) are called medium-chain fatty acids (MCFAs) [5–7]. FAs (SCFAs and MCFAs) are industrially important chemicals that are currently derived from petrochemicals causing

substantial GHG emissions [1,8–10]. These are widely used in pharmaceutical, chemical, food processing, cosmetic, textile, paint and other industries. Additionally, these FAs can be used as a substrate in the microbial production of bioplastics and biofuels [11–15]. Comparatively, MCFAs have higher economical value than SCFAs because of their higher carbon to oxygen ratio (C:O) and energy potential [16].

Dry fermenters such as leachate bed reactors (LBRs) are being widely studied as an energy-efficient and cost-effective bioreactor platform for the production of FAs. Unlike the commonly used continuously stirred tank reactors (CSTRs), LBRs can handle higher solid contents (30–40% of total solid). Consequently, no dilution of food waste is required (no process water). Furthermore, no mechanical stirring is required in LBRs, which significantly reduces energy consumption. An added advantage of the LBR design is the separation of the degraded food waste from FAs containing broth, which eliminates or reduces the downstream costs associated with solid–liquid separation [17,18].

The operating parameters of LBRs significantly impact FA production (yield) and the percentage composition of individual FAs (e.g., the composition of acetate, butyrate and propionate) during acidogenic fermentation. Amongst the different operating parameters, pH is one of the most crucial parameters affecting FA production and composition, since pH has an impact on the microbial community's composition and metabolic activity of the bacteria [12,18,19]. Many studies have investigated the impacts of pH on the production and composition of FAs from food waste in LBRs [18,20–24]. However, FA compositional analysis in these studies has been limited to primarily three SCFAs, namely, acetate, propionate and butyrate. The production of other SCFAs, such as iso-butyrate, n-valerate and iso-valerate, as well as MCFAs (i.e., n-caproate, iso-caproate, heptanoate), has not been extensively analyzed. In addition to limited compositional analysis, most of the studies on LBRs have tested food waste fermentation at temperatures above 35 °C. Maintaining LBRs at such temperatures (>35 °C) requires external heating, which can significantly impact the net energy gain as well as the reduction in GHG emissions. Therefore, the characterization of SCFAs and MCFAs under room conditions (i.e., without external heating) is of interest. However, there is limited information in the literature in this regard. This study was performed to fill these research gaps.

This study evaluated the impacts of five different pHs (uncontrolled, 5.5, 6.5, 7.5 and 8.5) on the full range of SCFAs (acetate, propionate, butyrate, iso-butyrate, n-valerate, iso-valerate) and MCFAs (n-caproate, iso-caproate, heptanoate) produced from food waste in an LBR at the room temperature of 22 °C. Firstly, the hydrolysis yields and rates were compared at different pHs. Secondly, the production and composition of SCFAs and MCFAs were analyzed to elucidate the impacts of pH on the range of acidogenic products obtained from food waste, and finally, the microbial community composition was analyzed to elucidate the hydrolytic and fermentative bacteria at different pHs.

## 2. Material and Methods

### *2.1. Characteristics of Food Waste and Inoculum*

Simulated food waste was used in this study because of the closure of restaurants, cafeterias and commercial centers due to the COVID-19 pandemic. The simulated food waste consisted of (weight basis) 13% apples, 7% bananas, 17% capsicums, 14% tomatoes, 26% potatoes and 22% guavas with a total solids (TS) content of 13.23 ± 0.20% and volatile solids (VS) content of 9.86 ± 0.25%. To prevent degradation, each component of the food waste was stored at −10 °C until the time of use for the experiments. The required quantity of an individual component of food waste was defrosted at room temperature (22 °C) for two hours before use for the experiments. The food waste was then immediately shredded to an average particle size of 5–10 mm.

Anaerobic digestion sludge (AD-sludge) was used as the inoculum in this study. The AD sludge was collected from an anaerobic wastewater treatment plant (Ulu Pandan Water Reclamation Plant, Singapore). The AD sludge was filtered to remove solid particles with a size of over 10 mm so as to prevent the clogging of the LBR and then stored at 4 °C until use.

To kill the methanogens, the AD sludge was heated at 75 °C for 15 min before addition to the LBR. The AD sludge contained a TS content of 1.68 ± 0.04% and VS content of 1.08 ± 0.02%.

### *2.2. LBR Design*

A cylindrically shaped LBR was used in this study, which was fabricated using acrylic materials. The LBR was cylindrical with a height of 620 mm and a diameter of 130 mm; thus, the total volume of the LBR was 8 L (volume of a cylinder). The LBR comprised three sections (Figure 1A): (1) a top section with a headspace of 2.5 L, (2) a middle section with a food-waste-holding basket of 1.5 L and (3) a bottom section with leachate-holding bed of 4 L. The headspace was equipped with a detachable cover with a customized sprinkler nozzle and a gas collection port. The gas produced during acidogenic fermentation was collected in the gas collection bag (Tedlar Multilayer Gas Sampling Bags, 10 L) connected to the gas collection port. The food-waste-holding basket was made of stainless steel with a height of 185 mm and a diameter of 100 mm. The side wall and base of the food-waste-holding basket were perforated with a pore size of 4 mm to enable the percolation of the leachate into the leachate-holding bed while preventing food waste particles from entering the leachate-holding bed (Figure 1B). The leachate-holding bed had side sampling ports to collect the leachate samples for different analyses. The pH probe was installed on the retaining wall of the leachate-holding bed to monitor the pH using a pH controller (MODEL MC122, Milwaukee, WI, USA). The pH controller was connected to a dosing pump to adjust the pH of the leachate to the desired level by injecting 1 M NaOH. The leachate in the leachate-holding bed was gently mixed using a peristaltic pump (Masterflex Standard Digital Drive, Model 77200-62, Cole Parmer, Vernon Hills, IL, USA). Another peristaltic pump (Masterflex Digital Economy Drive, Model 77800-62, Cole Parmer, Vernon Hills, IL, USA) was used to recirculate and spray the leachate from the bottom of the leachate-holding bed to the food-waste-holding basket. The recirculation and mixing of the leachate with the peristaltic pumps were controlled using a timer (33 Multifunction timer relay, RS pro, Singapore). To ensure anaerobic conditions inside the LBR, the joints of the LBR had O-rings and rubber gaskets.

### *2.3. LBR Experimental Procedure*

All LBR experiments were performed using the same procedure unless otherwise specified. The LBR was operated in batch mode at room temperature (22 °C) for a fixed period of 14 days. For each run, 1.5 kg of food waste was loaded into the food-waste-holding basket along with 0.6 L of heat-treated AD sludge in the leachate-holding bed, with an inoculum to substrate ratio (ISR) of 4%. This loading of food waste (VS of 98.63 g/kg) and AD sludge (VS of 10.81 g/kg) provided a volumetric organic loading of 19 g VS/$L_{reactor}$ for each LBR run. Nitrogen gas was purged from the LBR to ensure anaerobic conditions inside the LBR. The impacts of pH on hydrolysis and acidification (SCFA and MCFA production) were evaluated by operating the LBR at different pHs: uncontrolled pH (designated as LBR-UC), 5.5 ± 0.5 (designated as LBR-5.5), 6.5 ± 0.5 (designated as LBR-6.5), 7.5 ± 0.5 (designated as LBR-7.5) and 8.5 ± 0.5 (designated as LBR-8.5). The pH for LBR-UC was measured to be in the range of 3.5–4. During the experiments, FA (SCFA and MCFA) generation causes the leachate to be acidic, which affects microbial activity. Therefore, it is crucial to use alkaline solution to maintain the pH. To maintain the pH at the required level in the LBR, 1 M solution of caustic soda (NaOH) was used during the experiments. The leachate was recirculated from the leachate-holding bed to the food-waste-holding basket at a leachate recirculation rate of 3 L/h in all the LBRs.

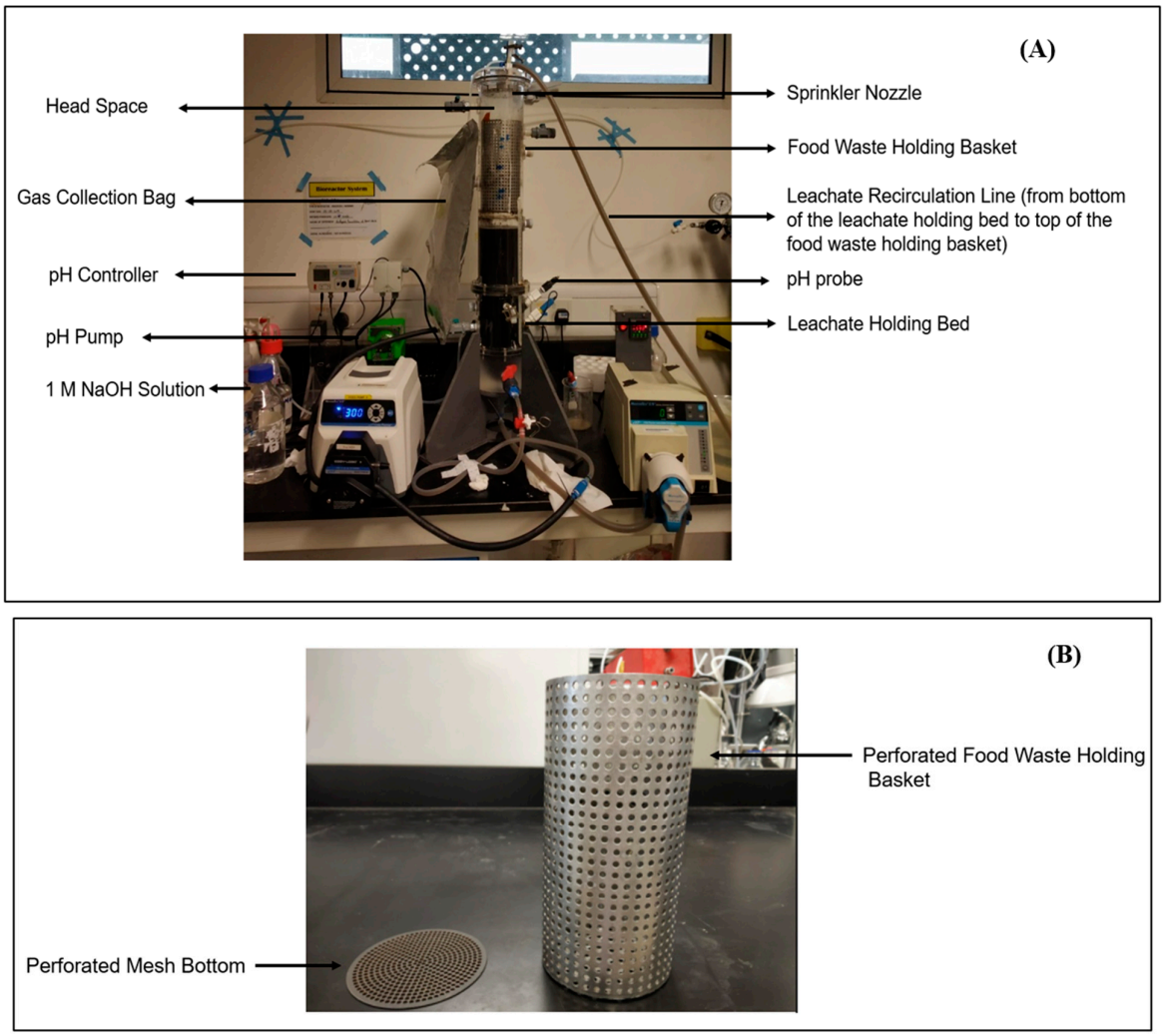


**Figure 1.** Image of (**A**) LBR and (**B**) Food-Waste-Holding Basket.

### *2.4. Sampling and Analytical Methods*

About 30 mL of leachate was sampled every second day for all the LBRs to analyze the soluble chemical oxygen demand (SCOD) and VFAs. TS and VS were analyzed at the beginning and the end of each experimental run. For the SCOD and VFA analyses, the leachate sample was centrifuged at 6000 rpm for 10 min to obtain the supernatant, and then the supernatant was filtered with a syringe filter with a 0.45 µm pore size filter membrane. Subsequently, the filtered sample was used to analyze the SCOD using a COD reagent tube (Hatch, Los Angeles, CA, USA). Fatty acids (FAs) in the leachate were analyzed by injecting the filtered sample into a gas chromatograph (GC 7890A, Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a DB-FFAP fused-silica capillary column. The injector and detector were both set to a temperature of 260 °C. The column temperature was initially adjusted to 80 °C for 1 min and then raised to 120 °C at a rate of 20 °C/min, and after that, it was increased to 205 °C at a rate of 10 °C/min and maintained at this temperature for 2 min.

In this study, the total FA (TFA) content was estimated as the sum of SCFAs (acetate, propionate, n-butyrate, iso-butyrate, iso-valerate and n-valerate) and MCFAs (isocaproate, n-caproate and heptanoate). The lactate in the leachate was quantified with a high-performance liquid chromatograph (HPLC, CA) equipped with a refractive index detector (RID) and an ion exclusion column (300 × 7.8 mm diameter, 9 µm particle size, Aminex HPX-87H, Biorad, CA, USA). The temperature of the column was maintained at 65 °C with a sample injection flow rate of 0.6 mL/min. The concentration of total FAs and

lactate was expressed as a chemical oxygen demand (COD) equivalent using half reactions for FAs and lactate with $O_2$ [25]. All analyses were performed in triplicate unless otherwise specified.

### *2.5. Calculations*

The impacts of pH on food waste degradation and acidification were evaluated based on the hydrolysis yield, acidification yield, FA yield, lactate yield and the ratios of FAs and lactate to SCOD [3,13,17,18,26]. The hydrolysis yield was calculated as the ratio of the mass of cumulative SCOD produced in the leachate to the initial amount vs. that added to the LBR (Equation (1)) [3]:

$$\text{Hydrolysis yield } (\text{g SCOD/kg VS}_{\text{added}}) = \frac{\text{cumulative SCOD produced (g SCOD)}}{\text{VS}_{\text{add edinitially}}(\text{kg})} \quad (1)$$

where

Cumulative SCOD produced (g SCOD) = Final SCOD of leachate(g SCOD) − Initial SCOD of the inoculum (g SCOD).

$VS_{added\ initially}$ (kg) = VS of food waste (kg) + vs. of inoculum (kg).

The FA yield was computed as the cumulative TFA (sum of SCFAs and MCFAs) produced to the initial, amount vs. that added to the LBR (Equation (2)) [17]:

$$\text{TFA yield } (\text{g COD}_{\text{FA}}/\text{kg VS}_{\text{added}}) = \frac{\text{cumulative TFA produced (g COD}_{\text{FA}})}{\text{VS}_{\text{added initially}}(\text{kg})} \quad (2)$$

where:

Cumulative TFA produced (g $COD_{FA}$) = Final total TFA of leachate (g $COD_{FA}$) − Initial total TFA of inoculum (g $COD_{FA}$).

$VS_{added\ initially}$ (kg) = VSof food waste (kg) + VSof inoculum (kg).

The TFA/SCOD ratio (%) was calculated as the ratio of the TFA yield to the hydrolysis yield.

The lactate yield was calculated based on the ratio of cumulative lactate produced (g $COD_{Lactate}$) to the initial amount vs. that added to the LBR (Equation (3)) [17]. The lactate/SCOD ratio (%) was calculated as the ratio of the lactate yield to the hydrolysis yield:

$$\text{Lactate yield } (\text{g COD}_{\text{Lactate}}/\text{kg VS}_{\text{added}}) = \frac{\text{cumulative lactate produced (g COD}_{\text{Lactate}})}{\text{VS}_{\text{added initially}}(\text{kg})} \quad (3)$$

where:

Cumulative lactate produced (g $COD_{Lactate}$) = Final total lacate of leachate (g $COD_{Lactate}$) − Initial total lactate of inoculum (g $COD_{Lactate}$).

$VS_{added\ initially}$ (kg) = VSof food waste (kg) + VSof inoculum (kg).

The acidification yield was calculated as the sum of the TFA yield and lactate yield (Equation (4)) [17]:

$$\text{Acidification yield } (\text{g COD/kg VS}_{\text{added}}) = \frac{\begin{array}{c}\text{cumulative TFA produced (g COD}_{\text{FA}}) + \\ \text{cumulative lactate produced (g COD}_{\text{Lactate}})\end{array}}{\text{VS}_{\text{added initially}}(\text{kg})} \quad (4)$$

where:

Cumulative TFA produced (g$COD_{VFA}$) = Final total TFA of leachate (g $COD_{VFA}$) − Initial total TFA of inoculum (g $COD_{VFA}$).

Cumulative lactate produced (g$COD_{Lactate}$) = Final total lacate of leachate (g $COD_{Lactate}$) 25 May 2023 Initial total lactate of inoculum (g $COD_{Lactate}$).

$VS_{added\ initially}$ (kg) = VSof food waste (kg) + VSof inoculum (kg).

### *2.6. Microbial Community and Statistical Analysis*

The residual food waste (in the food waste basket) and centrifuged biomass from the leachate in different LBRs were collected at the end of the batch cycle for microbial community analysis. As described by Xiong [18], genomic DNA (gDNA) was extracted from the food waste and the biomass samples using the Sox DNA Isolation Kit (Genewiz, Singapore) according to the protocol provided by the supplier. Polymerase chain reaction (PCR) for 16S rRNA genes was performed for each sample of gDNA (25 µL each) in triplicate, containing 0.5 µL of 10 mM dNTP, 2.5 µL of PCR buffer, 5.0 µL of 1 µM forward primer, 5.0 µL of 1 µM reverse primer, 0.25 µL of BSA (20 mg/mL), 5.0 µL DNA, 0.2 µL of Taq DNA polymerase (5u/µL) and 6.55 µL of PCR water. The forward and reverse primers were used to target 16S rRNA genes in both bacteria and archaea: Pro341F: CCTACGGGNBGCASCAG, Pro805R: GACTACNVGGGTATCTAATCC [27]. The PCR cycle included: (1) initial DNA denaturation at 95 °C for 5 min, (2) 35 cycles of DNA denaturation at 95 °C for 30 s, primer annealing at 30 °C for 30 s, primer extension at 72 °C for 50 s and then (3) a final extension at 72 °C for 10 min [27].

An equal amount of PCR amplicons were pooled and quantified using the NanoDrop 1000 (Thermo Fisher Scientific Inc.). The DNA sequences were produced in FASTQ files with a MiSeq Reagent Kit v2 (2 × 250 cycles) using an Illumina MiSeq sequencer (Illumina Inc, San Diego, CA, USA). The demultiplexing sequences, including the truncation of forward and reverse reads to 245 nucleotides, primer removal and the merging of paired reads, were processed using the DADA2 v1.6 tool [28] in QIIME 2 v.2018.2 [29].

After the chimera-containing sequences' removal, clustering was performed at 97% identity, and then taxonomy was assigned to representative sequences from each cluster using a naive Bayesian classifier implemented in QIIME 2 based on SILVA release 132.

Single-factor analysis of variance (ANOVA) analysis was used to verify the impacts of different pHs on food waste degradation and acidification ($p \leq 0.05$) using Microsoft Excel software version 2019.

## 3. Results and Discussion

### *3.1. Hydrolysis of Food Waste at Different pHs*

The hydrolysis of food waste was assessed based on the cumulative SCOD production. Figure 2 illustrates the impact of pH on cumulative SCOD (g SCOD) production in the LBR throughout the fermentation time of 14 days. The cumulative SCOD (g SCOD) production differed depending on the pH range. A nearly neutral to alkaline pH (i.e., 6.5–8.5 pH) resulted in statistically higher cumulative SCOD production than acidic pH ranges (uncontrolled pH–5.5). The highest cumulative SCOD production of 112.5 ± 2.6 g SCOD was obtained in LBR-7.5, followed by 111.7 ± 4.1 g SCOD in LBR-8.5, 110.8 ± 1.7 g SCOD in LBR-6.5, 89.0 ± 3.4 g SCOD in LBR-5.5 and 71.34 ± 0.9 g SCOD in LBR-UC. Notably, no statistical difference ($p \geq 0.05$) was found for the cumulative SCOD (g SCOD) production from pH 6.5 to 8.5 (i.e., LBR-6.5, LBR-7.5 and LBR-8.5) after 14 days of fermentation time; however, these values were statistically ($p \leq 0.05$) higher than the cumulative SCOD (g SCOD) obtained at pH 5.5 (LBR-5.5) and the uncontrolled pH (LBR-UC). A similar trend was also observed for the hydrolysis yields.

Table 1 summarizes the hydrolysis yields obtained in the LBRs at different pHs on day 14. A hydrolysis yield of 718–729 g SCOD/kg $VS_{added}$ was achieved in a pH range of 6.5–8.5 (LBR 6.5, LBR7.5 and LBR-8.5), which was 21–58% higher than those obtained at pH 5.5 (LBR-5.5; 577 g SCOD/kg $VS_{added}$) and the uncontrolled pH (LBR-UC; 462 g SCOD/kg $VS_{added}$). This result indicated that nearly neutral to alkaline pH ranges (pH 6.5–8.5) enhance the hydrolysis of food waste in LBRs. It has been reported that the hydrolysis of food waste in an LBR was improved when the pH was increased from an acidic pH (pH < 5.5) to a nearly neutral to alkaline pH [17,18,30,31]. Hussain [17] reported a 0.18–1.3-foldincrease in the hydrolysis yield obtained by increasing the pH from 4–5 (227–405 g SCOD/kg $VS_{added}$) to 6–7 (478–530 g SCOD/kg $VS_{added}$) during food waste fermentation in an LBR. Similarly, in another study treating food waste in an LBR, a 73%

higher hydrolysis yield was obtained at a pH of 6 (505 g SCOD/kg $VS_{added}$) compared to that obtained in uncontrolled pH conditions (292 g SCOD/kg $VS_{added}$) [31]. This enhanced hydrolysis of food waste at a nearly neutral to alkaline pH (i.e., 6.5–8.5 pH) can be attributed to better hydrolytic activity of the bacteria in these pH ranges, which results in the improved solubilization of particulate organic matter in the food waste [17,18].

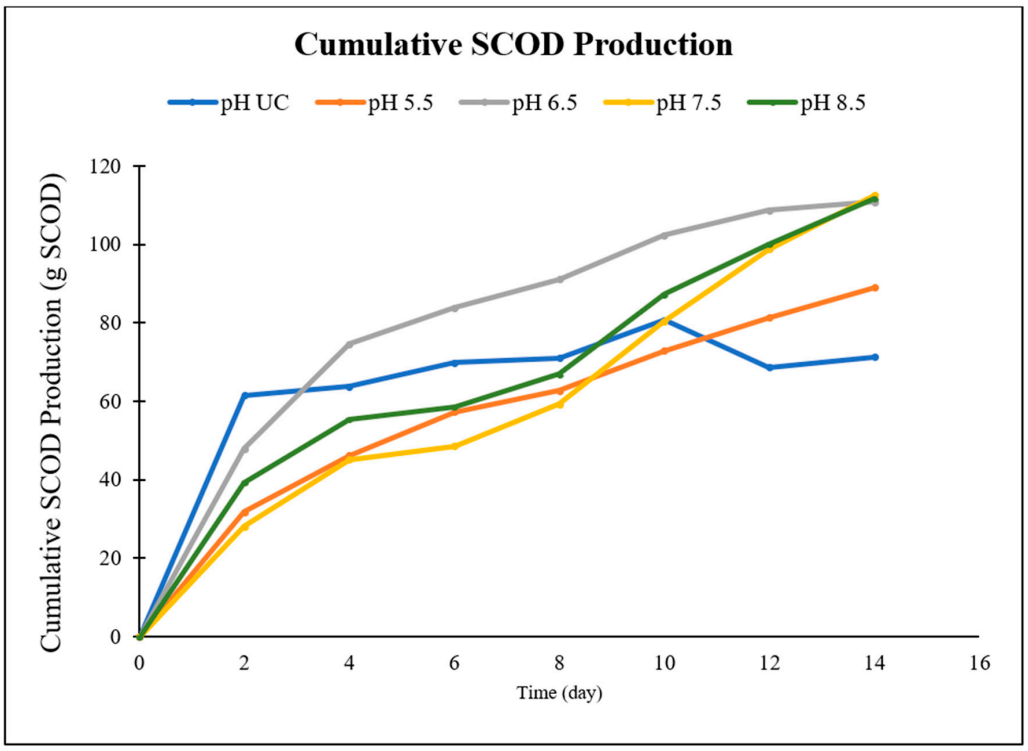


**Figure 2.** Cumulative SCOD production in LBRs at different operating pHs at room temperature (22 °C).

**Table 1.** Performance of LBRs at different operating pHs in this study.

| Parameters | LBR-UC | LBR-5.5 | LBR-6.5 | LBR-7.5 | LBR-8.5 |
|---|---|---|---|---|---|
| Cumulative SCOD production (g SCOD) | 71.3 ± 0.1 | 89.0 ± 3.4 | 110.8 ± 1.7 | 112.5 ± 2.6 | 111.7 ± 4.1 |
| Hydrolysis yield (g SCOD/kg $VS_{added}$) | 462 ± 6.0 | 577 ± 22.1 | 718 ± 11.2 | 729 ± 17.1 | 724 ± 26.6 |
| Acetate (g $COD_{FA}$/L) | 3.85 ± 0.0 | 4.5 ± 0.42 | 6.15 ± 0.52 | 7.43 ± 0.3 | 10.7 ± 0.75 |
| Propionate (g $COD_{FA}$/L) | 0.57 ± 0.05 | 1.76 ± 0.08 | 2.57 ± 0.22 | 2.6 ± 0.2 | 4.1 ± 0.2 |
| iso-Butyrate (g $COD_{FA}$/L) | - | 0.08 ± 0.0 | 0.09 ± 0.00 | 0.24 ± 0.01 | 0.23 ± 0.05 |
| n-Butyrate (g $COD_{FA}$/L) | 5.72 ± 0.0 | 9.72 ± 0.13 | 16.3 ± 1.4 | 5.9 ± 0.3 | 3.63 ± 0.1 |
| iso-Valerate (g $COD_{FA}$/L) | - | 0.13 ± | 0.15 ± 0.01 | 0.32 ± 0.02 | 0.31 ± 0.00 |
| n-Valerate (g $COD_{FA}$/L) | - | 0.76 ± 0.00 | 1.2 ± 0.09 | 1.3 ± 0.0 | 0.32 ± 0.01 |
| iso-Caproate (g $COD_{FA}$/L) | - | 0.09 ± 0.01 | 0.08 ± 0.00 | 0.1 ± 0.0 | 0.09 ± 0.0 |
| n-Caproate (g $COD_{FA}$/L) | - | 2.54 ± 0.01 | 2.83 ± 0.23 | 0.24 ± 0.1 | 0.11 ± 0.0 |
| Heptanoate (g $COD_{FA}$/L) | - | 0.29 ± 0.00 | 0 | 0.28 ± 0.0 | 0 |
| TVFA production (C2-C7) (g $COD_{FA}$) | 25.5 ± 0.2 | 71.4 ± 0.6 | 99.2 ± 3.0 | 67.4 ± 3.4 | 65.3 ± 4.2 |
| TVFA yield (g $COD_{FA}$/kg $VS_{added}$) | 165 ± 1.1 | 463 ± 4.3 | 643 ± 19.2 | 437 ± 22.1 | 423 ± 27.4 |

**Table 1.** *Cont.*

| Parameters | LBR-UC | LBR-5.5 | LBR-6.5 | LBR-7.5 | LBR-8.5 |
|---|---|---|---|---|---|
| TVFA (C2-C7):SCOD (%) | 36 | 80 | 90 | 60 | 58 |
| Lactate (g $COD_{Lactate}$) | 39.3 ± 0.3 | - | - | - | - |
| Lactate yield (g $COD_{Lactate}$/kg $VS_{added}$) | 255 ± 2.1 | - | - | - | - |
| Lactate: SCOD (%) | 55 | - | - | - | - |
| Acidification yield (g COD/kg $VS_{added}$) | 420 ± 5.8 | 463 ± 4.8 | 643 ± 19.2 | 437 ± 22.1 | 423 ± 27.4 |
| Acidification (%) | 91 | 80 | 90 | 60 | 58 |

Interestingly, the hydrolysis of the food waste was faster at the pH of 6.5 (LBR-6.5) than in the other pH conditions (LBR-UC, LBR-5.5, LBR-7.5, LBR-8.5). Notably, in LBR-6.5, the cumulative SCOD production on day 8 was 91 g SCOD, which was 82% of the cumulative SCOD produced on day 14 (Table 2). Comparatively, on the same day (day 8), the cumulative SCOD production for LBR-7.5 and LBR-8.5 was 53% and 60%, respectively, of the cumulative SCOD produced on day 14 in the reactor (Table 2). Additionally, the cumulative SCOD production on day 8 for LBR-6.5 was statistically ($p \leq 0.05$) higher (LBR-6.5) than that obtained in the other pH conditions on day 10 (LBR-UC, LBR-5.5, LBR-7.5, LBR-8.5). This faster hydrolysis in LBR-6.5 indicates that operating the reactor at a pH of 6.5 can significantly shorten the fermentation time to 10–12 days instead of the 14 days required at the pHs of 7.5 and 8.5 to achieve the same SCOD production/hydrolysis yield.

**Table 2.** Cumulative SCOD production in LBRs at different operating pHs in this study.

| Time | Cumulative SCOD Production (g SCOD) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Day | LBR-UC | | LBR-5.5 | | LBR-6.5 | | LBR-7.5 | | LBR-8.5 | |
| | g SCOD | % | g SCOD | % | g SCOD | % | g SCOD | % | g SCOD | % |
| 0 | 0.0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% |
| 2 | 61.5 | 86% | 31.8 | 36% | 47.9 | 43% | 28.1 | 25% | 39.3 | 35% |
| 4 | 63.8 | 89% | 46.2 | 52% | 74.6 | 67% | 45.1 | 40% | 55.5 | 50% |
| 6 | 69.8 | 98% | 57.3 | 64% | 83.9 | 76% | 48.5 | 43% | 58.6 | 52% |
| 8 | 71.1 | 100% | 62.7 | 70% | 91.1 | 82% | 59.3 | 53% | 66.9 | 60% |
| 10 | 80.6 | 113% | 72.8 | 82% | 102.3 | 92% | 80.3 | 71% | 87.2 | 78% |
| 12 | 68.6 | 96% | 81.3 | 91% | 108.6 | 98% | 98.8 | 88% | 100.1 | 90% |
| 14 | 71.3 | 100% | 89.0 | 100% | 110.8 | 100% | 112.5 | 100% | 111.7 | 100% |

### *3.2. TFA Production at Different pH*

TFA production was calculated as the sum of SCFAs (i.e., acetate, propionate, n-butyrate, iso-butyrate, n-valerate, iso-valerate) and MCFAs (i.e., n-caproate, iso-caproate, heptanoate) produced in a particular LBR (Equation (2)). The TFA production also varied depending on the operating pH. On day 14, the maximum TFA production of 99.2 ± 3.0 g $COD_{FA}$ was obtained in LBR-6.5, followed by 71.4 ± 0.6 g $COD_{FA}$ in LBR-5.5, 67.4 ± 3.4 g $COD_{FA}$ in LBR-7.5, 65.3 ± 4.2 g $COD_{FA}$ in LBR-8.5 and 25.5± 0.2 g $COD_{FA}$ in LBR-UC. These results showed that the TFA production in the LBR operated at pH 6.5 (LBR-6.5) was statistically ($p \leq 0.05$) higher than that obtained at the other pHs (LBR-UC, LBR-5.5, LBR-7.5, LBR-8.5) (Table 1). Moreover, the TFA production at pH 6.5 (LBR-6.5) was 47–52% higher than that obtained at pH 7.5–8.5 (LBR-7.5, LBR-8.5), even when the cumulative SCOD production was statistically the same at pH 6.5–8.5 (Table 1). The lower TFA production at pH 7.5–8.5 could be attributed to alcohol production under alkaline pH conditions [4]. Higher alcohol production (ethanol, butanol, etc.) at neutral and slightly alkaline pHs has been reported in previous studies [4,17,18]. It is due to enhanced hydrolysis in these alkaline pH ranges of 7.5–8.5 (Table 1), resulting in a greater availability of carbon as a

source for alcohol production during the solventogenesis phase of the metabolic pathway in bacteria [4,17,18].

The positive impact of pH 6.5 on TFA production can be further determined from the TVFA yield and TVFA/SCOD ratio (Table 1). The highest TFA yield of 643 ± 19.2 g $COD_{FA}$/kg $VS_{added}$ was obtained for LBR-6.5, followed by 463 ± 4.3 g $COD_{FA}$/kg $VS_{added}$ for LBR-5.5, 437 ± 22.1 g $COD_{FA}$/kg $VS_{added}$ for LBR-7.5, 423 ± 27.4 g $COD_{FA}$/kg $VS_{added}$ for LBR-8.5 and 165 ± 1.1 g $COD_{FA}$/kg $VS_{added}$ for LBR-UC. This implies that a nearly neutral pH (pH 6.5) resulted in higher TFA production, along with improved hydrolysis yields (Table 1). Yu [32] reported a 34% increase in the TFA yield during the acidogenic fermentation of food waste when the pH was increased from pH 5.5 to pH 6.5. Likewise, Cysneiros [30] reported a high TFA yield of 720 g $COD_{FA}$/kg $VS_{added}$ at a pH of 6.5 in an LBR treating maize, which was 76% higher than that obtained under uncontrolled pH conditions (410 g $COD_{VFA}$/kg $VS_{added}$). This higher TFA production at pH 6.5 could be due to better acidogenic activity at pH 5.5–6.5 [33].

The maximum TFA/SCOD of 90% was achieved at pH 6.5 in LBR-6.5 (Table 1), followed by 80% at pH 5.5 (LBR-5.5) and between 58 and 60% for a pH of 7.5–8.5. A low TFA/SCOD of 36% was obtained in LBR-UC due to the transformation of solubilized matter into lactate rather than FAs at an uncontrolled pH (Table 1). Lactate production of 39.3 ± 0.3 g $COD_{Lactate}$, a lactate yield of 255 ± 2.1 g $COD_{Lactate}$/kg $VS_{added}$ and a lactate/SCOD of 55% were obtained for LBR-UC. Notably, no lactate was produced at the controlled pH of 5.5–8.5. The higher lactate production at the uncontrolled pH was due to a shift in the microbial community from FA-producing bacteria to lactate-producing bacteria (discussed in Section 3.4). Lactate-producing bacteria such as *Lactobacillus* can thrive under acidic conditions (pH 3.5–4.5) [19,34]. High lactate production at an uncontrolled pH was also observed in other studies. For instance, Kim [35] reported significantly higher lactate production from food waste at a pH of 3.3–3.4 than a pH of 7.2–7.9. Similarly, Ye [36] obtained higher lactate production at an uncontrolled pH as compared to a controlled pH of 6–8 during the acidogenic fermentation of vegetable waste.

### *3.3. TFA Composition at Different pH*

The TFA composition at different pHs is shown in Figure 3. Butyrate (56% of TFA) was the most dominant FA at the uncontrolled pH (LBR-UC), followed by acetate (38% of TFA). At the pH of 5.5 (LBR-5.5), butyrate (50% of TFA) and acetate (23% of TFA) were the prevalent FAs and, together, constituted 73% of the TFA. Similarly, the produced amounts of butyrate (56% of TFA) and acetate (21% of TFA) were higher than those of the other FAs at pH 6.5 in LBR-6.5. The higher production of acetate and butyrate at pH 5.5–6.5 implies that bacteria follow the acetate–butyrate metabolic pathway at pH 5.5–6.5 [18]. On the other hand, the main FA content shifted from butyrate to acetate, along with a comparatively higher production of propionate at pH 7.5 (LBR-7.5). Acetate constituted 36% of the TFA, being the most dominant FA, followed by butyrate (29% of TFA) and propionate (27% of TFA). At pH 8.5, acetate (57% of TFA) was still the dominant FA, but the production of propionate (22% of TFA) increased significantly. Butyrate constituted 19% of the TFA at pH 8.5. Overall, an alkaline pH of 7.5–8.5 promotes the production of acetate and propionate, which is in agreement with the findings of other research studies. Bacteria will conserve their energy by producing acetate and balance their intracellular reducing power by producing propionate at the alkaline pH of 7.5–8.5 [18].

Caproate was the main MCFA (Table 1). A relatively high composition of caproate was observed at pH of 5.5, forming 13% of the TFA produced (Figure 3). Other MCFAs, such as heptanoate, constituted a very low fraction of the TFA (0–1.5%). Overall, the results demonstrate that the operating pH is a key parameter that impacts the production and composition of SCFAs and MCFAs during the acidogenic fermentation of food waste.

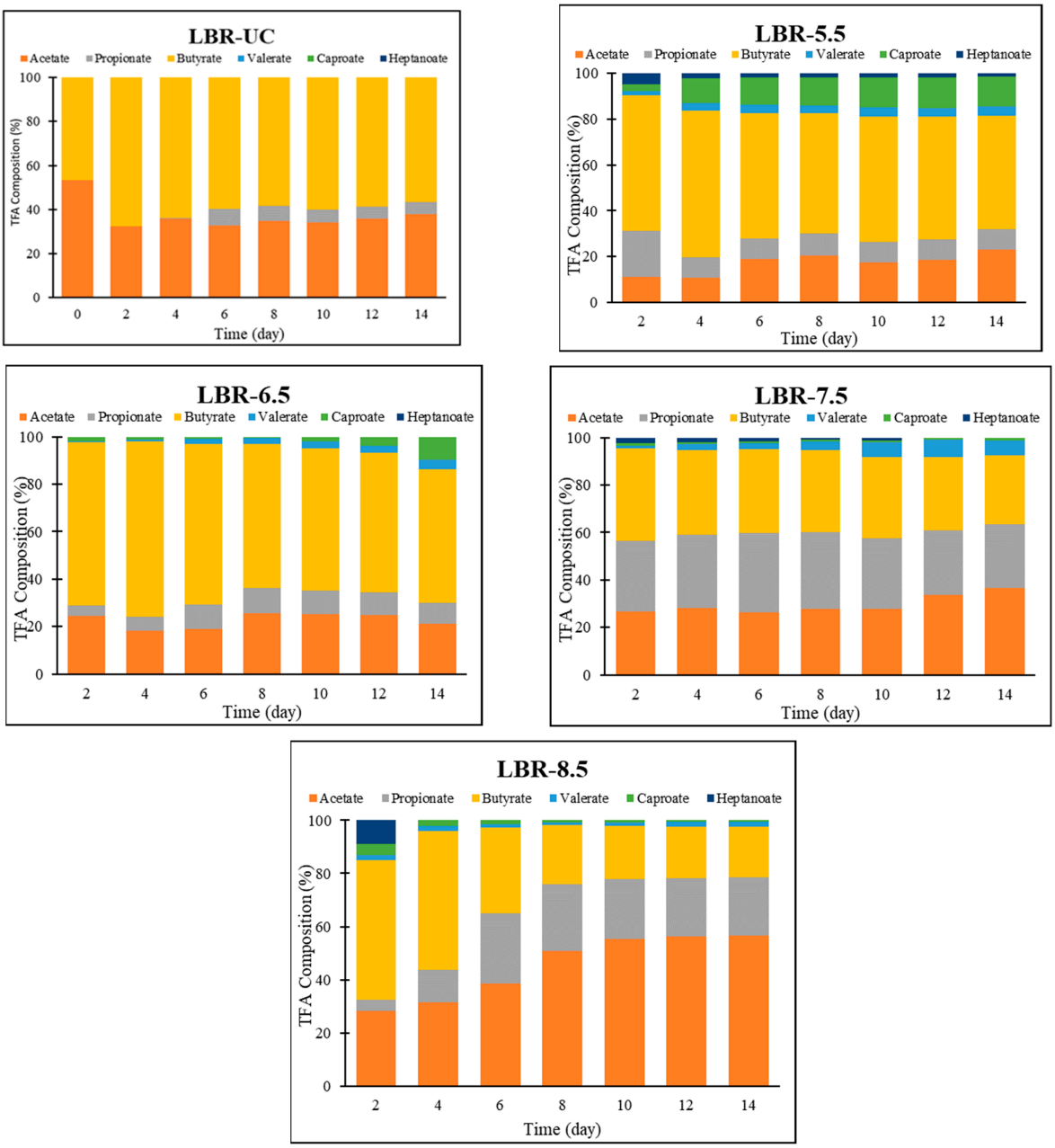


**Figure 3.** TFA compositions in the LBRs at different pHs. Butyrate refers to the sum of n-butyrate and iso-butyrate. Caproate refers to the sum of n-caproate and iso-caproate. Valerate refers to the sum of n-valerate and iso-valerate.

### *3.4. Microbial Community Composition*

Figure 4 shows the microbial community at the genus level of the leachate and food waste for LBRs at different pHs. In the inoculum, *Clostridium* (43%) and *Marcellibacteroides* (34%) were mostly dominant. While the composition of *Marcellibacteroides* became significantly smaller throughout all the pH conditions, *Clostridium* was consistently found at all pHs in both the leachate and food waste. *Clostridium* is known for acetate, butyrate and hydrogen production through organic fermentation [18].

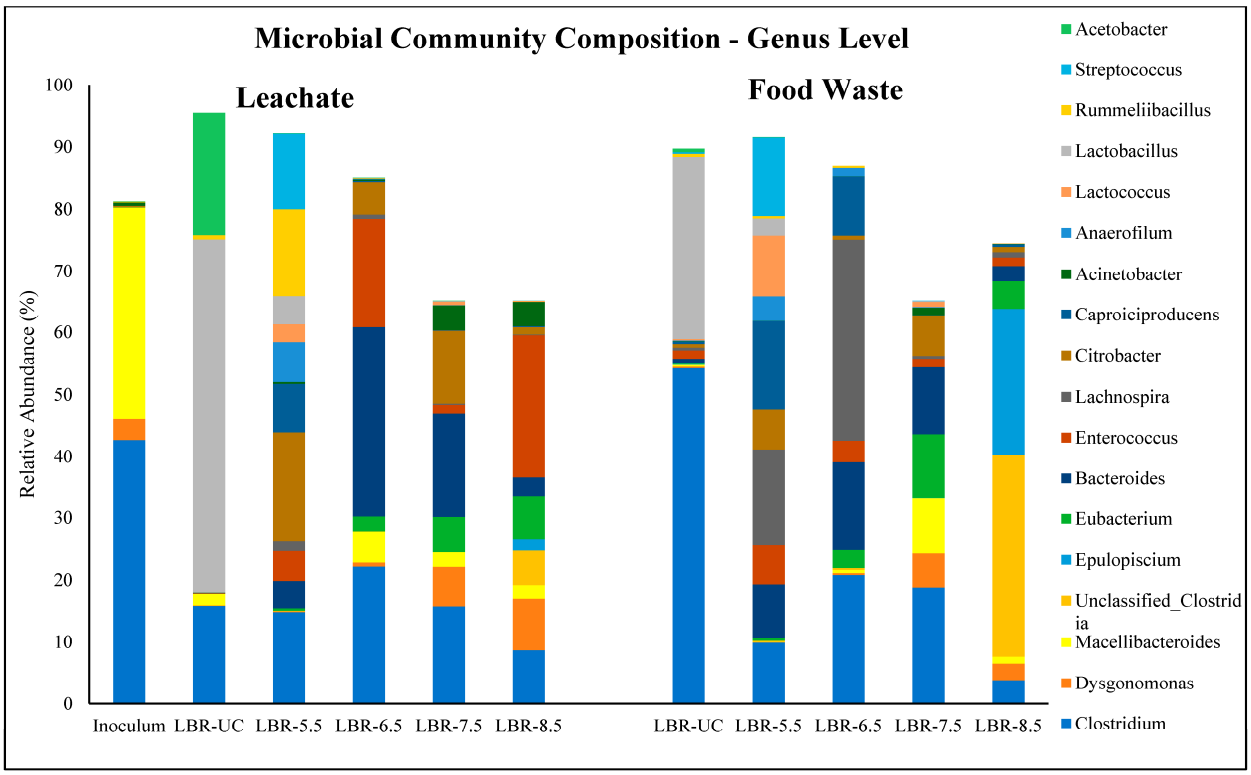


**Figure 4.** Microbial community compositions of food waste and leachate for LBRs operated at different pHs.

Different microbial communities were found in the leachate in response to different pHs. For the uncontrolled pH (LBR-UC), *Lactobacillus* was the most dominant (57%). *Lactobacillus* are lactate-producing bacteria and have been found under acidic conditions (pH 3.5–4.5) [34], which corresponds with the pH measured for LBR-UC. The high relative abundance of *Lactobacillus* supports high lactate production in LBR-UC. LBR-5.5 had the most diverse genera composition in its leachate, including *Clostridium* (15%), *Citrobacter* (18%) and *Rummeliibacillus* (14%). Among them, *Citrobacter* is a known fermentative bacteria, especially for hydrogen production through acetogenesis [37]. Recently *Rummeliibacillus suwonensis*, one of the species of *Rummeliibacillus* which is known for caproic acid production, was isolated [38]. Given that caproic acid was produced at a high rate at pH 5.5, this genus is suggested to be a major player in the production of caproic acid. In LBR-6.5 at pH 6.5, *Bacteroides* (31%) and *Enterococcus* (18%) were predominantly found with *Clostridium*. *Enterococcus* was reported as a fermentative bacteria producing butyrate and acetate, and it is also known to be a fermenter of carbohydrate and lignocellulose [39,40]. Bacteroides generates butyrate, acetate and propionate [41,42]. This genus was also found predominantly in LBR-7.5 at pH 7.5 (17%). In addition, the composition of *Dysgonomonas* became larger when the pH increased to 7.5 and 8.5. *Dysgonomonas* ferments glucose and produces propionate, acetate, lactate and succinate [43]. This result is consistent with our previous study that reported an abundance of *Dysgonomonas* at a high pH [18].

The bacterial communities in the food waste exhibited different compositions from those in the leachate, except for the uncontrolled conditions, in which *Lactobacillus* and *Clostridium* were dominant. At both pH 5.5 and 6.5, *Lachnospira* (16 and 33%, respectively) and *Caproiciproducens* (14 and 10%, respectively) were predominantly found, while these genera were less abundant in the leachate. *Lachnospira* is known as a type of pectin- and glucose-fermenting bacteria [44]. In our previous study, *Caproiciproducens* was mostly found in food waste and not in leachate [18]. Based on the literature, *Caproiciproducens* can hydrolyze cellulose using extracellular enzymes [45], and it also ferments fatty acids [46]. While LBR-7.5 at pH 7.5 showed a similar microbial composition, pH 8.5 exhibited a

different composition, leading us to identify unclassified *Clostridia* (32.7%) and *Epulopiscium* (24%). Unclassified *Clostridia* were only identified in LBR-8.5 at pH 8.5. *Clostridia* is a class-level bacterium, and *Clostridium* also belongs to the *Clostridia* class. Thus, it is assumed that these populations would be involved in hydrolysis and fermentation at a specific high pH. However, it is challenging to fully comprehend their roles due to the limitation of identification to the lower phylogenetic level.

## 4. Conclusions

The operating pH significantly affects the solubilization and formation of fermentative products from food waste. High hydrolysis and acidification yields of 718 g SCOD/kg $VS_{added}$ and 643 ± 19.2 g $COD_{FA}$/kg $VS_{added}$, respectively, were obtained at pH of 6.5. The acidification yield at pH 6.5 was 47–52% higher than that obtained at pH 7.5–8.5, even when the hydrolysis yields were statistically similar in a pH range of 6.5–8.5. A higher TFA/SCOD ratio of 90% was also achieved at pH 6.5. Butyrate was the dominant fermentative product at the pH of 5.5–6.5, whereas acetate formed the major proportion of the TFA composition at pH 7.5–8.5. Lactate-producing bacteria were the most prevalent at the uncontrolled pH, thus resulting in a high lactate production in LBR-UC. The pH of 5.5 (LBR-5.5) resulted in the highest level of MCFA production, constituting 13% of the TFA produced.

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