



Effect of pH on lactic acid production from acidogenic fermentation of food waste with different types of inocula



Jialing Tang^{a,b,c}, Xiaochang C. Wang^{a,b,c,*}, Yisong Hu^{a,c}, Yongmei Zhang^{c,d}, Yuyou Li^e

^a School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an 710055, China

^b Key Lab of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an 710055, China

^c International Science & Technology Cooperation Center for Urban Alternative Water Resources Development, Xi'an 710055, China

^d Taiyuan University of Technology, Shanxi Province, Taiyuan 030054, China

^e Department of Civil and Environmental Engineering, Tohoku University, Sendai 9808579, Japan

HIGHLIGHTS

- LA was produced from food waste using three inocula.
- The highest LA yield was obtained at pH 5 using fresh food waste as the inoculum.
- LA was observed to degrade to VFAs at pH 6.
- VFA compositions varied significantly with different inocula supplied.
- *Lactobacillus* could be enriched (83.4–98.5%) during the fermentation process.

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ABSTRACT

Effect of acidic pH (4, 5, 6 and uncontrolled) on lactic acid (LA) fermentation from food waste was investigated by batch fermentation experiments using methanogenic sludge, fresh food waste and anaerobic activated sludge as inocula. Results showed that due to the increase of hydrolysis, substrate degradation rate and enzyme activity, the optimal LA concentration and yield were obtained at pH 5, regardless of the inoculum used. The highest LA concentration (28.4 g/L) and yield (0.46 g/g-TS) were obtained with fresh food waste as inoculum. Moreover, after the substrate was completely utilized, the lactic acid bacteria population sharply decreased, and the LA produced was converted to volatile fatty acids (VFAs) at pH 6 within a short period. The VFA components varied with the inoculum supplied. Microbial community analysis using high-throughput pyrosequencing revealed that diversity decreased and a high abundance of *Lactobacillus* (83.4–98.5%) accumulated during fermentation with all inocula.

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1. Introduction

The Food and Agriculture Organization (FAO) estimates that approximately 1.6 gigatons of food waste are wasted annually. In China, due to the rapid urbanization, population growth and industrialization, a large quantity of food waste has been generated, increasing to approximately 90 million tons in 2010 (Wen et al., 2016). Taking Beijing as an example, approximately 2000 tons of food waste was produced each day (Chen et al., 2016), which poses serious environmental challenges. Until now, cattle feeding, burning, and landfills have remained the most common methods to dis-

pose of food waste, which cannot meet the more stringent environmental standards, as they would cause air, soil and water contamination (Wu et al., 2016; Tang et al., 2016; Jiang et al., 2013). Moreover, carbohydrate, protein and lipids have been noted as the main components in food waste and are regarded as potential resources; thus, there is a need to explore alternative technologies to convert these valuable resources into value added chemicals and fuels (Jiang et al., 2013; Tang et al., 2016; Liang et al., 2015; Liang and McDonald, 2015).

Anaerobic digestion (AD) of organic wastes, e.g., food waste, for recovering energy and producing valuable chemicals (e.g., biogas, VFAs and alcohols) has been investigated to reduce environmental stress (Jiang et al., 2013; Shen et al., 2013). Lactic acid (LA) is also an important intermediate in the AD processes. It is a chemical precursor that has been widely applied in food, pharmaceutical, textile

* Corresponding author at: School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an 710055, China.

E-mail address: xcwang@xauat.edu.cn (X.C. Wang).

and leather production, especially in the plastic sector in the production of biodegradable polylactic acid (PLA) plastic and polyhydroxyalkanoates (PHAs) (Kim et al., 2012; Liang et al., 2016) and has exhibited increasing demand in recent years (Tang et al., 2016; Liang et al., 2016). To reduce LA production cost, various organic wastes such as organic municipal solid waste (MSW), fruit and vegetable wastes, potato peel waste and food waste have been tested as substrates in LA fermentation (Liang et al., 2014, 2016; Wu et al., 2015; Dreschke et al., 2015). Due to its high organic content and large yield, food waste has proven to be a superior substrate for LA fermentation (Li et al., 2015; Tang et al., 2016).

AD processes consist of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. LA is produced in the first two steps. To obtain a high LA yield, operating conditions such as temperature, substrate, C/N ratio, inoculum and pH should be optimized (Tang et al., 2016; Wu et al., 2015; Liang et al., 2014). As an important parameter, pH shows significant influence on hydrolysis and acidogenesis, microbial communities and fermentation products during anaerobic LA fermentation process (Tang et al., 2016; Wu et al., 2015; Dreschke et al., 2015; Itoh et al., 2012). Increasing pH from 4 to 5 can clearly promote the hydrolysis rate using food waste as a substrate for LA production (Wu et al., 2015; Dreschke et al., 2015). Li et al. (2014) fermented waste activated sludge and found that alkaline conditions benefit the hydrolysis processes. In contrast, Wang et al. (2014) digested food waste with activated sludge as inocula and found that acidic conditions (pH < 4) could also enhance hydrolysis. Differences primarily resulted from differing microbial communities and the substrates utilized. In addition, researchers noted that pH adjustment from 4 to 5 would effectively enhance hydrolysis and acidification processes, largely improving the lactic acid yield (Wu et al., 2015). Further, increasing pH > 6 would convert the produced LA into VFAs or biogas (Kim et al., 2003; Itoh et al., 2012; Probst et al., 2015), possibly due to pH playing an important role in determining dominant microbial communities, further affecting metabolic pathways and leading to different products (Dreschke et al., 2015; Probst et al., 2015; Wu et al., 2015). Although effort has been made, investigations of the effects of pH, especially acidic pH on lactic acid fermentation and related microbial communities properties are insufficient and further research is still needed.

The inoculum is another important factor affecting the evolution of fermentative pathways (Wang et al., 2014; Liang et al., 2015, 2016). A relative high yield and optically pure lactic acid can be biosynthesized using axenic microbial cultures such as *Lactobacillus* and *Lactococcus* with refined sugars and starch (Wakai et al., 2014) and some complicated substrates such as lignocellulosic feedstock, food waste and green biomass (Abdel-Rahman et al., 2011; Wang et al., 2015; Tashiro et al., 2016). While specific criteria such as nutrients, temperature, pH, microbial cell density, and aseptic conditions have to be maintained to keep a reasonable fermentation productivity (Liang et al., 2014, 2016), which increase the LA fermentation cost to some extent (Wang et al., 2011). Mixed

microbial cultures such as aerobic or anaerobic activated sludge could also be alternatives, as they can be widely obtained with low cost, handle complex conditions with abundant microbial diversity, and have great adaption and high self-evolution abilities (Liang et al., 2014, 2016; Wang et al., 2014). However, the characteristics of fermentation processes and variations in microbial communities with different inocula have been scarcely considered under acidic conditions (Liang et al., 2016).

In this study, the effects of pH on lactic acid fermentation supplied with different undefined mixed cultures (methanogenic sludge, fresh food waste and anaerobic sludge) as inocula were investigated. Lactic acid yield under acidic pH (uncontrolled, 4, 5 and 6) was discussed, and the evolution of microbial communities was also investigated to provide some useful information for lactic acid fermentation.

2. Materials and methods

2.1. Inocula

Three types of inocula were chosen in this study. Methanogenic sludge was collected from an anaerobic continuous stirring tank reactor (CSTR) in which food waste and waste activated sludge were utilized as substrates with a biogas yield of approximately 300 mL-CH₄/g-VSS. Anaerobic sludge was obtained from a full-scale wastewater treatment plant with an anaerobic/anoxic/oxic plus membrane bioreactor (A-A-O-MBR) system in Xi'an, China (Hu et al., 2013). Sludge was collected from an anaerobic tank and stored in a refrigerator for 24 h. Then, the supernatant was drained, and the sludge was collected. In addition, fresh food waste was utilized as inoculum. Fresh food waste collected from a cafeteria was crushed and sieved (1 mm), and then the total solid content (TS) was adjusted with tap water. Characteristics of the three types of inocula are presented in Table 1.

2.2. Food waste substrate

Food waste was collected from the canteen of a university campus in Xi'an, China, primarily consisting of rice, vegetables and meat. It was homogenized with an electrical blender after sorting out animal bones and clamshells, and the resulting slurry was sieved (1 mm) and stored in a refrigerator (4 °C). To avoid effects of the viable indigenous microbiota in the food waste substrate on fermentation, the food waste slurry was sterilized at 121 °C for 30 min to kill microorganisms. Characteristics of the sterilized food waste slurry are shown in Table 1.

2.3. Batch fermentation experiments

Sixteen identical batch reactors (250 mL) were divided into four groups, as shown in Table 2. The TS content in each reactor was

Table 1
Characteristics of inocula and sterilized food waste substrate.

Parameter	Units	Methanogenic sludge	Fresh food waste	Anaerobic sludge	Sterilized food waste slurry
pH	–	8.0	4.3	7.3	3.8
TS*	%	3.6 ± 0.4	4.3 ± 0.3	2.1 ± 0.5	6.5 ± 0.4
VS/TS	%	61.5 ± 10.2	96.4 ± 7.6	68.5 ± 15.3	95.7 ± 8.4
Total COD (TCOD)	g/L	38.7 ± 1.3	47.8 ± 5.2	26.0 ± 0.7	71.6 ± 1.0
Soluble COD (SCOD)	g/L	13.2 ± 1.3	11.0 ± 1.4	1.3 ± 0.5	42.2 ± 0.6
Total carbohydrate	g/L	1.7 ± 0.1	31.4 ± 2.9	2.1 ± 0.01	41.9 ± 0.8
Total protein	g/L	8.8 ± 0.2	2.9 ± 1.1	4.7 ± 0.4	3.6 ± 0.2
VFAs	g/L	2.5 ± 1.1	3.6 ± 0.9	0.09 ± 0.01	3.8 ± 1.2
Lipids	g/L	–	1.2 ± 0.2	–	1.9 ± 0.5
Ash*	%	38.5 ± 7.8	3.6 ± 1.1	31.5 ± 11.6	4.3 ± 1.5

* TS is wet basis, Ash is dry basis. Data are means of at least three samples, ± represents the standard deviation, – represents no detection.

Table 2
The operation conditions of batch fermentation experiments.

Group	Reactor	pH	Inocula	Substrate
Control	C1-C4	uncontrolled (Un), 4, 5, 6	–	Sterilized food waste slurry
1	M1-M4	uncontrolled (Un), 4, 5, 6	Methanogenic sludge	Sterilized food waste slurry
2	F1-F4	uncontrolled (Un), 4, 5, 6	Fresh food waste	Sterilized food waste slurry
3	A1-A4	uncontrolled (Un), 4, 5, 6	Anaerobic activated sludge	Sterilized food waste slurry

adjusted to $6 \pm 0.5\%$ and the substrate was composed of $85 \pm 2\%$ sterilized food waste slurry (Section 2.2) and $15 \pm 4\%$ inoculum (dry weight) (Wang et al., 2014). The four reactors in each group were operated at pH 4, 5, and 6 and uncontrolled pH by adding NaOH or HCl (5 M) every 4 h according to the previous study (Tang et al., 2016; Tashiro et al., 2016). Four reactors without inocula were set as the control group. Agitators (120 rpm) were installed on the reactors which were placed in a water bath at 37 °C. Headspace of each reactor was flushed with nitrogen gas. Samples were obtained from each reactor periodically to analyze physical and chemical parameters. Fermentation tests were carried out for 7 days.

2.4. Lactic acid bacteria counts

Viable lactic acid bacteria (LAB) in the broth during fermentation were detected using De Man–Rogosa–Sharpe (MRS) agar (Ye et al., 2008). A mixture of 14 g agar, 20 g glucose, 20 g peptone, 10 g beef extract, 5 g yeast extract, 2 g K_2HPO_4 , 2 g diammonium citrate, 5 g CH_3COONa , 0.58 g $MgSO_4 \cdot 7H_2O$, 0.25 g $MnSO_4 \cdot 4H_2O$, 2 g $CaCO_3$ and 1 mL Tween-80 was diluted in 1 L pure water. The pH was adjusted to 6.5 ± 0.2 . The solution was sterilized at 121 °C for 15 min and cooled to 50 °C. Samples obtained from the fermentation reactors were serially diluted, and 1 mL of diluted solution was added evenly to the prepared solid MRS agar and then overlaid with the MRS agar solution. Colony-forming units (cfu) were determined by incubating the MRS agar at 36 °C for 48 h in an incubator. Each sample was tested in triplicate, and lactic acid bacteria cell counts were averaged.

2.5. Analytical methods

Samples obtained from the reactor were used to analyze total chemical oxygen demand (TCOD), total nitrogen and phosphate, total carbohydrate and protein immediately. After centrifugation (6000 r/min for 10 min at 4 °C), the supernatant was filtered through 0.45 μ m filters. The filtrate was analyzed for total organic carbon (TOC), soluble COD (SCOD), volatile fatty acids (VFAs), soluble protein and carbohydrate, and lactate. Measurements of SCOD and TCOD were according to standard methods (APHA, 1998). Soluble proteins were detected by the Lowry-Folin method with BSA as the standard (Lowry et al., 1951). Carbohydrates were measured by the phenol-sulfuric method with glucose as the standard (Herbert et al., 1971). Food waste substrate elements were assayed by elemental analyzer according to Li et al. (2015). Analyses of α -glucosidase and protease activity were made according to reported methods (Li et al., 2015).

To analyze VFAs, the filtrate was collected in a 1.5 mL gas chromatography (GC) vial, and 3% H_3PO_4 was added to adjust the pH to approximately 4.0. A gas chromatograph (GC2014, Shimadzu, Japan) with flame ionization detector and equipped with a 30 m \times 0.32 mm \times 0.25 μ m CPWAX52CB column was utilized to analyze VFA compositions. Nitrogen was the carrier gas at 50 mL/min. The injection port and detector were maintained at 200 and 220 °C, respectively. The GC oven was programmed to begin at 100 °C, remain there 2 min, increase at a rate of 10 °C/min to

200 °C, and then hold at 200 °C for 2 min. The sample injection volume was 0.5 μ L.

Lactate concentration was determined using a liquid chromatograph (LC-10AD, Shimadzu, Japan) equipped with an ultraviolet detector (210 nm). Separation was achieved using a COSMOSIL 5C18-AR-II column at 40 °C and elution with 0.05 M phosphoric acid buffer liquid (50 mM NaH_2PO_4 :50 mM $H_3PO_4 = 9:1$, pH = 3) at 1.0 mL/min.

2.6. Microbial community analysis

To explore bacterial community change, samples of the inocula and fermentation mixture were sent to Sangong, Inc. (Shanghai, China) for DNA extraction and next-generation sequencing processes. As discussed in our previous study, the extracted DNA was amplified by PCR using the primer 27F (5'-AGAGTTT GATCCTGGCTCAG-3') and 533R (5'-TTACCGCGGCTGCTGGCAC-3') for the V1-V3 region (Tang et al., 2016; Li et al., 2015). Pyrosequencing was conducted using a Roche 454 GS FLX+ Titanium platform. The homologous or ambiguous sequences or those with a length shorter than 200 bp were trimmed to obtain high-quality sequences with an average length larger than 400 bp (Table S1, Supporting information).

3. Results and discussion

3.1. Effect of pH on hydrolysis

3.1.1. Soluble COD

Fig. 1a–c describes the effect of pH on SCOD content during fermentation, while the TSS removal rate is shown in Fig. 1d. In Fig. 1a, with the methanogenic sludge as inoculum, SCOD shows a slight increase during the entire fermentation period. This could be due to two reasons: microorganisms in the inocula might utilize solubilized organics in the mixture, or the solubilization rate in these reactors was very low. As indicated in Fig. 1d, TSS removal rates in these reactors were approximately 40%, indicating a high solubilization rate. Thus, consumption of soluble organics was the main cause of the unapparent SCOD increase in the broth. In addition, as shown in Fig. 1a, SCOD in the reactor at uncontrolled pH decreased to 27.2 g/L, even lower than the initial value (38.1 g/L). Soluble organics such as VFAs, carbohydrates or proteins in the broth can be utilized by microorganisms in methanogenic sludge to produce biogas under acidic conditions, which was observed in this work and consistent with previous work (Karadag and Puhakka, 2010).

Reactors inoculated with fresh food waste showed an obvious increase in SCOD concentration within the first 48 h (Fig. 1b), indicating a relatively higher solubilization rate than the consumption rate. Additionally, it could also be found that the SCOD at steady state were approximately 41.7 g/L, 43.3 g/L, 48.9 g/L and 51.2 g/L at uncontrolled pH, pH 4, 5 and 6, respectively, reflecting a higher hydrolysis rate under higher pH conditions. However, after 120 h, the SCOD in the reactor at pH 6 decreased to 45.2 g/L, which was different from other reactors and further indicated the degradation of soluble organic matter at higher pH conditions.

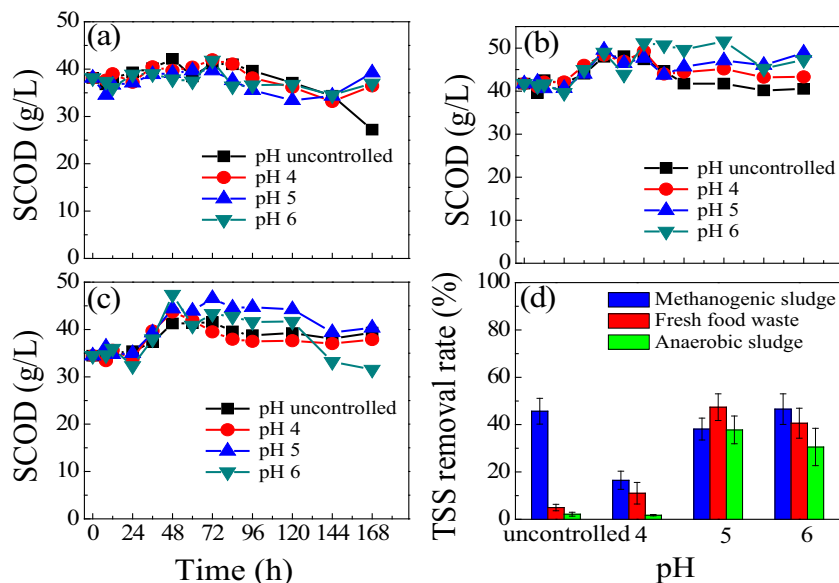


Fig. 1. SCOD content and TSS removal rate during food waste fermentation using the three inocula (a) methanogenic sludge, (b) fresh food waste, (c) anaerobic sludge and (d) TSS removal rate.

In the reactors seeded with anaerobic sludge (Fig. 1c), the SCOD also sharply increased in the first 48 h. The peak value (47.4 g/L) was obtained at pH 6, but gradually decreased with fermentation and reached 31.5 g/L at 168 h. However, at pH 4 and uncontrolled pH, the SCOD maintained an almost constant value during fermentation, indicating the weak hydrolysis under low pH conditions.

The TSS removal rate increased with pH (Fig. 1d), indicating higher pH conditions benefit the solubilization processes, which was consistent with previous studies (Jiang et al., 2013; Wu et al., 2015; Tang et al., 2016). pH adjustment relieves the free lactic acid toxicity to microorganisms, improving enzyme activity and promoting hydrolysis processes (Stenberg et al., 2000; Aljundi et al., 2005; Wang et al., 2014). Thus, the TSS removal rate sharply increased to 30–50% at pH 5 and 6 regardless of the inocula used. However, the reactor inoculated with methanogenic sludge also achieved TSS removal of 45.7% at uncontrolled pH, which might be due to the presence of facultative anaerobic microorganisms in the inoculum.

3.1.2. Soluble carbohydrates

Soluble carbohydrates in the fermentation broth were the result of a net balance between competing rates of release and degradation. The concentrations of carbohydrates clearly decreased with fermentation time and showed different profiles with pH (Fig. 2). At uncontrolled pH, in the reactor inoculated with methanogenic sludge, carbohydrates sharply decreased in the first 60 h and showed a relatively lower decline rate thereafter (Fig. 2a). However, in reactors with fresh food waste and anaerobic sludge as inoculum, carbohydrate also decreased in the first 60 h at uncontrolled pH, while it maintaining stability thereafter (Fig. 2b and c), which primarily resulted from the inhibition caused by accumulated acids and low pH in the reactors.

As shown in Fig. S1 (Supporting information), pH in reactors seeded with fresh food waste and anaerobic sludge as inocula at uncontrolled pH decreased from 4 to 3.3 in the first 60 h. Without pH adjustment, the accumulated free acid restricted bacterial enzyme activity and hydrolysis processes (Itoh et al., 2012); thus, neither the carbohydrate concentrations nor the pH values was not further reduced. However, pH in the reactor inoculated with methanogenic sludge showed a rapid decrease in the first 60 h, and fluctuated at 4, which alleviated the free lactic acid feedback

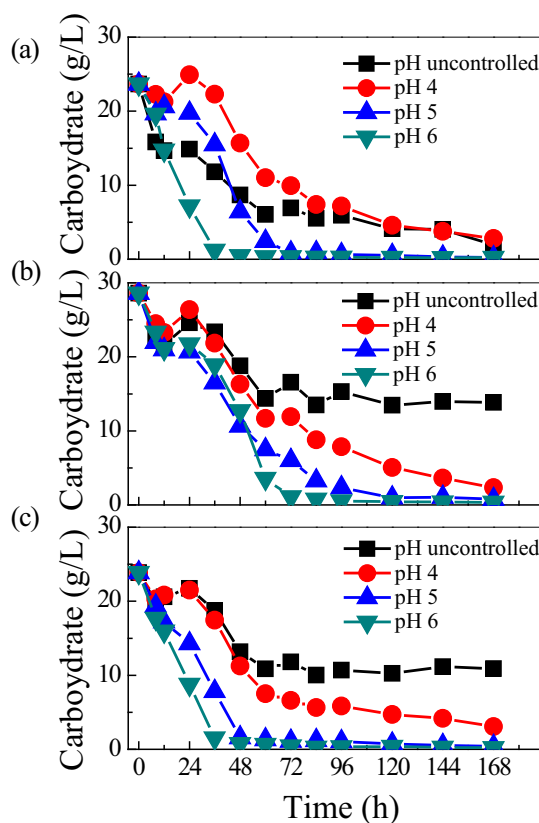


Fig. 2. Effect of pH on carbohydrates degradation during fermentation with different inocula (a) methanogenic sludge, (b) fresh food waste, and (c) anaerobic sludge.

effect. Thus, as shown in Fig. 2a, after 60 h, the carbohydrate variations in the reactor with uncontrolled pH were extremely similar to that at pH 4.

It has been reported that lactic acid bacteria (LAB) such as *Lactobacilli* can exist at extremely low pH conditions and produce lactic acid at pH 4 (Itoh et al., 2012). Soluble carbohydrates in the

Table 3
Carbohydrate degradation rate and α -glucosidase activity during fermentation at different pH (48 h).

Inoculum	Methanogenic sludge			Fresh food waste			Anaerobic sludge					
	Un	4	5	6	Un	4	5	6	Un	4	5	6
Residual carbohydrate (g/L)	12.6	20.7	12.8	5.2	28.9	25.6	25.3	22.1	22.5	19.6	10.6	3.2
Degradation rate (mg/L.h)	392.5	223.8	389.4	548.0	253.5	323.8	328.2	395.9	203.3	263.3	450.5	605.5
α -Glucosidase activity (U/g-VSS)	87.2 \pm 7.7	114.7 \pm 10.4	260.3 \pm 55.7	293.8 \pm 39.4	34.9 \pm 3.9	71.5 \pm 10.8	76.7 \pm 4.3	417.6 \pm 75.8	87.1 \pm 16.3	180.4 \pm 14.0	290.5 \pm 23.7	504.4 \pm 47.1

broth decreased with fermentation time and achieved low residual concentrations (2.3–3.1 g/L) in all reactors at pH 4, which reinforces the importance of pH adjustment on bacterial activity and fermentation processes. When pH increased to 5 and 6, carbohydrates degraded faster and reached an extremely low concentration (0.2–0.7 g/L) within a short period (72 h) because higher pH relieves the acid feedback inhibition and significantly promotes bacterial activity (Itoh et al., 2012; Tang et al., 2016; Wu et al., 2015).

Interestingly, a slight carbohydrate increase was observed in reactors during the first 24 h, especially in lower pH conditions, which might be attributed to a lower acidogenesis rate than hydrolysis rate or the acidogenic bacteria activity was not as high as hydrolytic bacteria at the initial fermentation stage.

The carbohydrate degradation rate and α -glucosidase activity are presented in Table 3. These results indicate that α -glucosidase activity increased with pH, demonstrating a higher microbial enzyme activity under higher pH conditions. Additionally, α -glucosidase activity was much higher in reactors with methanogenic sludge and anaerobic sludge as inoculum, showing a higher hydrolysis rate of the sludge, which might be due to the existence of large populations of hydrolytic bacteria in the inocula. It is well known that methane production is achieved through hydrolysis, acidogenesis and methanogenesis processes (De la Rubia et al., 2009); thus, hydrolysis is a necessary stage of methanogenesis and methanogenic sludge should be rich in hydrolytic bacteria. However, hydrolytic bacteria were rarely found in fresh food waste, as food waste was immediately obtained from the restaurant and had low pH; therefore, bacterial growth would be limited. For this reason, α -glucosidase activity in reactors seeded with fresh food waste was much lower than the other two inocula under the same pH conditions. However, when pH increased to 6, the enzyme activity sharply increased to 417.6 U/g-VSS and was more than 10 times higher than that at uncontrolled pH, reinforcing the strong carbohydrate decline at pH 6 (Fig. 2b). Bouallagui et al. (2004) also reported that the optimal pH for better hydrolytic bacteria activity was between 5.0 and 6.0, which was in agreement with this study.

Additionally, the degradation rate of carbohydrates clearly increased with pH (Table 3), indicating that higher pH significantly improves reaction rate, and shortens the fermentation period. Except for the reactor with methanogenic sludge, degradation rates were lowest at uncontrolled pH and significantly improved with pH. The highest degradation rate was obtained at pH 6 in all three inocula, consistent with α -glucosidase activity and results of other researchers indicating optimum hydrolytic enzymes activities were observed at pH 6.0 (Wang et al., 2014).

3.2. Effect of pH on acidogenesis

3.2.1. Lactic acid

The effect of pH on lactic acid production is shown in Fig. 3. When methanogenic sludge was used as inoculum, LA concentration increased with time. After 48 h, LA in the reactor at uncontrolled pH was 8.4 g/L. While at pH 4, LA continuously increased and reached a peak of 19.0 g/L and a yield of 0.33 g/g-TS (Table 4), almost 2-fold higher than that in the reactor at uncontrolled pH. At pH 5, LA first increased to 20.7 g/L at 72 h, showing a higher yield (0.36 g/g-TS), but it gradually reduced to 0.88 g/L at 168 h. The decreased LA was converted into VFAs, as shown in Fig. S2 (Supporting information). However, by increasing pH to 6, a lower LA concentration (9.1 g/L) and yield (0.15 g/g-TS) were obtained. Thus, pH adjustment clearly improved LA production. However, pH 6 was not suitable for LA fermentation due to the lowest LA concentration and earliest degradation, in agreement with a study reporting that lactate can be converted to VFAs at pH 6 (Kim et al., 2003).

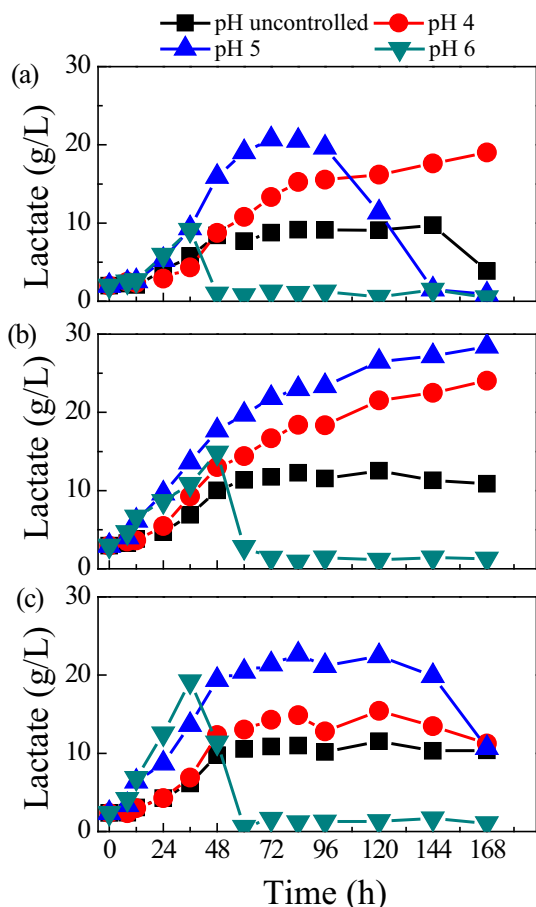


Fig. 3. Effect of pH on lactic acid production with different inocula (a) methanogenic sludge, (b) fresh food waste, and (c) anaerobic sludge.

Inoculated with fresh food waste, reactors showed much higher LA production (Fig. 3b). The highest LA concentrations were 12.3, 24.0 and 28.4 g/L at uncontrolled pH, pH 4 and pH 5, respectively. Similar to that in Fig. 3a, the reactor at pH 6 achieved the maximal LA concentration of 14.8 g/L at 48 h and sharply decreased to 2.8 g/L at 60 h, further confirming that pH 6 causes early LA degradation, as discussed in the following section. However, unlike the reactor inoculated with methanogenic sludge (Fig. 3a), LA showed no decline at pH 5, which might be a result of higher substrate content or differences in microbial communities between the two inocula.

Similarly, LA in the reactors inoculated with anaerobic sludge increased to 10.5 g/L after 60 h then maintained stability thereafter at uncontrolled pH (Fig. 3c). Increasing pH indeed promoted LA

production, but the concentration increased to 19.2 g/L in 36 h and sharply decreased to 0.6 g/L at the pH 6. In addition, the maximal LA concentrations were 15.4 and 22.6 g/L at pH 4 and 5, respectively, but they gradually decreased after 120 h.

Due to a feedback inhibition established by accumulated free acids, the cell membrane, metabolic system and substrate transport pathways were damaged (Aljundi et al., 2005; Dalie et al., 2010), LA concentration and yield at uncontrolled pH were relatively low, showing a high content of residual substrate (e.g., carbohydrate) in the broth (Table 4). Although pH 6 could improve hydrolysis, it also leads to LA degradation. Thus, LA yield was relatively low, which was consistent with the results of previous studies (Tang et al., 2016; Jiang et al., 2013; Kim et al., 2003).

Although the highest LA concentration and yield were obtained at pH 5 in all inocula (Table 4), the time needed to achieve the highest yield differed with inocula. Longer fermentation time (168 h) was required when using fresh food waste as inoculum than the other two types of inocula, which might be a result of different microbial communities and enzyme activity.

The number of lactic acid bacteria (LAB) in the broth was measured and is shown in Table 5. The numbers of LAB increased with pH increase from uncontrolled pH to pH 5, which was in accord with the LA increase. In addition, the sharp decrease in LAB populations from 48 h to 96 h in reactors at pH 6 further reinforced the drastic LA reduction, potentially caused by low substrate content (e.g., carbohydrates) in the reactors (Fig. 2) and/or resulted from the evolution of microbial communities in such conditions. In addition, reactors with methanogenic sludge as inoculum exhibited larger populations than those with fresh food waste and anaerobic sludge at uncontrolled pH, possibly due to favorable conditions in the reactors, such as the strong alkalinity. Compared with two other types of inocula, reactors inoculated with anaerobic sludge showed relatively lower LAB populations, but LA in the slurry was not very low, possibly reflecting different types of LAB or metabolic pathways. Additionally, the lower C/N ratio of the substrates provided by the anaerobic sludge might also contribute to high LA production (Li et al., 2015). Lower LAB populations in reactors inoculated with food waste and anaerobic sludge at uncontrolled pH further indicated that uncontrolled pH (pH < 4) obviously restricted LAB growth and lactic acid production.

3.2.2. Volatile fatty acids

As mentioned earlier, LA concentrations sharply decreased after achieving a peak with all inocula at pH 6; converting LA to VFAs was hypothesized as a potential reason. To investigate these phenomena, VFAs in the broth were measured, as shown in Fig. 4. A sharp increase in VFAs matched the quick decrease in LA (shown in Fig. 3) indicating that LA can be transformed into VFAs, also in accordance with the previous studies (Kim et al., 2003; Itoh et al., 2012; Tang et al., 2016).

Table 4
Maximal lactic acid yield with different pH and inocula.

	pH	Time (h)	LA (g/L)	LA yield (g/g-TS)	Lactate %SCOD	VFAs %SCOD	Carbohydrate %SCOD	Protein %SCOD
Methanogenic sludge	Un	144	9.7	0.16	30.1	36.7	12.5	2.63
	4	168	19.0	0.33	55.3	17.4	8.12	2.78
	5	72	20.7	0.36	55.3	15.5	2.36	1.65
	6	36	9.12	0.15	24.8	54.2	3.24	1.61
Fresh food Waste	Un	120	12.5	0.20	31.8	12.1	34.2	2.49
	4	168	24.0	0.39	63.2	13.4	6.17	2.85
	5	168	28.4	0.46	61.6	17.1	1.62	1.47
	6	48	14.8	0.24	32.1	11.6	27.5	1.76
Anaerobic sludge	Un	120	11.5	0.21	31.2	16.5	27.7	1.40
	4	120	15.4	0.28	43.5	17.5	13.3	1.90
	5	84	22.6	0.41	53.8	23.6	1.75	1.26
	6	36	19.20	0.34	53.5	16.4	4.22	1.29

Table 5
Cell counts of lactic acid bacteria during fermentation.

pH	Methanogenic sludge				Fresh food waste				Anaerobic sludge			
	0 h	48 h	96 h	120 h	0 h	48 h	96 h	120 h	0 h	48 h	96 h	120 h
Un	2	440	310	140	15	60	40	40	1	40	47	12
4	3	670	640	340	21	350	240	330	1	130	30	5
5	2	630	720	280	10	430	420	410	1	90	50	14
6	1	340	87	20	17	400	220	10	1	70	40	1

Unit: $\times 10^8$ cfu/L.

Moreover, compositions of VFAs showed large differences between inocula. The reactors seeded with methanogenic sludge appeared to produce more butyrate from the beginning of the fermentation (Fig. 4a), as butyrate sharply increased from 6.0 g/L to 12.3 g/L while the lactic acid decreased from 9.1 g/L to 1.0 g/L at 48 h. However, propionate was very low during the entire fermentation period, and acetate in the broth maintained stability at 3.0–5.0 g/L. However, the fresh food waste inoculum tended to convert lactate into acetate, propionate and butyrate (Fig. 4b), as the content of these organic acids increased from 3.9 to 8.0 g/L, 0.9 to 5.1 g/L and 0.1 to 3.9 g/L, respectively. This result might be attributed to diverse microbial communities in the inoculum. Additionally, anaerobic sludge was more favorable for producing acetate and propionate with lactate, while butyrate in the broth consistently maintained a low content (Fig. 4c). VFA concentrations in other reactors at lower pH were relatively low and stable during fermentation (Fig. S2, Supporting information).

Accordingly, it can be concluded that increasing culture pH could promote lactate production, but when pH was adjusted to

6, lactate will degrade to VFAs at the early stage. Both pH 4 and 5 can relieve the low pH restriction and free acids, but stronger hydrolysis at pH 5 resulted in higher LA yield making pH 5 more suitable for lactic acid fermentation.

3.3. COD balance analysis

The COD balance at the optimal condition of each inoculum is presented in Fig. 5. Although COD components varied with culture pH, some interesting results were obtained. In Fig. 5a, with metha-

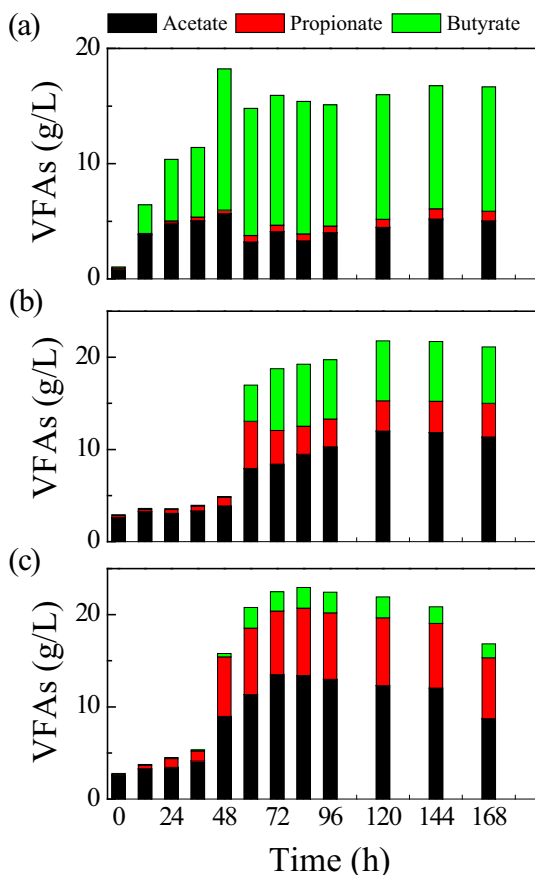


Fig. 4. Variation of VFA concentrations during fermentation at pH 6 with three types of inocula (a) methanogenic sludge, (b) food waste, and (c) anaerobic sludge.

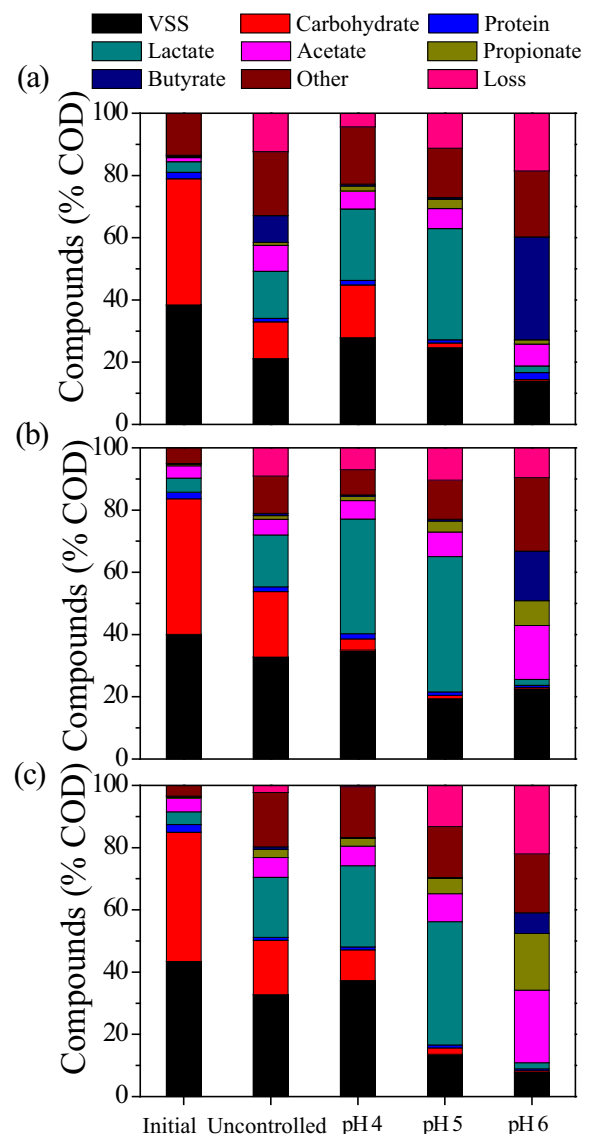


Fig. 5. COD balance analysis at different pH with different inocula (a) methanogenic sludge at 72 h, (b) fresh food waste at 168 h, and (c) anaerobic sludge at 84 h.

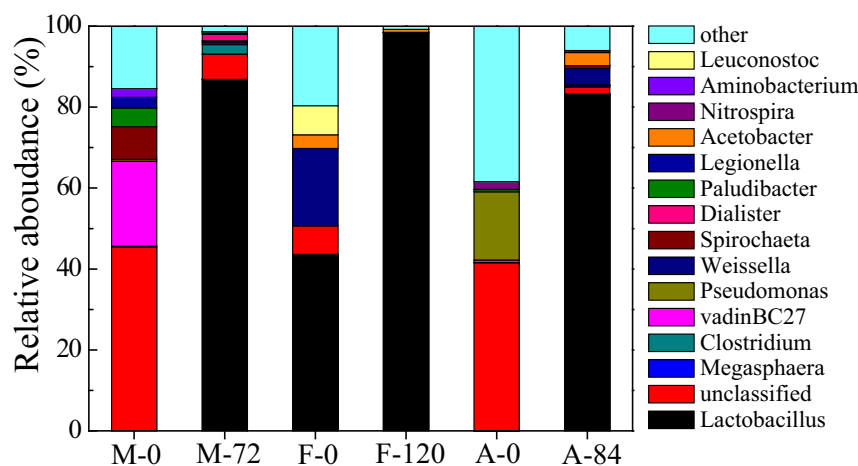


Fig. 6. Microbial community analysis of inocula and samples in reactors at pH 5. (M-0: inoculum methanogenic sludge; M-72: reactor with methanogenic sludge at 72 h; F-0: inoculum fresh food waste; F-120: reactor with fresh food waste at 120 h; A-0: inoculum anaerobic sludge; A-84: reactor with anaerobic sludge at 84 h).

nogenic sludge as inoculum, an obvious COD loss is observed in all pH conditions, which reinforced the slight SCOD increase (Fig. 1a). The highest COD loss was found in the reactor at pH 6, which accounted for 18.5% of the TCOD supplied, indicating a large amount of substrate was utilized to produce biogas or consumed through other pathways (Karadag and Puhakka, 2010). The proportion of soluble carbohydrates was 40.6% at the initial stage, while it decreased to 1.5% and 0.5% at pH 5 and 6, respectively. However, the fate of degraded carbohydrates was completely different. At pH 5, carbohydrates were mainly converted to lactic acid, but at pH 6, the VFAs, especially butyrate, were the dominant products. However, soluble carbohydrates at uncontrolled pH and pH 4 remained approximately 11.9% and 17.0%, reflecting a low carbohydrates degradation rate as mentioned previously. Particulate organics decreased with pH increase and achieved the lowest percentage (13.9%) at pH 6, indicating the strongest solubilization rate, in agreement with the TSS removal rate (Fig. 1d).

Similarly, reactors seeded with fresh food waste and anaerobic sludge showed the highest VSS reduction to VFAs and other unidentified soluble organics at pH 6 (Fig. 5b and c). LA proportions in the TCOD were 43.6% and 39.7% at pH 5 with these two inocula, respectively. In addition, it was found that although the proportion of lactic acid at pH 4 was not as high as that at pH 5, the percentage of by-products such as VFAs and other unidentified matter were lower. These results might be due to the fact that lactic acid bacteria could grow, but other bacteria could not survive in such a low pH environment (Itoh et al., 2012; Wu et al., 2015).

The highest proportions of lactic acid and satisfactory particulate reduction were obtained at pH 5 with all the inocula and can be regarded as the optimal condition for lactic acid fermentation. In addition, the proportion of lactic acid in the broth was in the following order: food waste > anaerobic sludge > methanogenic sludge. Methanogenic sludge tended to produce butyrate, while anaerobic sludge was more favorable for producing acetate and propionate. The difference might be related to differing microbial communities in the inocula at the initial stage and/or during the fermentation process, and further research is required to clarify this point.

3.4. Effect of pH on microbial communities

To investigate the effect of pH on the microbial communities, samples representing the inocula and highest LA yield were collected and analyzed using a molecular biological technique, and

the results are shown in Fig. 6. High microbial diversity existed in all inocula (M-0 (methanogenic sludge), F-0 (fresh food waste), and A-0 (anaerobic sludge)), and microorganisms in the inocula were significantly distinct. The *Lactobacillus* accounted for a large proportion (43.6%) in fresh food waste inoculum, followed by the genus of *Weissella* (19.2%). However, the relative abundance of *Lactobacillus* in methanogenic sludge and anaerobic sludge were only 0.02% and 0.04%, respectively. The *vadinBC27* (21.0%) and *Spirochaeta* (8.1%) were the main identified genera in methanogenic sludge, while *Pseudomonas* (16.9%) was the main identified microorganism in anaerobic sludge.

However, when reactors reached their own highest LA yield, *Lactobacillus* dominated microbial communities in all three reactors (86.9% in M-72 (methane sludge at 72 h), 98.5% in F-120 (fresh food waste at 120 h) and 83.4% in A-84 (anaerobic sludge at 84 h)), which further verified the high LA concentration in the fermentation broth. Similar results were also reported with mixed culture fermentation to produce LA (Liang et al., 2016; Tang et al., 2016). These results revealed a selection of bioreactor microbes, and the unique LAB group function. It also demonstrated the suitability of food waste as a useful substrate for lactic acid fermentation initiated by different inocula containing mixed bacterial cultures.

4. Conclusions

The optimal LA concentration (20.7–28.4 g/L) and yield (0.36–0.46 g/g-TS) were obtained at pH 5 with all three inocula, showing a higher TSS removal rate, substrate degradation rate and microbial enzyme activity. The highest LA yield (0.46 g/g-TS) was achieved using fresh food waste as inoculum. Low microbial enzyme activity, LAB populations and LA yield were observed at uncontrolled pH. LA was degraded to VFAs at pH 6 at early stages and the VFA compositions differed with the inocula used. *Lactobacillus* was enriched (83.4–98.5%) during the fermentation process, although abundant microbial diversity existed in the initial inocula.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2016.11.111>.

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