Waste Management xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR

Jialing Tang [a,*], Xiaochang Wang [a,**], Yisong Hu [a], Yongmei Zhang [a], Yuyou Li [b]

[a] School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an 710055, China
[b] Department of Civil and Environmental Engineering, Tohoku University, Sendai 9808579, Japan

ARTICLE INFO

Article history:
Received 21 December 2015
Revised 1 March 2016
Accepted 18 March 2016
Available online xxxx

Keywords:
Lactic acid fermentation
pH adjustment
Temperature
Organic loading rate (OLR)
Lactobacillus

ABSTRACT

The effects of pH, temperature and high organic loading rate (OLR) on lactic acid production from food waste without extra inoculum addition were investigated in this study. Using batch experiments, the results showed that although the hydrolysis rate increased with pH adjustment, the lactic acid concentration and productivity were highest at pH 6. High temperatures were suitable for solubilization but seriously restricted the acidification processes. The highest lactic acid yield (0.46 g/g-TS) and productivity (278.1 mg/L h) were obtained at 37 °C and pH 6. In addition, the lactic acid concentration gradually increased with the increase in OLR, and the semi-continuous reactor could be stably operated at an OLR of 18 g-TS/L d. However, system instability, low lactic acid yield and a decrease in VS removal were noticed at high OLRs (22 g-TS/L d). The concentrations of volatile fatty acids (VFAs) in the fermentation mixture were relatively low but slightly increased with OLR, and acetate was the predominant VFA component. Using high-throughput pyrosequencing, *Lactobacillus* from the raw food waste was found to selectively accumulate and become dominant in the semi-continuous reactor.

1. Introduction

Lactic acid (LA) is an important chemical commodity that is widely used in the food, pharmaceutical and chemical industries (Jiang et al., 2011; Mazzoli et al., 2014). The continuing increase in demand for lactic acid has led to a 15% annual growth rate of the global market (Ye et al., 2008). Lactic acid fermentation through anaerobic digestion (AD) has been widely applied for LA production in which separation and purification processes and raw material are the two main factors determining the production cost. Economical purification methods such as electrodialysis, membrane filtration, liquid surfactant extraction have been explored by many researchers (Vijayakumar et al., 2008; Aljundi et al., 2005). Cheap agricultural and renewable resources such as barley, corn, whey, potato peel waste, fruit and vegetable wastes, and hardwood pulp have been used for lactic acid fermentation (Hama et al., 2015; Eom et al., 2015; Wu et al., 2015; Liang et al., 2014). Additionally, many studies have claimed that food waste could be a potential material for LA fermentation due to its high starch content and large quantities (Li et al., 2015; Ye et al., 2008).

Anaerobic digestion consists of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. LA was produced in the first two steps. Other products such as VFAs, ethanol (Jiang et al., 2013; Komemoto et al., 2009; Wang et al., 2013; Lim et al., 2008; Chen et al., 2013; Zhang et al., 2016) and biogas (Chu et al., 2012; Lee et al., 2014; Wang et al., 2015a, 2015b) could also be produced during the AD processes by controlling the operation parameters such as pH, temperature and OLR. In this context, to obtain a high LA yield, it is necessary to control and optimize the operation conditions to minimize the production of other intermediate products.

The AD process can be influenced by several important operation parameters such as pH, temperature and organic loading rate (OLR). It is well known that pH significantly affects enzyme activities and bacterial metabolism. Parawira et al. (2005) found that the optimum pH of hydrolytic enzyme activity was approximately 6.0, but wider pH ranges from 4 to 11 were obtained by other researchers with different substrates during lactic acid fermentation (Li et al., 2015; Wang et al., 2014; Akao et al., 2007; Zhang et al., 2008). Under low pH conditions, LA could be produced (Wang et al., 2014; Itoh et al., 2012; Wu et al., 2015), but the yield was low, because the undissociated LA would inhibit the metabolism of bacteria (Amrane and Prigent, 1994; Aljundi et al., 2005; Dalie et al., 2010). Thus, adjusting the pH to neutralize the free LA becomes necessary as reported by researchers (Li et al., 2014,

* Corresponding author.
** Corresponding author.
E-mail addresses: tangjialing88@126.com (J. Tang), xcwang@xauat.edu.cn (X. Wang).

http://dx.doi.org/10.1016/j.wasman.2016.03.034
0956-053X/

2015; Wu et al., 2015). However, high pH levels adversely affect the LA yield, as the produced LA would be partially degraded into SCFAs or CH_4 (Kim et al., 2003; Li et al., 2014; Komemoto et al., 2009); hence, more attention needs to be warranted on the optimization of the pH control strategy for LA fermentation. Moreover, temperature as another important operation parameter influences the microbial activity, conversion rate of substrate, and economic analysis (Kim et al., 2003; Mahmoud et al., 2004) during LA fermentation. Liang et al. (2014) compared the LA fermentation under different temperature conditions and found that although the hydrolysis was promoted at high temperature, the LA yield at 55 °C was only 0.088 g/g-TS which was much lower than that at 37 °C (0.22 g/g-TS). This might be because high temperature was not suitable for lactic acid bacteria (LAB) growth (Zhang et al., 2007). However, Kim et al. (2012) obtained higher LA production at high temperatures (50–55 °C) by selectively enriching thermophilic LAB over long term operation in a CSTR. In addition, some researchers have attempted to obtain high LA production through increasing OLR (Zhang and He, 2014). As reported, although a high OLR could reduce the reactor volume and decrease the operating cost, it would also lead to imbalanced osmotic pressure and negatively affect the growth of LAB (John et al., 2009; Shen et al., 2013), which further decreases LA productivity notably. These parameters simultaneously influenced the LA fermentation, while some controversial results were still presented in the aforementioned work. Accordingly, it is necessary to explore the proper pH control strategy, temperature and OLR for maximizing LA production and stabilizing the long-term operation.

Microbial communities are also widely studied in LA fermentation. Specific lactic acid bacteria (LAB) species such as *Lactobacillus brevis* and *Lactobacillus plantarum* have been widely used as inoculation bacteria (Yang et al., 2015; Eom et al., 2015; Zhang and Vadlani, 2015), but their real application in LA fermentation could be limited because they are fragile and easily influenced by nutrient imbalance or other operation parameters. Compared with the pure genus fermentation, the indigenous microflora accumulated during fermentation benefits the degradation and utilization of macromolecules (John et al., 2007) and increases the tolerance of LA yields or decreases the need for nutrient supplements (Secchi et al., 2012). Clearly, LA fermentation with indigenous microflora is more advantageous; therefore, the variations in microbial structures during fermentation need further investigation.

The objectives of this study were firstly to investigate the effects of pH and temperature on lactic acid fermentation with indigenous microbiota as inoculators to find the optimal operational conditions and then to explore the stability of the long-term operation with a high organic loading rate (OLR). Finally, the variations in microbial communities before and after fermentation were discussed.

2. Methods and materials

2.1. Food waste substrate

Food waste was collected from a canteen of a university campus in Xi'an, China. The food waste mainly comprised rice, vegetables and meat. It was crushed with an electrical blender after animal bones and clamshells were sorted out; afterward, the resulting slurry was sieved (1 mm) and stored in a refrigerator (4 °C). Before adding the slurry into the reactors, it was adjusted to a proper TS content. The characteristics of food waste slurry were shown in Table 1.

2.2. Effects of pH and temperature

The effects of pH on lactic acid fermentation were evaluated in four identical reactors (P1–P4, in Table 2). According to the results of some researchers and of our previous studies, the TS of fermentation substrate ranged from 2% to 13% (Liang et al., 2014; Wang et al., 2014; Zhang et al., 2016); the TS of the slurry in this study was firstly adjusted to 7% with tap water. Then, 20 L of slurry was separated into the 4 batch fermentation bioreactors (working volume 5 L). All reactors were mechanically stirred at 120 rpm, and maintained at ambient temperature (25 ± 2 °C). The pH was adjusted intermittently by sodium hydroxide (5 M) or hydrochloric acid (5 M) to 6, 8, and 10 every 12 h. A reactor without any pH adjustment was operated as a control. The variations in chemical oxygen demand (COD), carbohydrates and lactic acid were detected and compared to select a proper pH for LA fermentation.

According to the pH results, the highest lactic acid yield was obtained when the pH was adjusted to 6 intermittently (every 12 h). Thus, a series of experiments were conducted to investigate the effects of temperature on lactic acid fermentation. The temperatures of 37 °C (mesophilic) and 55 °C (thermophilic) were chosen. At first, 20 L of food waste slurry (TS = 7%) was completely mixed and separated in four anaerobic fermentation reactors (T1–T4, in Table 2). The temperature in T1 and T2 was 37 °C, and it was 55 °C in T3 and T4 with an automatic water recycling bath. The pH in T1 and T3 was not controlled, whereas it was adjusted to 6 every 12 h in T2 and T4 using sodium hydroxide (5 M) or hydrochloric acid (5 M). All reactors were stirred at 120 rpm with electric agitators. The fermentation products were sampled regularly and analyzed to explore the effect of temperature on lactic acid fermentation.

Table 1
Characteristics of raw food waste.

Parameter	Units	Mean	S.D.
pH	–	4.5	0.1
Total solid content (TS)	% of wet weight	20	1.2
Volatile solid content (VS)	% of wet weight	19.2	1.5
VS/TS	%	96.4	0.3
Total COD (TCOD)	g/L	257.9	5.2
Soluble COD (SCOD)	g/L	71.9	3.5
Soluble carbohydrate	g/L	56.8	2.3
Soluble protein	g/L	6.2	1.6

Note: S.D. represents standard deviation.

2.3. Effects of OLR

To investigate the effects of high OLR on lactic acid production and stability over long-term operation, an up-scaled semi-continuous fermentation reactor (10 L, once-a-day feeding and draw-off) was set up. According to the results of the batch fermentation, the experiments were conducted at 37 °C. The pH in the reactor was adjusted by sodium hydroxide (5 M) or hydrochloric acid (5 M) to 6 every 12 h. The retention time was kept at 5 days. On the first day, 10 L of the fresh food waste mixture (TS = 7%) was added into the reactor and fermented. The stirrer at 120 rpm was installed to mix the substrates. Every morning, 2 L of fermentation mixture was drained from the reactor and replaced by the same volume of fresh food waste slurry. At day 15, the OLR was

Table 2
Conditions of batch fermentation reactors.

	Reactor	TS (%)	Temperature (°C)	pH
TEST1	P1	7	25	Uncontrolled (UN)
	P2		25	6
	P3		25	8
	P4		25	10
TEST2	T1	7	35	Uncontrolled (UN)
	T2		35	6
	T3		55	Uncontrolled (UN)
	T4		55	6

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increased to 18 g-TS/L d, and 10 days later the OLR of 22 g-TS/L d was investigated by adjusting the TS content of the feed.

2.4. Analytical methods

Samples were used to analyze the total chemical oxygen demand (TCOD), total nitrogen and phosphate, and total carbohydrate and protein immediately. After being centrifuged (6000 r/min for 10 min), the supernatant was filtered through 0.45-μm filters. The filtrate was utilized to measure the total organic carbon (TOC), soluble chemical oxygen demand (SCOD), volatile fatty acids (VFAs), soluble proteins and carbohydrates and lactic acid. The measurement of SCOD and TCOD was according to the standard methods (APHA, 1998). Carbohydrates were measured by the phenol–sulfuric method with glucose as a standard (Herbert et al., 1971). Soluble protein was determined by the Lowry-Folin method with BSA as a standard (Lowry et al., 1951). The elements of the food waste substrate were assayed by an elemental analyzer according to Li et al. (2015).

To analyze the VFAs, the filtrate was collected in a 1.5-mL gas chromatograph (GC) vial, and 3% H_3PO_4 was added to adjust the pH to approximately 4.0. A gas chromatograph (GC2014, Shimadzu, Japan) with a flame ionization detector and equipped with a 30 m × 0.32 mm × 0.25 mm CPWAX52CB column was utilized to analyze the composition of VFAs. Nitrogen was the carrier gas, and the flux was 50 mL/min. The injection port and the detector were maintained at 200 and 220 °C, respectively. The oven of the GC was programmed to begin and remain at 100 °C for 2 min, then increase at a rate of 10 °C/min to 200 °C, and remain at 200 °C for 2 min. The sample injection volume was 0.5 μL.

The concentration of lactate was determined using a liquid chromatograph (LC-10AD, Shimadzu, Japan) equipped with an ultraviolet detector. COSMOSIL 5C18-AR-II was used for the column, and 0.05 M phosphoric acid buffer liquid (50 mM NaH_2PO_4:50 mM H_3PO_4 = 9:1, pH 3) was used for the carrier liquid. The analysis was performed at a detector temperature of 40 °C, flow velocity of 1.0 mL/min, and UV of 210 nm.

2.5. Microbial community analysis

To explore the change in bacterial communities before and after fermentation, samples of the raw food waste and fermentation mixture (at Day 13) were sent to Sangong, Inc. (Shanghai, China) for DNA extraction and the next-generation sequencing processes. To conduct the pyrosequencing, the extracted DNA was amplified by PCR using the primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) for the V1–V3 region (Li et al., 2015). Pyrosequencing was conducted using a Roche 454 GS FLX+ Titanium platform, and homologous or ambiguous sequences or those with a length shorter than 200 bp were trimmed to obtain high-quality sequences. A total of 12,074 (in the raw food waste) and 13,585 (from the sample on the 13th day) high-quality 16S rRNA gene sequences were obtained with an average length larger than 400 bp.

2.6. Calculation

The hydrolysis and acidification can be calculated according to SCOD, COD_{VFA} and $COD_{lactic\ acid}$, respectively. All calculations were determined by previous research, and the following equations were used (Zhang et al., 2016):

$$\text{Hydrolysis}\ (\text{COD}) = \frac{\text{SCOD} - \text{SCOD}_0}{\text{TCOD}_{in}} \quad (1)$$

$$\text{Acidogenesis}\ (\text{COD}) = \frac{\text{COD}_{VFA} + \text{COD}_{lactic\ acid}}{\text{SCOD}} \quad (2)$$

where $SCOD_0$ and $TCOD_{in}$ were the soluble and total COD of the influent, respectively. SCOD was the soluble COD at the end of fermentation.

3. Results and discussion

3.1. Effects of pH and temperature

3.1.1. Effect of pH

As shown in Fig. 1, pH had significant effects on hydrolysis and acidification. Due to the accumulation of acidic products (VFAs and lactic acid), the pH in the uncontrolled reactor decreased to 3.5 in 36 h. Such a low pH severely restricted the activities of hydrolytic enzymes and the growth of microorganisms. Thus, the SCOD increased slowly from 24.5 g/L to 40.0 g/L until the end of fermentation. However, after adjusting the pH to 6 intermittently (every 12 h), the SCOD reached 46.1–49.4 g/L. The pH adjustment benefited the avoidance of the inhibition of acid matter (e.g., lactic acid) on the activities of microorganisms and enzymes. Therefore, it could be concluded that the intermittent pH adjustment did improve hydrolysis. However, it can be seen from Fig. 1a that the SCOD concentrations were very close for the pH adjustments to 6, 8 or 10, which might be a result of the fast decrease in pH due to the acid production. Although the pH was adjusted to 8 or 10, it decreased to 4–6 in 4–5 h due to the accumulation of acid produced (Fig. S1, Supporting information). Moreover, the SCOD increased faster in the first 48 h and remained stable thereafter. The solubilization reaction is closely modeled as a first-order reaction (Feng et al., 2009; Kim et al., 2003), and the rates of reaction based on the SCOD variations in the first 48 h was investigated. According to the results of simulation (Fig. S2, Supporting information), the rate of solubilization for uncontrolled pH and for a pH of 6, 8 and 10 were approximately 177.4, 334.6, 351.3 and 397.7 mg-SCOD/(L h), respectively, which indicated that a slightly greater solubilization rate could be obtained when the pH was adjusted to higher levels.

The soluble carbohydrates detected were the results of a net balance between competing rates of release and degradation. As shown in Fig. 1b, the soluble carbohydrates increased gradually in the first 48 h, which might be attributed to the higher rate of hydrolysis than acidogenesis. More particulate carbohydrates were solubilized than could be consumed by acidifying bacteria which led to the accumulation of soluble carbohydrates. However, the rate of acidification was faster after 48 h; thus, the concentration of soluble carbohydrate decreased gradually. This might be due to the accumulation of hydrolysis bacteria being easier and faster than that of acidogenesis bacteria. In the reactor with uncontrolled pH, the soluble carbohydrate concentration stabilized after 144 h, the lactic acid concentration also remained unchanged (Fig. 1c). Meanwhile, the pH in the reactor also decreased to 3.0 ± 0.3 due to the free lactic acid accumulation, which inhibited the hydrolysis. Low pH not only affects cell growth but also inhibits biochemical reactions for lactic acid production (Iyer and Lee, 1999). In addition, lactic acid is known to be a strong inhibitor of cell growth, enzymatic hydrolysis and microbial activity production in lactic acid fermentation (Huang et al., 2005). However, through adjusting the pH to 6 and 8 intermittently, the carbohydrates decreased to 1.54 g/L and 0.61 g/L at 288 h respectively. The carbohydrates in both reactors were degraded completely. The LA produced was neutralized through pH control and preventing its self-inhibition on LAB. However, when the pH was adjusted to 10, the degradation of carbohydrates was slower, and the residual carbohydrate content was 11.1 g/L. Under alkaline conditions, the activities of acidifying bacteria were restricted or the cell membrane was damaged (Anderson et al., 2015), which resulted in incomplete carbohydrate degradation (Zhang et al., 2016).

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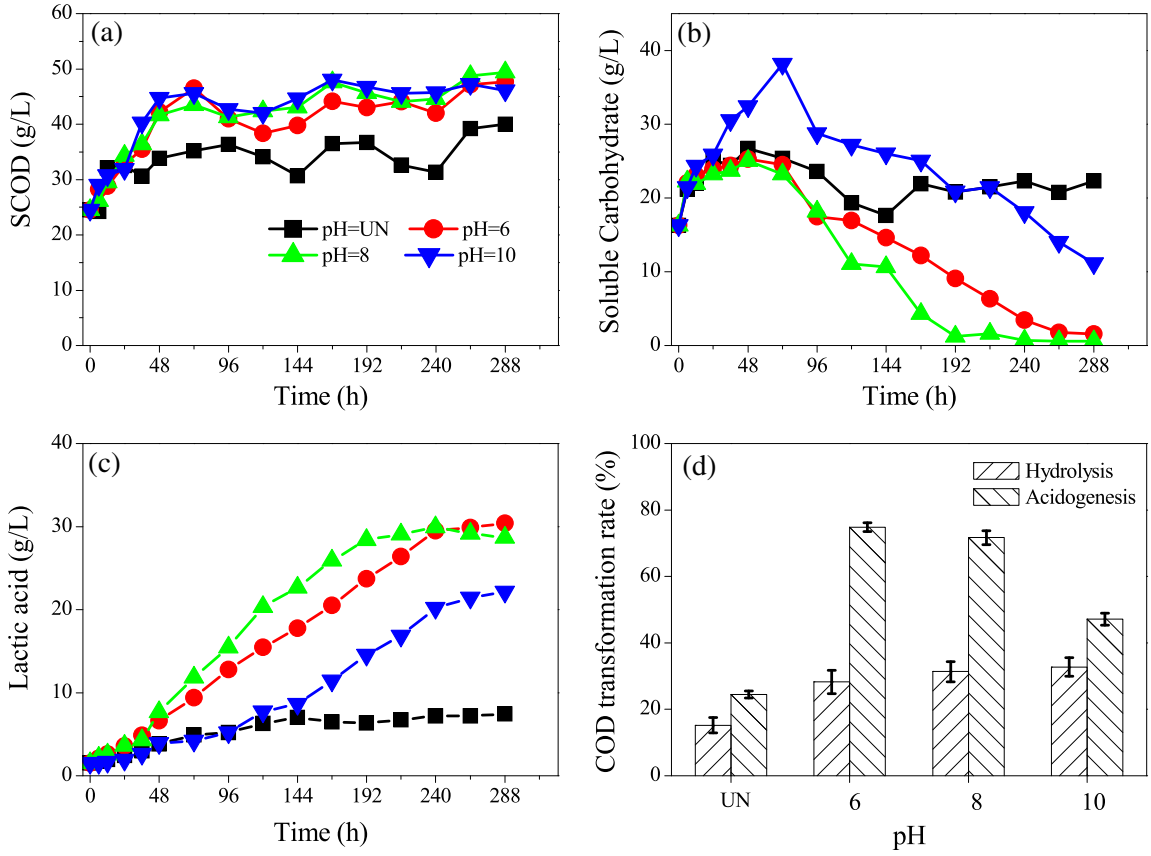

Fig. 1. Effects of pH on lactic acid fermentation under ambient temperature: (a) SCOD concentration, (b) soluble carbohydrate concentration, (c) lactic acid concentration, and (d) hydrolysis and acidogenesis rate.

The variations in lactic acid concentration are shown in Fig. 1c. Without pH control, the lactic acid concentration was the lowest. The lactic acid reached a steady state after 144 h, and remained constant at 6.3 g/L. Although some types of lactic acid bacteria (LAB) can produce lactic acid in extremely low pH (pH = 3.5) conditions (Itoh et al., 2012), most LAB are not resistant to low pH and hardly produce lactic acid at pHs below 4 (Hano et al., 1996). The extremely low pH in our reactor severely retarded hydrolysis and acidogenesis, which further reduced lactic acid production. However, by adjusting the pH to 6 and 8, lactic acid increased gradually and achieved a maximal content (30.4 and 29.5 g/L, respectively) at 240 h. These results are consistent with those of Kim et al. (2003) who concluded that the greatest degree of hydrolysis and acidogenesis occurred when the pH was controlled at 6.5. This was because the acid components in the products were neutralized by the sodium hydroxide, avoiding the negative effects on hydrolysis and further strengthening the hydrolysis and acidogenesis. However, when the pH was increased to 10, the highest lactic acid concentration was only 22.1 g/L, which was much lower than those at pH 6 and 8. This might be due to the inhibition of bacteria activities by higher pH. The optimal pH for lactic acid fermentation was approximately neutral (Akao et al., 2007; Zhang et al., 2008).

The hydrolysis rate increased with the increase in pH adjustment. As seen in Fig. 1d, the hydrolysis rate was only 15.20% when the pH was not controlled. On the other hand, it increased to 28.25% as the pH was adjusted to 6 intermittently. However, when the pH was increased to 8 and 10, the hydrolysis rate showed a slight increase to 31.34% and 32.72%, respectively, depicting the same trend as the variations in SCOD. The acidogenesis rate increased significantly from 24.49% at uncontrolled pH to 74.85% at pH 6. However, when pH was 10, the acidogenesis decreased to 47.18%, which also accorded with the lactic acid variations.

From the results of the batch experiments, it can be concluded that pH showed significant effects on both hydrolysis and acidification. The maximal acidogenesis rate and lactic acid yield were attained at both pH 6 and 8, but the proportion of LA in fermentation products (LA-SCOD/SCOD) at pH 6 was 68.1% which was higher than that at pH 8 (61.9%) and less alkali was needed at pH 6. Hence, in this present study, pH 6 was chosen as the optimal pH for lactic acid fermentation because it involves less operational costs.

3.1.2. Effect of temperature

The effects of temperature on lactic acid fermentation are shown in Fig. 2. Under thermophilic conditions (55 °C), the lactic acid productivity was much slower. The final lactic acid in the reactor without pH control was only 5 g/L, and it was only 14.6 g/L when the pH was adjusted to 6, which was obviously lower than that under methophilic conditions (37 °C). This might be because some lactic acid functional bacteria in the influent might not acclimate to thermophilic conditions (Zhang et al., 2007; Liang et al., 2014), or they may require a longer adaption time. Without pH adjustment, the hydrolysis rate under thermophilic conditions was higher than that under mesophilic conditions, but when the pH was adjusted to 6, the hydrolysis rates were very similar. However, the acidogenesis rate was much higher under mesophilic conditions regardless of pH adjustment. These results implied that hydrolysis could be enhanced and may not need a long running time at a higher temperature, but acidogenesis could be retarded at higher temperatures (Kim et al., 2003).

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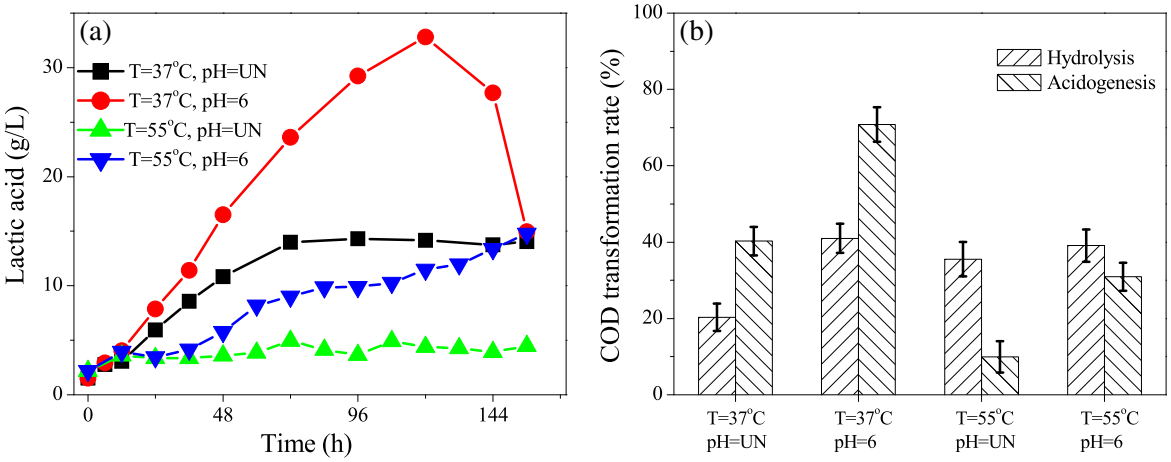

Fig. 2. Effects of temperature on lactic acid fermentation at pH 6: (a) lactic acid concentration and (b) hydrolysis and acidogenesis rate.

As mentioned above, the accumulation of acid in the reactor inhibited the activity of microorganisms. The lactic acid concentration at 37 °C without pH adjustment was only 13.98 g/L, and it remained stable thereafter (Fig. 2(a)). However, compared with the lactic acid content at stable state under ambient conditions, the lactic acid yield at 37 °C was higher, which was mainly attributed to the stronger hydrolysis or the higher tolerance of microorganisms to low pH in mesophilic conditions. When the pH was adjusted to 6, lactic acid in the reactor accumulated rapidly and achieved a maximal content of 32.8 g/L at 120 h. This might have resulted from two reasons: firstly, pH neutralization can effectively reduce the negative effects of low pH on hydrolysis; secondly, lactic acid was transformed into lactate which avoided the self-inhibition of free lactic acid on LAB (Amrane and Prigent, 1994; Aljundi et al., 2005). The lactic acid was transformed into VFAs after 120 h in 37 °C at a pH of 6 (Fig. S3, Supplementary Information), consistent with Kim et al. (2003) and Komemoto et al. (2009) who found a similar phenomenon at a pH of 6.5.

Without pH adjustment, the pH in the reactors remained at 3.2 ± 0.4, and the hydrolysis rate at 37 °C was much lower than that at 55 °C (Fig. 2b), indicating that higher temperatures could improve hydrolysis even under low-pH conditions. Meanwhile, by intermittently adjusting the pH to 6, the hydrolysis rate under the two temperature conditions was very close. However, because some LA functional microorganisms might be not suited to thermophilic conditions or require a longer adaption time (Zhang et al., 2007; Liang et al., 2014), the acidogenesis rate was lower at 55 °C, which was only 9.9% and 30.9% at uncontrolled pH and a pH of 6, respectively. These results were consistent with the variations in lactic acid (Fig. 2a).

It can also be seen in Table 3 that the SCOD at 55 °C was slightly higher than that at 37 °C regardless of the pH adjustment, indicating that a higher solubilization rate could be obtained under higher temperatures. Temperature had a significant influence on the hydrolysis of proteins, carbohydrates and lipids (Mahmoud et al., 2004; Zhang et al., 2009). The reason was presumably due to the accelerated growth rate and lower substrate affinity of some thermophilic bacteria (Rehm et al., 2000; Kim et al., 2002). In addition, thermophilic bacteria exhibit higher activity than mesophilic bacteria (Harris and Dague, 1993; Kim et al., 2003).

High temperatures accelerated hydrolysis and retarded acidogenesis, which resulted in more residual carbohydrates in the fermentation mixture. It can be seen in Table 3 that soluble carbohydrates were still the main component under 55 °C after 120 h. The accumulation of soluble carbohydrates might have resulted from two reasons: firstly, high temperatures stimulate hydrolysis, and more particulate carbohydrates were transformed into soluble forms; secondly, acidification was restricted by high temperatures, and fewer carbohydrates were consumed. However, the carbohydrate content decreased to 39.9% and 4.4% at 37 °C, and most carbohydrates were transformed into lactic acid and VFAs. The acidogenesis rate was higher than that of hydrolysis, which demonstrated that mesophilic temperature was more suitable for lactic acid fermentation even under conditions of uncontrolled pH.

The VFA content in the fermentation mixture were very low, which might be due to the low pH in the reactors. Although the pH was adjusted every 12 h, it decreased to 3–4 in just 4 h with the accumulation of acid products. Such low pH conditions were not suitable for VFAs production. Meanwhile, the VFAs production at 37 °C and a pH of 6 was the highest, accounting for 16% of the soluble COD. VS reduction reached the highest value of 45.83% at a mesophilic temperature by adjusting the pH to 6 intermittently. Meanwhile, the lactic acid productivity at pH 6 was twice that at uncontrolled pH at mesophilic temperatures, and 3–15 times higher than that at thermophilic temperatures.

3.1.3. The interactions of pH, temperature

Lactic acid yield is an important indicator of lactic acid fermentation. Thus, the interaction of pH and temperature on LA yield was further investigated based on the response surface methodology (RSM) using Design Expert software. The results of ANOVA for the LA yield are shown in Table S1 (Supplementary Information), which indicated the statistical significance and reliability of the models. The fit summary recommended the quadratic model as the appropriate one among other polynomial models for the response with low P value (<0.05), and the following mathematical regression in terms of coded factors was generated:

$$R = -1.478 + 0.316A + 0.052B - 0.0014AB - 0.0193A^2 - 0.0006B^2$$

where R indicates the predicted response (LA yield, g/g-TS) and A and B are the two independent factors: pH value and temperature (°C), respectively. The 3D surface graphs and the contour plots of RSM for the responses are shown in Fig. 3. A higher LA yield was obtained at neutral pH; conditions with too high or low pH were not fit for lactic acid fermentation. In addition, temperatures higher than 37 °C showed inhibition to LA fermentation. Based on the regression modeling, the maximal LA yield was 0.49 g/g-TS at the optimized pH of 6.8 under a set temperature of 37 °C (Fig. 3). By adjusting the pH to 6 intermittently (every 12 h) at the temperature of 37 °C, the satisfactory LA yield of 0.47 g/g-TS was achieved and quite close to the above mathematically calculated result (0.49 g/

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Table 3
Effects of temperature on lactic acid fermentation (at 120 h).

	Influent	Tem = 37°C pH = UN	Tem = 37°C pH = 6	Tem = 55°C pH = UN	Tem = 55°C pH = 6
Total COD (mg/L)	102,421	98,105	96,178	98,286	98,067
Soluble COD (mg/L)	20,387	32,889	49,416	47,831	51,612
Carbohydrate (% SCOD)	84.6	39.9	4.4	75.1	64.0
Protein (% SCOD)	6.1	8.5	2.8	4.5	6.0
Lactic acid (% SCOD)	7.9	46.0	70.8	9.8	23.7
VFAs (% SCOD)	1.4	4.2	16.0	2.3	3.9
Others (% SCOD)	0.0	1.5	6.0	8.2	2.3
VS reduction (%)	–	4.9	45.8	1.5	23.4
LA Productivity (mg/L h)	–	180.4	278.1	14.6	93.1

g-TS). Due to the acceptable LA yield and low alkali addition, the pH of 6 and temperature of 37 °C were chosen as the predetermined conditions during the next continuous operation experiment.

3.2. Effects of OLR

Based on the results of the batch tests, the stability and effects of high organic loading rate (OLR) on lactic acid fermentation were explored at 37 °C. The pH in the reactor was adjusted to 6 every 12 h, the OLR of the reactor was maintained at 14, 18, and 22 g-TS/L d by adjusting the TS contents of the feed. HRT was fixed at 5 days according to the batch fermentation.

As seen in Fig. 4, at the start-up stage, lactic acid concentration increased sharply to 29 g/L in the first 6 days and then remained stable. At day 14, lactic acid increased gradually to 37.6 g/L with the increase in OLR to 18 g-TS/L d. However, when OLR was increased to 22 g-TS/L d, the lactic acid concentration decreased to 26.4 g/L at day 35. High OLR increased the viscosity of the fermentation broth (Lim et al., 2008), which restricted the mass transfer and further influenced the fermentation processes (Li et al., 2011; Zhang et al., 2013). The decrease in lactic acid indicated that the fermentation was not stable at high OLR.

More residual carbohydrates existed in the fermentation mixture at higher OLR. As seen in Fig. 4, the soluble carbohydrate content was only 0.9 g/L at the OLR of 14 g-TS/L d, but it increased to 5.3 g/L when the OLR increased to 22 g-TS/L d. The protein content in the fermentation mixture also increased slightly with OLR. VFAs were produced in our reactor, but the concentrations were relatively low (Table 4). It was approximately 5.1 g-COD/L at an OLR of 14 g-TS/L d, whereas it increased to 9.5 and 13.3 g-COD/L with the increase of OLR to 18 and 22 g-TS/L d, respectively. In the VFAs, acetate was the main component under all OLR feeding conditions, accounting for 44.2% of the VFAs. The acetate concentration increased from 2.1 g/L to 5.5 g/L when the OLR increased from 14 to 22 g-TS/L d. Meanwhile, propionate and butyrate showed little

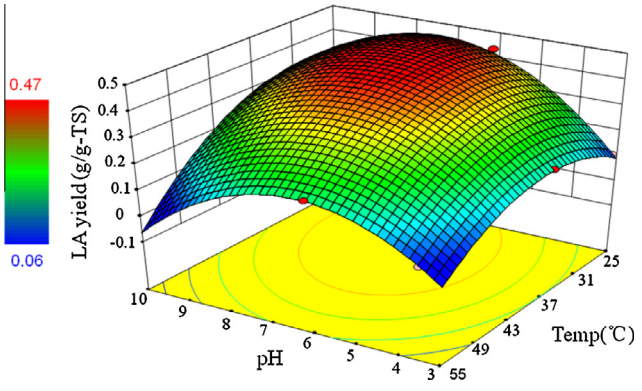

Fig. 3. The interaction of pH and temperature on LA yield using RSM.

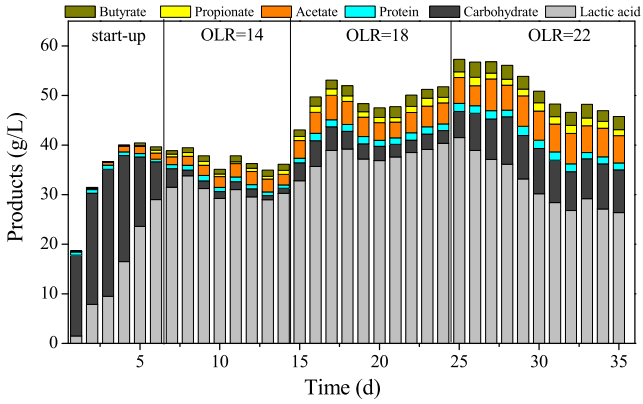

Fig. 4. Effects of OLR on lactic acid fermentation.

Table 4
Effects of OLR on lactic acid fermentation from food waste.

OLR (g-TS/L d)	14	18	22
SCOD (g/L)	42.6	56.4	61.3
S-Carbohydrate (g/L)	2.3	3.4	8.2
S-Protein (g/L)	0.8	1.4	1.6
Lactic acid (g/L)	30.5	37.6	32.3
Acetate (g/L)	2.1	4.0	5.5
Propionate (g/L)	0.6	1.2	1.4
Butyrate (g/L)	1.1	1.9	2.9
LA/SCOD (%)	76.4	71.1	56.2

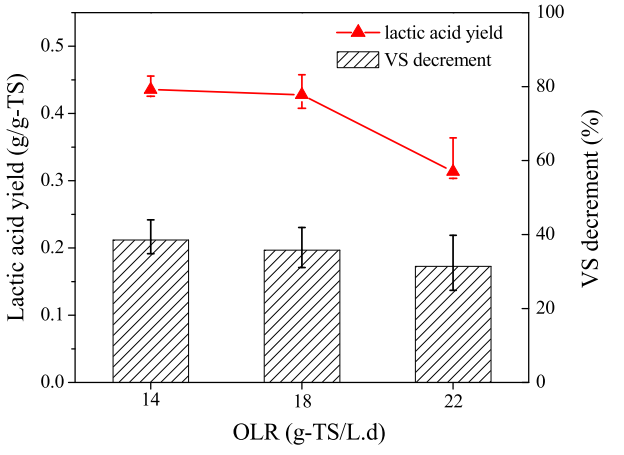

Fig. 5. Lactic acid yield and VS decrement under different OLRs.

variation with the increase in OLR. In the reactor, although the pH was adjusted to 6, it decreased to approximately 4 in 6 h with the accumulation of lactic acid. Under conditions of such a low pH,

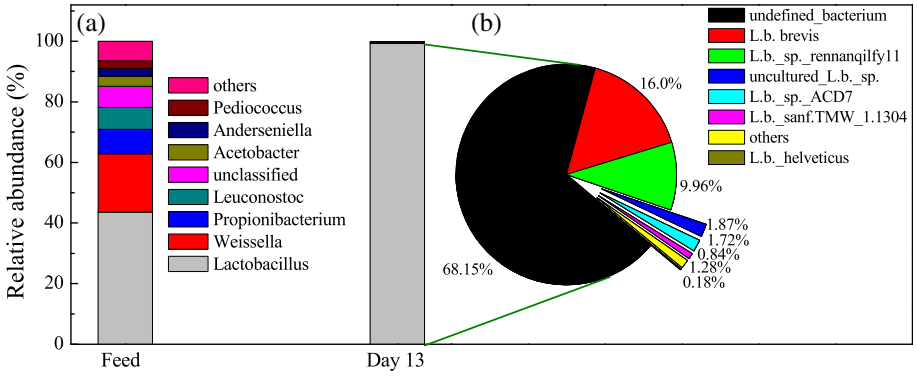

Fig. 6. The structure of bacterial community in raw and fermented (Day 13) mixture based on high throughout pyrosequencing (a) and bacterial community by clone library analysis in the fermented food waste on Day 13 (b).

VFAs could hardly be produced as the optimal pH for VFAs production was approximately 6 (Jiang et al., 2013).

The yield of lactic acid decreased with the increase in OLR. It can be seen from Fig. 5 that the yield decreased slightly from 0.44 to 0.43 g/g-TS when the OLR increased from 14 to 18 g-TS/L d. However, increasing OLR to 22 g-TS/L d clearly decreased the lactic acid yield to 0.31 g/g-TS. The decrease in lactic acid yield was due to the decreased hydrolysis at higher OLR. The VS reduction also decreased from 38.5% to 31.4% as the OLR increased to 22 g-TS/L d. The low hydrolysis and acidogenesis at high OLR might be due to the weak transfer of fermentation products, which in turn influenced the enzyme activity. In addition, a high substrate content may cause imbalanced osmosis pressure and damage the metabolism of bacteria as mentioned in the former context.

3.3. Bacterial community

Anaerobic microorganisms play key roles in lactic acid fermentation. To explore the microbial mechanisms of high lactic acid production in our reactor, 454 high-throughput pyrosequencing was conducted to reveal the changes in bacterial community structure before and after fermentation. As clearly seen in Fig. 6(a), before fermentation the communities were more diverse. *Lactobacillus* and *Weissella* were the dominant fermentative bacteria (accounted for 43.64% and 19.18%, respectively), followed by *Propionibacterium* (8.13%), *Leuconostoc* (7.22%) and *Acetobacter* (3.32%). The *Lactobacillus* in the feed provided a prerequisite for the quick initiation of lactic acid fermentation. However, *Lactobacillus* was the dominant bacteria in the fermentation mixture, accounting for 99.29% of the abundance. The main product of *Lactobacillus* was lactic acid (Carr et al., 2002), which was the reason for the high lactic acid productivity in the reactor. In Table 4, lactic acid accounted for a larger proportion of soluble COD than other components in the fermentation mixture. In addition, *Acetobacter* also existed in the reactor despite an abundance of only 0.41%, which might be the reason why the acetate content in the effluent was higher than other VFA components. The selective accumulation of *Lactobacillus* in our reactor benefited from the intermittent pH adjustment. After adjusting the pH to 6, lactic acid was produced in reactor and decreased the pH to lower than 4 in 4–5 h, which seriously inhibited the growth of other bacteria.

The results of the clone library also indicated that *Lactobacillus* was the dominant bacteria in the semi-continuous reactor. It can be seen from Fig. 6(b) that in addition to the undefined bacterium, the main *Lactobacillus* in this study was *Lactobacillus brevis* (16%) and *Lactobacillus* sp._*rennanqilfy11* (9.96%), which are the main lactic acid producers. Other *Lactobacillus* such as *Lactobacillus* sp. *ACD7* (1.72%), *Lactobacillus helveticus* (0.18%) *and Lactobacillus sanfranciscensis TMW 1.1304* (0.84%) also coexisted in the fermentation mixture. The high proportion of lactic acid bacteria further explained the high LA yield in our reactor.

4. Conclusions

The effects of pH, temperature and OLR on lactic acid fermentation with indigenous microbiota were investigated in this study. Using batch fermentation experiments with intermittent pH adjustments to 6, 8 and 10 at ambient temperature, the highest lactic acid concentration and yield was achieved at a pH of 6. At 37 °C and 55 °C and a pH of 6, a similar hydrolysis rate was obtained, but at 37 °C the acidogenesis rate was much higher with higher lactic acid productivity and yield. When OLR was gradually increased from 14 g-TS/L d to 22 g-TS/L d in the semi-continuous fermentation bioreactor, the stable production of lactic acid could be achieved at OLR = 18 g-TS/L d, while the lactic acid concentration and yield as well as VS removal decreased as with higher OLR. As a result of high-throughput pyrosequencing, *Lactobacillus* from raw food waste was found to perform a key role in the quick start-up of lactic acid fermentation, and through selective accumulation in the fermentation process, *Lactobacillus* finally became the dominant species accounting for 99.29% of the microbial community and facilitating high lactic acid productivity.

Acknowledgments

This work was supported by the National Program of Water Pollution Control in China (Grant no. 2013ZX07310-001), the Natural Science Foundation of China (No. 51508450), the Program for Innovative Research Team in Shanxi Province (Grant No. 2013KCT-13) and the Fund for Postdoctoral Scientific Research Project of China (Grant no. 2015M582760XB).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.wasman.2016.03.034.

References

Akao, S., Tsuno, H., Horie, T., Mori, S., 2007. Effects of pH and temperature on products and bacterial community in-lactate batch fermentation of garbage under unsterile condition. Water Res. 41 (12), 2636–2642.

Aljundi, I.H., Belovich, J.M., Talu, O., 2005. Adsorption of lactic acid from fermentation broth and aqueous solutions on Zeolite molecular sieves. Chem. Eng. Sci. 60 (2005), 5004–5009.

Amrane, A., Prigent, Y., 1994. Lactic acid production from lactose in batch culture: analysis of the data with the help of a mathematical model; relevance for

nitrogen source and preculture assessment. Appl. Microbiol. Biotechnol. 40 (5), 644–649.
Anderson, C., Malambo, D.H., Perez, M.E.G., et al., 2015. Lactic acid fermentation, urea and lime addition: promising faecal sludge sanitizing methods for emergency sanitation. Int. J. Environ. Res. Publ. Health 12, 13871–13885.
APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC.
Carr, F.J., Chill, D., Maida, N., 2002. The lactic acid bacteria: a literature survey. Crit. Rev. Microbiol. 28 (4), 281–370.
Chen, Y.G., Li, X., Zheng, X., Wang, D.B., 2013. Enhancement of propionic acid fraction in volatile fatty acids produced from sludge fermentation by the use of food waste and Propionibacterium acidipropionici. Water Res. 47, 615–622.
Chu, C.F., Xu, K.Q., Li, Y.Y., Inamori, Y., 2012. Hydrogen and methane potential based on the nature of food waste materials in a two-stage thermophilic fermentation process. Int. J. Hydrogen Energy 37, 10611–10618.
Dalie, D.K.D., Deschamps, A.M., Richard-Forget, F., 2010. Lactic acid bacteria–potential for control of mould growth and mycotoxins: a review. Food Control 21, 370–380.
Eom, I.Y., Oh, Y.H., Park, S.J., Lee, S.H., Yu, J.H., 2015. Fermentative L-lactic acid production from pretreated whole slurry of oil palm trunk treated by hydrothermolysis and subsequent enzymatic hydrolysis. Bioresour. Technol. 185, 143–149.
Feng, L.Y., Yan, Y.Y., Chen, Y.G., 2009. Kinetic analysis of waste activated sludge hydrolysis and short-chain fatty acids production at pH 10. J. Environ. Sci. 21, 589–594.
Hama, S., Mizuno, S., Kihara, M., Tanaka, T., et al., 2015. Production of D-lactic acid from hardwood pulp by mechanical milling followed by simultaneous saccharification and fermentation using metabolically engineered *Lactobacillus plantarum*. Bioresour. Technol. 187, 167–172.
Hano, T., Matsumoto, M., Hirata, M., Miura, S., 1996. Recovery of lactic acid by extraction from fermented broth. In: ISEC, 144752.
Harris, W.L., Dague, R.R., 1993. Comparative performance of anaerobic filters at mesophilic and thermophilic temperatures. Water Environ. Res. 65 (6), 764–771.
Herbert, D., Philipps, P.J., Strange, R.E., 1971. Chemical Analysis of Microbial Cells Methods in Microbiology, vol. 5b. Academic Press Ltd., London, pp. 249–252.
Huang, L.P., Jin, B., Lant, P., Zhou, J.T., 2005. Simultaneous saccharification and fermentation of potato starch wastewater to lactic acid by Rhizopus oryzae and Rhizopus arrhizus. Biochem. Eng. J. 23, 265–276.
Itoh, Y., Tada, K., Kanno, T., Horiuchi, J.I., 2012. Selective production of lactic acid in continuous anaerobic acidogenesis by extremely low pH operation. J. Biosci. Bioeng. 114 (5), 537–539.
Iyer, P.V., Lee, Y.Y., 1999. Product inhibition in simultaneous saccharification and fermentation of cellulose into lactic acid. Biotechnol. Lett. 21 (5), 371–373.
Jiang, J.G., Zhang, Y.J., Li, K.M., Wang, Q., et al., 2013. Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate. Bioresour. Technol. 143, 525–530.
Jiang, Y., Marang, L., Kleerebezem, R., Muyzer, G., van Loosdrecht, M.C.M., 2011. Polyhydroxybutyrate production from lactate using a mixed microbial culture. Biotechnol. Bioeng. 108 (9), 2022–2035.
John, R.P., Anisha, G.S., Madhavan Nampoothiri, K., Pandey, Ashok, 2009. Direct lactic acid fermentation: focus on simultaneous saccharification and lactic acid production. Biotechnol. Adv. 27, 145–152.
John, R.P., Nampoothiri, K.M., Pandey, A., 2007. Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. Appl. Microbiol. Biotechnol. 74, 524–534.
Kim, D.H., Lim, W.T., Lee, M.K., Kim, M.S., 2012. Effect of temperature on continuous fermentative lactic acid (LA) production and bacterial community, and development of LA-producing UASB reactor. Bioresour. Technol. 119, 355–361.
Kim, M., Ahn, Y.H., Speece, R.E., 2002. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. Water Res. 36, 4369–4385.
Kim, M., Gomec, C.Y., Ahn, Y., Speece, R.E., 2003. Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion. Environ. Technol. 24 (9), 1183–1190.
Komemoto, K., Lim, Y.G., Nagao, N., Onoue, Y., Niwa, C., Toda, T., 2009. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. Waste Manage. 29 (12), 2950–2955.
Lee, D.Y., Xu, K.Q., Kobayashi, T., Li, Y.Y., Inamori, Y., 2014. Effect of organic loading rate on continuous hydrogen production from food waste in submerged anaerobic membrane bioreactor. Int. J. Hydrogen Energy 39, 16863–16871.
Li, X., Chen, H., Hu, L.F., Yu, L., Chen, Y., Gu, G., 2011. Pilot-scale waste activated sludge alkaline fermentation, fermentation liquid separation, and application of fermentation liquid to improve biological nutrient removal. Environ. Sci. Technol. 45 (5), 1834–1839.
Li, X., Chen, Y.G., Zhao, S., Wang, D.B., Zheng, X., Luo, J.Y., 2014. Lactic acid accumulation from sludge and food waste to improve the yield of propionic acid-enriched VFA. Biochem. Eng. J. 84, 28–35.
Li, X., Chen, Y., Zhao, S., Chen, H., Zheng, X., Luo, J.Y., Liu, Y., 2015. Efficient production of optically pure L-lactic acid from food waste at ambient temperature by regulating key enzyme activity. Water Res. 70, 148–157.
Liang, S.B., Mcdonald, A.G., Coats, E.R., 2014. Lactic acid production with undefined mixed culture fermentation of potato peel waste. Waste Manage. 34 (11), 2022–2027.
Lim, S.J., Kim, B.J., Jeong, C.M., Choi, J., Ahn, Y.H., Chang, H.N., 2008. Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. Bioresour. Technol. 99 (16), 7866–7874.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.
Mahmoud, N., Zeeman, G., Gijzen, H., Lettinga, G., 2004. Anaerobic stabilisation and conversion of biopolymers in primary sludge – effect of temperature and sludge retention time. Water Res. 38 (4), 983–991.
Mazzoli, R., Bosco, F., Mizrahi, I., Bayer, E.A., Pessione, E., 2014. Towards lactic acid bacteria-based biorefineries. Biotechnol. Adv. 32, 1216–1236.
Parawira, W., Murto, M., Read, J.S., Mattiasson, B., 2005. Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste. Process Biochem. 40 (9), 2945–2952.
Rehm, H.J., Reed, G., Puhler, A., Stadler, P.J.W., 2000. Environmental Processes I, . second ed. Biotechnology second ed., vol. 11A Wiley, New York.
Secchi, N., Giunta, D., Pretti, L., Garcia, M.R., Roggio, T., Mannazzu, I., Catzeddu, P., 2012. Bioconversion of ovine scotta into lactic acid with pure and mixed cultures of lactic acid bacteria. J. Ind. Microbiol. Biotechnol. 39, 175–181.
Shen, F., Yuan, H., Pang, Y.Z., Chen, S.L., et al., 2013. Performances of anaerobic co-digestion of fruit & vegetable waste (FVW) and food waste (FW): single-phase vs. two-phase. Bioresour. Technol. 144, 80–85.
Vijayakumar, J., Aravindan, R., Viruthagiri, T., 2008. Recent trends in the production, purification and application of lactic acid. Chem. Biochem. Eng. Q. 22 (2), 245–264.
Wang, D.B., Chen, Y.G., Zheng, X., Li, X., Feng, L.Y., 2013. Short-chain fatty acid production from different biological phosphorus removal sludges: the influences of PHA and gram-staining bacteria. Environ. Sci. Technol. 47, 2688–2695.
Wang, D.B., Zeng, G.M., Chen, Y.G., Li, X.M., 2015a. Effect of polyhydroxyalkanoates on dark fermentative hydrogen production from waste activated sludge. Water Res. 73, 311–322.
Wang, D.B., Zhao, J.W., Zeng, G.M., Chen, Y.G., Bond, P.L., Li, X.M., 2015b. How does poly(hydroxyalkanoate) affect methane production from the anaerobic digestion of waste-activated sludge? Environ. Sci. Technol. 49, 12253–12262.
Wang, K., Yin, J., Shen, D.S., Li, N., 2014. Anaerobic digestion of food waste for volatile fatty acids (VFAs) production with different types of inoculum: effect of pH. Bioresour. Technol. 161, 395–401.
Wu, Y.Y., Ma, H.L., Zheng, M.Y., Wang, K.J., 2015. Lactic acid production from acidogenic fermentation of fruit and vegetable wastes. Bioresour. Technol. 191, 53–58.
Yang, P.B., Tian, Y., Wang, Q., Cong, W., 2015. Effect of different types of calcium carbonate on the lactic acid fermentation performance of *Lactobacillus lactis*. Biochem. Eng. J. 98, 38–46.
Ye, Z.L., Lu, M., Zheng, Y., Li, Y.H., Cai, W.M., 2008. Lactic acid production from dining-hall food waste by *Lactobacillus plantarum* using response surface methodology. J. Chem. Technol. Biotechnol. 83, 1541–1550.
Zhang, B., He, P., Ye, N., Shao, L., 2008. Enhanced isomer purity of lactic acid from the non-sterile fermentation of kitchen wastes. Bioresour. Technol. 99 (4), 855–862.
Zhang, B., He, P.J., 2014. Performance assessment of two-stage anaerobic digestion of kitchen wastes. Environ. Technol. 35 (10), 1277–1285.
Zhang, P., Chen, Y., Zhou, Q., Zheng, X., Zhu, X., Zhao, Y., 2013. Understanding short-chain fatty acids accumulation enhanced in waste activated sludge alkaline fermentation: kinetics and microbiology. Environ. Sci. Technol. 44 (24), 9343–9348.
Zhang, P., Chen, Y.G., Zhou, Q., 2009. Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: effect of pH. Water Res. 43, 3735–3742.
Zhang, Y.M., Wang, X.C., Cheng, Z., Li, Y.Y., Tang, J.L., 2016. Effect of fermentation liquid from food waste as a carbon source for enhancing denitrification in wastewater treatment. Chemosphere 144, 689–696.
Zhang, Y.X., Vadlani, P.V., 2015. Lactic acid production from biomass-derived sugars via co-fermentation of *Lactobacillus brevis* and *Lactobacillus plantarum*. J. Biosci. Bioeng. 119 (6), 694–699.
Zhang, Z.Y., Jin, B., Kelly, J.M., 2007. Production of lactic acid from renewable materials by Rhizopus fungi. Biochem. Eng. J. 35, 251–263.

Please cite this article in press as: Tang, J., et al. Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. Waste Management (2016), http://dx.doi.org/10.1016/j.wasman.2016.03.034