Graphic abstract

**Efficient production of optically pure L-lactic acid from food waste at
ambient temperature by regulating key enzyme activity**

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13 **Abstract:**

14 Bio-production of optically pure L-lactic acid from food waste has attracted much interest as it can treat
15 organic wastes with simultaneous recovery of valuable by-products. However, the yield of L-lactic
16 acid was very low and no optically pure L-lactic acid was produced in the literature due to (1) the lower
17 activity of enzymes involved in hydrolysis and L-lactic acid generation, and (2) the participation of other
18 enzymes related to D-lactic acid and acetic and propionic acids production. In this paper, a new
19 strategy was reported for effective production of optically pure L-lactic acid from food waste at ambient
20 temperature, i.e. via regulating key enzyme activity by sewage sludge supplement and intermittent
21 alkaline fermentation. It was found that not only optically pure L-lactic acid was produced, but the
22 yield was enhanced by 2.89-fold. The mechanism study showed that the activities of enzymes relevant
23 to food waste hydrolysis and lactic acid production were enhanced, and the key enzymes related to
24 volatile fatty acids and D-lactic acid generations were severally decreased or inhibited. Also, the
25 microbes responsible for L-lactic acid production were selectively proliferated. Finally, the pilot-scale
26 continuous experiment was conducted to testify the feasibility of this new technique.

27 **Key Word:** Food waste; waste activated sludge; L-lactic acid fermentation; key enzyme activity

1. Introduction

Lactic acid has been widely used in medical, food, and general chemical industries. It has two optical isomers, i.e., L- and D-lactic acid. L-lactic acid is the precursor of poly-L-lactate (PLLA), a promising biodegradable plastic (Hofvendahl and Hahn-Hägerdal, 2000). The physical property and biodegradability of PLLA are highly depended on the L-isomer purity of lactic acid (Lunt, 1998). Nevertheless, chemical synthesis of lactic acid only generates the racemic mixture, whereas microbial fermentation is an alternative approach to produce L-lactic acid (Ilmen et al., 2007).

With the rapid growth of human population in the world, food waste and its environmental impact have become a major issue. Anaerobic treatment of food waste has become an interest as it can recover energy and valuable by-products (Carballa et al., 2011; Levis and Barlaz, 2011). Although lactic acids could be biologically produced from organic waste by anaerobic fermentation, the lactic acid was the mixture of L- and D-isomers (Gao et al., 2011). Also, the documented methods were usually complicated as they were conducted under sterile and thermophilic or mesophilic conditions and the lactic acid producing bacterial strains need be inoculated (Nakasaka et al., 1999). In addition, the yield was only 0.227 gram per initial total chemical oxygen demand of the substrate (g/g TCOD) (Akao et al., 2007).

During anaerobic fermentation the organic compounds are firstly hydrolyzed by enzymes (such as protease and α -glucosidase, Figure 1). It is well known that food waste is usually rich in carbohydrate. When a carbohydrate-enriched matter was anaerobically fermented to produce volatile fatty acids (VFA), the activity of hydrolysis enzyme and the yield of VFA were observed to be significantly improved by the addition of a certain amount of waste activated sludge (Feng et al., 2009). Thus, it can be speculated that the production of lactic acid from food waste could be enhanced by the addition of waste activated sludge. Also, as shown in Figure 1, the hydrolyzed products can be bio-converted to L-lactic acid, D-lactic acid, acetic acid or propionic acid. The enzymes responsible for L- and D-lactic acid production are respectively NAD-dependent L-lactate dehydrogenase (L-LDH) and NAD-dependent D-lactate dehydrogenases (D-LDH) (Garvie, 1980). Obviously, Optically pure L-lactic acid could be produced by improving the activity of L-LDH and simultaneously inhibiting that of D-LDH. In addition, if the activities of enzymes (acetate kinase (AK), phosphotransacetylase (PTA), succinyl CoA

transferase (CoAT) and oxaloacetate transcarboxylase (OAATC)) relevant to the generations of acetic and propionic acids were decreased, more substrate could be used to produce L-lactic acid. Also, supposing that NAD-independent lactate dehydrogenase (iLDH) attributed to the consumption of L-lactic acid declined in activity, the yield of L-lactic acid would be further enhanced. Until now, however, no any reference is available regarding the efficient production of optically pure L-lactic acid from wastes at ambient temperature by regulating the activities of these enzymes.

In this paper a new method, i.e., by sewage sludge supplement and intermittent alkaline fermentation to regulate the activity of key enzymes, for effectively producing optically pure L-lactic acid from food waste at ambient temperature was reported. Firstly, the effect of sludge supplemented to food waste fermentation system on L-lactic acid production was studied. In order to increase the optical purity of L-lactic acid the intermittent alkaline fermentation strategy was developed. Then, the mechanisms for remarkably high optically pure L-lactic acid being produced by sewage sludge supplement and intermittent alkaline fermentation were explored. Finally, the feasibility of continuously and effectively producing optically pure L-lactic acid from food waste by sewage sludge supplement and intermittent alkaline fermentation at ambient temperature was testified in a pilot-scale reactor.

2. Materials and methods

2.1. Food Waste and Waste Activated Sludge

After the removal of facial tissue, chopsticks, bones, and inorganic particles, the food waste, which was collected from a dinning restaurant in Shanghai, was milled to slurry state and diluted with tap water. The final characteristics of the food waste were as follows (average data plus standard deviation of triplicate measurements): total suspended solids (TSS) 97.42 ± 3.62 g/L, volatile suspended solids (VSS) 94.68 ± 3.28 g/L, total chemical oxygen demand (TCOD) 137.62 ± 5.76 g/L, soluble chemical oxygen demand (SCOD) 26.67 ± 3.92 g/L, total carbohydrate 68.31 ± 5.97 g COD/L, and total protein 14.31 ± 0.74 g COD/L. The waste activated sludge (WAS) was withdrawn from a municipal wastewater treatment plant in Shanghai, and settled for 24 h. The main characteristics of the concentrated sludge were pH 6.8 ± 0.2 , TSS 17.85 ± 2.45 mg/L, VSS 12.58 ± 1.45 g/L, TCOD 19.82 ± 3.52 g/L, SCOD 0.22 ± 0.01 g/L, total carbohydrate 2.43 ± 0.07 g COD/L, and total protein $10.88 \pm$

84 0.62 g COD/L.

85 **2.2. Batch Experiments of Sludge Addition and Intermittent pH Control Affecting L-lactic Acid** 86 **Production**

87 Food waste and sludge were mixed at different volatile solid mass ratios (g/g) as 1.5, 3.0, 6.0, 9.0 and
88 12.0, respectively, and then put into the fermentation reactors with working volume of 1.2 L each.
89 Unless otherwise noted, tap water was added to make the initial TCOD of the fermentation substrate in
90 all tests to be 25.0 ± 1.1 g/L. All reactors were mechanically stirred at 120 rpm and maintained at
91 ambient temperature (21 ± 1 °C). Since in the literature the reported fermentation pH for lactic acid
92 production was around neutral (Akao et al., 2007; Zhang et al., 2008), the pH value in this section was
93 adjusted by sodium hydroxide (3 M) or hydrochloric acid (3 M) to pH 7 every 5 h. The fermentation
94 of sole food waste at pH 7 was set as control in the current study. Samples were taken to assay the
95 contents of L-lactic acid and D-lactic acid. The influences of pH on the optical purity and production
96 of L-lactic acid were studied in several identical reactors. The ratio of food waste to sludge in all
97 reactors was 6/1 (g/g), and every 5 h, the pH was intermittently adjusted to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
98 and 12, respectively. All other operations were the same as described above. The contents of
99 L-lactic acid, D-lactic acid, and VFA were measured during the fermentation.

100 **2.3. Batch Experiments of Identifying the Biological or Chemical Effects Responsible for** 101 **Optically Pure L-lactic Acid Production**

102 To examine whether the formation of L-lactic acid during intermittent alkaline (pH 11) fermentation
103 was caused mainly by the chemical or biological effect, the following experiments were conducted.
104 Food waste and sludge were mixed at ratio of 6/1 (g/g), and then divided equally into two parts. One
105 part was autoclaved at 121 °C for 30 min to eliminate the bio-activity before being put into the reactor,
106 and another one was set as the control. The pH in two reactors was adjusted to pH 11 every 5 h.
107 After fermentation for 6 d the activities of the key enzymes relevant to hydrolysis (α -glucosidase and
108 protease) and L-lactic acid production (L-LDH) were assayed.

109 **2.4. Effect of Sludge Addition on Key Enzyme Activity**

110 Batch experiments were conducted in several identical reactors as follows. The different volatile solid
111 mass ratios affecting key enzyme activity were firstly studied: different mass ratios (g/g) of 1.5, 3.0, 6.0,

9.0, 12.0 as well as sole food waste and sole sludge were prepared accordingly. The initial carbon to nitrogen ratios of the fermentative matter from all reactors were assayed by the elemental analyzer. All reactors were mechanically stirred and adjusted to pH 11 every 5 h at ambient temperature. The activities of α -glucosidase, protease, L-LDH, D-LDH, iLDH, AK, PTA, CoAT, and OAATC were recorded when the maximal L-lactic acid was achieved. To evaluate the effect of sludge supplement on the efficiency of hydrolysis, the molecular weight (M_w) distribution in the liquid phase from sole food waste, sole sludge, and mixture of food waste and sludge with mass ratio of 6.0 (g/g) was recorded initially and after 5 d of fermentation.

2.5. Effect of Intermittent pH Control on Key Enzyme Activity

The different intermittent pH adjustment affecting key enzyme activity was investigated: food waste and sludge were all mixed at volatile solid mass ratio (g/g) 6/1. But pH was intermittently control at 7, 8, 9, 10 and 11, individually. Soluble carbohydrate and protein were recorded with fermentation time. The activities of α -glucosidase, protease, L-LDH, D-LDH, iLDH, AK, PTA, CoAT and OAATC were recorded when the maximal L-lactic acid was achieved respectively. Additionally, Design Expert Software (version 8.05, Stat-Ease, Inc., USA), a five-level-three-variable central composite design (CCD) was used to evaluate the interaction effect of pH adjusting interval and pH value on the optical purity of lactic acid according to the literature (Kavitha et al., 2013). Several identical reactors with food waste and sludge mass ratio (g/g) 6/1 were fermented for 6 d, and the pH was adjusted according to Table S1 (Supplementary Information). The activity of L-LDH and D-LDH were assayed every day, and the relative activity compared to the maximal one was recorded. According to the regression analysis of the software, the fit summary recommended a 2FI model for both responses: the relative activity of L-LDH and D-LDH. With the regression of the model, the intermittent pH adjustment on the optical purity was illustrated by means of surface plots.

2.6. Pilot-scale Semi-continuous Fermentation Experiment

To testify the feasibility of effectively producing optically pure L-lactic acid from food waste by sewage sludge supplement and intermittent alkaline fermentation at ambient temperature, the pilot-scale semi-continuous experiments were conducted. In the initial phase, 90 L of the mixture of food waste and sludge, with ratio of 6/1 (g/g) and TCOD of 25 ± 3 (g/L), were fermented for 5 d, and the pH in the fermentation reactor was intermittently adjusted to 11 every 5 h. Then, the semi-continuous operation

was conducted, i.e., 18 L of the fermentation mixture were pumped out from the reactor every morning, which were replaced by 18 L of the fresh mixture with the ratio of food waste to sludge of 6/1 (g/g) and TCOD of 25 ± 3 (g/L). The pH in the reactor was intermittently adjusted to 11 every 5 h. It was observed that after operation for 46 d, the concentration of L-lactic acid in the reactor reached relatively stable, and then the operation time was recorded and the data were reported. To investigate the microbial community structure from neutral and alkaline, a parallel reactor fermented at pH 7 was also conducted according to the above steps. Both samples were assayed by 454 high-throughput pyrosequencing method when fermentation was stabilized.

2.7. Analytical Methods

L- and D-lactic acids were assayed by high-performance liquid chromatography (Agilent 1200, USA) equipped with Astec CLC-D (5 μ m, 15 cm $\times$ 4.6 mm) column and detected at the wavelength of 254 UV. 5 mM of copper sulfate solution (pH range of 3.0-4.7) was used as mobile phase at a flow rate of 1.0 mL/min. Samples were diluted to a proper concentration (less than 5g/L) and filtered through 0.22 μ m-pore-size filters with an injection volume of 5 μ L for analysis. The elements of carbon (C), hydrogen (H), and nitrogen (N) of the fermentation mixture (treated according to the Supplementary Information) were determined by Elemental Analyzer Vario EL III (Elementar, German). The methods for analyzing Molecular weight (M_w) distributions, disaccharide, monosaccharide and VFA were elaborated in Supplementary Information. The analyses of TSS, VSS, TCOD, SCOD, total carbohydrate and total protein, carbon dioxide, methane were the same as those described in our previous publications (Li et al., 2011; Yuan et al., 2006). The analyses of α -glucosidase and protease were according to the reported method (Goel et al., 1998). The activities of intracellular enzymes (L-LDH, D-LDH, iLDH, AK, PTA, CoAT and OAATC) were determined according to the references (Feng et al., 2009; Ferain et al., 1994), and the pretreatment of the sample was explained in Supplementary Information. Bacterial community structures from reactors operated at pH 7 and pH 11 were investigated using 454 high-throughput pyrosequencing on a Roche 454 GS FLX+ Titanium platform, and the related process can be found in the Supplementary Information. All other analyses were detailed in Supplementary Information.

2.8. Calculation

The calculation for carbon mass analysis was explained in the Supplementary Information. The

optical activity (OA) % of L-lactic acid was defined as enantiomeric excess (ee) value according to the following equation (eq 1):

$$OA(\%) = \frac{[L] - [D]}{[L] + [D]} \times 100\% \quad (eq\ 1)$$

Where [L] and [D] were the concentration of L- and D-lactic acid, respectively.

2.9. Statistical Analysis.

All tests were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation. An analysis of variance (ANOVA) was used to test the significance of results and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effect of Activated Sludge Addition and pH on L-Lactic Acid Production and Optical Purity

In the literature wastes were used directly as the substrate to produce fermentative L-lactic acid, and the fermentation pH value was usually at around neutral pH (Akao et al., 2007; Sakai et al., 2006). In the current study, as seen from Figure 2, the maximal L-lactic acid production was only 3.37 g/L when sole food waste was fermented at pH 7, and the OA was 34.5%. The maximal L-lactic acid production was observed to be affected by the addition of activated sludge. After the addition of sludge with the volatile solid mass ratio of food waste to sludge of 12/1 (g/g), the maximal L-lactic acid was increased to 4.55 g/L. With the increase of sludge, more L-lactic acid was produced. The production reached the greatest (5.78 g/L) at food waste to sludge ratio of 6/1, but the OA was only 61.5%. Further increasing the addition of sludge, however, decreased the generation of L-lactic acid. Accordingly, the supplemented amount of sludge should be controlled at the volatile solid mass ratio of food waste to sludge of 6/1 (g/g). It was noted that no lactic acid was observed as sole activated sludge was fermented for even 14 d. Also, it can be seen that the highest L-lactic acid production from the mixture of food waste and sludge (5.78 g/L) was much greater than the sum of that from sole food waste and sole sludge ($3.37 + 0 = 3.37$ g/L), which suggested that the synergistic effect between food waste and sludge occurred when they were fermented together. Its mechanism has been elaborated in the following text.

In order to increase the OA and further improve the production of L-lactic acid, the influence of pH was studied. The data in Figure 3A indicated that the OA of L-lactic acid was rather low and

198 decreased with fermentation time between pH 4 - pH 8. At other pHs (pH 9, pH 10 and pH 11) the
199 OA was relatively stable during the entire fermentation process. Nevertheless, the OA at pH 9 was
200 only around 63%, which was much lower than that at pH 10 (around 99.1%). At pH 11, optically pure
201 L-lactic acid (OA 100%) could be produced. It was observed that there was no lactic acid produced at
202 pH 2, 3 and 12 even if the fermentation time was prolonged to 14 d. From Figure 3B it can be seen
203 that at pH 11 the production of L-lactic acid was enhanced from 0.13 to 13.11 g/L with the increase of
204 fermentation time from 2 to 5 d. When the fermentation time was further prolonged to 6 d, the
205 production was only slightly increased to 13.18 g/L. Obviously, for efficiently producing optically
206 pure L-lactic acid from food waste at ambient temperature the optimal pH and fermentation time should
207 be pH 11 and 5 d, respectively. Apparently, under conditions of food waste to sludge ratio of 6/1, pH
208 11, and fermentation time of 5 d, the L-lactic acid production, compared with the sole food waste
209 fermentation at pH 7 (control), was enhanced by 2.89 folds (13.11 versus 3.37 g/L).

210 It was reported in the literature that L-lactic acid could be produced from wastes, but the highest OA
211 of L-lactic acid was 99% when the artificial garbage was fermented at mesophilic temperature, and the
212 yield of L-lactic acid was only 0.277 g/g-TCOD (Akao et al., 2007). In the current study the optimal
213 L-lactic acid production was 0.52 g/g-TCOD. Clearly, by using the method reported in this study not
214 only the optically pure L-lactic acid was produced from food waste at ambient temperature, but the
215 production was improved by 87.7% compared with the literature (Akao et al., 2007). In the coming
216 text the mechanisms for sewage sludge supplement and intermittent alkaline fermentation resulting in
217 optically pure and significantly high L-lactic acid production were investigated.

218 **3.2. Mechanisms for Optically Pure L-lactic Acid Production Enhanced by Sludge Addition and** 219 **Alkaline Fermentation**

220 In the literature the production of L-lactic acid from food waste was usually conducted at neutral pH, and
221 it has been proved to be the biological effect (Akao et al., 2007; Zhang et al., 2008). However, in the
222 current study the intermittent alkaline fermentation was observed to be favorable to produce optically
223 pure L-lactic acid, which had never been reported previously. It is therefore necessary to investigate
224 whether the production of optically pure L-lactic acid by intermittent alkaline fermentation was caused
225 by chemical or biological effect. As shown in Table S2 (Supplementary Information), the production of

L-lactic acid was respectively 0.52 and 0.01 (g/g TCOD) when the un-autoclaved and autoclaved mixture of sludge and food waste was used as the substrates. Also, the un-autoclaved fermentation system showed much higher activities of key enzymes relevant to hydrolysis (α -glucosidase and protease) and L-lactic acid production (L-LDH). It is obvious that the enhanced production of optically pure L-lactic acid from food waste by sludge addition and intermittent alkaline fermentation was attributed to the biological effect instead of chemical one.

In this study carbohydrate and protein are the two main organic components of fermentation substrates (i.e., the mixture of food waste and sludge), and they accounted for 49.6% and 10.4% of the total COD, respectively. Thus, the biological production of L-lactic acid from food waste and sludge was relevant to the metabolism of these compositions (Figure 1). Protein and carbohydrate are firstly hydrolyzed by protease and α -glucosidase, respectively (Goel et al., 1998). Then the hydrolyzed products are converted to pyruvic acid, which is further converted to L-lactic acid (6.2 ± 0.5 g COD/L), D- lactic acid (1.5 ± 0.1 g COD/L), and other by-products, like acetic acid (2.5 ± 0.1 g COD/L), propionic acid (3.3 ± 0.1 g COD/L), ethanol (1.5 ± 0.2 g COD/L), butyric acid (0.2 ± 0.1 g COD/L) and valeric acid (0.1 ± 0.1 g COD/L) etc (Table S3, Supplementary Information).

The measurement of molecular weight (M_w) has been applied to assess the anaerobic fermentation efficiency of organic wastes and the smaller M_w of the organic carbon in the liquid phase, the easier to be utilized by the anaerobic microorganisms (Zhao and Chen, 2011; Eskicioglu et al., 2006). As shown in Figure S1 (Supplementary Information) and Figure 4(A) and (D), when food waste was used as the sole substrate for intermittent alkaline fermentation, little variations of M_w distribution were exhibited after 5 d of fermentation, and the percentage of $M_w < 1000$ was slightly increased from 8.4% to 15.7%. L-lactic acid was only 1.56 g/L, which was less than half of that from sole food waste fermentation at neutral pH. In Figure 4(B) and (D), although the variations of M_w distribution changed apparently when sole sludge was fermented, the organic compounds with larger molecular dominated in the liquid phase, and no lactic acid was produced even prolonged the fermentation time to 14 d, which was same as that obtained in neutral condition. In Figure 4(C) and (D), when food waste was fermented with the supplement of sludge, the M_w distribution in the smaller region ($M_w < 1000$) was significantly expanded after 5 d of fermentation, and the percentage of $M_w < 1000$ was remarkably improved from 12.9% to 54.1%.

255 The activities of α -glucosidase and protease were enhanced with the addition of sludge in the range
256 of food waste to sludge ratio of 12/1 – 6/1, which, however, were not significantly affected by further
257 increasing sludge supplement (Table S4, Supplementary Information). Apparently, the microbial
258 hydrolysis of both carbohydrate and protein reached the maximum at food waste to sludge ratio of 6/1.
259 It can be seen from Table S4 that the activity of L-LDH increased from sole food waste to the mass ratio
260 of 6/1 (food waste to sludge), where the maximal activity of L-LDH was observed. Nevertheless,
261 further increasing sludge addition decreased the activity of L-LDH. Since no lactic acid was observe
262 from sole sludge fermentation, the increase of sludge fraction, i.e., the decrease of food waste fraction,
263 caused inadequate fermentative substrate for lactic acid production, which might undermined the activity
264 of L-lactic acid generation enzyme. On the other hand, D-lactic acid generation, which competed
265 against L-lactic acid production for fermentative substrate, was completely inhibited in all tests due to
266 the intermittent pH 11 fermentation strategy being adopted. Therefore, no competition for substrate
267 was occurred from D-lactic acid generation, and the mechanism will be explained in the following
268 paragraph. Meanwhile, pyruvic acid can also be converted to VFA, mainly acetic and propionic acids
269 in the current study (Table S3, Supplementary Information). As shown in Table S4 (Supplementary
270 Information) the activities of iLDH (consumption of lactic acid), AK and PTA (for acetic acid
271 production), and OAATC and CoAT (for propionic acid generation) in all tests showed almost the same
272 level, which suggested that the supplement of sludge caused negligible influence on pyruvic acid
273 transformation to acetic and propionic acids.

274 In our previous study, the synergistic effect between carbohydrate-rich matter and sludge on
275 fermentation was relevant to the balance of carbon to nitrogen (C/N) ratio (Feng et al., 2009). The
276 suitable C/N ratio for anaerobic microbes led to the improvement of enzymes activities both in
277 hydrolysis and acidification (Parkin et al., 1986). The food waste contains plenty of carbohydrate with
278 C/N around 32/1, while sludge had the C/N of 6.8/1, which contains EPS (extracellular polymeric
279 substance), mainly composed of protein, carbohydrate and lipid as well (Kavitha et al., 2014). When
280 they were mixed together at a proper ratio (mixed ratio of 6/1 in the current study), C/N was balanced to
281 an optimal ratio ($12.6 \pm 0.3/1$) for lactic acid generation, which was similar to the our previous findings
282 (Chen et al., 2013). Additionally, sludge contained plenty of lactic acid bacteria (Maeda et al., 2009),

which led to the increase of L-LDH with the addition of sludge to food waste fermentation. Further increasing sludge fraction led to the decline of food waste content, which decreased the fermentative substrate for lactic acid generation. From this study it can be seen that the greatest activity of L-LDH occurred at food wastes to sludge ratio of 6/1.

As shown in Table 1, the hydrolysis of polysaccharide (indicated by α -glucosidase) and protein (by protease) was improved with the intermittent fermentation pH being increased from 7 to 11, which resulted in the maximal concentration of soluble carbohydrate and protein at intermittent pH 11 fermentation (Figure S2, Supplementary Information). The activity of L-LDH in the intermittent pH 11 fermentation reactor was not lower than any other reactors, while that of D-LDH was severely inhibited (non-detectable), which was in good agreement with Table S4 (Supplementary Information). Moreover, with the increase of fermentation pH, less fermentation substrate and lactic acid were converted to other organic acids, which could be conformed by the VFA generation in Figure S3, Supplementary Information.

In the literature lactic acid fermentation from food waste was conducted at around neutral pH, and more focus had been paid on the temperature-based or single-strain-cultured strategies (Akao et al., 2007; Ferain et al., 1994). However, both L- and D-lactic acids were generated according to the documented methods. In the current study optically pure L-lactic acid with no D-lactic acid was produced by intermittent alkaline fermentation. It is therefore necessary to dig out the reason for selectively inhibiting D-LDH but without affecting L-LDH by this new method. Figure 5 exhibited the 3D surface graphs and the contour plots of the interaction effect of pH adjustment interval and pH value on the relatively activity of L-LDH and D-LDH. The adequate precision, an indicator of signal-noise with a desirable value of greater than 4, was large enough to support the application of this model. The predicted and adjusted R-squared values were respectively 0.8857 and 0.9535. The statistical significance of the models and their terms were evaluated by ANOVA (Table S5, Supplementary Information), and the following mathematical regressions could be obtained.

$$R_{L-LDH} = 91.66 - 0.42 \times A + 7.15 \times B + 6.75 \times AB \quad (eq\ 2)$$

$$R_{D-LDH} = 49.06 - 23.33 \times A + 15.74 \times B - 1.50 \times AB \quad (eq\ 3)$$

Where R means the predicted response (L-LDH or D-LDH), and A , B are the two independent factors:

pH value and pH adjusting interval (h), respectively. Figure 5 and the above equations both showed that the increase of the adjusting interval to 5 h at alkaline condition significantly improved the activity of L-LDH, while the activity of D-LDH rose up only with the decrease of pH and the increase of adjusting interval. Obviously, L-LDH exhibited stronger alkali-resistance (coefficient -0.42) compared to that of D-LDH (coefficient -23.33), and the intermittent alkaline fermentation (5 h interval) benefited exclusive L-lactic acid generation.

Currently, 454 high-throughput pyrosequencing is one of the preferable sequencing technologies to explore the environmental microbial community structure due to the long read length (Zheng et al., 2013, Luo et al., 2014). Thus, microbial community structures from the reactors operated at pH 7 and pH 11 were analyzed. The Shannon diversities at the 3% dissimilarity level were not obviously increased from two reactors when the numbers of bacterial sequence sampled was up to 4000, which indicated that the sequencing depth above 11400 in this study was satisfied to fully characterize the bacterial communities (Figure S4, Supplementary Information). The pyrosequencing data (Table S6, Supplementary Information) revealed much more bacterial populations existed from reactor operated at pH 7 compared to that at pH 11 according to the richness estimators such as OTUs, ACE and Chao indices, which could be confirmed by the rarefaction curves (Figure S5, Supplementary Information). Therefore, a large fraction of anaerobic organisms would compete with lactic acid bacteria for the fermentative substrate at pH 7, which was in accordance with Figure 3(B) that less lactic acid was produced at neutral. Meanwhile, Figure 6 depicted the genus level distributions of bacterial OTUs from the reactor of pH 7 and pH 11, which was elaborated by a detail hierarchical cluster heatmap (Figure S6, Supplementary Information). It can be seen in Figure 6(A) *Lactococcus* sp. (abundance to 85.5%) dominated in the neutral pH fermentation, which were responsible for both L-, and D-lactic acid production (Cock and de Stouvenel, 2006). This was in good agreement with Figure 3(A) that OA of L-lactic acid was less than 40% at neutral pH. The bacterial community was altered significantly after intermittent alkaline fermentation compared to the neutral one. As seen from Figure 6 (B), *Aerococcus* sp. (90.3%), *Enterococcus* sp. (5.0%) and *Trichococcus* sp. (3.5%) were enriched after intermittent pH 11 fermentation. In the literature, *Aerococcus* sp., *Enterococcus* sp., and *Trichococcus* sp. were reported to exclusively produce L-lactic acid (Liu, 2003; Yang et al., 2007). Apparently, the current intermittent alkaline fermentation selectively proliferated the L-lactic acid producing microbes.

3.3. Production of Optically Pure L-lactic Acid in Pilot-scale Semi-continuous Reactor

The pilot-scale semi-continuous experiment was conducted to examine the feasibility of effectively producing optically pure L-lactic acid from food waste by sewage sludge supplement and intermittent alkaline fermentation at ambient temperature. The average concentration and OA of L-lactic acid was 12.05 (g/L) and 100% (Figure 7). Although slightly decrease of L-lactic acid production was observed compared to the batch test (13.11 g/L), optically pure L-lactic acid with a high yield of 0.48 (g/g TCOD) could be continuously obtained from this method. The fermentative kinetics of semi-continuous reactor was different from the batch fermentation, and inadequate utilization of the substrate was normally occurred in a bigger scale continuous fermentation system due to the inhomogeneous mass transfer (Li et al., 2011; Zhang et al., 2013). According to the batch and pilot-scale experimental data, the carbon mass balance was analyzed (see Figure S7, Supplementary Information). Obviously, the carbon amount of L-lactic acid was the highest (5.24 at batch and 4.82 g carbon/L at pilot-scale) fraction in the liquid phase. Several by-products, like acetic acid, propionic acid and ethanol, were existed in the liquid phase: which only contributed 0.61 and 0.45 g carbon/L in total from batch and pilot-scale works respectively. Very few carbons were escaped to the gas phase (only 0.26 g carbon/L (batch) and 0.21 g carbon/L (pilot-scale) of carbon dioxide were generated) during the fermentation. Also, methane was non-detectable in this study, which was consistent with our previous reports (Feng et al., 2009; Yuan et al., 2006).

4. Conclusions

In this study, sewage sludge supplement and intermittent alkaline fermentation to the food waste obviously improved the hydrolysis steps, and inhibited the enzymes of lactic acid consuming and D-lactic acid producing. Thus, the effective production of optically pure L-lactic acid (yield of 0.52 g/g TCOD, OA=100%) from food waste at ambient temperature was achieved in batch experiment. Furthermore, the current fermentation system selectively proliferated the L-lactic acid producing microbe (i.e., *Aerococcus* sp., *Enterococcus* sp. and *Trichococcus* sp.), and OA 100% of L-lactic acid (yield of 0.48 g/g TCOD) was then achieved.

368 Figures S1–S7.

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374 **References**

- 375 Akao, S., Tsuno, H., Horie, T., Mori, S., 2007. Effects of pH and temperature on products and bacterial
376 community in-lactate batch fermentation of garbage under unsterile condition. *Water Research* 41
377 (12), 2636-2642.
- 378 Carballa, M., Duran, C., Hospido, A., 2011. Should we pretreat solid waste prior to anaerobic digestion?
379 An assessment of its environmental cost. *Environmental Science and Technology* 45 (24),
380 10306-10314.
- 381 Chen, Y., Li, X., Zheng, X., Wang, D., 2013. Enhancement of propionic acid fraction in volatile fatty
382 acids produced from sludge fermentation by the use of food waste and *Propionibacterium*
383 *acidipropionici*. *Water Research* 47 (2), 615-622.
- 384 Cock, L., de Stouvenel, A., 2006. Lactic acid production by a strain of *Lactococcus lactis* subs *lactis*
385 isolated from sugar cane plants. *Electronic Journal of Biotechnology* 9 (1), 40-45.
- 386 Eskicioglu, C., Kennedy, K., Droste, R., 2006. Characterization of soluble organic matter of waste
387 activated sludge before and after thermal pretreatment. *Water Research* 40 (20), 3725–3736.
- 388 Feng, L., Chen, Y., Zheng, X., 2009. Enhancement of waste activated sludge protein conversion and
389 volatile fatty acids accumulation during waste activated sludge anaerobic fermentation by
390 carbohydrate substrate addition: the effect of pH. *Environmental Science and Technology* 43 (12),
391 4373-4380.
- 392 Ferain, T., Garmyn, D., Bernard, N., Hols, P., Delcour, J., 1994. *Lactobacillus plantarum* *ldhI* Gene:
393 overexpression and deletion. *Journal of Bacteriology* 176 (3), 596-601.
- 394 Gao, C., Ma, C., Xu, P., 2011. Biotechnological routes based on lactic acid production from biomass.
395 *Biotechnology Advance* 29 (6), 930-939.

396 Garvie, E. I., 1980. Bacterial lactate dehydrogenases. *Microbiology Review* 44 (1), 106-139.

397 Goel, R., Mino, T., Satoh, H., Matsuo, T., 1998. Enzyme activities under anaerobic and aerobic
398 conditions inactivated sludge sequencing batch reactor. *Water Research* 32 (7), 2081-2088.

399 Hofvendahl, K., Hahn-Hägerdal, B., 2000. Factors affecting the fermentative lactic acid production from
400 renewable resources. *Enzyme and Microbial Technology* 26 (2-4), 87-107.

401 Ilmen, M., Koivuranta, K., Ruohonen, L., Suominen, P., Penttilä, M., 2007. Efficient production of
402 L-lactic acid from xylose by *Pichia stipitis*. *Applied and Environmental Microbiology* 73 (1),
403 117-123.

404 Iwata, T., Doi, Y., 1999. Crystal structure and biodegradation of aliphatic polyester crystals.
405 *Macromolecular Chemistry and Physics* 200 (11), 2429-2442.

406 Kavitha, S., Jayashree, C., Kumar, S., Yeom, I., Banu, J., 2013. Effect of enzyme secreting bacterial
407 pretreatment on enhancement of aerobic digestion potential of waste activated sludge interceded
408 through EDTA. *Bioresource Technology* 150, 210 -219.

409 Kavitha, S., Jayashree, C., Kumar, S., Yeom, I., Banu, J., 2014. The enhancement of anaerobic
410 biodegradability of waste activated sludge by surfactant mediated biological pretreatment,
411 *Bioresource Technology* 168, 159-166.

412 Levis, J., Barlaz, M., 2011. What is the most environmentally beneficial way to treat commercial food
413 waste? *Environmental Science and Technology* 45 (17), 7438-7444.

414 Li, X., Chen, H., Hu, L. F., Yu, L., Chen, Y., Gu, G., 2011. Pilot-scale waste activated sludge alkaline
415 fermentation, fermentation liquid separation, and application of fermentation liquid to improve
416 biological nutrient removal. *Environmental Science and Technology* 45 (5), 1834-1839.

417 Liu, S., 2003. Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food
418 and beverage fermentations. *International Journal of Food Microbiology* 83 (2), 115-131.

419 Lunt, J., 1998. Large-scale production, properties and commercial applications of polylactic acid
420 polymers. *Polymer Degradation and Stability* 59 (1-3), 145-152.

421 Luo, J., Feng, L., Chen, Y., Li, X., Chen, H., Xiao, N., Wang, D., 2014. Stimulating short-chain fatty
422 acids production from waste activated sludge by nano zero-valent iron. *Journal of Biotechnology*
423 187 (10), 98–105.

424 Maeda, T., Yoshimura, T., Shimazu, T., Shirai, Y., Ogawa, H., 2009. Enhanced production of lactic acid
425 with reducing excess sludge by lactate fermentation. *Journal of Hazardous of Materials* 168 (2-3),
426 656-663.

427 Nakasaki, K., Akakura, N., Adachi, T., Akiyama, T., 1999. Use of wastewater sludge as a raw material
428 for production of L-lactic acid. *Environmental Science and Technology* 33 (1), 198-200.

429 Nguyen, D., Van Hoorde, K., Cnockaert, M., De Brandt, E., De Bruyne, K., Le, B., Vandamme, P., 2013.
430 A culture-dependent and -independent approach for the identification of lactic acid bacteria
431 associated with the production of nem chua, a Vietnamese fermented meat product. *Food Research*
432 *International* 50 (1), 232-240.

433 Parkin, G., Owen, W., 1986. Fundamental of anaerobic digestion of wastewater sludge. *Journal of*
434 *Environmental Engineering* 112, 867-920.

435 Sakai, K., Fujii, N., Chukeatirote, E., 2006. Racemization of L-lactic acid in pH-swing open
436 fermentation of kitchen refuse by selective proliferation of *Lactobacillus plantarum*. *Journal of*
437 *Bioscience and Bioengineering* 102 (3), 227-232.

438 Yang, G., Tian, J., Li, J., 2007. Fermentation of 1,3-propanediol by a lactate deficient mutant of
439 *Klebsiella oxytoca* under microaerobic conditions. *Applied Microbiology and Biotechnology* 73 (5),
440 1017-1024.

441 Yuan, H., Chen, Y. G., Zhang, H., Jiang, S., Zhou, Q., Gu, G., 2006. Improved bioproduction of
442 short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. *Environmental*
443 *Science and Technology* 40 (6), 2025-2029.

444 Zhang, B., He, P., Ye, N., Shao, L., 2008. Enhanced isomer purity of lactic acid from the non-sterile
445 fermentation of kitchen wastes. *Bioresource Technology* 99 (4), 855-862.

446 Zhang, P., Chen, Y., Zhou, Q., Zheng, X., Zhu, X., Zhao, Y., 2013. Understanding short-chain fatty acids
447 accumulation enhanced in waste activated sludge alkaline fermentation: kinetics and microbiology.
448 *Environmental Science and Technology* 44 (24), 9343-9348.

449 Zheng, X., Su, Y., Li, X., Xiao, N., Wang, D., Chen, Y.G., 2013. Pyrosequencing reveals the key
450 microorganisms involved in sludge alkaline fermentation for efficient short-chain fatty acids
451 production. *Environmental Science Technology* 47 (9), 4262-4268

452 Zhao, Y., Chen, Y., 2011. Nano-TiO₂ enhanced photofermentative hydrogen produced from the dark
453 fermentation liquid of waste activated sludge. Environmental Science and Technology 45 (19),
454 8589-8595.

455 **Figure captions**

456 Fig. 1 - Proposed metabolic pathway for lactic acid production from organic wastes (Garvie, 1980; Feng
457 et al., 2009).

458 Fig. 2 - The effect of activated sludge addition on the maximal L-lactic acid production at pH 7. The
459 fermentation of sole food waste at pH 7 was set as control in this study. There was no lactic acid
460 produced as sole activated sludge was fermented for even 14 d. The standard deviations, optical
461 purity of L-lactic acid, and fermentation time required to reach the maximal L-lactic acid were
462 respectively 0.16 g/L, 34.5% and 6 d (sole food waste), 0.26 g/L, 36.2% and 4 d (food waste/sludge
463 = 12/1), 0.29 g/L, 40.6% and 4 d (9/1), 0.51 g/L, 61.5% and 2 d (6/1), 0.26 g/L, 58.6%, and 2 d
464 (3/1), and 0.21 g/L, 84.3% and 1 d (1.5/1).

465 Fig. 3 - Effect of intermittent pH adjustment on the OA (A) and production (B) of L-lactic acid at the
466 ratio of food waste to sludge of 6/1. At any fermentation time lactic acid was not detectable at pH
467 2, 3 and 12. Error bars represent standard deviations of triplicate tests.

468 Fig. 4 - The gel-filtration chromatograph of Mw distributions (A-C) and percentage of Mw < 1000 (D) in
469 the sole food waste (A), sole sludge (B), and the mixture of food waste and sludge with ratio of 6/1
470 (C) in the intermittent pH 11 fermentation tests. Error bars represent standard deviations of
471 triplicate tests.

472 Fig. 5 - 3D surface plots and contour plots for relative activity of L-LDH (A) and D-LDH (B).

473 Fig. 6 - Figure 6 Genus level distributions of bacterial populations from reactors operated at pH 7 (A)
474 and pH 11 (B). The detail information from the two reactors was elaborated in Figure S6
475 (Supplementary Information).

476 Fig. 7 - Performance of the continuously operated reactor after the production of L-lactic acid reached
477 relatively stable. Error bars represent standard deviations of triplicate tests.

497 **Table captions**

498 Table 1. Effect of intermittent fermentation pH on the relative activities of key enzymes involved in
499 hydrolysis and generations of acetic, propionic and lactic acids

Table 1. Effect of intermittent Fermentation pH on the Relative Activities of Key Enzymes Involved in Hydrolysis and Generations of Acetic, Propionic and Lactic Acids.^a

Key enzyme	pH 7	pH 8	pH 9	pH 10	pH 11
α -glucosidase	89.3 $\pm$ 9.5	100.0	102.2 $\pm$ 1.8	116.3 $\pm$ 4.3	115.4 $\pm$ 4.1
protease	79.2 $\pm$ 8.1	100.0	102.8 $\pm$ 1.7	106.4 $\pm$ 2.4	115.8 $\pm$ 3.6
L-LDH	85.6 $\pm$ 6.2	100.0	103.4 $\pm$ 1.5	101.2 $\pm$ 1.1	105.9 $\pm$ 1.2
D-LDH	115.1 $\pm$ 4.6	100.0	59.9 $\pm$ 7.6	2.3 $\pm$ 0.3	0
iLDH	99.5 $\pm$ 1.4	100.0	79.4 $\pm$ 8.7	43.5 $\pm$ 5.2	32.5 $\pm$ 4.2
AK	98.5 $\pm$ 1.2	100.0	76.4 $\pm$ 9.3	50.4 $\pm$ 6.2	47.5 $\pm$ 5.4
PTA	99.6 $\pm$ 3.3	100.0	98.5 $\pm$ 2.1	79.6 $\pm$ 8.1	12.4 $\pm$ 4.1
OAATC	99.5 $\pm$ 2.2	100.0	104.1 $\pm$ 3.5	104.3 $\pm$ 3.1	104.5 $\pm$ 3.4

^a The experiments were conducted at food waste to sludge ratio of 6/1 (g/g). The data reported are the averages and their standard deviations in triplicate tests as maximal L-lactic acid was observed. Since the production of L-lactic acid at pH 4-6 was much lower than that at other pHs (see Figure 2), only the data at pH 7-11 were provided. The activities at pH 8 were set as 100.0, and the unit of the relative activities was %.

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Figure 2. The effect of activated sludge addition on the maximal L-lactic acid production at pH 7. The fermentation of sole food waste at pH 7 was set as control in this study. There was no lactic acid produced as sole activated sludge was fermented for even 14 d. The standard deviations, optical purity of L-lactic acid, and fermentation time required to reach the maximal L-lactic acid were respectively 0.16 g/L, 34.5% and 6 d (sole food waste), 0.26 g/L, 36.2% and 4 d (food waste/sludge = 12/1), 0.29 g/L, 40.6% and 4 d (9/1), 0.51 g/L, 61.5% and 2 d (6/1), 0.26 g/L, 58.6%, and 2 d (3/1), and 0.21 g/L, 84.3% and 1 d (1.5/1).

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