

Acidogenic properties of carbohydrate-rich wasted potato and microbial community analysis: Effect of pH

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Vegetable waste is one of the major organic solid residues available for sustainable biogas production. The aim of this study was to investigate the possibility and optimal controlling strategy for acidogenic fermentation of wasted potato (WP). Three leaching bed reactors (LBRs) were operated at various pH values (6.0, 7.0 and 8.0) with an organic loading rate (OLR) of 6.7 g volatile solid/(L·d) and hydraulic retention time of 6 d. Butyric acid-type fermentation with butyric acid as predominant volatile fatty acid (VFA) was observed with a concentration and proportion (of total VFAs) of butyric acid, which were 7.8 g/L, 49.7 % and 9.6 g/L and 52.2 % at pH 6.0 and 7.0, respectively. Conversely, at pH 8.0, mixed acid-type fermentation was observed with acetic and butyric acid as the major VFAs. Control experiment without pH manipulation didn't perform well in VFAs production at first 6 days and then VFAs concentration increased as pH value was adjusted to 8. It was indicated that the inhibition was caused by high undissociated VFAs concentration due to low pH and the VFAs production could be improved through pH control strategy to regulate the undissociated VFAs concentration. According to the bacterial analysis, the microbial community was diverse and Firmicutes were the most important bacteria at different pH conditions. Therefore, the results suggested that a process of pH control might be feasible for stable and efficient acidogenic fermentation.

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[Key words: Vegetable waste; Volatile fatty acids; Acidification; pH control; Bacterial community]

Vegetable waste, as an organic solid waste, is generated in substantial quantity in China. However, no more than 20% of the waste is properly treated (1,2). Potato is an essential vegetable in Chinese diet. During transportation and storage, a certain quantity of wasted potatoes like rotten or germinated ones are generated. Given the potential treatment by anaerobic digestion, carbohydrate-rich potato is an alternative as the feedstock. Based on the properties of wasted potato (WP), most of them can be readily degraded and the intermediate compounds such as volatile fatty acids (VFAs) which are inhibitory to the microbial populations at high concentrations, resulted in the failure of methanogenesis. It was indicated that the range of organic loading rate (OLR) in the two-phase anaerobic digestion of vegetable wastes was 5.7–7.7 kg volatile solid (VS)/(m³·d) (3). Therefore, optimized treatment of two-phase anaerobic digestion of vegetable waste is required, which has the advantages of high organic load in the acidogenic reactor and separated optimum conditions for acidogenesis and methanogenesis.

The hydrolysis/acidification of many kinds of feedstocks have been investigated. However, the research focuses were different due to the variations in material properties (4,5). There was little acidogenesis information about the carbohydrate-rich WP. Therefore, it is necessary to optimize conditions of acidification in the first stage reactor and to produce more suitable acid metabolites for

methanogens, which would consequently improve the overall system performance. The hydrolysis/acidification rates as well as fermentative types in the acid reactor determine the subsequent acetogenesis and methanogenesis efficiencies (6). The study of the VFA production is quite essential not only for methane production but also for other applications. The VFAs are known to be used in other processes such as polyhydroxyalkanoate (PHA) production (7), biological nutrient and phosphorous removal (8).

The objective of this work was to evaluate the possibility and controlling strategy of acidogenic fermentation of potato at different pH values. In order to improve the acidification biologically, the microbial diversity was analyzed to provide useful bacterial information. Batch experiments with a pH-control strategy and insight into the bacterial community were of great importance to identify the optimum conditions for efficient and stable VFAs fermentation. The combinations of acidogenic and methanogenic phases were preliminarily discussed to prove the feasibility of two-phase anaerobic digestion of WP.

MATERIALS AND METHODS

Substrate and inoculum The germinated potatoes were collected from a vegetable distribution center in Jinan, China. The WP were shredded to 3–5 cm before feeding. The inoculum of anaerobic sludge was obtained from Boxing Wastewater Treatment Plant, Binzhou, China. The general properties of the feedstock and inoculum are listed in Table 1. The inoculum was incubated at 35°C for 7 days to deplete the residual nutrients.

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TABLE 1. Characteristics of feedstock and inoculum.

	Potato	Inoculum
Total solid (TS, %)	18.2 ± 0.7	7.4 ± 0.4
Volatile solid (VS, %-TS)	94.6 ± 1.4	21.3 ± 0.4
Carbon (%)	40.6 ± 2.1	—
Nitrogen (%)	1.9 ± 0.1	—

Experimental set-up Leaching bed was double-walled organic glass reactor with a diameter of 9 cm and height of 40 cm. The leaching bed was vertical and the perforated plate was fitted at the bottom to support the substrate. The top had a port connected to a gas bag. It was maintained at 35°C by water bath. Each reactor was equipped with a leachate reservoir tank with a holding capacity of 5 L. At the beginning of the experiments, WP of 100 g VS (OLR of 6.7 g VS/L·d) was added into the reactors, and a mixture of the sludge and tap water (total volume of 2 L) was utilized as the initial leachate. The inoculum to substrate ratio of 1:10 on VS/VS basis was adopted. The recirculation was conducted automatically at constant intervals during the entire period. Anaerobic batch experiments were performed to study the acidogenesis of WP at different constant pH values of 6, 7, 8. The pH of leachate was adjusted throughout the experiment with a pH automatic controller by adding 6 N NaOH or HCl solution. A control group was carried out at the same time.

Up-flow anaerobic sludge blanket (UASB) was used as methanogenic reactor with a working volume of 2.7 L, which was integrated with LBR. UASB reactor was inoculated with granular sludge and acclimated with sodium acetate solution (2 g/L) at OLR of 1.0 g chemical oxygen demand (COD)/L·d for 1 month. Simulated acidic leachate (acetic acid of 10.47 mg/L, propionic acid of 1.75 g/L, butyric acid of 6.75 g/L, iso-butyric acid of 0.32 g/L, valeric acid of 0.18 g/L, iso-valeric acid of 0.44 g/L) was adopted according to genuine VFAs compositions and concentrations obtained in the acidification at pH 8. The COD concentration of influent was diluted to 4 g/L and the OLR was adjusted by changing the hydraulic retention time (HRT). The UASB reactors were continuously fed with synthetic medium at an initial OLR of 2.0 g COD/L·d for 7 days, and then increased to 3, 4.5, 7, 10, 12, and 15 g COD/L·d at 7 days intervals. Sodium nitrate and potassium dihydrogen phosphate were supplemented to maintain the COD:N:P at the ratio of 100:4:1. The daily gas was collected and COD of effluent was utilized to evaluate the conversion efficiency of VFAs.

Analytical method TS and VS content were evaluated according to the standard methods (9). COD concentration was determined with COD analyzer (5B-3B, Lianhua, Beijing, China). The methane content was detected by biogas analyzer (Geotech, Biogas, UK). The concentrations of VFAs (acetic-, propionic-, butyric-, valeric-, isobutyric-, and isovaleric acid) were measured with Agilent 7890 series gas chromatograph (GC). Pretreatments were conducted before VFAs measurements. The samples were centrifuged and then acidified with 3% phosphoric acid to a pH less than 2, in order to convert the fatty acids to their undissociated forms. Then the samples were diluted with deionized water to assure the VFAs concentration to be in the range of standard curve and filtered through 0.22 μm pore-sized filters. The GC was operated under the following conditions: inlet temperature of 250°C, flame ionization detector of 300°C; nitrogen as carrier gas with a flow rate of 30 mL/min. The oven temperature was programmed to increase. It started from 60°C to 100°C at 10°C/min, and then further increase to 250°C at 10°C/min, holding for 2 min. The bacterial analysis was conducted by high throughput sequencing. The procedures were as follows: DNA was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) according to protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 regions of the bacteria 16S rRNA gene were amplified with primers (5'-ACTCCTACGGGAGGCAGCAG-3')/806R (5'-GGACTACHVGGGTWTCTAAT-3') for the V3 and V4 region of 16S rRNA and archaeal primer pairs Arch349F Arch806R. The PCR program consisted of an initial 5 min denaturation step at 94°C, and a total of 25 cycles (each including 30 s at 95°C, 30 s at 50°C, and 40 s at 72°C) was followed by a final extension step of 7 min at 72°C. Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw fastq files were demultiplexed.

The undissociated acid concentration was calculated as described in Eqs. 1 and 2 (10).

$$\text{pH} = \text{pK}_a + \log(\text{A}^-/\text{HA}) \quad (1)$$

$$\text{A}^- + \text{HA} = \text{Total acid} \quad (2)$$

HA (mg/L) and A⁻ (mg/L) are the concentrations of dissociated and undissociated VFAs, respectively. pK_a values of acetic acid and butyric acid were 4.76 and 4.81 at 35°C, respectively.

RESULTS AND DISCUSSION

Effect of pH on acidification and types of acidogenic fermentation in batch reactors The effects of pH on the yields and compositions of VFAs during the acidification of WP are shown in Fig. 1. Under the acidic and alkaline conditions, three levels of pH (pH 6.0, 7.0, and 8.0) were investigated. As shown in Fig. 1, the cumulative acidification products significantly increased during the retention time of 5 days, but the concentration increased more quickly at pH 8.0 than those at pH 6.0 and 7.0. The total concentrations of acidification products were 15.72, 18.44, and 19.92 g VFAs/L at pH 6.0, 7.0, and 8.0, respectively. According to previous study, pH had a great influence on the type of fermentation. The fermentation types based on the proportion

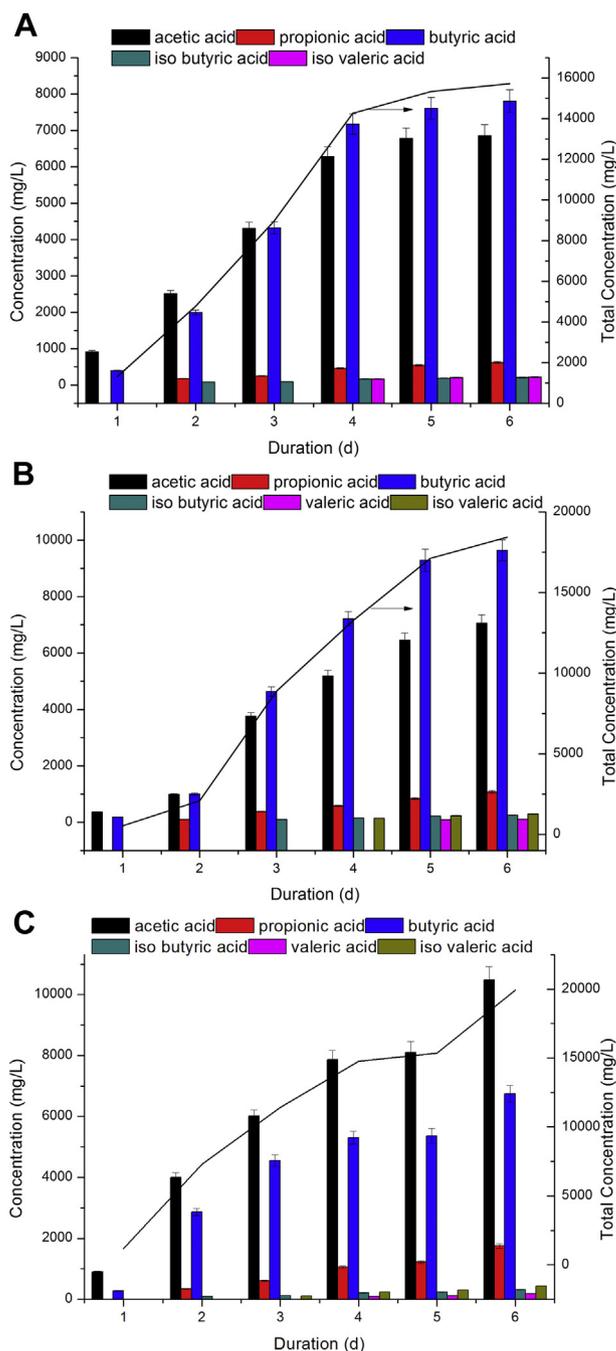


FIG. 1. Distribution and total concentration of VFAs at pH of 6 (A), 7 (B), and 8 (C).

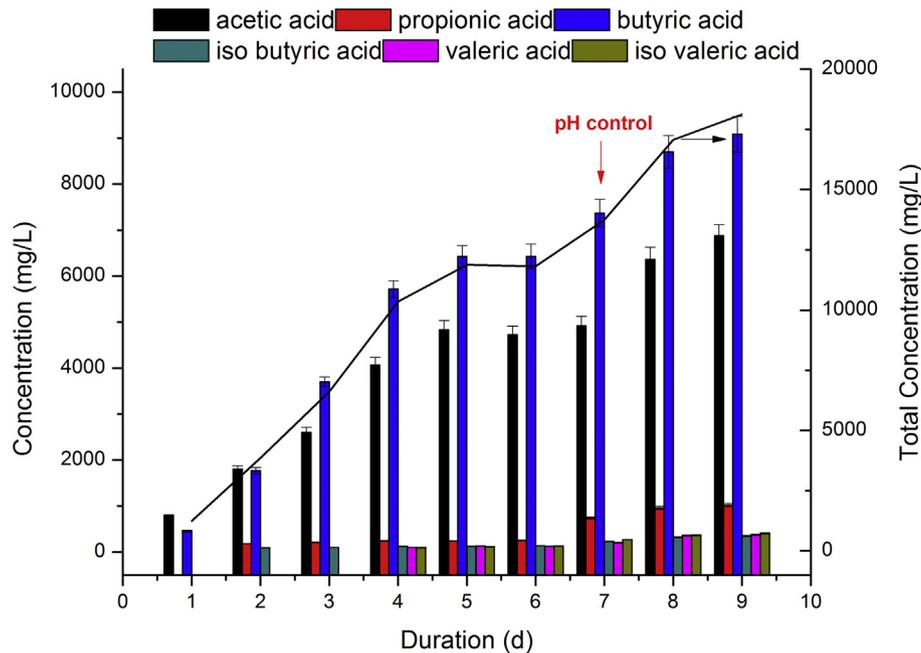


FIG. 2. Distribution and total concentration of VFAs in control experiment (pH without control at first 6 days and then pH controlled at 8).

of acidogenic products were distinguished (11). The acetic acid concentration was up to 10.5 g/L at pH 8.0, which accounted for 52.6% of the acidification products, thereby indicating typical ethanol-type fermentation according to Ren et al. (12). At pH 6.0 and 7.0, the dominant acids were both butyric acid. The percentage of butyric acid (49.7% and 52.3%) revealed its butyric acid-type fermentation characteristic. The sum of acetic and butyric acid reached 86.4% at pH 8.0, which was the mixed acid-type fermentation. These results showed that pH considerably affected the acidification intermediate products and type of WP. One of the most important factors in digestion systems is pH. It could be explained that different metabolic routes possibly occurred because of microbial growth and changes in microbial population when operational and environmental conditions varied, thereby resulting in different fermentation types.

In this study, carbohydrate-rich WPs could be easily acidified. In addition, a constant pH of 8.0 was conveniently beneficial for enables acidogenic fermentation. Although NaOH was needed to adjust the initial pH to 8.0, the VFAs concentration significantly increased which might compensate the operation cost. Further investigation and optimization of the conjunction with the succeeding methanogenesis during a two-phase anaerobic digestion will be performed.

Fig. 2 shows the VFAs output and compositions in control experiment. The pH was not controlled at first 6 days and the

VFAs concentration of 11.8 g/L was the lowest compared to those with pH control. Based on the above-mentioned results, the pH of control experiment was adjusted to 8 from 7th day on. It was found that there was an obvious increase in VFAs concentration. The maximum concentration could reach 18.1 g/L at 9th day, increasing by 53.4% compared with that without pH control. Combined with Table 2, the variation should be well correlated with the undissociated VFAs concentration. As can be seen in Table 2, undissociated acid concentration increased as pH decreased as a result of dissociation due to low pH. The undissociated acetic and butyric acid concentration reached 2.25 g/L and 3.25 g/L in control, approximately 74.5 mM. After pH adjustment, the undissociated VFAs concentration significantly decreased to a low level. It has been reported that undissociated acid forms of VFA caused inhibition. There should be some certain concentration of undissociated acids which influenced the fermentation. van Ginkel and Logan (10) found that the inhibition occurred when the acetic or butyric acid concentration was 60 mM at pH 5 and 30°C. The critical inhibitory concentration of undissociated butyric acid of 50 mM was determined by van den Heuvel et al. (13). Undissociated acid concentrations in this range are considered to greatly increase the energy requirements of cell maintenance (14). The comparison further demonstrated the importance of pH control in acid metabolism, which had a great effect on the production of organic acids. It was also indicated

TABLE 2. Free acetic and butyric acid concentration at different pH values.

	pH 6		pH 7		pH 8		pH without control		
	Acetic acid (mg/L)	Butyric acid (mg/L)	Acetic acid (mg/L)	Butyric acid (mg/L)	Acetic acid (mg/L)	Butyric acid (mg/L)	pH value	Acetic acid (mg/L)	Butyric acid (mg/L)
1	49.5	24.1	2.0	1.2	0.5	0.2	6.45	15.8	10.3
2	137.1	120.9	5.6	6.4	2.3	1.8	5.35	369.1	395.5
3	234.5	262.1	21.5	29.7	3.5	2.9	4.89	1107.2	1683.6
4	341.9	435.2	29.7	46.3	4.5	3.4	4.76	2032.5	3024.3
5	368.8	461.4	36.9	59.6	4.7	3.5	4.76	2416.0	3400.5
6	373.1	473.5	40.4	61.8	6.0	4.3	4.80	2253.4	3245.9
7	—	—	—	—	—	—	8.00	2.8	4.7
8	—	—	—	—	—	—	8.00	3.7	5.6
9	—	—	—	—	—	—	8.00	4.0	5.9

TABLE 3. Soluble COD variation and VFA production profile under different conditions.

	Equivalent COD _{VFA} (mg/L)	Soluble COD (sCOD) (mg/L)	Equivalent COD _{VFA} /sCOD (%)	VFA yield (g/gVS-added)
pH 6	23263.1	25136	92.5	0.36
pH 7	27946.3	29418	95.0	0.42
pH 8	27926.8	29140	95.8	0.46
Control 6th day	17887.0	20145	88.8	0.27
9th day	27609.9	29065	95.0	0.42

from Table 2 that the final concentrations of free acetic and butyric acid were around 0.85 g/L, 0.10 g/L, and 0.01 mg/L at pH 6, 7, and 8, respectively, which was negatively associated with the VFAs yield. This could further explain the difference of VFAs yield resulting from the effect of pH on the presence of acids form.

The VFAs included acetic-, propionic-, butyric-, iso valeric-, and valeric acids, which were converted into equivalent COD to obtain the degree of acidification products which was equal to equivalent COD_{VFA} divided by soluble COD. Equivalent COD_{VFA} was based on the total COD of individual VFA. The conversion coefficients of acetic-, propionic-, butyric-, and valeric acid were 1.066, 1.512, 1.816, and 2.036, respectively.

The ratio of equivalent COD_{VFA} to sCOD is very important, as it shows the amount of sugars converted into VFAs. The soluble substances were produced from macro-molecule in WP such as starch. Higher equivalent COD_{VFA}/sCOD ratios were achieved at pH 7.0 and 8.0. However, at pH 6.0 and uncontrolled pH, the ratios of VFA/sCOD were relatively low. Due to the end products inhibition effect, the sCOD was affected under different conditions. Most of the soluble COD was contributed by the VFAs at pH 7 and 8, while some other soluble substances still existed except for VFAs at pH 6 and pH without control, which indicated that free acids under acidic conditions inhibited the activity of acidogenic bacteria and decrease the degradation of substrate. The VFA yield was analyzed to evaluate the acidification rate, which was calculated based on the VS-added (Table 3). Higher VFAs yield was obtained at pH 8 in comparison with those at pH of 6 and 7. It was also observed that VFAs concentration was higher at pH 8 than that of pH 7. However, there is little difference on the equivalent COD_{VFA}. This was because of the different VFAs compositions. The butyric acid content was higher at pH 7 and accordingly corresponded to higher COD due to high coefficient.

As can be seen from this study, higher VFAs yield was obtained at pH 8.0, which was not similar to the results reported by Jiang et al. (15). The highest activities of hydrolytic enzymes and highest concentration of VFAs were observed at pH 6.0. The investigated pH value was in the acidic range, not including the alkaline condition. Carbohydrate was the one of the organic matter in food waste and also the proteins accounted for a part of the organic materials. The differences may be resulted from the substrate distinction. The factors affecting the VFAs production involved pH, the substrate, inoculum and environment and so on. Therefore, the results could not be directly compared. Taking the acidic and alkaline pH into

TABLE 4. Performance of UASB reactors under different OLRs.

OLRs (g/L·d)	HRT (h)	COD of influent (g/L)	COD of effluent (g/L)	COD removal efficiency (%)	Methane yield (ml CH ₄ /g COD)
2	24	2	0.04	98.0	340
3	16	2	0.08	96.0	337
4.5	10.7	2	0.13	93.5	332
7	6.9	2	0.20	90.0	326
10	4.8	2	0.29	85.5	319
12	4	2	0.33	83.5	315
15	3.2	2	0.38	81.0	310

consideration, the pH control of 8 during the acidification of WP was determined to be the optimum.

Bacterial community analysis

The performance of acidification was influenced not only by fermentation conditions and environmental factors, but also the microbial community. VFAs accumulation led to products inhibition to metabolic pathways. Under high VFAs concentration circumstances, the microbial community distribution was critical for the subsequent regulation of acidogenic efficiency from the biological point of view. The acidogenic microbe mainly consisted of bacteria, and the relative abundances of bacterial groups were analyzed at phylum level for all samples (Fig. 3). The dominant bacteria in the anaerobic sludge involved Bacteroidetes, Proteobacteria, Chloroflexi and Firmicutes. These groups had similar abundance and together accounted for 62% of the total bacteria. The result were consistent with the report of Lee et al. (16). It has been found that Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria were the most abundant bacteria in a full-scale digester (17,18). The bacteria in WP-based acidogenic leachate were some efficient species commonly present in anaerobic digestion.

Fig. 4 shows that the obviously enriched bacteria at phylum level in anaerobic sludge were Firmicutes, Bacteroidetes, Proteobacteria and Chloroflexi. The Firmicutes and Proteobacteria are widely distributed and indicate the acidogenic ability to metabolize many macromolecules such as proteins and carbohydrates (19,20). The bacteria of Chloroflexi could use different carbohydrates and amino acids as substrates for their growth (21,22). Therefore, VFAs production was closely correlated with the presence of Firmicutes and some other acidogens.

At the end of the fermentation under different pH conditions, the bacterial diversities were almost identical. However, the abundances of each bacteria at pH 6, 7, and 8 were distinct. Firmicutes and bacteroidetes were the most abundant bacteria as the acidification of WP was operated at pH 7 and 8, while Firmicutes and proteobacteria were the major ones at pH 6. Despite the same substrate and inoculum, there were great changes in fermentation environments and pH variation could influence the microbes distribution. Firmicutes that are able to utilize various carbon sources, i.e., hexoses, pentoses, and saccharides, were the most important group in all samples (23). Therefore, it could be concluded that Firmicutes played an important role in the acidogenesis of

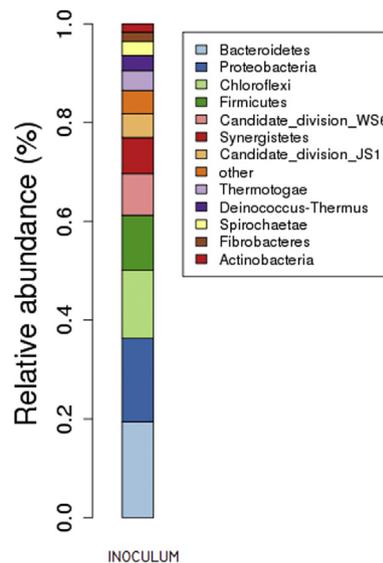


FIG. 3. The bacterial community analysis of inoculum at phylum level.

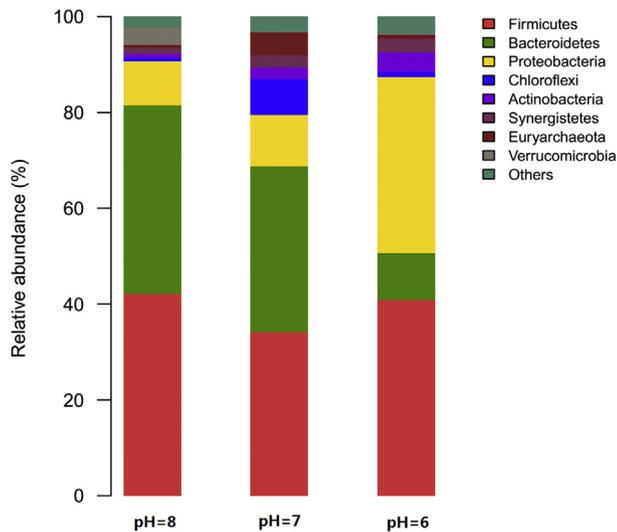


FIG. 4. The bacterial community analysis of digestate obtained at different pH at phylum level.

carbohydrate-rich hydrolysate. The VFAs distribution and output were directly affected by the bacterial structure.

At genus level (Fig. 5), the bacteria was specifically classified from phylum to genus. Despite the changes in the community composition, the most functional bacterial operational taxonomic units (OTUs), represented as relative abundance, were observed including *Bacteroides*, *Lactobacillus*, *Lactococcus*, *Clostridium sensu stricto*, *Lachnospiraceae* and *Ruminococcaceae*. Their proportion varied drastically in different reactors, related with the different pH environments. *Lactobacillus* is a well-known lactic acid bacterium (LAB) typically used in dairy fermentation processes. *Lachnospiraceae* and *Ruminococcaceae* is important for degrading lignocellulosic feedstock, such as cellulose and hemicellulose (24).

All the genus bacteria showed synergistic effects on the WP acidogenesis.

Conversion of VFAs into methane in UASB reactor There are several kinds of VFAs with different concentrations in WP leachate. To verify the conversion of VFAs in methanogenic stage, UASB was employed as methanogenic reactor to be combined with the acidogenic process. Of the several intermediate steps, methane production from VFAs is often the critical pathway that limits the whole process rate, therefore, stepwise OLR increase was implemented in methanogenic stage. The methane yield was evaluated in the methanogenic process. Methane yield is equal to the daily methane volume divided by the total COD (influent COD concentration multiplied by the effective reactor volume). Acclimatization of sludge under different OLRs was within 7 days. Stable performances under different OLRs are observed in Table 4. It could be seen that with OLR increasing, the COD of effluent rose and COD removal efficiency were reduced due to short HRT. However, the COD removal efficiency exceeded 80%, which met the basic UASB operation requirement. It has been reported by Parawira et al. (25) that a stable conversion process of OLR of 6.1 g COD/L·d could be performed in UASB. In the present study, the OLR reached 15 g COD/L·d with specific VFAs compositions, which achieved a final COD removal efficiency (81%) throughout the operational periods. Based on the daily COD input, the methane yield for the test could be above 310 mL CH₄/g COD. The results demonstrated the effective combination of acidogenic and methanogenic stage with WP as feedstock.

It was demonstrated that VFAs output could be significantly improved with carbohydrate-rich WP as feedstock and different compositions of VFAs were obtained using the same feedstock and acidogenic culture just by regulating the process pH. Overall, there were two effects of pH: (i) manipulation of the undissociated VFAs concentration to alleviate the inhibition to substrate degradation; (ii) regulation of metabolic pathways to obtain some specific VFAs. The change in pH also had an impact on the microbial population. The result of microbial analysis indicated the diversity of bacterial

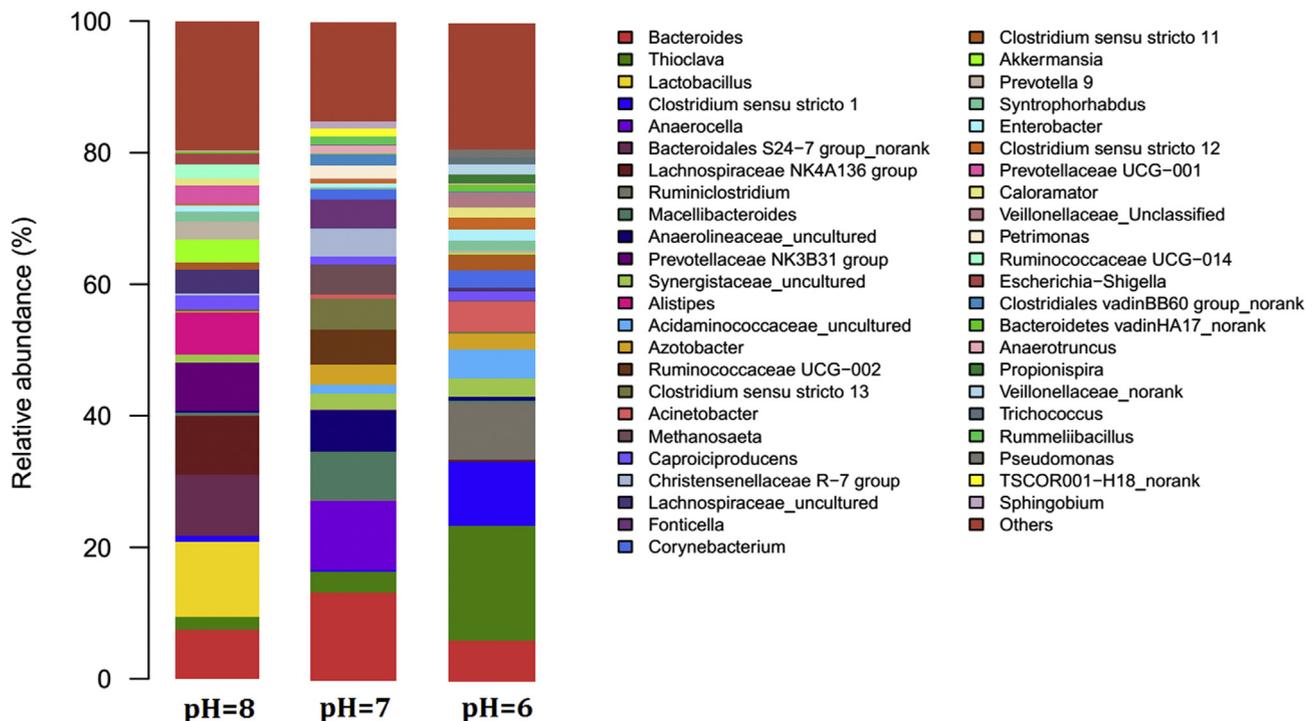


FIG. 5. The bacterial community analysis of digestate obtained at different pH at genus level.

composition shifted with environmental conditions. Evidently, Proteobacteria, Firmicutes, and Bacteroidetes were the key bacterial communities in the acidogenic process. The microbial community information would play an important role in the guidance of microbe enrichment and biological regulation, which should be closely associated with the VFAs accumulation. Finally, the fermentation products in acidogenic stage were efficiently transformed into methane in UASB reactor. The effective combination of both processes could prove the feasibility of two-stage anaerobic digestion process.

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References

- Liu, Y. and Tanaka, S.: Ethanol fermentation from biomass resources: current state and prospects, *Appl. Microbiol. Biotechnol.*, **69**, 627–642 (2006).
- Shen, F., Yuan, H., Pang, Y., Chen, S., Zhu, B., Zou, D., Liu, Y., Ma, J., Yu, L., and Li, X.: Performances of anaerobic co-digestion of fruit & vegetable waste (FWW) and food waste (FW): single-phase vs. two-phase, *Bioresour. Technol.*, **144**, 80–85 (2013).
- Ganesh, R., Torrijos, M., Sousbie, P., Lugardon, A., Steyer, J. P., and Delgenes, J. P.: Single-phase and two-phase anaerobic digestion of fruit and vegetable waste: comparison of start-up, reactor stability and process performance, *Waste Manag.*, **34**, 875–885 (2014).
- Cirne, D. G., Lehtomäki, A., Björnsson, L., and Blackall, L. L.: Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops, *J. Appl. Microbiol.*, **103**, 516–527 (2007).
- Nizami, A. S., Thamsiroj, T., Singh, A., and Murphy, J. D.: Role of leaching and hydrolysis in a two-phase grass digestion system, *Energy Fuel*, **24**, 4549–4559 (2010).
- Zhang, B. and He, P. J.: Performance assessment of two-stage anaerobic digestion of kitchen wastes, *Environ. Technol.*, **35**, 1277–1285 (2014).
- Albuquerque, M. G. E., Eiroa, M., Torres, C., Nunes, B. R., and Reis, M. A. M.: Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses, *J. Biotechnol.*, **130**, 411–421 (2007).
- Rajagopal, R. and Béline, F.: Anaerobic hydrolysis and acidification of organic substrates: determination of anaerobic hydrolytic potential, *Bioresour. Technol.*, **102**, 5653–5658 (2011).
- American Public Health Association: Total and volatile suspended solids. Standard operating procedure, revision 2. American Public Health Association, Baltimore, MD, USA (2005).
- van Ginkel, S. and Logan, B. E.: Inhibition of biohydrogen production by undissociated acetic and butyric acids, *Environ. Sci. Technol.*, **39**, 9351–9356 (2005).
- Wu, Y., Wang, C., Zheng, M., Zuo, J., Wu, J., Wang, K., and Yang, B.: Effect of pH on ethanol-type acidogenic fermentation of fruit and vegetable waste, *Waste Manag.*, **60**, 158–163 (2017).
- Ren, N. Q., Wang, B. Z., and Huang, J.: Ethanol-type fermentation from carbohydrate in high rate acidogenic reactor, *Biotechnol. Bioeng.*, **54**, 428–433 (1997).
- van den Heuvel, J. C., Beeftink, M. H., Verschuren, P. G., and de Beer, D.: Determination of the critical concentration of inhibitory products in a repeated fed-batch culture, *Biotechnol. Tech.*, **6**, 33–38 (1992).
- Maddox, I. S., Steiner, E., Hirsch, S., Wessner, S., Gutierrez, N. A., Gapes, J. R., and Schuster, K. C.: The cause of acid crash and acidogenic fermentations during the batch acetone–butanol–ethanol (ABE-) fermentation process, *J. Mol. Microbiol. Biotechnol.*, **2**, 95–100 (2000).
- Jiang, J. G., Zhang, Y. J., Li, K. M., Wang, Q., Gong, C. X., and Li, M. L.: Volatile fatty acids production from food waste: effect of pH, temperature, and organic loading rate, *Bioresour. Technol.*, **143**, 525–530 (2013).
- Lee, S. H., Kang, H. J., Lee, Y. H., Lee, T. J., Han, K., Choi, Y., and Park, H. D.: Monitoring bacterial community structure and variability in time scale in full-scale anaerobic digesters, *J. Environ. Monit.*, **14**, 1893–1905 (2012).
- Guo, J., Peng, Y., Ni, B.-J., Han, X., Fan, L., and Yuan, Z.: Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing, *Microb. Cell Fact.*, **14**, 33 (2015).
- Li, Y., Hua, D., Mu, H., Xu, H., Jin, F., and Zhang, X.: Conversion of vegetable wastes to organic acids in leaching bed reactor: performance and bacterial community analysis, *J. Biosci. Bioeng.*, **124**, 195–203 (2017).
- Wiegel, J., Tanner, R., and Rainey, F. A.: An introduction to the family *Clostridiaceae*, pp. 654–678, in: Dworkin, M. (Ed.), *The prokaryotes: an evolving electronic resource for the microbial community*, 3rd ed. Springer-Verlag, New York (2005).
- Lim, J. W., Chiam, J. A., and Wang, J. Y.: Microbial community structure reveals how microaeration improves fermentation during anaerobic co-digestion of brown water and food waste, *Bioresour. Technol.*, **171**, 132–138 (2014).
- Sekiguchi, Y., Yamada, Y., Hanada, S., Ohashi, A., Harada, H., and Kamagata, Y.: *Anaerolinea thermophile* gen. nov., sp. nov. and *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain *Bacteria* at the subphylum level, *Int. J. Syst. Evol. Microbiol.*, **53**, 1843–1851 (2003).
- Yamada, T., Sekiguchi, Y., Imachi, H., Kamagata, Y., Ohashi, A., and Harada, H.: Diversity, localization, and physiological properties of filamentous microbes belonging to *Chloroflexy* subphylum I in mesophilic and thermophilic methanogenic sludge granules, *Appl. Environ. Microbiol.*, **71**, 7493–7503 (2005).
- Xin, X., He, J., and Qiu, W.: Volatile fatty acid augmentation and microbial community responses in anaerobic co-fermentation process of waste-activated sludge mixed with corn stalk and livestock manure, *Environ. Sci. Pollut. Res.*, **25**, 4846–4857 (2018).
- Sträuber, H., Schröder, M., and Kleinstüber, S.: Metabolic and microbial community dynamics during the hydrolytic and acidogenic fermentation in a leach-bed process, *Energy Sustain. Soc.*, **2**, 13 (2012).
- Parawira, W., Murto, M., Zvauya, R., and Mattiasson, B.: Comparative performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate, *Renew. Energy*, **31**, 893–903 (2006).