

pH-adjustment strategy for volatile fatty acid production from high-strength wastewater for biological nutrient removal

Li Xie, Hui Liu, Yin-guang Chen and Qi Zhou

ABSTRACT

Volatile fatty acid (VFA) production from three types of high-strength organic wastewater (cassava thin stillage, starch wastewater and yellow-wine processing wastewater) were compared. The results showed that cassava thin stillage was the most suitable substrate, based on its high specific VFA production (0.68 g chemical oxygen demand (COD)/g initial soluble chemical oxygen demand (SCOD)) and yield (0.72 g COD/g SCOD) as well as low nutrient content in the substrate and fermented liquid. The acid fermented cassava thin stillage was evaluated and compared with sodium acetate in a sequencing batch reactor system. Total nitrogen removal efficiency was higher with fermented cassava thin stillage than with the sodium acetate. The effects of pH and a pH-adjustment strategy on VFA production and composition were determined using cassava thin stillage. At an initial pH range of 7–11, a relatively high VFA concentration of about 9 g COD/L was obtained. The specific VFA production (g COD/g initial SCOD) increased from 0.27 to 0.47 to 0.67 at pH 8 and from 0.26 to 0.68 to 0.81 at pH 9 (initial pH, interval pH, and constant pH adjustment, respectively). The dominant VFA species changed significantly with the increasing frequency of the pH adjustment. Further studies will examine the metabolic pathways responsible for VFA composition.

Key words | cassava thin stillage, external carbon source, pH-adjustment strategy, short-chain fatty acids

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INTRODUCTION

Volatile fatty acids (VFAs) and carboxylic acids are commonly recognized as high value-added metabolic products of different organic wastes that have undergone fermentation by mixed microbial cultures. Currently, the short-chain VFAs generated by these processes are used as fuels or biochemicals, for example, as alternatives to traditional external carbon sources in municipal wastewater treatment (Dionisi *et al.* 2005; Li *et al.* 2011). Naturally-produced mixed VFAs were shown to be an excellent carbon source during denitrification, as they were more efficient than other forms of soluble organic carbon (Elefsiniotis *et al.* 2004). In the integrated management of wastewater and sludge treatment at a wastewater treatment plant (WWTP), sewage sludge was treated as a substrate for the generation of VFAs, which were then used as an internal carbon source for nitrogen removal (Pavan *et al.* 2000; Li *et al.* 2011). The results from a pilot-scale study of the acidogenic

fermentation of sewage sludge showed that the sludge fermentation liquid, after recovery of the contained ammonia and phosphorus, was effective in improving biological nitrogen and phosphorus removal (Li *et al.* 2011). However, the amount of VFAs generated by sludge is usually insufficient to meet the carbon source requirements of WWTPs, and the dissolved ammonia and phosphorus must be recovered or removed from the fermented liquid before it can be used further. Consequently, research into other options as external carbon sources, such as high-strength organic wastewater and its fermented liquid, has been conducted. Some researchers applied the unfermented wastewater directly, to enhance the biological nutrient removal (BNR) process. Rodriguez and his co-workers evaluated the feasibility of the addition of winery wastewater for the removal of nitrogen in wastewater treatment plants and the results showed that the continuous addition of the winery

wastewater not only improved the denitrification process, but also improved the settling properties of the activated sludge. (Rodriguez *et al.* 2007). However, some shortcomings have been revealed, such as the availability and consistency of these wastes (Cappai *et al.* 2004). The fermented waste was also used in laboratory and pilot-scale studies. Lee and co-workers examined the fermented leachate of food waste as an external carbon source for BNR. They found that addition of the fermented leachate improved total nitrogen (TN) and total phosphorus (TP) removal efficiency (Lee *et al.* 2002; Shin *et al.* 2004). Alternatively, hydrolyzed molasses was shown to be an economical and effective external carbon source for improving nitrogen removal efficiency (Quan *et al.* 2005).

To ensure the suitability of the substrates in VFA production, their appropriate evaluation and screening are of great importance, as are methods to improve the concentration of VFAs in the acidified bioreactor to obtain the desired VFA species (Onnis-Hayden & Gu 2008; Soares *et al.* 2010). Lee and his co-workers suggested that specific VFA production, expressed as VFAs produced per gram chemical oxygen demand (COD)/g total COD (TCOD) of the substrate, could be used as an indicator of the VFA production potential of different substrates (Lee *et al.* 2000). The ideal substrate as external carbon source is defined as one with a high VFA production potential and generating few nitrogen- and phosphorus-based nutrients; that is, complex-carbohydrate-containing organic wastes with minimal nitrogen and phosphorus contents.

The accumulation of acidogenic bacteria in an anaerobic system is influenced by many operating parameters, especially pH, resulting in different types of anaerobic digestion (Horiuchi *et al.* 2002; Yang *et al.* 2003; Li *et al.* 2013). Most studies on the effects of pH have been conducted on sludge fermentation, whereas the effects of pH and pH-adjustment on a high-strength wastewater, such as high-carbohydrate/low-protein distillery wastewater, have not been determined.

In the present work, VFA production during the acid fermentation of three industrial wastewaters, cassava thin stillage, starch wastewater, and yellow-wine processing wastewater, was investigated with the aim of evaluating the use of these substrates in wastewater treatment. The fermentation liquid of the most suitable substrate was then tested as an external carbon source for nitrogen removal and its performance was compared with that of a commonly used carbon source, sodium acetate. We conclude this paper with a discussion of the effects of pH and a pH-adjustment strategy on VFA yields and composition.

MATERIALS AND METHODS

Feedstock and inocula

The cassava thin stillage used in this study was obtained from a cassava alcohol plant in Jiangsu, China. Starch wastewater was collected from a starch plant and yellow-wine processing wastewater from an industrial source, both in Shanghai, China. All three types of wastewater were first centrifuged at 4,000 rpm for 10 min. The resulting pellets were discarded to ensure that all the substances for further use were soluble and to avoid the hydrolysis of particulates. The inocula was granule sludge taken from the bottom of an upflow anaerobic sludge blanket reactor of a paper WWTP in Jiangsu, China. The concentrations of volatile suspended solids (VSS) and total suspended solids (TSS), as well as the sludge retention time (SRT) of the inocula were 112, 177 g/L, and 30 d, respectively.

Experimental design and procedure

Carbon-source evaluation

Batch VFA production tests were performed in 500 mL vials under mesophilic conditions. Granule sludge of 40 and 360 mL cassava thin stillage, starch wastewater or yellow-wine processing wastewater was added to each bottle, and operated at $35(\pm 1)$ °C. The pH was adjusted to 8 every 12 h with 5 M NaOH or 5 M HCl stock solutions. The bottles were then flushed with nitrogen gas for 1 min to provide anaerobic conditions. The reactors were mixed with a water-bath shaker at a rate of 120 rpm to sustain homogeneous mixing during fermentation. Samples were taken for analysis after 3 days of fermentation. Each test was carried out in duplicate.

Effects of pH and a pH-adjustment strategy on the VFA concentration

Granule sludge of 30 mL and cassava thin stillage of 270 mL were added to each reactor. The initial pH of each reactor was adjusted to 4, 5, 6, 7, 8, 9, 10 and 11, respectively. According to the results of initial pH values from 4 to 11, VFA concentration was relatively higher under alkaline conditions than in acid conditions. Thus, pH 8 and 9 were chosen to investigate the effects of a pH-adjustment strategy on VFA concentration and composition. The effect of the pH-adjustment strategy was determined at: (1) initial pH

values of 8 and 9; (2) an interval pH in which the system was adjusted to pH 8 or 9 in every 12 h; (3) a maintenance pH of 8 or 9, in which the pH was constantly adjusted by an automatic pH control device. Identical reactors, each with a working volume of 300 mL and a temperature maintained at $35(\pm 1)^\circ\text{C}$, were used. The mixing rate was controlled at 120 rpm. Each experiment was conducted in duplicate, unless otherwise specified.

Nitrate utilization rate (NUR)

The tests were conducted in a sequencing batch reactor (SBR) with a working volume of 3 L. The reactor was set up to compare the denitrification rate of sodium acetate with that of cassava thin stillage and the fermented cassava thin stillage after 3 days of anaerobic fermentation. The operational cycle for the SBR consisted of 10 min of fill, a 90 min anaerobic period with mixing, a 210 min anoxic period with mixing, a 40 min aerobic period with mixing, 60 min of settling followed by a 10 min decant, and 70 min of idling. The mixing speed was 50 rpm and pH was about 7. Both pH and dissolved oxygen (DO) profiles were monitored. The freshly returned activated sludge was used as inocula and VSS was 15 g/L. At the beginning of the run, 400 mL of freshly returned activated sludge and sodium acetate or fermentation liquid were mixed to obtain 3 L of mixed liquor, with a mixed-liquor VSS concentration of about 2,000 mg/L. Nitrate and soluble chemical oxygen demand (SCOD) concentrations were 35.0 and 300 mg/L, respectively. Samples were withdrawn every 8–30 min, filtered through a Whatman GF/C filter (Whatman, Kent, UK), and analyzed for nitrate and SCOD.

Analytical methods

COD, SCOD, TSS, VSS, ammonia, TN, and soluble orthophosphate were analyzed by *Standard Methods* (APHA 1998). Liquor samples were centrifuged at 12,000 rpm for 10 min and then filtered through a Whatman GF/C glass microfiber filter (0.45 μm pore size). The filtrate was collected in a 1.5 mL gas chromatography (GC) vial, and 3% H_3PO_4 was added to adjust the pH to approximately 3.0. The VFA composition was analyzed by means of gas chromatography (Agilent 6890N GC (Agilent, California, USA)) with a flame ionization detector (FID) and DB-WAXETR column (30 m \times 1.0 μm \times 0.53 mm). The temperature of the injector and FID were 200 and 220 $^\circ\text{C}$, respectively. Nitrogen was the carrier gas with a flow rate of 50 mL/min. The GC oven was programmed to begin at 110 $^\circ\text{C}$ and

remain there for 2 min, then increase at a rate of 10 $^\circ\text{C}/\text{min}$ to 220 $^\circ\text{C}$, and to stay at 220 $^\circ\text{C}$ for an additional 2 min. The filtrate was also used to analyze soluble protein and carbohydrate, determined, respectively, by the Lowry–Folin method with bovine serum albumin (BSA) as standard (Lowry *et al.* 1951). The carbohydrates were analyzed using the phenol-sulfuric method (Herbert *et al.* 1971). Lactic acid concentration was measured using an Agilent 1200 high-pressure liquid chromatography (HPLC) set-up equipped with an Aminex HPX-87H column (Bio-Rad, California, USA) at 54 $^\circ\text{C}$ and a refractive index detector. The eluting solution was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min. Nitrate and nitrite were analyzed by ion chromatography, with two conductivity detectors, and two sets of columns (Dionex ICS-3,000). The anions were separated and eluted on an IonPac AG11-HC (4 \times 50 mm) guard column and an IonPac AS11-HC (4 \times 250 mm) analytical column (Dionex, California, USA). The eluent was 18 mM KOH at an isocratic flow rate of 1.2 mL/min. Auto suppression mode was used during the detection.

Statistical analysis

All assays were conducted in duplicate 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.

Calculation

In this study, the concentration of ethanol and VFAs were expressed in terms of COD and the ratio of 2.01 for ethanol, 1.07 for acetic acid, 1.51 for propionic acid, 1.82 for butyric acid and 2.04 for valeric acid were used.

RESULTS AND DISCUSSION

VFA production from three sources of organic wastewater

The VFA production potential of three sources of high-strength organic wastewater, cassava thin stillage, yellowwine processing wastewater and starch wastewater, was compared. As shown in Table 1, all three wastewaters had an acidic pH (3.5–4.0). Cassava thin stillage and starch wastewater contained high concentrations of carbohydrates, but low concentrations of protein, as indicated by their SCOD/TN ratios of 144 and 125, respectively. Conversely,

Table 1 | Characteristics of different organic wastewaters^a

	Cassava thin stillage	Yellow-wine processing wastewater	Starch wastewater
pH	4.0 ± 0.5	3.5 ± 0.5	4.0 ± 0.2
SCOD	36,000 ± 283	24,480 ± 255	19,400 ± 114
Carbohydrate	11,190 ± 212	980 ± 28	5,200 ± 170
Protein	2,540 ± 57	10,600 ± 198	1,530 ± 42
Ethanol	457 ± 16	1,083 ± 24	48 ± 6
Acetate	526 ± 13	652 ± 14	7 ± 1
Lactate	8,244 ± 60	8,087 ± 31	2,943 ± 24
Ammonia	3.2 ± 0.3	45.5 ± 1.1	8.0 ± 0.4
TN	250 ± 14	1,450 ± 71	240 ± 28
Soluble orthophosphate	16.6 ± 0.4	114.6 ± 2.8	120 ± 2.4
SCOD/TN	144 ± 5	17 ± 1	81 ± 5

^aEthanol and acetate expressed as mg COD/L, others expressed as mg/L except for pH and SCOD/TN.

the carbohydrate content of the yellow-wine processing wastewater was relatively low whereas the protein content was relatively high (about 10 g/L), resulting in a SCOD/TN of only 17. Both cassava thin stillage and yellow-wine processing wastewater typically contain small amounts of lactic acid. Based on their low nutrient contents, which indicated the low VFA consumption, cassava thin stillage and starch wastewater were considered to be the most suitable substrates as external carbon sources.

Table 2 summarizes VFA production and composition together with carbohydrate and protein degradation,

determined at the end of the acid fermentation (3 days). Of the three wastewater sources, the VFA content of acid-fermented cassava thin stillage was highest, followed by yellow-wine processing wastewater and starch wastewater, respectively ($p < 0.05$). Specific VFA production (g COD/g initial SCOD) was significantly higher from the fermentation of cassava thin stillage than from the fermentation of the other two substrates tested (0.68, 0.47, and 0.44, respectively) ($p < 0.05$), probably because of its higher carbohydrate content. The final VFA composition of each of the three organic wastewater types is presented in Table 2. The dominant acid fermentation products of yellow-wine processing wastewater and starch wastewater were acetate and propionate, whereas for cassava thin stillage they were n-butyrate, acetate, and propionate (40, 36.5, and 20%, respectively). Previous reports showed that acetate, propionate, and butyrate are the best carbon sources for BNR (Chen *et al.* 2004; Kulikowska 2012).

The VFA yield, that is, the percentage VFA content with respect to SCOD. For cassava thin stillage, the yellow-wine processing, and the starch wastewaters, the VFA yields were 72, 67, and 68%, respectively. Thus, VFAs accounted for most of the SCOD in all effluents. The carbohydrate and protein remaining in the samples were also detected.

Interestingly, the degradation of organic matter under anaerobic conditions resulted in the generation of ammonia and phosphorus rather than VFAs. Thus, in fermentation liquids, the ammonia and phosphorus concentrations could be indicators of VFA consumption. After fermentation, the ammonia concentration of cassava thin stillage, yellow-wine processing wastewater and starch wastewater were 16.8,

Table 2 | Characteristics of the acid fermentation products of the tested substrates^a

	Cassava thin stillage	Yellow-wine processing wastewater	Starch wastewater
SCOD	34,075 ± 35	17,375 ± 106	12,500 ± 198
Carbohydrate	634 ± 7	255 ± 28	810 ± 7
Protein	1,460 ± 24	2,540 ± 14	1,260 ± 28
VFAs	24,371 ± 484	11,577 ± 590	8,554 ± 320
Acetate	8,901.8 ± 526.9	4,944.5 ± 342.0	5,846.1 ± 254.8
Propionate	4,847.1 ± 140.0	3,855 ± 226.3	1,328.8 ± 20.5
n-Butyrate	9,763.4 ± 174.0	1,259.4 ± 17.7	1,182.2 ± 41.2
Lactate	35.0 ± 1.5	78.5 ± 2.5	36.4 ± 2.9
Ammonia	16.8 ± 1.1	1,085.0 ± 49.5	9.1 ± 0.3
TN	375 ± 7	1,285 ± 35	235 ± 7
Soluble orthophosphate	2.5 ± 0.1	34.4 ± 0.6	20.7 ± 0.2
VFAs/SCOD	0.72 ± 0.03	0.67 ± 0.03	0.68 ± 0.01

^aVFAs expressed as mg COD/L, others expressed as mg/L except for pH and VFAs/SCOD.

1085 and 9.1 mg/L, respectively ($p < 0.05$). The soluble orthophosphate in cassava thin stillage was the lowest ($p < 0.05$). The results above might explain the low VFA consumption of cassava thin stillage. With cassava thin stillage and starch wastewater, the high specific VFA production and low VFA consumption were indicative of the suitability of these substrates as carbon sources, while the acid fermentation products of yellow-wine processing wastewater might also be used as a carbon source in some industrial WWTPs, such as dyeing wastewater. With this substrate, VFA and protein will improve the biodegradability of the industrial wastewater and be additionally required as supplemented nutrients.

Effect of pH and a pH-adjustment strategy on VFA production from cassava thin stillage

One of the important parameters affecting VFA yield and composition is pH, as determined from studies of the acid fermentation of sewage sludge. Therefore, in this work we examined the effects of pH and a pH-adjustment strategy using cassava thin stillage as the fermentation substrate. As shown in Figures 1(a) and (b), the VFA yield increased as the initial pH was increased from 4 to 7 and then remained at a relatively stable level as the pH reached 11. Neutral and alkaline conditions enhanced VFA production, resulting in concentrations 90% higher than those obtained at the initial pH of 4. However, at an initial pH range of 7–11 both stable VFA production and VFA species distribution were attributable to the system pH. As shown in Figure 2, despite adjustment of the initial pH to between 7 and 11, the system pH decreased to 5.8 and remained constant after 24 h of fermentation, in parallel with maximum VFA production. Thus, the VFA production potential seems to be related to the system pH.

The dominant VFAs from all samples with initial pH adjustment were n-butyrate, acetate, and propionate, accounting for 70–80, 8–18, and 12–20% of the total VFAs, respectively (Figure 1(b)). The especially large butyrate contribution in the VFA metabolic pathway can be explained by the high carbohydrate content of the cassava thin stillage (Fu et al. 2012).

Considering the higher VFA concentration at alkaline than at acidic pH, the changes in VFA production and distribution achieved at pH 8 and pH 9 with different pH-adjustment strategies were examined. Figure 3 shows the VFA concentrations and the distributions of the individual VFA species under different pH-adjustment conditions. The pH-adjustment strategy significantly influenced VFA

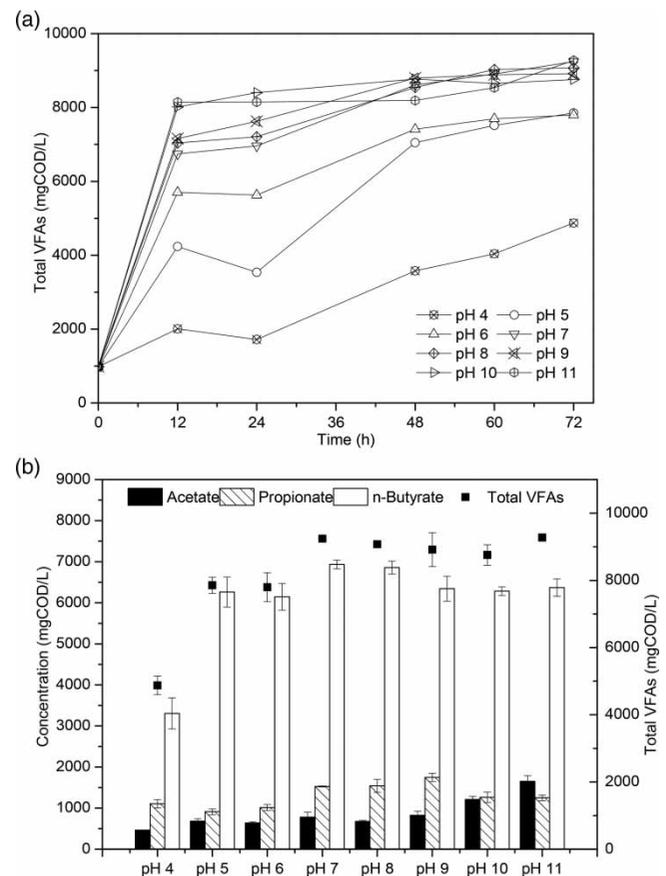


Figure 1 | (a) Time profiles of VFA generation at different initial pH values. (b) VFA composition at different initial pH values.

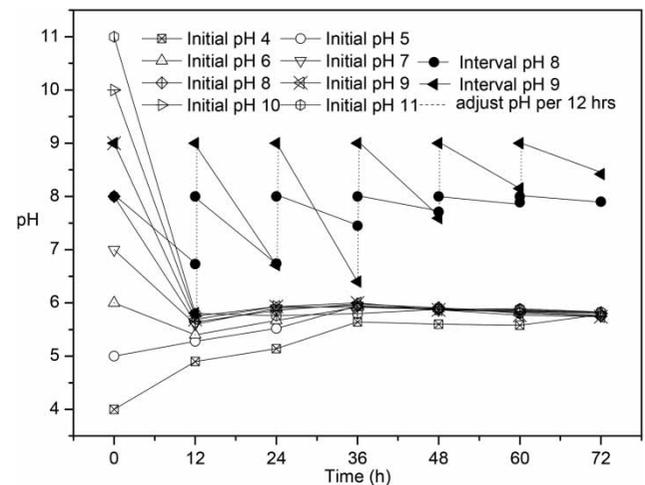


Figure 2 | The pH tendency in response to the different pH-adjustment strategies.

production, with the highest production obtained with the constantly adjusted pH mode, followed by the interval pH and initial pH modes. For example, at pH 9, specific VFA production (g COD/g initial SCOD) was highest in the

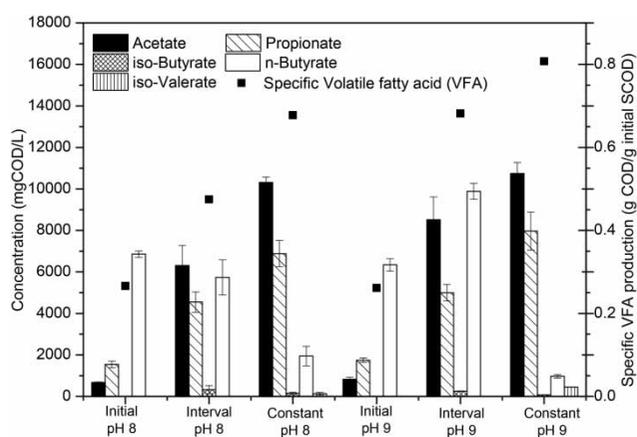


Figure 3 | VFA concentrations in response to the different pH-adjustment strategies.

constant pH mode (0.81), followed by the interval (0.68), and initial (0.26) pH modes. At a constant pH of 9, the dominant VFAs were acetate and propionate, while at an interval pH of 9 the main VFAs were butyrate, acetate, and propionate.

Figure 4 further describes the effect of pH and the pH-adjustment strategy on the percentages of the individual VFA species. With a change in the pH-adjustment strategy, the percentages of individual VFA species also changed. Thus, while at an initial pH of 8, butyrate, acetate, and propionate accounted for 75.6, 17.0, and 7.4% of the VFA species, respectively; at an interval pH of 8, the percentages of acetate and propionate increased to a total of 63.5% whereas the butyrate contribution decreased to 33.5%. If the pH was maintained at 8, the combined percentage of acetate and propionate was higher (87.5%). A similar tendency was observed at pH 9. At the end of the

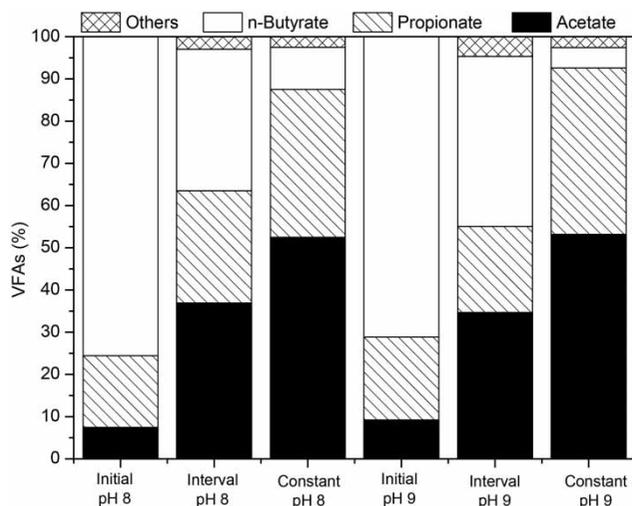


Figure 4 | Contributions of the individual VFAs to the total VFAs in response to the different pH-adjustment strategies.

fermentation, the specific VFA production (g COD/g initial SCOD) increased from 0.27 (initial pH 8) to 0.47 (interval pH 8) and 0.68 (constant pH 8). As for pH 9, the specific VFA production increased from 0.26 (initial pH 9) to 0.68 (interval pH 9) and 0.81 (constant pH 9).

Thus, pH is of vital importance to VFA concentration and composition. The influence of the different pH-adjustment strategies can be attributed to the change in the system pH by the controlling mode. VFA production potential and species distribution were similar under neutral and alkaline conditions because of the similar system pH of 5.8 after fermentation. However, when the pH was adjusted to 8 or 9 in every 12 h, the VFA concentration increased but the composition changed significantly, probably because of the increase in the system pH to about 7. Under alkaline conditions, the dominant bacteria were likely to be *Clostridium* species, which would have contributed to the abundance of acetate and propionate, thereby improving their respective percentages as the pH-adjustment frequency increased (Liu *et al.* 2012). In addition to being used as carbon sources, VFAs are regarded as a favorable source for microbial production of biodegradable plastic. Since the composition of VFAs in the medium influences biological phosphorus removal and the quality of the biodegradable plastic produced by fermentation, it is important to control the product spectrum during anaerobic acidogenesis (Chen *et al.* 2004; Horiuchi *et al.* 2002). It was also notable that acid fermentation was more sensitive to the pH-adjustment strategy than to the system pH (pH 8 and 9). This observation will be explored further later in 2014.

Nitrogen removal in an SBR using fermented cassava thin stillage as carbon source

The nitrogen reduction efficiencies of fermented cassava thin stillage and sodium acetate as external carbon sources were compared in an SBR. Table 3 shows the average nitrogen removal efficiency over 30 days of system operation. In the reactor containing sodium acetate, the nitrate concentration in the effluent was 15.50 ± 0.52 vs. 6.63 ± 0.71 mg/L for fermented cassava thin stillage. With the latter substrate, the denitrification efficiency was $68.3 \pm 2.4\%$. Furthermore, TN removal efficiency was higher with fermented cassava thin stillage, which accounted for the abundance of VFA species and metabolic products (e.g., propionate, butyrate). In addition, the denitrification of the fermented cassava thin stillage is better than that of the unfermented. The reason that fermented cassava was better than the acetate might be explained in terms of biological yield. For acetate more

Table 3 | Performance of the fermentation liquid in the N removal process

	$\text{NO}_x^- \text{-N}$ (end of anoxic stage) (mg/L)	Denitrification efficiency (%)	TN (end of aerobic stage) (mg/L)	TN removal (%)
Sodium acetate	15.50 ± 0.52	47.8 ± 2.0	21.92 ± 1.21	55.2 ± 2.0
Fermentation of cassava thin stillage	6.63 ± 0.71	68.3 ± 2.4	13.24 ± 1.52	73.1 ± 1.3
Cassava thin stillage	12.83 ± 0.77	57.5 ± 3.1	18.84 ± 1.73	61.6 ± 0.9

COD goes into the growth of biomass, while for fermented cassava the yield of biomass is lower.

As seen in Table 4, a comparison of the nitrate reduction rate of fermented cassava thin stillage and the corresponding literature values of other external carbon sources showed that the nitrate utilization rate (NUR) of the fermentation liquid was similar to or even higher than that of other carbon sources. The denitrification rate of the cassava thin stillage fermentation liquid was 6.63–6.81 mg N/(g VSS · h) (12.8–13.1 °C), which is higher than that of many other carbon sources, such as beet-sugar (2.72 mg N/(g VSS · h)) and brewery (2.4–6.0 mg N/(g VSS · h)) wastewaters, but lower than that of sodium acetate (16.69–13.62 mg N/(g VSS · h) at 18.9–23.1 °C), probably because of the difference in the reaction temperatures. It is also notable that the denitrification rate of the fermentation liquid was similar to that of the commercial product, MicroC, which effectively supports denitrification and is used throughout the north-eastern United States in 92 WWTPs because it meets the stringent effluent nitrogen limits mandated by law (Cherchi *et al.* 2008). However, fermented cassava thin stillage would be a more economical source of carbon. Up to 20 liters of stillage may be generated per liter of ethanol production and the pollution potential of stillage can exceed a COD

of 100 g/L (Wilkie *et al.* 2000). If cassava stillage can be used as substrate for VFA generation, it can also be used as a substitute for commercial carbon sources, which at the same time would reduce the amount of stillage waste.

CONCLUSION

In this study, VFA production during the acid fermentation of cassava thin stillage, starch wastewater, and yellow-wine processing wastewater was compared and evaluated. Among these three substrates, cassava thin stillage was the most suitable substance for application to BNR, based on its highest specific VFA production (0.68 g COD/g initial SCOD) and VFA yield (0.72 g COD/g SCOD). The low concentrations of ammonia and phosphorus contained in fermented cassava thin stillage determined the low level of VFA consumption. A comparison of the cassava thin stillage, the acid fermentation products of cassava thin stillage and sodium acetate with respect to nitrogen removal in an SBR system showed that the former not only had a higher TN removal efficiency but also that the denitrification rate (~6.63–6.81 mg N/(g VSS · h)) was comparable to the rate of other external carbon sources (Table 4).

Table 4 | Comparison of different external carbon sources on denitrification

Carbon source	Temperature (°C)	$\Delta \text{COD}/\Delta \text{N}$ (g COD/g N)	NUR [mg N/(g VSS · h)]	Reference
Sodium acetate	18.9–23.1	9.20–9.84	16.69–13.62	This study
Cassava stillage fermentation	12.8–13.1	10.07–10.38	6.63–6.81	This study
Cassava thin stillage	19.6–21.9	11.11–11.56	5.49–5.99	This study
Dairy effluent	–	–	3.4–8	Sage <i>et al.</i> (2006)
Anaerobically fermented leachate of food waste	20	–	8.2	Lee <i>et al.</i> (2002)
Ice cream production industrial wastewater	20	–	3.28	Cappai <i>et al.</i> (2004)
Beet-sugar industrial wastewater	20	–	2.72	
Distillery wastewater	23.1–24.0	11.1–12.7	4.8–5.1	Swinarski <i>et al.</i> (2009)
Brewery wastewater	19.7–22.7	6.5–20.8	2.4–6.0	
Fish-harvesting wastewater	21.4–23.0	11.7–13.1	4.0–6.0	
MicroC TM	20	6.4	4.7–6.37	Cherchi <i>et al.</i> (2008)

To enhance VFA production and to achieve the desired VFA distribution, we examined the effects of pH and a pH-adjustment strategy, using cassava thin stillage as the substrate. Neutral and alkaline conditions were shown to promote VFA production, which was 90% higher than that obtained at an initial pH of 4. In contrast, total VFAs were similar in the pH range of 7–11. For all samples with pH adjustment, at the initial pH, n-butyrate was the dominant acid. The change in the pH over the course of the reaction showed that the system pH self-adjusted to about 5.8 after fermentation for 12 h and stayed relatively constant to the end of fermentation, even at an initial pH of 11, suggesting that the metabolic pathway was actually influenced by the system pH. The implemented pH-adjustment strategy significantly influenced VFA production and distribution, as specific VFA production (g COD/g initial SCOD) increased from 0.27, then to 0.47, 0.67 at pH 8 and 0.26, and then to 0.68, 0.81 at pH 9 with three adjustment conditions, initial pH, interval pH, and constant pH, respectively. The percentages of acetate and propionate increased with increasing pH-adjustment frequency. At an initial pH of 8 or 9, the dominant VFA was butyrate, followed by acetate and propionate; at a constant pH of 8 or 9, acetate and propionate were dominant, with only a small percentage of butyrate (5%). Regarding application, VFAs can be used not only as an external carbon source, but also as the material for other high-value added chemicals, such as polyhydroxyalkanoates and long-chain fatty acids.

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