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Improved Bioproduction of Short-Chain Fatty Acids (SCFAs) from Excess Sludge under Alkaline Conditions

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The production of short-chain fatty acids (SCFAs) from excess sludge was conducted in batch fermentation tests at different pH values ranging from 4.0 to 11.0. Experimental results of the impacts of different pHs on SCFAs production showed that during the first 8-day fermentation time the total SCFAs production at either pH 9.0 or pH 10.0 was much greater than that at acidic or neutral pH, and the maximal yield of 256.2 mg SCFAs−COD per gram of volatile suspended solids (VSS) was at pH 10.0, which was, respectively, over 3 and 4 times that at pH 5.0 and uncontrolled pH. Clearly, SCFAs production from excess sludge could be significantly improved and maintained stable by controlling the fermentation pH at 10.0. The composition of SCFAs and the percent distribution of individual SCFAs accounting for total SCFAs at pH 10.0 were analyzed. The SCFAs consisted of acetic, propionic, iso-butyric, *n*-butyric, iso-valeric, and *n*-valeric acids, and acetic acid was the most prevalent product with a fraction of 40−55%. Because the results of this study were different from those of previous studies of SCFAs production, the mechanism of increased SCFAs production under alkaline conditions was investigated. Results showed that as soluble COD increased, more soluble protein was provided as the substrate for producing SCFAs. In addition, less or even no SCFAs were consumed by methanogens at alkaline pH, so the SCFAs production was therefore remarkably improved. Further investigation revealed that the formation of SCFA at pH 10.0 was dominated by biological effects rather than by chemical hydrolysis.

Introduction

Concentrations of readily biodegradable COD, such as short-chain fatty acids (SCFAs), in wastewater strongly affect the efficiency of biological nutrient (phosphorus and nitrogen) removal processes. SCFAs, such as acetic and propionic acids, have been demonstrated to be the most suitable substrates to support enhanced biological phosphorus removal (EBPR) (*1*−*3*). It has been reported that 6−9 mg of SCFAs is required for biological removal of 1 mg of phosphorus (*4*−*5*). These amounts of SCFAs, however, are not always available in wastewater, particularly when influent COD is low. In addition, phosphorus removal is limited by the available SCFAs supply due to consumption of SCFAs by other organisms competing with phosphate accumulating organisms (PAO). Thus, additional supply of SCFAs becomes necessary in order to keep high and stable phosphorus removal efficiency.

Two strategies have been applied to increase wastewater influent SCFAs concentration for improving biological nutrient removal. The first method is by the addition of chemically synthesized SCFAs, such as acetic and/or propionic acid, to wastewater for improving nutrient removal (*1*, *6*). For minimizing the operating cost of supplementary carbon dosing while achieving the effluent quality requirements, another way of increasing SCFAs concentration in a biological nutrient removal facility is by using an internal carbon source, i.e., by fermentation of waste sludge generated in wastewater treatment plants (*7*, *8*).

Primary and secondary sludges are the two different wastes generated in municipal wastewater treatment plants. Primary sludge is produced through a mechanical wastewater treatment process. It occurs after the screen and the grit chamber, and amasses at the bottom of the primary sedimentation basin with a high portion of organic matter, as feces, vegetables, fruits, textiles, paper, etc. In contrast, excess secondary sludge is a settling material produced at the secondary sedimentation tank of the wastewater treatment plant after biological treatment. It contains non-hydrolyzable particulate materials and biomass due to biological metabolism. As the excess sludge contains high levels of organic matter, it may become a plentiful source of inexpensive organic substrate for fermentative SCFAs production, by which reduction and stabilization of organic wastes can also be accomplished. Until now, however, most of the studies on SCFAs production from sludge focused on the controlled digestion of primary sludge or its mixture with excess sludge under acidic or near neutral pH conditions (*7*−*10*).

The anaerobic digestion process usually includes three stages: hydrolysis, acidification, and methane production. By controlling the operational conditions it is possible to confine sludge anaerobic digestion to the acidogenic phase (*11*), with the processes involved described by the terms hydrolysis and/or anaerobic fermentation. By increasing the hydrolysis and acidification rates and meanwhile blocking the methane generation pathway, the SCFAs production could be improved. The purpose of this study was to efficiently produce SCFAs from excess sludge by controlling the anaerobic fermentation conditions at pH 10.0, at which the excess sludge hydrolysis and acidification rates were increased without methane production. Also, the composition of SCFAs was examined, and the mechanisms of SCFAs production in this case were discussed.

Materials and Methods

Excess Sludge. The excess sludge used in this study was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Shanghai, China. The sludge was concentrated by settling at 4 °C for 24 h, and its characteristics are shown in Table 1. As shown in Table 1, proteins and carbohydrates are the two predominant types of organic compounds in excess sludge, and they account for approximately 90% of the volatile suspended solids.

Batch Fermentation Experiments. Nine identical reactors, each with working volume of 1.5 L, internal diameter of 100 mm, and height of 250 mm, were maintained at room temperature (20−22 °C). A 13.5 L portion of excess sludge was divided equally into the 9 reactors. Each reactor was mechanically stirred at a speed of 80 rpm (revolutions per

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TABLE 1. Characteristics of the Excess Sludge[a]

parameter	mean	SD[b]
pH	6.8	0.1
TSS (total suspended solids)	13808	743
VSS (volatile suspended solids)	10815	159
SCOD (soluble chemical oxygen demand)	41	21
TCOD (total chemical oxygen demand)	13407	573
BOD_5	5417	440
total carbohydrate (as COD)	1522	332
total protein (as COD)	8180	103
lipid and oil(as COD)	131	8

[a] All values are expressed in mg/L except pH. [b] SD: standard deviation.

minute). From reactors 1 to 8, the pH was controlled at 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0, respectively, by adding 2 M sodium hydroxide (NaOH) or 2 M hydrochloric acid (HCl). The pH in reactor 9 was not adjusted and used as a blank control.

A batch fermentation experiment with bovine serum albumin (BSA, model protein compound used in this study) and glucose (model carbohydrate compound) was conducted, respectively, to study whether the production of SCFAs was directly related to the fermentation of protein and carbohydrate under acidic or alkaline conditions. A certain amount of the model compound (BSA or glucose) was dissolved into 900 mL of tap water, and the pH was adjusted to the set point of experiments. A 100 mL aliquot of excess sludge was then added to each reactor as an inoculum, and the batch reactors were maintained at 21±1 °C. At different fermentation times, the concentrations of SCFAs, glucose, and BSA in different batch reactors were measured.

Batch tests using autoclaved sludge were compared with the un-autoclaved sludge to examine whether the formation of SCFAs during sludge fermentation at pH 10.0 was caused mainly by the chemical or biological effects. The experiments were duplicated. A 2 L portion of excess sludge were divided equally into four 1 L Erlenmeyer flasks. Two of them were autoclaved at 121 °C for 20 min, and the pH values in both were adjusted to pH 10.0 after cooling. Another two flasks were not autoclaved, but their pHs were also adjusted to pH 10.0. Oxygen in the flasks was removed from the headspace by nitrogen gas sparging for 30 s. The flasks were capped with rubber stoppers. The four flasks were then placed in an air-bath shaker (120 rpm) at 21±1 °C. Every 4 h, the pH in each flask was adjusted to 10.0. The adjustment of pH with the autoclaved sludge was conducted in a sterilized cabinet.

Analytical Methods. Sludge samples from the reactors were immediately filtered through a Whatmann GF/C glass microfiber filter (1.2 μm pore size). The filtrate was immediately analyzed for SCFA, SCOD, carbohydrate, and protein, and the filter was assayed for TSS and VSS. The analyses of SCOD, TSS, and VSS were conducted in accordance with Standard Methods (*12*). Carbohydrate was measured by the phenol−sulfuric method with glucose as standard (*13*). Soluble protein was determined by the Lowry−Folin method with BSA as standard (*14*).

Sludge lipid was extracted by the Bligh−Dyer method from the acidified sample, and was then measured gravimetrically after the solvent was evaporated at 80 °C (*12*). The total protein content of sludge was estimated from the corresponding TKN concentration by subtracting the inorganic nitrogen concentration and dividing the difference by 0.16, then multiplying the result by 1.5 (*15*). The activity of sludge enzymes (alkaline and acid phosphatases, α-glucosidase and protease) was measured according to Miron et al. (*15*).

To analyze SCFAs the filtrate was collected in a 1.5 mL gas chromatography (GC) vial, and 3% H_3PO_4 was added to adjust the pH to approximately 4.0. A HP5890 GC with flame ionization detector and equipped with a 30 m × 0.32 mm × 0.25 mm CPWAX52CB column was utilized to analyze the composition of SCFAs. Nitrogen was the carrier gas and the flux was 50 mL/min. The injection port and the detector were maintained at 200 and 220 °C, respectively. The oven of the GC was programmed to begin at 110 °C and to remain there 2 min, then to increase at a rate of 10 °C/min to 200 °C, and to hold at 200 °C for 2 min. The sample injection volume was 1.0 μL.

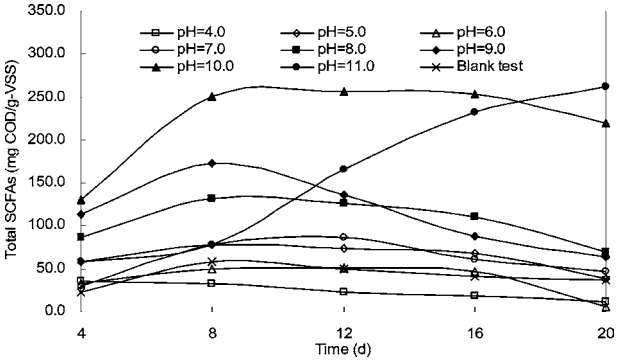

FIGURE 1. Effects of pH values and fermentation time on total SCFAs production.

Methane concentration was measured by a gas chromatograph (GC-14B, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD) and a 3 m stainless column. The temperatures of the injection, column, and detector were set at 40, 50 and 90 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 30 mL/min.

Results and Discussion

Effects of pH on Total SCFAs Production. The effects of pH and fermentation time on the total SCFAs production are shown in Figure 1. The initial total SCFAs was about 1.5 mg COD/g VSS (data not shown in Figure 1). During the initial 4-days of fermentation the production of total SCFAs was as follows: pH 10.0 (130.53 mg COD/g VSS) > pH 9.0 (113.46) > pH 8.0 (86.10) > pH 11.0 (58.16) ≈ pH 5.0 (57.43) > pH 4.0 (34.76) > pH 6.0 (32.15) > pH 7.0 (30.18) > blank test (22.29). With the increase of fermentation time to 8 days, the SCFAs increased except at pH 4.0, and almost the same order of pH values was observed with respect to the production of total SCFAs as that on the 4th day, i.e., pH 10.0 (250.39 mg COD/g VSS) > pH 9.0 (173.22) > pH 8.0 (131.38) > pH 5.0 (78.08) ≈ pH 7.0 (77.86) ≈ pH 11.0 (77.33) > pH 6.0 (50.17) > blank test (58.58) > pH 4.0 (32.78). Further investigation revealed that the total SCFAs production on the 8th days linearly increased with pH between pH 6.0 and 10.0 ($y_{\text{total SCFAs}} = 49.58\text{pH} - 260.05$, $R^2 = 0.97$). After 8 days, however, further increasing the fermentation time in most cases did not result in the increase of total SCFAs production apart from pH 11.0. As seen in Figure 1, the SCFAs at pH 11.0 continued to increase with time, and reached 165.22, 231.78, and 262.30 mg COD/g of VSS on the 12th, 16th, and 20th day, respectively, but the increase slowed in the latter stage of fermentation.

The above results showed that the production of SCFAs could be significantly improved and maintained stable by controlling fermentation pH at 10.0. Although high SCFAs production might also be achieved at pH 11.0, it took a much longer time to produce the same amount of SCFAs as that produced at pH 10.0. It seems that the optimum conditions for SCFAs production were pH 10.0 and a fermentation time of 8 days. The reason for less SCFAs produced at pH 11.0 during the initial 8 days might be attributed to the toxic effects of stronger alkaline conditions to acidogenic bacteria. Also, as shown in Figure 1, an obvious SCFAs consumption was

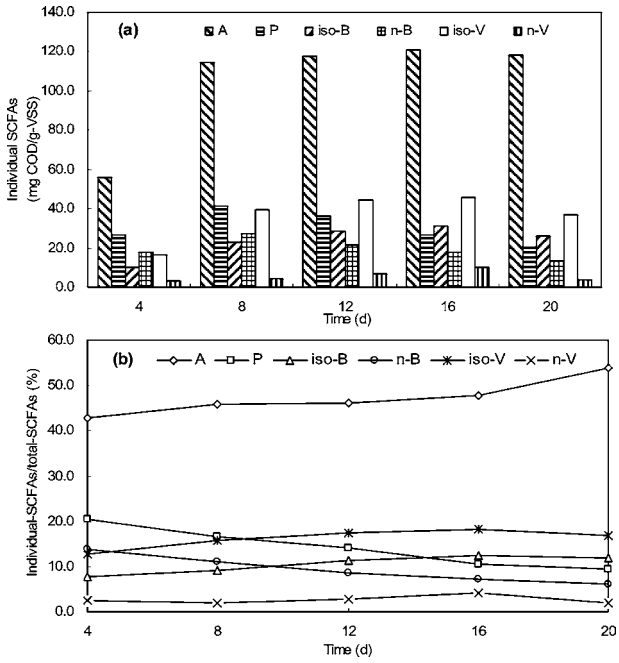

FIGURE 2. Composition of SCFAs at pH 10.0 (a) individual SCFAs production and (b) percentage of individual SCFAs accounting for total SCFAs (acetic (A), propionic (P), iso-butyric (iso-B), *n*-butyric (n-B), iso-valeric (iso-V), and n-valeric (n-V)).

observed in the range of pH 4.0−9.0 and in the blank test after 12 days of fermentation due to the participation of SCFAs consumers, such as methanogens. However, higher pH values, such as pH 10.0 and 11.0, could reduce or prevent these bacterial activities, which will be discussed in the following text.

Composition of SCFAs Produced at pH 10. The detectable SCFAs produced from excess sludge mainly include straight- or branched-chain SCFAs from 2−5-carbon atoms, i.e., acetic, propionic, iso-butyric, *n*-butyric, iso-valeric, and *n*-valeric acids, either in pH controlled (pH 4.0−11.0) experiments or in the blank test. At pH 10 the distribution of individual SCFAs in the fermentation system is as shown in Figure 2. Acetic acid was the most prevalent product at any fermentation time. Its production increased significantly from 56.09 mg COD/g VSS on the 4th day to 114.77 mg COD/g VSS on the 8th day, but there was only a very little increase in SCFAs production with further increasing fermentation time (117.98 and 120.95 mg COD/g VSS, respectively, on the 12th and 16th days). As shown in Figure 2 the percentage of acetic acid accounting for total SCFAs increased gradually from 42.97 to 53.83% during the whole fermentation process, which was much greater than any other SCFAs at any fermentation time.

Propionic acid was the second major SCFA during the first 8-day fermentation. Its maximum production occurred on the 8th day (41.30 mg COD/g VSS), which accounted for 16.49% of the total SCFAs. Iso-valeric acid, however, became the second one after 8 days. It continued to increase with time, reaching a maximum production of 45.88 mg COD/g VSS with a percentage of 18.12% on the 16th day. Butyric and iso-butyric acids only accounted for approximately 10% of total SCFAs between a fermentation time of 4 and 20 days. Valeric acid was the SCFA present in the smallest amount in the fermentation process. Its maximum production was approximately 11.0 mg COD/g VSS, which was around 4.10% of the total SCFAs.

Acetic, propionic, iso-butyric, and *n*-butyric acids may be formed directly from the fermentation of carbohydrates and proteins, but the higher molecular weight SCFAs, such as iso-valeric and *n*-valeric acids, are largely relevant to the fermentation of protein (*17*). The protein content in the excess sludge of this study was 61%, which indicated that fermentation of protein-enriched substrates should produce a great deal of iso-valeric and *n*-valeric acids. In addition, it may be expected that propionic, iso-butyric, *n*-butyric, iso-valeric, and *n*-valeric can accumulate in the fermentation process since SCFAs with more than 3 carbon chain cannot be used directly by methanogens. However, these SCFAs are easily biodegraded to form acetic acid in the anaerobic fermentation system. Thus, there was not much propionic, iso-butyric, *n*-butyric, iso-valeric, or *n*-valeric acids accumulated.

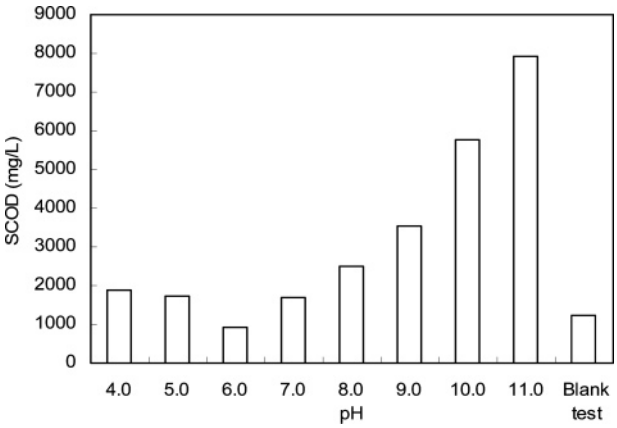

FIGURE 3. Effects of pH on sludge hydrolysis in terms of SCOD variations at 8-day fermentation time.

It is well-known that acetic acid can be degraded into CH_4 and CO_2 directly by methanogens, which should result in little net acetic acid production. However, the opposite results were observed in this study. In the following text we will discuss the reasons for large amounts of acetic acid accumulated under alkaline conditions, especially at pH 10.0.

Mechanism of Improved SCFAs Production under Alkaline Conditions. In this study higher SCFAs yields were obtained at higher pH of 8.0−11.0 (optimal pH 10.0) as compared with those at pH 4.0−7.0. In the literature, the production of SCFAs from sludge was conducted under acidic or near neutral pH values (*7*−*10*). It is therefore necessary to investigate the reasons for improved SCFAs production under alkaline conditions.

During the anaerobic fermentation of sludge three stages are usually included: hydrolysis, acidification, and methane generation. For accumulating more SCFAs, the following two strategies can be adopted: (a) increasing the hydrolysis rate to produce more soluble substrates for acidification, and (b) decreasing or preventing the activity of methanogens. Sludge hydrolysis can be expressed by the change of SCOD (*18*). Figure 3 presents the effect of pH on SCOD at an 8-day fermentation time. With the increase of pH in the range pH 7.0−11.0, the SCOD linearly increased (y_{SCOD} = 1382pH − 8021, R^2 = 0.94). Also, it is obvious that the SCOD production under alkaline conditions, such as pH 10.0 or 11.0, was much greater than that under acidic hydrolysis. Thus, alkaline sludge hydrolysis was more efficient than acidic one.

It has been reported that protein, carbohydrate, and lipid are the main constituents of domestic sludge (*19*), and the formation of SCFAs might be associated with the fermentation of these organic compounds (*10*, *17*). In this study the excess sludge consisted of 61% protein, 11% carbohydrate, less than 1% lipid, and over 27% unknown components on the basis of sludge TCOD. Protein was the largest constituent of the excess sludge, and the lipid content was neglected in this study. Thus, the SCOD composition was further analyzed by focusing on soluble protein and carbohydrate. Figure 4 illustrates the changes of soluble protein and carbohydrate

TABLE 2. Comparison between SCFAs Production from Glucose and BSA

substrate	pH	initial COD[a]	SCFAs concentration at different time (*d*) (as COD)[b] 1	3	5	8
glucose	5.0	283	0	21.1	1.0	0
BSA	5.0	1764	0	101.2	334.7	5.2
glucose	10.0	989	0	55.6	51.0	48.8
BSA	10.0	4642	0	172.7	666.5	596.1

[a] The initial COD values of glucose and BSA were set respectively close to the COD values of soluble carbohydrate and protein at pH 5.0 and 10.0 as shown in Figure 4. Units: mg/L. [b] Units: mg/L

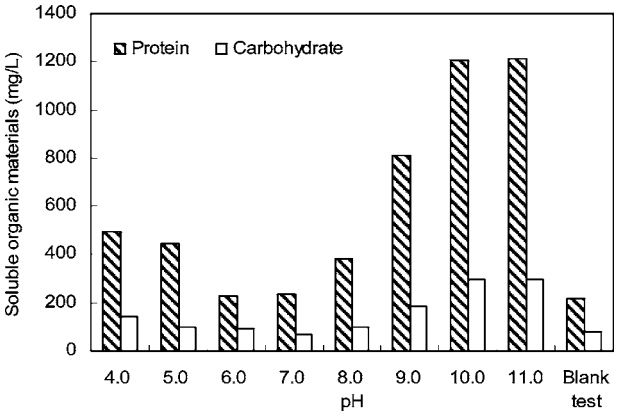

FIGURE 4. Effects of pH on concentrations of protein and carbohydrate in aqueous phase at sludge fermentation time of 8 days.

concentrations with pH. Obviously, both the soluble protein and carbohydrate under alkaline conditions were higher than those under acidic conditions, and the soluble protein was much greater than carbohydrate. These observations could be made at any other fermentation time (data not shown). It seems that more soluble protein and carbohydrate were provided for acidification to produce more SCFAs under alkaline conditions.

To further study whether the production of SCFAs was directly related to the consumption of protein and carbohydrate, batch tests with a model protein compound (BSA) and model carbohydrate compound (glucose) were conducted, respectively. It can be seen from Figure 5 that both glucose and BSA decreased with time, which indicated that they were consumed by the sludge microbes. As can be seen in Table 2, no matter whether the substrate was glucose or BSA, SCFAs were produced, and the SCFAs production at pH 10.0 was significantly higher than that at pH 5.0. Also, the results in Table 2 showed that BSA caused much greater SCFAs production than glucose at either pH 5.0 or 10.0. All these results revealed that even at pH 10.0 the production of SCFAs from sludge was related to its protein and carbohydrate fermentation, but protein played a more important role due to its higher content in the sludge.

As mentioned above, the production of SCFAs can also be improved by reducing or preventing the conversion of

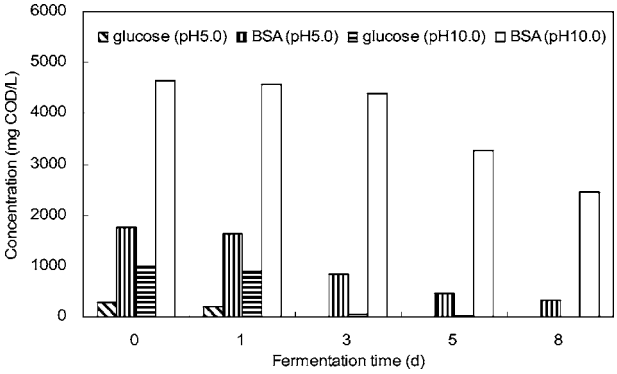

FIGURE 5. Changes of glucose and BSA concentrations with time at pH 5.0 and pH 10.0 in the batch tests using glucose or BSA as the sole substrate (the initial COD values (at time 0) of glucose and BSA were set respectively close to the COD values of soluble carbohydrate and protein at pH 5.0 and 10.0 as shown in Figure 4).

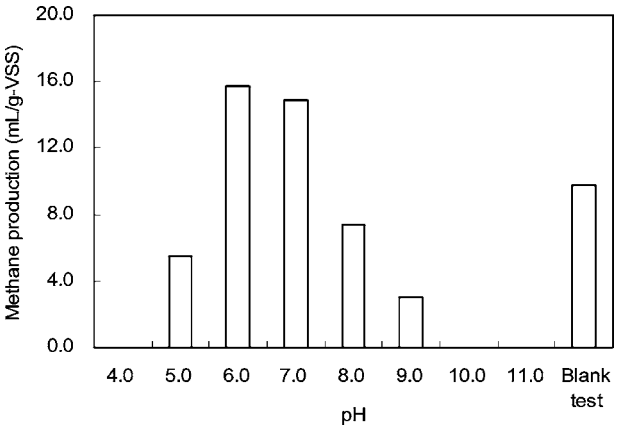

FIGURE 6. Effects of pH on methane production at 8-day fermentation time.

SCFAs to methane during sludge fermentation. Figure 6 is the profiles of methane production at different pHs. As can be seen in Figure 6 the methane production linearly decreased with the increase of pH from 6.0 to 10.0 ($y_{methane} = -4.34pH + 42.91$, $R^2 = 0.96$), and there was no methane generated at pH 10.0 and 11.0. Apparently, at a higher pH value, the activity of methanogens decreased, which has also been observed by other researchers (*20*). Thus, less SCFAs were consumed, which resulted in the improvement of SCFAs production.

The batch fermentation experiments with autoclaved and unautoclaved sludge were conducted to examine whether the formation of SCFAs was caused directly by alkaline (chemical) hydrolysis or biological effects. Table 3 shows the production of total SCFAs and the activities of four types of hydrolytic enzymes (protese, α-glucosidase, alkaline, and acid phosphatases) in the autoclaved and unautoclaved tests at pH 10.0 and at a time of 8 days. The concentration of total SCFAs in the unautoclaved sludge test was much greater than that in the autoclaved sludge (1010.81 versus 45.87 mg COD/L). It can also be seen in Table 3 that all four enzymes were active with the unautoclaved sludge, but they lost activity

TABLE 3. Comparison of SCFAs Concentration and Enzyme Activites between Autoclaved and Unautoclaved Fermentation Tests at pH 10.0 and Fermentation Time of 8 Days

	total SCFAs	enzyme activity[a]			
	mg(COD)/L	protease	α-glucosidase	alkaline phosphatase	acid phosphatase
un-autoclaved sludge	1010.81	0.02	3.70	5.79	30.26
autoclaved sludge	45.87	0	0	0	0

[a] The unit of protease activity was Δabs/mL·h, and the units of all other enzyme activities were μg-nitrophenol/mL·h.

when sludge was autoclaved. It seems that the formation of SCFAs at pH 10 was caused mainly by microbiological activity.

Acknowledgments

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