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Revealing the Underlying Mechanisms of How Sodium Chloride Affects Short-Chain Fatty Acid Production from the Cofermentation of Waste Activated Sludge and Food Waste

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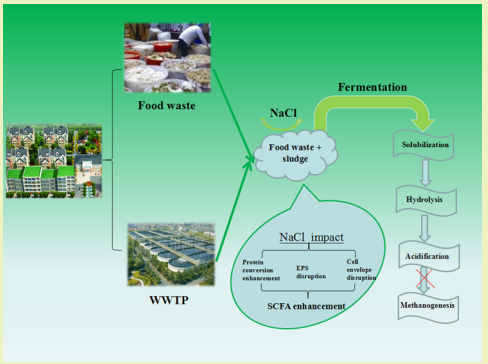
Supporting Information

ABSTRACT: Recently, anaerobic cofermentation of waste activated sludge (WAS) and food waste for short chain fatty acid (SCFA) production has drawn growing attention. However, the details of how sodium chloride (NaCl) in food waste affects SCFA generation from the cofermentation remain largely unknown, which provides limited understanding of the cofermentation process. This work therefore aims to provide such support. Experimental results showed that the effect of NaCl on SCFA production was dosage dependent. With the increase of NaCl level from 0 to 8 g/L SCFA production increased from 367.6 to 638.5 mg chemical oxygen demand (COD)/g of volatile suspended solids (VSS). However, further increase of NaCl caused severe inhibition of SCFA production. The presence of NaCl not only accelerated the release of soluble substances from food waste and disruption of both extracellular polymers and cell envelopes in sludge but also promoted the conversion of protein released from the disintegration process, thereby causing more substrates for SCFA production. It was also found that low NaCl levels improved hydrolysis and acidification processes but inhibited methanogenesis while both acidification and methanogenesis processes were seriously inhibited by high NaCl levels. Further investigation with enzyme analysis showed that the activities of protease and α-glucosidase were in the order of high NaCl > low NaCl > blank while the activities of oxaloacetate transcarboxylase and CoA transferase were in the sequence of low NaCl > blank > high NaCl. However, the activity of coenzyme F420 decreased with increasing NaCl level. This work reveals the underlying mechanism of how NaCl affects SCFA production from the cofermentation process and might be of significance for the operation of the cofermentation system.



KEYWORDS: *Sodium chloride, Waste activated sludge, Food waste, Short-chain fatty acid production, Anaerobic cofermentation*

INTRODUCTION

Waste activated sludge (WAS), as a byproduct of biological wastewater treatment, is inevitably generated with large quantities daily. On one hand, WAS will cause environmental pollution if treated and disposed inappropriately. On the other hand, WAS consists of high levels of organic substrates, which makes it a potentially renewable resource. Usually, WAS is used to produce methane by the anaerobic digestion process. Recently, short-chain fatty acid (SCFA) production from WAS anaerobic fermentation has attracted increasing attention, because the value added SCFA, a crude material for microbial production of biodegradable plastics and a preferred substrate for biological nutrient removal microorganisms, is produced and the volume of sludge is reduced simultaneously.[1−3]

The main component of WAS is protein, and the anaerobic bioconversion of protein to SCFA is always limited due to the low carbohydrate levels in raw sludge.[4,5] It is documented that the suggested carbon to nitrogen (C/N) mass ratio of anaerobic digestion should be in the range of 20/1−30/1,[6] whereas the C/N ratio in raw sludge is roughly around 7/1.[4,7] To enhance the bioconversion of protein of WAS, cofermentation of WAS and other C-rich wastes to balance C/N ratio is often used. Food waste is another largely produced waste, which contains high levels of carbohydrate.[4,5] In China, food waste was produced at an increasing rate (higher than 10%) every year and accounted for 40−50% of the municipal solid waste.[8] Therefore, anaerobic cofermentation of WAS and food waste has recently gained growing attention.[5,8−10]

Salt (e.g., NaCl) is one type of food flavoring, which is inevitably accumulated in food waste at high levels. It is

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Table 1. Main Characteristics of WAS, Food Waste, and Cofermentation Substrates[a]

parameter	WAS	food waste	WAS + food waste
pH	6.9 ± 0.1	6.5 ± 0.1	6.7 ± 0.1
TSS	12560 ± 160 mg/L	25.9 ± 1.2%	21.3 ± 1.1%
VSS	9420 ± 90 mg/L	18.6 ± 0.8%	15.3 ± 0.8%
total COD	13420 ± 150 mg/L	262.6 ± 4.1 g/L	192.9 ± 8.4 g/L
total protein	8560 ± 130 mg COD/L	38.4 ± 2.4 g COD/L	17.5 ± 1.1 g COD/L
total carbohydrate	1420 ± 80 mg COD/L	128.5 ± 5.2 g COD/L	65.8 ± 3.4 g COD/L
SCFA	21.2 ± 0.3 mg COD/L	102.2 ± 5 mg COD/L	58.9 ± 2.7 mg COD/L
C/N	7.2/1	28.6/1	22.8/1

[a]Error bars represent standard deviations of triplicate tests.

reported that the concentration of NaCl in food waste is in the range of 7−12 g NaCl/L.[11] Previous research showed that the presence of NaCl posed a significant impact on the anaerobic fermentation/digestion of food waste.[12,13] For example, Rinzema et al. demonstrated that 5 g Na^+/L decreased methane production by 10%, and 10 g Na^+/L resulted in methane reduction by 50%.[14] Kim et al.[15] found that a high concentration of sodium (such as 6 g/L) suppressed fermentative hydrogen production. Jeison et al. reported that high inhibition on methanogen activity was observed at sodium concentrations over 7 g/L in up-flow anaerobic sludge bed reactor.[16] Despite this significant progress, the effect of NaCl on the cofermentation of WAS and food waste has been seldom investigated before.

Unlike the monofermentation of food waste, cofermentation of WAS and food waste is a more complex system which contains several different biotransformations. For instance, the microbial cells (e.g., extracellular polymeric substances (EPS) and cell envelopes) in WAS must be disintegrated before they are fermented to produce SCFA. Due to the low C/N ratio in the raw sludge, one important aim of the cofermentation system is to enhance the bioconversion of protein released from WAS by the addition of carbohydrate-rich food waste, which thereby provides more substrate for the subsequent SCFA production. To date, however, it is unknown whether the presence of NaCl causes significant impacts on these biotransformations. Besides, the presence of NaCl may also affect other biochemical metabolism reactions such as hydrolysis, acidification, and methanogenesis involved in the cofermentation system, but details of how NaCl affects these processes are also poorly understood.

The aim of this work is to investigate the influence of NaCl on SCFA production from the cofermentation of WAS and food waste and to reveal the underlying mechanisms of how NaCl affects the cofermentation. First, the SCFA production from the cofermentation system at the presence of different NaCl levels is compared. Then, the underlying mechanisms of NaCl affecting SCFA production are explored by analyzing the impacts of NaCl on the disruption of both EPS and cell envelopes in WAS, the release of soluble substances from food waste, the bioconversion of released protein, the biotransformations of hydrolysis, acidification, and methanogenesis processes, and the activities of key enzymes relevant to SCFA production. As the impact of NaCl on SCFA production was found to be dosage dependent (i.e., low levels of NaCl promotes SCFA production whereas high levels of NaCl inhibits SCFA production), the feasibility of further enhancement of SCFA production from the cofermentation by combining the positive role of low NaCl levels with other pretreatment strategies was finally confirmed. To the best of our knowledge, this is the first study to reveal the underlying mechanisms of how NaCl affects SCFA production from the cofermentation of WAS and food waste. The findings obtained in this work provide insights into the cofermentation system and might be of significance for its operation in real situations.

EXPERIMENTAL SECTION

Sources of WAS and Food Waste. WAS was collected from the secondary tank of a municipal wastewater treatment plant in Changsha, China. The obtained WAS was concentrated by settling in a 4 °C-controlled refrigerator for 24 h before use. The inherent NaCl contained in WAS was less than 5 mg/L. The main characteristics of the concentrated sludge are shown in Table 1.

Food waste was withdrawn from a canteen in Hunan University, Changsha, China. After removal of indigestible substrates such as inorganic particles, bones, and chopsticks, the food waste was crushed and homogenized to slurry state using an electrical blender. Before use, the food waste was washed three times with tap water to eliminate the inherent NaCl. The main characteristics of food waste are also shown in Table 1.

The typical cofermentation substrates were variable with different ratios of food waste to WAS in different publications, and their C/N ratios of the cofermentation system were generally maintained in the range of 20/1−30/1 to facilitate anaerobic fermentation. In this study, food waste was mixed with WAS at the ratio of 6 g/g according to the literature,[17] and the corresponding C/N was 22.8/1, which was in the suitable ratio range of C/N. To reduce the viscosity of the mixed substrates, a certain volume of tap water was added to make the TSS of cofermentation substances around 21%. After mixture, the main characteristics of the cofermentation substrates are also shown in Table 1.

Effect of NaCl on SCFA Production from the Anaerobic Cofermentation of WAS and Food Waste. Batch tests were carried out in five replicate reactors with a working volume of 1.0 L each. First, 5 L of the mixture, as mentioned above, were evenly divided into five reactors. Then, different dosages of NaCl were added into those reactors to obtain the designed NaCl concentrations of 0, 4, 8, 12, and 16 g/L. Afterward, all anaerobic reactors were flushed with nitrogen gas for 10 min to remove oxygen, sealed with rubber stoppers, and fermented in an air-bath shaker (100 rpm) for 15 d. Temperature is a vital factor affecting the anaerobic fermentation of sludge. Thermal fermentation (e.g., 55 °C) and mesophilic fermentation (e.g., 35 °C) require high energy input, which are not economic in a full scale plant. Low temperature (e.g., 10 °C) could reduce the activities of key enzymes or biochemical metabolic reactions, which decrease the efficiency of sludge anaerobic fermentation. In this work, room temperature (20 °C) is therefore selected. The samples of fermentation mixtures were taken periodically for the analysis of soluble COD, protein, carbohydrate, SCFA.

Effect of NaCl on the Disruption of WAS and the Solubilization of Food Waste. According to our preliminary assessment, it was found that the presence of NaCl led to increased release of soluble organic matters from the cofermentation system. Since the cofermentation system includes two original substrates, i.e.,

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WAS and food waste, it is necessary to clarify the effect of NaCl on the disruption of WAS and the solubilization of food waste, respectively.

A batch test with nine replicate serum bottles was carried out. These bottles were divided evenly into three groups (Group-I, Group-II, and Group-III) with three each. The first two groups were used to assess the effect of NaCl on the disintegration of microbial cells (i.e., EPS and cell envelops) in WAS while Group-III was employed to examine the response of food waste to the presence of NaCl. Each bottle of Group-I, Group-II, and Group-III was received 500 mL of WAS, WAS with EPS extraction, and food waste, respectively. It should be noted that Group-I and Group-II were respectively used to evaluate the impact of NaCl on the disintegration of EPS and cell envelopes. To minimize the influence of EPS, WAS used in Group-II should contain no or little EPS, thus EPS extraction was performed before the sludge was applied in the test of Group-II. EPS extraction was conducted by the extraction method, and the procedure of extraction were described in the Supporting Information.[18] Adenosine-triphosphate analysis was also used to detect the release of adenosine-triphosphate. Experimental results showed no significant adenosine-triphosphate release was measured after the extraction, as compared with the blank ($p > 0.05$). This observation indicated that the cell envelopes were not damaged significantly by the EPS extraction method applied. After the addition of designed substrates, the NaCl concentration in three serum bottles of each group was maintained at 0, 8, and 16 g/L, respectively. All other operational conditions were the same as described above.

Effect of the Presence of NaCl on Bioconversion of Released Protein. This batch test was conducted in three replicate anaerobic fermentation reactors (working volume of 1 L each). Each reactor first received 900 mL same synthetic wastewater containing 11.6 g bovine serum albumin (BSA, average molecular weight 67 000, a model protein compound)/L and 62.6 g dextran (average molecular weight 23 800, a model polysaccharide compound)/L. The protein and carbohydrate contents in the synthetic wastewater were similar to those in the cofermentation substrates. After that, 100 mL of identical inoculum, which was taken from an anaerobic fermentation reactor in our laboratory, was added into each reactor. Inoculum was employed to decompose the released protein. The main characteristics of the inoculum are as follows: pH 7.2 ± 0.1, TSS 6100 ± 260 mg/L, VSS 4950 ± 210 mg/L, total COD 9850 ± 320 mg COD/L, total protein 3126 ± 125 mg COD/L, total carbohydrate 658 ± 23 mg COD/L. The anaerobic fermentation reactor was also fed with 900 mL of cofermentation mixture of WAS and food waste. The NaCl concentration in the three reactors was controlled at 0, 8, or 16 g/L. After removal of oxygen with nitrogen gas all the reactors were capped, sealed, and stirred at 100 rpm. All other operation conditions were the same as described above.

Effect of NaCl on the Processes of Acidification and Methanogenesis. The following two batch experiments (named Batch-I and Batch-II) were performed to evaluate the effect of NaCl on the processes of acidification and methanogenesis, respectively. Three replicate reactors with a working volume of 1 L each were operated in Batch-I. Each reactor received 100 mL same inoculums, as mentioned above, and 900 mL same synthetic wastewater containing 2.3 g L-alanine (a model amino acid compound)/L and 5.3 g glucose (a model monosaccharide compound)/L. The initial NaCl concentration of these three reactors was respectively controlled at 0, 8, and 16 g/L. All other operational conditions were the same as those described above. Each reactor in Batch-II was operated the same as those in Batch-I, except that 1 g acetate/L was used to substitute for L-alanine and glucose.

SCFA Production from the Anaerobic Cofermentation Containing Pertinent NaCl when Pretreatment Strategies Were Applied. Three identical reactors (working volume of 1.0 L each) were operated in this batch experiment. The NaCl concentration in each reactor was maintained at 8 g/L. The mixture in reactor 1 was not pretreated and set as the control while the mixtures in the other two reactors were pretreated either by incubation with 1.8 mg free nitrous acid (FNA)/L for 48 h or by microwave at the special energy input of 38400 kJ/kg TS for 15 min.[19−22] All other anaerobic fermentation conditions were the same as those depicted above.

Analytical Methods. Fermentation mixtures sampled were first centrifuged at a speed of 4000 rmp and then filtered through 0.45 μm disposable Millipore filter units. Soluble COD, TSS, VSS, NH_4^+−N, and PO_4^{3-}−P were measured according to the standard methods.[23] SCFA was measured using Agilent 6890 DB-MAXETR, and the detailed measurement was described in previous publications.[24−26] Briefly, the filtrate was collected in a gas chromatography vial (1.5 mL), and then 3% H_3PO_4 was added to adjust the pH to 4.0. The sample injection volume wascontrolled at 1.0 μL. The temperature of injection port and the detector was maintained at 200 and 220 °C, respectively. The carrier gas was nitrogen at the flux of 25 mL/min. The total content of SCFA was calculated as the sum of acetic, propionic, *n*-butyric, *iso*-butyric, *n*-valeric, and *iso*-valeric acid. Protein was determined by Lowry−Folin method with BSA as the standard,[27] and carbohydrate was determined by the phenol-sulfuric method with glucose as the standard.[28] The measurements of BSA, dextran, L-alanine, glucose, are conducted in accordance with the methods documented in ref 3. The levels of Mg^{2+} and Ca^{2+} in fermentation liquid were determined using a 761 Compact Ion Chromatograph (Metrohm, Switzerland). The major functional groups of fermentation liquid in the cofermentation systems were determined by Fourier transform infrared spectroscopy (FTIR), and the procedure was detailed in the literature.[29,30] EPS structure was characterized by Excitation emission matrix (EEM) fluorescence spectroscopy. EEM fluorescence spectroscopy was detected via a luminescence spectrometry (Fluoromax-4 Spectrofluorometer, HORIBA Scientific, France) with 450 W Xe arc lamp, and the method was detailed in the literature.[31] EPS extraction method was described in the Supporting Information. The activities of key hydrolytic enzymes (protease and α-glucosidase) and propionic-forming enzymes (oxaloacetate transcarboxylase (OAATC), CoA transferase) were determined according to the method developed by Goel et al.[32] The measurement of coenzyme F420 was the same as described in the literature.[33]

Statistical Analysis. All experiments were conducted in triplicate, and the results shown in this paper were expressed as means ± standard deviation. An analysis of variance was employed to assess the significance of the results, and $p < 0.05$ was recognized to be statistically significant.

RESULTS AND DISCUSSION

Effect of NaCl Concentration on SCFA Production from the Cofermentation of WAS and Food Waste. The curve of SCFA generation from the anaerobic cofermentation at different NaCl levels is displayed in Figure 1. It can be seen

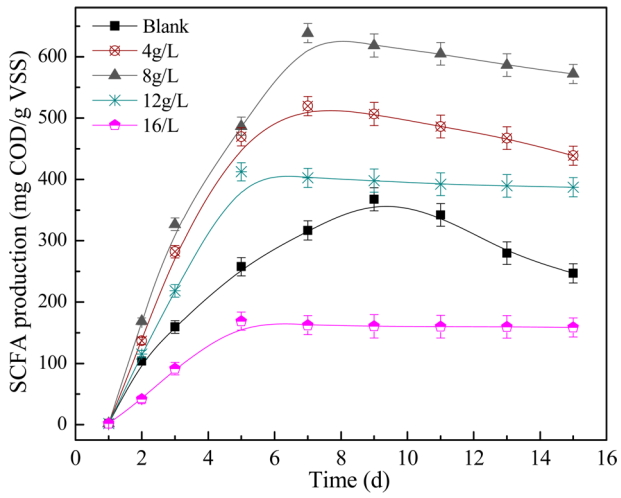

Figure 1. Effect of NaCl concentration on total SCFA production from the anaerobic cofermentation of WAS and food waste. Error bars represent standard deviations of triplicate tests.

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that a satisfied level of SCFA was achieved in the cofermentation of WAS and food waste in the absence of NaCl (i.e., the blank), and the highest SCFA generation of 367.6 mg COD/g VSS was obtained at the fermentation time of 9 d, which was similar to that reported in the literature.[4] When NaCl appeared in the cofermentation system, SCFA generation was largely affected. When the NaCl level increased from 0 to 8 g NaCl/L, the maximum SCFA production increased from 367.6 to 638.5 mg COD/g VSS. It should be noted that the total production of SCFAs decreased from 7 d when NaCl concentration was 4 and 8 g/L. The decrease of SCFA production was ascribed to their consumption for methane generation. However, further increase of the NaCl level led to the decrease of SCFA production. For example, the maximal SCFA generation was only 168.9 mg COD/g VSS when the NaCl level reached at 16 g/L. The results showed that the effect of NaCl on SCFA production from the cofermentation of WAS and food waste was dosage dependent. Relatively low levels (e.g., 0−8 g/L) enhanced SCFA production whereas relatively high levels (e.g., 16 g/L) inhibited SCFA production.

The percentage of individual SCFA in different reactors under their optimal conditions was also determined. Unlike the monofermentation of WAS or food waste where acetic acid is the major individual SCFA,[3,26] propionc acid instead of acetic acid ranked the first in all investigated reactors (Figure S1, Supporting Information). Further analysis exhibited that the average order of individual SCFA in all cofermentation systems was as follows: propionic > acetic > *n*-butyric > *iso*-valeric > *iso*-butyric > *n*-valeric.

Effect of NaCl on the Disruption of WAS and the Solubilization of Food Waste. Protein and carbohydrate, which are originally in the solid phase, are the two major organic substances in the cofermentation system.[34−36] Before they are fermented to produce SCFA, they need to be released in the fermentation liquid. Figure 2 presents the effect of NaCl level on the released substrates (i.e., soluble COD) in the initial 6 fermentation days. The soluble COD concentration increased with the increasing NaCl in the range of 0−16 g/L at any fermentation time investigated. To characterize the effect of NaCl presence on the chemical functional groups in the fermentation liquid, FTIR was applied. It is seen from Figure 3 that compared with the blank there are some decreases of light transmittance in the bands of 1050 and 1540 cm^{-1} in the reactor with 16 g NaCl/L, which means that the specific absorption peaks of 1050 and 1540 cm^{-1} in the presence of NaCl was greater than those in the blank. These two bands are assigned to be polysaccharidelike (C−O−C) and proteinlike (C−N and N−H) substrates (Table S1, Supporting Information). Similar observations were also made in other NaCl presence reactors. This result suggested that the presence of NaCl benefited the release of soluble substrates such as protein and carbohydrate. Table 2 further summarizes the released protein and carbohydrate in the initial 6 fermentation days with and without NaCl addition. As shown in Table 2, soluble levels of protein and carbohydrate in both NaCl presence reactors were higher than those in the blank, which was consistent with the results shown in Figures 2 and 3. Moreover, the VSS reduction of cofermentation substances in the presence of NaCl was also higher than that in blank (Figure S2, Supporting Information), which further confirmed that the presence of NaCl was beneficial to the release of soluble substrates.

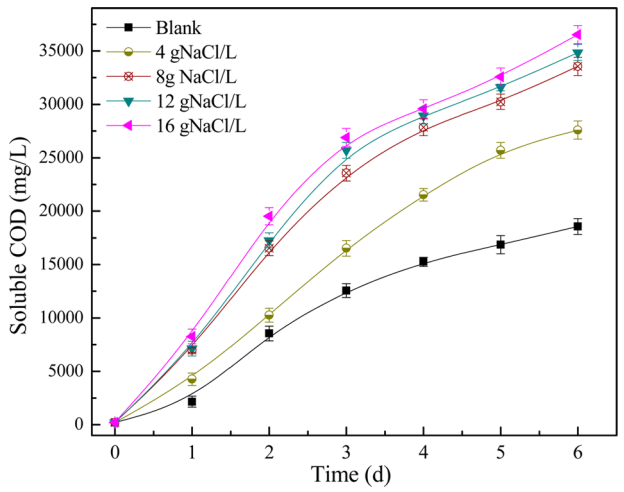

Figure 2. Effect of NaCl on soluble COD variations from the cofermentation system of WAS and food waste. Error bars represent standard deviations of triplicate tests.

As the cofermentation system includes two original solid substrates, i.e., WAS and food waste, it is necessary to clarify whether the presence of NaCl accelerated the disintegration of either WAS, or food waste, or both of them. EPS and cell envelopes are the main substances protecting microbial cells against adverse environments. To confirm whether NaCl benefits the disintegration of WAS, the effect of NaCl addition on the disruption of EPS and cell envelopes was first evaluated.

Figure 4 presents the variations of soluble protein and carbohydrate in the initial 20 h of fermentation with and without NaCl addition. On the basis of the analysis of COD mass balance (Table S2, Supporting Information), it was found that cell envelopes did not disintegrated significantly in the initial 16 h of fermentation in all reactors investigated, as the sum of soluble COD and extracted COD showed an insignificant change in comparison to the total amount of COD determined in the initial EPS ($p > 0.05$). However, when 8 or 16 g NaCl/L presented, the sum of soluble COD and extracted COD was significant higher than the total amount of COD determined in the initial EPS ($p < 0.05$) at the fermentation time of 20 h, which indicated that the disruption of cell envelope happened. Therefore, the soluble protein and carbohydrate in the initial 16 h of fermentation were used to indicate EPS disruption. It was observed from Figure 4 that both the soluble protein and carbohydrate in the reactors with NaCl addition were much higher than those in blank, which revealed that the presence of NaCl accelerated the disruption of EPS. Verification of this result was further provided by the EEM fluorescence spectroscopy. The EEM fluorescence spectroscopy is often employed to characterize the changes of EPS structure.[37] It was documented that the main peak of tryptophan proteinlike substances was located at the Ex/Em of 275/340 nm.[37,38] As shown in Figure 5, the presence of 16 g/L NaCl decreased the fluorescence intensity to 59.2% of the blank, suggesting again that the presence of NaCl resulted in more disruption of EPS.

Figure 6 exhibits the effect of NaCl on the disruption of cell envelops. Since sludge without EPS extraction was used here, the release of soluble COD can be applied to indicate the disruption of cell envelops. In addition, adenosine-triphosphate analysis was also conducted to confirm that cell envelop was not damaged with EPS extraction. Experiment results showed

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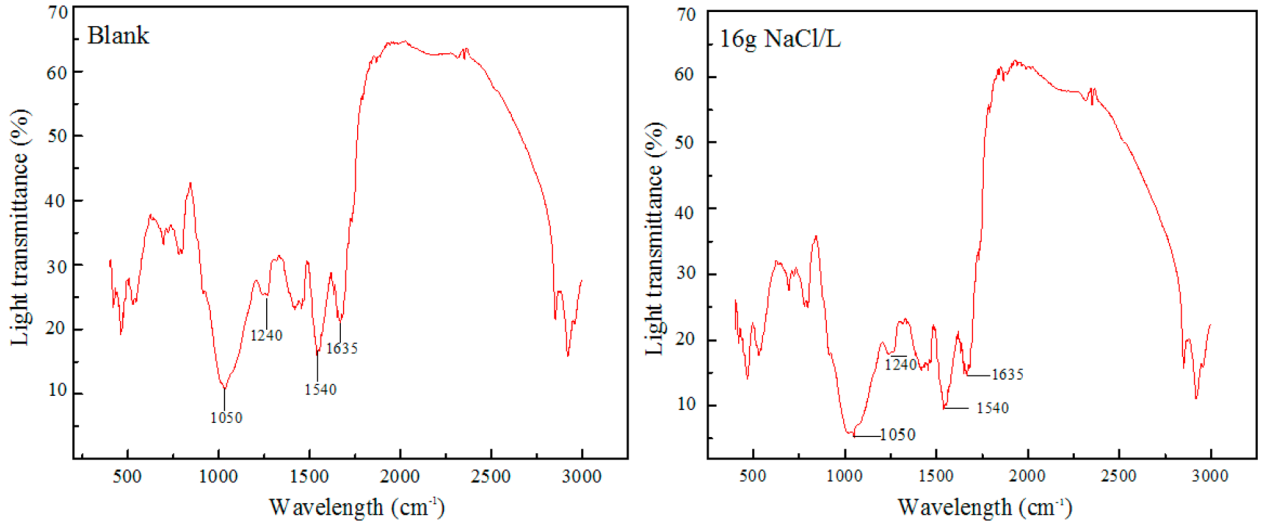

Figure 3. FTIR analysis of the soluble carbon from the cofermentation of WAS and food waste with different NaCl levels on 2 d.

Table 2. Variations of Soluble Protein and Soluble Carbohydrate with Fermentation at Different NaCl Concentrations in the First Experiment[a]

		2 d	4 d	6 d
blank	soluble protein	1569 ± 29	2256 ± 35	3274 ± 39
	soluble carbohydrate	3256 ± 56	5269 ± 49	7200 ± 65
8 g/L	soluble protein	2596 ± 24	4126 ± 41	3458 ± 56
	soluble carbohydrate	5968 ± 51	9850 ± 50	8254 ± 68
16 g/L	soluble protein	2613 ± 24	4219 ± 34	3468 ± 36
	soluble carbohydrate	5972 ± 51	9862 ± 57	8267 ± 68

[a]The units of soluble protein and soluble carbohydrate are milligrams COD per liter. Error bars represent standard deviations of triplicate tests.

that as compared with the blank, insignificant release of adenosine-triphosphate was detected after this EPS extraction ($p > 0.05$), which confirmed that the cell envelope was not significantly destroyed by this EPS extraction method. From Figure 6, it can be seen that the presence of NaCl gave rise to greater disruption of cell envelopes. For example, 16 g/L NaCl caused 1.64-fold soluble COD release at 12 h of fermentation, as compared with the blank. According to the above analysis, it can be concluded that the presence of NaCl promoted the disruption of both EPS and cell envelops. As a result, compared with the blank, more soluble substrates would be released from the sludge in the presence of NaCl. Figure 7 displays the variations of Mg^{2+} and Ca^{2+} in the sludge fermentation liquid with fermentation time. It is known that divalent cations (e.g., $Ca^{2+}$and Mg^{2+}) are either absorbed onto the surface of EPS or located in the intracellular cells, e.g., as composition of polyphosphate.[39] Thus, if more $Ca^{2+}$and Mg^{2+} are measured in the sludge fermentation liquid, higher levels of sludge disintegration are obtained. It is seen from Figure 7 that both the released levels of $Ca^{2+}$and Mg^{2+} in the NaCl presence reactors were much higher than those in the blank no matter what level of NaCl was. Moreover, 16 g/L NaCl caused higher release of $Ca^{2+}$and Mg^{2+} in comparison to 8 g/L, which was in agreement with the results shown in Figures 4 and 6. The increased releases of Mg^{2+} and Ca^{2+} indicated the disruption of sludge flocs, and higher NaCl caused greater level of sludge disintegration. It was suggested that the presence of high salt resulted in huge osmotic pressure between microorganisms and the ambient environment, which gave rise to cell plasmol-

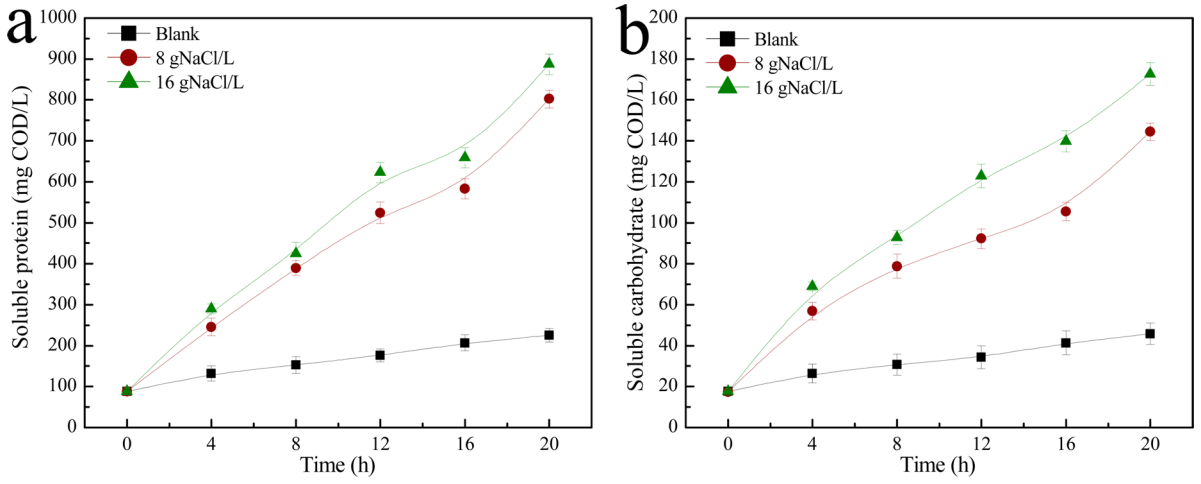

Figure 4. Effect of NaCl addition on the release of soluble protein and soluble carbohydrate in the initial 20 h fermentation. Error bars represent standard deviations of triplicate tests.

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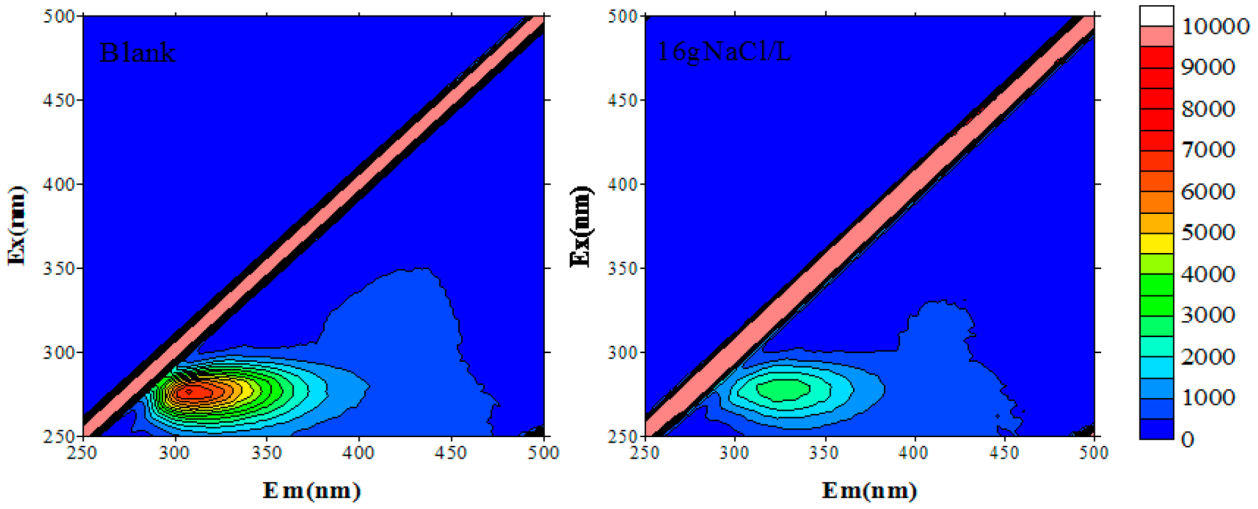

Figure 5. Effect of NaCl addition on EEM profile of sludge EPS at the fermentation time of 16 h.

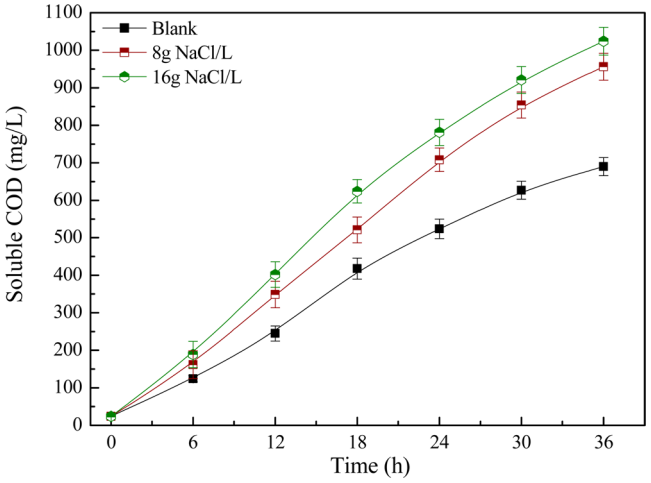

Figure 6. Effect of NaCl on the disruption of cell envelopes. Error bars represent standard deviations of triplicate tests.

ysis.[40,41] Plasmolysis further accelerated the release of EPS from WAS.

Besides WAS, food waste is the other original solid substrate in the cofermentation system, thus the potential role of NaCl on the disintegration of food waste was also assessed. Table 3 summarizes the effect of NaCl on variations of soluble protein and soluble carbohydrate during anaerobic fermentation of food waste. As the food waste employed in this batch experiment was the same, the released protein and carbohydrate were used to represent the disintegration of food waste. From Table 3, it can be found that with the increase of fermentation time the released protein and carbohydrate levels increased in all reactors investigated. Compared with the blank, 8 g/L NaCl led to more release of protein and carbohydrate. When 16 g/L NaCl was applied, the levels of released protein and carbohydrate further increased. It is clear that the presence of NaCl also enhances the disintegration of food waste. It can be therefore understood that more soluble COD was determined in the reactors with the presence of NaCl (Figure 2) since the presence of NaCl accelerated the disintegration of both WAS and food waste.

Effect of NaCl on Bioconversion of Protein. One major purpose of the cofermentation of WAS and food waste is to

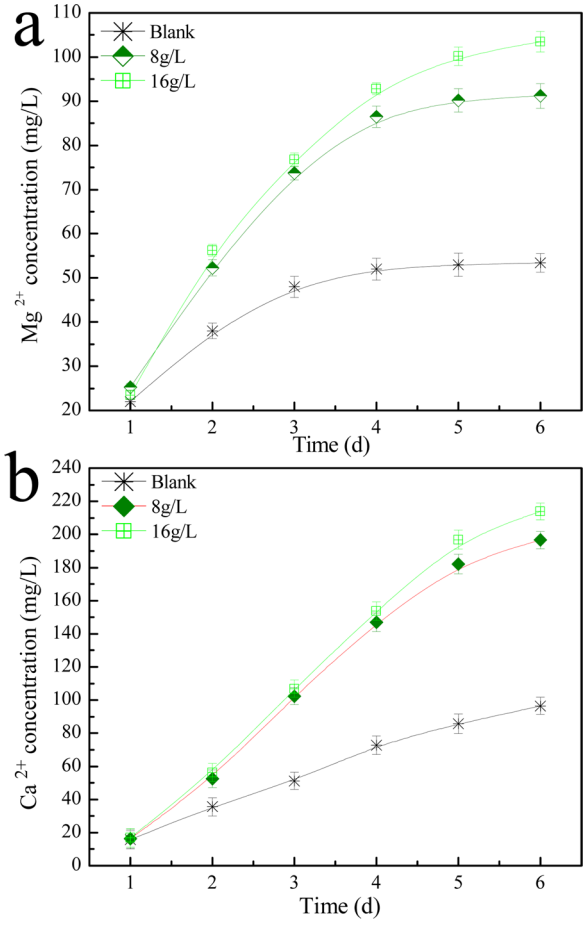

Figure 7. Effect of NaCl addition on the variations of Mg^{2+} (a) and Ca^{2+} (b) in the sludge fermentation liquid. Error bars represent standard deviations of triplicate tests.

enhance the bioconversion of released protein to provide more substrates for subsequent acidification. However, it remains unknown whether the presence of NaCl affects this bioconversion process. Table 4 presents the effect of NaCl on the bioconversion of protein. With the increase of fermentation time from 1 to 5 d, the converted protein in the blank increased from 852.6 to 3241.4 g COD/L. When 8

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Table 3. Effect of NaCl on Variations of Soluble Protein and Soluble Carbohydrate during Anaerobic Fermentation of Food Waste[a]

		0 h	6 h	12 h	18 h	24 h
blank	soluble protein	235.6 ± 10.2	335.8 ± 12.6	403.4 ± 18.7	458.9 ± 20.6	518.2 ± 21.3
	soluble carbohydrate	568.9 ± 19.3	741.6 ± 21.4	823.7 ± 26.8	912.3 ± 31.5	1024.7 ± 42.4
8 g NaCl/L	soluble protein	235.6 ± 10.2	389.4 ± 15.7	446.3 ± 18.4	501.7 ± 20.7	567.8 ± 22.9
	soluble carbohydrate	568.9 ± 19.3	842.6 ± 20.8	951.4 ± 24.6	1014.2 ± 26.7	1213.4 ± 35.2
16 g NaCl/L	soluble protein	235.6 ± 10.2	412.8 ± 15.6	499.7 ± 18.4	548.1 ± 20.6	623.5 ± 24.3
	soluble carbohydrate	568.9 ± 19.3	912.6 ± 24.6	1029.4 ± 28.4	1234.6 ± 29.8	1356.2 ± 41.5

[a]The units of soluble protein and soluble carbohydrate are milligrams COD per liter. Error bars represent standard deviations of triplicate tests.

Table 4. Effect of the Presence of NaCl on Bioconversion of Protein[a]

	1 d	2 d	3 d	4 d	5 d
blank	852.6 ± 26.4	1567.8 ± 31.7	1985.6 ± 34.8	2958.7 ± 39.4	3241.4 ± 41.2
8 g NaCl/L	957.2 ± 34.7	1986.4 ± 35.7	2546.9 ± 42.9	3819.6 ± 45.1	4526.7 ± 50.9
16 g NaCl/L	1021.5 ± 34.7	2115.6 ± 34.7	2682.4 ± 34.7	4021.2 ± 34.7	4882.2 ± 34.7

[a]The unit of protein is milligrams COD converted per liter. Error bars represent standard deviations of triplicate tests.

Table 5. Effect of NaCl Addition on Degradation Rate of Model Compounds with Time[a]

		degradation rate (%)				
	time (d)	BSA	dextran	L-alanine	glucose	acetate
blank	2	13.5 ± 1.8	40.3 ± 1.7	48.9 ± 2.1	41.1 ± 2.3	51.3 ± 2.4
	4	25.5 ± 2.1	60.8 ± 2.3	64.1 ± 2.7	63.4 ± 2.1	82.7 ± 2.7
	6	48.6 ± 2.6	79.4 ± 2.9	73.8 ± 3.2	82.6 ± 2.8	91.6 ± 4.2
8 g NaCl/L	2	17.2 ± 1.4	51.6 ± 2.1	59.8 ± 2.8	50.5 ± 2.6	24.5 ± 1.9
	4	32.9 ± 2.3	81.6 ± 2.8	74.6 ± 3.4	75.6 ± 3.4	33.9 ± 2.7
	6	62.8 ± 3.5	92.3 ± 3.4	89.9 ± 3.9	92.3 ± 3.8	42.7 ± 3.1
16 g NaCl/L	2	18.3 ± 1.1	52.9 ± 2.7	31.2 ± 1.7	34.9 ± 1.7	12.6 ± 1.5
	4	34.6 ± 2.4	83.4 ± 3.1	42.5 ± 2.6	47.6 ± 2.6	20.7 ± 1.9
	6	64.1 ± 3.6	93.7 ± 3.8	56.4 ± 3.1	52.3 ± 2.4	26.1 ± 2.3

[a]Error bars represent standard deviations of triplicate tests.

and 16 g/L of NaCl were added into the fermentation systems, the converted protein were respectively increased from 957.2 and 1021.5 g COD/L to 4526.7 and 4882.2 g COD/L with the increasing fermentation time from 1 to 5 d. The results clearly showed that the presence of NaCl improved the conversion of protein released in the cofermentation system.

Na^+ is an essential element for biochemical metabolism of anaerobic microorganisms, probably because of its role in the formation of adenosine triphosphate or in the oxidation of NADH.[42] It was reported that the range of 100−350 mg Na^+/L was beneficial to the growth and metabolism of mesophilic anaerobes.[43] Although 8 g Na^+/L was found to seriously inhibit methanogens,[43] hydrolytic bacteria could tolerate high levels of NaCl (e.g., 16 g/L, Table 5). Therefore, it is likely that the presence of NaCl in the fermentation systems enhances the activities of hydrolytic bacteria, which thereby leads to the increased bioconversion of protein.

Effect of NaCl on the Processes of Hydrolysis, Acidification, and Methanogenesis Involved in the Cofermentation. Apart from solubilization, the following three biotransformation processes: hydrolysis, acidification, and methanogenesis, are generally included in anaerobic digestion.[44] All the three processes are closely relevant to SCFA accumulation, thus the potential effects of NaCl on these processes were also evaluated via a series of batch tests using synthetic wastewaters containing either BSA and dextran, or L-alanine and glucose, or acetate. From Table 5, it is found that the presence of NaCl led to higher degradation rate of BSA and dextran than those in blank no matter what treatment time was, suggesting the presence of NaCl promoted the hydrolysis step of anaerobic fermentation. The degradation rates of L-alanine and glucose, however, were highly influenced by NaCl dosage. With the NaCl level from 0 increased to 8 g/L, the degradation rates of L-alanine and glucose increased from 73.8% and 82.6% to 89.9% and 92.3% on 6 d of fermentation, respectively. However, when 16 g/L of NaCl was added, the degradation rates of L-alanine and glucose were 56.4% and 52.3% at the same time, respectively, which were only 0.76- and 0.63-fold of those in blank. This result indicated that relative low levels (e.g., 8 g NaCl/L) enhanced the acidification process, but relatively high levels (e.g., 16 g NaCl/L) inhibited the acidification process.

As to the influence of NaCL on the methanation step, the degradation rate of acetate was seriously inhibited by the presence of NaCl. When 8 and 16 g NaCl/L were applied, the degradation rate decreased from 91.6% (the blank) to 42.7 and 26.1% at the fermentation time of 6 d, respectively. Similar observations were also made at other fermentation times. This result indicated that the presence of NaCl significantly inhibited the methanogenesis process. NaCl is a vital factor affecting the activities of methanogenes. It was documented that the optimum Na^+ concentration for methanogens was in the range of 0.23−0.35 g/L and 10 g Na^+/L decreased the activities of methanogens by 50% while 25 g Na^+/L completely inhibited the methanogenic activity.[14,45] In addition, the presence of NaCl also affected pH variation in the fermentation process

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(Figure S3, Supporting Information). The decrease of pH was detected in all reactors, but the reactor containing higher NaCl level showed greater pH drop. For example, pH was 6.2 at the fermentation time of 6 d in the blank, whereas the corresponding data were respectively 5.4 and 5.2 in the reactors with the addition of 8 and 16 g NaCl/L. It is known that the optimum pH for methanogens is around 7, thus the relative low pH caused by the presence of NaCl posed a negative influence on methanogenic activity, which may be another reason for NaCl inhibiting the methanogenesis process.

Effect of NaCl on the Activities of Key Enzymes Responsible for SCFA Generation. SCFA production from the cofermentation of WAS and food waste is dominated by biological effects, and measurement of the activities of key enzymes is an alternative approach to reflect the activities of microbial cells in the cofermentation systems.[4] Protease and α-glucosidase are the key enzymes responsible for the hydrolysis of protein and polysaccharide, respectively.[4,32] As propinoic acid was the main SCFA in all fermentation systems, thus the key enzymes (i.e., OAATC, and CoA transferase) relevant to its generation were selected to be assayed here.[4] In addition, coenzyme F420 is the key enzyme for methane production. Thus, these five enzyme activities, as mentioned above, were measured in this study. It can be seen from Figure 8 that the

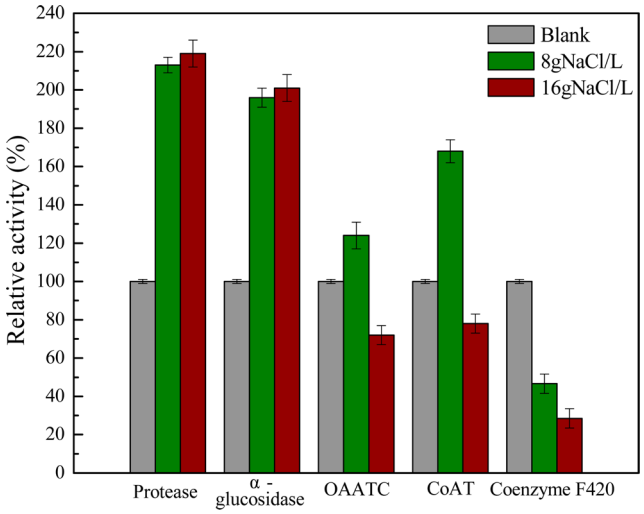

Figure 8. Comparison of the relative activities of key enzymes responsible for SCFA generation on 6 d. The unit for these enzymes is units per milligram of VSS. Error bars represent standard deviations of triplicate measurements.

activities of protease and α-glucosidase were in the sequence of 16 g/L NaCl > 8 g/L NaCl > the blank, which was in accordance with the data shown in Figure 2. The activities of OAATC and CoA transferase were in the order of 8 g/L NaCl > the blank >16 g/L. However, the activity of coenzyme F420 was severely inhibited by the presence of NaCl. The higher NaCl level, the greater inhibition of coenzyme F420 was. These results revealed that pertinent presence of NaCl was beneficial to the growth of acid-producing organisms but high level of NaCl inhibited the activities of acid-producing organisms. This may be the main reason for the reactor containing 16 g/L NaCl showing lower SCFA production than the blank, though it contained much higher released substrates (Figure 2).

Improved SCFA Production When Other Pretreatment Strategies Were Applied. As discussed above, the presence of NaCl is an important factor affecting the cofermentation of WAS and food waste, and low levels of NaCl improved SCFA generation by modifying the characteristics of cofermentation substances and enhancing the bioconversion of protein. Because SCFA, potentially renewable carbon sources, can be used to produce biogas, generate polyhydroxyalkanoates, and remove biological nutrient from wastewater. Maximization of SCFA recovery from cofermentation substrates is one main aim for cofermentation of WAS and food waste. Thus, it is possible to further enhance the production of SCFA when other pretreatment approaches are applied. To confirm this hypothesis, two pretreatment methods, microwave and FNA, were tested in this study. It was found that both VSS reduction and SCFA production were greatly promoted when these two pretreatment approaches were applied (Table S3, Supporting Information). For instance, VSS reduction (or SCFA production) increased from 34.8% (638.5 mg COD/g VSS) to 38.4% (824.1 mg COD/g VSS) and 36.9% (759.1 mg COD/g VSS), respectively, when microwave and FNA pretreatments were applied. Apparently, enhancement of SCFA production from the cofermentation of WAS and food waste containing pertinent NaCl is feasible when pretreatment strategies are employed.

Implications for the Treatment of WAS and Food Waste. Cofermentation of WAS and food waste is a promising method to produce the value added product, SCFA, because this method not only improves balance of nutrients but also dilutes toxic chemicals. NaCl is one representative food flavorings, and its influence on solo digestion of WAS or food waste has been largely investigated.[14,46] Although several researchers have recently started to pay attention to its potential effect on mono or cofermentation,[11,46] the underlying mechanism of how NaCl affects SCFA production from cofermentation remains poorly understood. This work, for the first time, reveals the details of how the presence of NaCl affects SCFA production from the cofermentation system, which thereby fills this knowledge gap.

The findings obtained in this work may have significant implications for future treatment of WAS and food waste. Usually, WAS and food waste are treated by the anaerobic digestion process to produce methane.[11,46] However, it is widely found that the relatively low levels of NaCl severely inhibit the activity of methanogenic *Archaea*, which diminishes the value of the codigestion system. For example, Rinzema et al. demonstrated that 5 g Na^+/L decreased methane production by 10% while 10 g Na^+/L reduced methane production by 50%.[14] A previous publication pointed out that the concentration of NaCl in food waste was generally in the range of 7−12 g NaCl/L.[11] When it is mixed with WAS, the level of NaCl in the mixture should be less than 12 g/L. According to the results achieved in this work (Figure 1), it can be observed that NaCl levels below 12 g/L did not inhibit, but promoted, SCFA production. The findings achieved in this study therefore erase the concern regarding NaCl inhibition on the cofermentation of WAS and food waste. Considering the threshold of NaCl inhibition on codigestion and cofermentation and the value of the fermentation product, SCFA (e.g., a raw material for production of biodegradable plastics), it is suggested that cofermentation of WAS and food waste to produce SCFA might be a better choice in comparison to codigestion to generate methane.

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CONCLUSION

This study evaluated the influence of NaCl on SCFA production from the anaerobic cofermentation of WAS and food waste. Experimental results showed that the effect of NaCl on SCFA production was dosage dependent. With the increase of NaCl level from 0 to 8 g/L SCFA production increased from 367.6 to 638.5 mg COD/g of VSS. However, further increase of NaCl caused inhibition on SCFA production. Mechanism studies showed that the presence of NaCl not only accelerated the release of soluble substance from food waste and disruption of both EPS and cell envelopes but also promoted the conversion of protein released from the cofermentation system. It was also observed that low levels of NaCl enhanced hydrolysis and acidification processes but inhibited the methanogenesis process, whereas both acidification and methanogenesis processes were seriously inhibited by high NaCl levels. All these observations were in accordance with the SCFA production determined.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.6b00816.

Additional analytical method, Tables S1−S3, and Figures S1−S3 (PDF)

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Notes

The authors declare no competing financial interest.

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