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COMMUNICATION

Optimization of ultrasonic pretreatment and substrate/inoculum ratio to enhance hydrolysis and volatile fatty acid production from food waste

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A new method of combinating ultrasonic (US) pretreatment and substrate/inoculums ratio (S/I) adjustment was applied to enhance hydrolysis and volatile fatty acid (VFA) production from food waste. The maximum VFA production was obtained at US of 1 W/mL and S/I 6, which was four times compared to that without pretreatment and S/I adjustment.

Introduction

Nowadays, the unavoidable high-yield food waste (FW) has already became the main source of decay, odor, toxic gas, and groundwater contamination,[1] that severely threats to the environment health and security. On the other hand, FW can also be regarded as an ideal substrate to generate energy by anaerobic fermentation owing to its characteristics of high moisture, high digestibility, well balanced carbon and nutrient contents, and abundant organic composition.[2] Through anaerobic fermentation, the large quantities of organic matters inside FW can be converted into high-valued products such as volatile fatty acid (VFA), methane, hydrogen and so on.[3-4]

VFA, which mainly contains acetate, propionate, iso-butyrate, n-butyrate, iso-valerate, and n-valerate, is a potentially renewable energy and carbon source, and has been considered as a vital raw material for various usage.[3] Based on previous studies, it was successfully used for nutrients removal enhancement,[3] biodegradable plastic production,[5] biogas and biodiesel bioconversion,[6] polyhydroxyalkanoate (PHAs) biosynthesis[7] and electricity generation.[1] However, VFA is mainly extracted and obtained from fossil resources through chemical synthesis route,[3,8] which largely consumes the non-renewable resources and makes VFA costly for industrial use. Therefore, VFA production from FW is a more eco-friendly and cost effective way for sustainable development. To date, the low efficiency of VFA production from FW is an urgent problem for practical application. It is well known that the hydrolysis of organic matter is the rate-limiting step for the production of VFA.[9,10] Moreover, the VFA produced by anaerobic acidogenesis can be consumed by methanogens.[11] Therefore, if the hydrolysis rate is accelerated, and the methanogenesis is prevented or reduced, VFA production can be improved.[12,13]

In order to accelerate the hydrolysis of organic matters, FW is pretreated by various pretreatment methods, including heat, acid, alkaline and ultrasonic.[9] Among these pretreatment methods, ultrasonic (US) pretreatment is proved to be non-hazardous to environment and high-efficiency to disintegrate organic structure.[12] As mentioned above, in addition to enhance hydrolysis rate, the suppression of methanogens is also equally significant for higher VFA production. The studies about inhibiting methanogens mainly focused on adjusting pH to alkaline in the reactors.[12] The pH adjustment by adding chemicals is effective in laboratory to enhance VFA production, but is not economically viable in large-scale application. Based on the studies of Kawai et al.[4] and Zhou et al.[14], it was reported that adjusting the initial substrate/inoculum ratio (S/I) to higher than 2.0 could effectively prevent VFA converting into methane, and thus enhance the VFA production. The main principle is that the initial pH is lower due to the effect of FW, and the growth rate of acidogens is faster than methanogens under the higher S/I. Thus, acidogens can rapidly proliferate at the startup of anaerobic fermentation, leading to significant accumulation of VFA, which will further reduce the pH levels and inhibit the growth and activity of methanogens.[14-15] Previous studies have showed that adjusting proper S/I is feasible to suppress methanogens.[4,14,16]

Although US pretreatment is favourable for FW hydrolysis and suitable S/I is effective for methanogens inhibition, the combined enhancement effects of US pretreatment and S/I adjustment to promote VFA production has not even been investigated. Our pre-research also found that both US pretreatment and S/I adjustment played important roles in FW hydrolysis and VFA production. Thus, the objectives of this study were to investigate the effects of combined US pretreatment and S/I on FW hydrolysis and VFA production. Moreover, the mechanisms of S/I to enhance VFA composition were also discussed.

Materials and methods

FW, which consisted of mainly rice, noodles, vegetables and meat, was obtained from a cafeteria in Harbin Institute of Technology (Harbin, China). The FW after removing the superficial oil was crushed by an electrical blender and stored at 4 ^{o}C in a refrigerator for experimental use. The excess sludge obtained from the secondary setting tank of Harbin Wenchang sewage treatment plant (Harbin, China) was used as inoculums. Prior to inoculation, the sludge sample was washed three times

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with tap water to remove impurities. The characteristics of the FW and inoculums used in this experiment are shown in Table 1.

Table 1 Characteristics of FW and inoculums

Parameter	FW	Inoculum
Total solid (mg/L)	31788 ± 133.11	13381 ± 238.51
Volatile solid (mg/L)	29704 ± 102.56	9255 ± 71.33
Total chemical oxygen demand (mg/L)	62320 ± 233.12	12110 ± 216.74
Soluble chemical oxygen demand (mg/L)	31400 ± 142.53	572 ± 23.35
Soluble carbohydrate (mg/L)	7061 ± 115.12	\
Soluble protein (mg/L)	2453 ± 75.33	\
Initial pH	5.13 ± 0.21	6.96 ± 0.14

To obtain the optimal US pretreatment conditions for disintegrating FW, an ultrasonicator (FS-300, 20 kHz, Shengxi Ultrasonic Instrument Co., Shanghai, China) with a frequency of 20 kHz was carried out, the sonoprobe (diameter 8 mm) dipped 2 cm into 300 mL samples. The US energy densities were controlled at 0.25, 0.5, 0.75, 1.0 and 1.25 W/mL, respectively. During the pretreatment tests, each experiment was operated for 30 min with sampling every 5 min. The disintegration degree (DD), as the indicator of the effects of US pretreatment, was shown in Eq. (1):

$$\text{Disintegration degree } (\%) = \frac{\mathrm{SCOD_{after}} - \mathrm{SCOD_0}}{\mathrm{TCOD_0} - \mathrm{SCOD_0}} \times 100 \qquad (1)$$

where $SCOD_{after}$ is the soluble chemical oxygen demand (SCOD) of the pretreated FW, $SCOD_0$ is the SCOD of the non-pretreated FW, and $TCOD_0$ is the total COD of the non-pretreated FW.

In the subsequent batch fermentation experiments, adjusting the initial FW and sludge quantities (based on the volatile solid (VS) content) to keep each designed S/I in each reactor. The anaerobic fermentation experiments were carried out in a series of 250 ml reactors with the working volume of 180 mL. To evaluate the influence of US pretreatment, two types of substrates were prepared: Substrate 1 was non-pretreated FW, Substrate 2 was US pretreated FW. Ten identical reactors in this study were divided into two groups: with Substrate 1 and Substrate 2 used evenly. Before anaerobic fermentation experiments, each group was inoculated with designed FW and sludge, and controlled the S/Is at 2.0, 4.0, 6.0, 8.0 and 10.0 g-$VS_{substrate}$/g-$VS_{inoculums}$, respectively. At the same time, blank 1# (only excess sludge) and blank 2# (only US pretreated FW) were also operated for comparison. Finally, all the reactors were flushed with nitrogen gas (99.9%) for 20 min to remove oxygen and stirred in a water-bath shaker (180 rpm) at 35 ± 1 ^{o}C.

In this study, total solid (TS) and volatile solid (VS) were measured using standard methods (APHA, 2005). Total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were measured by COD analyzer (DR1010, HACH, USA). Protein was determined by BCA Protein Assay Kit (P0012, Beyotime Institute of Biotechnology, China). Carbohydrate was determined by the phenol-sulfuric acid method with UV wavelength of 490 nm using glucose as standard. The pH value was measured by a pH probe (Germany WTW Company pH meter). The concentrations of VFA (acetate, propionate, butyrate, valerate) were analyzed using a gas chromatograph (GC) (HP 4800, Agilent Technologies®, USA) with a flame ionization detector (FID). By measuring the VFA concentrations, the injector and detector temperatures were programmed at 200 and 250 ^{o}C, and the sample injection volume was 1.0 µL. The COD conversion factors of acetate, propionate, butyrate, valerate were 1.066, 1.512, 1.816 and 2.036, respectively. Soluble parameters were determined after centrifugation (10000 rpm, 10 min) and filtration of the samples through a 0.45 µm filter. Methane contents were analyzed using a GC (GC-SC2, Shanghai Analytical Apparatus, Shanghai, China), equipped with a thermal conductivity detector and a 2.0-m stainless steel column packed with TDS-01 (60/80 mesh). All experiments were carried out in parallel triplicates and average values were determined for each test to minimize random.

Results and discussions

In this study, the disintegration degree (DD), soluble carbohydrate and protein concentrations were used as indicators of US pretreatment effects. As shown in Fig. 1a, the DD increased rapidly at the first 20 min sonication, and then kept stable regardless of extending pretreatment time. This variation trend was similar to previous studies,[4,17] which was attributed to the rapid cavitation effect arising from transient bubbles in fractions of microseconds.[17] Therefore, the optimal US processing time was selected as 20 min. Furthermore, during this pretreatment time, the DD increased from 35.12% to 57.38% as the US energy densities increased from 0.25 to 1 W/mL, and further increase in the US energy density showed a small increase in DD. As shown in Fig. 1b, the concentrations of soluble carbohydrate and protein increased rapidly with US energy density increase to 1 W/mL. However, only slight increase was observed in the amount of soluble substances with further US energy density increase, indicating that the breakdown of FW has become saturated. The maximum soluble carbohydrate and protein concentrations were 19150.04 and 5696.59 mg/L, markedly increased by 171.21% and 132.25% compared with non-pretreatment. Apparently, US pretreatment was effective to release large amounts of soluble substances into the supernatant, which could be easily used by the microorganisms and contributed to higher anaerobic acidification performance in subsequent experiments.[9]

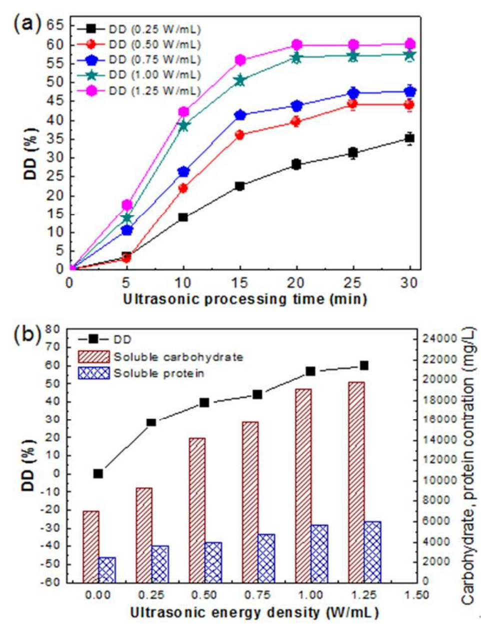

Fig. 1 Effect of US pretreatment on (a) disintegration degree (DD); (b) soluble organic matters.

Considering both the disintegration effects and the economic factors, an ultrasonic time of 20 min and energy density of 1 W/mL were considered as the best conditions for the dissolution of organic matter from FW. And then, the US pretreated FW under best conditions was applied to produce VFA in the subsequent anaerobic fermentation.

The first step of hydrolysis during FW anaerobic fermentation process can be characterized by the changes in SCOD concentrations.[3] As shown in Fig. 2, the increased SCOD concentrations in Substrate 2 were higher than Substrate 1 at all S/Is. The maximum SCOD concentrations for Substrate 1 and 2 were 865.4 and 1403.7 mg/g-VS, which increased 1.15 and 1.61 times compared with the initial SCOD before fermentation, respectively. The obvious increase of SCOD concentration in Substrate 2 indicated that the hydrolysis of FW has been significantly enhanced by US pretreatment. The higher hydrolysis degree was attributed to the loose solid organic compound induced by pretreatment, making undissolved organic matters easily dissolve into liquid under the action of microorganisms. In addition, the hydrolysis efficiencies were also closely related with S/Is that directly influenced pH levels, which will be discussed in detail in following section. For Substrate 1 and 2, the concentrations of SCOD increased quickly at S/I of 4 and 6 at the beginning of 30-48 h fermentation, and then kept stable, which indicated that these S/Is were beneficial to the solubilisation of insoluble organic matters such as carbohydrates, proteins and fats, and thus led to the increase in the output dissolved organic compounds such as simple sugars, fatty acids, and amino acids. However, the concentrations of SCOD in blank 1#, blank 2# and S/I of 2 were lower and decreased with the fermentation time. The possible reason might due to the pH conditions (Table 2) that would not benefit substrate hydrolysis, at the same time, these pH levels also could not inhibit the consumptions of organic matters from methanogens. As is shown in Table 2, the inoculum (sludge) used in this study had high activities of methanogens as indicated from the methane production of blank 1#. For both Substrate 1 and 2, higher methane yields obtained at the lower S/I of 2, and at S/I of 2, the methane production was partially inhibited in the first 24 h and then methane production raised obviously, the highest methane production was 27.71 ml/g-VS. However, higher S/I of 4-10 presented the suppression of methanogens, especially at S/I of 4 and 6, which could explain the changes in SCOD concentration.

Compared with SCOD concentration, the variations in VFA production and component directly reflect the result of acidogenesis. Fig. 3 shows the effect of S/I on the total VFA production. It was observed that the positive effect of S/I adjustment for VFA production was more evident under US pretreatment conditions. For example, the maximum VFA productions in Substrate 2 (967.12 mg COD/g-VS) was 4.29 times of the maximum VFA production in Substrate 1 (225.45 mg COD/g-VS). This result highlighted the importance of the combination of S/I adjustment and US pretreatment to obtain higher VFA production. At a given fermentation time, both Substrate1 and 2 obtained higher VFA productions at S/I of 4 and 6 than those at other S/I conditions. The maximum VFA production of 967.12 mg COD/g-VS was obtained at S/I of 6, and followed by 751.20 mg COD/g-VS at S/I of 4 from Substrate 2 (Fig. 3b). The higher VFA productions were attributed to the following two reasons. One is that US pretreatment and the suitable S/I effectively enhanced the hydrolysis of organic matters, which has been discussed above. The other is that the suitable pH (5.3-6.3) inhibited the activity of methanogens (Table 2).[15,16] Moreover, the fermentation time for the maximal VFA production without US pretreatment (72 h) was much longer than that with US pretreatment (48 h). Therefore, the combination of US pretreatment and S/I adjustment not only significantly enhanced VFA accumulation but also shortened the lag time for the maximal VFA production. It should also be noted that whether pretreatment or not and whatever S/Is were applied, further increasing fermentation time could not gave more VFA production. On one hand, FW hydrolysis has reached the maximum at the experimental conditions in this study. On the other hand, due to the reaction rates of methanogenesis is slower than acidogenesis, with the prolonged fermentation time, the generated VFA could be consumed by methanogens,[18,19] which can be seen from the methane production in Table 2. For example, for Substrate 1, methanogenesis were inhibited at S/I of 4 and 6 at the first 48 h, and then methane production increased gradually. For Substrate 2, no methane was produced at S/I of 6 during the whole fermentation process, and little methane was detected at S/I of 4 after 96 h fermentation.

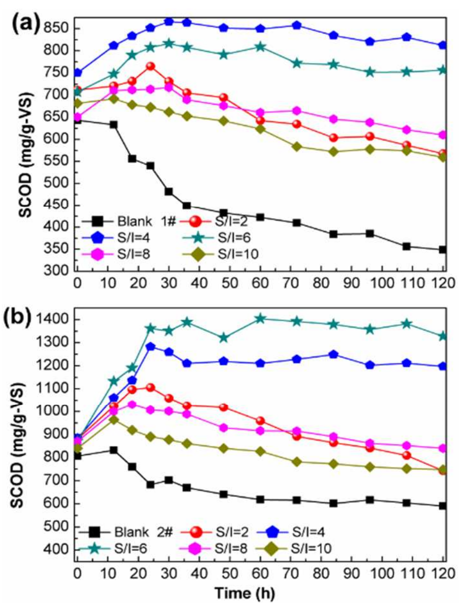

Fig. 2 Effect of S/I and fermentation time on SCOD: (a) non-pretreatment, (b) US pretreatment

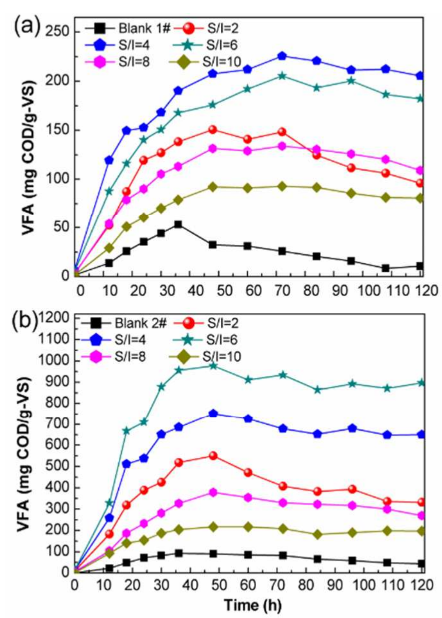

Fig. 3 VFA variations at different S/I and fermentation time: (a) non-pretreatment, (b) US pretreatment

Table 2 Methane production and pH variations at different S/Is

Substrate 1	Methane production (ml/g-VS)					pH		Substrate 2	Methane production (ml/g-VS)					pH	
	24h	48h	72h	96 h	120h	Initial	Final		24h	48h	72h	96 h	120h	Initial	Final
Blank 1#	20.32	32.57	43.21	41.68	37.77	6.85	6.68	Blank 2#	5.88	10.95	11.90	10.61	9.20	5.17	4.52
2	9.67	25.59	26.78	25.66	24.43	6.57	6.32	2	10.23	21.86	27.71	26.90	27.11	6.51	6.15
4	-	-	1.27	2.19	3.16	6.33	5.52	4	-	-	-	1.03	1.51	6.25	5.39
6	-	-	1.67	4.24	4.11	6.18	5.45	6	-	-	-	-	-	6.13	5.32
8	1.57	4.74	6.02	7.83	7.21	6.05	5.44	8	1.69	5.29	7.04	7.70	8.18	5.95	5.37
10	2.20	7.86	7.97	8.66	8.92	5.84	5.19	10	3.58	7.69	8.01	8.79	9.28	5.79	5.02

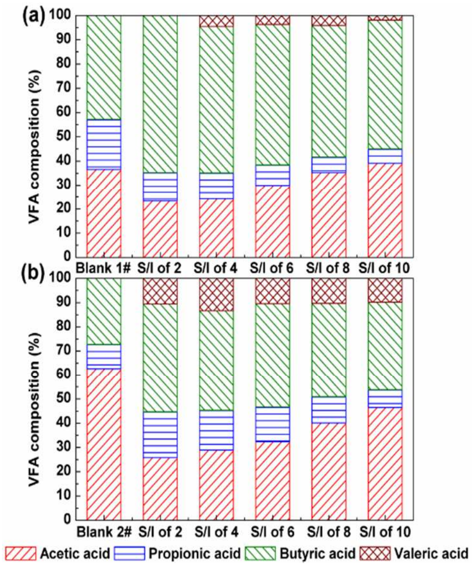

Fig. 4 VFA composition variation: (a) Substrate 1 with S/I of 4 and fermentation time of 72 h, (b) Substrate 2 with S/I of 6 and fermentation time of 48h

Table 3 Comparison of VFA production with different treatment

Substrate	Treatment method	VFA production	References
Kitchen waste	Control pH 8.0	0.69 g-COD/g-VS	1
Food waste	Control pH 6.0	0.316 g/g-VS	2
Food waste	Temperature 45ºC	47.89g/L	2
Food waste	Control pH 6.0	0.918 g/g-VSS	3
Food waste	Adjust ISR 80%	11.8 g COD/L	15
Food waste	US-acid pretreatment	16.9 g COD/L	20
Food waste	US-base pretreatment	14.4 g COD/L	20
Food waste	US-heat pretreatment	12.1 g COD/L	20
Food waste	US-S/I adjustment	0.967 g COD/g-VS	This study

The VFA to SCOD ratio (VFA/SCOD) shows how much soluble substance is converted into VFA,[2] which can be also referred to as the acidification degree (%).[15] Higher VFA/SCOD was achieved by US pretreatment, regardless of which S/I was applied. The maximum VFA/SCOD of 72.27% was observed at S/I of 6, which was comparable to the maximum value of 72.8% obtained at pH controlled 6.0 by adding NaOH during fermentation,[2] followed by 61.61% at S/I of 4 with US pretreatment. However, under non-pretreatment conditions, the VFA/SCOD was relatively low, the maximum VFA/SCOD was only 26.28% occurred at S/I of 4, which was similar with the previous studies without any pretreatment.[11,15,21] The results suggested that US pretreatment well assisted the anaerobic acidogenic bacteria to convert soluble substance to VFA.

VFA composition is also an useful information to represent the degree of hydrolysis and acidification.[3] VFA were mainly composed of acetate, propionate, butyrate, and valerate. The distribution of VFA at different S/Is from Substrate 1 and 2 is presented in Fig. 4. The difference on VFA composition mainly caused by pH variation and the substrate type.[2,3] Butyric and acetic acids were the predominant products in all reactors, which comprising of 70%-90% of total VFA. The results suggested that butyrate type fermentation was achieved from FW in this study. It was demonstrated that the higher S/I resulted in lower pH levels of 5.3-6.5 in the reactors (Table 2), which was the optimal ecological niche for butyric acid fermentation.[23] Both the percentages of propionic acid (Fig. 4) and pH in the reactors (Table 2) decreased with the increases of S/I, which were consistent with the report from Hawkes et al.[24] They suggested that the conversion of acetate and butyrate to propionate would be inhibited as the pH decreased. On the other hand, the main composition of FW used in this study was carbohydrates, which also contributed to the production of acetic and butyric acids.[3] As shown in Fig. 4a and b, with the S/I increased from 2 to 10, the percentage of acetic acid went up, whereas butyric acid showed decreased trends. These observations were consistent with the result of previous study, and the significant accumulation of acetic acid could greatly inhibited methane production.[14] At the same time, it was noted that US pretreatment also obviously improved the production of valeric acid, which mainly produced from fermentation of protein via reductive deamination or the stickland reaction.[22] Thus, it is likely that the cavitation effect from US may increase the dissolution of soluble protein and at the same time promote reductive deamination or stickland reaction. Consequently, according to the type of needed VFA, appropriate S/I could be chosen to obtain the target products.

Discussion

The results described above indicated that it was feasible to improve VFA production from FW anaerobic fermentation by the combination of US pretreatment and S/I adjustment. Both the US pretreatment and S/I adjustment were able to benefit FW hydrolysis with more soluble organic matters release into the fermentation liquid, which in turn resulted in an enhanced microbial activity.[25] The methanogens present in the inoculums could be effectively inhibited by adjusting proper S/I levels. This combined method successfully provided more moderate operation condition compared to the preheated or acid or base pretreatment, which could benefit the microbial survival and activity. As shown in Table 2, Fig. 2 and 3, the blank 1# (only inoculum) and S/I of 2 presented high methane productions, and as for VFA, it decreased with fermentation time, indicating that high methanogens activity inoculum used in the present study had negative effect on VFA production. Therefore, the suppression of methanogens in initial excess sludge is significant for higher VFA production using FW as raw material.

Increasing the S/I led to lower pH levels in the reactors (Table 2). The initial pH in the reactors with S/I 4-10 were not artificially adjusted and it was fluctuated between 5.7 and 6.5, which was reported optimum for hydrolysis and acidogenesis, at the same time, inhibited for methanogenesis.[15] During anaerobic fermentation process in these S/Is, methane production obviously lower than which at S/I of 2 and blank 1#, and there was little reduction in VFA production. Suggesting that the methanogens have been successfully suppressed due to the lower pH levels. Accordingly, weak acidic pH condition was preferred to simultaneously suppress methanogenic acitivity and to keep acidogens activity. Many researchers used alkali to adjust pH value within a satisfactory range. Although it worked well, the side effect is that artificial alkali agent addition greatly increases operation cost. The high cost and complex operation prohibit the widespread use of the method in most developing countries, like China. The combined US pretreatment and optimal S/I adjustment used in this study have successfully solved these problems. The best S/Is were 4 and 6 under US pretreatment condition, with the initial pH of 6.1-6.4 and final pH of 5.3-5.6 (Table 2), methanogens were successfully suppressed and little methane was detected. Therefore, higher VFA production and suitable composition can be achieved, which are important for subsequent treatment process or utilization. It should be noted that, for blank 2# (only FW) and higher S/I of more than 8, the final pH were as low as 4.5-5.5, and VFA productions turned much lower than those under S/I of 4 and 6. The reason might be attributed to that higher substrates than microorganisms caused the food overloading which slowed down the hydrolysis and acidogenesis processes.[26] Moreover, the low pH could inhibit the growth and activity of microorganisms and go against the further dissolution of soluble organic matters, thus VFA production were obviously lower (Fig. 2 and 3).[27] The different pH can result in different VFA production, VFA composition and fermentation rates, because the pH not only affect the anaerobic bacteria community structures, activities and growth rates, but also metabolic pathways.[26] This viewpoint also can be confirmed by our experimental results.

Increasing pH to alkaline conditions by adding NaOH or KOH can improve VFA production. However, this method has some unavoidable disadvantages, such as increased operation cost and complexity, unmanageable fermentation liquor with high concentrations of chemicals, and the potential negative impacts of chemicals on microorganism.[16] There has been some studies shown that VFA production could be effectively enhanced by uncontrolled pH in the reactors.[11,28] The maximum VFA production obtained from FW anaerobic fermentation in this study without pH control was comparable with other papers (Table 3), which applied alternative strategies to improve VFA production from FW, including pH adjustment, temperature control, and inoculum and substrates pretreatment. Among these literatures, the maximum VFA production (0.918 g/g-VSS) was obtained by controlling pH 6.0 by adding NaOH, which was lower than our maximum VFA production (0.967 g COD/g-VS). Therefore, the combination of US pretreatment and suitable S/I is an effective and promising method to achieve higher VFA production from FW by fermentation.

Conclusions

A new strategy to obtain high VFA production from FW using anaerobic fermentation without pH control was proposed. The improved VFA production by combined US pretreatment and S/I adjustment was attributed to two points: (i) increase of FW hydrolysis mainly caused by US pretreatment; (ii) inhibition of methanogens activity by optimal S/I. It was found that the optimal S/Is of 4 and 6 resulted in the pH levels of 5.3-6.4 in the reactors, which were favorable for acidogenesis, and inhibited for methanogenesis. The maximal VFA production (967.12 mg COD/g-VS) was obtained under US energy density of 1 W/mL and S/I of 6. Whereas, under non-pretreatment condition, VFA production was in a lower level and the advantage of S/I adjustment was unobvious, with a maximum VFA production of 225.45 mg COD/g-VS. Therefore, the combined US pretreatment and S/I adjustment have significantly enhanced VFA production.

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