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# Enhancement of volatile fatty acid production by semi-continuous food waste anaerobic fermentation without pH control

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This study proposed a cost-effective and high-yield volatile fatty acid (VFA) production strategy from food waste (FW) anaerobic fermentation without pH control, which could be recommended for practical scale VFA production and FW treatment. Efficient hydrolysis, high VFA production (867.42 mg COD per g-VS) and VFA/SCOD (88.65%), and slight methane production (20.56 ml per g-VS, only 5.34% of theoretical value) were obtained by FW semi-continuous anaerobic fermentation under the optimized conditions, which were substrate to inoculum ratio 5, temperature 40 $^{o}$C, solid retention time 7 d and organic loading rate 9 g VS per L-d. The high-efficient VFA production was mainly contributed to the proper pH, which was not adjusted artificially and maintained in 5.2-6.4 by the neutralization effect of ammonia release. And both kinetic model and carbon mass balance analysis showed that the substrate was adequately utilized to produce VFA under the optimized conditions. These results were of great guiding significance for practical continuous VFA recovery from FW.

## 1. Introduction

One of the biggest challenges of the human development in 21$^{st}$ century is to meet the food supply for the increasing population meanwhile reduce the negative effect of unavoidable food waste (FW) on the environment.[1] As the largest fraction of municipal waste, FW is extremely fast increasing every year in China,[2] and it has already become the main sources of decay, odour, toxic gas, and groundwater contamination, which severely threaten environmental health and security.[3] Thus, the efficient, sustainable and eco-friendly treatment and management on FW has become an urgent task. At the same time, FW also has been regarded as an ideal substrate to generate energy due to its natures of high moisture, easily biodegradation, well balanced carbon and nutrient content, and abundant organic composition.[4] As an economical and eco-friendly strategy, anaerobic fermentation has attracted widespread attention for treating FW and meanwhile harvesting biogas and volatile fatty acid (VFA).[5] Therein, VFA is generally regarded as a better product than biogas owing to its high yield and wide application. Based on previous studies, VFA has been successfully used for nutrients removal enhancement,[6] biodegradable plastic production,[7] biogas and biodiesel bioconversion,[6] polyhydroxyalkanoate biosynthesis[8] and electricity generation.[9] Given the high practical application value of VFA, the study on high-efficiency VFA production from FW is of great significance.

Three steps of hydrolysis, acidogenesis and methanogenesis are involved in anaerobic fermentation, and VFA is produced in the first two steps. Complex organic substances are solubilised by hydrolysis, and then the dissolved organic components are utilized by acidogenic bacteria for their growth meanwhile accumulating VFA.[10] Thus, to enhance VFA production, the hydrolysis must be accelerated to produce more soluble substrates, meanwhile acidogenesis rate should be promoted to furthest convert these substrates to VFA, and equally important, the methanogenesis should be prevented to reduce the consumption of generated VFA.[11] In addition, the effect of anaerobic fermentation is greatly affected by the relevant operational conditions. The specific operation conditions for hydrolytic-acidogenic stages have been widely studied in batch tests,[9, 12] but relevant research in semi-continuous mode is still scarce although it will better guide the future large-scale application. The key operation parameters on semi-continuous VFA production mainly include temperature (T), solid retention time (SRT), organic loading rate (OLR), and pH etc.[13] Recently, *Jiang et al.*[4] has found that the optimal operating conditions for VFA production from FW anaerobic fermentation were pH 6, T 35 $^{o}$C and OLR 11 g per L-d, and *Chen et al.*[9] has reported that pH 8, C/N 22, T 37 $^{o}$C and a fermentation time of 6 d were optimum to enhance VFA accumulation. However, these studies mentioned above only focused on the maximizing VFA production but did not detailedly investigate their influences on hydrolysis, acidification and methanation. Besides, these studies improve VFA production by adding a large amount of NaOH/HCl to obtain alkaline or weak acidity of pH, which caused some unavoidable disadvantages such as higher operation cost and

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complexity, unmanageable fermentation liquid with high chemicals concentration, and potential negative impacts of chemicals on microorganisms.[3] To date, few studies reported that VFA could be efficiently produced without controlling pH. Our previous work from batch FW anaerobic fermentation showed that, by adjusting an appropriate substrate to inoculum ratio (S/I), the self-formed buffer action system could automatically control the pH at a suitable range (5.7-6.5), which was beneficial for hydrolysis and acidogenesis and inhibiting for methanogenesis.[3] In this way, the operation complexity and production cost could be greatly reduced without the additional addition of acid or alkali in practical application. However, whether this strategy can be successfully carried out in semi-continuous mode has not been verified, and the effect of optimum S/I on VFA production under specific optimal conditions has not been investigated. Meantime, aiming at the practical application of continuous VFA production without artificial pH adjustment, it is also necessary to study the optimal conditions of the key influencing factors including T, SRT and OLR in semi-continuous mode.

The main objectives of this work were therefore (1) to investigate the optimum operation conditions (S/I, T, SRT and OLR) for VFA production from FW semi-continuous anaerobic fermentation by response surface methodology, and verify the optimized model; (2) to analyze the effects of optimum S/I on hydrolysis, acidification and methanation; (3) to discuss the reasons of efficient VFA production under optimum conditions without artificial pH control.

## 2. Materials and Methods

### 2.1 FW and inoculum

FW, which mainly consisted of rice, noodles, vegetables and meats, was obtained from a cafeteria in Harbin Institute of Technology (Harbin, China). After removing the superficial oil, the FW was crushed by an electrical blender and stored at 4 $^{o}$C for experimental use. The waste activated sludge (WAS) obtained from the secondary setting tank of Harbin Wenchang sewage treatment plant (Harbin, China) was used as inoculum. The characteristics of FW and inoculum used in this study are shown in Table 1.

**Table 1** Characteristics of FW and inoculum

| Parameters | FW | Inoculum |
|---|---|---|
| Total solid (TS) (g $L^{-1}$) | 66.17 ± 0.81 | 27.63 ± 0.53 |
| Volatile solid (VS) (g $L^{-1}$) | 65.15 ± 1.32 | 15.96 ± 0.74 |
| Total chemical oxygen demand (TCOD) (g $L^{-1}$) | 104.37 ±10.40 | 25.10 ± 3.41 |
| Soluble chemical oxygen demand (SCOD) (g $L^{-1}$) | 50.64 ± 6.63 | 1.19 ± 0.37 |
| Total biological oxygen demand (TBOD) (g $L^{-1}$) | 72.46 ± 5.34 | 9.67 ± 0.55 |
| Total carbohydrate (g $L^{-1}$) | 56.72 ± 5.75 | 3.58 ± 0.58 |
| Total protein (g $L^{-1}$) | 10.47 ± 1.04 | 21.14 ± 2.33 |
| Soluble $NH_4^+$-N (mg $L^{-1}$) | 340.38 ± 9.45 | 160.76 ± 1.07 |
| pH | 5.62 ± 0.10 | 7.03 ± 0.13 |

### 2.2 Optimization of operation conditions

Response surface methodology (RSM) is always used to analyze the mutual relationships between the response and independent variables, and obtain the optimum operation conditions.[14] The RSM based on a five-level-four-variable central composite design (CCD) by Design Expert 8.0 was carried out in this study. The variations of S/I (based on the volatile solid content), T, SRT and OLR, and their levels are shown in Table S1 (Supplementary Information, SI). The ratio of VFA to SCOD (VFA/SCOD), indicating the amount of soluble substance converts into VFA, is often regarded as an evaluation of successful acidogenesis.[4] Thus, VFA/SCOD was regarded as a dependent output variable. The response variable was fitted by the response surface regression procedure, and the second order polynomial equation and the analysis of variance (ANOVA) were described in Section S1 (SI).

Thirty identical fermentation reactors (working volume of 500 mL) designed by RSM were operated in semi-continuous mode (once-a-day draw-off and feeding) for VFA production, and the fermentation equipments were shown in Fig. S1 (SI). Each reactor was inoculated with designed amount of FW and inoculum, and the final VS concentrations in all reactors were 17820 ± 635 mg $L^{-1}$. During anaerobic fermentation, according to the designed SRT and OLR, everyday and each reactor withdrew a certain amount of fermentation mixture, and then added the same volume fresh mixture of FW and inoculum correspondingly. For most anaerobic microorganisms could not tolerate the hostile environment such as alkaline or acidic, the pH in all reactors were not controlled. All the reactors were flushed with nitrogen gas (99.9%) for 5 min to remove oxygen after daily feeding, and then the reactors were stirred in a water-bath shaker (180 rpm). The liquid samples were taken from reactors daily for the SCOD and VFA concentrations analyses. When the SCOD and VFA concentrations remained relatively stables, the reactors were considered to be in a steady state and then the related investigations were carried out.

### 2.3 Operation of semi-continuous reactors under optimal conditions

Some previous studies have investigated the effect of SRT, OLR, T and pH on VFA production, but fewer researches devoted to S/I especially in the fermentation process without pH control. Thus, three identical semi-continuous reactors were operated to observe the effects of optimum S/I on hydrolysis efficiency, VFA and methane productions. Reactor 1# contained sole FW, reactor 2# was inoculated with sole WAS, and reactor 3# was seeded with FW and inoculum according to the optimal S/I obtained by RSM. In the three reactors, T, SRT and OLR were controlled with the optimum conditions obtained by RSM, and the final VS concentrations were 17820 ± 635 mg $L^{-1}$.

### 2.4 Analytical methods

The analyses of TS, VS and ammonia were conducted in accordance with standard methods.[15] TCOD and SCOD were measured by COD analyzer (DR 1010, 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 glucose as standard. The pH value was measured by a pH probe (Germany WTW Company pH meter). VFA was analyzed by a gas chromatograph (GC) (HP 7890, Agilgent Technologies, USA) with a flame ionization detector (FID). The injector and detector temperatures were programmed at 200 and 250 °C, respectively. The oven of GC began at 80 °C, then increased to 180 °C, and finally held at 200 °C. The sample injection volume was 1.0 μL, and the carrier gas was nitrogen at the flux of 25 mL $min^{-1}$. The COD conversion factors of acetic, propionic, butyric and valeric acids were 1.066, 1.512, 1.816 and 2.036, respectively. Methane production was determined using a GC (GC-SC2, Shanghai Analytical Apparatus, Shanghai, China) with a thermal conductivity detector and a 2.0 m stainless steel column packed with TDS-01 (60/80 mesh). Three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy analysis was conducted by a fluorescence spectrometer (FP-6500, JASCO, Japan). Scanning emission (Em) spectra from 220 to 650 nm were obtained at 1 nm increments by varying the excitation (Ex) wavelength from 220 to 450 nm at 5 nm increments. The scan speed was 2000 nm $min^{-1}$, and slit widths were 5 nm for both excitation and emission monochromators. The EEM plots were generated from fluorescence spectral data by Origin 8.0. All the soluble parameters were determined after centrifugation (10000 rpm, 10 min) and filtration (0.45 μm). All tests were carried out in parallel triplicates and the average values were determined to minimize random.

### 2.5 Kinetic analysis

The VFA production was related to hydrolysis, acidogenesis and methogenesis, thus the kinetic model presented here described the above three stages. The hydrolysis step was described by the first-order kinetic, which has been commonly selected as an appropriate model to evaluate the hydrolysis rate of complex fermentation substrates.[16, 17] The conversion of hydrolyzate and the transformation of VFA to methane were described by Michaelis-Menten kinetic models, which were widely applied to evaluate the anaerobic fermentation process.[18] Accordingly, the anaerobic fermentation was presented by the following four equations:[19]

$$\frac{dS_p}{dt} = -kS_p \quad (1)$$

$$\frac{dS_h}{dt} = kS_p - \frac{k_h S_h}{k_{s,h} + S_h} \quad (2)$$

$$\frac{dS_v}{dt} = \frac{k_h S_h}{k_{s,v} + S_h} - \frac{k_v S_v}{k_{s,m} + S_v} \quad (3)$$

$$\frac{dS_m}{dt} = \frac{k_v S_v}{k_{s,m} + S_v} \quad (4)$$

Where $k$ is the hydrolytic rate of particular organic matter ($d^{-1}$); $k_h$ is the maximum utilization rate of hydrolysis products (g SCOD $d^{-1}$); $k_v$ is the maximum utilization rate of VFA (g VFA $d^{-1}$); $k_{s,h}$, $k_{s,v}$ and $k_{s,m}$ are the saturation constants (g SCOD). All concentrations of particular organic matter ($S_p$), hydrolysis products ($S_h$), VFA ($S_v$) and methane ($S_m$) were expressed as g COD $L^{-1}$. The value of the constants $k$, $k_h$, $k_v$, $k_s$, $h$, $k_{s,v}$ and $k_{s,m}$ were determined by experimental data and Mathcad 15.0.

## 3 Results and discussion

### 3.1 Optimization of fermentation conditions

#### 3.1.1 ANOVA and model fitting

The effects of four independent variables i.e., S/I ($A$), SRT ($B$), OLR ($C$) and T ($D$) on VFA/SCOD obtained from FW anaerobic fermentation were investigated by RSM. The CCD experiments with 30 runs were randomly conducted to minimize the negative effect of uncontrolled variable. The experimental and predicted responses of VFA/SCOD are presented in Table S2. Obviously, the experimental values were close to predicted values. A second-order polynomial equation for VFA/SCOD ($Y$) fitted in terms of actual factor obtained by Design Expert 8.0 is shown in Eq. (5).

$$\begin{aligned} Y = {} & -579.24 + 94.43A + 26.77B + 23.76C + 10.73D - 0.61AB \\ & + 0.35AC + 0.22AD - 0.26BC + 0.05BD + 0.05CD - 9.76A^2 \\ & - 1.58B^2 - 1.50C^2 - 0.15D^2 \end{aligned} \quad (5)$$

The ANOVA was used for the regression analysis of the experimental data and the response surfaces, which was shown in Table S3. And the further analyses and model fitting were described in Section S2 (SI).

#### 3.1.2 Response surface plots

The four independent variables (S/I, SRT, OLR and T) and the effects of their interactions on VFA/SCOD against any two variables holding other factors at the zero level were investigated to determine the optimum operation conditions by plotting 3D response surfaces (Fig. 1). Each response surface plot showed a clear peak, suggesting that the optimum condition was inside the design boundary. The optimum conditions of the four variations obtained by "Point optimization" tool of Design Expert 8.0 for maximum VFA/SCOD were as follows: S/I 5, SRT 7 d, OLR 9 g VS per L-d and T 40 °C, which were reasonable and similar with some previous literatures. It has been reported that SRT shorter than 8 d was favorable to acidogenic bacteria while longer than 8 d was beneficial for methanogens.[20] Lim *et al.*[21] and Chen *et al.*[12] showed a similar OLR (9 and 8.31 g VS per L-d, respectively) and variation trends with this paper. That was VFA concentrations increased with OLR increment, whereas a higher OLR was unstable to operate reactor for the viscous fermentation broth. Jiang *et al.*[4] reported the maximal VFA/SCOD (82.6%) could be achieved at a fermentation temperature of 45 °C, which was in favour of efficient solubilization and acidification of FW, whereas the VFA/SCOD obtained at 35 °C and 55 °C were only 75.6% and 26.4%, respectively. Improper fermentation temperature could suppress the growth of hydrolytic and acidogenic bacteria and relevant enzymatic activities. In addition, it was worth noting that the S/I used in this study not only provided microbial biomass but affected pH in fermentation reactors. Thus, the optimum S/I obtained in this paper might be different with others, and the detailed discussions will be shown in the following section.

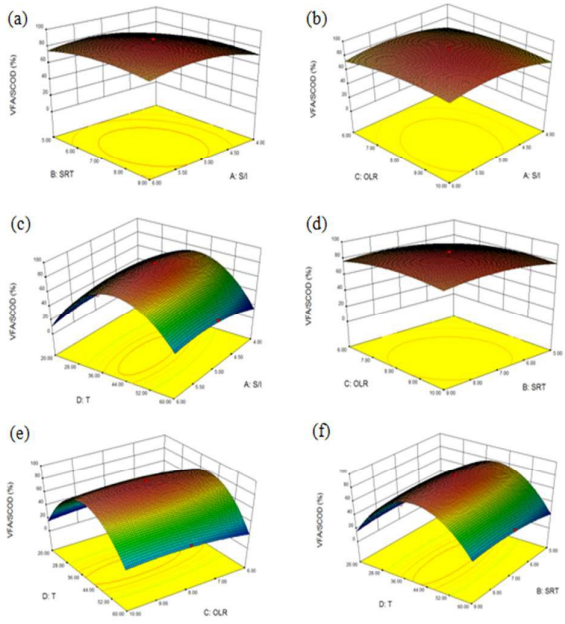


**Fig. 1** Three-dimensional response surface plots for VFA/SCOD. Effects of (a) S/I and SRT at OLR 8 g VS per L-d and T 40 °C; (b) S/I and OLR at SRT 7 d and T 40 °C; (c) S/I and T at SRT 7 d and OLR 8 g VS per L-d; (d) SRT and OLR at S/I 5 and T 40 °C; (e) SRT and T at S/I 5 and OLR 8 g VS per L-d; (f) OLR and T at S/I 5 and SRT 7 d.

### 3.1.3 Verification of the model

The fermentation experiments under above optimum conditions were performed in triplicate, and the average value of VFA/SCOD was 88.65%, which was in good agreement with the predicted value (89.79%). The maximum VFA/SCOD was compared with some semi-continuous tests (Table 2), which applied other operation parameters or treatment methods. As seen in Table 2, Zhang *et al.*[22] obtained a higher VFA/SCOD (90.36%), which was mainly contributed to the constant pH control of 6.5 by manually adding NaOH/HCl every day. In this way, chemical agents addition cost would hinder its scale-up application. Although the VFA/SCOD (88.65%) obtained in this study ranked a bit lower, the advantage was that pH self-adjust mode enabled this method cost-effective use. Besides, it was noted that the optimum conditions used in this study were more advantageous to gain a higher VFA/SCOD and VFA production compared with the fermentation conditions in other literatures.

**Table 2** Comparison of VFA/SCOD with other relevant literatures

| Substrates | Operation conditions | pH | VFA/SCOD | Reference |
|---|---|---|---|---|
| FW | SRT 5 d, OLR 11 g TS per L-d, 35 °C | 6 | 72.8% | 4 |
| FW | S/I 4, 30 °C | 6 | 50-70% | 5 |
| FW | SRT 9 d, 35 °C | 9 | 61.0% | 8 |
| FW | SRT 8 d, OLR 9 g TS per L-d, 35 °C | 6 | 63.29% | 21 |
| FW | S/I 9, SRT 8 d, OLR 4 g VS per L-d, 35 °C | 6.5 | 90.36% | 22 |
| FW | S/I 5, SRT 7 d, OLR 9 g VS per L-d, 40 °C | - | 88.65% | This study |

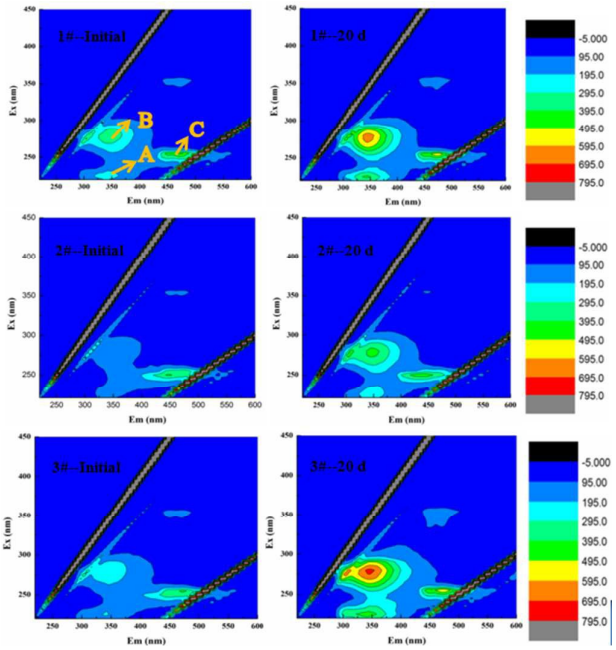


**Fig. 2** EEM fluorescence spectroscopy analysis of DOM.

### 3.2.2 EEM fluorescence spectroscopy analysis of soluble substrates

The composition of dissolved organic matters (DOM) plays a significant role in the microbial growth and VFA production. EEM can provide an overall view of fluorescent properties of DOM over a selected spectral range by locations and intensities of fluorescence peaks.[14] Thus, EEM analysis was introduced to further investigate the component variations of soluble substrates and clearly reveal the effects of FW hydrolysis and utilization. The EEM fluorescence spectra of the initial supernatant before fermentation and the fermentation liquid after 20 d operation are shown in Fig. 2. And the variations of peak locations and intensities are shown in Table S5. Three main fluorescence peaks marked as Peak A, B and C were observed, which were associated with tryptophan-like protein (moderate biodegradability), soluble microbial by-product (high biodegradability) and humic-acid like organics (low biodegradability), respectively.[23, 24] It can be seen from Fig. 2 and Table S5 that, the specific fluorescent intensities of peak A, B and C were strengthened after 20 d operation compared with those in initial supernatants, especially in 3#. Indicating that plentiful fluorescence materials such as protein-like, humic-like and so on were dissolved out by anaerobic fermentation. The shifts of peak location are always associated with the variations of soluble organic matters in the fermentation liquid.[25] Previous literatures reported that a red shift was connected with the solubilization of complex substrate,[24, 26, 27] while a blue shift was mainly contributed to the hydrolysis or degradation of organic matter.[25,26] The fluorescent intensities of Peak A and B in 3# enhanced 118.3% and 159.6%, respectively, while Peak C only increased by 59.4%, indicating that the substrates in 3# produced abundant high and moderate biodegradable organic matters. Besides, Peak B in 3# had an obvious blue shift, while Peak A and C appeared the tendencies of red shifts. Suggesting 3# not only

obtained a higher hydrolysis degree in biodegradable organics but also significantly enhanced the solubilization of hard-biodegradable substrates. Therefore, considerable soluble organic fragments were released into fermentation liquid of 3# including carboxyl, amino, hydroxyl and alkoxy groups,[27] which provided enough substrates for higher VFA production. In addition, it was noted that all of the fluorescent intensities in 1# and 2# were much lower than 3#, and expect Peak B in 1# showed a blue shift tendency, all of the Peaks appeared a slight red shift. The phenomenon demonstrated the substrates in 1# and 2# obtained incomplete dissolution and insufficient biodegradable organics, thus led to lower hydrolysis efficiencies.

### 3.2.2 The production and composition of VFA

VFA/SCOD, VFA production and VFA composition were often used to represent the degrees of hydrolysis and acidification.[11] It was observed that the VFA/SCOD in 1#, 2# and 3# were 20.32%, 14.65% and 88.65%, and the corresponding VFA productions were 314.57, 168.38 and 867.42 mg COD per g-VS, respectively. Moreover, the variations of VFA productions with fermentation time were presented in Fig. S2 and relevant analyses were shown in S3 (SI). Obviously, both of the VFA/SCOD and VFA production in 3# were much higher than 1# and 2#. The different acidic abilities were mainly contributed to the different pH, which directly influenced the hydrolysis and acidification efficiencies. And the reason will be further investigated in following section.

The detectable VFA mainly included acetic, propionic, iso-butyric, n-butyric, iso-valeric and n-valeric acids, and the individual VFA concentrations and percentages were shown in Fig. 3. It can be seen that acetic acid was the most predominant VFA (31.52%, 43.43% and 34.42% for 1#, 2# and 3#, respectively, and corresponding concentrations were 99.15, 73.13 and 298.556 mg COD per g-VS, respectively), and propionic acid ranked the second (22%-30%), followed by n-butyric acid, which was similar with the result of Chen *et al.*[16]. However, Feng *et al.*[28] reported that the top three VFA were propionic > acetic > n-butyric acids, the different observation might be attributed to their higher pH (6.0-9.0), which could promote the conversion of acetic and butyric acids to propionic acid.[29] In addition, the SRT 7 d in this study also gave positive influence on the acetic acid accumulation but negative effect on that of propionic acid, owing to plenty of propionic acid could be converted to acetic acid by acetogenic bacteria in continuous fermentation with a SRT 7 d.[13] Moreover, the concentrations and percentages of propionic acid increased while acetic acid decreased in 1# (sole FW) and 3# (S/I 5) compared with 2# (sole WAS). It might be due to the higher carbohydrate/protein in FW than that in WAS. The carbohydrate acidification mainly produced propionic acid, while acetic acid was the top fraction of protein fermentation in FW and WAS treatment.[9] The higher protein concentration in 2# also contributed to higher percentages of iso-valeric and n-valeric acids, which were mainly associated with protein fermentation by deamination of single amino acids or oxidation-reduction of a pair of amino acids.[30]

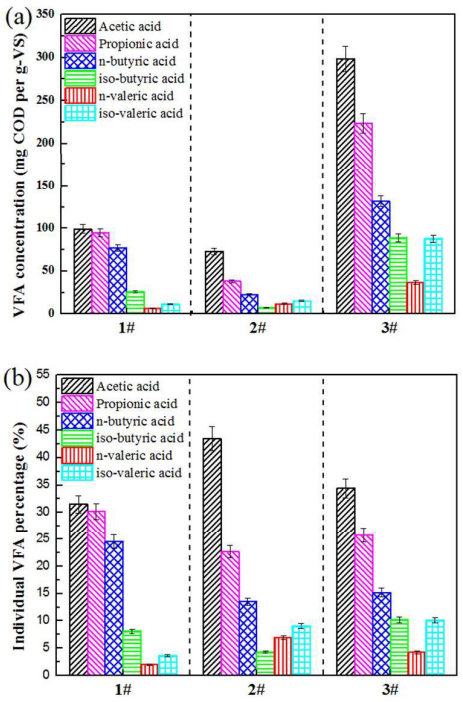


**Fig. 3** Comparison of individual VFA in 1#, 2# and 3#: (a) concentrations, and (b) percentages.

### 3.2.3 The production of methane

It is well known that, the generated VFA can be further converted to methane by methanogens during anaerobic fermentation.[3, 9, 11] As reported by Rittmann *et al.*[31], 1 g BOD could generate 0.35 L methane under standard temperature and pressure. The fermentation substrate in 3# had an initial BOD concentration of 19.684 g $L^{-1}$, and the theoretical methane production should be 3.43 L (0.35 L × 19.684 g $L^{-1}$ × 0.5 L = 3.43 L) if BOD was fully utilized. But in fact, the average methane production was 183.19 mL, which only accounted for 5.34% of the theoretical methane production. Therefore, only a small part of VFA was consumed by methanogens to produce methane, and more generated VFA was accumulated. However, for 2#, it presented relatively higher methane production of 480.16 mL, while only obtained a relatively lower VFA production (168.38 mg COD per g-VS) compared with 3# (867.42 mg COD per g-VS). Indicating that high methanogens activity in inoculum had negative effect on VFA production. Therefore, the suppression of methanogens in inoculum was of great significance. It was also noted that 1# represented low VFA and methane productions simultaneously, and along with a low pH range of 3.7-4.8 during the whole fermentation process (Fig. 4). There were two likely reasons. On one hand, too much FW accumulation and insufficient microorganisms caused the food overloading, which slowed down the hydrolysis and acidogenesis processes.[32] On the other hand, the low pH inhibited the growth and activity of microorganisms, and prevented the further dissolution of organic matters and the subsequent correlative reactions.[33]

**Table 3** The comparison of methane production in three reactors

| Parameters | 1# | 2# | 3# |
|---|---|---|---|
| Methane production (mL per g-VS) | 34.77 | 53.89 | 20.56 |
| Practical methane yield (mL) | 309.80 | 480.16 | 183.19 |
| Theoretical methane yield (L) | 3.47 | 1.89 | 3.43 |
| Practical/Theoretical (%) | 8.93% | 25.41% | 5.34% |

In fact, the methane productions obtained in the three reactors were far below the theoretical values (Table 3). In the literature, Luo *et al.*[13] and Miron et al.[20] reported SRT shorter than 8 d was favourable to the acidogenic bacteria, whereas SRT exceeded 8 d would enhance the activities of methanogens. Thus the low methane productions could be partly contributed to the opportune SRT 7 d. And as a main inhibitor in aceticlastic methanogenesis (one of the main methanogenic pathways), the high acetic acid concentrations obtained in this study especially in 3# also led to low methane productions.[2] In addition, the methane production was significantly influenced by the pH variations. The pH drop caused by the rapid VFA accumulation could directly lead to the activity loss of acid-sensitive glycolytic enzymes and the inhibition of methanogen, but too low pH was also adverse to acidification bacteria.[34] Therefore, the different VFA and methane production were also greatly contributed to the different pH levels in the three reactors.

**3.2.4 Kinetics analysis of hydrolysis, acidification and methanation**

The optimized kinetic parameters in the three fermentation stages are shown in Table 4. The hydrolysis rate ($k$) of 0.0667 $d^{-1}$ in 3# was higher than the value (0.058 $d^{-1}$) reported by Xu et al.,[16] which used a similar S/I with this study. Indicating that the optimal conditions used in the study was more beneficial to improve hydrolysis efficiency. Firstly, the suitable OLR 9 g VS per L-d provided a stable fermentation performance,[35] and opportune fermentation T 40 $^{o}$C could effectively enhance the activities of hydrolytic bacteria and relevant enzymes, at the same time SRT 7 d provided sufficient time for the degradation of particular substrates into dissolved forms and the conversion of complex VS into simple compounds.[21] Moreover, it was noticed that the hydrolysis rate ($k$) and maximum utilization rate of hydrolysis products ($k_h$) in 3# were higher than the values obtained in 1# and 2#. Suggesting the S/I 5 played a significant role both in enhancing the hydrolysis of particular organic matters and the conversion of soluble hydrolysis products to VFA. The opportune microbial biomass provided by S/I 5 prevented the excessive accumulation of FW and made plenty of solid organics further dissolve into liquid. Besides, the appropriate pH range for hydrolytic-acidogenic bacteria and relevant enzymes caused by S/I 5 was also closely related to the higher hydrolysis and acidification efficiencies. It was also noted that the relatively lower maximum utilization rates of VFA ($k_v$) were found in the three reactors especially in 3#, which were in accordance with the practical methane productions (Table 3), and the reasons have been detailedly discussed in 3.2.3. Overall, the high VFA production in 3# could be well explained by the higher $k$ and $k_h$, and the lower $k_v$.

**Table 4** Kinetic parameters of anaerobic fermentation

| Parameters | 1# | 2# | 3# |
|---|---|---|---|
| $K$ ($d^{-1}$) | 0.0329 | 0.0161 | 0.0667 |
| $k_h$ (g SCOD $d^{-1}$) | 4.25 | 2.68 | 11.65 |
| $k_v$ (g VFA $d^{-1}$) | 0.76 | 1.19 | 0.37 |

**3.2.5 Carbon mass balance analysis**

Based on the experimental results, the carbon mass balance analyses were established as COD mass to further understand the carbon conversion efficiencies, and the variations and contributions of different organic matters could also be clearly revealed. In generally, the carbon in FW would be converted into VFA, protein, carbohydrate, lactic acid, alcohol, methane and carbon dioxide by anaerobic fermentation.[11] To simplify calculation, the carbon contents in the soluble protein, carbohydrate, VFA and methane were determined. The other carbon-containing compounds, such as lactic acid, alcohol and carbon dioxide were refered to "other". The carbon mass balance analyses of the three reactors after 20 d operation were conducted, and the results are shown in Fig. 4.

The COD mass represented by carbohydrate, "other" and VFA were the three dominant substances in 1#, 2# and 3#, respectively. And the COD masses of "other" occupied considerable proportions both in 1# and 2# (31.35% and 62.77% of the total carbon, respectively), which simultaneously accompanied with the relatively higher methane masses and lower VFA masses compared with 3#. Besides, carbohydrate mass in 1# and protein mass in 2# also showed greater percentages than 3#, which were 34.23% and 11.31% of total carbon, respectively. These data clearly indicated that the fermentation substrates in 1# and 2# were not adequately utilized to produce VFA. However, the COD masses of "other" and methane in 3# were only 0.57 g and 0.32 g, which were consistent with negligible methane production reported above. And the COD mass from carbohydrates and proteins only accounted for a small part of the total COD mass compared with the VFA mass. Meanwhile the VFA mass increased significantly and reached a highest value (7.73 g) in 3# compared with 1# (2.83 g) and 2# (1.50 g). Therefore, the optimum S/I 5 combined with other optimized condition (SRT 7 d, OLR 9 g VS per L-d and T 40 $^{o}$C) were conducive for the sufficient utilization of particular organic matters and the conversion of hydrolysis products into VFA.

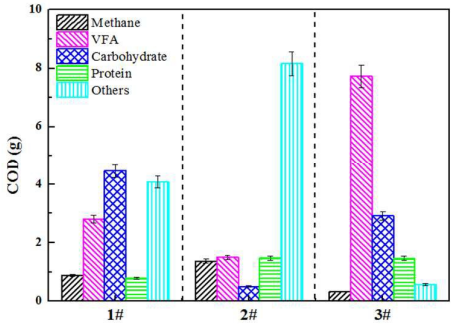


**Fig. 4** Carbon balance analysis of the fermentation substrates.

### 3.3 Discussion

The pH plays a crucial role in VFA production due to its effects not only on the anaerobic bacteria community structures, activities and growth rates, but also on metabolic pathways.[32] VFA accumulation would affect the pH in fermentation liquid during anaerobic fermentation, and the pH variation directly influenced hydrolysis, acidification and methane generation. Veeken *et al.*[36] reported that hydrolysis of bio-waste was inhibited at pH 5.0, and Wang *et al.*[11] observed that the activity of acidogenic bacteria was suppressed at pH below 4.0. Some previous studies reported that the optimal pH for effective hydrolysis and suppressive methanogenesis was 5.0-6.5,[11, 16] while higher pH (exceed 6.5) could bring about high methanogen activity.[37] As shown in Fig. 5, fast drops of pH in the start phase caused by the rapid acidification of substrate were observed in all reactors, which meanwhile generated a certain inhibitory effect on methanogens.[2] Followed by a slight increase and then a stabilization due to the neutralization of fresh substrate and ammonia release. The pH in 1#, 2# and 3# were not adjusted artificially in the whole fermentation processes, which were maintained in 3.7-4.8, 6.1-6.9, and 5.2-6.4, respectively. This phenomenon was mainly attributed to the buffering capacity of the system itself caused by the released ammonia.[38]

The variations of ammonia concentrations during anaerobic fermentation are also shown in Fig. 5. It can be seen that, there was a significant release of ammonia together with the production of VFA, and the ammonia concentrations were 240-580, 740-1310, and 820-1190 mg $L^{-1}$ in 1#, 2# and 3#, respectively. The difference of ammonia concentration in different reactor was in line with their composition of the substrate, which was produced by the biological degradation of protein and amino acid.[11] The high ammonia level in 2# was attributed to the fact that WAS was a material rich in protein as opposed to FW (1#), which was principally composed of carbohydrate. It should be noted that the variation of pH was closely connected with the variation of ammonia concentration. The release of ammonia provided a high alkalinity, which could compensate for the decreased pH.[25] Particularly, during the whole fermentation, the pH in 3# fluctuated between 5.2 and 6.4, a range that was mentioned above to be optimum for hydrolysis and acidification, and inhibited for methanogenesis, resulting in a high-efficiency hydrolysis, higher VFA production and minimum methane production compared with 1# and 2# (Table 3). The favorable pH range in 3# was mainly attributed to the optimum S/I 5, which provided opportune amount of ammonia source to form high buffering capacity. At the same time, it was noted from Table 3 together with Fig. 5 that, the pH in 1# was less than 5.0, and the particulate substrates could not be thoroughly dissolved out and hydrolyzed, which resulted in low VFA and methane productions. In addition, the vast release of ammonia in 2# resulted in a relatively higher pH range (6.1-6.9), which encouraged the production of methane through methanation while presented a low VFA production. Therefore, it can be concluded that the optimum proportion of FW and WAS addition can obtain a suitable pH range in semi-continuous fermentation reactor, which is in favour of hydrolysis, acidification and inhibited for methane generation, thus contributed to a higher VFA production.

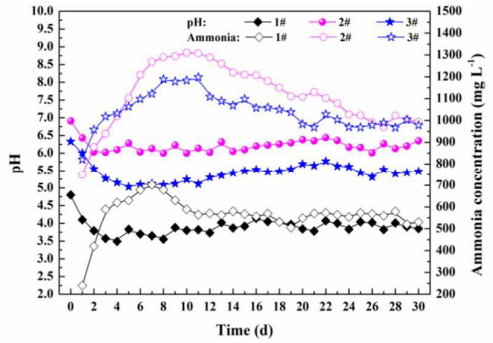


**Fig. 5** The variations of pH and ammonia.

## 4. Conclusions

A high VFA production was obtained from FW semi-continuous anaerobic fermentation without pH artificially control, which provided a cost-effective VFA production strategy for practical continuous production. The optimum conditions were S/I 5, T 40 °C, SRT 7 d and OLR 9 g VS per L-d, and the high VFA/SCOD (88.65%) and VFA production (867.42 mg COD per g-VS) were achieved. The high VFA production was mainly contributed to the advantageous pH (5.2-6.4) obtained by the buffering capacity of fermentation system, which was favorable for hydrolysis and acidogenesis of substrates, and inhibited for methanogenesis. And the main VFA compositions were acetic, propionic and n-butyric acids.

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