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Article

Mesophilic and Thermophilic Fermentation of Waste Activated Sludge for Volatile Fatty Acids Production: Focusing on Anaerobic Degradation of Carbohydrate and Protein

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Abstract: The volatile fatty acids (VFAs) productions, as well as particulate organics decomposition, soluble chemical oxygen demand (SCOD) yield, and the VFAs production pathways, of mesophilic and thermophilic anaerobic fermentation were investigated. Batch experiments showed that the decomposition rate of volatile suspended solids (VSS), particulate carbohydrate (P-C) and particulate protein (P-P) followed the first-order kinetic model at different temperature. However, the intermediates, accumulated in the process of protein or carbohydrate digestion had a more significant inhibitory effect on the production of VFAs during the mesophilic anaerobic acidification process. Thermophilic fermentation led to more accumulation of VFAs compared to mesophilic fermentation. Then ¹³C stable isotope labeling experiment demonstrated that, acetic (HAc), propionic (HPr) and butyric (HBu) could be produced from carbohydrate and protein digestion, but valeric (HVa) was only generated from the degradation of protein. Finally, the possible pathway of HVa synthesis was proposed.

Keywords: anaerobic hydrolysis and acidification; mesophilic; thermophilic; waste activated sludge; volatile fatty acids

1. Introduction

In recent decades, economic growth, urbanization and climate change require the development and implementation of more sustainable strategies to maximize resource recovery from waste (Fernández-Domínguez et al., 2020). Anaerobic fermentation for volatile fatty acid (VFA) production is one of the efficient method for resource utilization of waste activated sludge (WAS) (Yuan et al., 2019). Short-chain fatty acids (SCFAs) production by anaerobic fermentation of waste activated sludge has attracted growing attention in wastewater treatment plants (WWTPs) since SCFAs can be widely used in bioenergy, bioplastics and biological removal of nutrient from wastewater (Wang et al., 2017, Liu et al., 2019, Xu et al., 2019, Adeleye et al., 2020, Wang et al., 2022). In order to achieve a great VFAs yield, the adjust and control of fermentation temperature is a simple and practical strategy since temperature can exert tremendous influences on fermentation products (Fernández-Domínguez et al., 2020). Conventional mesophilic conditions (30 - 40°C) have long been adopted for anaerobic digestion with a good operational performance, but the accumulation of VFAs is limited due to the slow hydrolysis rate of large organic molecules associated with microbial cells (Eskicioglu et al., 2006). In contrast, a higher fermentation temperature (i.e., thermophilic regimes, 50 - 60°C) is more favorable because it can result in higher metabolic activities and substrate conversion rates (Cho et al., 2015). Several successful cases of sludge thermophilic treatment in full-scale digesters approved the stability of the thermophilic operation (Nielsen & Petersen, 2000, Ahring et al., 2002). However, few researchers are concerned about VFAs yield and composition during sludge thermophilic fermentation, particularly WAS treatment (Şentürk et al., 2010, Xiong et al., 2012) have also been

adopted for anaerobic digestion (Tian et al., 2015, Zhang et al., 2015) as thermophilic process derives from higher metabolic activities and substrate conversion rates than mesophilic.

The valeric (HVa) was beneficial to produce a polymer enriched in polyhydroxyvalerate (PHV) (Lemos et al., 2006) which improved more elastic and flexible and raise the price of polyhydroxyalkanoates (PHAs) (Kulkarni et al., 2010, Kulkarni et al., 2011, Tamis et al., 2014). Although HVa was found in several researches for anaerobic acidification and PHA synthesis (Wang et al., 2013, Zheng et al., 2013, Jie et al., 2014), no one proposed a possible pathway for HVa synthesis in the anaerobic acidification process. In this study, the sludge produced by the sewage treatment plant was used as the raw material, and the key parameters such as particle size, particle organic matter degradation, SCOD, and VFAs were analyzed to evaluate the hydrolysis and acidification effect of mesophilic and high-temperature anaerobic digestion. Further analysis of the source of VFA during carbohydrate and protein degradation. Finally, a possible pathway of HVa synthesis was proposed.

2. Materials and Methods

2.1. Source of waste activated sludge

The waste active sludge (Sludge A) used for anaerobic hydrolysis and acidification analysis was obtained from a drum thickener of Xiaohongmen municipal wastewater treatment plant in Beijing, China. This plant using typical anaerobic–anoxic–oxic (AAO) treatment process, with treatment capacity of 600,000 m³/day. The waste active sludge (Sludge B) used for stable isotopic labeling test was taken from a sludge thickening tank of the Ashbridges Bay treatment plant in Toronto, Canada. This plant have a treatment capacity of 576,000 m³/day. All collected samples were transported to laboratory within 2 h after sampling and stored at 4°C.

The characteristics of WAS were listed in Table 1. The ratio of VSS in TSS is more than 67.71%. This indicated that sludge A and B contained mainly activated biomass or organic matter. Furthermore, particulate protein (P-P), particulate carbohydrate (P-C) and particulate lipid (P-L) are the three predominant types of organic compounds in the two types of WAS, and they account for above 80% of TCOD. Obviously, P-P was the top organic compound in WAS. But the concentration of P-L and P-C were slightly different. It changes considerably, due to seasonal variations and human habits between two countries.

Table 1. Characteristics of thickened waste activated sludge sample.

Parameter	Unit	Sludge A Mean ^a ± SD ^b	Sludge B Mean ^a ± SD ^b
TSS (total suspended solids)	g/L	22.21±1.21	23.37±1.60
VSS (volatile suspended solids)	g/L	15.04±2.52	17.58±2.10
pH		7.02±0.01	6.97±0.01
SCOD (soluble chemical oxygen demand)	mg/L	561.79±11.03	590.81±21.32
TCOD (Total chemical oxygen demand)	mg/L	21326.72±125.62	24166.11±372.23
P-P (particulate protein) ^c	mg COD/L	12850.06±242.50	12202.50±142.65
P-C (particulate carbohydrate) ^c	mg COD/L	2369.56±47.39	4647.18±36.73
P-L (particulate lipid) ^c	mg COD/L	4066.57±86.63	3050.35±63.39
S-P (soluble protein) ^c	mg COD/L	73.20±1.45	31.22±2.36
S-C (soluble carbohydrate) ^c	mg COD/L	38.46±1.76	195.76±10.82
HA (humic acid) ^d	mg COD/L	14.73±0.82	71.31±3.26
TVFAs (total volatile fatty acids)	mg COD/L	423.32±16.93	222.05±21.63

a. Mean value of three individual determinations. b. Standard deviation. c. The COD conversion factors for protein, carbohydrate and lipid is described by Grady(Grady *et al.*, 1999). d. The COD conversion factors of HA was 1.2236, detailedly shown in Supplementary Information (Figure S1, Page 2).

2.2. Anaerobic hydrolysis and acidification batch experiment

The pretreatment was performed in six identical flasks, each was fed with 1500 mL WAS (Sludge A), and the pH was not controlled. The flasks capped with rubber stoppers were placed in two rotary shakers at 170 rpm. The operation conditions for each of the digestion systems are summarized in Table S1. The samples in all flasks were assayed every certain interval. During operation process, flasks maintained anoxic condition and experimental domestication adopted natural selection. All tests were performed in triplicate.

2.3. Kinetics analysis of mixed liquid volatile suspended solids (MLVSS) disintegration

As reported before, hydrolysis of organic polymers is often described by a first-order kinetic model [Eq. (1)] as the enzymatic activity is not directly coupled to the bacterial growth (Eastman & Ferguson, 1981, Sosnowski *et al.*, 2008, Sosnowski *et al.*, 2008).

$$-\frac{dX_x}{dt} = k_x X_x \quad (1)$$

where X_x was the concentration of X type particulate organics (g/L or mg/L), k_x was the hydrolysis constant of X type organics (h^{-1}) and t was time (h).

After merging polynomial, Eq. 1 can be shown as:

$$X_{x_t} = X_{x_0} \times \exp(-k_x t) \quad (2)$$

2.4. Stable isotopic labeling test

Stable isotopic labeling (SIL) test was prepared with 72 mL with different feed and 8 mL inoculum (Hydrolyzed Sludge B) in a 160 mL glass serum bottles, as shown in Table S2. The inoculated sludge is the mixed sludge obtained after 1.5 L of thickened sludge was incubated at 50 °C for 2 d. The feed included glucose, bovine serum albumin (BSA) and vegetable oil (VO) to simulate the elements of sludge and control the same total COD (TCOD) of feed for all SIL tests. TCOD was about 2.1g for 72 ml feed volume. D-Glucose- $^{13}\text{C}_6$ was the stable isotopic chemicals. All serum bottles were incubated at 50°C for 5 days, respectively in a rotary shaker at 170 rpm without pH control. Samples were taken at predetermined time for the analysis of VFAs and ^{13}C . All tests were performed twice.

2.5. Analytical methods

2.5.1. Excitation-emission matrix (EEM) fluorescence spectroscopy

All EEM spectra were measured on luminescence spectrometry (F-7000, Hitachi Co., Japan). The EEM spectra were recorded at 5 nm intervals for excitation spectra between 220 and 600 nm and emission spectra between 230 and 650 nm at a scanning speed of 1200nm/min. The spectrum of distilled water was recorded as the blank. The supernatant obtained after centrifuging the WAS at 4°C and 10,000 rpm for 10 min. The centrifuged supernatant could be used for EEM analysis after filtrating through a 0.22 mm-pore-size glass fibre filter. The software Origin Pro 8.5 (Origin Lab Inc., USA) was employed for handling EEM data.

EEM spectra are illustrated as the elliptical shape of contours. The X-axis represents the emission spectra from 250 to 550 nm, and the Y-axis is the excitation wavelength from 250 to 450 nm.

2.5.2. Determination of ^{13}C stable isotopic label

The negative ions of ^{13}C -VFAs stable isotopic label were determined by mass selective detection (MSD, Thermo Scientific Q Exactive, USA), such as CH_3COO^- and so on. The WAS before and after the digestion were centrifuged at 4°C and 10,000 rpm for 10 minutes to obtain the supernatants, which were filtered with the glass fiber filter ($0.22\ \mu\text{m}$) and stored at 4°C before testing.

2.5.3. Other items' detected methods

The measurement of pH, soluble COD, $\text{NH}_4\text{-N}$, TN, OP, TP, TSS, VSS and VFAs were the same as described in our previous work (Xiong et al., 2012). Soluble carbohydrate (S-C) was measured by the phenol-sulfuric method with glucose as standard (Herbert et al., 1971). Soluble protein (S-P) and humic acids (HA) were determined by the Modified Lowry method (Peterson, 1979, Frolund et al., 1995) with BSA (Prod # 23209, Thermo) and humic acid (53680 MSDS, Sigma-Aldrich) as standard.

Total carbohydrate (T-C) was according to standard of GB/T 15672-2009. Total protein (T-P) was according to standard of ISO1871:2009. Total lipid (T-L) was extracted by the Bligh-Dyer method from the acidified sample, and was then measured gravimetrically after the solvent was evaporated at 80°C (Bligh & Dyer, 1959).

Particle size analysis was carried out using the LS I3 320 (stirrer speed=350; use pump =50%; ultrasonic=off) at Beckman Instruments Ltd., USA. For analysis, each sample was not diluted. The results were then averaged to produce the particle size distribution, and recorded as the particle volume percent between 0.45 and $2000\ \mu\text{m}$.

2.6. Calculation

The COD conversion factors of VSS, proteins, carbohydrates, lipids, and VFAs, used in this study, were performed according to Grady's description (Grady et al., 1999). All formulars were shown in the Supplementary Information (Table S3, Page 5).

3. Results and discussion

3.1. Effects of mesophilic and thermophilic anaerobic digestion on sludge particle size

Sludge particles are approximately spherical, so average particle size can often be used as an indicator to characterize the physical properties of WAS (Brittain, 2001). Figure 1 shows the change of particle size distribution of WAS with time under mesophilic and thermophilic hydrolysis conditions. The particle size of WAS all decreased with time, and the mean particle sizes were $234\ \mu\text{m}$ (35°C) and $121\ \mu\text{m}$ (50°C) at 48 h, respectively. High temperature had significant decreased the particle sizes of the sludge floc. It can be inferred that the rate of VSS destruction at 50°C may be better than at 35°C .

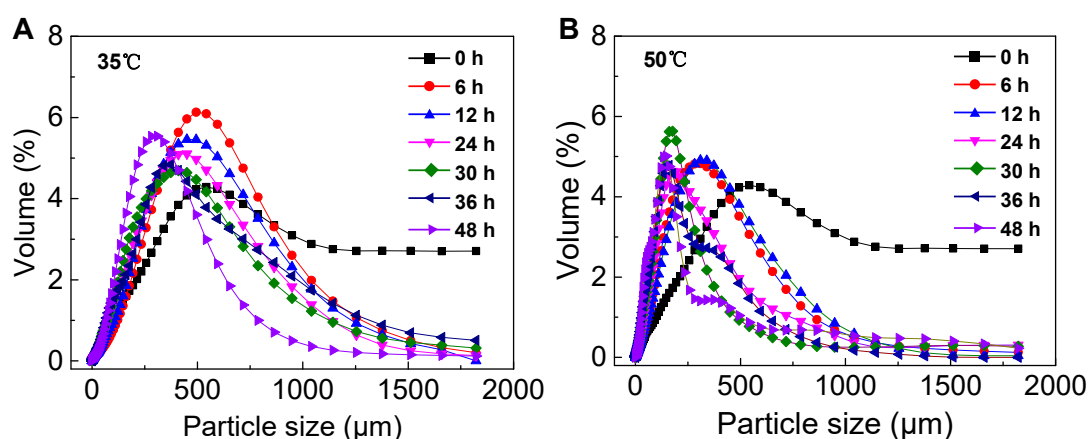


Figure 1. Particle size distribution of WAS during hydrolysis. (A) mesophilic digestion and (B) thermophilic hydrolysis.

3.2. Kinetics analysis of MLVSS, P-C and P-P hydrolysis

Particulate protein (P-P), particulate carbohydrates (P-C) and particulate lipid (P-L) are important particulate organic substances in waste activated sludge. During the mesophilic and thermophilic anaerobic hydrolysis, the main decomposed organics were P-P and P-C, while the degradation rate of P-L was less than 5%, as shown in Table 2. The reason for this phenomenon is that P-L is hardly disintegrated during hydrolysis and acidification. Some researchers have come up with similar results in their study (Eastman & Ferguson, 1981, Miron et al., 2000, Martínez et al., 2012). Therefore, P-L was not considered in the subsequent analysis process. The decomposition rates of MLVSS, P-C and P-P in thermophilic digestion were all higher than those in mesophilic digestion, indicating that higher temperature helps to improve the hydrolysis efficiency of WAS.

Table 2. The degradation rate of P-C, P-P and P-L at 48h.

Degradation Rate(%)	35°C	50°C
MLVSS		
P-C	32.98	54.70
P-P	22.10	42.52
P-L	3.27	4.26
MLVSS	32.69	41.10

As reported by Siegrist et al., the hydrolysis of particulate organic matter and dead biomass, converting carbohydrates, proteins, and lipids to sugars, amino acids, and long-chain fatty acids, follows first-order reaction kinetics (Siegrist et al., 2002). According to the equation. 2, $X_{MLVSS0}=21365.8$ mgCOD/L, $X_{P-C0}=2369.56$ mgCOD/L and $X_{P-P0}=12850.06$ mgCOD/L, and the degradation equations of MLVSS, P-C and P-P are obtained, see Table 3. The k_x value were found during thermophilic hydrolysis process in the following order: $k_{P-C} > k_{MLVSS} > k_{P-P}$. This means that P-C is more easily degraded by microorganisms than P-P. The composition of sludge are complicated, including P-P, P-C, dead microbes, etc. Then k_{VSS} value must be restricted by above mentioned organics and should locate in the middle range. The hydrolysis constant of MLVSS (k_{MLVSS}) will be affected by these organic substances, so k_{MLVSS} lies between k_{P-C} and k_{P-P} .

Table 3. The hydrolysis rate constant of P-C, P-P and MLVSS.

Temperature (°C)	Kinetic equation	k_x (h ⁻¹)	Coefficient R ²
35°C	$y=2369.54 \times \exp(-0.00915x)$	$k_{P-C}=0.00915$	0.9486
	$y=12850.06 \times \exp(-0.00372x)$	$k_{P-P}=0.00915$	0.8208
	$y=15.04 \times \exp(-0.0101x)$	$k_{MLVSS}=0.01018$	0.7697
50°C	$y=2369.54 \times \exp(-0.02060x)$	$K_{P-C}=0.02060$	0.6370
	$y=12850.06 \times \exp(-0.01220x)$	$k_{P-P}=0.01220$	0.9606
	$y=15.04 \times \exp(-0.0126x)$	$k_{MLVSS}=0.0126$	0.9474

3.3. Effects of mesophilic and thermophilic anaerobic digestion on particulate organics degradation

Particulate organics mainly composed of P-P, P-C, P-L and other unknown particulate organics. Through the analysis of the degradation ratio of various types of particulate organic matter, the degradation types of the mesophilic and thermophilic digestion processes can be revealed. The COD content of MLVSS, P-P and P-C was estimated by mass balance analysis, and the result was shown in Figure 2. Unknown particulate organics were main degradable matters in mesophilic digestion process. After 48 h of digestion, the degradation ratios of unknown particulate organic matter, P-P and P-C accounted for 48.14%, 40.67% and 11.19% of MLVSS, respectively. Mesophilic microorganisms can utilize unknown particulate organics as substrates, probably because mesophilic microorganisms are more diversity than thermophilic microorganisms (Kim et al., 2002). These mesophilic anaerobic microorganisms can use unknown particulate organic matter as a substrate for catabolism and microbial anabolism.

In the thermophilic digestion process, in the initial stage of the digestion (0-12 h), unknown particulate organics and P-C were the main degraded substances. However, in the late stage of the digestion (24-48 h), P-P was main degradable substance. After 48 h of digestion, the degradation ratios of unknown particulate organic matter, P-P and P-C accounted for 22.99%, 62.24% and 14.76% of MLVSS, respectively. It can be seen from Figure 2A,C, the degradation of P-P mainly occurred after 36h under the condition of mesophilic digestion, while that of P-P occurred after 12h under the condition of thermophilic digestion, which means that the activity of protein-degrading bacteria at high temperature is significantly better than that at mesophilic temperature Song *et al.* (2004).

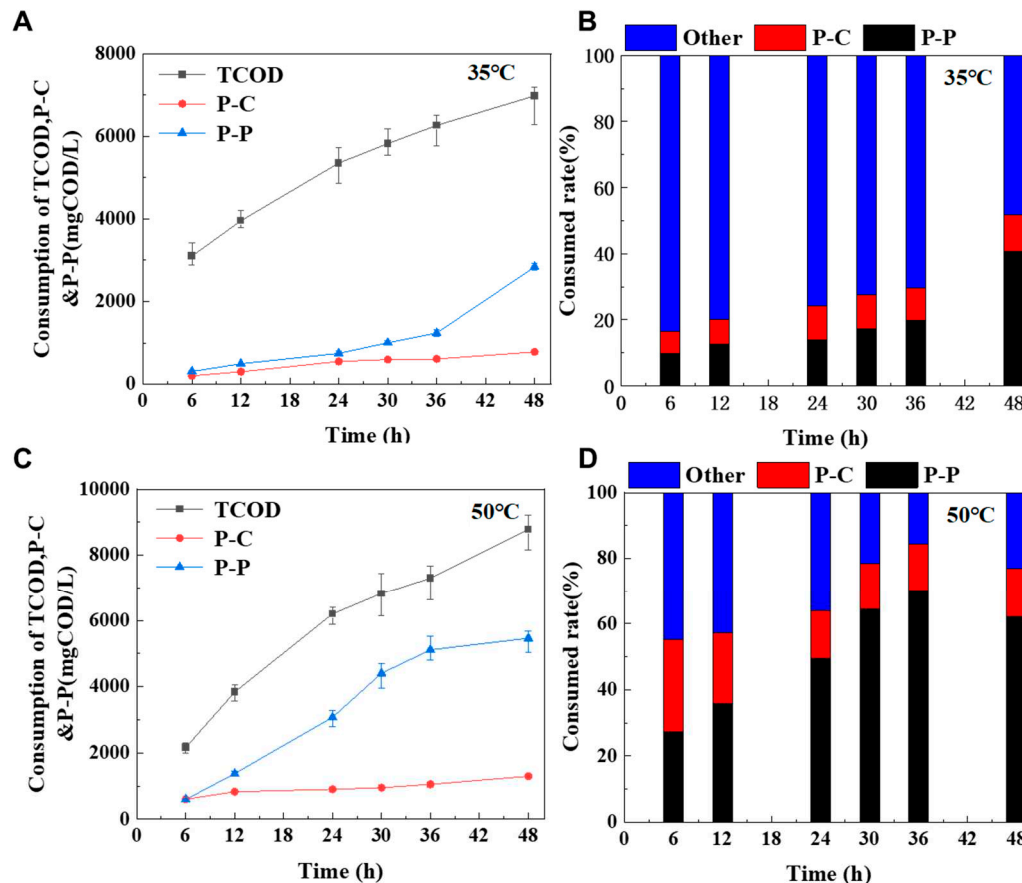


Figure 2. Particulate organics decomposition balance analysis of the digestion system. (A) and (B) mesophilic digestion; (C) and (D) thermophilic digestion.

3.4. Effects of mesophilic and thermophilic anaerobic digestion on hydrolysis and acidification

Hydrolysis and acidification are two steps in anaerobic digestion. Hydrolysis means both the solubilization of insoluble particulate matter and the biological decomposition of organic polymers to monomers or dimers, which can pass the cell membrane and enter into aqueous phase. Then SCOD will increase in hydrolysis process. During the acidification step, the dissolved organic matter is biodegraded mainly to VFAs and alcohols by a heterogeneous microbial population. The concentration of SCOD will keep balance as organic macromolecules degrade into smaller organic molecules in acidification step.

As shown in Figure 3, the SCOD value of mesophilic anaerobic digestion continued to increase from 0 h to 48 h. Then the reactor had been in hydrolysis step. From 0 h to 24 h, the SCOD value of thermophilic anaerobic digestion increased rapidly. After that, the SCOD value kept stably. Then thermophilic digestion had an obvious cut-off point: hydrolysis (0-24 h) and acidification (24-48 h). Thermophilic digestion was faster into acidification stage than mesophilic, as in Table 3, the hydrolysis constant values for thermophilic digestion were significantly higher than for mesophilic digestion.

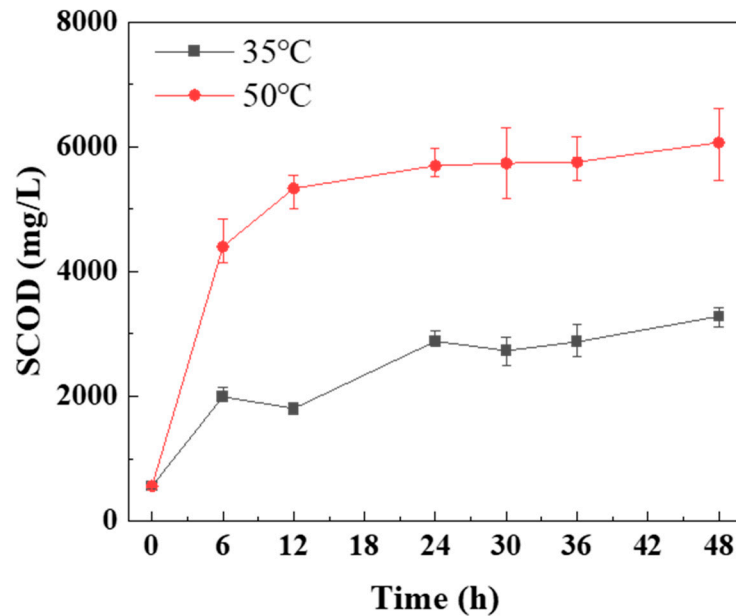


Figure 3. SCOD change between mesophilic and thermophilic anaerobic digestion.

SCOD mainly includes VFAs, humic acid (HA), soluble carbohydrate (S-C), soluble protein (S-P), and uncertain organics. The compositional changes of SCOD during hydrolysis and acidification are shown in Figure S2 (Supplementary Information, Page 6). VFAs, HA and uncertain organics were main soluble organic ingredients of mesophilic and thermophilic digestion. HA and uncertain organics were degraded after 12 h of thermophilic digestion with the raise of VFAs concentration. However, HA and uncertain organics had been accumulated in mesophilic digestion as the activity of microbes in mesophilic was lower than thermophilic digestion. This phenomenon suggests that the degradation of uncertain organic matter contributes to the acid production process of anaerobic systems.

Qualitative analysis of uncertain organics was detected by EEM fluorescence spectroscopy. As shown in Figure 4A,B, two peaks were readily identified from EEM fluorescence spectra of digestion supernatant. Peak A was identified at excitation/emission wavelengths (Ex/Em) of 220/350nm, while peak B was detected at Ex/Em of 275/350nm. These peaks had been inferred as protein-like peaks, in which the fluorescence is associated with the aromatic amino acid and tryptophan (Sheng & Yu, 2006, Wang et al., 2009). Therefore, uncertain organics might be intermediates of the decomposition of protein or carbohydrate. The VFAs yield of mesophilic digestion was less than thermophilic digestion because of the inhibitory effect of these intermediates.

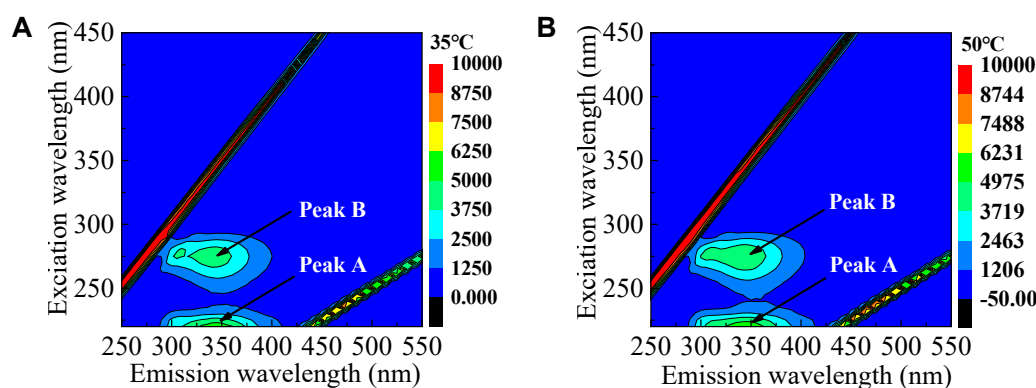


Figure 4. Fluorescence EEM spectra of the digestion supernatant. (A) mesophilic digestion and (B) thermophilic digestion.

3.5. Effects of mesophilic and thermophilic anaerobic digestion on VFAs yield

The production of six VFAs, acetic (HAc), propionic (HPr), iso-butyric (i-HBu), n-butyric (n-HBu), iso-valeric (i-HVa), and n-valeric (n-HVa), were investigated. As seen in Figure 5A–D, HAc was the predominant product at any time of digestion at mesophilic and thermophilic condition. During the mesophilic digestion, the content of HAc increased with digestion time and the maximum amount of HAc was 596.21 mgCOD/L at 48 hour. However, the concentration of HAc can reach a maximum of 1072.04 mgCOD/L within 24 h during thermophilic digestion. HAc concentration decreased slightly after 24 h and its concentration was 913.75 mgCOD/L at 48 h.

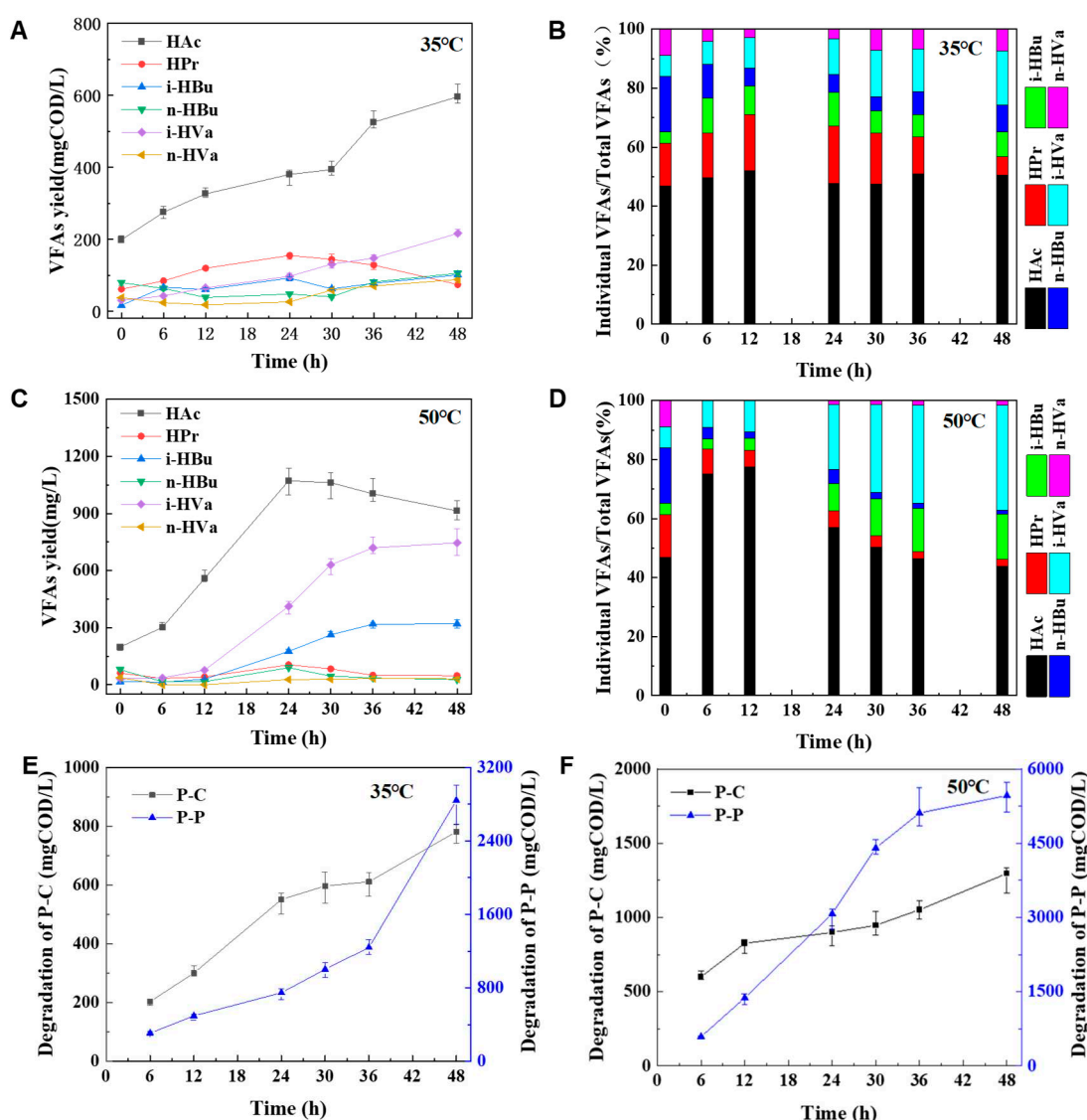


Figure 5. The relation among individual VFAs, P-C and P-P. A and B: VFAs production in mesophilic digestion, C and D: VFAs production in thermophilic digestion, E and F: P-C and P-P degradation.

In mesophilic digestion, HPr was the second predominant VFAs before 36 h. However, after 36 h, i-HVa acid became the second major VFA and reached a concentration of 216.55 mgCOD/L at 48 h. Compared to mesophilic digestion, i-HVa acid became the second predominant VFAs within 18 h, reaching a maximum content of 745.75 mgCOD/L at 48 h in the thermophilic digestion.

According to the degradation of P-C and P-P in Figure 5E,F, HAc and HPr increased with a large number of P-C consumption and a small amount of P-P degradation. It seemed that HAc and HPr mainly derived from P-C degradation (Zehnder, 1988). When a large amount of P-P was degraded during thermophilic digestion, the concentration of i-HBu and i-HVa also increased rapidly, while

the concentration of HAc also increased. This is because the conversion of amino acids to valeric, butyric, and propionic acids, also produces HAc (Ramsay & Pullammanappallil, 2001). So the conclusion is the lower molecular weight VFAs, such as HAc and HPr, are largely relevant to the digestion of P-C and P-P. But the higher molecular weight VFAs are only dependent on the degradation of P-P, just like i-HBu, n-HBu, i-HVa and n-HVa.

3.6. Concentration changes of VFAs in ^{13}C stable isotope labeling experiment

The concentrations of six VFAs (HAc, HPr, i-HBu, n-HBu, i-HVa, n-HVa) produced by using sludge B (Series 3#) were also analyzed, as shown in Figure S3 (Supplementary Information, Page 7). The concentration of i-HVa in VFAs was higher when P-P was the main particulate organic matter in the thickened sludge. To further verify this result, stable isotope labeling experiment were designed to reveal the proportions of different kinds of VFAs derived from P-P or P-C.

In the series 4#, the substrate used in anaerobic fermentation was glucose, so the fermentation product VFAs only contained three organic acids (n-HBu, HAc and HPr), as shown in Figure 6A,B. Three kinds of VFAs accounted for approximately 69%, 26% and 5% of total VFAs within 2 to 5 days of digestion. This result well matched with the butyric acid type digestion (BATF) without pH control when the substrate was only or mainly glucose during anaerobic acidification (Zoetemeyer et al., 1982, Cohen et al., 1984).

However, five types of VFAs can be produced in the series 5#, with the following order: n-HBu, HAc, i-HVa, HPr and i-HBu, as shown in Figure 6C,D. A significant lag in iso-valeric acid production, with a yield of only 16.76%. The reason for this difference may have been caused by obvious change from ^{12}C to ^{13}C in the substrate. The rate of microbe metabolism would be slightly varied when ^{13}C entered into the metabolic pathway of VFAs yield because of the atomic radius, electronic force and so on.

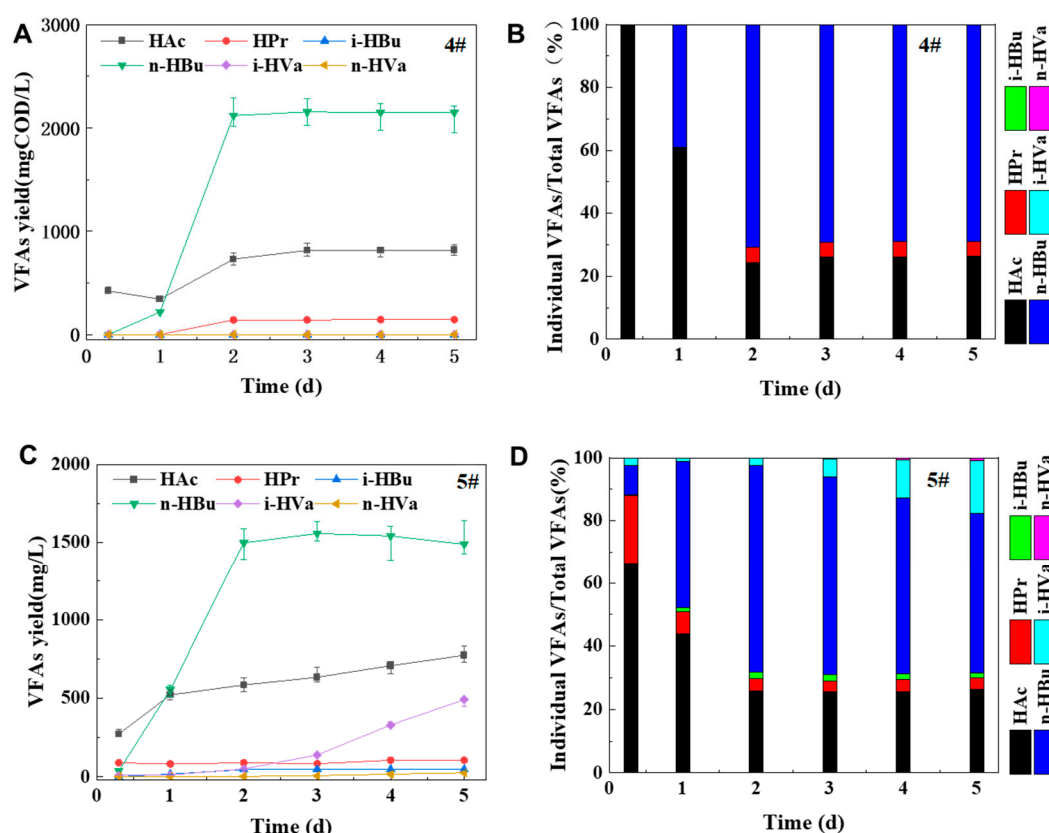


Figure 6. The concentration variation of VFAs under thermophilic anaerobic digestion. (A) and (B) Series 4#; (C) and (D) Series 5#.

3.7. The source of VFAs

The Thermo Q Exactivated MSD’s library has not the isomeride of butyric and valeric acids. Therefore, it should be defined that HBu contained iso-HBu and n-HBu. Similarly, HVa included iso-HVa and n-HBu. According to the different amounts of ¹²C and ¹³C atoms, HAc, HPr, HBu and HVa have lots of existing forms which were shown in Table S4.

In the stable isotopic trail, three kinds of VFAs would be simultaneously produced from carbohydrate and protein digestion, such as HAc, HPr and HBu, as shown in Figure 7A–F. Most of HAc and HBu were gained from the degradation of carbohydrate. But HPr was mainly acquired from the decomposition of protein (Figure 7C,D). Especially HVa was only generated from the degradation of protein, not from carbohydrate digestion, shown in Figure 7G,H.

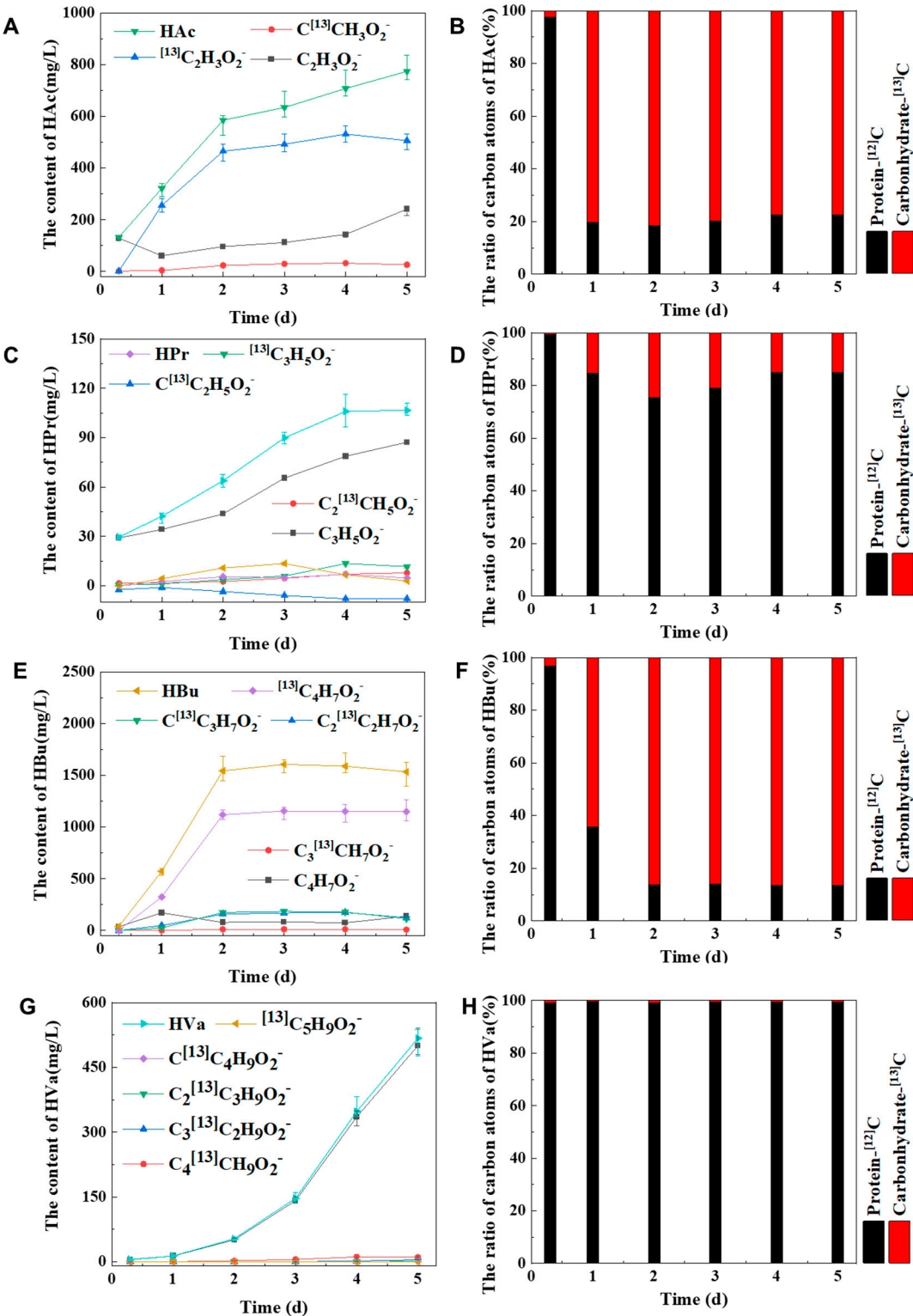


Figure 7. The source of carbon atoms for VFAs yield. (A) and (B) HAc; (C) and (D) HPr; (E) and (F) HBu; (G) and (H) HVa.

A simplified diagram of the proportion of carbohydrate and protein anaerobic production of VFAs was drawn, as shown in Figure S4. It was found that 77% of HAc and 86% of HBu were produced from carbohydrate degraded, low yields of HAc and HBu are produced by protein. And 85% of HPr and 99% of HVa were gained from protein, afterwards the remains of HPr and HVa were obtained from carbohydrate.

In the production of PHAs, HAc and HBu mainly synthesize PHB, and HPr and Hva mainly synthesize PHV (Lemos et al., 2006). It is well known that high-purity PHB tends to make PHAs plastic hard and brittle, but mixing a small amount of PHV can make PHAs plastic more elastic. Therefore, the ratio of carbohydrates and proteins in the fermentation matrix can be adjusted according to the needs for the production of PHAs.

3.8 The synthesis pathway of HVa

The metabolic pathway of carbohydrate and protein during anaerobic digestion proposed by Zehnder shown in Figure S5 (Zehnder, 1988). However, this metabolic pathway missed the key step in the formation of HVa. Based on the lipid metabolism (Hames & Hooper, 2010), Propionyl acyl carrier protein (Propionyl-ACP) which had three carbon atoms was regarded as the original compound for fatty acids with odd carbon atoms, not Malonyl acyl carrier protein (Malonyl-ACP) which add two carbon atoms into the original compound each time. Therefore, this study proposed a possible pathway of HVa yield, as shown in Figure 8. This pathway started from acetyl coenzyme A (Acetyl-CoA) and proionyl coenzyme A (Proionyl-CoA). Then HVa was gained by the process of condensation, reduction, dehydration and reduction.

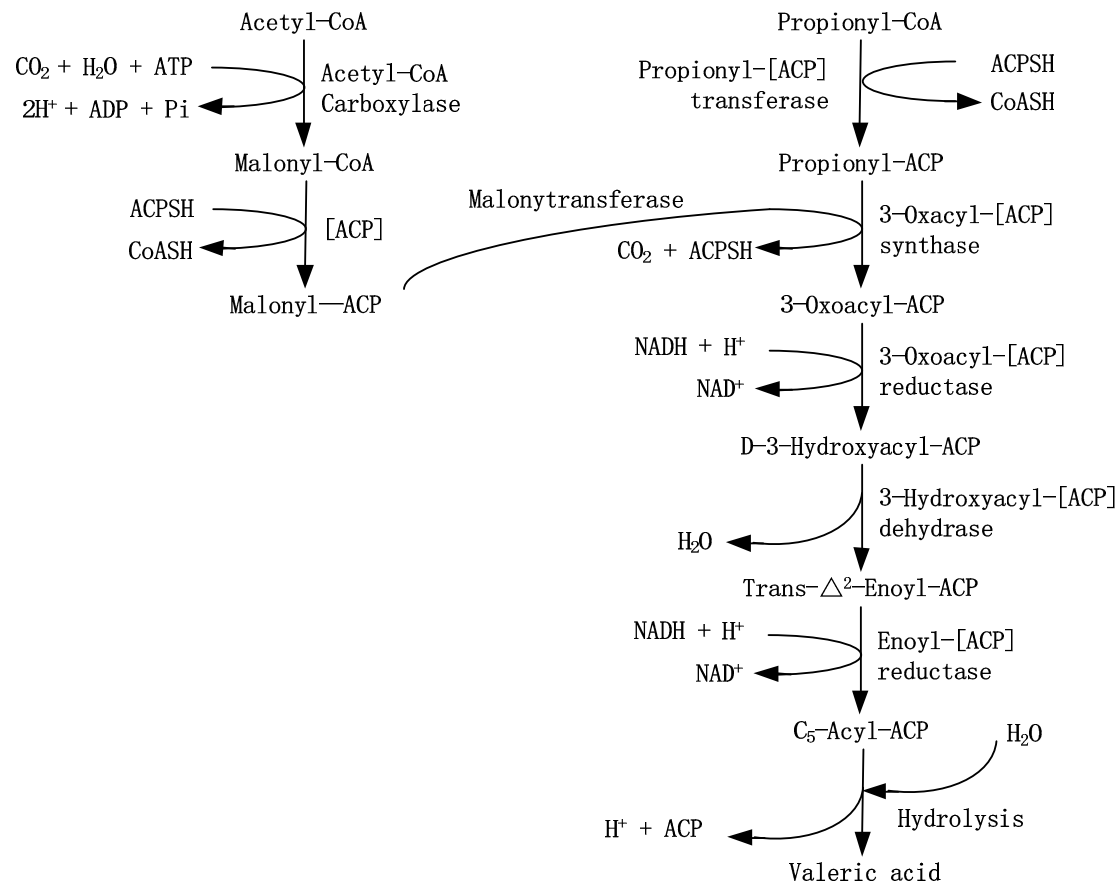


Figure 8. The digestion pathway for HVa yield.

4. Conclusions

Hydrolysis of VSS, P-C and P-P obeyed first-order kinetic model, and the k_x value was found in the following order: $k_{P-C} > k_{VSS} > k_{P-P}$ during thermophilic anaerobic process. Thermophilic fermentation resulted in greater VFA accumulation and higher percentages of acetic acid and iso-valeric acid compared to mesophilic fermentation due to intermediates accumulated during protein or carbohydrate breakdown. Then ^{13}C stable isotope labeling experiment demonstrated that, HAc, HPr and HBu were produced from carbohydrate and protein digestion, but HVa was only generated from the degradation of protein. Finally, the possible pathway of HVa synthesis was proposed.

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