RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

RSCPublishing

ARTICLE

Synthetic effect on volatile fatty acids disinhibition and methane production enhancement by dosing $FeCl_3$ in sludge thermophilic anaerobic digestion system

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Bao Yu,[a] Dongling Zhang,[a] Xiaohu Dai[b], Ziyang Lou,[a] Haiping Yuan,[a] and Nanwen Zhu*[a]

Anaerobic digestion (AD) is considered as an efficient way for sludge reduction and biogas generation simultaneously, while the excessive accumulation of volatile fatty acids (VFAs) often results in the process instability in the thermophilic AD system. In this study, ferric chloride ($FeCl_3$), as the additive was supplemented to the sludge thermophilic AD system directly. A dosage gradient was adopted and 9.92 mgFe/gDS was favorable for the disinhibition of VFAs with the maximum methane production of 236.75 mL/gVS under the test condition, which was 2.2 times higher than the control group. The results indicated that methane production could be successfully enhanced by disinhibiting the excessive VFAs. Further investigations of the composition of soluble organic compounds confirmed that acetic acid was the main inhibitor during the sludge thermophilic AD process, which was responsible for the acid inhibition. Meanwhile, dosing appropriate dosage of $FeCl_3$ into the thermophilic AD system would contribute to acetic acid utilization for methane production, which should be attributed to the synthetic effects, except the direct removal of acetic acid by $Fe(OH)(CH_3COO)$ precipitate. The dosage of $FeCl_3$ in the thermophilic AD system showed significant impact on process performance and certain dosage at low concentration (< 9.92 mgFe/gDS) showed positive effect on substrate environment. Based on the fitting analysis, the optimum dosage of 8.95 mgFe/gDS was recommended for the enhanced substrate utilization with the $\triangle$ cumulative methane production of 169.34 mL/gVS.

1. Introduction

As the byproduct of wastewater treatment, waste activated sludge (WAS) yield has increased continuously in the recent decades due to the increasing population in cities and towns and construction of new wastewater treatment plants.[1,2] Sludge disposal remains one of the biggest challenges in the wastewater treatment industry and accounts for up to 50% of a wastewater treatment plant's operating cost.[3] On the other side, the rapidly increasing energy demand, along with growing concerns for environmental protection, has fostered the search for alternative energy sources. Anaerobic digestion (AD), coupled with renewable-energy production in the form of biogas (mainly CH_4 and CO_2), is a viable option for the waste treatment, especially for sludge stabilization and reduction.[4]

Compared with mesophilic AD (35-40 ℃), thermophilic AD (55-60 ℃) achieves a better pathogen inactivation and yields more biogas, and is considered a highly-efficiency system.[5] In spite of these benefits, however, it has been reported that the thermophilic system is particularly susceptible to the accumulation of volatile fatty acids (VFAs), and inhibits the activity of methanogens and potentially decreases the pH-buffer system.[6] The acid forming and the methane forming microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions, and unbalance between these two groups of microorganisms is the primary cause of excessive VFAs.[7]

Investigations related to alleviating VFAs inhibition have mainly focused on controlling the feedstock to inoculum (F/I) ratio and carbon to nitrogen (C/N) ratio, providing proper pH buffering, employing a two-stage digestion system to separate methanogenesis stage from other stages of hydrolysis-acidification, and the adding of trace element (TEs) to accelerate the growth of methanogens.[8,9] The importance of TEs for methanogenesis is well known and related to the structure of enzymes such as Methyl-Coenzyme M reductase, Carbon monoxide dehydrogenase and Methyltransferase.[10] Particularly, Co, Ni and Fe are common additives to biogas processes due to their vital role in the metabolic machinery of a wide range of anaerobic microorganisms active in the conversion of complex organic substrates to biogas.[11]

Furthermore, more attentions have been paid on Fe supplementation due to its outstanding performance in sulfur control and low cost. Ivanov et al. investigated the effect of different iron compounds, i.e., ferric hydroxide, iron-containing clay, and iron ore, on the anaerobic treatment of fat-rich wastewater, and enhanced removal rate and biogas yield were observed;[12] Gustavsson et al. also reported the positive effect of dosing iron on the stable process in a wheat stillage fed mesophilic biogas production.[13] We previously demonstrated that $FeCl_3$ could enrich *Coprothermobacter* for proteins fermentation and *Methanosarcina* for methanogenesis from the perspective of microorganisms, compared with other ferric salts.[14] These observations may suggest the possibility for enhancing biomethanation through dosing $FeCl_3$, yet very limited information is available on this potential particularly for evaluating substrate environment.

Understanding the bioavailability of Fe for thermophilic AD system at different dosages could help to better control and optimize the sludge fermentation process, since the additive of $FeCl_3$ will influence the system conditions.[11] Besides, the residual product of AD (i.e. digestate) is widely used as biofertilizer in agriculture practices[15] and therefore, an optimum dosage of $FeCl_3$ to biogas reactors should be carried out in a way to ensure high quality of the biofertilizer products without causing negative effects on the environment.

The objective of the present study was to clarify how process performance and substrate environment respond to the addition of $FeCl_3$ in the thermophilic AD system treating WAS. To assess the extent of inhibition and the main VFA inhibition type during the thermophilic AD process, variations of soluble chemical oxygen demand (SCOD), VFAs and the composition of soluble organic compounds in the supernatant were investigated. Excitation-emission matrix (EEM) and fluorescence regional integration (FRI) technique were used to examine the dissolved organic matter (DOM) species. Aside from analyzing the bioavailability and distribution of Fe, the optimum dosage of $FeCl_3$ for enhanced methane production was determined.

2. Experimental

2.1 Characteristics of sludge and inoculum

WAS applied in this study was collected from the secondary sedimentation tank of a municipal wastewater treatment plant (MWWTP) in Shanghai, China, where wastewater was treated by the anaerobic-anoxic-aerobic process with a capacity of 50,000 m^3/d. The sludge obtained was screened with a 1.0-mm mesh to eliminate large particles and hair before thickening to required total solid (TS) of ~4%.Then the pretreated samples were stored at 4℃ for further analyses. The inoculum (seed sludge) was collected directly from a long-term continuous lab-scale anaerobic bioreactor in our lab.[14] The main characteristics of WAS and seed sludge are given in Table 1.

2.2 Batch experiments

The batch experiments were performed in double-walled cylindrical vessels with a working volume of 6 L, and each group has three parallel reactors. Initially, each reactor was sparged with nitrogen gas (99.99%) for 5 min after loading 5 L mixture of inoculum and raw sludge with a ratio of 1:9 (based on the total solids). All the reactors maintained at a thermophilic digestion temperature of 55±2 ℃ by water circulation, equipped with stainless-steel stirrers for mixing the contents. During the digestion, the biogas produced from each reactor was collected and stored in gasbag for analysis, and then the volume was measured using a calibrated sampling syringe. All samples from the reactors were analyzed in triplicate, and one-way analysis of variance (ANOVA) at 0.05 level was used to analyze the data.

Table 1 Characteristics of WAS and seed sludge used in experiments [a].

Parameters	WAS	Seed sludge
pH	6.60-6.72	6.90-6.97
TS (mg/L)	39,400-40,300	68,200-69,500
VS (mg/L)	28,900-30,200	48,200-51,800
TCOD (mg/L)	32,000-34,200	79,200-82,340
SCOD (mg/L)	670-780	8,420-9,120
Soluble proteins (mg/L)	10.8-12.4	363.2-406.2
Soluble carbohydrates (mg/L)	62.2-68.3	237.6-258.3
Fe (%)	1.40-1.58	1.62-1.90

[a] TS: total solid; VS: volatile solid; TCOD: total chemical oxygen demand; SCOD: soluble chemical oxygen demand; The content (%) of Fe was in dry sludge solid.

The whole cycle of digestion process occupied 43 days and samples were taken from each reactor at particular intervals (initial, 3rd, 6th, 9th, 12th, 15th, 18th, 23rd, 28th, 33rd, 38th and 43rd day) for analysis intermittently. In our previous study, it was found that not all the ferric salts contribute to methane production because the addition of anion could influence anaerobic condition, while $FeCl_3$ could create a favorable anaerobic environment; thus $FeCl_3$ was chosen as the additive[14]. Aim to investigate the effect of different dosage on methane production during thermophilic AD process, six groups with a dosage gradient of 100 mg/L (R2), 200 mg/L (R3), 400 mg/L (R4), 800 mg/L (R5) and 1200 mg/L (R6) were adopted, which based on the concentration of iron ion, that is, 2.48 mgFe/gDS, 4.96 mgFe/gDS, 9.92 mgFe/gDS, 19.84 mgFe/gDS and 29.76 mgFe/gDS, respectively in terms of the dry matter (DS) in unit. The control group (R1) was also carried out under the same operation conditions without $FeCl_3$. Since the serious unbalance of hydrolysis-acidification and methanogenesis often occurred in the initial stage of thermophilic AD, thus dosing time on 3rd day was applied in this study. No alkalinity or buffering agent was added into the system, and the pH value was not adjusted during the entire process.

2.3 Analytical methods

The parameters of total solids (TS), volatile solids (VS), total chemical oxygen demand (TCOD), SCOD, total nitrogen (TN), total phosphate (TP), soluble proteins and soluble carbohydrates were measured according to the Standard Methods,[16] and the values were subtracted with TS and VS that chemicals engendered by itself. The pH and oxidation reduction potential (ORP) of the sludge samples were determined by a pH meter (pHs-3C, Leici Co. Ltd., Shanghai) and an ORP meter (ORP-502, Ruosull Technology Co., Ltd., Shanghai), respectively. DOM extraction: The sludge was centrifuged at 12,000 rpm for 5 min and then a subsequent filtration through 0.45μm microfiber filter paper was carried out for the corresponding supernatant. The concentrations of CH_4 and CO_2 were quantified by a gas chromatograph (GC-14B, Shimadzu) equipped with a chromatographic column (TDX-02) and a thermal conductivity detector (TCD). VFAs (including acetic

acid, propionic acid, butyric acid, iso-butyric acid, valeric acid and iso-valeric acid) were analyzed by a gas chromatograph (GC-2010, Shimadzu) with a chromatographic column (DB-FFAP： 30 m×0.25 mm×0.25 mm) and a flame ionization detector (FID). The equivalent relationships between COD and substrates were as follows: 1.5 g-COD/g protein, 1.06 g-COD/g carbohydrate, 1.07 g-COD/g acetate, 1.51 g-COD/g propionate, 1.82 g-COD/g butyrate, and 2.04 g-COD/g valerate. The sludge solids were pretreated by microwave digestion system (ETHOS ONE, MILESTONE) with a mixture of $HNO_3/H_2O_2/HF$ followed by neutralization with H_3BO_3. Then the concentration of iron ion in the sludge and supernatant was analyzed with atomic absorption spectrometry method (spectrometer contrAA, Analytik Jena). The elemental analyzer (Vario Macro Cube, Elementar) was used for determining the contents of C, H, N and S in sludge solids with sulfanilamide ($C_6H_8O_2N_2S$) as reference material.

2.4 EEM fluorescence spectra and FRI analysis

Hitachi FL-7000 (Japan) fluorescence spectrophotometer was used at a room temperature (approximately 25 ℃) to obtain the EEM fluorescence spectra of DOM. The photomultiplier tube voltage, scanning speed and slit width were set to 600 mV, 1200 nm/min and 5 nm, respectively. The excitation wavelength (Ex) increased from 200 to 500 nm at 5 nm increments, while the emission wavelength (Em) varied between 250 and 600 nm at 5 nm intervals. The spectrum of deionized (DI) water was recorded as the blank and Raleigh scattering was subtracted according to Chen et al.[17]

A Fluorescence Regional Integration (FRI) technique was applied to analyze the results of EEM fluorescence,[17] and EEM peaks were divided into five regions (Region I, II, III, IV and V).[18] The corresponding organic matters were tyrosine, tryptophan, fulvic acid-like substances, soluble microbial by-product-like materials and humic acid-like organics respectively as shown in Table 2. The normalized Ex/Em area volumes ($\emptyset_{i,n}$, $\emptyset_{T,n}$) and percent fluorescence response ($P_{i,n}$,%) were calculated as Eqs. (1) - (3):

$$\emptyset_{i,n} = \mathrm{MF}_i \emptyset_i = \mathrm{MF}_i \sum_{ex} \sum_{em} I(\lambda_{ex}\lambda_{em}) \Delta\lambda_{ex} \Delta\lambda_{em} \quad (1)$$

$$\emptyset_{T,n} = \sum \emptyset_{i,n} \quad (2)$$

$$\mathrm{P}_{i,n} = \emptyset_{i,n} / \emptyset_{T,n} \times 100\% \quad (3)$$

In which $\emptyset_i$ is the volume beneath region ''i'' of the EEM. $\Delta\lambda_{ex}$ and $\Delta\lambda_{em}$ are the excitation wavelength interval (taken as 5 nm) and emission wavelength interval (taken as 5 nm). $I(\lambda_{ex}\lambda_{em})$ is the fluorescence intensity (au) at each excitation-emission wavelength pair. MF_i is multiplication factor, equal to the inverse of the fractional projected excitation-emission area. In addition, the fluorescence intensity had been multiplied by its dilution ratio (five-fold with DI water) before the statistical calculation to represent the actual content of each substance in the filtrates.

3. Results and discussion

3.1 Methane production at different dosages

The experimental reactors run in batch mode for 43 days, and the cumulative methane production is shown in Fig. 1a. All reactors performed stable process during the start-up phase and turning point occurred after the introduction of different dosages of $FeCl_3$ on 3rd day. Supplementation of $FeCl_3$ could enhance the methane production, and more $FeCl_3$ added, more methane generated, while the dosage should be below 9.92 mgFe/gDS. For example, with an increase of $FeCl_3$ from 0 mgFe/gDS (R1) to 2.48 mgFe/gDS (R2), methane yield exhibited a progressive climb from 73.18 to 177.31 mL/gVS, which was further enhanced ($P < 0.05$) as dosage increased to 4.96 mgFe/gDS (R3) and above. The highest methane production of 236.75 mL/gVS was obtained in R4 with 9.92 mgFe/gDS, which was 2.2 times higher than the control group (R1) under the test condition. The cumulative methane production under thermophilic condition in literatures[19-24] were compared and listed in Table 3. Obviously, the highest cumulative methane production in this study was still lower than that in some literatures, most probably attributable to the complexity of substrate and differences of operation conditions (e.g. inoculation proportion, reactor configuration and digestion time). When the dosage reached 19.84 mgFe/gDS (R5) and 29.76 mgFe/gDS (R6), the cumulative methane production decreased to 7.32 and 5.02 mL/gVS respectively, indicating the anaerobic process collapsed. These results suggested that the inhibition threshold for methane production as indicated was in the range from 9.92 mgFe/gDS to 19.84 mgFe/gDS in sludge thermophilic AD process, which was agreement with the researches using different substrates.[25] Interestingly, a clear lag phase was observed in R3 and R4, indicating that $FeCl_3$ had a disturbance effect on the thermophilic AD system. The effect was intensified as the dosage increased and a distinct rising trend occurred on 9th day and 15th day in R3 and R4, respectively, while that in R5 and R6 were irreversible. Compared with R1, the cumulative methane production in R2 had an immediate increase, which should be attributed to minor environment fluctuation and enhanced microbial activity with a low dosage of 2.48 mgFe/gDS.

Table 2 Excitation and emitting (Ex/Em) wavelengths of fluorescence region

Region	Substance	Assumed Biodegradability	Ex/Em wavelengths (nm)
I	Tyrosine-like protein	High	200-250/200-330
II	Tryptophan-like protein	Moderate	200-250/330-380
III	Fulvic acid-like organics	Low	200-250/380-500
IV	Soluble microbial by-product	High	250-280/200-380
V	Humic acid-like organics	Low	250-400/380-500

As expected, the pH (Fig.1b) of the substrate during the AD process dropped at the start-up phase, due to the hydrolysis and acidification phases of AD with the accumulation of VFAs.[10] After dosing $FeCl_3$ into the AD system, enhanced acidification degree was obtained and pH dropped sharply due to the rapid hydrolysis of $FeCl_3$ (Eq. (4)), especially that in R5 (5.30) and R6 (5.00).

$$FeCl_3 + 3H_2O \rightarrow Fe(OH)_3 \downarrow + 3HCl \quad (4)$$

The shock made the system collapsed, which was in accordance with the poor cumulative methane production. Compared with R1, pH in R2 achieved a significant increase and up to 7.05 on 9th day, and afterwards remained in the range of 7.20-7.90, almost in the desired pH range of 6.60-7.80 for high solid (4%-10% TS) AD. Similarly, pH in R3 and R4 had a recovery period with the postponed rising on 9th day and 15th day respectively, which was positively related to the trend of cumulative methane production.[26] These results indicated that appropriate dosage of $FeCl_3$ was beneficial to process stability in terms of desired pH, while excessive dosage will break the system balance.

Table 3 Comparison of cumulative methane production under thermophilic condition in literatures to that in this study.

Substrate	Treatment strategy	Operation mode	Solid retention time (d)	Cumulative CH4 production (mL/g VS)	Ref.
Sewage sludge	Co-phase anaerobic digestion	Semi-continuous	21	424.2~468.2 mL/g VS	19
Waste activated sludge	Ultrasound pretreatment	Semi-continuous	8~12	0.321~0.500 m^3/kg VS	20
Primary and secondary waste sludge	Thermal pretreatment	Semi-continuous	10	0.40~0.49 L/g VS	21
Primary and secondary waste sludge	Organic loading rate	Continuous	15~75	0.03~0.29 m^3/kg COD	22
Excess sludge and cassava stillage	Co-digestion	Batch	24	61.8~379.6 mL/g VS	23
Sewage sludge and crude glycerine	Balance C/N ratio	Continuous	20	0.18~0.39 Nm^3 m^{-3} d^{-1}	24
Waste activated sludge	Adding trace element	Batch	43	236.7 mL/g VS	This study

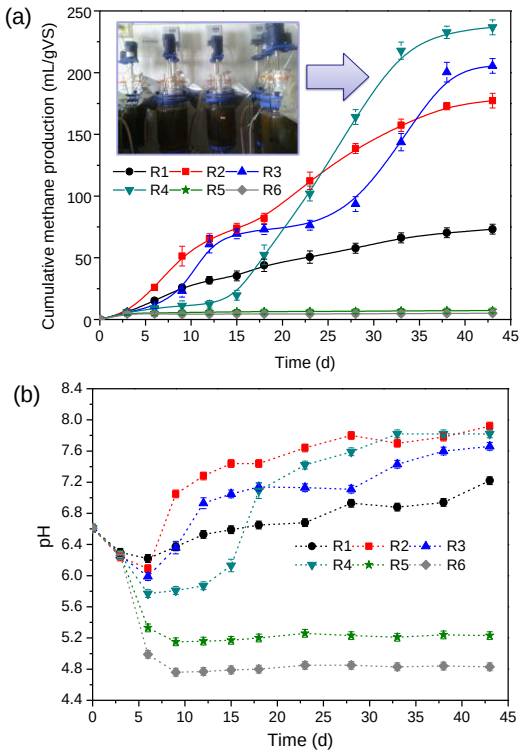


Fig. 1 (a) Cumulative methane production of the reactors at different dosages of $FeCl_3$; (b) Variations of pH at different dosages of $FeCl_3$.

3.2 Variations of SCOD and VFAs at different dosages

The solubilization of WAS is commonly regarded as a key step before hydrolysis. Variations of SCOD in the course of operational time were analyzed, and the corresponding results are presented in Fig. 2. As expected, SCOD in anaerobic fermentation kept increasing during the initial 6 days, due to the continued endogenous biomass decay and slow rate of methanogenesis. The former made a large amount of soluble compounds release while the later retarded the further conversion of these organics into methane.[27] The corresponding concentrations on 6th day were as follows: R3 (21,972 mg/L) > R5 (20,165 mg/L) > R4 (18,295 mg/L) > R2 (18,198 mg/L) >

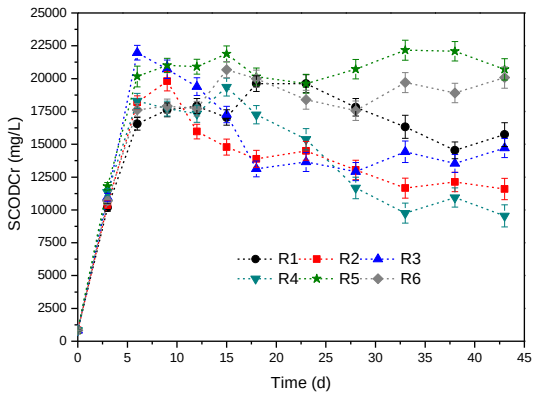


Fig. 2 Variations of SCOD concentration at different dosages of $FeCl_3$.

R6 (17,617 mg/L) > R1 (16,552 mg/L). The results suggested that supplementation of $FeCl_3$ in thermophilic AD could promote sludge solubilization, regardless of the correlation between the dosage and the increment of SCOD, which was in agreement with our previous study.[14] Afterwards, a declined trend was obtained in R2, R3 and R4 due to the balance established gradually between acidification process and methanogenesis process; while R5 and R6 kept in a high level (> 19,620 mg/L) until the end of the experiment. The consumed SCOD was in conjunction with methane production, and the minimum value was achieved in R4 (9540 mg/L), compared with that in R1 (15,736 mg/L), R2 (11,591 mg/L), R3 (14,711 mg/L), R5 (20,706 mg/L) and R6 (20,084 mg/L), respectively. The residual SCOD indicated that appropriate dosage of $FeCl_3$ could contribute to the decomposition of sludge and consumption of biodegradable organic matters with enhanced methane production.

VFA is a key index controlling the overall stability of AD. VFAs continuously produced in anaerobic fermentation and resulted in high VFAs concentrations in the system, which was inhibitory to AD microorganisms. Under conditions of overloading and in the presence of inhibitors, methanogenic activity cannot remove hydrogen and volatile organic acids as quickly as they are produced.[28] As shown in Fig. 3(a), the concentration of total volatile fatty acids (TVFA) achieved a rapid increase in the initial 6 days and then moved up to the peaks, which was consistent with SCOD. In terms of R1, its

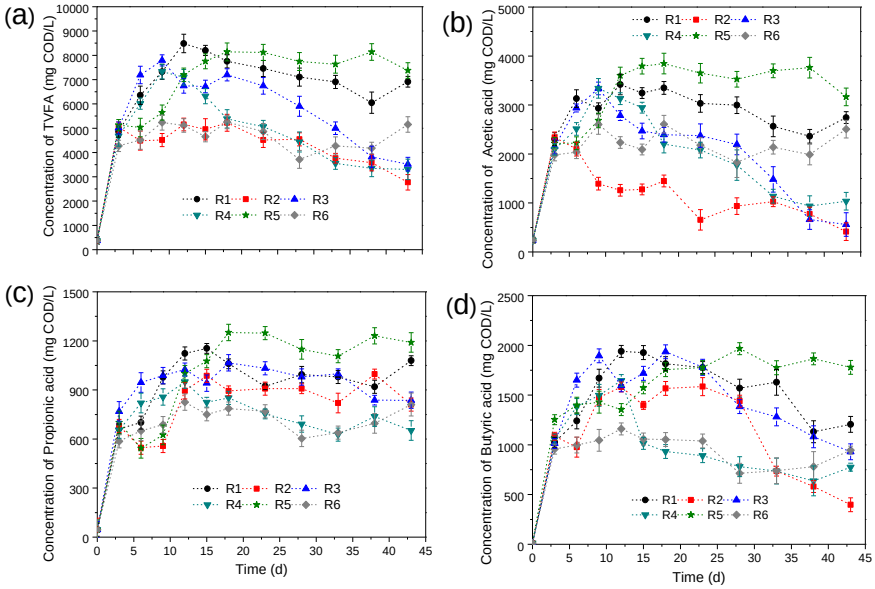


Fig. 3 Variations of (a) TVFA; (b) acetic acid; (c) propionic acid; (d) butyric acid concentrations in the supernatant at different dosages of $FeCl_3$.

accumulation started right away due to the hydrolysis of sludge particles and rapid growth of acidogenic and fermentative bacteria, and the peak value (8490 mg COD/L) was obtained on 12th day, exceeding the inhibition threshold for methanogens (~6900 mg COD/L);[29] Afterwards, the concentration of TVFA ranged from 6050 mg COD/L to 8202 mg COD/L at the end of experiment, contributing to the depression of pH (Fig. 1b) and the inhibition of methane production. By contrast, R3 and R4 with the dosages of 4.96 mgFe/gDS and 9.92 mgFe/gDS respectively decreased substantially on 9th day and 15th day with the residual values of 3519 mg COD/L and 3305 mg COD/L, indicating the adaption and activity recovery of methanogenic bacteria and the subsequent balance of VFAs generation and consumption in the reactors. The result had a positive correlation with the maximum methane production. When 19.84 mgFe/gDS was added, a higher concentration of TVFA was observed compared with other groups after 18th day, ranging from 7197 mg COD/L to 8140 mg COD/L; while as the dosage up to 29.76 mgFe/gDS, a relatively low concentration (< 5250 mg COD/L) was obtained in R6 after the rapid increase in the initial 6 days. These results implied that appropriate dosage of $FeCl_3$ (<9.92 mgFe/gDS) could alleviate the inhibition of excessive VFAs. Furthermore, the hydrolysis-acidification process could be promoted with the dosage of 19.84 mgFe/gDS accompanied with inhibited methanogenic process; while both the processes could be inhibited simultaneously when the dosage up to 29.76 mgFe/gDS.

The concentrations of main VFAs (i.e., acetic acid, propionic acid and butyric acid) in all the groups were shown in Fig. 3. As the trend of TVFA, a rapid accumulation was obtained in the initial 6 days, and the concentrations of individual VFA in the following order: acetic acid > butyric acid > propionic acid. As for acetic acid on 6th day, the maximum value (3130 mg COD/L) was observed in R1, compared with that in R2 (2100 mg COD/L), R3 (2939 mg COD/L), R4 (2512 mg COD/L), R5 (2219 mg COD/L), R6 (2036 mg COD/L), respectively. The results indicated that the concentration of acetic acid declined with the increasing dosage of $FeCl_3$ except to 2.48 mgFe/gDS. During the thermophilic AD process, acetic acid could be attached to the sludge particles as the precipitation through the following Eq.(5) and Eq. (6).[30]

$$Fe^{2+} + 2CH_3COO^- \rightleftharpoons Fe(CH_3COO)_2 \quad (5)$$

$$Fe(CH_3COO)_2 + 2H_2O \rightleftharpoons Fe(OH)(CH_3COO) + CH_3COOH \quad (6)$$

It should be emphasized that ferrous iron would be the main form in consideration of the high standard reduction potential ($Fe^{3+}+e \leftrightarrow Fe^{2+}$, E_h^0=0.771V) and reversible reaction of Eqs. (5) and (6); thus Fe^{3+} would be reduced to Fe^{2+} gradually under anaerobic environment and $Fe(OH)(CH_3COO)$ would be the main precipitation instead of $Fe(OH)_2(CH_3COO)$ precipitation. Besides, the relatively low concentration in R2 could be attributed to weak disturbance effect after dosing and stable consumption for methane generation. The sharp drop was obtained in R3 and R4 on 9th day and 15th day, which was well consistent with the methane production. Thus, dosing appropriate dosage of $FeCl_3$ into the thermophilic AD system would contribute to acetic acid utilization for methane production. On the contrary, propionic acid in all groups maintained a minor fluctuation (> 750 mg COD/L) after a rapid increase in the initial stage, and contributed to a considerable part of the VFAs. It could be explained by that the conversion is frequently difficult to occur in thermodynamics ($\triangle G$ = +76.1 kJ/mol) (Eq. (7))[31] because of the strict requirements for H_2 partial pressure (3×10^{-4} -10^{-6} atm)[32], acetate concentration and ORP (< -278 mV).

$$CH_3CH_2COO^- + 3H_2O \rightarrow HCO_3^- + CH_3COO^- + H^+ + 3H_2 \quad (7)$$
$$\Delta G = +76.1\ kJ/mol$$

Butyric acid in R2 and R4 obtained an effective conversion and decreased at a considerable level with the residual values of 398 mg COD/L and 775 mg COD/L, compared with that in R1 (1206 mg COD/L). Therefore, acetic acid could be the main

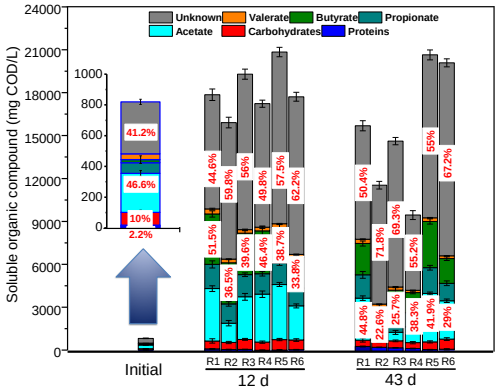


Fig. 4 Variations of the soluble organic compounds and corresponding percentages of VFAs and unknown matters after the digestion for 12d and 43d (acetate, propionate, butyrate and valerate were summed as VFAs).

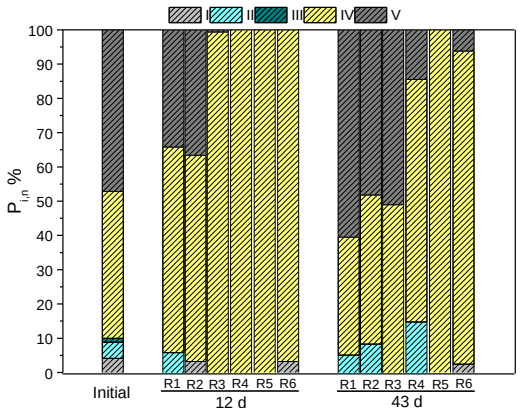


Fig. 5 Effects of different dosages of $FeCl_3$ on the distribution of FRI in DOM.

inhibitor in thermophilic AD, which was responsible for the acid inhibition, and meanwhile effective disinhibition of excessive VFAs could be obtained with the supplementation of $FeCl_3$.

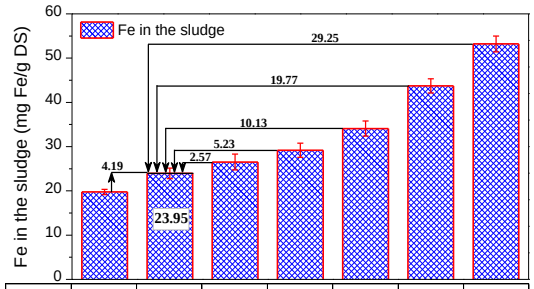
3.3 Evaluation of the substrate environment

3.3.1 Composition of soluble organic matters

Converting sludge particles to the supernatant is the first step; those soluble matters would be finally utilized by anaerobic microbes, accompanied with the sludge reduction.[33] To determine the mechanisms by which substrate was degraded during the thermophilic AD, we examined the changes in soluble organic compounds composition and the levels of intermediates formed. The soluble organic compounds and the corresponding percentages in the fermentation supernatant on 0^{th} day (initial stage), 12^{th} day (when highest VFAs accumulation was achieved) and 43^{rd} day (terminal stage) were shown in Fig. 4. In the initial stage, VFAs, carbohydrates and unknown organic matters (such as ethanol, amino acids and long chain fatty acid (LCFA) etc.) were the main component in SCOD (820 mg/L), which contributed to about 46.6%, 10% and 41.2%, respectively. After 12 days' digestion, the contribution proportion of VFAs in R1 increased from 46.6% to 51.5% (P <0.05), while that in other groups with dosing $FeCl_3$ declined along with the enhanced contribution proportion of unknown matters, indicating the presence of acid inhibition. Furthermore, the minimum proportion of acetate was achieved in R2 with 2.48 mgFe/gDS, which was relative to the week disturbance effect and stable methane generation. At the end of the digestion (43 days), the soluble organic compounds in R1, R2, R3 and R4 had been removed at different levels, while that in R5 and R6 increased instead. The corresponding percentages of VFAs were 44.8% (R1), 22.6% (R2), 25.7% (R3), 38.3% (R4), 41.9% (R5) and 29% (R6), respectively. Particularly, acetate in R2, R3 and R4 was converted effectively with the proportion of 3.8%, 4.1% and 11.6 %, respectively, compared with that in R1 (18.7%). The results confirmed that acetic acid was the main inhibitor and could be disinhibited by the supplementation of $FeCl_3$.

3.3.2 Metabolic advantages analysis of DOM by EEM

DOM could be released from sludge into supernatant during the fermentation process, which is the direct source of available carbon source and energy for microbes.[34] Thus compositional and structural characteristics of DOM are particularly useful for investigating the transformation and fate of sludge substrate in the following AD process. EEM fluorescence spectroscopy can provide an overall view of the fluorescent properties of DOM over a selected spectral range by characterizing the locations and intensities of fluorescence peaks.[35] A variation or shift of peak location is often associated with the changes of soluble organic matters in the fermentation liquid,[36] and the distribution of biodegradable (tyrosine-like and soluble microbial by-product-like) and non-biodegradable (tryptophan-like, fulvic acid-like, and humic acid-like) materials in DOM could be characterized.[37] To understand the EEM characteristics of these DOM samples and clarify the bioavailability of the unknown organic matters,the fluorescence regional integration technique was used to analyze the five Ex/Em regions as shown in Fig. 5. In the initial stage, Region V (humic acid-like organics) presented in the highest contribution proportion of percent fluorescence response ($P_{i,n}$) of 47.13%, followed by Region IV (soluble microbial by-product) and with the values of 42.89%, while Region I (tyrosine-like), Region II (Tryptophan-like protein) and Region III (Fulvic acid-like organics) accounted for small fraction. After 12 days' digestion, the $P_{i,n}$ value of Region IV in R3, R4, R5 and R6 increased to almost 100%, which indicated the increase of biodegradable materials in DOM after dosing $FeCl_3$ (> 4.96 mgFe/gDS). However, the fluorescence intensity (23,812 au) in R6 was lower than that in other groups as shown in Fig. S1, which was in agreement with the low-concentration SCOD. The declined $P_{i,n}$ of Region IV in R1 (42.89%) an R2 (60.04%) demonstrated the conversion of soluble microbial by-product with the incremental methane production; meanwhile the elevated $P_{i,n}$ of Region V in R1 (34.04%) and R2 (36.50%) implied the accumulation of humic acid-like organics, which was the unfavorable substrate for methane production.[38] At the end of digestion (43^{rd} day), the substrate environment in R1 turned into be worse with an increased $P_{i,n}$ of Region V of 60.38%; while that in R4 with 9.92 mgFe/gDS was only 14.35%. Furthermore, more tyrosine-like matters (14.89%) and soluble microbial by-product-like matters (70.76%) in R4 were observed, indicating that the unknown organic matters in soluble organic compounds were biodegradable and contributed to the better substrate environment. Since the methanogenesis process in R5 and R6 was cut off by the excessive $FeCl_3$, the fluorescence intensity and $P_{i,n}$ had no significant change, which was in accordance with the methane production. These results above demonstrated that appropriate dosage of $FeCl_3$ (2.48-9.92 mgFe/gDS) had an effective promotion of metabolism, resulting in enhanced



	R0	R1	R2	R3	R4	R5	R6
C (%)	35.72	28.63	28.31	27.71	26.91	28.82	28.83
H (%)	5.21	4.39	4.44	4.49	4.48	4.81	4.78
N (%)	7.04	4.39	4.54	4.58	4.73	5.56	5.74
S (%)	1.078	1.103	1.179	1.197	1.225	1.26	1.29

Fig. 6 Concentration of iron ion in the sludge phase (mgFe/DS), and the contents (%) of C, H, N and S in dry sludge solid before and after AD at different dosages of $FeCl_3$ (DS: dry solids).

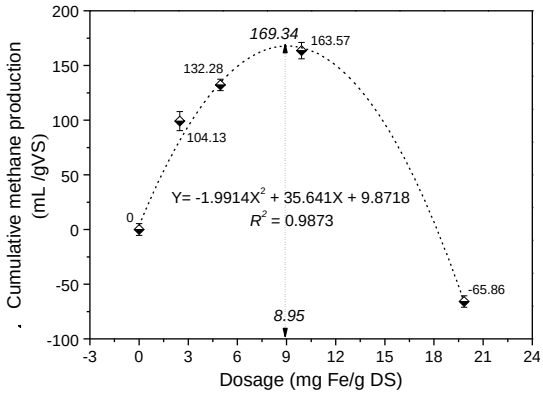


Fig. 7 Relationship between △cumulative methane production and dosages of $FeCl_3$ under the test conditions.

substrate utilization and higher methane production in the thermophilic AD system.

3.4 Bioavailability assessment of Fe

To investigate the fate of Fe, the content of Fe in the solid phase and liquid phase was investigated before the supplementation and after 43 days' digestion in all groups. As shown in Fig. 6, Fe content increased from 19.76 mgFe/gDS to 23.95 mgFe/gDS in R1, which could be owing to the reduction of VS and the relative increase proportion of Fe. Compared with R1, different increments of Fe content were observed in the solid phase from 2.57 mgFe/gDS to 29.25 mgFe/gDS, which was in accordance with the dosages. Meanwhile, Fe content in R2, R3 and R4 were slightly higher than the supplementation (2.48 mgFe/gDS, 4.96 mgFe/gDS and 9.92 mgFe/gDS, respectively), while that in R5 and R6 were lower than the supplementation instead, which could be attributed to the low conversion of VS in R5 and R6. Comparatively, Fe (ferrous ion) in the supernatant (data not shown) was very low than that in the sludge, implying the content of Fe in the sludge was dominant. The contents (%) of C, H, N and S in DS were also investigated (Fig. 6). Compared with R1, carbon percentage obtained a declined trend with the increasing dosage from 2.48 mgFe/gDS to 9.92 mgFe/gDS, and the minimum value of 26.91% was achieved in R4. In terms of sulfur percentage, 1.103% was observed in R1, while that in $FeCl_3$ added groups had a rising trend with the increasing dosage, confirming the effect of Fe for sulfur control.[39]

For the best performance case of R4 with the dosage of 9.92 mgFe/gDS, 1.22‰ sulfur was immobilized in the digested residue if the fraction in R1 was deducted. After 43 days' digestion, the residual DS was ~105.3 g, and the corresponding mass of immobilized sulfur in the form of iron sulfide (FeS) was ~0.128 g. The consumption of Fe in this part was ~0.224 g (Part 1) as shown in Fig. S2. Phosphate would react with iron ion to precipitate as hydrogen phosphate ferrous ($FeHPO_4$), [40] and the reduction of phosphate (Fig. S3) was ~1.296 g based on the final concentrations of R1 (665 mg/L) and R4 (377 mg/L). The consumption of Fe in this part was ~0.432 g (Part 2). Regardless of the sampling loss and consumption of enzymatic synthesis, the residue Fe in R4 was ~1.8 g. Thus, 1.144 g Fe could be left for acetic acid control (Part 3), and the corresponding reduction of acetic acid was ~470 mg/L (calculated as Eq. 6), which was far from the decrement of acetic acid (~1706 mg COD/L). Therefore, the disinhibition of excessive VFAs in the thermophilic AD system should be attributed to the synthetic effects (e.g. enhanced microbial activity and enriched methanogens), except the direct removal of acetic acid by Fe(OH)(CH_3COO) precipitate in the system.

Based on the increment of cumulative methane production (△cumulative methane production) and dosage of $FeCl_3$, a strong correlation (R^2=0.9873) was found under the test conditions as depicted in Fig. 7. The peak value (169.34 mL/gVS) could be obtained when 8.95 mgFe/gDS was added into the thermophilic AD system, that is, when the dosage was below 8.95 mgFe/gDS, the cumulative methane production ascended with the increasing dosage, while a falling limb occurred if surpassed. In addition, it was assumed that the inhibition threshold of methane production was 19.84 mgFe/gDS and the methane production would be inhibited completely with the excessive dosage. Therefore, the dosage of 8.95 mgFe/gDS was recommended, which would be the optimum dosage for hydrolysis-acidification and methanogenesis processes in the thermophilic AD system.

4. Conclusions

Thermophilic anaerobic digestion of waste activated sludge was undertaken. The highest methane production of 236.75 mL/gVS was obtained with 9.92 mgFe/gDS, increasing by 2.2 times relative to the control group. Appropriate dosage of $FeCl_3$ could contribute to the disinhibition of excessive VFAs, while elevated concentration (> 19.84 mgFe/gDS) would result in the system collapse. The disinhibition of excessive VFAs along with the favorable substrate environment in the thermophilic AD system should be attributed to the synthetic effects, except the direct removal of acetic acid. The potential ability to create favorable substrate environment will open new doors for better understanding and application of $FeCl_3$ in sludge thermophilic AD system.

Acknowledgements

This study was financially supported by National Natural Science Foundation of China (No. 51178261), the Key project of Science and Technology Commission of Shanghai Municipality (No. 12231202101, 14DZ1207306) and Open Funding Project of National Key Laboratory of Human Factors Engineering, Grant No. HF2012-K-05.

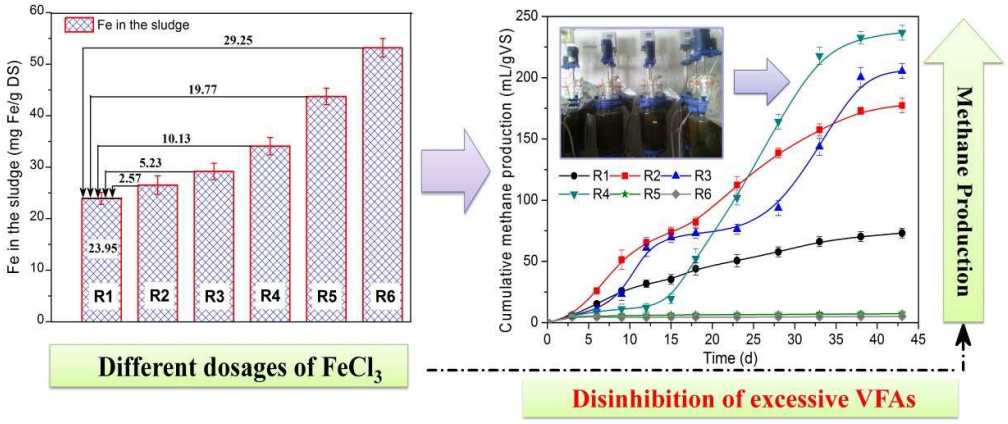
Notes and references

[a] School of Environmental Science and Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P. R. China. Fax: +86 021 54743710; Tel: +86 021 54743710; E-mail: nwzhu@sjtu.edu.cn
[b] National Engineering Research Center for Urban Pollution Control, School of Environmental Science and Engineering, Tongji University, Shanghai 200092, P. R. China

† Electronic Supplementary Information (ESI) available: The EEM spectra of DOM during the thermophilic AD process; The fate of Fe in R4 with the dosage of 9.92 mgFe/gDS; Variations of TP concentration in the supernatant at different dosages. See DOI: 10.1039/b000000x/

1 A. Dave, Y. Huang, S. Rezvani, D. M. Wright, M. Novaes and N. Hewitt, *Bioresour. Technol.*, 2013, **135**, 120-127.
2 Y. H. Feng, Y. B. Zhang, S. Chen and X. Quan, *Chem. Eng. J.*, 2015, **259**, 787-794.
3 L. Appels, J. Baeyens, J. Degrève and R. Dewil, *Prog. Energy Combust.*, 2008, **34**, 755-781.
4 G. Baek, J. Kim and C. Lee, *Bioresour. Technol.*, 2014, **166**, 596-601.
5 Q. Li, W. Qiao, X. Wang, K. Takayanagi, M. Shofie and Y. Y. Li, *Waste Manage.*, 2015, **36**, 77-85.
6 I. A. Nges and J. Liu, *Renew. Energ.*, 2010, **35**, 2200-2206.
7 Y. Chen, J. J. Cheng and K. S. Creamer, *Bioresour. Technol.*, 2008, **99**, 4044-4064.
8 Y. Xia, H. H. Fang and T. Zhang, *RSC Adv.*, 2013, **3**, 15528-15542.
9 J. P. Sheets, X. Ge and Y. Li, *Bioresour. Technol.*, 2015, **180**, 293-303.
10 T. Schmidt, M. Nelles, F. Scholwin and J. Pröter, Bioresour. Technol., 2014, **168**, 80-85.
11 X. M. Feng, A. Karlsson, B. H. Svensson and S. Bertilsson, *FEMS Microbiol. Ecol.*, 2010, **74**, 226-240.
12 V. N. Ivanov, E. V. Stabnikova, V. P. Stabnikov, I. S. Kim and A. Zubair, *Appl. Biochem. Micro.*, 2002, **38**, 255-258.
13 J. Gustavsson, B. Svensson and A. Karlsson, *Water Sci. Technol.*, 2011, **64**, 320-325.
14 B. Yu, D. L. Zhang, A. D. Shan, Z. Y. Lou, H. P. Yuan, X. T. Huang, W. X. Yuan, X. H. Dai and N. W. Zhu, *RSC Adv.*, 2015, **5**, 38538-38546.
15 K. Möller and T. Müller, *Eng. Life Sci.*, 2012, **12**, 242-257.
16 APHA, *Standard methods for the examination of water and wastewater*, 20st ed, 1998.
17 W. Chen, P. Westerhoff, J. A. Leenheer and K. Booksh, *Environ. Sci. Technol.*, 2003, **37**, 5701-5710.
18 M. Bahram, R. Bro, C. Stedmon and A. Afkhami, *Chemometrics*, 2006, **20**, 99-105.
19 Y. C. Song, S. J. Kwon, J. H. Woo, *Water Res.*, 2004, **38**, 1653-1662.
20 S. L. Trouqué, C.F. Forster, *Bioresour. Technol.*, 2002, **84**, 113-118.
21 I. Ferrer, S. Ponsá, F. Vázquez, X. Font, *Biochem. Eng. J.*, 2008, **42**, 186-192.
22 M. A. Rubia, M. Perez, L. I. Romero, D. Sales, *Process Biochem.*, 2006, **41**, 79-86.
23 W. Wang, L. Xie, J. R. Chen, G. Luo, Q. Zhou, *Bioresour. Technol.*, 2011, **102**, 3833-3839.
24 G. Silvestre, B. Fernández, A. Bonmatí, *Bioresour. Technol.*, 2015, **193**, 377-385.
25 R. Che, L. Huang and X. Yu, *Bioresour. Technol.*, 2015, **192**, 795-798.
26 L. Liu, T. Zhang, H. Wan, Y. Chen, X. Wang, G. Yang and G. Ren, *Energ. Convers. Manage.*, 2015, **97**, 132-139.
27 G. Y. Zhen, X. Lu, Y. Y. Li, Y. Liu and Y. C. Zhao, *Chem. Eng. J.*, 2015, **263**, 461-470.
28 Y. Xi, Z. Chang, X. Ye, R. Xu, J. Du and G. Chen, *Bioresour. Technol.*, 2014, **172**, 91-96.
29 J. D. Vrieze, L. D. Lathouwer, W. Verstraete and N. Boon, *Water Res.*, 2013, **47**, 3732-3741.
30 N. B. Jin, Y. W. Shao, H. P. Yuan, Z. Y. Lou and N. W. Zhu, *Chem. Eng. J.*, 2015, **265**, 9-15.
31 R. K. Thauer, K. Jungermann and K. Decker, *Bacteriol. Rev.*, 1977, **41**, 100-180.
32 F. Kus and U. Wiesmann, *Water Res.*, 1994, **29**, 1437-1443.
33 G. Silvestre, J. Illa, B. Fernández and A. Bonmatí, *Appl. Energy*, 2014, **117**, 87-94.
34 P. Caricasole, M. R. Provenzano, P. G. Hatcher and N. Senesi, *Bioresour. Technol.*, 2010, **101**, 8232-8236.
35 S. M. Wan, B. D. Xi, X. F. Xia, M. X. Li, D. D. lv, L. Wang and C. H. Song, *Bioresour. Technol.*, 2012, **123**, 439-444.
36 X. Huang, C. Shen, J. Liu and L. Lu, *Chem. Eng. J.*, 2015, **264**, 280-290.
37 G. Y. Zhen, X. Q. Lu, B. Y. Wang, Y. C. Zhao, X. L. Chai, D. J. Niu, A. H. Zhao, Y. Y. Li, Y. Song and X. Y. Cao, *Bioresour. Technol.*, 2012, **124**, 29-36.
38 J. Jimenez, E. Gonidec, J. A. C. Rivero, E. Latrille, F. Vedrenne, J. P. Steyer, *Water Res.*, 2014, **50**, 359-372.
39 L. H. Zhang, P. D. Schryver, B. D. Gusseme, W. D. Muynck, N. Boon, W. Verstraete, *Water Res.*, 2008, **42**, 1-12.
40 Y. B. Zhang, Y. H. Feng, Q. L. Yu, Z. B. Xu and X. Quan, *Bioresour. Technol.*, 2014, **159**, 297-304.

Graphical Abstract



A dosage gradient of $FeCl_3$ was adopted and 9.92 mgFe/gDS was favorable for the disinhibition of VFAs in sludge thermophilic digestion system.