Mechanisms of free nitrous acid and freezing co-pretreatment enhancing short-chain fatty acids production from waste activated sludge anaerobic fermentation

Yuqi Wu [a], Kang Song [a, *], Xiaoyan Sun [a], HuuHao Ngo [b], Wenshan Guo [b], Long D. Nghiem [b], Qilin Wang [b]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, PR China
[b] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Ultimo, NSW, 2007, Australia

* Corresponding author.
E-*mail address:* sk@ihb.ac.cn (K. Song).

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Free nitrous acid (FNA) or freezing has been recently utilized as an efficient pretreatment method to increase short-chain fatty acids (SCFAs) yield from waste activated sludge (WAS) anaerobic fermentation (AF). But until now, the performances and mechanisms of the co-pretreatment for SCFAs production are unknown. This research aimed to investigate the AF mechanisms through studying its influence on sludge solubilization and related bioprocesses. WAS was pretreated for 48 h with FNA (1.07 mg N/L), freezing (−5 °C) and combination of FNA and freezing (0.53–2.13 mg N/L FNA at −5 °C), respectively, then conducted batch AF. Experimental results indicated that the optimal total SCFAs yield of 391.19 ± 5.54 mg COD/g VSS was achieved after 1.07 mg N/L FNA + freezing pretreatment at 9 days of AF, which was 2.2, 1.6 and 1.3-fold of the blank, freezing and FNA pretreated samples, respectively. The mechanisms analysis showed that co-pretreatment showed synergetic effects on sludge disintegration and solubilization, which could release more soluble substrates for SCFAs production. The co-pretreatment resulted in slight inhibition to hydrolysis and negligible inhibition to acidogenesis but severe inhibition to methanogenesis, maybe due to less endurance of methanogens.

1. Introduction

The treatment of waste activated sludge (WAS) has become one of great challenges in many wastewater treatment plants (WWTPs), especially in China. It was reported that more than 11.2 million tons of dry sludge was generated annually in China (Hao et al., 2010;

Yang et al., 2019). It is time to find a cost-effective technology to treat and dispose WAS.

Anaerobic fermentation (AF) has been attracting more and more attention because resource recovery (such as short-chain fatty acids and hydrogen) and sludge reduction could be achieved simultaneously (Duan et al., 2019; Luo et al., 2019; Wang et al., 2017, 2019). During AF, short-chain fatty acids (SCFAs) are main intermediates, which could be applied as carbon-sources for biological nutrients removal (BNR) or substrates to produce bioenergy (Wang et al., 2018; Wu et al., 2018). Sludge disintegration and solubilization is the rate-limiting step in AF (Zhang et al., 2018), and different kinds of pretreatment methods have been studied to accelerate this process and increase SCFAs production, including physical, chemical, biological and combined methods (Lee et al., 2014; Khan et al., 2016; Wang, 2017).

Among the above methods, free nitrous acid (FNA or HNO_2) pretreatment has been studied widely to increase SCFAs yield from WAS anaerobic fermentation (Xu et al., 2018; Zhao et al., 2015, 2016). FNA, at parts per million (mg N/L) levels, is a strong biocide that can disintegrate sludge flocs and cells structure and provide large quantity of substrate for acidogens (Wang et al., 2014, 2016; Zhang et al., 2019). In addition, FNA pretreatment is an environmental-friendly and low-cost technology due to that FNA could be recycled in-situ by partial nitrification of sludge AF supernatant (Xu et al., 2018). It was reported that FNA combining with alkali, sodium dodecylbenzene sulfonate, tea saponin or alkyl polyglucose could achieve large quantity of SCFAs accumulation (Zhao et al., 2015, 2016; Xu et al., 2018; Liu et al., 2018). However, the addition of alkali or surfactant might increase the chemical expense for WWTPs and have negative effects on the following sludge treatment and disposal. Freezing is a green and low-cost technology to promote sludge solubilization in the areas where natural freezing is feasible (Gao et al., 2006; Hu et al., 2011; Montusiewicz et al., 2010). Since combination of freezing and NO_2^--N could increase the FNA concentration and then cause synergy on sludge disintegration and solubilization (Sun et al., 2018), we hypothesized that FNA + freezing pretreatment could increase SCFAs yield and reduce the influence on the environment.

This study aimed at confirming the impacts of FNA + freezing on SCFAs production from WAS fermentation and explaining the related mechanisms. To resolve these questions, a batch experiment was conducted with real WAS or model compounds as AF substrates. Firstly, the effect of WAS pretreatment with different FNA levels (0, 0.53, 1.07, 1.59 and 2.13 mg N/L) + freezing (−5 °C) for 48 h on SCFAs yield and components was investigated. Then, the mechanisms of co-pretreatment promoting SCFAs accumulation were studied by analyzing its effects on sludge solubilization, hydrolysis, acidogenesis and methanogenesis processes. At last, pH, oxidation reduction potential (ORP) and dewaterability of the fermented sludge were tested to further understand the AF performances.

2. Method and material

2.1. WAS source

WAS was sampled from a full-scale WWTP in Wuhan, China, and the main physicochemical characteristics were as follows: pH 6.91 ± 0.01, total protein 3,763.32 ± 9.44 mg/L, total carbohydrate 1,119.6 ± 15 mg/L, total suspended solids (TSS) 18,145 ± 25 mg/L, volatile suspended solids (VSS) 8,125 ± 85 mg/L, total chemical oxygen demand (TCOD) 11,000 ± 15 mg/L and soluble chemical oxygen demand (SCOD) 75.4 ± 5.7 mg/L.

2.2. WAS pretreatment and batch AF test

Firstly, WAS pretreatment was conducted in seven identical plastic bottles, and 450 mL WAS was added. The pretreatment conditions were displayed in Table 1. 2 M $NaNO_2$ stock solution was dosed to achieve 100, 200, 265, 300 and 400 mg NO_2^--N/L, respectively. The starting pH for test samples was adjusted at 6.0 by dosing 3 M HCl or 3 M NaOH. Subsequently, test samples were continuously frozen at −5 °C for 48 h in a refrigerator according to our previous study (Wu et al., 2019). Freezing temperature, initial NO_2^--N level and pH led to 0, 0.53, 1.07, 1.59 and 2.13 mg N/L FNA, respectively, as shown in Table 1.

After pretreatment, the test samples were thawed for 4 h at ambient temperature. Then seven identical 550 mL saline bottles were utilized to carry out batch AF experiment, and 350 mL pretreated sample was dosed to the reactor. Each bottle was purged with 5 L high purity N_2 to achieve anaerobic condition, sealed and placed in a shaker (180 rpm) at 35 ± 1 °C for 14 d, during which the pH was not controlled.

2.3. The effects of pretreatment on sludge disintegration and solubilization test

In order to test the influences of FNA + freezing on WAS disintegration and solubilization, the particle size of sludge, and the contents of SCOD, soluble protein, carbohydrate and DNA were determined after 48 h of pretreatment. In addition, three-dimensional excitation-emission (Ex-Em) matrix (3D-EEM) fluorescence spectroscopy was utilized to evaluate the structure variation of the fermentation supernatant at 3 d of AF.

2.4. The effects of pretreatment on sludge hydrolysis and acidogenesis test

Apart from sludge disintegration and solubilization during the AF, the specific microbe activities related to hydrolysis and acidogenesis also have significant influence on SCFAs yield. Therefore, the impacts of FNA, freezing and FNA + freezing on these specific microbe activities were determined according to the following method.

Table 1
WAS pretreatment conditions.

NO.	Temperature (°C)	Initial pH	Freezing time (h)	FNA (mg N/L)	NO_2^--N (mg/L)	Note
1	4	6.9 [a]	0	0	0.04 [a]	Blank
2	−5	6.0	48	0	0.04 [a]	Test
3	4	6.0	0	1.07	265	
4	−5	6.0	48	0.53	100	
5	−5	6.0	48	1.07	200	
6	−5	6.0	48	1.59	300	
7	−5	6.0	48	2.13	400	

[a] Background value.

Two model substrates, dextran and glucose, were used to estimate the influences of pretreatment on hydrolysis and acidogenesis during AF. Eight identical 250 mL saline bottles were prepared and split into two groups (i.e. Group I: dextran test and Group II: glucose test) with four in each group. The detailed process of the test was shown below. For each group, 60 mL inocula collected from a long-term steady fermenter in the lab were distributed into four plastic containers. The main characteristics of the inocula were: TSS 15,770 ± 180 mg/L, VSS 6,665 ± 115 mg/L and pH 7.14 ± 0.01. Among these samples, one without any pretreatment acted as blank. The inocula for other three samples were pretreated by freezing (−5 °C), FNA (1.07 mg N/L at 4 °C) and FNA + freezing (1.07 mg N/L at −5 °C) for 48 h, respectively. After that, the inocula were washed thrice with pure water to remove soluble organics and residual FNA, and then re-suspended in pure water with a finial volume of 15 mL.

Group I: This test was conducted for determining the influence of freezing, FNA and FNA + freezing pretreatment on hydrolysis process. 135 mL synthetic wastewater with 15 mL inoculum was dosed into each reactor. The synthetic wastewater contained 1200 mg/L dextran (a model polysaccharide, average molecular weight 20,000). All other procedures were the same as depicted in Section 2.2. The AF process continued for 3 days, during which the content of dextran in each reactor was tested daily. The calculation method for biomass specific degradation rate of dextran was shown in Supplementary materials.

Group II: This test was carried out for determining the influence of different pretreatments on acidogenesis process. The procedure was the same with Group I except that 1000 mg/L glucose (a model monosaccharide compound) was acted as the fermentation substrate.

2.5. The effects of pretreatment on sludge methanogenesis test

The effects of pretreatment on methanogenesis were evaluated by the methane (CH_4) accumulation during the AF conducted as Section 2.2. The calculation method for biomass specific production rate of CH_4 was shown in Supplementary materials.

2.6. Analytical methods

COD, NO_2^--N, TSS, VSS, pH and ORP were tested according to the standard method (SEPA, 2002). Protein, carbohydrate and DNA concentrations were tested using Lowry-Folin, Anthrone and Diphenylamine method, respectively. The analysis and test methods of EEM spectroscopy, sludge particle size, CH_4 production, SCFAs yield and sludge dewaterability were presented in Supplementary material.

3. Results and discussion

3.1. Effects of pretreatment on WAS disintegration and solubilization

Sludge disintegration and solubilization is the rate-limiting step during AF. Thus the influence of pretreatment on WAS solubilization was investigated in detail, and the results were shown in Fig. 1. After 48 h of pretreatment, SCOD, protein and carbohydrate in the

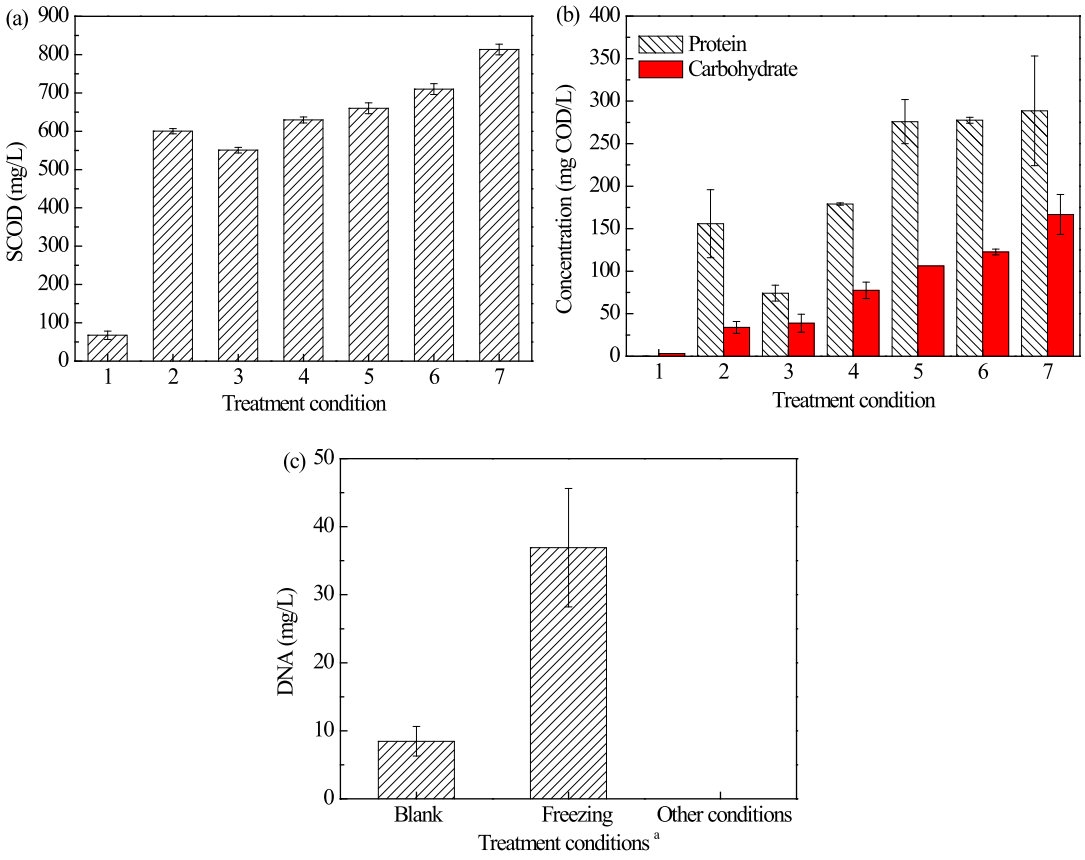
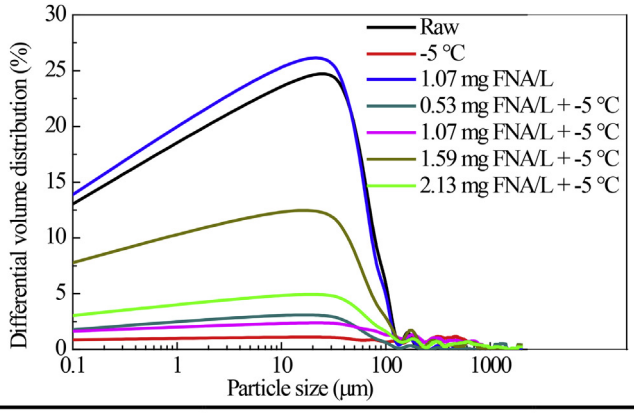


Fig. 1. SCOD (a) and soluble protein and carbohydrate (b) and soluble DNA (c) concentration after 48 h of pretreatment. The treatment conditions were: 1. Blank, 2. Sole freezing, 3. Sole 1.07 mg FNA/L, 4. Freezing + 0.53 mg FNA/L, 5. Freezing + 1.07 mg FNA/L, 6. Freezing + 1.59 mg FNA/L, 7. Freezing + 2.13 mg FNA/L.[a] Other conditions included NO. 3 to NO. 7, and soluble DNA under these five conditions cannot be detected.

fermentation supernatant from different reactors all increased remarkably, and the co-pretreatment resulted in the highest increment, showing synergy on WAS solubilization. For example, in the fermenter after 1.07 mg N/L FNA + freezing pretreatment, 659.78 mg/L SCOD was measured, which was 8.7 times of that in the blank. Soluble protein and carbohydrate concentration increased to 275.89 mg COD/L and 106.25 mg COD/L, respectively, while they were merely 0 and 3.12 mg COD/L in the blank, indicating that more biodegradable substrates could be utilized for acidogenesis. Soluble DNA was 36.9 mg/L after single freezing pretreatment, which was 4.34 times of the blank, showing remarkable cells breakage. In other reactors, soluble DNA could not be detected by the diphenylamine method due to that the chromogenic agent was interfered by high level of NO_2^--N (more than 100 mg/L). However, from the results of Fig. 1(a) and (b), it could be deduced that sludge cells disintegration also occurred after FNA + freezing co-pretreatment.

After pretreatment, the variation of sludge particle size may affect the AF performances and solid-liquid separation performance of the fermented sludge. It was well known that freezing and FNA showed different impacts on the variations of sludge particle size (Gao, 2011; Wang et al., 2013), however, the effects of co-pretreatment were rarely studied. In Fig. 2, it could be seen that sole FNA pretreatment slightly reduced the particle size, for example, dp 90 and dp 50 of the sludge particles decreased from 102.0 to 48.5 μm (blank) to 99.9 and 46.6 μm, respectively, and the specific surface area increased from 120.4 m^2/kg (blank) to 125.0 m^2/kg accordingly. On the contrary, much more flocs with larger size than that of the blank occurred after sole freezing or FNA + freezing pretreatment, which may be beneficial to the dewatering of fermented sludge, and it would be discussed in Section 3.4.

Apart from above analysis, 3D-EEM fluorescence spectra were utilized to determine sludge solubilization performances after pretreatment. According to the previous studies, there were five regions in the EEM spectra of sludge fermentation supernatant depended on the specific ranges of Ex and Em wavelength (see Supplementary material). Fig. S1 showed the EEM fluorescence spectra of the fermentation supernatant at 3 d of AF. For raw sample, only Peak A (region V) belonging to humic acid-like compounds was observed. However, there were two peaks (Peak A and Peak B) in other seven samples, which represented humic acid-like (region V) and soluble microbial by-product-like compounds (biodegradable, region IV), respectively. Compared with the blank,



Condition	Particle size distribution (μm)			Specific surface area (m^2/kg)
	dp 10	dp 50	dp 90	
Blank	16.9	48.5	102.0	120.4
Freezing	239	2530	3240	11.09
1.07 mg FNA/L	16.4	46.6	99.9	125.0
Freezing + 0.53 mg FNA/L	89.0	2560	3190	16.16
Freezing + 1.07 mg FNA/L	100	2300	3150	18.15
Freezing + 1.59 mg FNA/L	23.6	1820	2880	67.00
Freezing + 2.13 mg FNA/L	46.6	2100	3140	82.64

Fig. 2. Sludge particle size variation after 48 h of pretreatment.

FNA and (or) freezing pretreatment caused the increase of fluorescence intensities (FIs), red-shift of Em wavelength and occurrence of biodegradable substances, which indicated that more soluble organics were released in the supernatant after pretreatment and was favorable to the subsequent hydrolysis and acidogenesis. This result supported the data well shown in Fig. 1.

3.2. Effects of pretreatment on SCFAs production from WAS anaerobic fermentation

Fig. 3(a) showed the total SCFAs (TSCFAs) yield during WAS fermentation process. TSCFAs production for the blank without any pretreatment was the lowest (<200 mg COD/g VSS) during the whole fermentation process, which was mainly because of the low WAS solubilization performances and rapid SCFAs consumption by methanogens (Yang et al., 2019). In contrast, all the pretreatments resulted in the increment of TSCFAs. For example, after sole freezing and sole 1.07 mg N/L FNA pretreatment, the TSCFAs yield increased to 241.8 mg COD/g VSS and 292.3 mg COD/g VSS after 9 days of AF, respectively. FNA + freezing co-pretreatment showed synergy. For example, after 2.13 mg N/L FNA + freezing pretreatment, TSCFAs yield rose to 400.4 mg COD/g VSS at 9 d, which was 1.37, 1.66 and 2.24 times of the single FNA, freezing and blank, respectively. TSCFAs yield from other co-pretreatment systems showed the similar results. Meanwhile, it could be found that the contribution of FNA for TSCFAs yield was larger than that of freezing.

The constitution of SCFAs in wastewater could affect the activities of microbes, such as denitrifying bacteria and phosphorus-accumulating bacteria, and thus have effects on the BNR performances. An increasing of acetic and propionic acids content in the influent could lead to better nitrogen and phosphorus removal performances, respectively (Lee et al., 2014; Gao et al., 2011). Therefore, it is essential to analyze the fraction of individual SCFA after various pretreatments. The percentages of six individual SCFA, including acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric acids, were exhibited in Fig. 3(b) and Fig. S2. After FNA + freezing pretreatment, the total percentage of acetic and propionic acids reached the maximum of about 50% after 9 days of AF, and declined with further fermentation, especially for acetic acid. It was reported that 65%–95% CH_4 was directly produced from acetic acid (Khan et al., 2016), therefore, the decline of acetic acid percentage after 9 days may due to the methanogenesis process. It was also noticed that the TSCFAs yield after 1.07 mg N/L FNA + freezing pretreatment was 391.19 ± 5.54 mg COD/g VSS on 9 day, and further increasing FNA level to 2.13 mg N/L did not lead to a significant increase ($p > 0.05$). In order to balance TSCFAs production and distribution of individual SCFA, reduce NO_2^--N dosage and increase sludge treatment capacity, the optimal pretreatment condition and sludge retention time for this study was determined as 1.07 mg N/L FNA + freezing and 9 days, respectively.

To further determine the influences of FNA and freezing pretreatment on AF performances, COD mass balance analysis was conducted based on the determination of major intermediates and final production, as shown in Table 2. The percentages of VSS and CH_4 to TCOD all decreased for the pretreated sludge, and the samples from co-pretreatment reactors showed lower percentages than that from single pretreatment reactors, which indicated that co-pretreatment enhanced sludge disintegration and inhibited methanogens activity more effectively. On the other hand, samples from co-pretreatment reactors showed higher percentages of TSCFAs, soluble protein and carbohydrate to TCOD than that under single pretreatment conditions, indicating that more organics were transferred from solid phase to liquid phase and led to more SCFAs production. Above facts confirmed that besides WAS solubilization

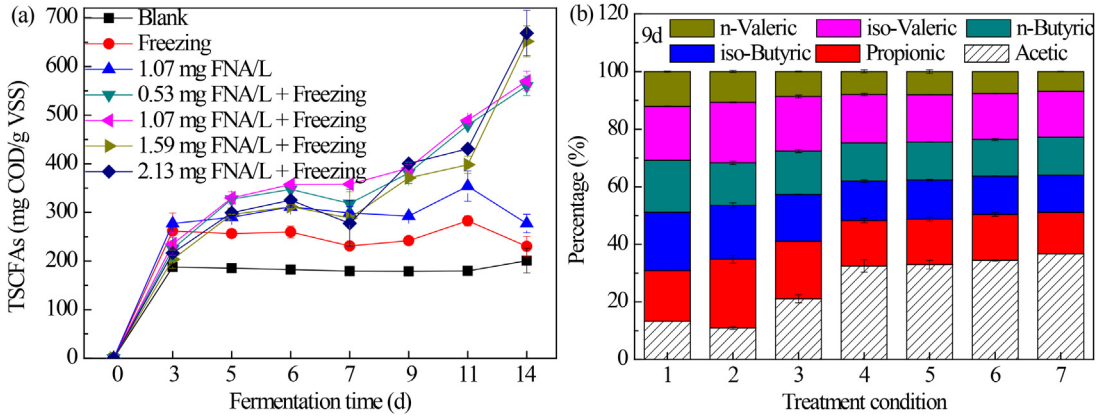


Fig. 3. TSCFAs yield (a) and the fraction of individual SCFA after 9 days of AF (b). The treatment conditions were: 1. Blank, 2. Sole freezing, 3. Sole 1.07 mg FNA/L, 4. Freezing + 0.53 mg FNA/L, 5. Freezing + 1.07 mg FNA/L, 6. Freezing + 1.59 mg FNA/L, 7. Freezing + 2.13 mg FNA/L.

Table 2
COD mass balance analysis of the AF systems after various pretreatments at 5 d of fermentation [a].

Condition	Percentage of various components to TCOD (%)					
	VSS	CH_4	TSCFAs	Protein	Carbohydrate	Others
Blank	66.28 ± 0.61	5.17 ± 0.12	13.21 ± 0.00	0.36 ± 0.05	0.15 ± 0.07	14.83 ± 0.72
Freezing	58.94 ± 1.65	3.21 ± 1.43	18.28 ± 0.07	1.40 ± 0.16	0.32 ± 0.06	17.84 ± 3.04
1.07 mg FNA/L	60.40 ± 088	0.39 ± 0.04	20.68 ± 0.06	2.10 ± 0.23	0.43 ± 0.05	16.00 ± 1.08
Freezing + 0.53 mg FNA/L	49.61 ± 5.07	0.19 ± 0.07	23.34 ± 0.14	2.68 ± 0.09	0.41 ± 0.01	23.77 ± 5.18
Freezing + 1.07 mg FNA/L	48.29 ± 0.50	0.14 ± 0.01	23.52 ± 0.67	2.90 ± 0.08	0.47 ± 0.01	24.69 ± 1.08
Freezing + 1.59 mg FNA/L	48.55 ± 1.21	0.14 ± 0.02	21.10 ± 0.02	3.07 ± 0.36	0.64 ± 0.06	26.50 ± 0.79
Freezing + 2.13 mg FNA/L	51.62 ± 0.11	0.01 ± 0.00	21.35 ± 0.05	3.40 ± 0.11	0.73 ± 0.01	22.89 ± 0.7

[a] TCOD in the AF system was 3,850 ± 5.25 mg. The COD conversion coefficients were consistent with the study of Yang et al. (2019).

and methanogenesis, other bioprocesses involved in the AF, including organics hydrolysis and acidogenesis, were affected by FNA and freezing pretreatment as well, which would be discussed in detail in Section 3.3.

During AF process, nearly 99% NO_2^--N in different reactors was removed after 7 days of treatment due to denitrification (Table S2) (Xu et al., 2018), and the low level of NO_2^--N, around 0.03–0.05 mg/L, showed negligible effects on the SCFAs reuse in the subsequent BNR process.

3.3. Effects of pretreatment on sludge hydrolysis, acidogenesis and methanogenesis

To determine the effects of FNA, freezing and co-pretreatment on bioprocesses involved in AF, two batch experiments using dextran and glucose as AF substrates were conducted, respectively. In addition, CH_4 production was measured during the AF conducted as Section 2.2.

In Table S3, it was found that the degradation efficiency of dextran decreased after pretreatment. For example, the degradation efficiency for the blank after 1d of AF was 37.21%, but decreased to 6.16% after co-pretreatment. Similar results were obtained on other fermentation days and for other pretreatments, indicating that pretreatment could inhibit hydrolysis process. As for the glucose degradation performances, diverse conditions showed little difference after 3 days of AF, showing that pretreatment caused negligible inhibition to acidogenesis.

In addition, CH_4 production during AF conducted as Section 2.2 was measured, as shown in Fig. 4. It was found that all pretreatments decreased CH_4 production during the first 7–9 days of AF, especially for that of the co-pretreatment. As a result, more SCFAs could be accumulated in the FNA + freezing reactors. After 9 day of AF, CH_4 production from pretreated reactors increased gradually, leading to acetate decrease, which was consistent with the result of acetate percentage shown in Fig. 3(b) and Fig. S2.

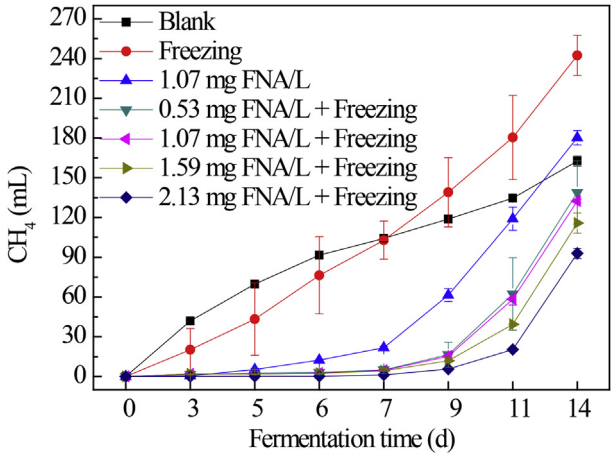


Fig. 4. CH_4 production during the AF process.

Table 3 further calculated the specific degradation rates of dextran and glucose and specific production rate of CH_4 during AF. The specific degradation rate of dextran for the blank was 23.2 mg/(g VSS·h), and this was set as the initial activity of hydrolytic microbes. However, it decreased to 20.7, 19.8 and 20.4 mg/(g VSS·h) after freezing, FNA and FNA + freezing pretreatment, respectively, which indicated that pretreatment by freezing, FNA and FNA + freezing decreased the relative activity of the hydrolytic microbes by 10.78%, 14.66% and 12.07%, respectively (indicated by % of the original). After 3d of AF, there was negligible difference for the relative activity of acidogens among the blank and pretreated samples. However, the inhibition of methanogens activity by pretreatment was more remarkable than that of the hydrolytic microbes and acidogens. The CH_4 specific production rate in blank sample was 0.2 mL/(g VSS·h), but decreased to 0.13 mL/(g VSS·h), 0.02 mL/(g VSS·h) and 0.01 mL/(g VSS·h) in freezing, FNA and FNA + freezing reactors, respectively, indicating that CH_4 production rate after 5 days of AF decreased by 37.84%, 92.47% and 97.31%, respectively. The severe inhibition to methanogenesis may be due to the less tolerance and more sensitivity of methanogens, comparing with hydrolytic microbes and acidogens (Wang et al., 2018; Zhang et al., 2018; Song et al., 2019). In this study, FNA showed strong inhibition effect on methanogens, especially at the early stage of AF. On the other hand, the weak methanogens activity of the pretreated samples could also result from the lower initial pH (6.0) than that of the blank (6.9), due to that the optimal pH for methanogens was 6.8–7.2 (Xu et al., 2018). From the above analysis, it could be inferred that co-pretreatment could inhibit hydrolysis and methanogenesis in the AF process, but the inhibition to SCFAs consumers was more remarkable than that of the SCFAs producers, leading to more SCFAs accumulation in the system.

3.4. pH, ORP and dewaterability variations

Apart from sludge solubilization and microbial activities, many other parameters could affect SCFAs accumulation, such as pH and ORP, which was shown in Fig. S3. During the whole AF, the pH showed increasing tendency due to ammonium accumulation (data not shown) (Zhang et al., 2019), and it varied from 6.15 to 7.72. As for ORP, it changed from −342.9 to −269.4 mV in different reactors. Both of these parameters were suitable for acidogens survival, which was beneficial to SCFAs production (Lee et al., 2014).

During the process of fermented sludge treatment and disposal, dewatering is an important factor, which accounts for high cost for a WWTP and affects the final outcome (Zhen et al., 2017; Wei et al., 2018; Song et al., 2016a, b). It has been demonstrated that sole freezing or freezing + FNA pretreatment could enhance WAS dewaterability remarkably (Hu et al., 2011; Gao, 2011). However, the dewaterability of sludge after AF was rarely studied. Fig. S4 showed the dewaterability variation without any flocculant addition. It could be found that the dewatering rate was deteriorated sharply after pretreatment, for example, the time to filter (TTF_{50})

Table 3
The specific degradation rates of dextran and glucose, and specific production rate of CH_4 during AF.

Substrate	Conditions			
	Blank	Freezing	1.07 mg FNA/L	Freezing + 1.07 mg FNA/L
Dextran [a]	23.2 ± 0	20.7 ± 0.03	19.8 ± 0.03	20.4 ± 0.01
Glucose [a]	20.6 ± 0	20.6 ± 0	20.5 ± 0	20.6 ± 0
CH_4 [b]	0.20 ± 0.01	0.13 ± 0.05	0.02 ± 0.0	0.01 ± 0.0

[a] Unit is mg/(g VSS ·h), after 3 days of AF.
[b] Unit is mL/(g VSS ·h), after 5 days of AF.

increased from 265s (blank) to 1,225 s after 1.07 mg N/L FNA + freezing pretreatment. The co-pretreatment resulted in more significant increment of TTF_{50} in comparison with the sole FNA or sole freezing pretreatment. As for the dewatering extent, it was observed that the water content of fermented sludge cake increased for all the pretreated samples excepted for that in the sole freezing reactor. It could be concluded that the increment of particle size after single freezing or FNA + freezing pretreatment (see in Fig. 2) showed minimal benefits to fermented sludge dewatering. The above analysis indicated that AF process had such a predominant effect on sludge dewaterability that the obvious positive effects created by FNA + freezing or sole freezing pretreatment prior to AF were inhibited severely, leading to dewaterability deterioration. This conclusion was similar to that of Apul et al. (2010). Therefore, how to enhance the dewaterability of fermented sludge after FNA + freezing co-pretreatment still needed to be researched in the future.

3.5. Implication for WWTPs

The research demonstrated that FNA + freezing showed synergetic effects on SCFAs production from WAS fermentation, and the related mechanisms were explained in detail. FNA + freezing had synergy on WAS solubilization and organics release, supplying more soluble substrates for SCFAs production. On the other hand, co-pretreatment caused much more significant inhibition to methanogenesis than that to hydrolysis and acidogenesis. As a consequence, more SCFAs accumulation after co-pretreatment occurred due to more acidogenesis substrates releasing and less SCFAs consuming compared with single pretreatment. The results revealed in current study enhanced the understanding of WAS fermentation process by FNA + freezing pretreatment, which could guide engineers to conduct the AF process in the real WWTPs.

FNA pretreatment before AF has been widely studied (Xu et al., 2018; Zhao et al., 2015, 2016). In these studies, FNA was combined with other methods, such as heat, alkali or surfactant addition, which needed extra energy input or chemicals. In current study, freezing as a low-cost and environmental-friendly method was integrated with FNA, which showed higher SCFAs yield than that of the previous studies (Xu et al., 2018; Zhao et al., 2015, 2016). For example, Zhao et al. conducted the AF under the condition of 1.54 mg N/L FNA combination with pH 10 pretreatment for 2 days, and the maximal TSCFAs yield was 370.1 mg COD/g VSS, which was lower than that of the current study under the optimal condition (391.19 mg COD/g VSS). In addition, it should be highlighted that microbial community and enzymatic activities also affect AF performances, and the related analysis could further reveal the AF mechanisms. Thus the related research about microbial activity should be performed in the future.

At last, a concept of "nutrients removal and SCFAs recovery" was proposed for the manipulation of WWTPs with FNA + freezing pretreatment, as shown in Fig. S5. Though this combined treatment, magnesium ammonium phosphate recovery, BNR performances enhanced and sludge reduction could be achieved simultaneously.

4. Conclusions

In this research, SCFAs production from WAS anaerobic fermentation remarkably increased after FNA + freezing pretreatment. The optimal SCFAs production, 391.19 ± 5.54 mg COD/g VSS, occurred after 1.07 mg N/L FNA + −5 °C pretreatment at 9 days of AF. The co-pretreatment showed positive synergetic effects on sludge solubilization, releasing more soluble protein and carbohydrate for SCFAs producers. Besides, co-pretreatment inhibited hydrolysis and methanogenesis processes to some extents, while the inhibition to methanogenesis was more remarkable than the former. This co-pretreatment could be acted as a high-efficiency technology to enhance SCFAs production in WWTPs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.05.107.

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