Chemical Engineering Journal 336 (2018) 28–37

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Role of free nitrous acid in the pretreatment of waste activated sludge: Extracellular polymeric substances disruption or cells lysis?

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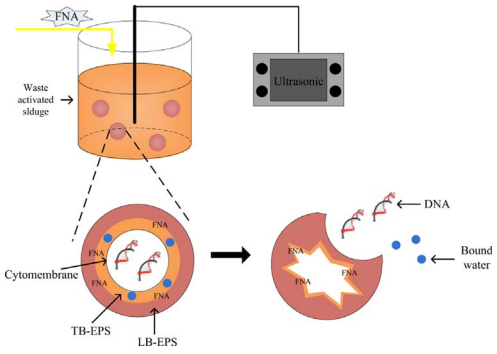
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Waste activated sludge
Freenitrous acid
Ultrasonic
Mechanism
Dewaterability

ABSTRACT

Free nitrous acid (FNA) has positive effect on the solubilization of waste activated sludge (WAS), but the mechanism is uncertain. In this study, five different pretreatments were compared to elucidate the enhanced mechanism through the variation of sludge physicochemical properties, including capillary suction time (CST), extracellular polymeric substances (EPS) and bound water. The CST values showed a significant positive correlation with soluble protein (PN) and polysaccharide (PS) under different FNA concentration and contact time. After being pretreated, the CST values increased from 156.5 (raw sludge) to 335.4 (ultrasonic), 237.5 (FNA), 258.7 (ultrasonic/FNA) and 214.4 (ultrasonic (washed)/FNA), respectively. The soluble extracellular polymeric substances (S-EPS) and loosely bound EPS (LB-EPS) increased for all pretreatment, but the tightly bound EPS (TB-EPS) decreased. The sludge filterability was in connection with the existence of humic acid in LB-EPS. Contrary to other pretreatments, bound water decreased from 82% to 79% after FNA treatment. This study showed that FNA was very effective in disintegrating cell membrane, which was indicated by increasing percentage of DNA in solution, however, its role in disintegration of EPS fractions was limited.

1. Introduction

With the rapid growth of wastewater treatment plants (WWTPs) worldwide, the yield of waste activated sludge (WAS) also increases significantly. According to statistics, the cost of WAS treatment in WWTPs accounts for up to approximately 60% of their total operating

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https://doi.org/10.1016/j.cej.2017.11.038
Received 9 August 2017; Received in revised form 5 November 2017; Accepted 6 November 2017
Available online 10 November 2017
1385-8947/

costs [1]. So the treatment/disposal of WAS has become one of the main challenges for WWTPs management. Anaerobic [2] and aerobic [3] sludge digestion were the main technologies of sludge stabilization and reduction for further disposal, but the effectiveness of these methods is constrained by the weak solubilization of WAS. Plenty of methods including mechanical [4], chemical [5,6], thermal [7] and their combination [8,9] had been used to intensify the WAS hydrolysis and solubilization before the sludge digestion process. Nevertheless, these ways all have some shortcomings, such as intensive energy input, large chemical consumption and considerable expenditure.

Free nitrous acid (FNA, i.e. HNO_2), which can be generated in situ by the nitritation of anaerobic digestion liquor, is a low cost and renewable chemical production. Previous researches had demonstrated that FNA could effectively make cell dissolve and improve the biodegradability of sludge even at per billion (ppb) levels [3,10]. It was verified that FNA has a forceful sterilization on microorganism in anaerobic sewer biofilms. After exposure to 0.2–0.3 mg N/L FNA for 6–24 h, the viable microorganisms decreased from approximately 80% to 5–15% [11]. Recently, the feasibility of FNA-based technology to reduce the sludge production was explored [12]. When WAS was treated by 2.0 mg N/L FNA for 1–2 days, sludge yield was 28% lower compared to the blank test. Furthermore, FNA pretreatment can stimulate the production of short-chain fatty acid (SCFA) from anaerobic digestion of WAS. Our previous research indicated that the maximum yield of SCFA from WAS anaerobic digestion was 3.7-fold of the blank after being pretreated by 1.8 μg FNA/mL [13]. So FNA pretreatment seemed to be a promising technique to enhance the degradability of WAS.

Extracellular polymeric substances (EPS), a natural polymers of high molecular weight secreted by microorganisms, are thought to be the colloid layer that attached to microorganisms and play a considerable role in the physical chemistry properties of the sludge flocs [14].The physical, chemical and biological properties of WAS, such as dewaterability, settleability, disintegrating and flocculability characteristics, are connected with the presence and the formation of EPS [15,16]. FNA could cause the crack of macromolecules in EPS, then resulting in the disintegration of WAS [17]. Therefore, FNA maybe affect the physicochemical properties of sludge, such as dewaterability. To give in-depth insight into the effect of FNA, the change of the compositions and structures of EPS should be confirmed. Fluorescence excitation-emission (EEM) spectroscopy had received much attention on studying of environmental samples including EPS due to its effective and sensitive nature in analysis of the fluorescence organic matter fractions (fluorophores), based on their characteristic fluorescence intensities and differences in the wavelength maxima [18,19]. The EEM graphs are scrutinized to build a thorough fluorescence fingerprint (i.e. number of peaks, the intensities and location of these peaks) of this substance as same as to identify potential correlation between properties and features of samples.

So far, it's still unclear that the effect of FNA pretreatment to WAS is EPS disruption or cells lysis? The aim of this work were to explore the effect and mechanism of FNA pretreatment on WAS in term of the variation of sludge physicochemical properties, including capillary suction time (CST), EPS and bound water. Meanwhile, the relationship between these physicochemical properties changes and sludge dewaterability was discussed.

2. Materials and methods

2.1. Waste activated sludge and chemicals

The WAS were collected from a secondary clarifier of WWTPs in Changsha, China. Sludge samples were sifted through a 1.2-mm sieve, then preserved at 4 °C in a refrigerator and used within 2 days. The main characteristics of sludge are as follows: total suspended solids (SS) 47.1 ± 1.0 g/L, volatile suspended solids (VSS) 16.1 ± 0.8 g/L, water content of sludge 82 ± 0.17%, pH 6.8 ± 0.1, CST 101.4 ± 2.0 s (Error bars represent 95% confidence intervals). Sodium nitrite was produced by Sinopharm Chemical Reagents Co. (Shanghai, China). All chemical reagents used in this study were of analytical pure without further purification.

2.2. Experimental procedure

Three series batch experiments being comprised of six beakers fed with 300 mL sludge sample were carried out to investigate the impact of FNA on sludge dewaterability and physicochemical properties. The initial pH for three series experiments was adjusted to 4.5, 5.5 and 6.5 (acid environment), respectively. And in each series, six nitrite levels (0, 100, 200, 300, 400 and 500 mg/L) were set by adding 0, 0.5, 1, 1.5, 2 and 2.5 mL sodium nitrite stocking solution (30 g/L), respectively, to achieve designed FNA concentration. FNA concentration was calculated by the acid-base-equilibrium [20]:

$$c_{FNA} = c_{NO_2^- - N}/(K_a \times 10^{pH}) \quad (1)$$

where c_{FNA} and $c_{NO_2^- - N}$ is the concentration of FNA and nitrite in the solution, the $K_a$value deems to a function of temperature T (°C) by $K_a = e^{-2300/(273+T)}$ (25 °C in this study).

The CST values, protein and polysaccharide were measured at 24 h and 48 h after the addition of sodium nitrite solution.

In order to evaluate the mechanism of FNA and/or its derivatives on sludge solubilization, five groups contrast experiment were designed and carried out in 500 mL conical flasks with 300 mL sludge sample. Group 1 was as the control using raw sludge without any treatment. Group 2 (FNA) was designed to evaluate the FNA effect, where the pH was maintained at 5.5 by 1 M hydrogen chloride or 1 M sodium hydroxide and 2 mL sodium nitrite stock solution (30 g/L) was added. It has been demonstrated that ultrasonic treatment could totally disintegrate EPS at ultrasonic intensity 2.0 W/mL and ultrasonic time 15 min [21]. In this study, we used ultrasonic treatment as a pretreatment method to elute EPS attaching to cells so as to determine that whether FNA and/or its derivatives could directly affect cytomembrane. So Group 3 (US) was to assess the ultrasonic effect, where 300 mL sludge was pretreated by an ultrasonic processor (VC 130 PB, Sonics & Materials, Inc., US) with a titanium probe operated at 20 kHz under optimum condition: ultrasonic intensity 2 W/mL and ultrasonic time 5 min. During this process, the ultrasonic probe was dipped 1.5 cm into the center of sludge and operated at 600 W with the interval of 4 s on and 5 s off to avoid the excessive temperature. Group 4 (US/FNA) was to explore the combined effect of ultrasonic/FNA treatment, where 2 mL sodium nitrite stock solution (30 g/L) was added into 300 mL raw sludge at pH 5.5 and then ultrasonically pretreated for 5 min under ultrasonic intensity 2 W/mL. Different from Group 4, the ultrasound was adopted to wash EPS from WAS in Group 5 (US(W)/FNA). In this experiment, the sludge firstly pretreated by ultrasound, then washed three times with deionized water and centrifuged at 4000*g* for 10 min to separate the supernate and pellet. The pellet was dissolved to 300 mL and then added 2 mL sodium nitrite stock solution (30 g/L). Sludge samples were taken at 24 h to measure CST, soluble chemical oxygen demand (SCOD), proteins, polysaccharides, SS, VSS, bound water, DNA and EEM fluorescence spectroscopy of EPS. Before the analyses of SCOD, protein and polysaccharide, samples were filtered by a 0.45 μm PTFE membrane syringe filter, and the filtrates were used. Triplicates were conducted for each test and the average values with standard deviations will be reported.

2.3. EPS analysis

The procedure of EPS extraction was in agreement with previous research [22]. The polysaccharide (PS) and protein (PN) in extracted EPS were measured according to our previous study [16]. The three-

dimensional EEMs were determined in a luminescence spectrometry (RF-5301pc, Shimadzu, Japan). The scanning emission spectra (Em) were collected from 210 to 500 nm at 10 nm intervals, with the excitation wavelengths (Ex) from 200 to 500 nm at 10 nm intervals. The spectra with a scan rate of 12,000 nm/min, were recorded using excitation and emission slit bandwidths of 5 nm.

2.4. CST analysis

All CST were measured in a CST instrument (model 304B, Triton, UK) using Whatman No.17 chromatography-grade paper with circular funnel of 18 mm in diameter. The filterability of sludge was expressed by the ratio of initial CST value (CST_0) to real-time CST values (CST). Higher CST_0/CST value usually represents the better filterability.

2.5. LIVE/DEAD bacterial viability analysis

Live and dead bacterial viability was analyzed by confocal laser scanning microscopy (CLSM) with a staining method. LIVE/DEADTM BacLightTM Bacterial Viability kit (Invitrogen by Thermo Fisher Scientific) was used to stain the microorganism matrix. Briefly, interfering components from bacterial suspension was removed through cell-wash step. Then 30 µL dye mixture was added in 10 mL re-suspended bacterial suspension. Then the suspension was mixed cautiously and hatched in the dark at room temperature for 15 min. After that, 5 µL stained bacterial suspension was moved between a slide and an 18 mm optical petri dish. A series of optical section were obtained with a Fluoview FV1000 Laser Scanning Biological Microscope (OLYMPUS, Japan). The number of viable and non-viable cells was defined as the fluorescence intensity proportional of Green and Red coloring agent.

2.6. Bound water

In this study, bound water was defined as the gross amount of internal water, interstitial water and surface water. 40 mL sludge were poured to a 9 cm standard Buchner funnel, whose bottom was filled with 1.2 µm prewet glass fiber filter paper and filtered at a constant vacuum pressure (P) of 50 kPa for 30 min. The filtered sludge was dried at 105 °C for 8 h in an oven. The deviation of sludge weight before and after drying was defined as bound water content and was calculated according to the equation as follow [23]:

$$\text{Bound Water} = \frac{W_1 - W_2}{W_1} \times 100\% \quad (2)$$

where W_1 and W_2 means the weight of wet and dry filter cake, respectively.

2.7. Other analysis

The total suspended solids (SS), volatile suspended solid (VSS) and soluble chemical oxygen demand (SCOD) were measured according to the standard method [24]. The pH were determined using Orion® ion selective pH meter (Thermo Electron rion®, 720A). The DNA in the supernatant was analyzed by Burton diphenylamine method [25].

2.8. Statistical analysis

Correlation coefficient (R) is applied to reflect the degree of the linear correlation between two parameters. The value of correlation R ranges from −1 to +1. −1 reveal a perfect negative correlation, +1 show a perfect positive correlation and 0 represent the irrelevant. The correlations are considered to be statistically significant at a 95% confidence interval (p, .05).

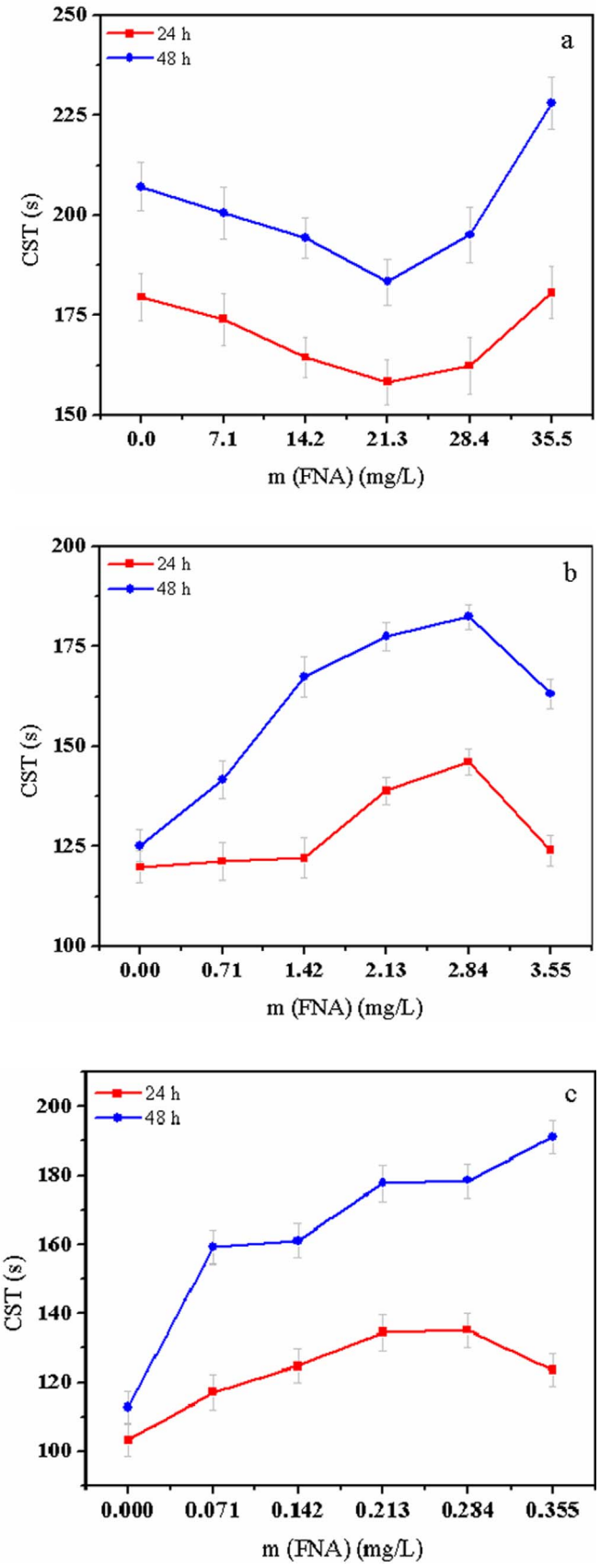

Fig. 1. Effect of initial pH, FNA concentration and exposure time on sludge dewaterability. (a) pH = 4.5, (b) pH = 5.5 and (c) pH = 6.5.

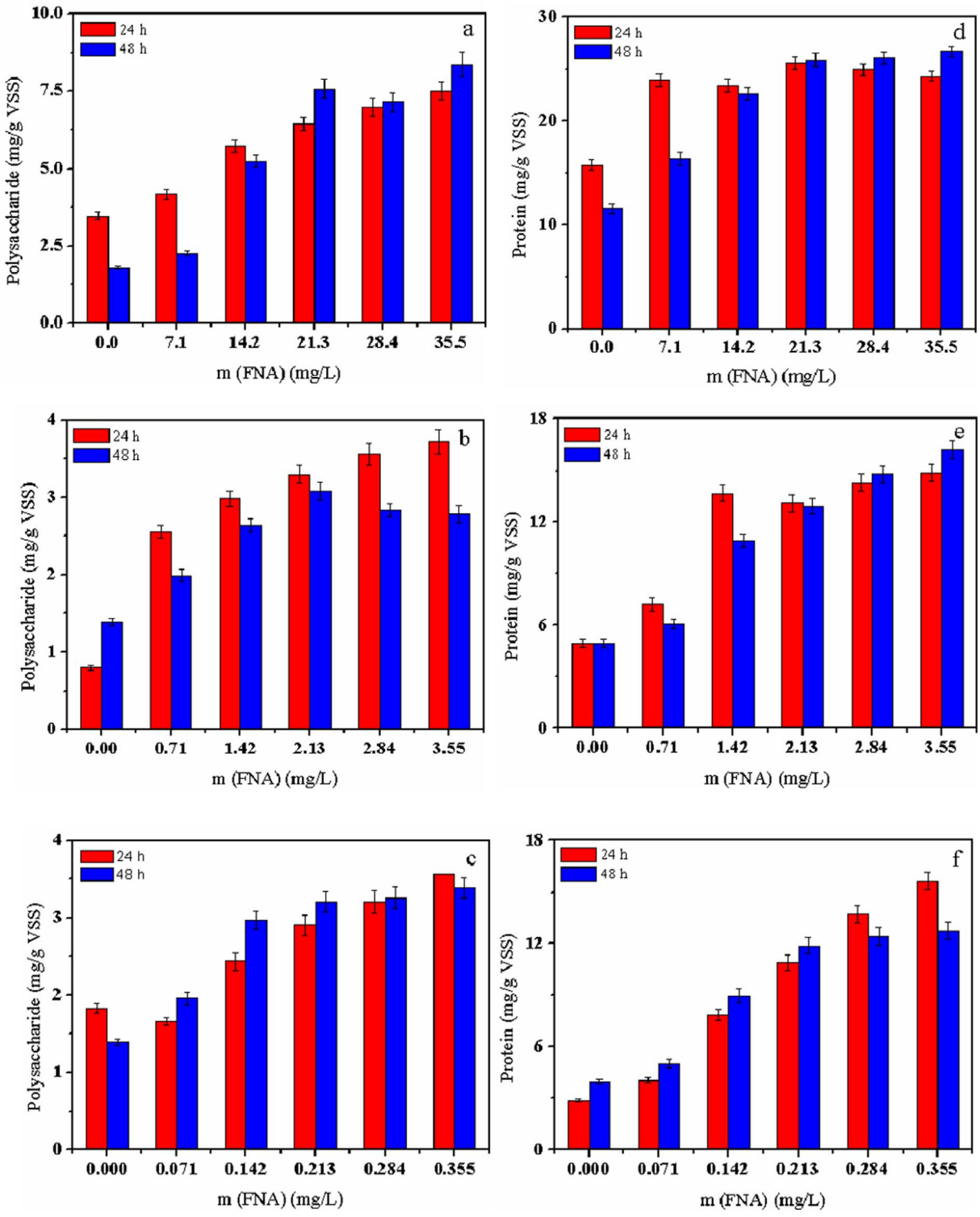

Fig. 2. Effect of initial pH, FNA concentration and exposure time on soluble polysaccharide and protein. (a), (d) pH = 4.5; (b), (e) pH = 5.5 and (c), (f) pH = 6.5.

3. Results and discussion

3.1. Effect of FNA on the dewaterability and physicochemical properties of WAS

Fig. 1 presents the effect of FNA concentration and contact time on sludge dewaterability in term of CST value. Based on the empirical formula (Eq. (1)), the initial pH determine the FNA concentration, thereby indirectly affect the sludge properties. From Fig. 1, it can clearly be found that the CST values gradually increased and then had a declining trend with the rising of FNA concentration at pH 5.5 and 6.5. However, the trend of CST was contrary at pH 4.5. The average CST value at pH 4.5 was much higher than that of pH 5.5 and pH 6.5. Contact time of FNA also had an obviously effect on sludge dewaterability. CST values after 24 h were lower than that after 48 h, which suggested long time of FNA treatment was adverse to the improvement of sludge filterability. Variations of soluble polysaccharide and protein with different FNA concentration and contact time at different pH levels

Table 1
Coefficients of Person correlation between PN, PS, PN/PS and CST.

Sludge sample		CST		
		PN	PS	PN/PS
pH = 4.5	24 h	−0.58	−0.36	0.17
	48 h	−0.06	0.05	0.01
pH = 5.5	24 h	−0.05	−0.03	−0.21
	48 h	0.86*	0.96**	0.63
pH = 6.5	24 h	0.85*	0.71	0.68
	48 h	0.9*	0.92**	0.66

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

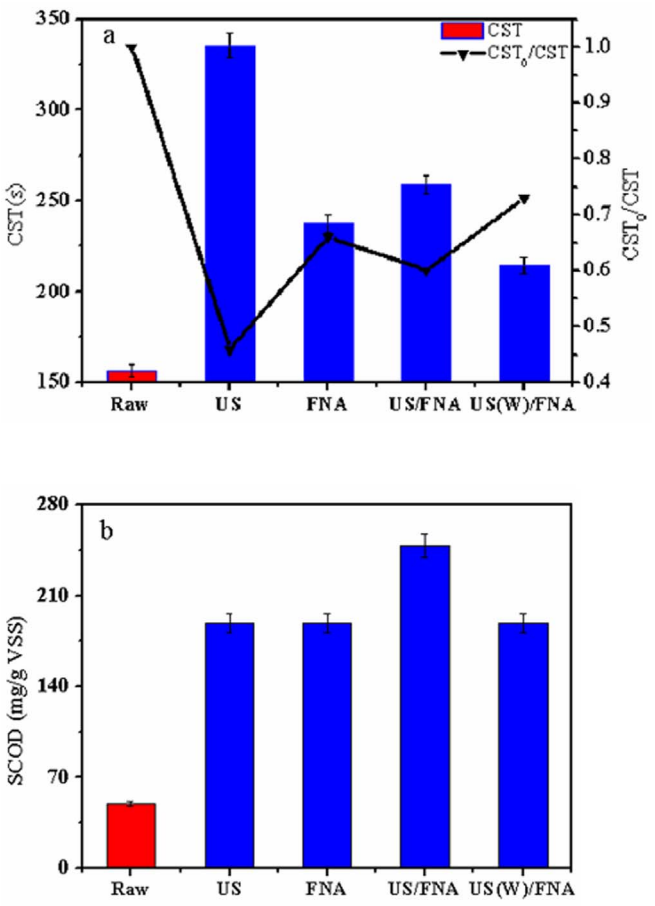

Fig. 3. Variation of sludge filterability and SCOD under different pretreatment.

are illustrated in Fig. 2. With the increase of FNA concentration, increasing soluble polysaccharide and protein concentrations were observed at all pH, which was likely caused by the solubilization of the elution of organics and sludge constituents under FNA treatment [17,26].

The Pearson correlation between PS, PN, PN/PS of the different production in the CST was listed in Table 1. Obviously, CST values had a positive correlation ($R > 0.85$, $p < .01$) with PS concentration at 48 h of pH 5.5, 24 h and 48 h of pH 6.5. The CST values also had a remarkable positive correlation ($R > 0.92$, $p < .05$) with PN concentration at 48 h of pH 5.5 and pH 6.5. It had been proved that PN was more important than PS in affecting the dewaterability of activated sludge [27]. Higgins and Novak indicated that the regression of PN could cause the disintegration of activated sludge [28]. The reason was that PN in sludge proportion owned a more forceful water binding capability than PS or other organic compounds, hence it greatly

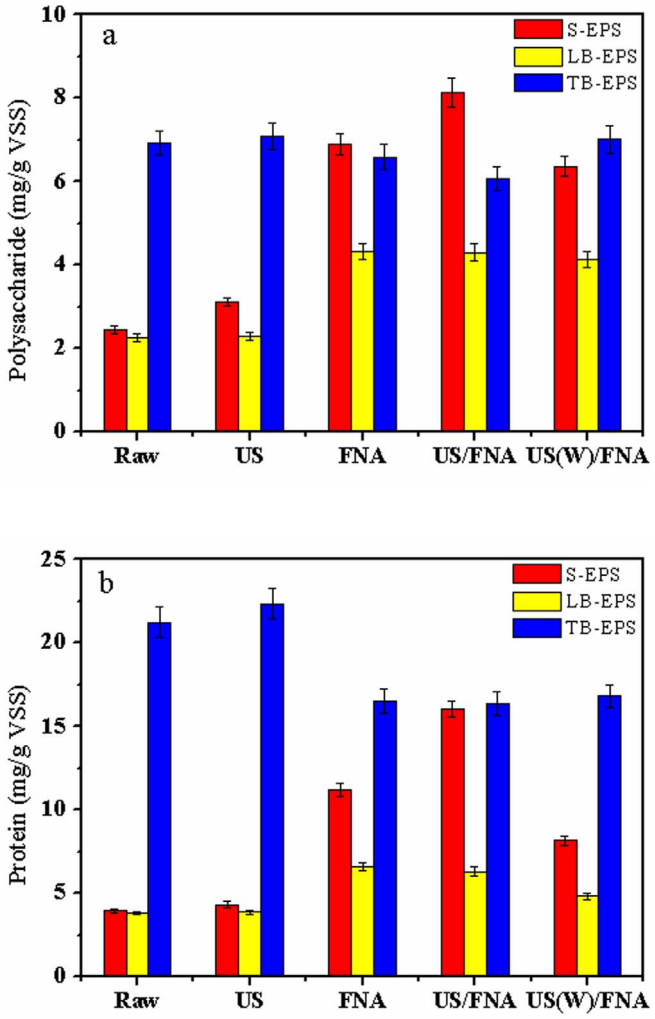

Fig. 4. Variation of polysaccharide (a) and protein (b) in S-EPS, LB-EPS and TB-EPS.

contributed to the sludge dewaterability [29]. Some publications also found that the sludge dewaterability was significantly enhanced with the decrease of PN concentration [16,30]. Compared to at 5.5 and 6.5, the FNA concentration at pH 4.5 increased one or two order of magnitude, resulting in stronger oxidation. Sludge flocs were separated into smaller flinders under strong oxidizing effect and the small sludge particle may plug up the filter paper, causing a reduction of the sludge filterability [31]. Additionally, the relationship between PN/PS and CST was obscure in this study. These results were implicit that FNA pretreatment enhanced the disintegration of sludge floc, which led the increase of PN and PS or other soluble organic compounds. The positive correlation between PS or PN and CST well explained the deterioration of sludge dewaterability induced by FNA pretreatment.

3.2. Enhanced mechanism of FNA pretreatment for WAS solubilization

3.2.1. Variation of WAS filterability

The CST values after different pretreatment are illustrated in Fig. 3a. The CST value of the raw sludge was 156.5 s, but the CST value increased rapidly to 335.4, 237.5, 258.7, 214.4 s after ultrasonic, FNA, ultrasonic/FNA and ultrasonic (wash)/FNA pretreatment, respectively. Correspondingly, CST_0/CST decreased to 0.46, 0.66, 0.60 and 0.72. It was noted that the sludge treated by ultrasound has the worst filterability. This may be relative to the agglomeration effect of EPS by ultrasonic treatment. Compared with ultrasonic treatment, whether FNA or ultrasonic (wash)/FNA treatment all achieved better filterability

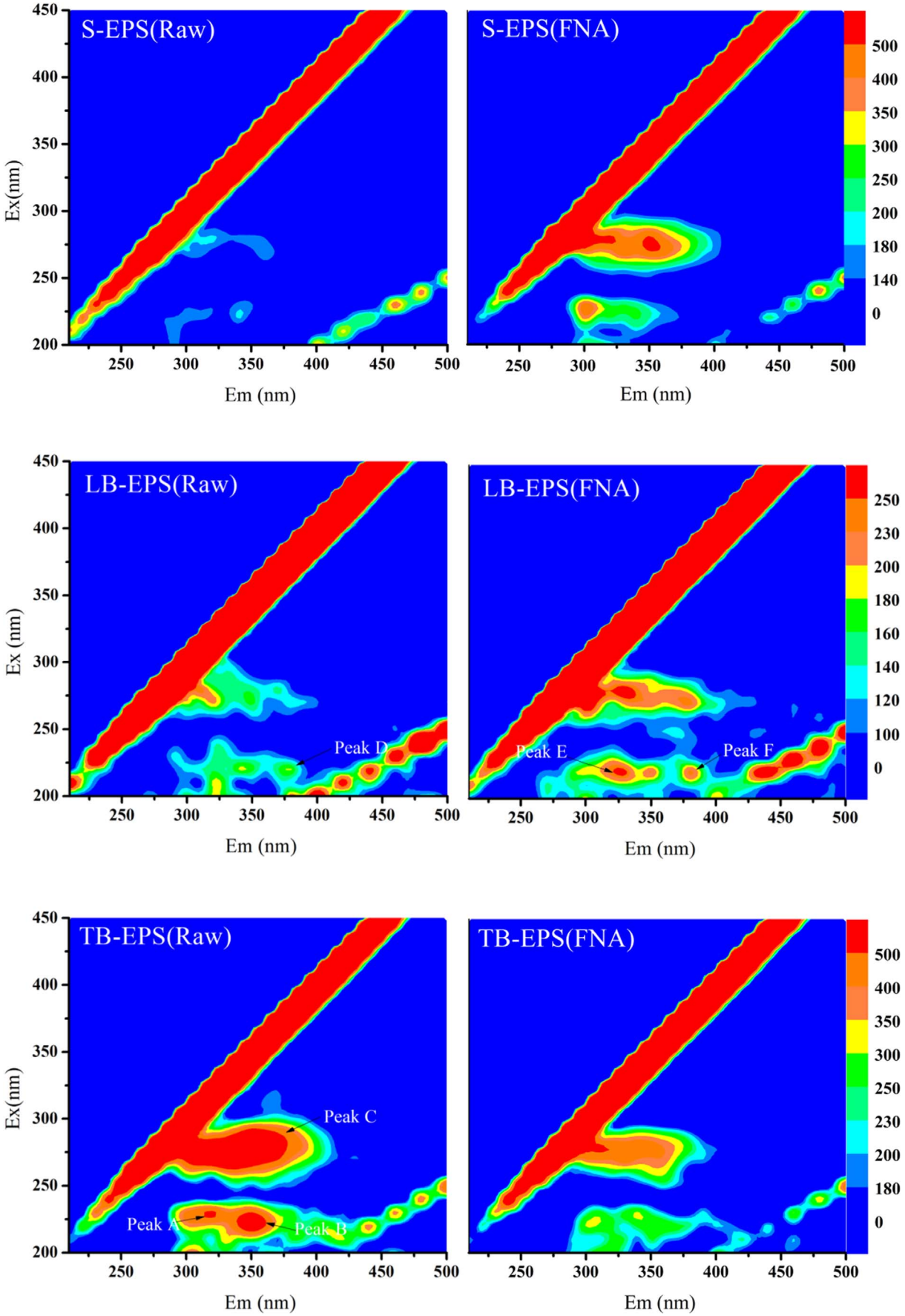

Fig. 5. EEM spectra of EPS fractions (S-EPS, LB-EPS and TB-EPS) before and after FNA treatment: 25 °C, pH = 5.5, FNA = 2.84 mg/L.

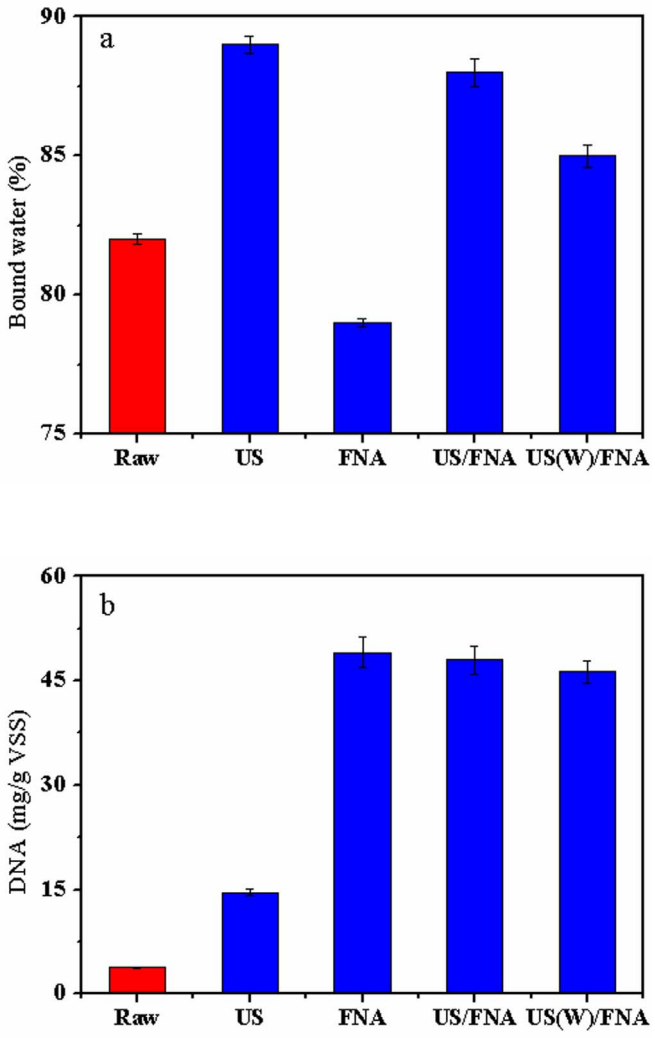

Fig. 6. Variation of bound water (a) and DNA (b) before and after different pretreatment.

improvement, suggesting that FNA can not only disrupt the EPS, but also destruct the cell membrane. This point will be proven in the next discussion.

3.2.2. Response of EPS

3.2.2.1. The content of polysaccharide and protein in EPS. It is well-known that FNA and/or its derivatives had a forceful impact on sludge disintegration [26,32].

The WAS aggregates and flocs were destructed after different pretreatments, a large amount of organic matters were released into the liquid phase, representing an increasing SCOD (Fig. 3b). The SCOD raised from 49.7 mg/g VSS (raw sludge) to 188.8, 188.8, 248.5, 188.8 mg/g VSS after ultrasonic, FNA, ultrasonic/FNA and ultrasonic (wash)/FNA pretreatment, respectively. The effect of FNA treatment alone on sludge solubilization was comparative to the ultrasonic treatment alone. However, their combination could further enhanced the destruction of WAS aggregates and flocs, which led more SCOD. It was worthwhile to note that during ultrasonic (wash)/FNA pretreatment, even ultrasonic pretreatment washed most of EPS, subsequent FNA still produced increased SCOD, which was equivalent to ultrasonic or FNA treatment. We speculated that ultrasonic treatment washed the EPS enwrapping the outside of biologic cells, so FNA could directly destroy the cell membrane and caused the release of intracellular organic matters.

EPS secreted by a variety of microbes were the crucial components of WAS flocs matrixes, basically making up of polysaccharide and protein [33]. Fig. 4 shows the polysaccharide and protein in three EPS layers including soluble EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) after different pretreatments. Their variations also are considered as one of the most important factors influencing sludge dewateribility. Comparing raw sludge, polysaccharide and protein in S-EPS and LB-EPS became more after FNA treatment, however, their content slightly decreased in TB-EPS, which suggested that FNA had stronger ability to disrupt outer EPS, but the effect to TB-EPS was weak. Furthermore, it was clear that more protein were released from the WAS than the polysaccharide, which was mainly because FNA and/or its derivatives has stronger oxidizing and can depolymerize variety of proteins, such as tryptophan and tyrosine [34]. The similar phenomena were found in the sludge treatment with $KMnO_4$, in which more proteins were released than polysaccharides due to the strong oxidizing of $KMnO_4$ [35].

Comparing FNA treatment alone, protein and polysaccharide in S-EPS increased 43% and 18% after with ultrasonic/FNA treatment, respectively, but a slightly decrease was found in LB-EPS and TB-EPS. EPS attaching to cells was eluted by the combined cavitation by ultrasonic and FNA treatment [21].

3.2.2.2. EEM spectra of different EPS fraction. The fluorescence EEM spectra for the EPS before and after FNA treatments were compared to get deep insight into the enhanced mechanism and the results are illustrated in Fig. 5.

Three main fluorescence peaks, situating at the excitation/emission wavelengths (Ex/Em) of (219, 231)/320 nm (Peak A), (210, 230)/(340, 360) nm (Peak B) and (275, 280)/(300, 350) nm (Peak C) in EEM spectra, were picked from fluorescence spectra of TB-EPS from raw sludge. Except for Peak A, a new peak, Ex/Em of (220,223)/(375,380) nm (Peak D), was found in LB-EPS, but no obvious peaks existed in the S-EPS. According to the five regions of EEM divided in previous study [36], Peak A was referred to tyrosine (Region I), Peak B and Peak D indicate aromatic protein (Region II) and Peak C, which had a strong fluorescence intensity, is related to the protein-like compounds [22,37]. After FNA treatment, four main peaks (Peak A, B, C and D) with higher fluorescence intensity were all found in LB-EPS layers, in addition, two new peaks, Ex/Em of (272, 281)/(375, 385) nm (Peak E) and (220, 224)/(380, 385) nm (Peak F) were observed in the LB-EPS. Peak E was referred to as soluble microbial by-product (SMP)-like substances (Region VI) and Peak F was related to humic-like substance [36].

The location of Peak A, Peak B and Peak C for S-EPS and LB-EPS after FNA treatment migrated to red region to a certain degree. The migration should attribute to the shift of alkomyl, hydroxyl, amino, and carbonyl in the fluorophores structures [38]. However, the fluorescence intensities of three peaks were significantly weakened in the TB-EPS. In general, the shifts of fluorescence peak intensity after FNA treatment is an indicator to the oxidation of flourescencing compounds. It can be seen from Fig. 5 that the intensities of fluorescence peaks increased for the S-EPS and LB-EPS fractions, but it decreased for TB-EPS. It represented that FNA treatment affected the fluorescence materials (mostly protein or protein-like) in S-EPS and LB-EPS. Liu had proved that the fluorescence materials in three different EPS layers had influence on the sludge dewaterability [22]. This research indicated that enhanced sludge dewaterability was mainly associated with the removal as well as degradation of organic matters exist in WAS. The fluorescence EEM spectra for the EPS suggested that the oxidation of FNA and/or its derivatives effectively disrupted the functional groups of organic compounds in EPS, especially outer EPS. Then the FNA broke the cytomembrane, which displayed as the weaken fluorescence intensity of TB-EPS. Additionally, the presence of Peak F illustrated that there was humic acid in LB-EPS, which showed a positive correlation with sludge dewaterability. Some researchers had pointed that fulvic and humic acids influenced the filtration resistance of activated sludge

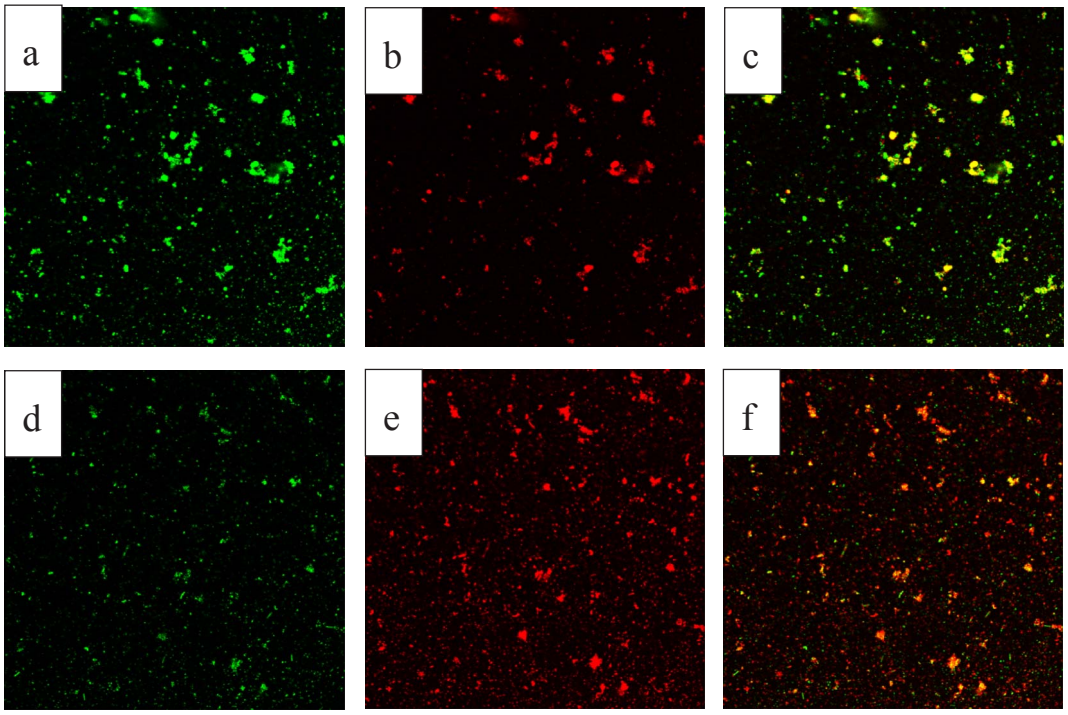

Fig. 7. CLSM images of raw sludge (a, b, c) and FNA pretreatment sludge (d, e, f). Viable cells (Green) and non-viable cells (Red) are stained by LIVE/DEAD™ BacLight™ Bacterial Viability kit (a, b, d, e); the combination of viable cells (Green) and non-viable cells (Red) is revealed in Yellow (c, f). Magnification ×20. *Scale bar* = 250 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

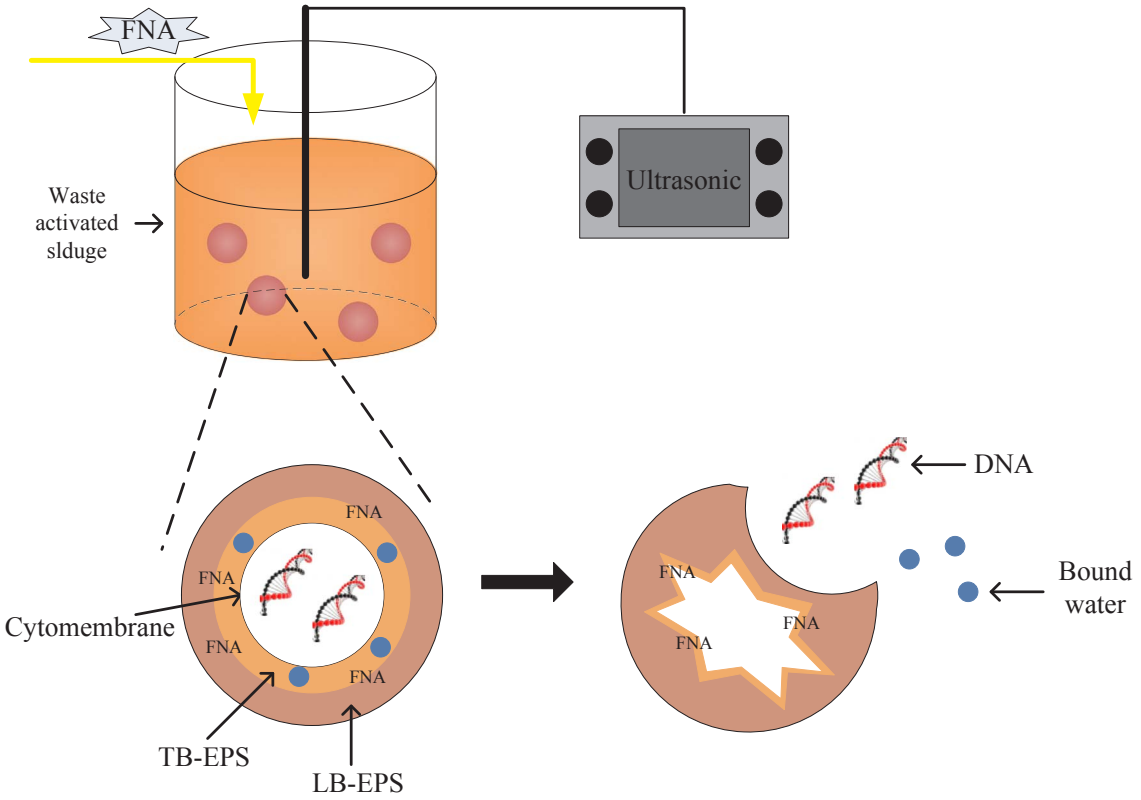

Fig. 8. The mechanism of FNA treatment on waste activated sludge.

[39,40]. Humic and humic acid-like compounds combined with cations and then constituted the intermolecular bridges, which significantly altered the filtration [41].

3.2.3. Bound water and cell viability analysis

The variation of bound water and DNA percentage after different pretreatments is shown in Fig. 6. Compared with raw sludge, the percentage of DNA in the supernatant increased 3.89, 13.08, 12.80 and 12.34 times after ultrasonic, FNA, ultrasonic/FNA and ultrasonic

(wash)/FNA pretreatment, respectively. DNA not only presented in living cells, but also in EPS matrix [42]. It seemed to be a not appropriate method to indicate the DNA from cell lysis or EPS disruption. Ultrasonic treatment served as an effective method to elute EPS matrix (Fig. S3), hence DNA the supernatant after the pretreatments involved FNA could be regarded partly or completely from cell lysis. FNA has been demonstrated that it could result in oxidative deamination of NH_2 group in cytosine or adenine to ether group [43]. Moreover, FNA caused the fragmentation of DNA structure by transforming guanine to xanthine, adenine to hypoxanthine and cytosine to uracil was the other [44]. Therefore, it could be found that the percentage of DNA in the supernatant had significantly increases after the pretreatments involved FNA. In order to clarify the effect by FNA treatment, Live and dead bacterial viability before and after FNA treatment was analyzed by confocal laser scanning microscopy (CLSM) with a staining method and the results are displayed in Fig. 7. As seen from Fig. 7, the Green fluorescence intensity, which reflects the number of viable cells, decreased dramatically after FNA pretreatment. These results further confirmed our above speculation that FNA could actually destroy the cytomembrane, which enhance the solubilization of WAS.

Bound water usually stay at the particle surface or is held in capillaries by adsorption [45]. As seen from Fig. 6a, it could be found that bound water of sludge was low 80% after FNA treatment, suggesting this part of water was lost through the disruption of S-EPS and LB-EPS. FNA and/or its derivatives could breakdown TB-EPS and caused the transfer of it. Bound water involved in this layer was released. On the other hand, the water held in the cells, accompany with the intracellular content, was discharged and then been evaporated. The bound water was trapped in the S-EPS and LB-EPS and the TB-EPS content was the main factor that influenced the bound water content. However, when WAS were treated by involved ultrasonic pretreatment, the bound water of sludge all rose, which increased from 82% (raw sludge) to 89% (ultrasonic), 88% (ultrasonic/FNA) and 85% (ultrasonic (wash)/FNA), respectively. It may be attributed to the formation of smaller pellets under ultrasonication, which resulted in poor filterability.

3.3. The enhanced mechanism

This study revealed that the FNA could not only disrupt outer EPS, but also destroy the cytomembrane. The main role of FNA in the pretreatment of WAS was cell lysis accompanying with weak EPS disruption. This viewpoint was evidenced by the change of protein and polysaccharide in different EPS layers, EEM spectra analysis for EPS and DNA percentage in the supernatant. The dewaterability of sludge became worse as the consequence of more organic matters existed in the supernatant. Moreover, the use of ultrasonic treatment before FNA treatment also provided evidence that FNA could effectively break cytomembrane. Previous publication demonstrated that the proteins in sludge and cells were damaged by FNA itself and/or its derivatives through the reaction between them [44]. Recently, it had been reported than FNA was able to break down EPS by changing the chemical structure and molecular weight distribution of EPS. Moreover, some studies focused on the chemistry between synthetic pure macromolecules and FNA [17]. And it also had been proven that the disruption of cell envelope happened at 12 h when WAS were treated by FNA, through the variation of the sum of soluble COD and extracted COD [46].

A schematic view of enhanced mechanism of FNA on WAS solubilization was illustrated in Fig. 8. FNA could break down outer EPS but the effect was weak. However, it was confirmed through washing EPS by ultrasonic pretreatment that TB-EPS could be really destroyed by FNA treatment. Polysaccharide and protein in TB-EPS reduced after FNA treatment (Fig. 4) and the fluorescence intensities of peaks in TB-EPS happened observably blue shift (Fig. 5). FNA can directly destroy the cytomembrane and caused the degradation of biological cells (Figs. 6b, 7). When WAS contacted with FNA or/and its derivatives, sludge flocs disintegrated and then EPS dissolved into the solution and translated into the soluble organic matters (Fig. 2), simultaneously, bound water (including interstitial water and vicinal water) in the outer EPS was released (Fig. 6a). On the other hand, the rupture of cell membrane also was accompanied by the release of TB-EPS. Moreover, the release of humic acid, tryptophan-like as well as the PN and PS existed in EPS resulted in the deterioration of sludge filterability (Fig. 1).

The FNA pretreatment technology on activated sludge/waste activated sludge has been proved to be an economically and effective method to enhance sludge biodegradability [3,10]. Our study confirmed the mechanism of FNA on sludge solubilization, which contributed to deeply understand the use and generalization of this technology in real application.

4. Conclusions

The goal of this study was to investigate that the role of FNA in the pretreatment of WAS is cell lysis or EPS disruption? Experimental results demonstrated that FNA had a weaker influence on disruption of S-EPS and LB-EPS matrix except TB-EPS but a strong destruction to the cytomembrane. The percentage of DNA in the supernatant increased rapidly and the viability of cells decreased after involved FNA treatment. The release of organic matters as well as humic acid in LB-EPS caused the deterioration of sludge dewaterability.

Acknowledgements

This research was financially supported by the project of National Natural Science Foundation of China (Nos. 51779088, 51508178, 51478170) and Hunan Provincial Natural Science Foundation of China (No. 2017JJ2051).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cej.2017.11.038.

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