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Free ammonia pretreatment assists potassium ferrate to enhance the production of short-chain fatty acids from waste activated sludge: Performance, mechanisms and applications

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ABSTRACT:

Anaerobic fermentation of waste activated sludge (WAS) is often limited by low disintegration rates of WAS and quick consumptions of short-chain fatty acids (SCFAs) by methanogens. This work proposed a novel, efficient and harmless pretreatment approach i.e., using potassium ferrate (PF) combined with free ammonia (FA), to enhance the disintegration of WAS and the accumulation of SCFAs from WAS anaerobic fermentation. It was found in this work, the best pretreating condition of WAS is 0.10 g PF/g VSS + 180 mg FA/L, under which the SCFAs consistence reached its maximum value (342.5 mg COD/g VSS), which was respectively 7.45-fold, 3.50-fold and 2.06-fold of that from the Blank, FA and PF reactor. Further in-depth mechanism study showed that the disintegration of WAS promoted and the biodegradability of fermentation broth enhanced under the PF + FA pretreatment condition. Moreover, the populations of fecal coliforms in WAS reduced significantly when PF + FA was adopted. Considering that FA could be produced *in situ* during the ammoniating process, the findings in this work promoted the utilization of PF-based pretreatment method in actual operations.

Keywords: Waste activated sludge; anaerobic fermentation; short-chain fatty acids; potassium ferrate; free ammonia

1. Introduction

Activated sludge process (ASP), as a method with many advantages for the treatment of wastewater, has been wildly adopted by wastewater treatment plants (WWTPs) (He et al., 2021). Although ASP has made a remarkable contribution to the protection of the water environment, waste activated sludge (WAS), a main terminal product in wastewater treatment, has also brought great challenge to the operation of WWTPs since its large quantities and inconvenience of treatment and disposal (Xu et al., 2020a). With the deeper realizing about the sustainable development, it is increasingly recognized that sludge is not a “waste” but a reusable resource due to the abundant available organic matter (Liu et al., 2020b). Therefore, a series of technologies, which could recover energy and/or resource from sludge or reduce sludge amount, have been developed and applied in recent years (Li et al., 2015).

Among them, anaerobic fermentation, which could not only reutilize WAS to generate valuable substance i.e., short-chain fatty acids (SCFAs) but also simultaneously realize sludge reduction, has aroused wide attention as a sludge treatment technology to recycle resources (Chen et al., 2018). Especially in recent years, it was demonstrated in full-scale that the generated SCFAs could be supplemented as a preferred carbon source into influent of WWTPs to solve the problem of insufficient carbon source, improving performance of biological nutrients removal in WWTP (Qian et al., 2021). Attention in the anaerobic fermentation of WAS has therefore continuously increased. Nevertheless, even at laboratory scale, the operational performance of the WAS anaerobic fermenters was usually not satisfactory. For instance, not only the production process of SCFAs was often limited by the rate-limiting of WAS solubilization but the accumulation of SCFAs was also disrupted by the rapid consumption of methanogens, jointly resulting in unsatisfactory SCFAs yield. Moreover, excluding substantial available organic substrates (e.g., proteins and polysaccharides), various toxic and hazardous pollutants (e.g., heavy metals, endocrine disrupting compounds (EDCs) and antibiotics) were also concentrated in the WAS (Xu et al., 2020b; Zhang et al., 2021; Zhao et al., 2021). These persistent organic pollutants not only were hard to biodegrade during the traditional anaerobic fermentation but also might induce negative effects on human health due to disruption of the endocrine system (Liu et al., 2020a). Meanwhile, these non-degradable pollutants would inevitably enter the environment along with the further disposal of WAS, posing potential risks to the ecological environment and even human health (Gong et al., 2020). Thus, pertinent pretreatment for WAS was usually required before anaerobic fermentation (Liu et al., 2021; Luo et al., 2020a).

Potassium ferrate (K_2FeO_4, PF), a novel disinfectant with high reactivity and selectivity, could decompose into harmless substances (i.e., iron hydroxide and oxygen) after a series of reactions in hydrous medium (Deng et al., 2018). Therefore, PF has been wildly used in wastewater treatment and the remediation of soil or groundwater (Antošová et al., 2021; Han et al., 2018). In recent years, PF was also found to have wide application potential in another new direction i.e., as a mean of pretreatment of WAS, after which the accumulation of SCFAs, sludge dewatering performance and the removal of persistent pollutants (e.g., heavy metal, nonylphenol and hormones) in WAS could be enhanced (Luo et al., 2021; Luo et al., 2020b). It has been experimentally demonstrated that PF markedly enhanced the disintegration of sludge cells and extracellular polymeric substance (EPS), thereby benefiting the production of SCFAs (Li et al., 2018b). These findings not only expanded the application field of PF but also provided a novel technique to pretreat sludge to produce SCFAs from anaerobic fermentation (Li et al., 2018b). Combined the findings about the degradation of pollutants in sludge, PF-based sludge pretreatment method could contribute the resource recovery and reduction of potential environmental risks from sludge, having important ecological benefit.

However, the high dosages of PF (such as 0.5 g/g VSS) reported will lead to excessive costs, which will not only greatly diminish its application value, but also frustrate its further applications in the real world. Therefore, any promotion that could significantly reduce the dosage of PF in an economical and sustainable manner would cause important benefits in both economy and ecology.

Free ammonia (FA), a renewable chemical recently used in sludge treatment, could achieve ~20% of sludge reduction at ~16 mg FA/L (Yang et al., 2018). Additionally, it was experimentally demonstrated that FA had powerful biocidal effect on microorganisms widely involved in wastewater and sludge treatment unit, including primary settler, bioreactor, secondary sedimentation tank, and sludge thickener (Liu et al., 2019). For example, the activities of *Nitrobacter* in nitrifying reactor were completely inhibited when the FA exposure concentration is greater than was more than 6 mg N/L (Zhang et al., 2018b). Similar inactivation on methanogens was also observed when the level of FA reached 150 mg N/L, resulting in severe suppression of methane production during the WAS treatment (Wang et al., 2018a). Recently, researchers applied the bactericidal effect induced by FA to sludge treatment and found that FA pretreatment enhanced the disruption of sludge cells, benefiting the WAS solubilization (Liu et al., 2018). These findings reported inspire us to consider whether FA pretreatment could be combined with PF to reduce the dosage of PF and even improve the generation of SCFAs from WAS. Considering that FA could be produced during the ammoniating process in sludge fermentation broth, if combining PF with FA pretreatment is feasible, it will not only bring economic benefit but also increase the application potential of PF in real-world situations. However, to date, the feasibility of enhancing SCFAs production at lower dosage of PF with the aid of FA pretreatment is unknown.

This work aims to find out whether the enhancement of SCFAs yields could be achieved by using this combined method (i.e., PF and FA pretreatment) during WAS anaerobic fermentation. Specifically, the SCFAs yields with different PF dosage combined with FA pretreatment were firstly compared. After that, to further understand how this combined method works, its effects on the processes of anaerobic fermentation relevant to SCFAs concentration changes were explored. Finally, the inactivation of different treatments (i.e., blank, PF, FA pretreatment and PF + FA pretreatment) on the coliform flora of fermented sludge was assessed. The experimental phenomena found in this work not only increase the appliance potential of PF-involved sludge treatment, but also may also provide a promising method for recovering bioenergy and resources from sludge and simultaneously removing toxic and hazardous pollutants.

2. Materials and methods

2.1. WAS and PF

The WAS for this work was taken from a WWTP in Changsha, China. The sewage treatment process of primary sedimentation tank + biological aerated filter was adopted by this WWTP. The sludge was filtered to remove sediment, and then kept in the refrigerator at 4 °C for 24 h prior to use. The specific characteristics were shown in Table 1. PF (over 90 wt%), provided by Macklin Biochemical Reagent Co., Ltd (Shanghai, China). It is in powder form and has a deep purple color with metallic luster.

2.2. Details of WAS pretreatment and batch tests for SCFAs production

The pretreatment experiment was conducted in 10 glass bottles (volume = 500 mL) and numbered 1-10. Firstly, 400 mL of sludge was poured into each bottle. Secondly, different dosages of PF and NH_4Cl were respectively added into the bottles, causing the PF dosage of 0, 0.05, 0.1 and 0.2 g/g VSS and the initial

NH_4^+ -N level of 19.2 and 477.6 mg/L (See Table 2 for details). Herein, 19.2 mg/L represented the background level of ammonium in sludge. Reactor 1 (i.e., blank) did not adjust pH or add any chemical substances. Reactor 2 and 3 were respectively set to evaluate the contribution of solo initial pH 9 and solo initial FA to the SCFAs generation. The impact of solo doses of PF (i.e., 0.05-0.2 g/g VSS) was verified by reactor 4-6 and reactor 7 was performed to test the influence of 0.1 g PF/g VSS combined with initial pH 9 on the generation of SCFAs, while reactor 8-10 were used for investigating the impact of different doses of PF combined with FA pretreatment on SCFAs production. The specific experimental conditions adopted by above reactor were presented in Table 2.

Among these reactors, the tested WAS in reactor 1, 4, 5 and 6 did not require pH pretreatment, while the initial pH was 9 in the remainder reactors prior to anaerobic fermentation. All reactors were at a temperature of 25 °C. Above pH adjustment was performed using 2.0 M NaOH or HCl. The factors including NH_4^+ -N concentration, pH value and temperature determined the initial FA levels of 0.07, 6.96 and 180 mg/L, which was calculated by the formula $S_{(NH3 - NNH4+- N)} \times 10^{pH} / (K_b/K_w + 10^{pH})$, where $S_{(NH3-N,NH4+-N)}$ means the sum of NH_3-N + NH_4^+-N, K_b and K_w represents the ionization constant of the ammonia equilibrium equation and ionization constant of water. The formula of $K_b/K_w = e^{6344/(273+T)}$ was used to calculate the value of K_b/K_w (El Hadj et al., 2009). The concentration of 180 mg/L FA pretreatment was reference to the previous work (Zhang et al., 2018a). At the end of pretreatment, 10 reactors were filled with nitrogen gas with purity of 99.99% for 6 min to maintain an anaerobic environment. The whole fermentation process was carried out in a water bath incubator (25°C, 120 rpm).

2.3. Evaluating the effects of PF, FA and PF + FA on the processes involved in anaerobic fermentation

It is recognized by most researchers that anaerobic fermentation mainly includes following processes: solublization, hydrolysis, acidogenesis and methanogenesis (Guo et al., 2015). Among of them, the first three processes are believed to be related to generation of SCFAs, while methanogenesis is considered as a SCFAs-consuming process (Yang et al., 2020). Hence, mechanism analysis of how PF + FA obtain higher SCFAs production starts with assessing the effects of PF, FA and PF integration with FA on solubilization process. Generally, most of the organic matters in the sludge is in the solid phase, thus by evaluating the content of organics (e.g., soluble COD and protein) in the sludge supernatant (i.e., liquid phase), the solubilization rate of sludge could be determined (Li et al., 2020b).

As for hydrolysis, acidogenesis, and methanogenesis, they are biochemical processes involving specific microbes. Therefore, the effects of PF, FA and PF integration with FA on these three processes can be indicated by comparing the specific activities of these relevant microbes. Herein, several batch tests used by many studies were conducted to support this part of the research (Li et al., 2020a). In these tests, 12 glass bottles of the same specification as Section 2.2 were divided into 3 groups (recorded as Hydrolysis-test, Acidogenesis-test, and Methanogenesis-test, respectively).

The general steps of these three groups of tests were as follows: 1) each reactor fed with 30 mL inoculum, domesticated in advance of the experiment. 2) 270 mL synthetic wastewater containing specific model compound were respectively added. 3) In each group of test, one reactor was set to blank without any treatment, and the experimental condition in the other three reactors was respectively set to 0.10 g PF/g VSS, 180 mg FA/L and the combination of 0.10 g PF/g VSS with 180 mg FA/L according to the results from Section 2.2. The detailed experimental conditions for three tests were shown in Table 3. Other procedures of test were the same as in Section 2.2.

Three tests were all lasted for 3 d, and specific model compound concentration, that is, dextran, glucose and sodium acetate, among these reactors of above tests was measured each day. By comparing the degradation rates of these specific model compounds, the influences of PF, FA and PF + FA on the activities of the microbes responsible for hydrolysis, acidogenesis, and methanogenesis were revealed. The calculation of degradation rate of three specific model compounds was analyzed by linear regression (shown in Supporting Information).

2.4. Analytical methods

TCOD, SCOD, TSS, VSS, and NH_4^+-N were measured with reference to standard methods (APHA, 2005). The density and component of SCFAs were gauged by gas chromatograph (GC 2010-plus, Shimadzu), with detailed procedures described in Text S1. The method of extracting EPS form WAS is refer to which documented in literature (Zahedi et al., 2016), and the calculation method for COD mass balance was described in literatures (Li et al., 2020c). The determination of soluble proteins was performed through Lowry-Folin (Lowry et al., 1951). The changes in degradable characteristics of soluble organic matter in fermentation broth was analyzed by three-dimensional excitation emission matrix (EEM) fluorescence spectroscopy (Hitachi F-4600 FL, Japan) (Li et al., 2020a), and the details were depicted in Text S2 (Supporting Information). The Coliert-18 Test kit (IDEXX Laboratories, USA) was utilized to measure the fecal coliforms in sludge, detailed procedures being depicted in Text S3 (Supporting Information).

3. Results and discussion

3.1. SCFAs productions under different pretreatment conditions

Fig. 1a shows the changes of SCFAs yield of WAS anaerobic fermentation under different pretreatment conditions. It was found that SCFAs yield in the blank was low (<46.0 mg COD/g VSS) since the sludge remains a relatively complete structure without any pretreatment, and the organics in fermentation broth was consumed rapidly (Yuan et al., 2006). The addition of PF notably promoted the production of SCFAs, and as the amount of PF added increased from 0.05 to 0.20 g/g VSS, the maximum SCFAs yield increased from 74.3 to 297.5 mg COD/g VSS. Also like demonstrated previously, sludge pretreated with initial pH 9 and initial 180 mg FA/L obviously enhanced SCFAs production compared to the blank fermenter, with the maximum SCFAs yield increasing from 46.0 to 83.6 and 97.8 mg COD/g VSS.

When the sludge undergone initial FA pretreatment before PF addition, the corresponding SCFAs yield obviously increased compared with either sole PF addition or sole initial FA pretreatment, e.g., 342.5 mg COD/g VSS in the initial 180 mg FA/L + 0.10 g/g VSS PF reactor, which was respectively 3.50 and 2.06 times of that from the initial 180 mg FA/L and 0.10 g PF/g VSS reactors. At the same initial 180 mg FA/L pretreatment, this enhancement of SCFAs production in initial FA + PF reactors was also significantly affected by the dose of PF. With increasing of PF dosage from 0.05 to 0.10 g/g VSS, the maximum SCFAs yield was improved from 297.6 to 342.5 mg COD/g VSS. Meanwhile, it was 8 d to reach the optimal SCFAs production, and 10 d under the normal condition (i.e., blank). Nevertheless, for the optimal SCFAs productivity, continuing to increase the dosage of PF to 0.20 g/g VSS only slightly increased it to 358.4 mg COD/g VSS. Considering comprehensively PF dosage, SCFAs yield and optimal fermentation time, initial 180 mg FA/L + 0.10 g/g VSS PF was therefore chosen as a relatively optimal fermentation condition. Moreover, as can be seen that the maximum SCFAs production of the initial 180 mg FA/L + 0.10 g/g VSS

PF reactor was higher than that in the 0.2 g PF/g VSS reactor (342.5 vs. 297.5 mg COD/g VSS), indicating that this FA pretreatment assisted PF method to promote the production of SCFAs while reducing PF dosage was feasible.

The effects of different treatment methods on the proportion of individual SCFAs are exhibited in Fig. 1b. It was found that the ratio of several individual SCFAs in the fermentation broth in each reactor, except for the initial pH 9, changed significantly after different pretreatments. In all reactors, acetic acid and propionic acid were two major SCFAs produced, whose sum of the proportions was increased from 61% to 81% after 8 days of fermentation. As far as the acetic, it respectively accounts for 15.9%, 16.1%, and 31.9% of the total SCFAs in blank, pH 9, and 180 mg FA/L. In particular, the scale of acetic acid improved markedly along with the enhancement of the amount of PF, it was 28.4% in 0.05 g PF/g VSS, 51.6% in 0.10 g PF/g VSS, and 63.0% in 0.20 g PF/g VSS. This might be relevant to the acid-producing bacteria, because previous study certified that PF enhanced the acidification activity by affecting the key enzymes (including protease, α-glucosidase, acetate kinase, and phosphotransacetylase) relevant to hydrolysis and acidification (Li et al., 2018a). While in the three combined reactors (PF + FA), the ratios of acetic among them (58.6% in 0.05 g PF/g VSS + 180 mg FA/L, 65.0% in 0.10 g PF/g VSS + 180 mg FA/L, and 63.3% in 0.20 g PF/g VSS + 180 mg FA/L) were higher than that in the three corresponding PF reactors. Obviously, the PF + FA pretreatment method enhanced the acetic proportion of the total SCFAs and even in the yields of SCFAs. For example, taking 0.10 g PF/g VSS + 180 mg FA/L and sole 0.20 g PF/g VSS for comparison, the composition of individual SCFAs in the two reactors was similar, even the ratio of acetic acid in the combined pretreatment is slightly larger. From the perspective of SCFAs production ratio, FA combined with PF pretreatment is an alternative method, and it can greatly reduce the cost of chemical addition.

3.2. Mechanism investigation for promoted SCFAs production

3.2.1. Effect of FA pretreatment assisted PF on sludge disintegration

At the beginning of WAS anaerobic fermentation, the variation in soluble organics is an effective tool for characterizing many related indicators (Wu et al., 2020a). On the one hand, soluble organics can reflect the degree of sludge disintegration since most of the organic matters are contained in the solid phase of sludge cells. On the other side, the organics released from sludge cells to fermentation supernatant, for instance proteins and carbohydrates, can be used for the following steps of anaerobic fermentation (Wu et al., 2020b). Therefore, soluble organics can also reflect the amount of the substrates provided for the SCFAs production process. The variations of soluble COD and protein distribution of different pretreatment cases were revealed in Fig. 2, which are significantly different under different pretreatment conditions, wherein the concentration of soluble COD and protein in 0.10 g PF/g VSS + 180 mg FA/L reactor is higher than that of sole PF (0.10 g/g VSS) reactor, the initial FA 180 mg/L reactor and other pretreatment conditions during the first 24 h. For instance, the content of soluble COD in sole FA (180 mg/L) reactor is 1853 ± 71 mg/L at 24 h, 1780 ± 80 mg/L in sole PF (0.10 g/g VSS) reactor, and only 575.2 mg/L in Blank, while the corresponding concentration in the PF (0.10 g/g VSS) + FA (180 mg/L) reactor was 2005 ± 55 mg/L. Similar phenomenon was also found in the release of protein at 24 h. It can be concluded that both FA and PF can improve the disruption of sludge, but the combination of FA and PF can further enhance the degree of disruption of sludge. This causes more organics being transferred into the liquid phase, thereby providing the microbes with more substrates for SCFAs production.

As we all know, EPS and cell envelop are two barriers to protect sludge cells from harmful external environment (Wu et al., 2014). Thus, the sludge disintegration could be effectively reflected by the morphological changes of EPS and cell envelop. To further reveal the details of how PF + FA pretreatment enhance SCFAs production through sludge disintegration, the EPS structure changes and cell membrane integrity was compared to testify the influence of different pretreatment conditions (i.e., PF, FA and PF + FA).

When the cell membrane remains intact i.e., intracellular organics is not released, the sum of soluble COD in the fermentation liquor and extracted COD from sludge remains balance at different fermentation times, the change of the soluble COD and extracted COD therefore could be used to indicate the damage degree of EPS. On the contrary, if the sum of soluble COD and extracted COD increases significantly from the initial value at a certain pretreatment time, the release of intracellular organics is thought to happen, that is, the cell membrane is broken (Li et al., 2021). From Fig. 3a, it was found that no remarkable increase in the sum of soluble COD and extracted COD with pretreatment time from 0 min to 10 min was observed in the blank fermenter, the PF fermenter, or the FA fermenter. In addition, the phenomenon displayed in the release of lactate dehydrogenase (LDH, a cell membrane integrity marker) with pretreatment time from 0 min to 10 min in above three fermenters was similar to above results (Fig. 3b). This suggested that the cell membrane remained intact within the initial 10 min, and the soluble COD measured at above three fermenters majorly come from the disintegration of EPS. Nevertheless, in the PF + FA fermenter, no matter the sum of soluble and extracted COD and the release of LDH both significantly increased at 10 min compared with 0 min, which indicated that cell envelope was disrupted within the initial 10 min. Above phenomenon indicated that PF in combination with FA caused the destruction of cell envelope faster than either PF or FA. Because EPS is on the periphery of the cell membrane, this synergetic disruption should act on EPS first. The accelerated destruction of EPS and cell membrane indicated faster sludge cells lysis, which might be one reason for the combined method improving SCFAs generation.

3.2.2. Effect of FA pretreatment assisted PF on the biodegradability of the released organics

Although sludge cells contain a lot of organics, in fact, only part of the organics released from disrupted sludge cells is biodegradable (e.g., carbohydrate, protein and lipid), and the rest are non-biodegradable (e.g., humus and lignocellulose), which are generally considered difficult to utilize in anaerobic fermentation processes (Li et al., 2018a). Thus, the influence of different pretreatment conditions (i.e., PF, FA and their combination) on the biodegradability of released organics was further studied using the method of the 3D fluorescence spectroscopy that can characterize the biodegradability of dissolved organic compounds.

It is reported that the EEM spectra has been widely used to scan the changes of dissolved organics, and the method to distinguishing their types has gained widely consensus (Guo et al., 2014). The regions where the excitation/emission wavelength (Ex/Em) of 200-250/250-330 nm (Regions-I), 200-250/330-380nm (Regions-II), and 200-250/380-500 nm (Regions-III), indicate the existence of tyrosine-like proteins, tryptophan-like substances, and fulvic acid-like substances, respectively. The regions where the excitation/emission wavelength (Ex/Em) of 250-350/250-380 nm (Regions-IV), and 250-400/380-500nm (Regions-V), indicate the existence of soluble microbial by-product substances and humic acid-like substances, respectively. Those dissolved organic matters in different regions have different properties, among which are considered to be biodegradable in the regions I and IV, and the rest are non-biodegradable (Li et al., 2018a). In this work, the EEM spectra of organic matters in fermentation liquid of different fermenters at 12 h and the corresponding percent of fluorescence response ($P_{i,n}$) were shown in Fig.4. It could be clearly seen from the EEM fluorescence based calculation that the percentage fluorescence response of Region-I and Region-IV in PF + FA fermenter (23.53% and 33.59%) was higher than those of either the PF fermenter (17.38% and 28.71%) or FA fermenter (19.14% and 30.94%), and much greater than those of the Blank fermenter without any pretreatment (13.79% and 21.83%). However, contrary observations were made on the percentage fluorescence response of Region-III as well as Region-V. The sum of the Region-I and -IV shows that their percentages increased from 35.62% in the Blank fermenter to 46.09% in the PF fermenter, 50.08% in the FA fermenter and 57.12% in their combined fermenter, respectively. Above facts suggested that PF combined with FA caused more release of biodegradable organics than PF, FA and Blank, so that SCFAs-producing microorganisms could use more biodegradable substrates, which might be another reason for FA pretreatment assisted PF promoting SCFAs production.

3.2.3. Effect of FA pretreatment, PF and FA pretreatment assisted PF on hydrolysis, acidogenesis, and methanogenesis processes.

In addition to WAS solubilization, it is widely believed that WAS anaerobic fermentation would undergo the following bio-processes, including hydrolysis, acidogenesis and methanogenesis. Among them, the first two processes are relevant to generation of SCFAs while the last process is relevant to the consumption of SCFAs (Wang et al., 2021). To explore the potential influence of PF (0.10 g/g VSS), FA (180 mg/L) and PF + FA on above three processes, three batch tests simulating microbial metabolism were carried out according to the literature (Wang et al., 2018b). In order to better demonstrate the metabolic process of microorganisms in stages， the substrates on which they depend were replaced (i.e., dextran, glucose, and acetate). It can be found that PF, FA pretreatment, or PF + FA almost affected the degradation of tested model compounds (Fig. S2, Supporting Information). In comparison of the blank, either solo pretreatment or their combination caused decrease in the degradation of three model substrates to a certain extent, and the inhibition induced by PF + FA was severer than that of either PF or FA.

The specific degradation rates of these three model compounds were further calculated and summarized in Table 4. It could be found that the specific degradation rate of dextran was 13.90 ± 0.49 mg/g VSS·h in the blank reactor, and this data could be considered as the original activity of the hydrolytic microbes. When PF, FA pretreatment and their combination were respectively applied, their corresponding specific degradation rate of dextran were respectively 10.88 ± 0.46, 12.70 ± 0.51 and 9.09 ± 0.37 mg/g VSS·h, suggesting that the relative activity of the hydrolytic microbes after PF, FA and PF + FA pretreatment were respectively reduced by 21.73%, 8.63% and 34.6% (expressed as % of the original data). When similar calculations were made, it was also noted that PF + FA restrained the metabolic rate of microbes responsible for acidification by 22.33% (in terms of a specific degradation rate of glucose). Above facts showed that although these pretreatments had a certain inhibitions, more than 65% of the relative activities could be held by the hydrolytic microorganisms and SCFAs producer even with PF + FA pretreatment. This indicated that the bio-processes involved in the generation of SCFAs could be tolerant to this combined method to some extents.

The SCFAs produced in the acidogenesis process would be easily consumed by methanogens to generate methane, the methanogenesis process was therefore considered to hinder the effective accumulation of SCFAs (Wei et al., 2017). From the metabolic rate of acetate (presented in Table 4), it can been seen from that its degradation rate was decreased from 77.5 ± 3.5 in blank reactor to 72.5 ± 3.5 in PF reactor and 70.1 ± 3.2 mg/g VSS·h in FA pretreatment reactor, suggesting that the relative activities of methanogens (i.e. SCFAs consumers) was respectively suppressed by 6.4% and 9.5%. While their combination was conducted, the corresponding inhibition was further up to 14.1%, leading to a synergistic inhibitory effect to the activity of methanogens. More severe inhibitory effect means more SCFAs could be accumulated, this may explained the accumulation of relative high concentrations of SCFAs obtained in the PF + FA reactors (shown in Fig.1a).

3.3. Effect of FA pretreatment assisted PF on the inactivation of fecal coliform in the fermented WAS

Due to the increasingly complex sources of wastewater, WAS, as the main processing by-product, also concentrates a lot of hazardous pollutants and pathogens in addition to rich organic matter matters (Yu et al., 2019). Previous studies have demonstrated that pollutants and pathogens existed in WAS are difficult to remove during the traditional anaerobic fermentation, which may bring potential environmental risks when further disposal of fermented WAS (e.g., landfill) (Limam et al., 2018). Both of FA and PF have been reported to have strong biocidal effects on a great variety of microbes (Zhang et al., 2018b). Nevertheless, the impact of PF + FA on the removal of pathogens in WAS has not been reported. In this work, fecal coliform was selected as pathogen indicator according to the literature (Ding et al., 2017). Through the determination of the fecal coliforms in the WAS during the fermentation process, the impact of PF, FA and PF + FA on the pathogens was shown in Fig. 5. Obviously, the MPN of fecal coliform in the blank fermenter increased from 6.9 of raw sludge to 8.1 log MPN/g VSS, indicating that conventional anaerobic fermentation without any pretreatment may be beneficial to the proliferation of fecal coliform. When the WAS was treated by sole PF and FA pretreatment, the MPN of fecal coliform was decreased to 5.4 and 4.5 log MPN/g VSS, respectively, suggesting that both PF and FA have a certain inactivation effect on fecal coliform. In terms of solo PF pretreatment, the decrease on the MPN of fecal coliform may due to the strong oxidation of potassium ferrate (Diak and Rmeci, 2017). The other hand, the diffusion of hydrophobic FA into the cell could cause the proton imbalance and/or potassium deficiency, significantly inhibiting cell viability and leading to fecal coliform necrosis (Yang et al., 2018). More important, it is noteworthy that the MPN of fecal coliform in the combined pretreatment fermenter was further reduced to 3.6 log MPN/g VSS. Above result suggested that the sole PF has good inactivation effect on pathogens in WAS, but the FA pretreatment assisted PF further enhanced this effectiveness. Given the potential environmental risk of pathogens, the combination of PF and FA pretreatment reported in this work could be beneficial to further disposal of fermented WAS.

4. Implications

This study first proved that appropriate level of FA pretreatment assisted PF could effectively promote the generation of SCFAs from sludge anaerobic fermentation, as compared with the sole FA pretreatment or PF. PF + FA pretreatment not only benefited the lysis of both EPS and cell envelop, but also increased the biodegradability of the released organics, consequently supplying more biodegradable substrates for the production of SCFAs. FA pretreatment assisted PF caused inhibitions to the anaerobes responsible for methane production, thus enhancing the accumulation of SCFAs. At the same time, PF + FA enhanced the inactivation of fecal coliform, which contributed to decrease of environmental risk of further disposal of fermented WAS. The findings reported here could provide an alternative method to strengthen sludge resource recovery and environmental risk control.

As a multifunctional green oxidant, PF and its combined method have been applied in promoting the production of SCFAs from WAS. A simple comparison of the combined treatment of PF + FA with the PF-based pretreatment methods described in other documents from the perspective of experimental conditions and SCFAs performance was made (please see Table 5). Considering that the reported dosage of PF is often very high (e.g., 0.5 g/g VSS), this will inevitably bring additional costs, hindering its popularization and application under actual conditions. It can be seen that PF + FA pretreatment has a great advantage in the dosage of chemical compared to the sole PF pretreatment (e.g., 0.5 g/g VSS), with the assistance of FA (only 180 mg/L), the dosage of PF can be greatly decreased to 0.1 g/g VSS, however the SCFAs yield could still be increased from 176.7 to 342.5 mg COD/g VSS. Compared with several PF + alkaline pretreatment, it can be found that no matter the amount of PF used or alkalis, PF + FA pretreatment is relatively lower. Since FA could be *in situ* generated by recycling a part of anaerobic fermentation broth, this combined method was easy to implement and eliminates application concerns. In addition, other pretreatment methods need the fermentation temperature of 35 °C, while the experiment performed in this study were under the condition of 25 °C, which could save a lot of electrical and thermal energy.

Finally, an advanced concept of "nutrient removal-resource recovery" for the operation of a WWTP that adopt the PF + FA strategy was proposed (please see Fig. 6.). In this operational mode, it not only ensures the reasonable removal of wastewater nutrients, but also recovers considerable energy and resources, reducing a large amount of WAS. In this mode of operation, desirable removal performance of wastewater nutrient, considerable recovery of energy and resource from WAS, and massive reduction of WAS could be achieved. According to the findings documented here and the data reported in the previous study, it is estimated that the combination PF and FA strategy could save about $1.12 million per year in a WWTP (Q=100000 m^3/d) compared with the traditional operational mode (Table S2, Supporting Information), indicating that this combined strategy is economically attractive. Moreover, it should be pointed out that the parameters such as FA concentration and pretreatment time adopted in this paper were based on our previous work (Wang et al., 2018b). Therefore, technical optimization has not yet been carried out. This is due to the purpose of this article is to assess the feasibility of FA-assisted PF in improving SCFAs production from WAS. Therefore, a comprehensive technical optimization is needed in the future before it can be formally applied.

5. Conclusions

Free ammonia (FA) and potassium ferrate (PF) were innovatively combined for enhancing SCFAs production from WAS anaerobic fermentation and whose feasibility was evaluated by a series of experiments. The optimal pretreatment situation considering the economy and benefits was 180 mg FA/L and 0.1 g PF/g VSS, the SCFAs yield (342.5 mg COD/g VSS) from anaerobic fermentation was 7.45-fold compared with the control, and the acetic proportion (65%) of the total SCFAs was reached which facilitate the use of SCFAs for carbon resource or bio-plastic. Further investigations revealed that the combination of FA and PF can further enhance the degree of disruption of sludge, this synergetic disruption act on EPS first, and then accelerated the rupture of cell membrane. This method also caused more release of biodegradable organics from sludge rather increase the amount of organics simply, so that SCFAs-producing microorganisms get profuse biodegradable substrates. While the combination of FA and PF was conducted, the corresponding inhibition on methanogens was further up to 14.08%, means more SCFAs could be accumulated than other reactors. Besides, this microbe inhibitory effect also greatly inactivated the fecal coliforms, thereby reducing its populations in fermented WAS.

By comparing the methods recorded in other documents, it was believed that the FA assists PF pretreatment is a promising technology for SCFAs production form WAS anaerobic fermentation. In terms of the amount of PF used, which required in this method (0.1 g/g VSS) was much less than the previous method (e.g. 0.5g/g VSS). Some researchers have also tried to combine alkali and PF for anaerobic fermentation pretreatment, it reduced the amount of PF but inevitably require higher alkali dosage (e.g. pH 9.5 or 10) to achieve satisfactory SCFAs yields, while its initial pH in this study was pH 9 which greatly cut the alkali consumption. In addition, medium-temperature anaerobic fermentation (e.g. 35 °C) was widely adopted in most previous studies while the fermentation temperature set up in this study was 25 °C, which could undoubtedly save massive electrical power and thermal energy. All the contents discussed above are detailed in Section 4 and Table 5. More importantly, the FA used in this technology is a recyclable substance that can be obtained directly from the sludge fermentation broth.

Future research should focus on finding the most suitable concentration of FA and PF in the pretreatment stage to achieve maximum bio-resource recovery and minimum chemical consumption. In view of the fact that PF mainly exerts its oxidizability as a strong oxygenant in the pretreatment process, the mechanism of FA caused cell membrane rupture on wide variety of microorganisms for the advanced sludge dissolution needs to be further explored. Further efforts should be also put to investigation on the effect of ammonia concentration in the SCFAs liquor returned to the nutrient removal process on nitrification/denitrification or phosphorus accumulating bacteria for the considerations of the feasibility in its real-world application.

Acknowledgments

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Supporting Information

This file contains additional analytical methods and 1 figure.

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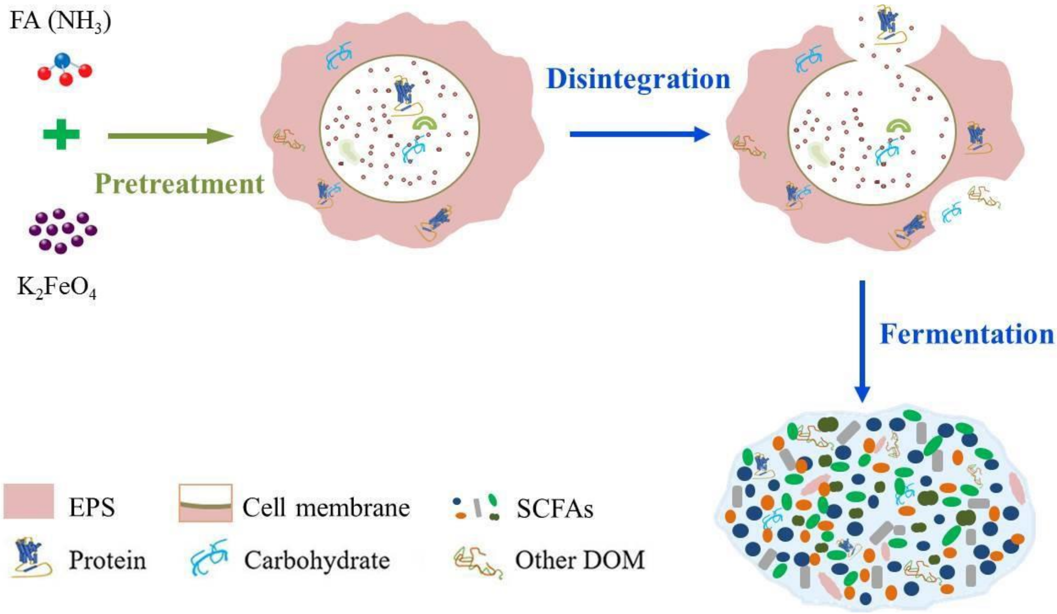
Graphical Abstract

Graphical Abstract



Highlights:

- Applicable addition of FA not only promoted SCFAs production but also reduced PF dosage.
- The combined method accelerated the disintegration of sludge cells and provided more biodegradable substrates.
- The combined method inhibited methanogenesis processes.
- The combined method benefited the inactivation of pathogens in fermented sludge.

Figure Legends

Fig. 1. The total SCFAs production from WAS anaerobic fermentation under different pretreatment conditions (a), and the percentage of individual SCFAs/total SCFAs at their optimal fermentation time (b). Error bars represent standard deviations of triplicate tests.

Fig. 2. The variations in soluble COD with treatment time under different pretreatment conditions (a), and the release of protein under pretreatment conditions at 24 h reaction time (b). Error bars represent standard deviations of triplicate determinations.

Fig. 3. The variations of the soluble COD and the extracted COD by heating method (a) and the release of LDH (b) with fermentation under different conditions. Error bars represent standard deviations of triplicate determinations.

Fig. 4. EEM spectra of fluorescence response ($P_{i,\ n}$) of the organics released under different pretreatment conditions at 12 h. All the samples were diluted by 100 times.

Fig. 5. The effects of PF, FA and their combination on fecal coliform in fermented sludge (The Fecal Coliform was present in the form of Log MPN). Error bars represent standard deviations of triplicate tests.

Fig. 6. The operating model diagram of a WWTP that adopt the PF + FA strategy.

Table Legends

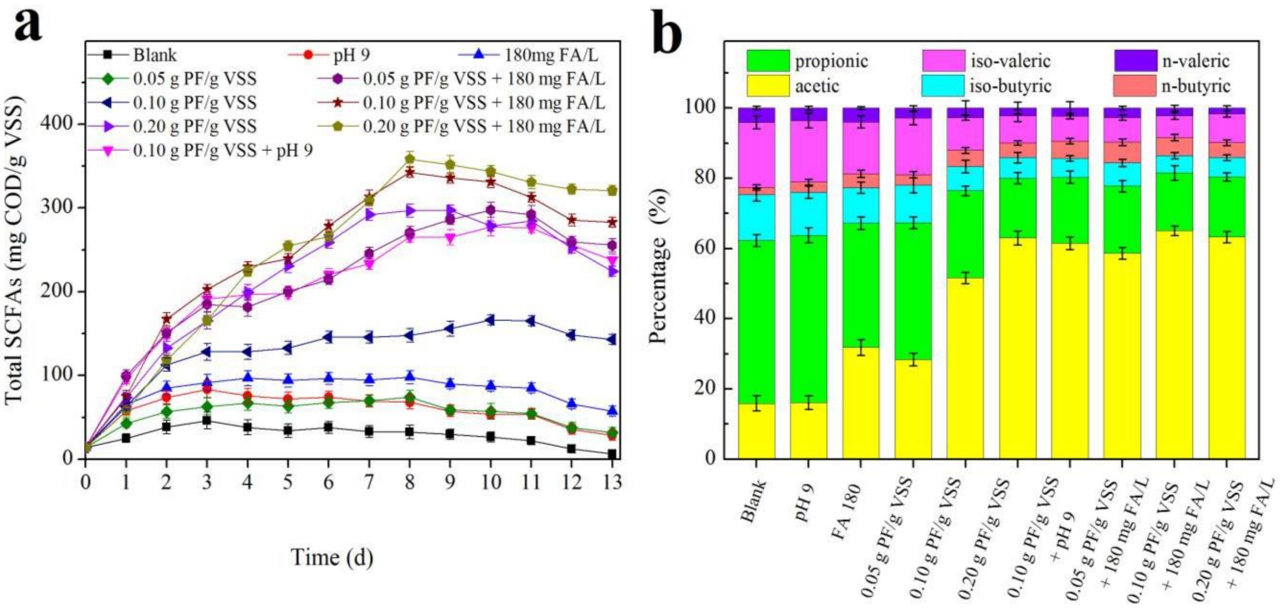
Table 1 Characteristics of the raw waste activated sludge samples.

Table 2 The experimental conditions applied in this work.

Table 3 Experimental conditions adopt in the batch tests for assessing the effects of different pretreatment method on each process of anaerobic fermentation.

Table 4 The impacts of PF, FA and their combinations on the specific activities of the microbes relevant to hydrolysis, acidogenesis, and methanogenesis.

Table 5 Comparison of different pretreatments for sludge SCFAs production.



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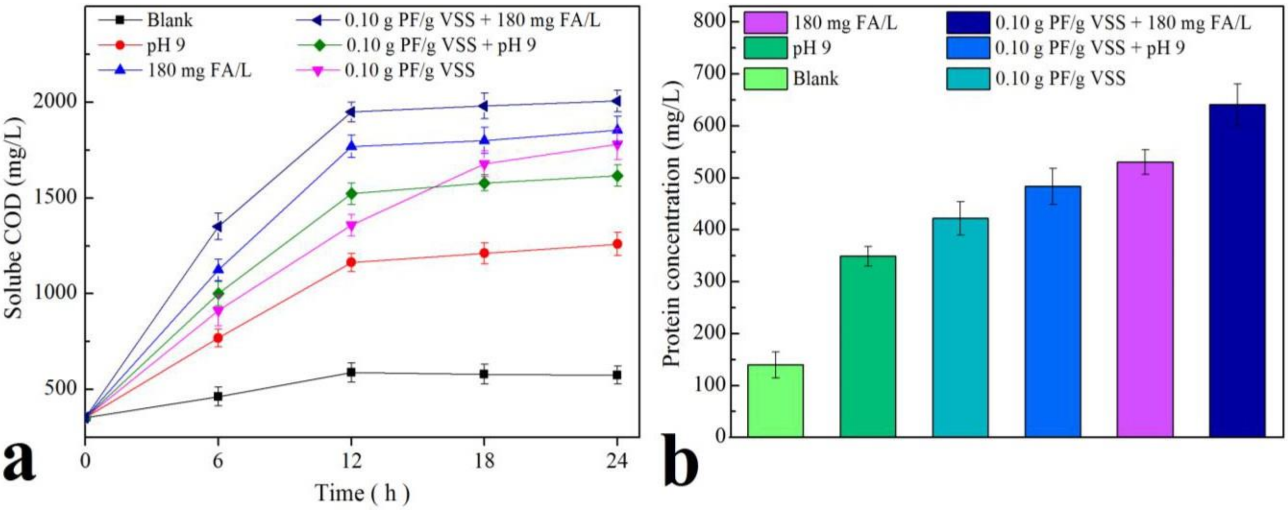

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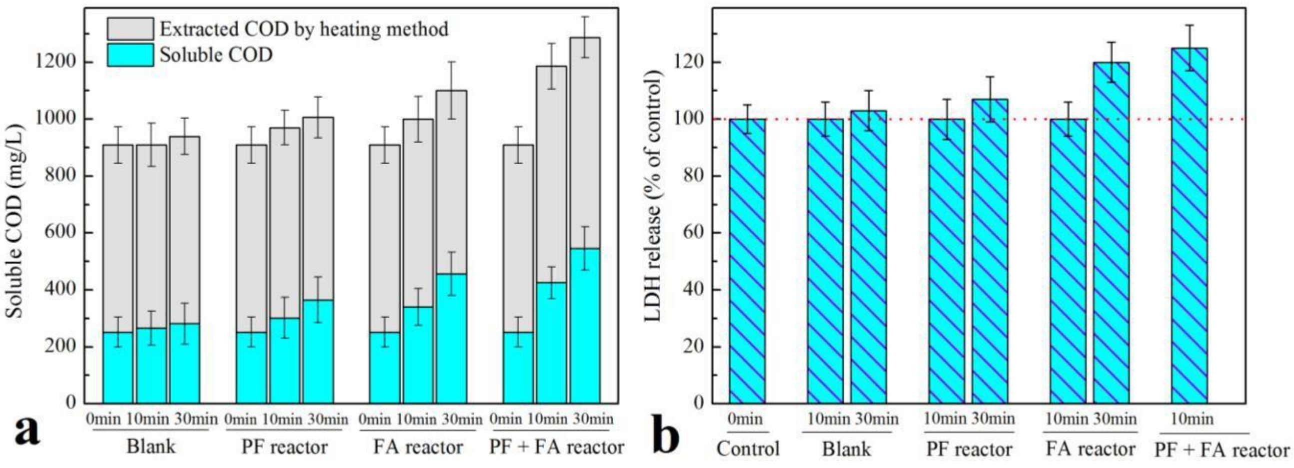

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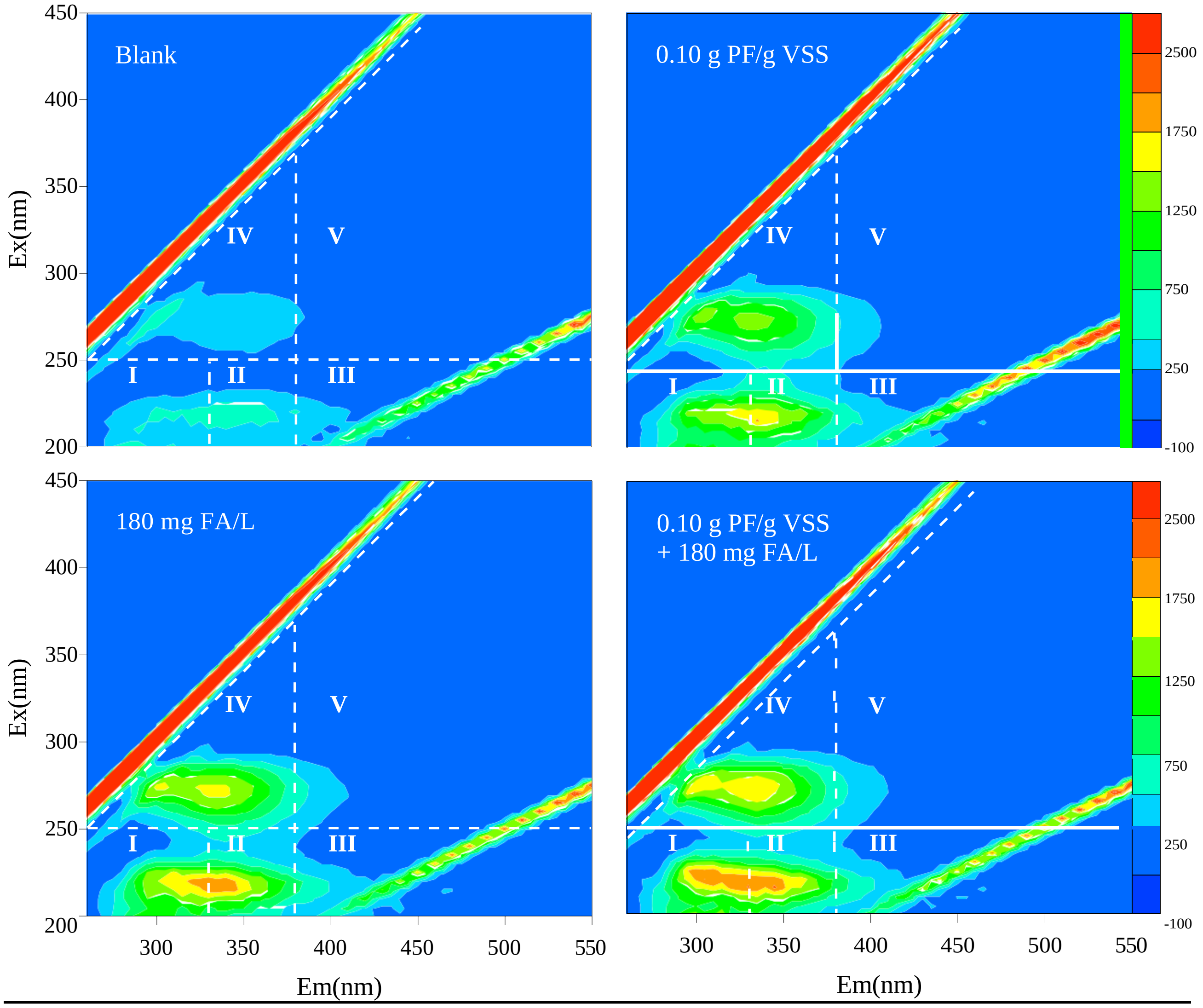
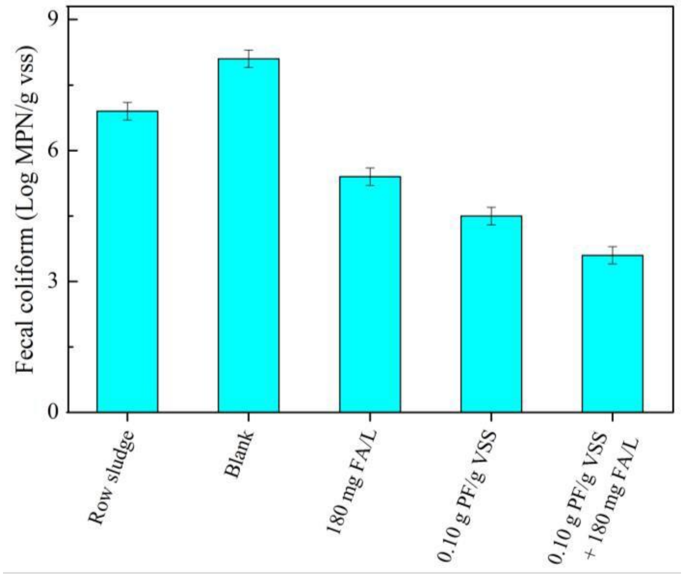


Region	**$P_{i,n}$ (%)**			
	Blank	0.10 g PF/g VSS	180 mg FA/L	0.10 g PF/g VSS + 180 mg FA/L
I	13.79	17.38	19.14	23.53
II	13.79	16.41	16.45	16.75
III	30.48	20.12	18.20	15.25
IV	21.83	28.71	30.94	33.59
V	20.11	17.38	15.27	10.88

Fig. 4. EEM spectra of fluorescence response ($P_{i,\,n}$) of the organics released under different pretreatment conditions at 12 h. All the samples were diluted by 100 times.



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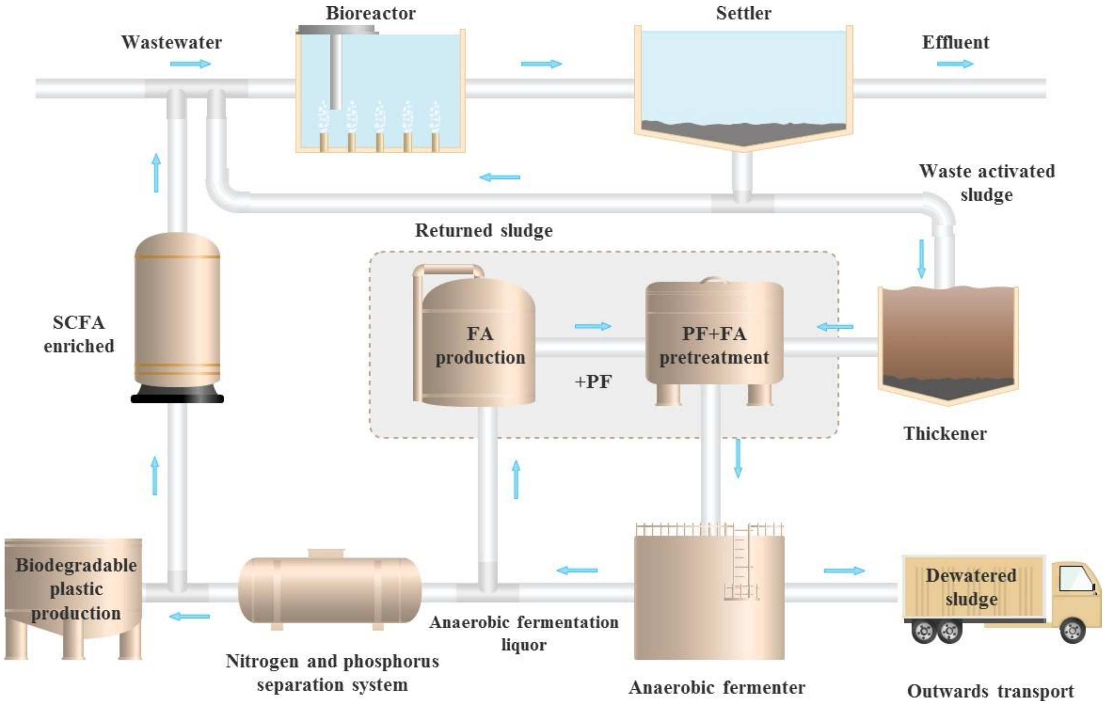

Fig. 6. The operating model diagram of a WWTP that adopt the PF + FA strategy.

Table 1

Characteristics of the raw waste activated sludge samples.

Parameters	Unit	Mean value [a]
pH		6.8 ± 0.1
TSS (total suspended solid)	mg/L	28340 ± 250
VSS (volatile suspended solid)	mg/L	12100 ± 70
TCOD (total chemical oxygen demand)	mg/L	16425 ± 130
SCOD (soluble chemical oxygen demand)	mg/L	353 ± 30
Soluble protein	mg/L	59 ± 2
NH_4^+-N	mg/L	24.72 ± 1.5
PO_4^{3-}-P	mg/L	9.1 ± 0.4
SCFAs	mg/L	25.2 ± 1.7

[a] Data are shown as arithmetic mean of three replicates ± standard deviation.

Table 2

The experimental conditions applied in this work.

Reactor	PF (g/g VSS)	Initial FA(mg/L)	NH_4^+-N (mg/L)	Temperature (°C)	pH
1	0	0.07	19.23[a]	25	NC[b]
2	0	6.96	19.23	25	9
3	0	180	477.57	25	9
4	0.05	0.07	19.23	25	NC
5	0.10	0.07	19.23	25	NC
6	0.20	0.07	19.23	25	NC
7	0.10	6.96	19.23	25	9
8	0.05	180	477.57	25	9
9	0.10	180	477.57	25	9
10	0.20	180	477.57	25	9

[a] The value was the background concentration of ammonium.

[b] NC means Non-controlled.

Table 3

Experimental conditions adopt in the batch tests for assessing the effects of different pretreatment method on each process of anaerobic fermentation.

Batch tests	Substrate	Reactors	Experimental conditions
Hydrolysis -test	Dextran	1	1.2 g/L dextran + 270 mL ultrapure water + 30 mL digested sludge
		2	1.2 g/L dextran + 0.10 g PF/g VSS + 270 mL ultrapure water + 30 mL digested sludge
		3	1.2 g/L dextran + 180 mg/L FA + 270 mL ultrapure water+ 30 mL digested sludge
		4	1.2 g/L dextran + 0.10 g PF/g VSS + 180 mg/L FA + 270 mL ultrapure water + 30 mL digested sludge
Acidogenesis -test	Glucose	5	1 g/L glucose + 270 mL ultrapure water + 30 mL digested sludge
		6	1 g/L glucose + 0.10 g PF/g VSS +270 mL ultrapure water + 30 mL digested sludge
		7	1 g/L glucose + 180 mg/L FA + 270 mL ultrapure water+ 30 mL digested sludge
		8	1 g/L glucose + 0.10 g PF/g VSS + 180 mg/L FA + 270 mL ultrapure water + 30 mL digested sludge
Methanogenesis -test	Sodium acetate	9	5 g/L sodium acetate + 270 mL ultrapure water + 30 mL digested sludge
		10	5 g/L sodium acetate + 0.10 g PF/g VSS +270 mL ultrapure water + 30 mL digested sludge
		11	5 g/L sodium acetate + 180 mg/L FA + 270 mL ultrapure water+ 30 mL digested sludge
		12	5 g/L sodium acetate + 0.10 g PF/g VSS + 180 mg/L FA + 270 mL ultrapure water + 30 mL digested sludge

Table 4

The impacts of PF, FA and their combinations on the specific activities of the microbes relevant to hydrolysis, acidogenesis, and methanogenesis.[a]

Substrate	Different pretreatment conditions			
	Blank	PF (0.10 g/g VSS)	FA (180 mg/L)	PF (0.10 g/g VSS) + FA(180 mg/L)
Dextran	13.9 ± 0.5	10.9 ± 0.5	12.7 ± 0.5	9.1 ± 0.4
Glucose	26.9 ± 1.2	23.0 ± 1.1	24.1 ± 1.1	20.9 ± 1.6
Acetate	77.5 ± 3.3	72.5 ± 3.5	70.1 ± 3.2	66.6 ± 3.0

[a] Results are the averages and their standard deviations of triplicate tests, and the unit is mg/g VSS·h.

Table 5 Comparison of different pretreatments for sludge SCFAs production.

Pretreatment method	Treatment parameters [a]	Fermentation condition	Fermentation time	SCFAs (mg COD/g VSS) [b]	References
PF	0.50 g PF/g VSS	35 ± 1°C under 135 rpm	8 d	176.7 mg/g VSS	(Li et al., 2018b)
PF + Alkaline	0.50 g PF/g VSS + pH 10	35 ± 1°C under 160 rpm	5 d	322.6 mg/g VSS	(Li et al., 2018a)
PF	0.35 g PF/g VSS	35 ± 1°C under 100 rpm	5 d	343 mg/g VSS	(He et al., 2018)
PF + Alkaline	0.175 g PF/g VSS + pH 10	35 ± 1°C under 100 rpm	4 d	382 mg/g VSS	(He et al., 2019)
PF + Alkaline	0.04 g PF/g VSS + pH 9.5	35 ± 1°C under 150 rpm	4 d	245 mg/g VSS	(Yang et al., 2020)
PF + FA	0.10 g PF/g VSS + 180 mg FA/L	25 ± 1°C under 120 rpm	8 d	342.5 mg/g VSS	This study

[a] The units of the treatment parameters from different research were converted to g/g VSS.

[b] The units of the experimental results from different research were converted to mg COD/g VSS.