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Understanding the Mechanisms of How Poly Aluminium Chloride Inhibits Short-Chain Fatty Acids Production from Anaerobic Fermentation of Waste Activated Sludge

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Abstract

Poly aluminum chloride (PAC) is accumulated in waste activated sludge at high levels. However, details of how PAC affects short-chain fatty acids (SCFA) production from anaerobic sludge fermentation has not been documented. This work therefore aims to fill this knowledge gap by analyzing the impact of PAC on the aggregate of sludge flocs, disruption of extracellular polymeric substances (EPS), and the bio-processes of hydrolysis, acidogenesis, and methanogenesis. The relationship between SCFA production and different aluminum species (i.e., Ala, Alb, and Alc) was also identified by controlling different OH/Al ratio and pH in different fermentation systems. Experimental results showed that with the increase of PAC addition from 0 to 40 mg Al per gram of total suspended solids, SCFA yield decreased from 212.2 to 138.4 mg COD/g volatile suspended solids. Mechanism exploration revealed that PAC benefited the aggregates of sludge flocs and caused more loosely- and tightly-bound extracellular polymeric substances remained in sludge cells. Besides, it was found that the hydrolysis, acidiogenesis, and methanogenesis processes were all inhibited by PAC. Although three types of Al species, i.e., Ala (Al monomers, dimer, and trimer), Alb ($Al_{13}(AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$), and Alc (Al polymer molecular weight normally larger than 3000 Da), were co-existed in fermentation systems, their impacts on SCFA production were different. No correlation was found between SCFA and Ala, whereas SCFA production decreased with the contents of Alb and Alc. Compared with Alb, Alc was the major contributor to the decreased SCFA production (R^2 = 0.5132 vs R^2 = 0.98). This is the first report revealing the underlying mechanism of how PAC affects SCFA production and identifying the contribution of different Al species to SCFA inhibition.

Keywords: Anaerobic fermentation, Short chain fatty acid production, Waste activated sludge

Ala	monomeric species
Alb	medium polymer species
Alc	species of sol or gel
AK	acetate kinase
BSA	bovine serum albumin
COD	soluble chemical oxygen demand
Da	Dalton, another name for atomic mass unit
EEM	excitation-emission matrix
EPS	extracellular polymeric substances
Ex/Em	excitation/emission
L-EPS	loosely-bound EPS
OAATC	oxaloacetate transcarboxylase
PAC	poly aluminum chloride
PAC_{10}	OH/Al=1.0
PAC_{25}	OH/Al=2.5
PACc	commercially purchased PAC
SCFA	short-chain fatty acids
TCOD	total chemical oxygen demand
T-EPS	tightly-bound EPS
TSS	total suspended solids
VSS	volatile suspended solid
WAS	waste activated sludge
WWTP	wastewater treatment plants

1. Introduction

As a byproduct of biological wastewater treatment, large amounts of waste activated sludge (WAS) are inevitably produced, which is becoming a big problem faced by wastewater treatment plants (WWTP) nowadays [1, 2]. WAS is one typical type of solid wastes and easily causes secondary pollution if it is treated inappropriately. Generally, the treatment and disposal of WAS accounts for up to 60% of total operating cost of a WWTP [3]. However, it contains high levels of organic matters such as protein and carbohydrate, which makes it a renewable bioenergy resource [4, 5]. Usually, WAS is used to produce methane by the anaerobic digestion process. SCFA are intermediate products of anaerobic digestion, which include acetic, propionic, iso-butytic, n-butyric, iso-valeric and n-valeric acids [6]. Thus, production of short-chain fatty acids (SCFA) from sludge fermentation has recently attracted growing interests, because the SCFA produced are not only preferred carbon sources for biological nitrogen and phosphorus removal but also raw materials for microbial production of biodegradable plastics [7].

The yield of SCFA from WAS fermentation is usually at low levels due to low rates of sludge disintegration and rapid consumptions by methanogens. Thus, most of the previous studies focused on the promotion of SCFA production through either accelerating sludge disintegration process or inhibiting the activities of methanogens [8]. Several pretreatment methods of WAS, such as enzyme, free nitrous acid, thermal, Fenton, ozone, and ultrasonic pretreatment [9-11], and operational conditions of the anaerobic reactor such as acid and alkaline controls [9], were documented to be effective. For example, free nitrous acid (1.54~1.80 mg/L) pretreatment on WAS for 2 d enhanced sludge disintegration substantially, which thereby led to a 1.5~3.7 fold increase in SCFA production [12]. Besides, increasing some organic matters in fermentation sludge, such as carbohydrate and intracellular polyhydroxyalkanoates, could also promote the SCFA generation [13].

Apart from protein and carbohydrate, WAS also contains lots of other substances such as persistent organic pollutants, heavy metals, and flocculants [14-16]. Previous investigations showed that these compositions affected sludge anaerobic fermentation and digestion as well [4, 6, 13, 14, 17].

Poly aluminum chloride (PAC), which is made by partial hydrolysis of acid aluminum chloride solution using a specific reactor, is an inorganic coagulant and generally consists of Al monomers such as Al^{3+}, $Al(OH)^{2+}$, and $Al(OH)_2^{+}$, dimer ($Al_2(OH)_2^{4+}$), trimer ($Al_3(OH)_4^{5+}$), $Al_{13}(AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+})$, and inert large polymer with molecular weight normally larger than 3000 Da [18]. The chemical species of hydrolyzed aluminum can be divided through different reaction rates with Ferron indicator into three types: monomeric species (Ala) (instantaneously reacted, i.e., Al monomers, dimer, and trimer), medium polymer species (Alb) (reacted within 120 min, i.e., $Al_{13}(AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+})$), and species of sol or gel (Alc) (no reaction, i.e., Al polymer molecular weight normally larger than 3000 Da). PAC was found to be superior to the traditional

Al-based coagulants (e.g., $AlCl_3$ and alum) for particulate and/or organic matter removal under some conditions, in which significant amounts of high-charged poly-nuclear aluminum hydrolysis products (Alb) are present [19].

Since1980s, PAC has been widely used in water and wastewater treatment for removal of small particles and heavy metals, precipitation of phosphate, and inactivation of virus throughout the world [20-22]. In wastewater treatment process, PAC is inevitably absorbed and concentrated by sludge, thereby resulting in its substantial accumulation in sludge. The level of PAC in sludge is highly dependent on the source of water quality and other used chemicals, and largely varies in different areas. It was reported that Al concentration in the municipal waste sludge was 2.6 -17.4 mg/g in Taiwan [23], 3-4 mg/g in Hong Kong [24], 81.6 mg/g in Hangzhou, China [25], 112.5 mg/g in Surat, India [26], 5.2-16.8 mg/g in Seville Spain [27], and 0.7-26.8 mg/g in Blacksburg, USA [16]. Besides being used in wastewater treatment, PAC is often used in sludge dewatering as well, thus PAC level in dewatered sludge would be much higher. For instance, it was found that Al concentration in dewatered sludge achieved at 104-320 mg/g in USA [16]. Usually, anaerobic fermentation or digestion of sludge is implemented in WWTPs before sludge dewatering. However, in small-scale WWTPs where in-stiu fermenting or digesting WAS in WWTPs is not economically feasible and in some counties such as China where most of the WWTPs has not already been configured with anaerobic digesters, fermenting or digesting the dewatered sludge in an assemble place will be executed. Cabirol et al. (2003) found that the specific activities of methanogenic and acetogenic microorganisms decreased by 50% and 72%, respectively, when they were exposed to 1000 mg/L $Al(OH)_3$ for 59 days [28]. Gossett and Evans (1978) verified that the sludge digestion performance was only 80% of the control when 325 mg/L alum (as $Al_2(SO_4)_3\cdot 18H_2O$) was present [29]. Using synthetic wastewater as the fermentation substrate, Kim and Jung (2007) found that PAC decreased SCFA production by more than 10% at the level of 46 mg Al/L [30]. Dentel and Gossett (1981) reported that the insoluble substrates in water were most affected with aluminum [31]. All these previous studies indicated that high levels of PAC in sludge might affect the sludge anaerobic fermentation process [27-30]. Despite these significant progresses above, the effect of PAC on anaerobic fermentation of real sludge has not been fully understood.

For example, compared with the fermentation systems using synthetic wastewaters, anaerobic fermentation of real sludge includes more complex reactions (e.g., sludge solubilization) and microbial community, but it is unknown whether PAC affects the anaerobic fermentation of real sludge. If it does, how does PAC affect WAS anaerobic fermentation such as solubilization, hydrolysis, acidogenesis, and methanogenesis? It is known that PAC consists of monomeric Al and polymeric Al in aquatic environments, and the performance of the coagulant is determined by the species present at the time during the coagulation process rather than those in the original reagents [18]. To date, however, the effect of different Al species (i.e., Ala, Alb, and Alc) on SCFA production has never been differentiated. Is there any different behavior among Ala, Alb and Alc? Which one is the major contributor?

Based on these questions above, the purposes of this study were therefore to investigate the influence of PAC on SCFA production from anaerobic WAS fermentation, and to reveal the underlying mechanisms of how PAC affects SCFA production. Firstly, the effect of different levels of PAC on SCFA production was investigated. Then, the mechanisms of PAC affecting SCFA production were explored by analyzing the impact of PAC on the aggregate of sludge flocs and disruption of extracellular polymeric substances (EPS), and its effect on the processes of solubilization, hydrolysis, acidiogenesis and methanogensis. Finally, the relationship between SCFA production and different aluminum species (i.e., Ala, Alb, and Alc) was identified in batch tests by controlling different OH/Al ratio (denoted as B value) and pH in fermentation systems. This is the first report revealing the details of whether and how PAC affects the production of SCFA from anaerobic WAS fermentation. The findings achieved in this work not only fill the knowledge gap in terms of PAC's impact on sludge fermentation, but also guide engineers to develop solutions to manipulate the PAC-involved sludge fermentation systems.

2. Materials and methods

2.1 The Sources of WAS and PAC

To minimize the background of PAC in WAS, the WAS used in this study was obtained from the secondary sedimentation tank of a municipal WWTP with sludge retention time of 18 d in Changsha, China, where PAC-based wastewater pretreatment was not performed. The collected sludge was concentrated for 24 h by settling at 4℃ before use, and the main characteristics of the concentrated WAS are as follows: pH 6.7 ± 0.1, total suspended solids (TSS) 17.5 ± 0.3 g/L, volatile suspended solid (VSS) 11.6 ± 0.2 g/L, soluble chemical oxygen demand (SCOD) 92.3 ± 2.7 mg/L, total chemical oxygen demand (TCOD) 12.4 ± 0.3 g/L, total protein 7.6 ± 0.2 g COD/L, and total polysaccharide 1.8 ± 0.1 g COD/L.

In this study, PAC, which contains 14.8% of Al (w/w), was provided by Tianjin GuangFu Fine Chemical Research Institute. All the reagents used were of analytical grade, except for those being specified.

2.2 Effect of PAC on SCFA Production from Sludge Fermentation

This batch experiment was carried out in five replicate reactors with a working volume of 1 L each. The five reactors were first fed with 600 mL of WAS. Then, different volumes of PAC stock solution were added in the five reactors to control sludge Al level at the preselected set-point (i.e., 0, 10, 20, 30, and 40 mg Al/g TSS). For comparison convenience, these values do not include the Al background value in sludge. Afterwards, the mixture volume of five reactors was all added to 800 mL using deionized water. To enhance sludge solubilization, all the sludge mixtures were pre-treated at 90 °C for 30 min [19]. When the reactors were cooled down to room temperature, 500 mL of seed sludge, which was collected from a sludge fermentation reactor in our lab, was equally divided and added into these five reactors. Finally, all reactors were flushed with nitrogen gas for 5 min to remove oxygen and capped with rubber stoppers, sealed, and placed in a water-bath shaker (120 rpm) at 30 ± 1 °C.

2.3 Effect of PAC on Hydrolysis, Acidogenesis, and Methonogenesis Processes using Model Substrates

Here, three tests were performed to assess the effect of PAC on hydrolysis (Test-I), acidogenesis (Test-II), and methanogenesis (Test-III), respectively. These tests were fed with synthetic wastewaters containing different model substrates.

Test-I: Four replicate reactors with working volume of 1 L each were carried out using synthetic wastewater. The synthetic wastewater contained bovine serum albumin (BSA, average molecular weight 67000, protein compound) and dextran (average molecular weight 23800, a model polysaccharide compound). Firstly, 3.6 L of synthetic wastewater with 6.66 g BSA/L and 2.07 g dextran/L was divided equally into the four reactors. Then, 100 mL of seed sludge was added into each reactor as inoculums. Finally, the four reactors received 0 (set as the control), 0.97, 1.93 or 2.9 g PAC (the amount of PAC was respectively equal to that in the 10, 20, 30 mg Al/g TSS reactors). All other fermentation conditions were the same as those described in section 2.2. At different fermentation time, the dextran concentration in the four reactors was measured.

Test-II: The operations of Test-II were conducted the same as described in Test-I except that the fermentation substrate (i.e., BSA and dextran) in synthetic wastewater was replaced by L-alanine (model amino acid compound, one of basic structural units of proteins) and glucose (model monosaccharide compound).

Test-III: This test was carried out with the same method described in Test-II except that 2.4 g/L sodium acetate was used to replace BSA and glucose in synthetic medium.

2.4 Identifying the Effect of Different Al Species on SCFA Production

As mentioned above, the chemical species of hydrolyzed aluminum can be divided into three types: Ala (instantaneously reacted, i.e., Al monomers, dimer, and trimer), Alb (reacted within 120 min, i.e., $Al_{13}(AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+})$), and Alc (no reaction, i.e., Al polymer molecular weight normally larger than 3000 Da). The three species of Al might have different effects on sludge fermentation, thus their respective impacts on SCFA production were therefore identified.

PAC with different species distributions in this experiment were either selected from commercially available products or prepared by the slow base titration method in our laboratory. Commercially purchased PAC (denoted as PACc) and $AlCl_3 \cdot 6H_2O$ were provided by Tianjin GuangFu Fine Chemical Research Institute. Two laboratory-prepared coagulants, PAC_{10} and PAC_{25} (OH/Al molar ratio of 1.0 and 2.5, respectively), were prepared by the base titration method at room temperature according to the literature[19]. Briefly, 50 mL of 0.5 mol/L $AlCl_3$ solution was transferred into a 300 mL glass reactor, and 0.5 mol/L NaOH was titrated at a rate of 0.5 mL/min into the Al solution using a peristaltic pump under rapid stirring and purging with nitrogen gas. The amount of NaOH added varied with the target OH/Al molar ratio (simplified as B values). The final concentrations of all PACs solutions are 0.1 mol Al/L.

The speciation of the different PACs solutions at pH 10, which was determined by the Ferron method, is shown in Table 1 (Table S1 presents the relevant data under pH uncontrolled condition). To assess these different Al species on SCFA production during anaerobic fermentation, the synthetic wastewater containing BSA and dextran was used. The operations and concentrations of substrates and Al were the same as described in Test-I.

2.5 Analytical Methods

COD, TSS,VSS were determined according to standard methods [32]. Soluble protein and carbohydrate were determined by the Lowry-Folin method with BSA as the standard and the phenol-sulfuric method with glucose as the standard, respectively [33, 34]. The COD conversion coefficients are 1.5 g COD/g protein and 1.06 g COD/g carbohydrate [5]. The concentration of SCFAs was measured with Agilent 6890N GC equipped with flame ionization detector, and the detailed method was provided in Supporting Information. The total SCFA content was calculated as the sum of measured acetic, propionic, n-butyric, iso-butyric, n-valeric, and iso-valeric acid. L-alanine was determined with Agilent 1100 HPLC, using Shim-pack amino-Na type column (10 cm × 6.0 mm, Shimadzu). The detailed method was provided in Supporting Information. In addition, the detailed analytical procedures of the activities of key hydrolytic enzyme (protease), acetate and propionic-forming enzymes (acetate kinase (AK), oxaloacetate transcarboxylase (OAATC)), and coenzyme F420 were also provided in the Supporting Information.

A heat extraction method was applied to extract different EPS, all excitation-emission matrix (EEM) fluorescence spectra were measured using a luminescence spectrometry (F-4600 FL spectrophotometer, Hitachi, Japan), and detailed method was provided in Supporting Information. Particle size was measured by a laser particle analyzer (Mastersizer 2000).

The measurement of Ala, Alb, and Alc was conducted according to the Ferron method documented in the literature [35]. Briefly, the Ferron solution was obtained by mixing reagent A (0.2% ferron), B (20% w/w CH_3COONa), and C (1:9 v/v HCl) according to a ratio of 2.5:2:1. During speciation experiments, 5.50 mL of the mixed reagent was transferred into 25 mL graduated glass tube, and then a certain amount of the sample was then added into the glass tube and quickly diluted to 25 mL. After mixing, the sample was quickly added to a 1 cm glass cuvette. The timed absorbance measurements (at 366 nm), using a shimadzu UV 2800 spectrophotometer, were carried out after 1 min and 120 min. It was operationally divided that the first 1min absorbance as Ala, and 1 min to 120 min as Alb, then Alc was obtained by Al_t minus Ala and Alb.

2.6 Assessing Correlation between SCFA and Different Al Species by Exponential Models

The correlation between SCFA and different Al species was modeled based on the experimental data. The MATLAB (Mathworks, Inc., Natick, MA) function “lsqcurvefit” was used to find the parameter values that resulted in the best fit between the models used and the experiment data.

2.7 Statistical Analysis

All measurements were performed in triplicate. An analysis of variance was used to evaluate the significance of results, and $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1 Effect of PAC Level on SCFA Production

Fig. 1a shows the variation of pH in the fermentation process in the presence of different PAC addition. It can be seen that the addition of PAC reduced the pH value. For example, the pH in the fermenter without PAC addition was in the range of 6.09-6.80, whereas it varied from 5.73 to 6.42 in the fermenter with 40 mg Al/g TSS PAC. Owing to the high charge on the Al ion, water molecules are polarised and might lead to a loss of one or more protons, resulting in the reduction of pH [36]. It was reported that pH affected SCFA production, and lower pH caused higher SCFA production in the range of 5-7 [37].

Fig. 1b presents the profiles of SCFA production from sludge fermentation in the presence of different PAC addition. The SCFA production profiles among the fermenters showed similar trends. SCFA yield first increased gradually with the fermentation time in the initial 2 or 3 days, and then remained almost constantly for a few days. Further increase of fermentation time resulted in decrease yields of SCFA in all fermenters. The maximal SCFA yield, however, was affected by the PAC addition. With the increase of PAC addition from 0 to 40 mg/g TSS, the maximal SCFA production decreased significantly from 212.17 to 138.43 mg COD/g VSS ($P<0.05$, Table S2 , Supporting Information). Kim (2007) found the conversion from organic matter to SCFAs decreased by >10% when the dosages of coagulant was >46 mg Al/L[30], which was consistent with our results. Further investigations showed that the addition of PAC did not affect the composition of SCFA (Fig. S1). The order of individual SCFA concentration (mg COD/g VSS) in all fermenters was in the sequence of acetic > propionic > n-butyric > iso-valeric > n-valeric > iso-butyric. Although the addition of PAC caused a decrease of pH value, the addition of PAC did not increase but decrease the SCFA production. Previous investigations demonstrated that the decrease of pH from 7 to 5 enhanced SCFA production, due to the inhibition of acidic condition to methanogenesis [37]. This paradox indicated that besides pH variation, the addition of PAC caused other inhibitions to sludge anaerobic fermentation. In the following text, the mechanisms of how PAC decreased SCFA production were explored.

3.2 Mechanisms of how PAC affects SCFA Production

During the anaerobic fermentation of sludge, four stages are usually included: solubilization, hydrolysis, acidogenesis, and methanogenesis. SCFA is generated in the acidogenesis step. If these stages are affected by PAC, SCFA accumulation will be affected inevitably. Therefore, the relevant mechanism explorations performed in this work mainly focused on these four stages.

In the literature, the degree of sludge solubilization is generally estimated by the determination of substrate release [6]. In this work, sludge solubilization degree was indicated by the ratios of soluble carbohydrate (protein) to total carbohydrate (protein). It can be seen from Fig. 2a that both the ratios of soluble carbohydrate to total carbohydrate and soluble protein to total protein after thermal pretreatment (i.e., 90 ℃ for 30 min) decreased with the increase of PAC addition. The variation of soluble carbohydrate with fermentation time is presented in Fig. S2. When PAC addition increased from 0 to 40 mg Al/g TSS, the ratio of soluble carbohydrate (protein) to total carbohydrate (protein) decreased from 5.43 ± 0.19% (8.21 ± 0.29%) to 2.27 ± 0.07% (5.53 ± 0.18%). These data could be further supported by the VSS reduction ratio, which is widely employed to indicate the degree of sludge solubilization as well. The higher the VSS reduction ratio, the greater the sludge solubilization. It was measured that the VSS reduction in the high PAC addition sludge was lower than that in the low PAC addition sludge (Fig. 2b). For example, VSS reduction ratio in the sludge without PAC addition was 7.30 ± 0.31% whereas the corresponding value was only 2.52 ± 0.09% in the sludge with 40 mg Al/g TSS. As a result, lower soluble COD was measured in the sludge with higher PAC addition (Fig. 2b). All these results indicated that the presence of PAC inhibited sludge solubilization stage, which was one reason for PAC addition decreasing SCFA production.

Sludge solubilization is mainly relevant to particle sizes and compositions of sludge flocs [6, 38]. Fig. 3 shows the distribution of particle size in the sludge with or without PAC addition at different fermentation time. It can be seen that particle size of sludge with PAC addition was larger than that without PAC addition. For instance, the average particle size in the sludge without PAC addition was 66.87 μm on 0 d fermentation whereas the corresponding value was 69.71μm in the sludge with 30 mg Al/g TSS PAC addition. Similar observations were also made on other fermentation days. These results suggested that the presence of PAC benefited the aggregates of sludge flocs, which thereby slowed the rate of sludge solubilization stage, the major rate-limiting step of sludge fermentation. Previous study also demonstrated that large particles with a low surface-to-volume ratio were hydrolyzed more slowly than small particles [39].

As the dosage of PAC in this work was low (0-40 mg Al/g TSS), its influence on the element composition of sludge could be ignored. However, the presence of PAC might cause the variations in EPS, the key substances (mainly composed by protein, carbohydrate, lipid, and humic acid) accounting for up to 80% of cells and protecting cells against detrimental environments, which thereby affected the rate of sludge solubilization. To further understand how PAC addition inhibits sludge solubilization, comparison of variation in EPS structure, which was indicated by EEM fluorescence spectroscopy according to the literature[6], between the sludge with 0 or 30 mg/g TSS PAC addition after heat pretreatment was made.

As shown in Fig. 4, four main peaks (signed as Peak A, Peak B, Peak C, and Peak D in this work, respectively) could be identified from fluorescence spectra of both loosely-bound EPS (L-EPS) and tightly-bound EPS (T-EPS) extracted from the sludge with 0 or 30 mg/g TSS PAC addition. Peak A and Peak B located at the excitation/emission wavelengths (Ex/Em) of 224/299-308 and 221-224/335-347 nm, respectively, which are assigned to simple aromatic proteins such as tyrosine. Peak C located at Ex/Em of 272-275/299-302 nm, which is related to tryptophan protein-like substances. Peak D was identified at 275-278/341-347 nm, which was assigned to soluble microbial by-product-like material such as tryptophan-like substance. It was reported that the variations in the location shift of fluorescence peak and the fluorescence intensity could be used to indicate the changes of EPS structure and amount [12]. A red-shift of fluorescence peak is related to the increase of carbonyl, hydroxyl, alkoxyl, amino, and carboxyl groups in the structure of fluorophores, while a blue-shift of fluorescence peak is ascribed to the elimination of particular functional groups such as carbonyl, hydroxyl and amine, the reduction in the degree of π-electron systems, and the decrease in the number of aromatic rings and conjugated bonds in a chain structure [40]. In addition, the fluorescence intensity of characteristic peaks on EEM plot has a good relationship with the concentration of fluorophores.

Compared with the control (i.e., the sludge without PAC addition), 30 mg Al/g TSS PAC addition caused not only red-shifts of fluorescence peaks but also increases of fluorescence intensity. It can be seen that the addition of PAC caused 3-12 nm of emission wavelength red-shift in Peak A, Peak B, Peak C, or Peak D in EEM spectra of L-EPS and T-EPS. Moreover, all the fluorescence intensities of the four peaks in the sample with PAC addition were much higher than those in the sample without PAC addition. The facts suggested that the sludge with PAC addition contained more EPS than the sludge without PAC addition. This can be further supported by the quantitative measurement of total proteins and carbohydrates in EPS (Fig. S3, Supporting Information). All the results indicated that the presence of PAC resulted in more EPS remained in sludge cells and thereby provided sludge cells a better protection against disruption. As a result, less soluble substrates were released for the subsequent hydrolysis and acidogenesis, which might be another reason for PAC addition decreasing SCFA production.

After sludge solubilization, the solubilized substrates will undergo hydrolysis and acidogenesis processes for SCFA production, and the SCFA produced will be further bio-converted to methane in the methanogenesis process. To evaluate the effects of PAC on these three processes, batch experiments were conducted using synthetic wastewaters containing BSA and dextran, L-alanine and glucose, or acetate. It can be seen from Table 2 that the presence of PAC largely decreased the degradation rates of both BSA and dextran no matter what fermentation time was. For instance, the degradation rates of BSA and dextran in the reactor without PAC addition were respectively 9.5 ± 1.6% and 45.1 ± 1.2% on 1 d fermentation whereas the corresponding data were only 3.3± 0.5% and 3.9 ± 0.1% in the reactor with 30 mg Al/g TSS of PAC addition, respectively. Similar results are also observed on other fermentation days and other PAC dosages. The impact of PAC on acidification was similar to that on hydrolysis. For example, it was found that, compared with the blank, 30 mg Al/g TSS inhibited the degradations of L-alanine and glucose at any fermentation time investigated. Similar observation was also made on other PAC dosages. The results suggested that the bio-processes of hydrolysis and acidogenesis were severely inhibited by the addition of PAC, thus it can be understood why PAC decreased SCFA production.

From Table 2, it can be found that the addition of PAC also affected methane production from acetate degradation. With the PAC addition increase from 0 to 30 mg Al/g TSS, the methane yield on 3 d fermentation decreased from 6.6 ± 0.2 to 1.9 ± 0.2 mL, indicating that the methanogenesis process was also inhibited by the presence of PAC, which was consistent with the results documented in the literature [28].

3.3 Identifying the Effect of Different Al Species on SCFA Production

As mentioned above, the chemical species of hydrolyzed PAC can be divided into three types: Ala (instantaneously reacted, i.e., Al monomers, dimer, and trimer), Alb (reacted within 120 min, i.e., $Al_{13}(AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+})$), and Alc (no reaction, i.e., Al polymer molecular weight normally larger than 3000 Da). These three species of Al might have different effect on sludge fermentation, thus their respective impacts on SCFA production were also identified in this work. It is known that these three types of Al species are generally co-existed in solutions, and solution pH has a significant effect on the species transformation, depending mainly on OH/Al ratio (denoted as B value). The PAC at low B ratios showed a significant change of species under the different pH conditions while PAC at high B ratios exhibited efficient speciation stability and tended to maintain the original speciation distribution. Therefore, to identify the effect of the different Al species on SCFA production, adding different types of Al-based chemicals to the same synthetic wastewater was performed under alkaline condition (i.e., pH 10) in this study. Table 1 shows the speciation of the different Al-based chemicals in solutions at pH 10.

Table 3 illustrates the total SCFAs production in different fermentation reactors fed with different Al-based chemicals. It can be seen that different Al-chemicals had different effects on SCFA production ($P<0.05$, Table S3, Supporting Information) except for $AlCl_3$ ($P>0.05$, Table S3, Supporting Information). For example, 4098.7 ± 89.2 mg COD/L of SCFA was produced in the control (i.e., without any Al-chemical addition) on 2 d fermentation, while the corresponding value decreased to 4019.0 ± 105.3 mg COD/L with $AlCl_3$ addition, 3159.1 ± 49.8 mg COD/L with PAC_{10} addition, 2195.0 ± 87.3 mg COD/L with PAC_{25} addition, and 2038.1 ± 64.8 mg COD/L with PAC_c addition (the individual SCFA in all fermenters are presented in Fig. S4). Similar observations were also made on other fermentation days. Clearly, the inhibition effect of different Al-chemicals on SCFA production was in the sequence of PACc > PAC_{25} > PAC_{10} > $AlCl_3$.

As all the Al-chemicals used above contained the three types of Al species in solutions (Table 1), it is necessary to correlate these three Al species in different fermenters with their SCFA yields to further assess their potential impacts on SCFA production. It can be seen that the correlation between the SCFA yield and the Ala concentration was not strong (Fig. 5a), suggesting that the species of Ala was not the major contributor to the decreased SCFA production. As shown in Fig. 5b and 5c, both Alb and Alc contents showed negative correlations with the SCFA production. The higher the Alb (or Alc), the lower the SCFA yield. However, compared with Alb, Alc exhibited a higher correlation coefficient (R^2 = 0.5132 vs R^2 = 0.98, Fig. 5b and 5c). The results indicated that although Alb and Alc could significantly inhibited SCFA production, the latter was the major contributor to the decreased SCFA production. It is reported that Ala could complex with organic matters, Alb is superior to other species in charge neutralization, and Alc could adsorb particle and organic matter to form larger flocs through patch coagulation [35]. Therefore, it can be inferred that charge neutralization and adsorption to form larger flocs were important reasons for the decreased SCFA production in sludge anaerobic fermentation, which would impede accessibility of microorganism to the substrates.

3.4 Effect of PAC addition on key enzyme activities

It is known that the production of SCFA from WAS fermentation was mainly attributed to biological effects, and determination of the activities of key enzymes is an alternative approach to assess the activities of microbial cells [6]. The key hydrolytic enzyme, i.e., protease, is related to the hydrolysis of protein. AK and OAATC are the key enzymes responsible for the acetic and propionic acid production, respectively [14]. Besides, coenzyme F420 is the key enzyme for methane production [6]. Therefore, these four enzyme activities were assessed in this study. The results showed that all the activities of protease, AK, OAATC and coenzyme F420 in the fermenter fed with 30 mg/g TSS PAC addition sludge were lower than those fed with sludge without PAC addition (Fig. 6), which was consistent with the data presented above. The results demonstrated again that the presence of PAC inhibited the hydrolysis, acidogenesis, and methanogenesis processes.

3.5 Implications for WWTPs

Although several efforts were dedicated to assessing the impact of Al-based coagulant on anaerobic fermentation or digestion, these studies only focused on either the impact of monomer Al (e.g., $Al_2(SO_4)_3$) on real sludge digestion or the impact of PAC on anaerobic digestion of model substrates. In this work, the details of whether and how PAC affects the production of SCFA from anaerobic fermentation of real sludge were revealed for the first time. It was verified through using either real WAS or synthetic wastewaters as the fermentation substrates. It was found that the addition of PAC benefited the aggregates of sludge flocs and caused more EPS remained in sludge cells, thereby providing sludge cells a better protection against harmful environments. It was also observed that the addition of PAC significantly restrained the hydrolysis, acidiogenesis, and methanogenesis processes. Although these three types of Al species, i.e., Ala, Alb, and Alc, are co-existed in fermentation systems, their impacts on SCFA production were different. Among them, no correlation was found between SCFA and Ala, whereas SCFA production decreased exponentially with the contents of Alb and Alc. Compared with Alb, Alc was the major contributor to the decreased SCFA production (R^2 = 0.5132 vs R^2 = 0.98). To the best of our knowledge, all the findings have not been documented before, which therefore deepen our understanding in terms of anaerobic fermentation of PAC-involved sludge.

Moreover, the findings obtained in this work might also guide engineers to further control the sludge anaerobic fermenter in real situations in the future. Due to the substantial levels of PAC in sludge (up to 320 mg/g TSS in dewatering sludge) [16], its negative impact was already enforced to sludge anaerobic fermentation systems (Fig. 7), but this impact was overlooked previously in real-world situations. Thus, this negative impact requires to be considered in the future manipulation of sludge fermenters, or even in the future operation of WWTPs. Although several physical-chemical methods such as ultrasonic and alkaline treatment might be used to mitigate the detrimental impacts of PAC, these methods are cost intensive due to high input of either energy or chemicals. According to the findings achieved in this work, an alternative strategy that is inexpensive and easy to control in practice is source control. From Fig. 7, it can be seen that PAC in sludge is usually originated from wastewater pretreatment and sludge dewatering processes. The results revealed that compared with the other two Al species, Ala did not inhibit SCFA production. Thus, if engineers use Al-chemicals with low B values such as $AlCl_3{\cdot}6H_2O$ and PAC_{10} instead of Al-chemicals with high B values (e.g., PAC_{25}) in these two processes and control the sludge fermenters under alkaline conditions (e.g., pH 10) to ensure Ala to be the dominant species of Al (Fig. 7), the negative impact of this coagulant on sludge anaerobic fermentation can be addressed in a simple way. Previous publications demonstrated that alkaline fermentation was an effective strategy to enhance SCFA production from sludge fermentation, as this strategy effectively promoted the sludge disintegration and strongly inhibited the methane production. As thus, integrating source control (i.e., Al-chemicals with low B values) with fermenter control (i.e., alkaline conditions) not only could mitigate the negative impact of PAC but also might further promote SCFA yield. However, this proposed strategy is indicative only, and its technical performance and economic evaluation need further assessment in the future.

4. Conclusions

This study assessed the effect of PAC on SCFAs production from the anaerobic fermentation of WAS and explored the underlying mechanisms of PAC affecting SCFA production for the first time. Experimental results showed the SCFA production was inhibited by PAC. Mechanism exploration revealed that the addition of PAC benefited the aggregates of sludge and offered a better protection of EPS against harmful environments, thereby slowing down the solubilisation process. Besides, hydrolysis, acidiogenesis and methanogenesis processes were all inhibited by PAC. Further investigation revealed that Ala did not affect SCFA production whereas SCFA production decreased exponentially with the contents of Alb and Alc. Compared with Alb, Alc was the major contributor to the decrease SCFA production.

Acknowledgement

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Appendix A. Supplementary data

This file contains Table S1-S3 and Figs. S1-S4.

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Figure captions:

Fig.1. The pH variations (a) and SCFA production (b) from anaerobic fermentation of sludge with different PAC addition. Error bars represent standard deviations of triplicate tests.

Fig. 2. Comparison of the soluble protein (carbohydrate) release ratios (a) and soluble COD concentration and VSS reduction (b) with different PAC addition after thermal pretreatment (90 ℃ for 30 min). Error bars represent standard deviations of triplicate tests.

Fig. 3. The distribution of particle size in sludge with or without PAC addition at different fermentation time.

Fig. 4. Comparison of EEM spectra of different EPS fractions (S-EPS, LB-EPS, TB-EPS) between the sludges with 0 and 30 mg/g TSS PAC addition after heat pretreatment.

Fig. 5. Correlation between SCFA production and Ala content (a), Alb content (b) and Alc content (c). SCFA data were obtained on 2 d fermentation. Error bars represent standard deviations of triplicate tests.

Fig. 6. Effect of PAC addition on relative activities of key enzymes on 3d fermentation. Error bars represent the standard deviations of triplicate tests.

Fig. 7. Schematic image illustrating the details of how PAC affects sludge anaerobic fermentation and the proposed strategy to mitigate its negative impact.

Table captions:

Table 1

The speciation distribution of coagulant PACs at pH 10

Table 2

Effect of PAC on the degradations of model compounds and the methane production from acetate degradation with time [a]

Table 3

The effect of different Al species on SCFA production during anaerobic fermentation at pH 10 [a]

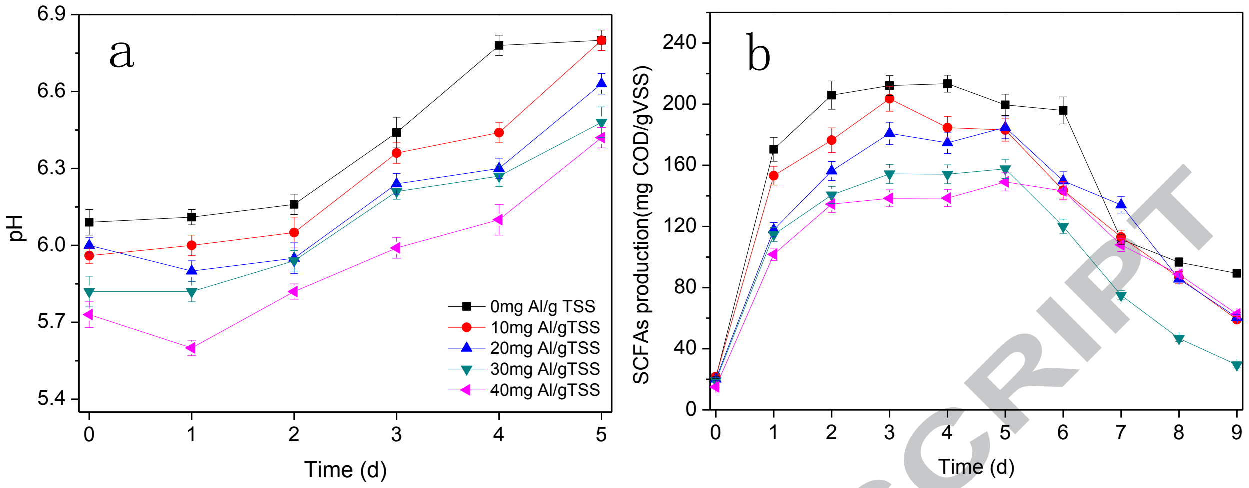
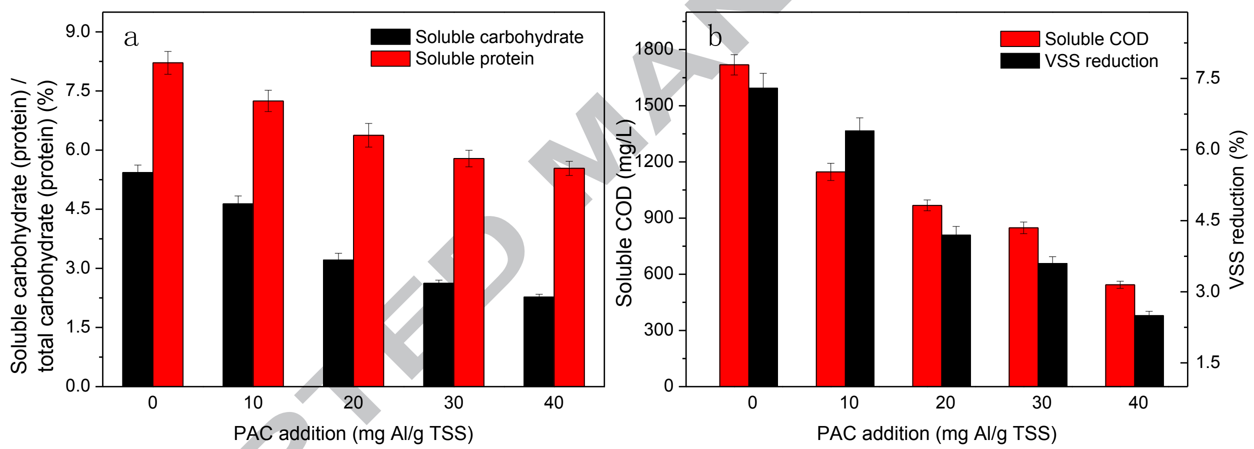


Fig. 1. The pH variations (a) and SCFA production (b) from anaerobic fermentation of sludge with different PAC addition. Error bars represent standard deviations of triplicate tests.



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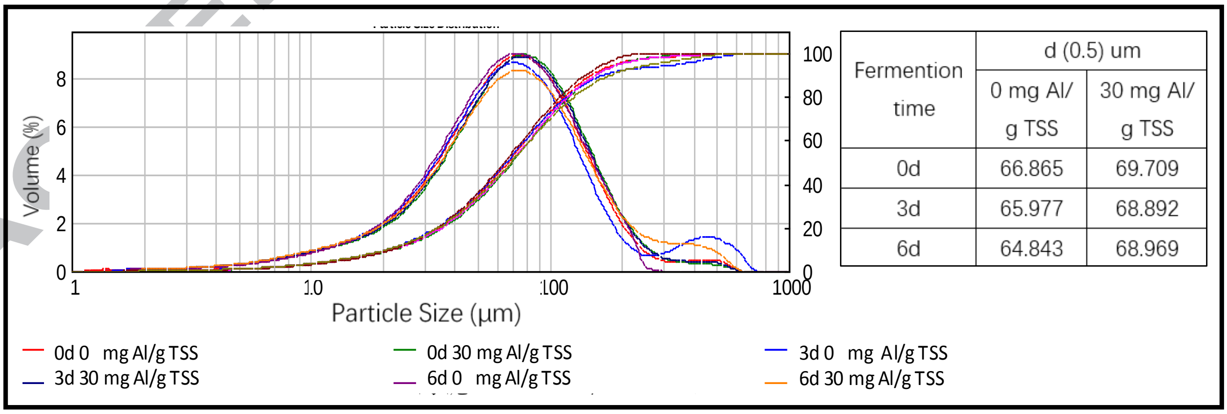
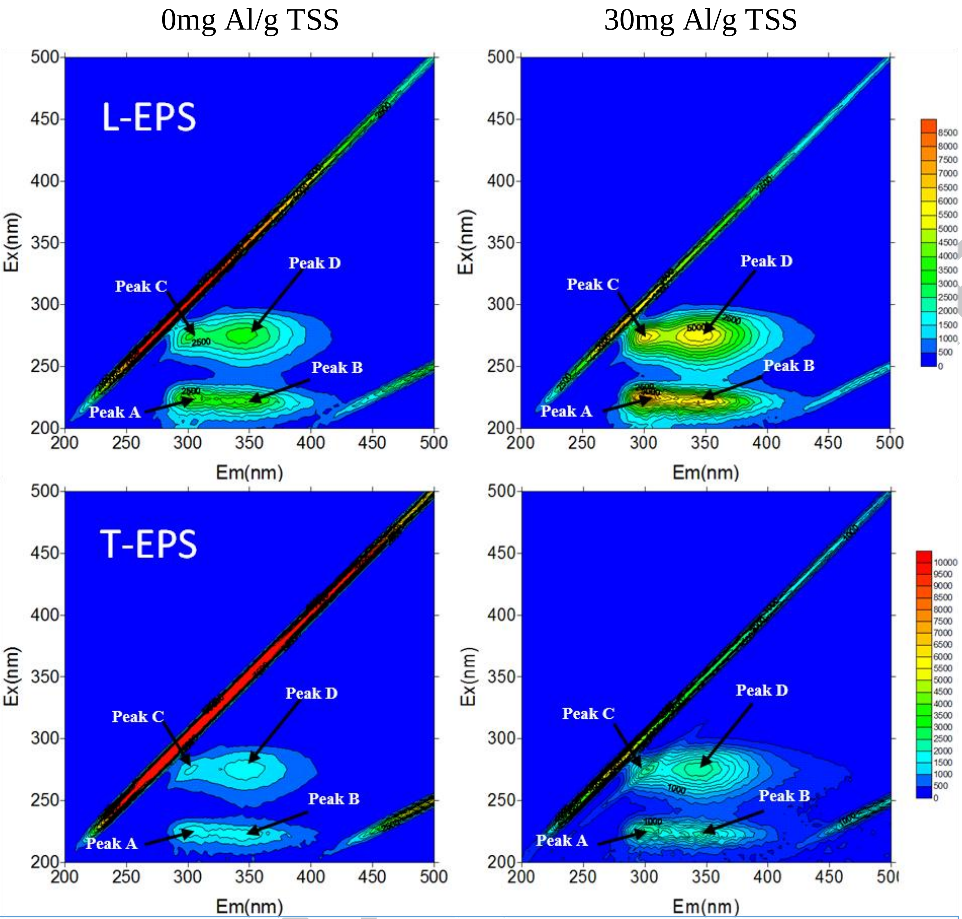


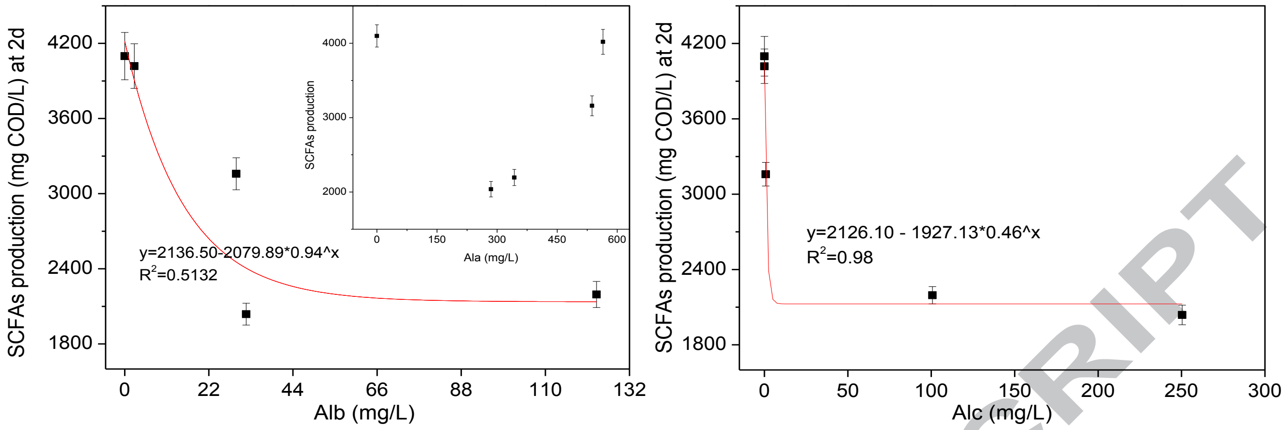
Fermention time	d (0.5) um	
	0 mg Al/ g TSS	30 mg Al/ g TSS
0d	66.865	69.709
3d	65.977	68.892
6d	64.843	68.969

Fig. 3. The distribution of particle size in sludge with or without PAC addition at different fermentation time.



Samples	PAC (mg Al/g TSS)	Peak A		Peak B		Peak C		Peak D	
		Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity
L-EPS	0	224/299	4094	221/344	4060	272/299	3138	278/344	3307
	30	224/305	8286	221/347	6740	275/302	5923	278/347	5754
T-EPS	0	224/299	1923	224/335	1890	272/299	1631	275/341	1768
	30	224/308	2038	224/347	2211	272/302	2307	275/344	2138

Fig. 4. Comparison of EEM spectra of different EPS fractions (S-EPS, LB-EPS, TB-EPS) between the sludges with 0 and 30 mg/g TSS PAC addition after heat pretreatment.



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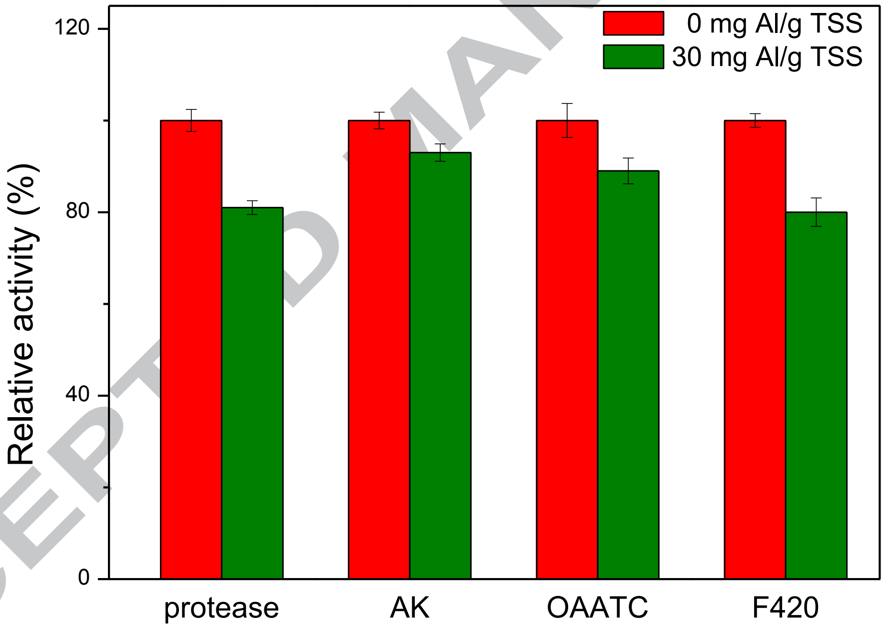

Fig. 6. Effect of PAC addition on relative activities of key enzymes on 3d fermentation. Error bars represent the standard deviations of triplicate tests.

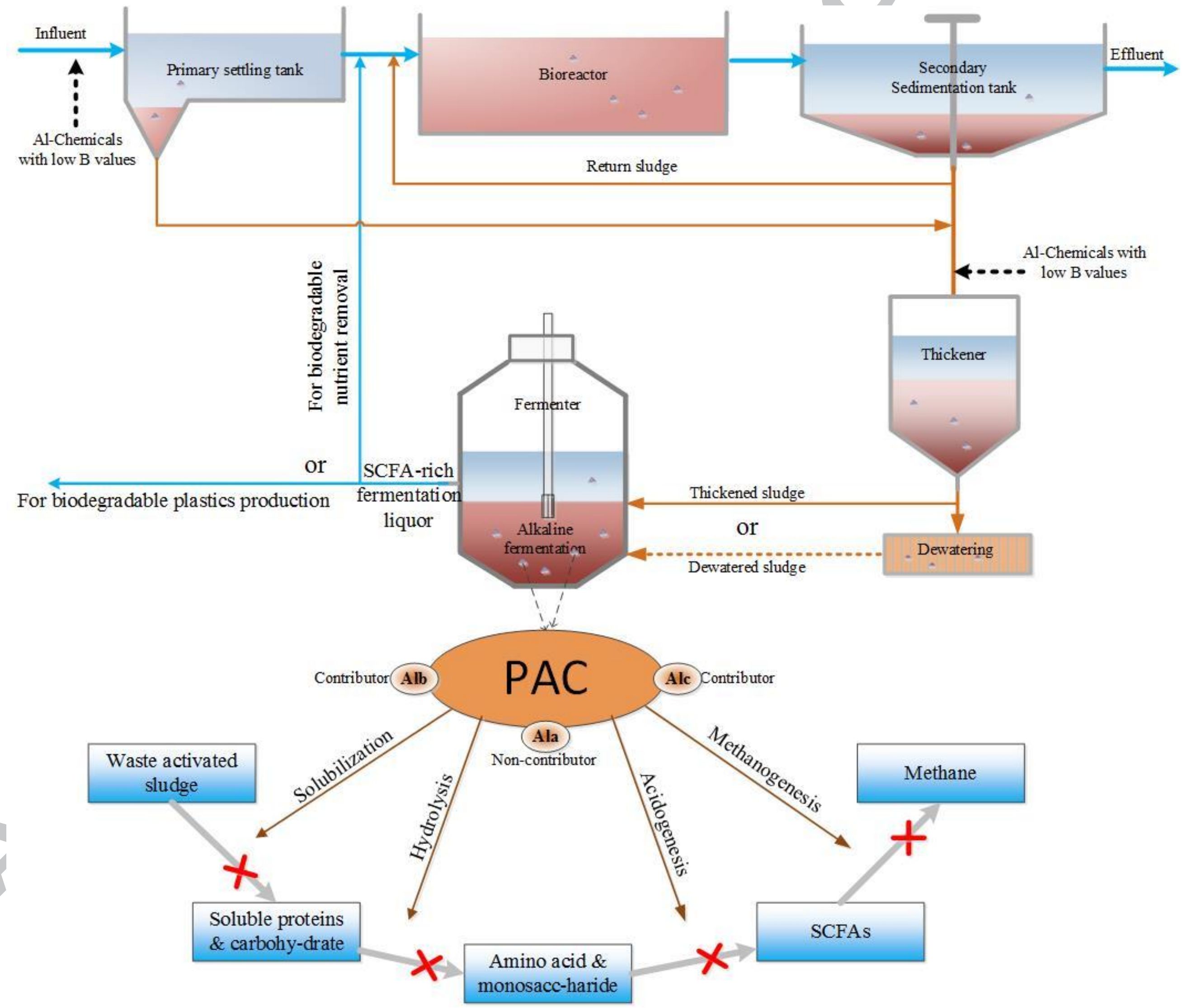

Fig. 7. Schematic image illustrating the details of how PAC affects sludge anaerobic fermentation and the proposed strategy to mitigate its negative impact.

Table 1

The speciation distribution of coagulant PACs at pH 10

PACs	B	Ala (%)	Alb (%)	Alc (%)
$AlCl_3 \cdot 6H_2O$	0	99.56	0.44	0
PACc	0.6	50.24	5.6	44.16
PAC_{10}	1	94.72	5.14	0.14
PAC_{25}	2.5	60.48	21.76	17.76

[a] The data reported are the averages and their standard deviations in triplicate tests.

Table 2

Effect of PAC on the degradations of model compounds and the methane production from acetate degradation with time [a]

Item	Time (d)	Hydrolysis		Acidogenesis		Methanongenesis
		BSA degradation (%)	Dextran degradation (%)	L-alanine degradation (%)	Glucose degradation (%)	Methane production (mL)
0 mg Al/g TSS	1 d	9.5±1.6	45.1 ± 1.2	11.3±0.9	33.6 ± 1.0	2.1 ± 0.1
	2 d	11.0±0.7	87.8 ± 2.3	13.9±1.5	95.1 ± 2.6	3.1 ± 0.2
	3 d	11.4±0.3	96.1 ± 3.1	14.1±1.3	97.4 ± 3.8	6.6 ± 0.2
10 mg Al/g TSS	1 d	7.7±0.9	32.9±0.8	9.7±0.8	28.7±2.1	1.8±0.1
	2 d	8.3±1.1	53.7±1.1	10.8±1.2	59.8±2.8	2.1±0.1
	3 d	8.8±0.5	62.8±0.9	11.3±0.9	93.4±3.2	2.8±0.2
20 mg Al/g TSS	1 d	5.3±0.4	16.8±1.5	7.6±0.4	15.8±0.8	1.6±0.1
	2 d	6.1±0.5	19.7±0.5	7.9±0.7	34.5±1.2	1.8±0.1
	3 d	6.8±0.1	23.9±0.7	8.5±0.4	84±2.6	2.3±0.2
30 mg Al/g TSS	1 d	3.3±0.5	3.9 ± 0.1	4.1±0.3	7.6 ± 0.4	1.6 ± 0.1
	2	4.4±0.2	7.4 ± 0.2	4.9±0.8	17.3 ± 0.8	1.7 ± 0.1

d					
3 d	4.5±0.1	8.5 ± 0.6	5.3±0.5	71.6 ± 1.8	1.9 ± 0.2

[a] The data reported are the averages and their standard deviations in triplicate tests.

Table 3

The effect of different Al species on SCFA production during anaerobic fermentation at pH 10 [a]

Time (d)	SCFA production mg COD/L				
	Control	$AlCl_3$	PAC_{10}	PAC_{25}	PAC_c
1d	251.6 ± 7.8	256.4 ± 4.9	176.0 ± 6.4	145.8 ± 5.4	126.3 ± 3.5
2d	4098.7 ± 89.2	4019.0 ± 105.3	3159.1 ± 49.8	2195.0 ± 87.3	2038.1 ± 64.8

[a] The data reported are the averages and their standard deviations in triplicate tests.

Highlights:

- PAC coagulant inhibited WAS fermentation largely
    - PAC benefited the aggregates of sludge flocs
    - PAC caused more EPS remained in sludge cells
    - Solubilization, hydrolysis, acidiogenesis, and methanogenesis were inhibited by PAC
    - Alb and Alc were the contributors to the decreased SCFA production

Graphic abstract