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Understanding the impact of cationic polyacrylamide on anaerobic digestion of waste activated sludge

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ABSTRACT

Previous investigations showed that cationic polyacrylamide (cPAM), a flocculant widely used in wastewater pretreatment and waste activated sludge dewatering, deteriorated methane production during anaerobic digestion of sludge. However, details of how cPAM affects methane production are poorly understood, hindering deep control of sludge anaerobic digestion systems. In this study, the mechanisms of cPAM affecting sludge anaerobic digestion were investigated in batch and long-term tests using either real sludge or synthetic wastewaters as the digestion substrates. Experimental results showed that the presence of cPAM not only slowed the process of anaerobic digestion but also decreased methane yield. The maximal methane yield decreased from 139.1 to 86.7 mL/g of volatile suspended solids (i.e., 1861.5 to 1187.0 mL/L) with the cPAM level increasing from 0 to 12 g/kg of total suspended solids (i.e., 0–236.7 mg/L), whereas the corresponding digestion time increased from 22 to 26 d. Mechanism explorations revealed that the addition of cPAM significantly restrained the sludge solubilization, hydrolysis, acidogenesis, and methanogenesis processes. It was found that ~46% of cAPM was degraded in the anaerobic digestion, and the degradation products significantly affected methane production. Although the theoretically biochemical methane potential of cPAM is higher than that of protein and carbohydrate, only 6.7% of the degraded cPAM was transformed to the final product, methane. Acrylamide, acrylic acid, and polyacrylic acid were found to be the main degradation metabolites, and their amount accounted for ~50% of the degraded cPAM. Further investigations showed that polyacrylic acid inhibited all the solubilization, hydrolysis, acidogenesis, and methanogenesis processes while acrylamide and acrylic acid inhibited the methanogenesis significantly.

1. Introduction

Biological wastewater treatment is widely used in the world, but large amounts of waste activated sludge (WAS) are produced, which is a big problem faced by wastewater treatment plants (WWTPs) nowadays (Chen et al., 2016; Wang et al., 2012; 2017a; Zhao et al., 2016a; 2017b). WAS would readily cause secondary pollution if it is treated and disposed inappropriately. However, WAS also contains high concentrations of carbon and nutrient substrates, which makes it a useful resource for energy recovery (Li et al., 2016a, 2016b; Wang et al., 2017b; 2017c, 2017d; Xie et al., 2016a). As anaerobic digestion is able to effectively reduce sludge volume, stabilize sludge characteristic, kill pathogenic microorganisms, and produce renewable energy, methane, it is considered the most promising method for WAS treatment and widely implemented in real situations (Jenicek et al., 2012; Xu et al., 2017).

As a major byproduct of wastewater treatment, WAS not only contains organic compounds such as protein, carbohydrate, and polyhydroxyalkanoates, but also concentrates a variety of pollutants present in wastewaters and biological/chemical additives

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added during wastewater treatment and sludge dewatering processes (Chen et al., 2014; Luo et al., 2016; Li et al., 2016a, 2016b; Qi et al., 2011; Yi et al., 2017). It is known that sludge anaerobic digestion includes several biological conversions (e.g., solubilization, hydrolysis, acidogenesis, and methanogenesis) executed by a series of microbes such as hydrolytic microorganisms, acid-producing microorganisms, hydrogenogens, and methanogens (Appels et al., 2008; Zhao et al., 2016b). Thus, these concentrated pollutants and additives might affect these bio-conversions, thereby affecting the performances of sludge anaerobic digestion.

Cationic polyacrylamide (cPAM), a linear water-soluble polymeric compound with a high molecular weight and cationic charges, is widely used in enhancing solid-liquid separation through charge neutralization and interparticle bridging (Aguilar et al., 2005; Moussas and Zouboulis, 2009). In WWTPs, cPAM is usually applied in wastewater pretreatment, leading to cPAM accumulation in WAS (Xia et al., 2005). Moreover, in small-scale WWTPs where digesting WAS in-situ in WWTPs is not economically feasible and in some developing counties like China where most of the WWTPs has not already been configured with anaerobic digesters, WAS is first required to be dewatered and then gathered together for further treatment (Duan et al., 2012; Guendouz et al., 2008; Zhai et al., 2012). As cPAM is substantially added into floc during mechanically dewatering process, cPAM contents in such sludges are inevitably at high levels. It was reported that cPAM level in dewatered sludge was in the range 2.5–10 g/kg dry sludge (Chu et al., 2002; Thornton et al., 2001).

Due to the significant level of sludge cPAM, its impact on sludge anaerobic digestion raised concerns in the past years. For example, Chu et al. (2003) found that compared with the control, 15 and 40 g/kg of cPAM resulted in 22% and 37% of methane yield reduction during anaerobic digestion, respectively. The increased size of the sludge caused by particle aggregation, which limited the disintegration rate of WAS, was considered the main reason for the deterioration of methane reduction (Campos et al., 2008; Chu et al., 2005; Dentel and Gossett, 1982). Recently, cPAM has been demonstrated to be degradable during anaerobic digestion (Chang et al., 2001; Dai et al., 2014). Furthermore, it could be utilized as nitrogen source to stimulate methanogenesis and even as a carbon source to produce volatile fatty acids (Dai et al., 2015; Haveroen et al., 2005). It is reported that cPAM could be anaerobically biodegraded via a series of biochemical processes (Bao et al., 2010; Chang et al., 2001; Dai et al., 2014, 2015; Haveroen et al., 2005). Long-chain cPAM is first transformed into short-chain cPAM by ectoenzymes. Then short-chain cPAM is activated by either ectoenzymes or amidases and hydrolyzed to acrylamide, acrylic acid, and polyacrylic acid. The generated acrylic acid and polyacrylic acid can be further bio-converted to pyruvic acid and acetyl-CoA, which are finally used to produce methane via acetic acid, hydrogen, and carbon oxidize. Despite these significant advances, details of how cPAM affects the process of anaerobic digestion of WAS are still poorly understood.

Since cPAM is degradable in the anaerobic digestion, it could be utilized as nitrogen and carbon sources to produce methane directly, but it is unknown whether cPAM has a lower biochemical methane potential on a unit VSS basis, as compared with other sludge compositions such as protein and carbohydrate. Previous studies demonstrated that cPAM slowed the sludge disintegration process, but the potential effect of cPAM on other processes of anaerobic digestion such as hydrolysis, acidogenesis, and methanogenesis have never been documented. Moreover, cPAM and its degradation metabolites such as polyacrylic acid, acrylic acid, and acrylamide would co-exist in the digestion system (Chang et al., 2001; Dai et al., 2015; Haveroen et al., 2005), thus the toxicity of cPAM and its degradation metabolites to the bio-conversion processes involved in anaerobic digestion is also needed to be comprehensively identified.

This work therefore aims to deeply understand the underlying mechanism of how cPAM affects methane production during anaerobic digestion of sludge. Firstly, the influence of cPAM at different dosages on methane production during WAS anaerobic digestion was investigated. Then, details of how cPAM affects the methane production were explored. Till now, most of the reported studies on WAS anaerobic digestion focused on the enhancement of methane yield, ignoring the effect of pollutants and additives such as cPAM contained in WAS Stuckey and Mccarty, 1978, Wang et al., 2013, Xie et al., 2016b, Zhang et al., 2011. To the best of our knowledge, this is the first work revealing the underlying mechanisms of cPAM inhibiting methane production during sludge digestion system. The findings obtained would not only fill a knowledge gap regarding cPAM's impact on sludge digestion but also could guide engineers to develop strategies to mitigate cPAM's negative impact in the future.

2. Materials and methods

2.1. WAS and cPAM

To minimize the background of cPAM in WAS, the WAS used in this study was obtained from the secondary sedimentation tank of a WWTPs with sludge retention time of 20 d in Changsha, China, where cPAM-based wastewater pretreatment was not implemented. Before use, the sludge was concentrated by settling at 4 °C for 24 h and screened with a 1 mm sieve to remove impurities. Its main characteristics are as follows: pH 6.9 ± 0.1, total suspended solids (TSS) 24655 ± 625 mg/L, volatile suspended solids (VSS) 16728 ± 342 mg/L, soluble chemical oxygen demand (COD) 82 ± 6 mg/L, total COD 21100 ± 420 mg/L, total carbohydrate (as COD) 1998 ± 120 mg/L, total protein (as COD) 11715 ± 90 mg/L, and ammonium nitrogen (NH_4^+-N) 22.4 ± 3 mg/L. The cationic PAM used in this study was purchased from Chongqing Reagent Company, which had a molecular weight of 6–8 million Da with a 30% charge density, and with a residual acrylamide content less than 10 mg/kg.

2.2. Methane production during WAS anaerobic digestion in the presence of different cPAM levels

This batch experiment was carried out in 5 replicate reactors, and each had a working volume of 1 L. A 4 L WAS was divided equally and added into the 5 reactors. Then different volumes of flocculant solution (0.3% w/w) were added into those reactors to achieve the predetermined dosage at the beginning of the experiment, followed by 200 rpm of stirring for 5 min and 50 rpm for 20 min. The predetermined dosages of cPAM addition were 0, 3, 6, 12, and 24 g/kg TSS, respectively. Afterwards, 400 mL of inoculum, which was collected from a laboratory anaerobic sludge digester, was equally divided and added to these five reactors. The TSS, VSS, and total COD concentrations of inoculum were respectively 12610 ± 360, 10120 ± 240, and 16500 ± 400 mg/L. Each reactor was diluted with Milli-Q water to 1 L, resulting in 0, 59.2, 118.3, 236.7, or 473.4 mg/L of initial cPAM in these digesters. Besides, one control reactor was also performed to assess the productivity of methane from the inoculum alone. This control reactor contained 80 mL of inoculum and 920 mL of Milli-Q water without either WAS or cPAM addition. All reactors were flushed with nitrogen gas for 5 min to remove oxygen. Finally, all reactors were capped with rubber stoppers, sealed, and placed in an air-bath shaker (150 rpm) with medium temperature of 35 ± 1 °C. The pH value in all reactors was controlled at 7.0 ± 0.1 in the whole digestion period by adding 4 M hydrochloric acid or 4 M sodium hydroxide with automatic

titrators. During the digestion period, the gas produced was determined periodically by releasing the pressure in the serum bottle using a 300 mL glass syringe to equilibrate with the room pressure according to the literature (Owen et al., 1979). The calculation of the cumulative volume of methane was detailed in our previous publications (Wang et al., 2015b, 2016). In this study, all tests, unless otherwise described, were conducted in triplicate with a control reactor containing 80 mL of inoculum and 920 mL of Milli-Q water, and the data reported below are net values with the values determined in the control reactor having already been subtracted.

2.3. Long-term operation of semi-continuous reactors for measuring key enzyme activities

Two semi-continuous reactors were performed in this work for the measurement of key enzyme activities. The two reactors were fed with 1000 mL of either 0 or 12 g/kg TSS cPAM-added sludge. According to the results of the experiment performed above, the maximum methane production occurred at 22 d in the reactor without cPAM addition and 26 d in the reactor with the addition of 12 g/kg TSS cPAM. Thus, the sludge retention time in the two reactors was controlled at 22 and 26 d, respectively. On each day, 45.5 and 38.5 mL of digestion mixtures were respectively withdrawn from the reactor without cPAM addition and the reactor with the addition of 12 g/kg TSS cPAM and were replaced with the respective same volume of new sludges. All other operations were the same as described in the above test. After 3 months operation, the methane yield in the two reactors did not change significantly with time, and then the measurement of key enzyme activities was made.

2.4. Comparison of biochemical methane potential among cPAM, protein, carbohydrate and WAS

In this batch experiment, five replicate reactors (working volume of 1 L each) were operated. Firstly, reactor 1–4 received 0.54 g cPAM, 0.54 g bovine serum albumin (BSA, a model protein compound with average molecular weight 67000), 0.54 g dextran (a model polysaccharide compound with average molecular weight 23800), or 32.3 mL WAS (0.54 g VSS). After that, these reactors were diluted with Milli-Q water to 920 mL. Reactor 5 received 920 mL Milli-Q water directly. Then, 80 mL of identical inoculum from the semi-continuous reactor fed with 12 g/kg TSS cPAM sludge, was added into each reactor. All other operations of the reactors were the same as described in Section 2.2.

2.5. Effect of cPAM on solubilization, hydrolysis, acidogenesis, and methanogenesis

In the literature, the effect of cPAM on the solubilization of particulate organic matters of sludge are usually obtained by batch tests using real sludge as substrates, evaluations of the reaction rates of hydrolysis, acidogenesis, and methanogenesis are usually made by batch tests using model substrates (Luo et al., 2016; Wang et al., 2015b; Zhao et al., 2016b). Thus, the following batch tests using real sludge or synthetic wastewaters were operated to assess the effect of cPAM on the processes of solubilization, hydrolysis, acidogenesis, and methanogenesis. Eight reactors with working volume of 1 L each were first divided into four groups (Test-I, Test-II, Test-III, and Test-IV) with two in each. Test-I, Test-II, Test-III, and Test-IV were respectively used to evaluate the impact of cPAM on solubilization, hydrolysis, acidogenesis, and methanogenesis. All these tests were lasted for 3 d.

Test-I: The two reactors received 920 mL 0 or 12 g/kg TSS cPAM-added sludge, and 80 mL of same inoculum withdrawn from the semi-continuous reactor fed with cPAM-added sludge. All other conditions were the same as those described above. By measuring the concentrations of soluble protein (carbohydrate) in fermentation liquor, VSS reduction and floc size of sludge, the effect of cPAM on solubilization process could be indicated.

Test-II: The two reactors received 920 mL synthetic wastewater and 80 mL of same inoculum withdrawn from the semi-continuous reactor fed with cPAM-added sludge. The synthetic wastewater contains 5.9 g BSA and 1.4 g dextran. One reactor was fed with 0.24 g cPAM (the amount of cPAM is equally to that in the 12 g/kg TSS cPAM digester) while the other reactor received no cPAM and was set as the control. All other conditions were the same as those described above. By measuring the degradation rates of protein and dextran, the effect of cPAM on hydrolysis process could be indicated.

Test-III: This test was operated the same as described in Test-II except that the substrates (i.e., BSA and dextran) in synthetic wastewater were replaced by 4.0 g L-alanine (model amino acid compound) and 0.6 g glucose (model monosaccharide compound), respectively.

Test-IV: The operation of this test was performed with the same approach as described in Test-II except that 3 g sodium acetate was employed to replace BSA and dextran in synthetic wastewater.

2.6. Effect of the main metabolites of cPAM degradation on anaerobic digestion

According to the pathway shown in Fig. S1, acrylamide, acrylic acid, and polyacrylic acid might co-exist in WAS anaerobic digestion. To assess the effect of acrylamide, acrylic acid, and polyacrylic acid on the bio-conversion processes involved in anaerobic digestion, the following batch test was operated. It was measured that the concentrations of acrylamide, acrylic acid, polyacrylic acid, and cPAM in the semi-continuous reactor fed with sludge with 12 g/kg TSS cPAM addition were 14.9 ± 2.1, 22.1 ± 1.6, 32.3 ± 3.2, and 158.8 ± 5.6 mg/L, respectively (Fig. S2), thus these concentrations were selected in the batch test. In this experiment, twenty 1 L reactors divided in four equal groups (G-I to G-IV) were run for three days.

G-I: This group experiment was used to evaluate the impacts of acrylamide, acrylic acid, and polyacrylic acid on sludge solubilization process. The five reactors were first fed with 600 mL concentrated sludge, respectively. Among them, one was set as the control without addition of any chemical, while the other four reactors received 14.9 mg/L acrylamide, 22.1 mg/L acrylic acid, 32.3 mg/L polyacrylic acid, or 158.8 mg/L cPAM. The impact of these chemicals on solubilization could be assessed by comparing soluble protein and soluble carbohydrate in the digestion liquid among the five reactors.

G-II: This group was used to assess the impacts of acrylamide, acrylic acid, and polyacrylic acid on hydrolysis process. In this experiment, each reactor received 920 mL of synthetic wastewater and 80 mL of digested sludge mixture as inoculums, which was withdrawn from the semi-continuous reactor fed with sludge with 12 g/kg TSS cPAM addition. The synthetic wastewater contains 5.9 g BSA and 1.4 g dextran. Among these reactors, one was set as the control, and the other four were fed with 14.9 mg/L acrylamide, 22.1 mg/L acrylic acid, 32.3 mg/L polyacrylic acid, or 158.8 mg/L cPAM. All other digestion conditions were the same as those described in Section 2.2.

G-III: This group was utilized to assess the impacts of acrylamide, acrylic acid, and polyacrylic acid on acidogenesis process. The operation of this test was performed with the same method as described in G-II except that the digestion substrates (i.e., BSA and dextran) in synthetic wastewater were replaced by 4 g L-alanine (model amino acid compound) and 0.6 g glucose (model

monosaccharide compound), respectively.

G-IV: The group was used to examine the impacts of acrylamide, acrylic acid, and polyacrylic acid on methanogenesis process. The operation of this experiment was also conducted the same as described in G-II except that 3 g acetate instead of BSA and dextran was added in synthetic wastewater.

2.7. Model-based analysis

Methane production was simulated by the modified Gompertz equation (Eq (1)) (Lay et al., 1997), and kinetic parameters (*Mm*, maximum methane yield potential, mL/g VSS or mL/L; *Rm*, methane production rate, mL/(g VSS·d) or mL/d or mL/(L·d); λ, lag-phase time of methane production, d; and t, digestion time, d; *e* is exp(1).) were calculated using Origin 7.0 software.

$$M = Mm \times \exp\left\{ -\exp\left[\frac{Rm \times e}{Mm}(\lambda - t) + 1\right]\right\} \quad (1)$$

The relationships of cPAM concentration with maximum methane yield potential (*Mm*, mL/g VSS or mL/L), methane production rate (*Rm*, mL/(g VSS·d) or mL/(L·d)) and lag phase time of methane production (λ, d) can be simulated by exponential equations using Origin 7.0 software.

The real impact of inhibitor (cPAM or its main metabolites) on each process of anaerobic digestion can be assessed by the inhibition constant, which can be obtained from Eq (2), where X is the reaction rate or removal efficiency, subindex "s" is the substrate and subindex "i" is the inhibitor, Ii is the concentration of inhibitor (mg/L), and Ks,i is the related inhibition constant (mg/L).

$$Xs,i/Xs,0 = 1/(1 + Ii/Ks,i) \quad (2)$$

2.8. Analytical methods

The analyses of TSS, VSS, COD and NH_4^+-N were conducted in accordance with Standard Methods (APHA, 1998). Carbohydrate was measured by the phenol-sulfuric method with glucose as standard. Protein was determined by the Lowry-Folin method with BSA as standard. The COD conversion coefficients are 1.5 g COD/g protein and 1.06 g COD/g carbohydrate (Zhao et al., 2015). The composition in biogas was analyzed by gas chromatograph equipped with a thermal conductivity detector according to the method documented in the literature (Wang et al., 2015a, 2017e; Zhao et al., 2017a). Quantifications of cPAM, acrylamide, acrylic acid, and polyacrylic acid in sludge digestion systems were performed according to the methods documented in the literature (Bao et al., 2010; Cotte et al., 2017; Czech and Pełech, 2008; Dai et al., 2014; Dowling and Fleming, 2011), and the detailed procedures were provided in Supporting Information.

The floc size distribution analysis was performed using a Malvern Moussas and Zouboulis, 2009 instrument with a detection range of 0.01–3500 μm. The capillary suction time was evaluated with a capillary suction time instrument (model 304B, Triton, UK) equipped with an 18 mm diameter funnel and Whatman No.17 chromatography-grade paper. The major functional groups of cPAM before and after anaerobic digestion were determined by Fourier transform infrared spectroscopy, and the preparation of the samples prior to spectroscopy was detailed in Supporting Information. Additionally, the activities of protease, acetate kinase, amidase and coenzyme F420 were also determined according to the approaches detailed in Supporting Information.

2.9. Statistical analysis

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

3. Results and discussion

3.1. The effect of cPAM on methane production

The cumulative methane yield from WAS during anaerobic digestion in the presence of different levels of cPAM addition is shown in Fig. 1. It can be observed that the methane yield in the digester without cPAM addition increased with the digestion time from 1 to 22 d, and no significant increase was observed after day 22 ($p > 0.05$). The optimum digestion time for the digester without cPAM addition was 22 d, and at this time the maximum methane yield of 139.1 ± 5.1 mL/g of VSS (i.e., 1861.5 ± 68.3 mL/L) was measured. Although similar tendencies of methane accumulation were also observed at other digesters with cPAM addition, the methane yield was significantly affected by cPAM. For example, when the sludges with 3, 6, 12, and 24 g cPAM/kg TSS (i.e., 59.2, 118.3, 236.7, and 473.4 mg/L) addition were digested, the maximum methane yields of 123.1 ± 4.7, 106.9 ± 4.6, 88.7 ± 3.9, and 67.4 ± 4.2 mL/g VSS (i.e., 1647.4 ± 62.9, 1430.6 ± 61.6, 1187.0 ± 52.29, and 902.0 ± 56.2 mL/L) were obtained, respectively (Fig. 1A). With the increase of cPAM addition from 0 to 24 g/kg, the methane production decreased exponentially ($R^2 = 0.9989$, Fig. S3).

To further understand the impact of cPAM on anaerobic digestion, the modified Gompertz equation was employed (Fig. 1). Three kinetic parameters, i.e., maximum methane yield potential (*Mm*), methane production rate (*Rm*), and lag phase time (λ), were determined (Fig. 1B–D and Table S1). It can be found that with the increase of cPAM addition, maximum methane yield potential (*Mm*) and methane production rate (*Rm*) decreased, whereas lag phase time (λ) increased. This suggested that the addition of cPAM not only decreased biochemical methane potential but also inhibited the rate of methane production. According to our calculation, it is further estimated that 50% inhibitory concentration of cPAM was around 164 mg/L, i.e., 8 g cPAM/kg TSS (Fig. 1C).

In the two long-term semi-continuous reactors, the methane produced from the digester with 12 g/kg cPAM addition was also higher than that from the digester without cPAM addition. After stable operation, 61.5 ± 2.5 and 52.3 ± 2.2 mL/d of methane was respectively measured from the digesters with 0 and 12 g/kg cPAM (i.e., 236.7 mg/L) addition (Fig. S3), suggesting the methane production rate of 95.5 mL/(g VSS·d) (i.e., 1278.0 mL/(L·d)) in the digester without cPAM addition and 68.7 mL/(g VSS·d) (i.e., 919.4 mL/(L•d)) in the digester with 12 g/kg cPAM (236.7 mg/L) addition. All these results demonstrated that the cPAM levels in sludge could be detrimental to methane production, which is in accord with the results reported previously (Chu et al., 2003). In the following text, the details of how cPAM affects the methane production rate were explored.

3.2. Comparison of biochemical methane potential among cPAM, protein, carbohydrate and WAS

Previous publications reported that cPAM could be partly biodegraded and used as nitrogen and carbon sources to produce volatile fatty acids and methane under anaerobic conditions (Chu et al., 2002; Dai et al., 2015; Haveroen et al., 2005). By measuring the concentrations of cPAM, and its main metabolic intermediates

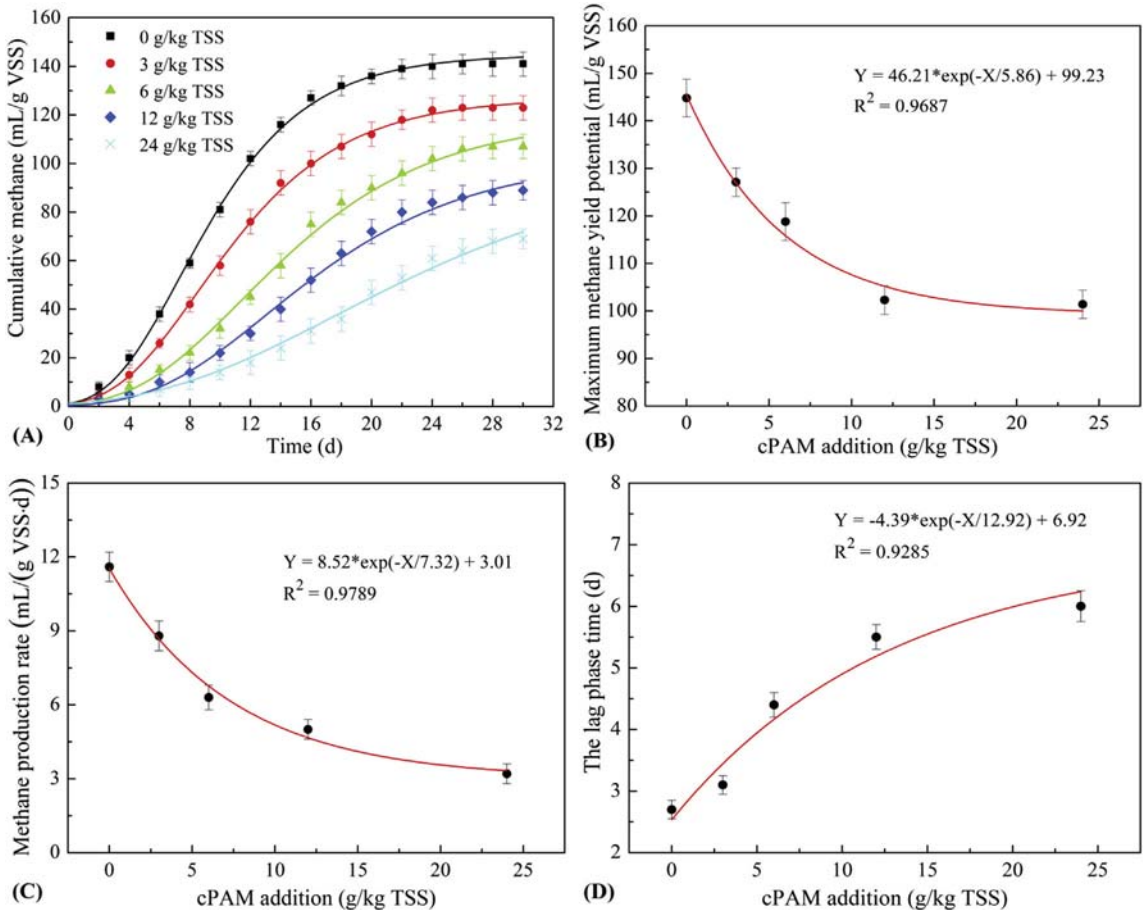

Fig. 1. Cumulative methane production during WAS anaerobic digestion in the presence of different levels of cPAM addition (A), correlation between cPAM addition and the maximum methane yield potential from model fit (B), and methane production rate from model fit (C), and lag phase time from model fit (D). 1 mL/g VSS is equal to 13.38 mL/L in this study. Symbols represent experimental measurements and lines represent model fit. Error bars represent standard deviations of triplicate tests.

(i.e., acrylamide, acrylic acid, and polyacrylic acid) in the long-term semi-continuous reactor fed with 12 g cPAM/kg TSS sludge, it was also found that ~46.3% of cPAM was degraded in such anaerobic digestion system (Fig. S2). However, it is unknown whether the exogenous additives cPAM has a lower methane production potential in comparison to protein and carbohydrate which are generally considered to be the major components of a sludge cell, with their contents accounting for approximately 65% of the total COD of sludge in this work. Thus, the biochemical methane potentials among cPAM, protein, carbohydrate, and WAS were first compared.

It is known that anaerobic digestion process includes internal redox reactions that convert organic substrates to methane and carbon dioxide, and the yields of these gases produced are relevant to the type of the digested substrates. The ideal stoichiometry of dextran, BSA, and WAS (cell) during anaerobic digestion can be theoretically expressed as Eqs (3)–(5). It can be calculated that for the digestion of 1 g of protein, dextran, or cell, 0.318, 0.296 or 0.354 g (i.e., 445.5, 414.4 or 495.6 mL) of methane will be theoretically produced. As cPAM is synthesized by its monomer acrylamide, the formula of acrylamide (C_3H_5ON) was utilized for theoretical calculation. Eq (6) shows the theoretical stoichiometry of acrylamide degradation. On the basis of this equation, it can be calculated that 0.343 g (or 480.5 mL) of methane will be produced per gram of cPAM if it is completely degraded.

$$C_6H_{10}O_5\ (\text{dextran}) + H_2O \rightarrow 3CH_4 + 3CO_2 \quad (3)$$

$$C_{10}H_{20}O_6N_2\ (\text{protein}) + 3.5H_2O \rightarrow 5.25CH_4 + 4.75CO_2 + 2\ NH_3 \quad (4)$$

$$C_5H_7O_2N\ (\text{cell}) + 3H_2O \rightarrow 2.5CH_4 + 2.5CO_2 + NH_3 \quad (5)$$

$$C_3H_5ON\ (\text{acrylamide}) + 2\ H_2O \rightarrow 1.5\ CO_2 + 1.5\ CH_4 + NH_3 \quad (6)$$

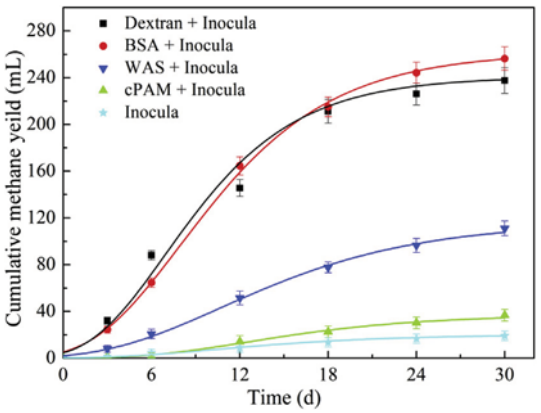

Fig. 2. Comparison of the methane production from anaerobic digestion of 0.54 g Dextran, 0.54 g BSA, 0.54 g WAS, and 0.54 g cPAM. Symbols represent experimental measurements and lines represent model fit. Error bars represent standard deviations of triplicate tests.

Fig. 2 shows the methane production from dextran, BSA, WAS, cPAM, and inocula during 30 d of digestion. Although no extra substrate was added into the sole inocula digester, ~20 mL methane was still measured. During the growth and decay of inocula in anaerobic digestion, soluble microbial products would be generated inevitably. These microbial products (proteins is the main component) may be used as substrates for methane production. By subtracting the amount of methane generated by inocula, it can be seen that the methane yield from cPAM was only 17.5 ± 1.2 mL (accounting for only 6.7% of the theoretical yield) after 30 d of digestion, whereas the corresponding yields from dextran, BSA, and WAS were 218.4 ± 12.5, 237.1 ± 16.4, and 91.8 ± 6.2 mL, respectively. Kinetic analysis revealed that the methane production rates of these substrates were in the sequence of dextran > BSA > WAS > cPAM (Table S2). Further investigations found that dextran and BSA were completely degraded in the anaerobic digestion of 30 d while more than 50% of cPAM was measured after 30 d digestion (data not shown). The results showed that although cPAM has a higher theoretical biochemical methane potential than protein and dextran (the dominant compositions of sludge cells), cPAM is much more reluctant to be degraded and utilized for methane production during anaerobic digestion, as compared with protein, dextran. The reluctant degradation characteristic of cPAM was also observed by other researchers (Chang et al., 2001; Chu et al., 2005).

3.3. How does cPAM affect the process of anaerobic digestion?

During WAS anaerobic digestion, the following four successive steps: solubilization, hydrolysis, acidogenesis, and methanogenesis are usually included. Methane is the terminal product in the anaerobic digestion. If these steps are affected, methane production will be affected inevitably. In this work, therefore, we examined whether and how cPAM affected solubilization, hydrolysis, acidogenesis, and methanogenesis secondly.

As mentioned above, protein and carbohydrate are the main compositions of WAS, thus their release is often employed to indicate the sludge solubilization step in the literature. It can be seen from Fig. 3A that cPAM addition affected the releases of both protein and carbohydrate. With an increase of cPAM addition, soluble protein and carbohydrate concentrations decreased. For example, the concentrations of soluble protein and carbohydrate in the reactor without cPAM addition on 3 d digestion were respectively 619.8 ± 14.1 and 113.9 ± 9.2 mg/L, whereas the corresponding data were only 290.2 ± 11.2 and 62.8 ± 7.6 mg/L in the reactor with 12 g/kg TSS cPAM addition (Please see Fig. S4 for more data of soluble protein and carbohydrate concentration on other digestion days in these two reactors). Fig. 3B shows the VSS reduction ratio among these reactors on 3 d digestion. It was found that the VSS reduction ratio decreased from 16.7 ± 1.4 to 5.5 ± 0.9% with cPAM addition increasing from 0 to 24 g cPAM/kg TSS. All the results indicated that the addition of cPAM restrained solubilization of sludge, which was one reason for the reactor with greater cPAM addition showing lower methane production.

Fig. 3C exhibits the profiles of floc size distribution among these reactors on 3 d digestion. It can be seen that with the increase of cPAM addition, the floc size of sludge increased. For example, the average sludge floc diameters in the digesters with 0, 6, 12 g/kg cPAM addition were respectively 51.29 μm, 58.88 μm, 64.57 μm, suggesting that the physical enmeshment increased with the increasing addition of cPAM dosage (Chu et al., 2005; Dai et al., 2015). Due to this physical enmeshment, it can be understood why the increase addition of cPAM inhibited the sludge solubilization process.

The effects of cPAM on the hydrolysis, acidogenesis, and methanogenesis processes were indicated by assessing the degradation

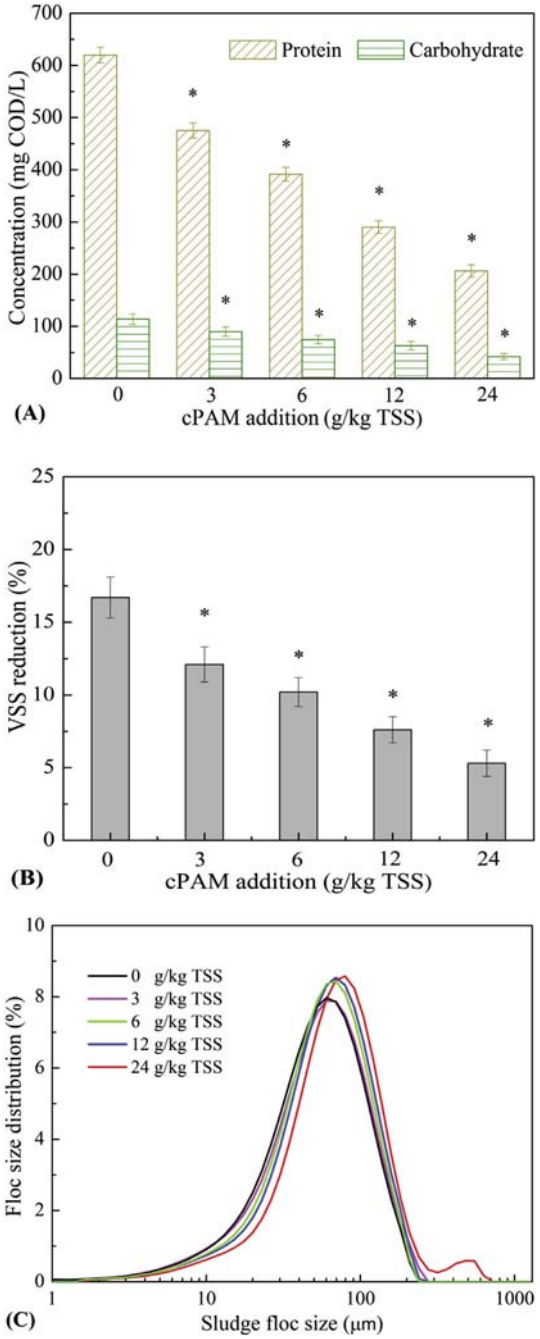

Fig. 3. Effect of cPAM dosage on soluble protein and carbohydrate concentrations (A), VSS reduction (B) and floc size distribution (C) on 3 d digestion. Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests.

efficiency and rate of model substrates in synthetic wastewaters (Fig. 3). Experimental results showed that the addition of cPAM decreased the degradation efficiency of all model substrates employed in this work. For instance, the degradation efficiency in the digesters without cPAM addition on 1 d digestion was 24.3 ± 1.3% in BSA, 42.5 ± 2.1% in dextran, 22.1 ± 1.5% in L-alanine, 24.3 ± 1.7% in glucose, and 17.1 ± 0.8% in acetate whereas these degradation efficiency were deceased to 10.6 ± 1.1, 31.2 ± 1.8, 12.2 ± 1.1, 16.4 ± 1.3, and 7.3 ± 0.3% in the digesters with 12 g/kg TSS cPAM addition, respectively. Similar observations were also made on other digestion days. Besides, the degradation rate of these

model substrates was also inhibited by cPAM. It was found that the inhibition of cPAM to the degradation rate of these substrates was in the order of acetate > BSA > L-alanine > glucose > dextran. The results suggested that although the addition of cPAM inhibited the processes of hydrolysis, acidogenesis, and methanogenesis, methanogenesis was more easily inhibited by cPAM than other reactions.

3.4. Effect of the main metabolites of cPAM degradation on anaerobic digestion

It has been reported that cPAM could be degraded in the process of anaerobic digestion, and the changes of the chemical functional groups reflected in fourier transform infrared spectroscopy could be used to indicate the degradation of cPAM (Bao et al., 2010; Dai et al., 2014; Zhao et al., 2016b). In this study, therefore, we analyzed fourier transform infrared spectroscopy spectra of cPAM before and after anaerobic digestion.

It can be seen from Fig. 5 that there were some transmittance changes in the spectra. The bands of 3415 cm^{-1} and 3251 cm^{-1}, which are assigned to free NH_2 and associating NH_2, became weaker after 30 d anaerobic digestion. The intensity of the bands at 1182 cm^{-1}, representing the C-N group, weakened after anaerobic digestion. Besides, it was also found that the transmittance of the band of 1667 cm^{-1}, which was related to the C═O on the amide group, decreased and shifted after anaerobic digestion, whereas the peak at 1564 cm^{-1} and 1253 cm^{-1} belonging to the C═O on the carboxyl group increased, indicating that part of carbonyl groups were degraded into carboxyl groups (Pi et al., 2015). In addition, the release of NH_4^+-N during WAS anaerobic digestion in the digesters with cPAM addition was higher than that in the digester without cPAM addition (Fig. S5). All the results demonstrated that the microorganisms involved in anaerobic digesters could degrade part of the cPAM, which was consistent with the results reported previously (Dai et al., 2014, 2015; Haveroen et al., 2005)

According to the metabolic pathways of cPAM degradation (Fig. S1), acrylamide, acrylic acid, and polyacrylic acid are supposed to be the main degradation metabolites. Further measurements showed that the concentrations of acrylamide, acrylic acid, and polyacrylic acid in the semi-continuous reactor fed with sludge with 12 g/kg TSS cPAM (i.e., 236.7 mg/L) addition were 14.9 ± 2.1, 22.1 ± 1.6, and 32.3 ± 3.2 mg/L, respectively (Fig. S2). According to mass balance analysis, these three metabolites accounted for ~50% of the degraded cPAM. Thus, the potential impacts of these metabolites at the relevant concentrations on anaerobic digestion were also evaluated, and the results were shown in Fig. 6.

It can be seen that polyacrylic acid inhibited all the solubilization, hydrolysis, acidogenesis, and methanogenesis processes significantly. Acrylamide and acrylic acid at the relevant levels in the tested digester did not significantly affect the processes of solubilization, hydrolysis, and acidogenesis, but both of them inhibited the methanogenesis significantly. The inhibition to the methanogenesis was in the sequence of cPAM > polyacrylic acid > acrylic acid > acrylamide. Similar observations were also made on the kinetics analysis of solubilization rate and degradation rate of different model substrates (Table S3). These results suggested that the degradation metabolites of cPAM in anaerobic digestion could inhibit the performance of sludge anaerobic digestion, and polyacrylic acid was the major contributor among these three metabolites. Previous studies demonstrated that acrylamide is highly toxic and could induce cancer and heritable mutations in lab animals and “probably carcinogenic to humans” (Pelucchi et al., 2006; Weiss, 2002). However, in this work, polyacrylic acid rather than acrylamide was found to be the major inhibitor to the solubilization, hydrolysis, acidogenesis, and methanogenesis, probably due to the higher concentration of polyacrylic acid, as compared with that of acrylamide (e.g., 32.3 ± 3.2 vs 14.9 ± 2.1 mg/L in the digester with 12 g/kg TSS cPAM (236.7 mg/L) addition).

The inhibition kinetic analysis, according to eq. (2), showed that Ks,i (inhibition constant) of these inhibitors to the degradation of acetate was lower than that to the degradation of other model substrate (Table S4). This suggested that methanogenesis was more sensitive to these inhibitors than other bioprocesses, which was in accord with the results presented in Fig. 4. For acetate, the inhibition constant of polyacrylic acid (108 mg/L), acrylic acid (216 mg/L), and acrylamide (266 mg/L) was all lower than that of cPAM (326 mg/L), indicating that cPAM's toxicity to methanogenesis was

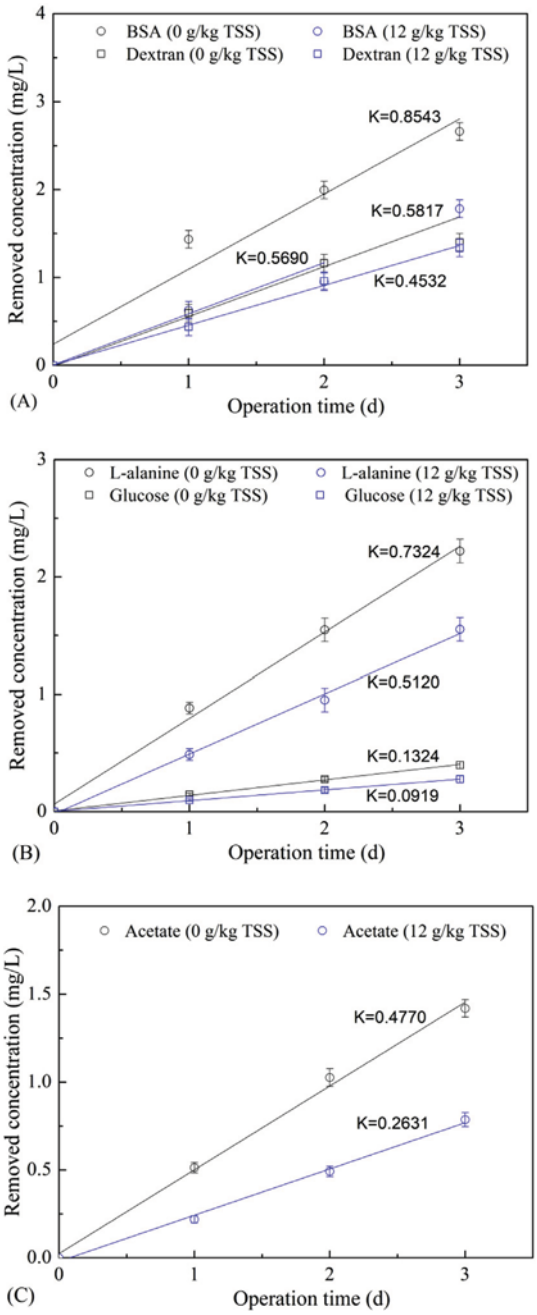

Fig. 4. Effect of cPAM on degradation rates of BSA and dextran (A), L-alanine and glucose (B), and acetate (C) with time. Error bars represent standard deviations of triplicate tests. A zero-order model was used to fit the removed concentration of model compounds with digestion time. Symbols represent experimental measurements and lines represent model fit.

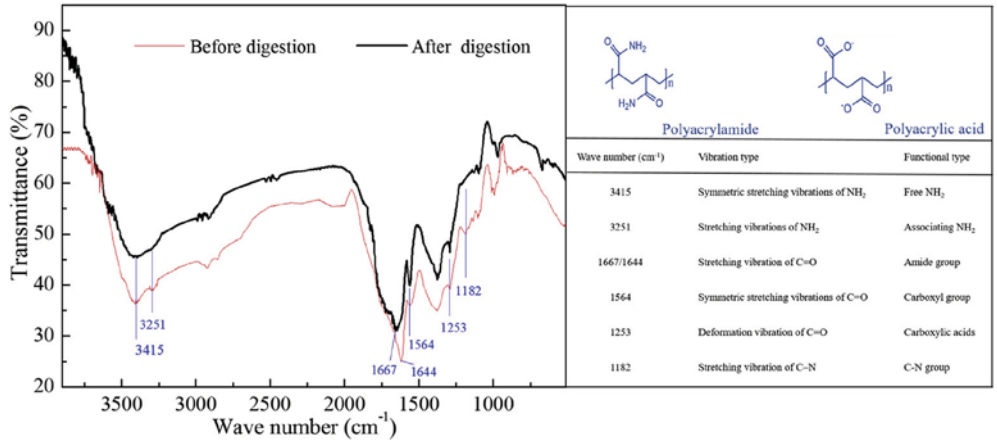

Fig. 5. Fourier transform infrared spectroscopy spectra of cPAM before and after 30 d anaerobic digestion and its main functional groups observed.

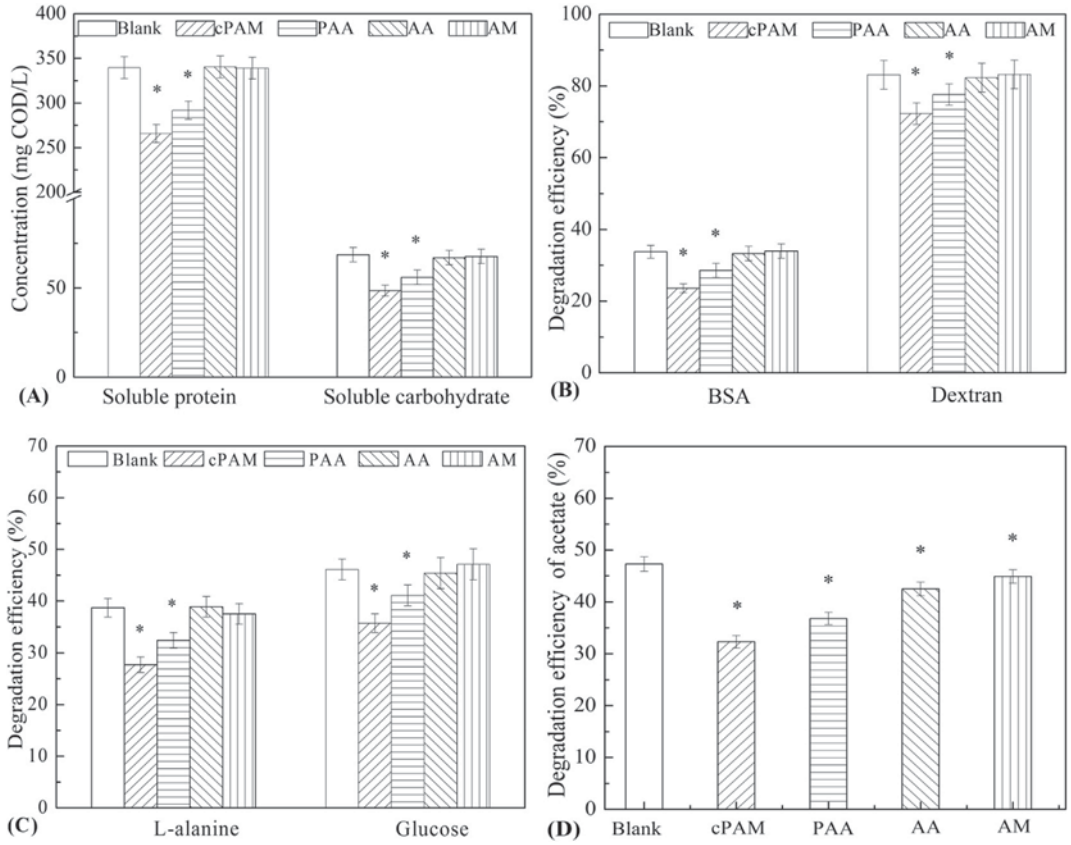

Fig. 6. Comparison of effect of cPAM and its main degradation metabolites on the solubilization (A), hydrolysis (B), acidogenesis (C), and methanogenesis (D) processes. AM: acrylamide; AA: acrylic acid; and PAA: polyacrylic acid. The data were obtained on 2 d digestion (similar observations were also made on 3 d), and error bars represent standard deviations of triplicate tests. Asterisks indicate statistical differences ($p < 0.05$) from the blank.

less than its major metabolites at the same concentration (please see Table S4 for the values of Ks,i of these inhibitors to other substrates). However, the inhibition constant of polyacrylic acid was also lower than that of acrylamide, suggesting that polyacrylic acid's toxicity to methanogenesis was still higher than acrylamide. The accurate reason is unknown and requires to be further investigated in the future.

It should be also noticed that the addition of cPAM caused higher ammonium release due to the hydrolysis of amide group (Fig. S5). Although some publications pointed out that ammonium could suppress the methane production from anaerobic digestion (Chen et al., 2008), our recent work demonstrated that the true contributor was free ammonia rather than ammonium. The ammonium at the fermentation or digestion levels did not affect significantly any process of anaerobic digestion.

3.5. Long-term operation of semi-continuous reactors for measuring key enzyme activities

The activities of key enzymes are directly relevant to the

methane yield (Figs. S1 and S6), and it can reflect the effects of cPAM addition on microbes involved in the digesters (Wang et al., 2015b). In this work, therefore, four enzymes, i.e., protease, acetate kinase, coenzyme F420 and amidase, which are respectively key enzymes for protein hydrolysis, acetate production, methane generation, and deamination, were selected to be measured. It was found that the relative activities of protease, acetate kinase, and coenzyme F420 in the digester with 12 g/kg cPAM addition decreased to 70.1%, 75.3%, and 85.2%, respectively, as compared with those in the digester without cPAM addition (Fig. 5B), which were in accord with the data shown above. However, the relative activity of amidase increased to 112.5% in the digester with 12 g/kg cPAM addition compared to the digester without cPAM addition. This indicated that more deamination occurred in the digester with cPAM addition, which was consistent with the results presented in Fig. 5.

3.6. Overall understandings and implications for WAS treatments

In this work, the mechanism of cPAM affecting the process of anaerobic digestion of WAS was revealed for the first time, filling a fundamental gap between cPAM and sludge anaerobic digestion. It was demonstrated through batch and long-term tests using either synthetic wastewaters or real sludge as the digestion substrates. It was found that the addition of cPAM significantly restrained the sludge solubilization, hydrolysis, acidiogenesis, and methanogenesis. It was measured that ~46% of cPAM was degraded in the digestion process, but only 6.7% of the degraded cPAM was transformed to the end product, methane. Acrylamide, acrylic acid, and polyacrylic acid were found to be the main degradation metabolites, and their amount accounted for ~50% of the degraded cPAM. Apart from cPAM, these metabolites also significantly inhibited the anaerobic digestion process. Polyacrylic acid inhibited all the solubilization, hydrolysis, acidogenesis, and methanogenesis processes significantly, while acrylamide and acrylic acid inhibited the methanogenesis significantly.

Moreover, the findings obtained in this work might also guide engineers to further control the sludge anaerobic digester in real situations in the future. Due to the substantial contents of cPAM in sludge (e.g., 2.5–10 g/kg DS) (Chu et al., 2002; Office for Official Publications of the European Communities: Thornton et al., 2001), its negative impact was already enforced to sludge digesters, but this impact was overlooked previously in field situations. Although some researchers raised concerns about the negative impact, they almost thought the particle aggregation caused by physical enmeshment was the main reason for the deterioration of methane reduction (Campos et al., 2008; Chu et al., 2005; Dentel and Gossett, 1982). As discussed above, besides physical enmeshment, cPAM also suppressed the bio-processes of hydrolysis, acidogenesis, and methanogenesis. Thus, these inhibitions are required to be considered in the future manipulation of sludge digesters. In addition, less than 10% of cPAM could be finally converted to methane, though its theoretically biochemical methane potential is higher than that of protein and carbohydrate (the major composition of sludge cells). Furthermore, the degradation metabolites such as acrylamide, acrylic acid, and polyacrylic acid would suppress the sludge anaerobic digestion process as well. Therefore, strategies are needed to be implemented to enhance the degradations of cPAM and its metabolic products in the anaerobic process in the future.

4. Conclusion

In this present study, the underlying mechanism of how cPAM affects methane production during anaerobic digestion of sludge was revealed. The main conclusions are:

(1) The addition of cPAM not only slowed the process of sludge anaerobic digestion but also decreased methane yield. With the increase of cPAM from 0 to 12 g/kg TSS (i.e., 0–236.7 mg/L), the maximal methane yield decreased from 139.1 to 86.7 mL/g VSS (i.e., 1861.5 to 1187.0 mL/L) while the methane production rate decreased from 155.2 to 66.9 mL/(L·d).
(2) The addition of cPAM significantly restrained the sludge solubilization, hydrolysis, acidogenesis, and methanogenesis processes.
(3) The addition cAPM could be degraded in the anaerobic digestion (~46%), and the degradation products significantly affected methane production.
(4) Acrylamide, acrylic acid, and polyacrylic acid were found to be the main degradation metabolites, and their amount accounted for ~50% of the degraded cPAM. Polyacrylic acid inhibited all the solubilization, hydrolysis, acidogenesis, and methanogenesis processes while acrylamide and acrylic acid inhibited the methanogenesis significantly.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2017.12.007.

References

Aguilar, M.I., Sáez, J., Lloréns, M., Soler, A., Ortuño, J.F., Meseguer, V., Fuentes, A., 2005. Improvement of coagulation–flocculation process using anionic polyacrylamide as coagulant aid. Chemosphere 58 (1), 47–56.
American Public Health Association (APHA), 1998. American Water Works Association, and Water Environment Federation: Washington, DC. Standard Methods for the Examination of Water and Wastewater, 20th ed.
Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34 (6), 755–781.
Bao, M., Chen, Q., Li, Y., Jiang, G., 2010. Biodegradation of partially hydrolyzed polyacrylamide by bacteria isolated from production water after polymer flooding in an oil field. J. Hazard. Mat. 184 (1–3), 105–110.
Campos, E., Almirall, M., Mtnezalmela, J., Palatsi, J., Flotats, X., 2008. Feasibility study of the anaerobic digestion of dewatered pig slurry by means of polyacrylamide. Bioresour. Technol. 99 (2), 387–395.
Chang, L.L., Raudenbush, D.L., Dentel, S.K., 2001. Aerobic and anaerobic biodegradability of a flocculant polymer. Water Sci. Technol. 44 (2–3), 461–468.
Chen, H., Liu, Y., Ni, B.J., Wang, Q., Wang, D., Zhang, C., Li, X., Zeng, G., 2016. Full-scale evaluation of aerobic/extended-idle regime inducing biological phosphorus removal and its integration with intermittent sand filter to treat domestic sewage discharged from highway rest area. Biochem. Eng. J. 113, 114–122.
Chen, H.B., Wang, D.B., Li, X.M., Yang, Q., Luo, K., Zeng, G.M., Tang, M.L., 2014. Effects of Cd(II) on wastewater biological nitrogen and phosphorus removal. Chemosphere 117 (1), 27–32.
Chen, Y., Cheng, J., Creamer, K., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol 99 (10), 4044–4064.
Chu, C.P., Lee, D.J., Chang, B.V., You, C.H., Liao, C.S., Tay, J.H., 2003. Anaerobic digestion of polyelectrolyte flocculated waste activated sludge. Chemosphere 53 (7), 757–764.
Chu, C.P., Lee, D.J., Chang, B.V., You, C.S., Tay, J.H., 2002. "Weak" ultrasonic pretreatment on anaerobic digestion of flocculated activated biosolids. Water Res. 36 (11), 2681–2688.
Chu, C.P., Tsai, D.G., Lee, D.J., Tay, J.H., 2005. Size-dependent anaerobic digestion rates of flocculated activated sludge: role of intrafloc mass transfer resistance. J. Environ. Manag. 76 (3), 239–244.
Cotte, J.F., Bouadam, A., Sordoillet, A., Jaudinaud, I., Chambon, V., Talaga, P., 2017. Determination of molecular size parameters and quantification of polyacrylic acid by high performance size-exclusion chromatography with triple detection. Anal. Bioanal. Chem. 409 (8), 1–10.
Czech, Z., Pełech, R., 2008. Use of pyrolysis and gas chromatography for the determination of acrylic acid concentration in acrylic copolymers containing carboxylic groups. Polym. Test. 27 (7), 870–872.
Dai, X., Luo, F., Zhang, D., Dai, L., Chen, Y., Dong, B., 2015. Waste-activated sludge fermentation for polyacrylamide biodegradation improved by anaerobic

hydrolysis and key microorganisms involved in biological polyacrylamide removal. Sci. Rep. 5, 11675.
Dai, X., Luo, F., Yi, J., He, Q., Dong, B., 2014. Biodegradation of polyacrylamide by anaerobic digestion under mesophilic condition and its performance in actual dewatered sludge system. Bioresour. Technol. 153 (2), 55–61.
Dentel, S.K., Gossett, J.M., 1982. Effect of chemical coagulation on anaerobic digestibility of organic materials. Water Res. 16 (5), 707–718.
Dowling, A.H., Fleming, G.J., 2011. The influence of poly(acrylic) acid number average molecular weight and concentration in solution on the compressive fracture strength and modulus of a glass-ionomer restorative. Dent. Mater. 27 (6), 535.
Duan, N., Dong, B., Wu, B., Dai, X., 2012. High-solid anaerobic digestion of sewage sludge under mesophilic conditions: feasibility study. Bioresour. Technol. 104, 150–156.
Guendouz, J., Buffière, P., Cacho, J., Carrère, M., Delgenes, J.P., 2008. High-solids anaerobic digestion: comparison of three pilot scales. Water Sci. Technol. 58 (9), 1757–1763.
Haveroen, M.E., Mackinnon, M.D., Fedorak, P.M., 2005. Polyacrylamide added as a nitrogen source stimulates methanogenesis in consortia from various wastewaters. Water Res. 39 (14), 3333–3341.
Jenicek, P., Bartacek, J., Kutil, J., Zabranska, J., Dohanyos, M., 2012. Potentials and limits of anaerobic digestion of sewage sludge: energy self-sufficient municipal wastewater treatment plant? Water Sci. Technol. 66 (6), 1277.
Lay, J.J., Li, Y.Y., Noike, T., 1997. Influences of pH and moisture content on the methane production in high-solids sludge digestion. Water Res. 31 (6), 1518–1524.
Li, X., Zhao, J., Wang, D., Qi, Y., Xu, Q., Deng, Y., Yang, W., Zeng, G., 2016. An efficient and green pretreatment to stimulate short-chain fatty acids production from waste activated sludge anaerobic fermentation using free nitrous acid. Chemosphere 144, 160–167.
Li, Y., Yuan, X., Wu, Z., Wang, H., Xiao, Z., Wu, Y., 2016b. Enhancing the sludge dewaterability by electrolysis/electrocoagulation combined with zero-valent iron activated persulfate process. Chem. Eng. J. 303, 636–645.
Luo, J., Chen, Y., Feng, L., 2016. Polycyclic aromatic hydrocarbon affects acetic acid production during anaerobic fermentation of waste activated sludge by altering activity and viability of acetogen. Environ. Sci. Technol. 50 (13), 6921–6929.
Moussas, P.A., Zouboulis, A.I., 2009. A new inorganic-organic composite coagulant, consisting of polyferric sulphate (PFS) and polyacrylamide (PAA). Water Res. 43 (14), 3511–3524.
Owen, W.F., Stuckey, D.C., Healy, J.B., Young, L.Y., Mccarty, P.L., 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res. 13 (6), 485–492.
Pelucchi, C., Galeone, C., Levi, F., Negri, E., Franceschi, S., Talamini, R., Bosetti, C., Giacosa, A., La, V.C., 2006. Dietary acrylamide and human cancer. Int. J. Cancer 118 (2), 467–471.
Pi, Y., Zheng, Z., Bao, M., Li, Y., Zhou, Y., Sang, G., 2015. Treatment of partially hydrolyzed polyacrylamide wastewater by combined Fenton oxidation and anaerobic biological processes. Chem. Eng. J. 273, 1–6.
Qi, Y., Thapa, K.B., Hoadley, A.F.A., 2011. Application of filtration aids for improving sludge dewatering properties – a review. Chem. Eng. J. 42 (41), 373–384.
Stuckey, D.C., Mccarty, P.L., 1978. Thermochemical treatment of nitrogenous materials to increase methane yield. Biotechnol. Bioeng. Symp. 8, 219–223.
Thornton, I., Butler, D., Docx, P., Hession, M., Makropoulos, C.K., McMullen, M., Nieuwenhuijsen, M., Pitman, A., Rautiu, R., Sawyer, R., Smith, S., White, D., Wilderer, P., Paris, S., Marani, D., Braguglia, C., Palerm, J., 2001. Pollutants in Urban Waste Water and Sewage Sludge. European Commission, pp. 1–11.
Wang, D., Li, X., Qi, Y., Wei, Z., Yan, W., Zeng, T., Zeng, G., 2012. Improved biological phosphorus removal performance driven by the aerobic/extended-idle regime with propionate as the sole carbon source. Water Res. 46 (12), 3868–3878.
Wang, D., Liu, Y., Ngo, H.H., Zhang, C., Yang, Q., Peng, L., He, D., Zeng, G., Li, X., Ni, B.-J., 2017a. Approach of describing dynamic production of volatile fatty acids from sludge alkaline fermentation. Bioresour. Technol. 238, 343–351.
Wang, D., Wang, Y., Liu, Y., Ngo, H.H., Yu, L., Zhao, J., Fei, C., Qi, Y., Zeng, G., Li, X., 2017b. Is denitrifying anaerobic methane oxidation-centered technologies a solution for the sustainable operation of wastewater treatment plants? Bioresour. Technol. 234, 456–465.
Wang, D., Fu, Q., Xu, Q., Liu, Y., Hao, N.H., Yang, Q., Zeng, G., Li, X., Ni, B., 2017c. Free nitrous acid-based nitrifying sludge treatment in a two-sludge system enhances nutrient removal from low-carbon wastewater. Bioresour. Technol. 244, 920–928.
Wang, D., Zeng, G., Chen, Y., Li, X., 2015a. Effect of polyhydroxyalkanoates on dark fermentative hydrogen production from waste activated sludge. Water Res. 73, 311–322.
Wang, D., Zhao, J., Zeng, G., Chen, Y., Bond, P.L., Li, X., 2015b. How does poly(-hydroxyalkanoate) affect methane production from the anaerobic digestion of waste-activated sludge? Environ. Sci. Technol. 49 (20), 12253–12262.
Wang, Q., Sun, J., Zhang, C., Xie, G.J., Zhou, X., Qian, J., Yang, G., Zeng, G., Liu, Y., Wang, D., 2016. Polyhydroxyalkanoates in waste activated sludge enhances anaerobic methane production through improving biochemical methane potential instead of hydrolysis rate. Sci. Rep. 6, 19713.
Wang, Q., Ye, L., Jiang, G., Jensen, P.D., Batstone, D.J., Yuan, Z., 2013. Free nitrous acid (FNA)-based pretreatment enhances methane production from waste activated sludge. Environ. Sci. Technol. 47 (20), 11897–11904.
Wang, Y., Wang, D., Yang, Q., Zeng, G., Li, X., 2017d. Wastewater opportunities for denitrifying anaerobic methane oxidation. Trends Biotechnol. 35 (9), 799–802.
Wang, Y., Wang, D., Liu, Y., Wang, Q., Chen, F., Yang, Q., Li, X., Zeng, G., Li, H., 2017e. Triclocarban enhances short-chain fatty acids production from anaerobic fermentation of waste activated sludge. Water Res. 127, 150–161.
Weiss, G., 2002. Cancer risks. Acrylamide in food: uncharted territory. Science 297 (5578), 27.
Xia, S., Wang, F., Fu, Y., Yang, D., Ma, X., 2005. Biodiversity analysis of microbial community in the chem-bioflocculation treatment process. Biotechnol. Bioeng. 89 (6), 656–659.
Xie, G.J., Chen, C., Hu, S., Yuan, Z., 2016a. Complete nitrogen removal from synthetic anaerobic sludge digestion liquor through integrating anammox and denitrifying anaerobic methane oxidation in a membrane biofilm reactor. Environ. Sci. Technol. 51 (2), 819–827.
Xie, G.J., Liu, B.F., Wang, Q.L., Jie, D., Ren, N.Q., 2016b. Ultrasonic waste activated sludge disintegration for recovering multiple nutrients for biofuel production. Water Res. 93, 56–64.
Xu, Q., Li, X., Ding, R., Wang, D., Liu, Y., Wang, Q., Zhao, J., Chen, F., Zeng, G., Yang, Q., Li, H., 2017. Understanding and mitigating the toxicity of cadmium to the anaerobic fermentation of waste activated sludge. Water Res. 124, 269–279.
Yi, K., Wang, D., Yang, Q., Li, X., Chen, H., Sun, J., An, H., Wang, L., Deng, Y., Liu, J., Zeng, G., 2017. Effect of ciprofloxacin on biological nitrogen and phosphorus removal from wastewater. Sci. Total Environ. 368, 605–606.
Zhai, L.F., Sun, M., Song, W., Wang, G., 2012. An integrated approach to optimize the conditioning chemicals for enhanced sludge conditioning in a pilot-scale sludge dewatering process. Bioresour. Technol. 121 (10), 161–168.
Zhang, D., Chen, Y., Zhao, Y., Ye, Z., 2011. A new process for efficiently producing methane from waste activated sludge: alkaline pretreatment of sludge followed by treatment of fermentation liquid in an EGSB reactor. Environ. Sci. Technol. 45 (2), 803–808.
Zhao, J., Liu, Y., Ni, B., Wang, Q., Wang, D., Yang, Q., Sun, Y., Zeng, G., Li, X., 2016a. Combined effect of free nitrous acid pretreatment and sodium dodecylbenzene sulfonate on short-chain fatty acid production from waste activated sludge. Sci. Rep. 6, 21622.
Zhao, J., Liu, Y., Wang, D., Chen, F., Li, X., Zeng, G., Yang, Q., 2017a. Potential impact of salinity on methane production from food waste anaerobic digestion. Waste Manag. 67, 308–314.
Zhao, J., Gui, L., Wang, Q., Liu, Y., Wang, D., Ni, B.J., 2017b. Aged refuse enhances anaerobic digestion of waste activated sludge. Water Res. 123, 724–733.
Zhao, J., Wang, D., Li, X., Qi, Y., Chen, H., Yu, Z., Zeng, G., 2015. Free nitrous acid serving as a pretreatment method for alkaline fermentation to enhance short-chain fatty acid production from waste activated sludge. Water Res. 78, 111–120.
Zhao, J., Zhang, C., Wang, D., Li, X., An, H., Xie, T., Chen, F., Xu, Q.X., Sun, Y., Zeng, G., 2016b. Revealing the underlying mechanisms of how sodium chloride affects short-chain fatty acid production from the Co-fermentation of waste activated sludge and food waste. ACS Sustain. Chem. Eng. 4 (9), 4675–4684.