Enhancement of activated sludge dewatering performance by combined composite enzymatic lysis and chemical re-flocculation with inorganic coagulants: Kinetics of enzymatic reaction and re-flocculation morphology

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ABSTRACT

The feasibility of combined process of composite enzymatic treatment and chemical flocculation with inorganic salt coagulants was investigated in this study. The evolution of extracellular polymeric substances (EPS) distribution, composition and morphological properties were analyzed to unravel the sludge conditioning mechanism. It was found that sludge filtration performance was deteriorated due to release of a large amount of biopolymers after enzymatic treatment. The change in EPS followed the pseudo-first-order kinetic equation well under enzymatic treatment. The feeding modes of enzymes had a significant influence on sludge lysis efficiency under compound enzymes treatment. Alpha amylase + protease was more effective in solubilization than other two addition modes (protease + α -amylase or simultaneous addition). The sludge floc re-formed and macromolecule biopolymers were effectively removed through coagulation process. At the same time, both of filtration rate and cake solid content of sludge treated with enzymes were improved with increasing dosage of coagulants, and ferric iron (FeCl_3) had better performance in sludge dewaterability enhancement than polyaluminium chloride (PACl). In addition, sludge filtration property was slightly deteriorated, while the cake moisture reduction was favored at the optimal dosage of inorganic coagulants.

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1. Introduction

The management of wastewater sludge, now, often referred to as biosolids, accounts for a major portion of the cost of the wastewater treatment process and represents significant technical challenges. Waste activated sludge from secondary settling tank generally contains over 99% water. The regulation sheet on landfills issued by the Ministry of Housing and Urban-Rural Development in China demands a minimum dry solids content of 40% for unlimited incorporation of dewatered sludge from municipal wastewater treatment plants (WWTPs). Therefore, the development of high

performance dewatering processes has been the most important research areas of environmental science and technology. In general, the moisture in sludge system can be classified into free water (accounted for about 70%), interstitial water (about 20%), adsorbed water (about 7%) and bound water (about 3%) (Vaxelaire and Cezac, 2004). Except for sludge properties, the dewatering performance was mainly dependent on selection of sludge devices and chemical conditioning methods.

Extracellular polymeric substances (EPS) account for about 60–80% of total sludge mass (Liu and Fang, 2003) and significantly affect other sludge physicochemical property, such as surface charge, floc stability and rhetorical behavior (Mikkelsen and Keiding, 2002). Many previous studies have demonstrated that EPS was the most crucial deciding factor for sludge dewatering (Mikkelsen and Keiding, 2002). Houghton et al. (2001) reported that the dewaterability was determined by the contents of EPS and there existed an optimal EPS content for each sludge, at this time, which exhibited the best dewatering property. Many researchers

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found that EPS composition (proteins and polysaccharides ratios) have more significant effects on sludge dewaterability (Higgins and Novak, 1997). Murthy and Novak (1999) reported that high proteins/polysaccharide ratio was detrimental for sludge dewatering performance. More recently, EPS was found to have double layers including the loosely bound EPS (LB-EPS) which is diffused from the tightly bound EPS (TB-EPS) that surrounds the cells (Poxon and Darby, 1997; Ramesh et al., 2006; Li and Yang, 2007). Moreover, some researchers reported that only the LB-EPS was related to the sludge dewaterability (Ramesh et al., 2006; Li and Yang, 2007; Raynaud et al., 2012). Yu et al. (2008) suggested that sludge floc was composed by five fractions: supernatant, slime, LB-EPS, TB-EPS, and pellet, and the sludge dewatering performance was mainly dependent on the soluble EPS characteristics.

In general, it is essential to precondition the sludge particles with chemicals properly before the dewatering in order to improve the operating performance. For example, addition of inorganic or organic flocculants can agglomerate fine sludge colloids to form large flocs through charge neutralization and bridging, which can be more easily separated from the water (Niu et al., 2013; Zhang et al., 2014). EPS fraction in activated sludge was highly hydrated, and the traditional chemical flocculants was ineffective to destroy EPS structure and convert bound water into free water. Therefore, the advanced sludge technologies (AST) were developed to improve sludge dewatering property and cake solid content by solubilizing EPS components (Neyens et al., 2004). The established AST includes photo-Fenton/Fenton oxidation technology (Tokumura et al., 2007; Liu et al., 2013; Neyens and Baeyens, 2003), acid and alkali treatment (Zhu et al., 2013), heat treatment (Neyens et al., 2004), enzymatic treatment (Thomas et al., 1993) and integrated process.

Biological enzymes cannot solubilize the EPS components of activated sludge under mild conditions, but also improve its subsequent treatment and disposal. Therefore, enzymatic treatment is very promising technique in sludge treatment (Ayol, 2005). Initially, Thomas et al. (1993) found that addition of the enzymes could improve the mechanical dewatering in common filter press or decanters in combination with flocculation. Dursun et al. (2006) compared the sludge conditioning efficiency of enzymatic treatment in laboratory and pilot scale. The results revealed that a significant increase in cake solid content can be reached by adding enzymes at low dose. In practical application, it was found that no obvious improvement of final cake solid content was observed using centrifuge as dewatering equipment, which can be attributed to floc deterioration in the high shear applied in centrifugation. Ayol (2005) reported that the enzyme additions enhanced dewaterability of the polymer conditioned samples in terms of CST and solids content of final product, demonstrating the possibility of significant volume reductions using enzymatic treatment at full-scale. Sesay et al. (2006) found that, with especially higher SRT values, extracting proteins was more effective after enzymatic treatment. In addition, Bonilla suggested that lysozyme aided in the flocculation of particles reducing the polymer demand from 11% to 6%, and conditioning mechanism of lysozyme was similar to that of a flocculant (Bonilla et al., 2015).

Few of previous studies have investigated the feasibility of combined enzymatic treatment and chemical re-flocculation on dewatering performance. Especially, the evolution of EPS and morphological properties under chemical conditioning was still not

clear. Thus, the objectives of this study were to: (1) investigate the effect of combined composite enzymatic lysis and chemical re-flocculation on sludge dewatering performance; (2) deeply understand the dynamic variation in distribution and composition of EPS under enzymatic hydrolysis reaction; (3) optimize the addition modes and operating parameters of composite enzymatic treatment; (4) observe the morphological properties under combined conditioning process.

2. Materials and methods

2.1. Waste sludge and reagents

Waste active sludge was obtained from the Beixiaohe WWTP in Beijing city. This WWTP treats approximately 1,000,000 m³ daily by combined membrane bioreactor (MBR) and ozonation process. The sludge characteristics can be found in Table 1. The characteristics of neutral protease and medium temperature alpha amylase (α -amylase) can be found in Table 2. Both of them were purchased from Tianjin Noao Sci & Tech Development Co., Ltd in China.

2.2. Batch test

2.2.1. Enzymatic hydrolysis procedure

100 mL sludge was placed into Erlenmeyer flasks under magnetic stirring at optimal temperature. Then, the enzyme was added to initiate the reaction. After 4 h of reaction, sludge suspension was used for EPS and dewaterability measurement. With regard to kinetic test, sludge of 600 mL were put into 1 L Erlenmeyer flasks with mixing. After enzymes were added, 50 mL sludge samples were taken out and placed into centrifuge tubes at different time intervals within 6 h. The reaction temperature for protease and α -amylase were 45 and 55 °C respectively.

2.2.2. Chemical re-flocculation

The sludge was mixed with a conventional jar-stirring device. Sludge samples of 500 mL in 1 L beakers were used in the experiments. The following mixing procedure was used: a rapid mix period for 30 s at 200 rpm followed by a slow-stir phase at 50 rpm for 10 min. The coagulants (FeCl₃, PACl) were added with agitation using a graduated finnpipette. At the end of mixing, the conditioned sludge was allowed to settle in the beaker for 30 min. Finally, the floc morphology and soluble EPS were determined.

2.2.3. Extraction of EPS

A heat extraction method was modified to extract the LB-EPS and TB-EPS from the sludge (Liyun Ge and Luming, 2006). A sludge suspension was firstly dewatered by centrifugation in a 50 mL tube at 3000 g for 10 min. The supernatant was collected as soluble EPS to analyze chemical oxygen demand (COD). The sludge pellet in the tube was then resuspended into 15 mL of 0.05% NaCl, sonicated at 20 KHz for 2 min, shaken horizontally at 150 rpm for 10 min and sonicated again for an additional 2 min. The liquor was centrifuged at 8000g for 10 min to separate solids and supernatant. The collected supernatant was regarded as the LB-EPS. The residual sludge pellet left in the centrifuge tube was resuspended in a 0.05% NaCl solution, sonicated for 3 min, then heated at 80 °C for 30 min, finally centrifuged at 12,000 g for 20 min to collect TB-EPS. The

Table 1
Characteristics of waste active sludge.

Indicator	Moisture content (%)	pH	VSS/TSS	CST (s)	d _{0.5} (μm)	Zeta potential (mV)	SCOD (mg/L)
Value	97.95	7.5	0.75	320	62	−15.2	130

Table 2
Characteristics of enzymes.

Enzymes	Enzyme activity (U/g)	Optimum reaction pH range	Optimum temperature (°C)
Neutral protease	200,000	7.0–8.0	40–50
α -amylase	4000	5.5–7.0	50–80

membrane filter was used to remove the particulates in EPS samples prior to analysis. The concentrations of different EPS components were determined for COD.

2.2.4. Determination of sludge dewaterability

Since the determination of specific resistance to filtration (SRF) has the similar physical process to the high pressure dewatering, it was used to evaluate the sludge dewaterability in this work. The SRF was conducted in a 250 mL Buchner funnel using a filter with 0.45 μm filter paper. The Buchner funnel was filled with 100 mL of the sludge suspension, and a constant pressure of 0.6 MPa was applied by an air pump. The volume of filtrate under pressure was continuously recorded every 10 s before surface cracking was detected. The SRF of the sludge was calculated by:

$$r = \frac{2PA^2b}{\mu\omega} \quad (1)$$

where P (kg/m^2) is the pressure applied, A (m^2) is the filter area, μ ($\text{Kg}\cdot\text{s}/\text{m}^2$) is the KV, ω (kg/m^3) denotes dry solid weight per unit volume sludge on the filtrate media, b is the time -to-filtration ratio, which is the slope of the curve that is obtained by plotting the ratio of the time of filtration to the volume of filtrate (t/V) versus the filtrate volume (V).

2.2.5. Analytical methods

COD was measured using HACH DR2800 spectrophotometer with 2 h digestion. Polysaccharide was determined by anthrone-sulfuric acid method with glucose as standard (Frolund et al., 1996). Protein was measured by Lowry-Folin method with bovine serum albumin (BSA) as standard (Frolund et al., 1996). The concentration of ammonia nitrogen was determined by Nessler's reagents spectrophotometer method according to APHA–AWWA (APHA, 1998). Three-dimensional excitation emission matrix (3-DEEM) was measured by a Hitachi F-4500 fluorescence spectrophotometer with an excitation range from 200 to 400 nm and an emission range from 220 to 550 nm. The spectra were recorded at a scan rate of 12,000 nm/min, using excitation and emission slit bandwidths of 5 nm. The EPS samples were diluted by 200 times prior to EEM analysis. Other sludge parameters, including TSS, volatile suspended solids (VSS) were determined according to standard methods (APHA, 1998). pH was measured by a pH-3C (Shanghai, China) pH meter.

Each jar test experiment was performed in duplicate. And each experimental parameters was analyzed in triplicate and the standard deviations of all analyses were always less than 5%, unless noted in the text.

3. Results and discussion

3.1. Effects of enzymatic treatment on sludge dewaterability

As depicted in Fig. 1, with the increase of two enzymes addition, the sludge SRF decreased firstly and then increased. It reached the minimum of $7.47 \times 10^{13} \text{ m kg}^{-1}$ and $1.34 \times 10^{15} \text{ m kg}^{-1}$ when the dosage of protease and amylase were 50 and 100 mg/gTSS. It was noted that SRF with α -amylase treatment was much higher than

Fig. 1. Effect of enzymatic treatment on SRF.

that after protease treatment at the same dosage. According to the value of SRF, sludge can be classified into poor dewaterability ($>1 \times 10^{13} \text{ m kg}^{-1}$), medium dewaterability ($0.5\text{--}0.9 \times 10^{13} \text{ m kg}^{-1}$) and good dewaterability ($<0.4 \times 10^{13} \text{ m kg}^{-1}$) (Zhang et al., 2014). Thus, further treatment was needed to improve sludge dewatering property.

3.2. Change in EPS properties under enzymatic treatment

3.2.1. Effects of enzymes dosage on EPS distribution and composition

It can be seen from Fig. 2 (a) and (b) that the contents of SEPS and LB-EPS were significantly increased, while no obvious change in TB-EPS was observed under enzymatic treatment. Overall, hydrolysis efficiency of amylase was higher than that of protease. With addition of protease and amylase, SEPS content increased from 90 to 78 mgCOD/gTSS to 254 and 319 mgCOD/gTSS after 4 h of reaction. At the same time, LB-EPS was increased from 11 to 8.5 mgCOD/gTSS to 52.5 and 46.5 mgCOD/gTSS. It is interesting to note that the amylase exhibited higher protein solubilization efficiency compared to protease. The protein concentration in SEPS increased from 23.65 mgCOD/gTSS to 34.2 mgCOD/gTSS under protease treatment, while it increased from 45.5 mgCOD/gTSS to 72.9 mgCOD/gTSS in α -amylase hydrolysis. Furthermore, the protein content in TB-EPS was decreased somewhat after addition of enzymes. As presented in Fig. 2 (e) and (f), the contents of polysaccharides in SEPS and LB-EPS of sludge were significantly increased after enzymatic treatment. The results indicated that EPS was more readily to be extracted after enzymatic treatment, producing looser floc structure. It should be noted that simultaneous release of proteins and polysaccharides was observed with single enzyme addition and the change in protein content showed a similar pattern to that of polysaccharide. In fact, protein and polysaccharide in activated sludge always existed in the form of complexes (Dignac et al., 1998), so both of them were released simultaneously under single enzyme treatment. As mentioned above, the SRF of sludge with amylase treatment was higher than that under protease hydrolysis. This can be attributed to more effective sludge solubilization of amylase; especially the higher content of protein was released. Many studies have demonstrated

Fig. 2. Effect of enzymes dosage on concentrations of different EPS fractions: (a) total EPS content-protease; (b) total EPS content-amylase; (c) protein-protease; (d) protein-amylase; (e) polysaccharide-protease; (f) polysaccharide-amylase.

that sludge filtration property was mainly dependent on SEPS properties, and the high content of protein in SEPS was always detrimental to sludge filterability (Zhang et al., 2014, 2015a, 2015b).

3.2.2. Enzymatic kinetics of different EPS components

The variation in EPS properties with reaction time can be found in Fig. 3. The sludge was effectively solubilized by addition of two enzymes. Enzymatic treatment resulted in reduction of TB-EPS while significant increase in content of LB-EPS and SEPS, indicating that the TB-EPS was converted into LB-EPS and SEPS. Therefore, the sludge floc became looser, which can be confirmed by the fact that sludge settleability was deteriorated and particles were floated on the top of the centrifugal tubes after enzymatic treatment (see Fig. S1 of supporting information (SI)). It should be pointed out that each EPS component reached the equilibrium after 3 h of reaction under protease treatment, while the hydrolysis process still wasn't complete for amylase hydrolysis. This

observation revealed that catalytic activity of amylase was more effective and lasting than protease in sludge solubilization.

Kinetic modeling (equations (2) and (3)) was used to get further insights into the kinetic variation in EPS characteristics under enzymatic hydrolysis.

$$-\frac{dx}{dt} = K_h x \quad (2)$$

$$\ln x = K_h t + b \quad (3)$$

Where, K_h denotes the solubilization rate constant, b is the intercept. By plotting $\ln x$ [$\ln (COD/COD_0)$] – t , the slope and the intercept can be obtained.

The fitting results were given in Fig. 4 and Table 3. It can be seen that the sludge hydrolysis rate of α -amylase was significantly higher than that of protease. This observation might be attributed

Fig. 3. Variation in different EPS content under enzymatic hydrolysis with reaction time: (a) total EPS content- α -protease; (b) total EPS content- α -amylase; (c) protein- α -protease; (d) protein- α -amylase; (e) polysaccharide- α -protease; (f) polysaccharide- α -amylase (Dosage of enzymes = 150 mg/g; hydrolysis temperature of protease and amylase were 45 and 55 °C).

to two reasons. At first, the polysaccharide was more soluble and thus its solubilization rate was higher than protein-like substances. On the other hand, temperature had a synergistical effect in sludge solubilization, the sludge lysis was enhanced by increasing the reaction temperature (Yu et al., 2014). In addition, it is well known that the enzymatic hydrolytic reactions are very dependent on temperature, and the enzymatic treatment could be improved by increasing reaction temperature (Sesay et al., 2006). Almost all cell reactions are affected by temperature, and high temperature may break the cell structure. This phenomenon could be explained according to the amended Arrhenius equation:

$$k = Ae(E_a/RT)^{\beta} \quad (4)$$

The reaction rate is always improved by 2–3 fold for every 10 °C rise in temperature (Yang et al., 2010). Moreover, proteins in EPS are actually extracellular enzymes which might enhance sludge

solubilization with increase of temperature. It should be mentioned that Guan et al. (2012) found that heating treatment had very limited influence on sludge EPS at the temperatures less than 55 °C, so the potential effects of temperature on sludge solubilization was marginal in the absence of enzymes addition.

As shown in Fig. 3, the curve of change in concentration of different EPS fractions reached the equilibrium after around 3 h of reaction. Again, the variation in EPS components can be fitted well with pseudo-first-order kinetic model, which was in agreement with the report of Yang et al. (2010). The reaction rates of SEPS, LB-EPS and TB-EPS were 0.146, 0.191 and -0.162 h^{-1} for protease and 0.306, 0.358 and -0.192 h^{-1} for α -amylase, thereby the production for LB-EPS was faster than that of SEPS. The process of enzymatic hydrolysis can be divided into three steps: (1) adsorption of hydrolytic enzymes on sludge surface; (2) diffusion of enzymes from out-layer to interior of sludge floc; (3) hydrolytic conversion of macromolecular substances (protein and polysaccharide). Feng

Fig. 4. Relationship between $\ln x$ and time during sludge hydrolysis.

et al. (2009) suggested that solubilization of particulate organic matters was the rate-limiting reaction of whole conversion from sludge particles to short-chain fatty acids. In the hydrolysis reaction stage, large protein was firstly decomposed into polypeptide and amino acids, which then converted into ammonium and organic acids; while the polysaccharide would be hydrolyzed into mono-saccharide and even reducing sugars (Ji and Brune, 2005). The decomposition of protein-like substances can be confirmed by the increase in ammonium concentration in sludge bulk. As depicted in Fig. S2 of SI, change in ammonia concentration in sludge supernatant showed a similar pattern with that of SEPS concentration, protein and polysaccharide. After 5 h of reaction, the ammonium concentration was increased from 23.24 mg L⁻¹ to 63.02 mg L⁻¹ for protease and 245.65 mg L⁻¹ for α -amylase treatment.

3-DEEM method is widely used for characterization of natural organic matters in various water bodies with the advantages of high sensitivity and selectivity (Henderson et al., 2009). According to the report of Sheng and Yu (2006), fluorescence intensity of EEM could be used to measure EPS content as dissolve organic carbon (DOC) content in samples was less than 10 mg L⁻¹. As depicted in Fig. S3 in SI, three dimensional fluorescence spectrum is divided into five major regions, which are soluble microbial byproducts (tryptophan-like protein), aromatic protein I, aromatic protein II, humic acid and fulvic acid respectively (Chen et al., 2003). Three dominant peaks were detected in all EPS fractions with the enzymatic hydrolysis: Peak A at excitation/emission (Ex/Em) of 275/335 (tryptophan-like protein), Peak B at Ex/Em of 225/310 (aromatic protein I) and Peak C at Ex/Em of 220/345 (aromatic protein II). Figs. S4 and S5 showed that fluorescent signals of all three peaks in SEPS and LB-EPS were intensified during reaction, while that of TB-EPS was weakened. For instance, the fluorescent intensities of Peaks A, B and C were increased from 122.5, 158.9 and 227.6 to 342.4, 542.1 and 604.5 respectively after amylase treatment (Fig. 5). This observation was consistent with Lowry-Folin analysis. At the

same time, no obvious change in fluorescence signals of humic and fulvic substances was detected, suggesting that enzymatic hydrolysis treatment had rather limited effect on these two substances.

3.3. Sludge solubilization with compound enzymes

Generally, most of biological enzymes are characterized by having a high degree of specificity, a specific enzyme is only able to catalyze for a defined substrate (Zhou et al., 2009). Meanwhile, protein and polysaccharide are the main components of sludge EPS in despite of its complexity (Roman et al., 2006). Therefore, the combined enzymatic hydrolysis (protease and amylase) was used to further enhance sludge solubilization efficiency. It can be seen from Fig. 6 (a) that the compound enzymes treatment performed better than single enzyme in sludge solubilization in terms of SEPS and LB-EPS. Furthermore, the addition mode had a significant influence on sludge lysis. α -amylase + protease was more effective in solubilization than other two addition modes (protease + α -amylase or simultaneous addition). This was because that the protein is the major component of α -amylase (Kurosawa et al., 2006), whose activity was very likely to be inhibited due to hydrolysis in presence of protease (Jung et al., 2002). Especially, the release of polysaccharide in protease + α -amylase treatment was much lower than other two addition modes and even single α -amylase treatment (see in Fig. 6(c)). Initially, the sludge EPS was solubilized with amylase addition, and then pre-dissolved substrates could make a more sufficient contact with protease, which improved whole sludge hydrolysis efficiency.

3.4. Effect of chemical re-flocculation on sludge properties

3.4.1. Effect of chemical re-flocculation on sludge dewatering performance

Sludge dewaterability can be reflected by filtration rate and cake solid content. According to the equation of SRF:

$$\frac{t}{V} = bV + a \quad (5)$$

Where b value is negatively linear with filtration rate. Bigger b value means lower filtration rate.

It was observed from Fig. 7 that sludge filtration rate was significantly decreased after enzymatic treatment. Our previous studies suggested that soluble EPS component was the major factor affecting sludge filtration behavior, and high soluble EPS content, especially protein, was detrimental to filtration rate (Zhang et al., 2014, 2015a, 2015b). As mentioned above, protein and polysaccharide in bound EPS was effectively solubilized and released into the sludge bulk under enzymatic hydrolysis, thereby the sludge treated with enzymes was more resistant to filtration (Mikkelsen and Keiding, 2002).

Addition of inorganic coagulants cannot only reconstruct floc structure through bridging, but remove the soluble EPS in sludge bulk, especially macromolecule organic compounds by adsorption process of hydrolyzed products (Niu et al., 2013). The

Table 3
Reaction rate of different EPS fractions under enzymatic hydrolysis.

Enzymes	Dynamic equation	Rate constants K_h (h ⁻¹)	Coefficients R^2
Protease (S-EPS)	$y = -0.146x - 0.014$	0.146	0.98
Protease (LB-EPS)	$y = -0.191x - 0.033$	0.191	0.99
Protease (TB-EPS)	$y = 0.162x + 0.010$	-0.162	0.96
α -amylase (S-EPS)	$y = -0.306x - 0.061$	0.306	0.91
α -amylase (LB-EPS)	$y = -0.358x - 0.090$	0.358	0.91
α -amylase (TB-EPS)	$y = 0.192x + 0.018$	-0.192	0.98

Fig. 5. Variation of fluorescent intensities in EEM contours under enzymatic hydrolysis with reaction time: (a) SEPS-protease; (b) SEPS-amylase; (c) LB-EPS-protease; (d) LB-EPS-amylase; (e) TB-EPS-protease; (f) TB-EPS-amylase (Dosage of enzymes = 150 mg/g; hydrolysis temperature of protease and amylase were 45 and 55 °C; EPS samples were diluted by 200 times).

macromolecule biopolymers were believed to be the major factor affecting sludge filtration behavior (Facchini et al., 2014). For the sludge treated with enzymes, both of filtration rate and cake solid content were improved with increasing dosage of inorganic salt coagulants. The optimal dosage of FeCl_3 and PACI was 15% (g/gTSS), and the b value was reduced from 1.78 to 0.17 for PACI and 0.1 for FeCl_3 . At the same time, sludge filtration property was very near to the sludge without enzymatic pretreatment, while the cake moisture reduction was favored after addition of enzymes. The cake moisture content was 82.6% for PACI and 81.5% for FeCl_3 under enzymatic treatment, which was lower than that without addition of enzymes.

It was noted that FeCl_3 performed better than PACI in improving sludge filterability and cake solid content. Since the floc strength

played an important role in sludge filtration dewatering process, high floc strength was always favored to filtration. According to report of Niu et al. (2013), the ferric ions exhibited higher affinity to EPS (especially protein-like substances), hence the sludge floc conditioned with ferric ions was denser than that of formed from PACI conditioning, resulting in a higher filtration rate.

3.4.2. Impact of chemical re-flocculation on sludge floc morphology

Fig. 8 presented that for the original sludge, sludge floc size was increased under chemical flocculation, and reached equilibrium of 134.33 μm and 97.97 μm when dosage of PACI and FeCl_3 were 5% and 10%. For the sludge after enzymatic treatment, the floc increased to 103.18 μm and 59.66 μm when dosage of both PACI and FeCl_3 were 20%. By comparison, the floc formed from PACI

Fig. 6. Addition mode of two enzymes on sludge solubilization efficiency: (a) total EPS content; (b) protein; (c) polysaccharide (The dosage of protease and α -amylase was 150 mg/g).

coagulation was larger than that of FeCl_3 at low coagulants dosage, while opposite trend was observed at high dose. PACI is typical inorganic polymer flocculants, it had more abundant binding sites with sludge particles and thereby producing larger flocs. However, the PACI generally was more positively charged, so overdosed PACI

Fig. 7. Effect of coagulants dosage on sludge (a) b value (filtration rate); (b) cake moisture content (The dosage of protease and α -amylase was 150 mg/g).

was more likely to result in colloidal re-stabilization and prevent further aggregation of sludge particles. It should be noted that coagulated sludge floc was smaller after enzymatic pretreatment. It was very likely that organic matters with high molecular weight (MW) in sludge EPS was converted into smaller organic compounds, and alleviated the electrostatic and hydrophobic interactions between hydrolyzed products of coagulants and EPS (Lee and Liu, 2001). As shown in Fig. S7, through enzymatic treatment (compound or single), original sludge floc was destroyed, and the particle became smaller, which was in agreement with the floc size analysis. Afterward, sludge floc grew and aggregated after addition of inorganic coagulants, and edge of sludge flocs were more uniform, revealing that more compact floc formed after chemical coagulation.

3.4.3. Influence of chemical flocculation on SEPS

As shown in Fig. 9 (a), the dosage for inorganic coagulants were 5% and 20%, the soluble COD (SCOD) content reached the minimum for original and composite enzyme treated sludge respectively. The SCOD concentration was increased with further increasing dosage of inorganic coagulants. This result could be attributed to re-stabilization of colloidal system in the presence of overdosed coagulants. It is worthy to note that since the charge density of PACI was higher than FeCl_3 , the phenomenon was more apparent for

Fig. 8. Impact of coagulants dosage on sludge particle size ($d_{0.5}$) (The dosage of protease and α -amylase was 150 mg/g).

PACl. Fig. 9 (b) clearly presented that FeCl_3 was more effective in removing tryptophan- and aromatic-proteins than PACl. This is consistent with the report of Yu et al. (2008), who also stated that ferric ions exhibited higher affinity to protein-like substances. In

Fig. 9. Variation in chemical characteristics of SEPS chemical re-flocculation with different inorganic coagulants: (a) SEPS content; (b) fluorescent intensities.

our previous studies, it was found that protein-like substances were the deciding factor of sludge filterability (Zhang et al., 2015b). Thus, this is another reason for higher performance of ferric iron in sludge dewaterability improvement.

4. Conclusion

This study attempted to use the enzymatic pretreatment to improve drying performance in sludge filtration dewatering process.

- Sludge filtration performance was deteriorated due to release of a large amount of biopolymers after enzymatic treatment. α -amylase exhibited better performance than protease in sludge solubilization. Moreover, EPS was more extractable after enzymatic treatment, producing looser floc structure.
- The change in EPS components can be fitted well with pseudo-first-order kinetic model. The reaction rates of SEPS, LB-EPS and TB-EPS were 0.146, 0.191 and -0.162 h^{-1} for protease and 0.306, 0.358 and -0.192 h^{-1} for α -amylase.
- The feeding modes of enzymes had a significant influence on sludge lysis efficiency under compound enzymes treatment. α -amylase + protease was more effective in solubilization than other two addition modes (protease + α -amylase or simultaneous addition).
- Both of filtration rate and cake solid content were improved with increasing dosage of inorganic salt coagulants. The optimal dosage of FeCl_3 and PACl was 15% (g/gTSS), the cake moisture reduction was improved after enzymatic pretreatment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2015.06.026>.

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