Improvement of sludge dewaterability by energy uncoupling combined with chemical re-flocculation: Reconstruction of floc, distribution of extracellular polymeric substances, and structure change of proteins

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Abstract

This study innovatively combines energy uncoupling and chemical re-flocculation helped to accelerate residual sludge dewatering. Ferric chloride ($FeCl_3$) and 3, 3', 4', 5-tetrachlor-osalicylanilide (TCS) were employed as the flocculant and uncoupler, respectively. The results showed that the specific resistance to filtration (SRF) and the water content of sludge filtered cake fell dramatically from 11×10^{12} m/kg and 80.2% to 1.1×10^{12} m/kg and 77.1% respectively, when the addition of TCS ranged from 0 to 0.12 g/g VSS with flocculation conditioning. The distribution of sludge extracellular polymeric substance (EPS) was altered radically after adding TCS, leading to the collapse and fragmentation of EPS, causing the reduction and formation of fragmentized sludge flocs. Meanwhile, the stretching and deformation vibrations of C=O and N-H bonds suggested the strong attack between TCS and EPS proteins, while variations of the main secondary structures of protein (i.e. α-helix, β-sheet and random coil) indicated the loose structure of proteins and enhanced hydrophobicity.

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Consequently, the cracked and loose structure of residual sludge resulted in the release of bound water. After TCS addition combined with chemical re-flocculation, the channels of sludge water discharge were widened, guaranteeing the discharge of sludge water. Therefore, the sludge dewaterability was elevated under the energy uncoupling combined with chemical re-flocculation. As well, the application of TCS would not destroy sludge cells, in which bioenergy (sludge carbon source) could be retained and effectively utilized in the subsequent disposal process. The findings reported here not only widen our perception of the energy uncoupling technology, but also encourage researchers to explore both effective and economic methods on the basis of energy uncoupling, aiming to achieve high-efficiency of reduction and dewatering in the future.

Key words: Sludge dewatering; Energy uncoupling; Extracellular polymeric substance; Bound water; Secondary structure of protein

1. Introduction

Sewage sludge is an inevitable by-product of biological wastewater treatment and contains various toxic substances, such as pathogens, heavy metals, and organic contaminants, which may cause serious pollution risks, if not disposed of properly (Wu et al., 2020). It is estimated that 60 million tons of wet sludge is produced per year in China, and needs extensive treatment facilities for proper handling (Maryam et al., 2021). Simultaneously reducing the biomass as well as the water from sludge is an essential step to diminish the volume of sludge, which can alleviate the pressure of the subsequent treatment and disposal. However, sludge is difficult to be dewatered directly due to the highly hydrated colloidal structures of microbial aggregates (Christensen et al., 2015).

Extracellular polymeric substance (EPS) including proteins and polysaccharides, is a type of complex polymer mainly produced by organic matters in the medium, the release of cellular products by cell lysis or different biochemicals secreted by microorganisms during

the dissolving and absorbing of organic components, and accounts for 70-80% of total biomass (More et al., 2014). Notably, proteinaceous components were closely related to sludge filterability, and the removal of proteins has a strong and positive correlation with the increased sludge filterability (Xiao et al., 2016; Xiao et al., 2017). Specifically, there is strong correlation between the surface hydrophilicity and spatial structure of proteins. The tight structure of protein has high water-holding capacity with a high ratio of α-helix and (β-Sheet + Random coil) (Zhang et al., 2019). Therefore, a decrease in the α-helix / (β-Sheet + Random coil) percentage in the secondary structure of the extracellular proteins is the key to eliminating the inhibitory effects of extracellular proteins on the bound water of the sludge (Wu et al., 2017).

In recent years, decreasing EPS content or destroying EPS structure has become the main means to improve sludge dewaterability. Using various oxidation methods which have strong oxidation capacity to disintegrate EPS therefore has garnered more scientific attention. Electrooxidation, the electro-Fenton process, molecular oxygen activation via zero valent iron (ZVI) and Fe (III) flocculation, has synergetic effects on the degradation of EPS, which enhances sludge dewaterability (Hu et al., 2018). In one study, a method of permanganate/bisulfate, and Mn (III) oxidation led to the cell disruption and EPS disintegration, releasing partial bound water (Guo et al., 2017). Conversely, free radicals such as hydroxyl and sulfate radicals could oxidize and penetrate the cell membrane, leading to the release of intracellular organic matter. Intracellular organics also have strong hydrophilicity, which could capture the cell entrapped in the network floc structure through hydrogen bonds combining with water molecules, and limit the dewatering process again (Yu et al., 2018; Zhen et al., 2013).

Apart from advanced oxidation process, energy uncoupling technology is very promising in reducing sludge EPS. Jiang and Liu (2012) first demonstrated that a chemical

uncoupler, 3, 3',4',5-tetrachlorosalicylanilide (TCS) inhibited adenosine triphosphate (ATP) synthesis, leading to significant reduction of acyl-homoserine lactones (AHLs) and EPSs production, which in turn prevented aerobic granulation. Nitrophenol compounds (metabolic uncouplers) could reduce the bound EPS of the mixed sludge, thus alleviated membrane fouling in a membrane bioreactor system (Liang and Hu, 2012). Our previous studies also pointed out that optimal dosage of TCS increased the stable flux of a gravity-driven membrane system, due to the control of the increase of sludge EPS (Ding et al., 2020). Additionally, it can inhibit sludge yield and reduce the biomass efficiently. It is worth noting that the sludge bacteria cell was not destroyed, because the intracellular ATP was not released (Lin et al., 2022). This is very important because the intracellular organic matter will not be released for reducing EPS compared with the advanced oxidation process (AOP) method, avoiding the hydrophilicity of intracellular organics to worsen sludge dewatering process again. Notably, uncouplers, as chemical reagents, are typically toxic. However, the toxicity could be eliminated quite rapidly by activated sludge (Gatidou et al., 2021).

Chemical flocculation is a traditional method of sludge conditioning to promote the aggregation of sludge flocs and further increase the size of sludge particles. Additionally, the method widens the channels of water outflow in sludge dewatering process, enhancing the capacity of sludge water discharge and improving sludge dewaterability (Wei et al., 2018). However, the increment of hydrophilicity limited the efficiency of using chemical flocculation alone on sludge dewatering process (Wu et al., 2020). Accordingly, nowadays, chemical flocculation is used as the re-flocculation process combines with other effective methods (which could decrease the negative effects of sludge EPS and hydrophilicity on sludge dewatering (i.e. advanced oxidation methods)) to further improve sludge dewaterability (Ai et al., 2021; Li et al., 2020b). Ferric chloride ($FeCl_3$), as a type of coagulants, which is used widely in the sludge dewatering process, could achieve the low

moisture content of sludge cake because of their high charge density that enabled the efficient compression of the double electric layer and skeleton builders of the hydrolysis products (Chen et al., 2016). Energy uncoupling has a similar effect on the degradation of EPS compared with oxidation methods, but the studies on energy uncoupling combines chemical re-flocculation are lacking.

As mentioned above, energy uncoupling can efficiently inhibit the production of sludge EPS. So, it may play a positive role in the sludge dewatering process. However, only a very few studies on it have been published and there is a need to ascertain: (i) how does the morphology and structure of sludge flocs change by adding uncouplers, as well as by adding subsequent flocculation conditioning; (ii) what is the distribution of sludge EPS (including tightly bound EPS, loosely bound EPS, and dissolved EPS) influenced by uncoupler, which can inform us about the transformation of sludge moisture; and (iii) how are the structure and functional groups of EPS protein changed by uncouplers. Knowing this can help us to further understand the mechanism of energy uncoupling on sludge dewaterability.

2. Materials and methods

2.1 Experimental setup

The sample of secondary sludge in this study was obtained from Wenchang sewage treatment plant in Harbin. The characteristics of the sludge sample were measured and calculated immediately after the sludge was transported to the laboratory. The calculated characteristics of the sludge sample were summarized in Table 1.

Experiments of sludge mixing process were achieved by using a six-link electric stirrer (MY3000-6G, MeiYu, China) and the temperature was controlled at 25 ℃ during mixing process. Five beakers were put into a group and the volume of each beaker was 1.0 L. During the experiment, deionized water was added to dilute the mixed liquor suspended solids (MLSS) concentration of sludge sample to 8000 mg/L and the concentration of dissolve

oxygen (DO) was measured about 0.4mg/L. The final working volume of each sludge sample was 500 mL. In the sludge dewatering experiment without flocculation conditioning, different concentrations of TCS (0, 0.03, 0.06, 0.09, 0.12 g/g VSS) were dosed to the beakers. The mixing time was 8 h with the rate of 100 rpm to ensure the dissolution of TCS. In the sludge dewatering experiment with flocculation conditioning, the difference was that 0.05 g/g VSS $FeCl_3$ was added to the beakers after TCS addition with 8 h mixing. After adding $FeCl_3$, the sludge samples were stirred at 200 rpm for 1 min to make sure the $FeCl_3$ was quickly dissolved. Then the speed was adjusted to 100 rpm for stirring for 4 min to guarantee the fully react between $FeCl_3$ and residual sludge. All tests were conducted in triplicates.

Table 1.

2.2 Analytical methods

2.2.1 SRF test

Specific resistance to filtration (SRF) was measured by a modified filter set-up (See Fig S1 in the Supporting Information (SI)) using a quantitative filter paper. The detailed calculated method can be found in that section as well. The gravimetric method was employed to measure the water content of the filtered cake which can be calculated using Eq. (1):

$$\mathrm{WC} = \frac{m_1 - m_2}{m_1 - m_0} \times 100\% \quad (1)$$

where: WC is the water content of the filtered cake (%), m_0 is the weight of the weighing vessels (g), m_1 is the weight of wet filtered cake and weighing vessels, (g), and m_2 is the weight of oven-dry filtered cake and weighing vessels (g). Every sample was measured three times and the average was obtained.

2.2.2 Proportions of free and bound water measurements

Bound water was quantified by using dilatometer with xylene as the indicator (Huo et al., 2014). The calculation process of the contents of free and bound water was done

according to the method used by Guo et al. (2019). In our study, the relationships between free water and bound water proportions were mainly explored, so that the proportion of free water is calculated through Eq. (2):

$$\omega = \frac{V_{free}}{m \times (1-C)} \quad (2)$$

where: ω is the proportion of free water, V_{free} is the calculated volume of free water (mL), m is the amount of sludge sample (g), and C is the solid content of sludge (%). Hence, the proportion of bound water can be calculated by (1-C). All samples were measured three times and the average was obtained.

2.2.3 EPS extraction

The thermal method was used for EPS extraction (Li and Yang, 2007). The 40 mL sludge samples were added to the 50 mL centrifugal tube and subsequently centrifuged for 15 min under 4000 rpm. The soluble EPS (S-EPS) was collected after filtering the soluble organic matter contained in the supernatant with the 0.45 μm micro-filtration membrane. After extracting S-EPS, the deionized water served to suspend the residual sludge samples to the volume of 50 mL, then centrifuged at 10,000 rpm for 15 min. The loosely bound EPS (LB-EPS) could be collected from the supernatant with organic substances. The sludge samples from the bottom of the centrifugal tube were resuspended to the volume of 50 mL and then shaken up in a water bath shaking table in the following scenario: 10 rpm, 80 ℃ for 30 min. After 30 min shaking, the sludge samples were centrifuged at 4000 rpm for 15 min. The organic materials in the supernatant were collected as tightly bound-EPS (TB-EPS).

After extracting EPS, the concentrations of protein, polysaccharide and total organic carbon (TOC) were quantified in S-EPS, LB-EPS and TB-EPS. The EPS concentration was the sum of S-EPS, LB-EPS and TB-EPS concentrations. The rapid Lowry method (Shanghai, China) was helped to detect the concentration of protein with bovine serum albumin (BSA) as the standard. Polysaccharide concentration was measured using the phenol-sulfuric acid

method with glucose being the standard substance (DuBois et al., 1956). The TOC concentrations were measured by an automatic TOC analyzer (multi N/C 2100, Jena, Germany). Every sample was measured three times and the average was obtained.

2.2.4 EEM analyses

Three-dimensional fluorescence excitation-emission matrix (EEM) spectra were generated to characterize S-EPS, LB-EPS and TB-EPS of the mixed liquor sludge by fluorescence spectrophotometer (F7000, Hitachi, Japan). The detailed method was based on the work done by Meng et al. (2011) and 3D EEM spectra were divided into four regions according to Table S1. Excitation and emission spectra were scanned from 220 to 450 nm in 5 nm increments and from 250 to 550 nm in 1 nm increments respectively.

2.2.5 SEM analyses

Sludge samples were oven-dried before scanning electron microscope (SEM) analyses. The surface morphology of residual sludge was acquired by a scanning electron microscope (SEM, SIGMA 500, Zeiss, Germany) with energy dispersive spectroscopy (EDS).

2.2.6 FTIR analyses

The functional groups of EPS were conducted by fourier transform infrared spectroscopy (FTIR) (Spectrum One PerkinElmer, USA), after EPS samples were oven-dried. The FTIR spectra were obtained over a frequency range from 4000 to 500 cm^{-1} and the resolution was set at 2 cm^{-1}. To further analyze the extracted secondary structures of EPS proteins, the amide I region of EPS at 1700-1600 cm^{-1} was analyzed. Peakfit software (Version 4.04, Software Inc., USA) functioned to resolve the overlapped peaks with the minimum residual.

2.2.7 Zeta potential analyses

Zeta potential was measured on Zeta sizer Nano-Z Instrument (Malvern, UK), and each sample was analyzed three times and the average was obtained.

2.2.8 Particle size analyses

Particle size is an important factor which influences how well sludge dewatering performs, expressing the form of sludge aggregation or sludge dispersion. The particle size was examined by the laser particle analyzer (Mastersizer 2000, Pennsylvania-based firm, UK). Each sample was measured at room temperature three times and the average was obtained.

2.2.9 The analysis of ATP

The ATP content of sludge samples was measured by BacTiter-Glo (Promega Corporation) method. The detailed measurement process could be found in the research of Hammes et al. (2010). Total ATP was measured directly by using the sludge samples, while extracellular ATP was measured after filtering the sample through using 0.45 μm membrane. The intracellular ATP was the difference between the total ATP and the extracellular ATP for each sample (Nescerecka et al., 2016). All ATP measurements were recorded by a microplate reader (SpectraMax M5, Molecular Devices, San Jose, CA). Each sample was made in triplicate.

2.2.10 Statistical analysis

All results of experiments were expressed as mean ± standard deviation. The error bars of all figures represent the standard deviation. The software used to process the data and graph was Origin 2018 (Version 9.5, Microcal Inc., USA).

3. Results

3.1 Effects of TCS addition on residual sludge bioactivity

The microbial bioactivity of sludge could be represented by ATP content (Zheng et al., 2019). ATP contents of each sample were measured immediately after 8 h mixing by TCS in this study. As shown in Figs. 1(a) and (b), after an increment of TCS addition from 0 to 0.12 g/g VSS, both total ATP content and extracellular ATP content of residual sludge decreased.

Total ATP content decreased from 36.5 to 12.8 μmol/g VSS and extracellular ATP content declined from 0.18 to 0.04 μmol/g VSS. Clearly, the addition of TCS significantly restrained the bioactivity of residual sludge. Similarly, in the study of Ferrer-Polonio et al. (2017), the synthesis of ATP was restrained obviously under the long-term (45 days) reaction conditions after adding TCS, leading to the breakup of sludge in the sequencing batch reactor (SBR) system. In our study, we explored the effects of energy uncoupling on residual sludge under the short-term (8 h) reaction conditions and the good performance was indicated by the better restriction of ATP content. The ratio of extracellular ATP to total ATP was shown in Table 2. It was obvious that the ratio was little difference between the treated samples and the control, with a maximum difference of less than 1%. Hence, it was proven that the effects of TCS addition on residual sludge bioactivity would not destroy sludge bacteria cells. Feng et al. (2013) also confirmed that the uncouplers TCS and 2,4,6-trichlorophenol (TCP) had no significant effect on the release of intracellular substances in sequential batch reactor.

Fig. 1.

Table 2.

3.2 Effects of TCS addition on sludge dewaterability (with and without chemical re-flocculation)

3.2.1 SRF analyses

As shown in Fig. 2(a), SRF rose from 45.3×10^{12} m/kg to 56.4×10^{12} m/kg with the dosage of TCS ranging from 0 to 0.12 g/g VSS without flocculation. Adding TCS significantly enhanced the resistance of sludge filtering, hence remarkably worsening sludge dewatering. The highest SRF increased by about 24.5% compared to the control. After flocculation, SRF diminished from 11×10^{12} m/kg to 1.1×10^{12} m/kg, in sync with adding TCS from 0 to 0.12 g/g VSS. The SRF of the control was approximately ten times higher than the sample conditioned by 0.12 g TCS/g VSS and $FeCl_3$. The variation trend was different from the experimental

group of no-flocculation. The reason was the promotion of TCS addition on the degradation of EPS and the transformation of bound water into free water, enhancing the content of easily removed water to discharge (See Section 4.1 and 4.3). Evidence shows that flocculation does greatly influence the decline of SRF. Above results clearly showed that adding TCS combined with flocculation markedly strengthened sludge dewaterability, which conversely was deteriorated by single TCS addition. Notably, the sludge dewatering showed the apparent advantage under the addition of TCS combined the conditioning of chemical re-flocculation compared with the flocculation conditioning alone.

3.2.2 Water content of the filtered cake

The water content of filtered sludge cake directly reflects sludge dewaterability. To realize less expensive subsequent disposal of sludge, low water content in the filtered sludge cake was urgently expected. As shown in Fig. 2(b), the water content of the filtered cake visibly expanded after the addition of TCS. The highest value of the water content was 86.6% under the TCS concentration of 0.03 g/g VSS, revealing a disadvantageous sludge dewaterability enhancement scenario. Meanwhile, the variation of the water content in the filtered cake was not obvious, even slightly decreased, despite the increased dosage of TCS from 0.03 g/g VSS to 0.12 g/g VSS. After the flocculation process, the opposite appeared, that is, the water content of sludge filtered cake diminished from 80.2% to 77.1% with the dosage of TCS increasing from 0 g/g VSS to 0.12 g/g VSS. The lowest value of the water content of the sludge filtered cake was observed with a TCS concentration of 0.12 g/g VSS combined the conditioning of $FeCl_3$. The flocculation conditioning altered the adverse effect of TCS addition and enhanced the sludge dewatering process. Accordingly, the addition of TCS promoted the improvement of dewaterability under the flocculation conditioning alone.

3.2.3 The percentage of bound water

Generally speaking, sludge consists of free water and bound water. It is easier to remove

free water from sludge than bound water (Vaxelaire and Cézac, 2004). It is crucial to reduce the proportion of bound water to achieve a large amount of drainable water and further enhance sludge dewaterability (Cai et al., 2018; Guo et al., 2017).

Fig. 2(c) shows the effects of TCS addition on the distribution of free and bound water. The proportion of bound water abated under the addition of TCS, with the lowest value of 0.232 under the TCS dosage of 0.09 g/g VSS. Simultaneously, the proportion of free water raised to the highest value of 0.768. Hence, adding TCS could promote the conversion of bound water into free water. Similarly, the fraction of bound water clearly declined under the method of flocculating after TCS was added. The value decreased from 0.237 to 0.197 with the concentration of TCS increasing from 0 g/g VSS to 0.09 g/g VSS. There was a slight growth in the proportion of bound water when the TCS concentration was 0.12 g/ VSS. In other words, the addition of TCS varied the distribution of bound water and free water, increasing the percentage of free water which is easier to remove. The flocculation process further intensified the conversion capacity of bound water to free water.

Fig. 2.

3.3 Effects of TCS on physicochemical properties of sludge floc (with and without chemical re-flocculation)

3.3.1 Particle size distribution

Fig. 3 displays the variations of particle size distribution of residual sludge under different treatments. Compared with the control, TCS addition signally reduced the average particle size of residual sludge. The average particle size of the control was 137.6 μm, and decreased to 70.9 μm under the TCS addition of 0.12 g/g VSS. The significant fall in particle size attributed to the addition of TCS. After flocculation, the average particle size of the control was 160.4 μm, which increased to 209.6 μm under the TCS dosage of 0.12 g/g VSS. These results indicated that TCS damaged the structure of sludge flocs, leading to a shrinkage

in particle size. As well, under the same flocculation conditions, a high dosage of TCS encouraged the enlargement of sludge flocs size.

Fig. 3.

3.3.2 Zeta potential

Zeta potential is essential in reflecting the aggregating or dispersing of sludge. It can be seen that the zeta potential of the control was -11.8 mV (Fig. 4). After an increment of TCS addition from 0.00 g/g VSS to 0.12 g/g VSS, the zeta potential decreased from -11.8 mV to -16.2 mV. The decrement of zeta potential could be explained by the increase of electrostatic repulsion among sludge flocs caused by the degradation of EPS which was electronegative (More et al., 2014). However, an opposite tendency was observed after flocculation conditioning (Fig. 4). The zeta potential heightened from -4.2 mV to -0.5 mV after flocculation. Therefore, the low absolute value of zeta potential was accomplished with the addition of TCS combined with chemical re-flocculation, compared to flocculation conditioning alone.

Fig. 4.

3.3.3 Morphology of sludge flocs

The morphology of residual sludge flocs was investigated through SEM. The distinctions in microtopography can be seen in Fig. 5. The highly smooth surface of raw sludge (i.e. the control) exhibited a complete and compact floc surface without large openings (Fig. 5(a)), which impeded the inner contained water from discharging. However, the flocs of residual sludge evolved into sparse and porous state after uncoupler TCS addition (Fig. 5(b)). A loose and block-shaped structure of sludge flocs with obvious cracks and pores was formed on the surface of treated residual sludge, leading to the formation of larger surface porosity (Table 3). The volumes of sludge flocs after flocculation conditioning were larger than the samples treated with/without uncoupler and without flocculation (Figs. 5(c) and 5(d)). These

results agree with the conclusions reached on average particle size in Section 3.3.1. Furthermore, the sizable volume of sludge flocs was recorded under the addition of TCS and flocculation conditioning. The reason for the formation of large sludge flocs can be explained by the mechanisms of charge neutralization and adsorption bridging of coagulant (Yan et al., 2013).

Fig. 5.

Table 3.

3.4 Effects of TCS addition on EPS distribution (with and without chemical re-flocculation)

It is well known that EPS consist of diverse polymers, such as proteins, polysaccharides, lipids, humic acids, etc. (Li and Yang, 2007). EPS are usually divided into three categories according to the structure and physical state: soluble EPS (S-EPS), loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Yuan et al., 2021). The existence of EPS triggers changes in the physicochemical and biological properties of activated sludge, which therefore impacted the sludge dewaterability (Basuvaraj et al., 2015; Wei et al., 2018). Hence, to understand the mechanism of TCS addition on enhancing sludge dewaterability, analyses of EPS were indispensable.

Fig. 6.

Fig. 6 displays the effect of TCS addition with/without flocculation on the variations of TOC in S-EPS, LB-EPS and TB-EPS. The TOC concentrations of both S-EPS and LB-EPS increased by adding TCS with/without flocculation. Compared to the variations of S-EPS and LB-EPS, TOC concentrations of TB-EPS decreased prominently after TCS addition. Additionally, the concentrations of TB-EPS under the coupled conditioning of TCS and flocculation were smaller than that under the single TCS addition.

As the main components of EPS, variety of proteins and polysaccharides concentrations

were investigated in our study. The proteins and polysaccharides concentrations of S-EPS and LB-EPS increased observably with the elevated dosage of TCS with/without flocculation. S-EPS proteins and polysaccharide increased from 1.7 mg/g VSS to 3.9 mg/g VSS and 1.8 mg/g VSS to 3.3 mg/g VSS, respectively, with TCS addition increasing from 0.00 g/g VSS to 0.12 g/g VSS. Similar phenomena were observed on the variations in S-EPS proteins and polysaccharides with flocculation and LB-EPS proteins and polysaccharides with/without flocculation (Figs. 6(b), 6(d) and 6(e)). Notably, the proteins and polysaccharides concentrations of S-EPS and LB-EPS decreased significantly after flocculation conditioning compared to the single TCS treatment (Figs 6(a), 6(b), 6(d) and 6(e)). These results could be ascribed to the adsorption of flocculants. Compared to the variations of S-EPS and LB-EPS, the proteins and polysaccharides concentrations of TB-EPS significantly diminished with increased TCS concentration.

After an increment of TCS addition from 0 g/g VSS to 0.12 g/g VSS, the concentrations of TB-EPS proteins and polysaccharides respectively decreased from 24.6 mg/g VSS to 14.4 mg/g VSS and 13.3 mg/g VSS to 8.4 mg/g VSS for conditioning without flocculation, and respectively decreased from 22.7 mg/g VSS to 12.7 mg/g VSS and 12.7 mg/g VSS to 6.6 mg/g VSS for conditioning with flocculation. These results suggested that TCS addition with flocculation conditioning could efficiently diminish the proteins and polysaccharides concentrations of TB-EPS. The total EPS contents refer to the sum of the proteins and polysaccharides concentrations of S-EPS, LB-EPS and TB-EPS. The EPS contents (i.e. EPS proteins (EPS_{pr}) and EPS polysaccharides (EPS_{ps})) clearly declined with increased TCS addition, and the flocculation process reduced the contents of EPS (Fig. 6(g)). Compared with chemical flocculation conditioning alone, the addition of TCS promoted the decrease of EPS. The highest value of the decrement was approximately 26% with the dosage of 0.09 g TCS/g VSS. The subsequent increase of EPS under the addition of 0.12 g TCS/g VSS indicated that

the further reduction of EPS would be limited under too high dosage of TCS, and reasonably selecting the optimal dosage was important. Reductions of EPS_{pr} and EPS_{ps} mean that sludge possesses minimal stickiness and high flocculating ability (Guo et al., 2017). Therefore, small amounts of EPS are favorable to improving sludge dewaterability.

Fig. 7.

To profoundly explore the effects of TCS addition on EPS distribution, EEM was applied to analyze the changes in aromatic proteins, tryptophan proteins, fulvic acid-like and humic acid-like substances contained in EPS. There are two main peaks in all samples (i.e. Peak A and Peak B), and Peak C and Peak D appeared in the samples conditioned by high dosage of TCS. It can be seen in Fig. 7, Fig. S3 (SI) and Table S2 (SI) that fluorescence intensities of Peak A and Peak B in S-EPS and LB-EPS increased with raised TCS dosage, while fluorescence intensities of Peak A and Peak B in TB-EPS decreased, which were consistent with the data above. The appearance of Peak C and Peak D suggested that adding TCS could promote the transformation of macromolecular organic matter (i.e. aromatic proteins and tryptophan proteins) into small molecular weight organic acids (i.e. fulvic acid-like and humic acid-like substances). Remarkably, the abatement of macromolecular organic matter facilitated the increment of relative hydrophobicity and the decrement of bound water content, while enhancing flocculation ability. Moreover, after flocculation, Peak C and Peak D disappeared in TB-EPS under the TCS dosage of 0.12 g/g VSS, indicating that small molecular weight organic matter was adsorbed and masked by flocculation conditioning.

3.5 Characterization of EPS protein

Except for the decrease of EPS content, the variation of EPS structure also determines the sludge dewatering, especially the protein structure of EPS (Li et al., 2020a; Yuan et al., 2021). The intrinsic connection between TCS addition and the structure of EPS protein, functional groups and secondary structures of protein were analyzed in this section.

3.5.1 Functional groups

FTIR was employed to generate information on variations of functional groups of EPS protein. EPS fractions consist of numerous functional groups. Notably, the stretching and deformation vibrations of C=O bond and N-H bond were related to protein (Fang et al., 2019; Li et al., 2020b). It is noted that the shape and intensity of several characteristic peaks changed after adding TCS. The narrow band at 3316 cm^{-1} dilated at 3300 cm^{-1} under the addition of 0.12 g TCS/g VSS without flocculation (Fig. 8(a)). Meanwhile, the band became relatively narrow at 3304 cm^{-1} after flocculation. In addition, the intensity of peak at 3315 cm^{-1} declined after adding TCS and then increased after flocculation. These results stated that the bond of N-H was stretched and the content of N-H bond decreased. The same changes of the intensity and stretching in C=O bond were discovered at 1655 cm^{-1} and 1386 cm^{-1}. These phenomena above illustrated that the functional groups (i.e. C=O bond and N-H bond) could be affected by TCS, motivating the reduction of EPS protein.

3.5.2 Secondary structures

Secondary structure of EPS protein generally sorted to seven types, and the main types are aggregated strands, β-sheet, random coil, α-helix, 3-turn helix and antiparalle β-sheet/aggregated strands (Fang et al., 2019). To get more information about the secondary structure, the band of amide I from 1700 cm^{-1} to 1600 cm^{-1} was characterized in FTIR spectra. As shown in Figs. 8(b), (c), (d) and (e), the peaks of the above seven types were separated under the wavelength of 1700 cm^{-1} to 1600 cm^{-1}, and the values of peak areas and the proportions of areas were calculated in Table 4. Results confirmed that adding TCS reduced the total areas of peaks, which increased after flocculation conditioning. The cutback in the total areas of peaks indicated the effective reduction of protein content. According to earlier studies, α-helix remains quite stable, owing to the formation of a hydrogen bond, while β-sheet is complex, and random coil is equipped with irregular characteristics (Wu et al., 2017;

Yin et al., 2015).

Hence, high content of α-helix and low content of β-sheet and random coil could lead to the tight structure of protein. Additionally, the low value of the ratio of α-helix and (β-Sheet + Random coil) strongly suggested that the loose structure of protein molecule promoted more hydrophobicity in EPS protein (Li et al., 2020b; Zhang et al., 2019). As shown in Table 4, the proportion of α-helix, β-sheet and random coil increased from 14.61%, 12.69% and 13.89% to 16.28%, 20.97% and 16.26% respectively, under the addition of 0.12 g TCS/g VSS. Conspicuously, there were no evident changes in the percentages of α-helix, β-sheet and random coil under combined conditioning of TCS and flocculation, compared to the TCS addition alone. Meanwhile, the value of α-helix / (β-Sheet + Random coil) decreased from 0.55 to 0.44 by adding 0.12 g TCS/g VSS, but increased to 0.53 after flocculation. It was noted that the value of α-helix / (β-Sheet + Random coil) increased from 0.55 to 0.57 under single flocculation conditioning. These results demonstrated that adding TCS could provoke the formation of loose structure in the EPS protein and sludge flocs. On the other hand, flocculation could promote the combination of EPS protein and produce a tight protein structure.

Fig. 8.

Table 4.

4 Discussion

4.1 The roles and potentials of energy uncoupling on sludge conditioning

As demonstrated in previous studies, small amounts of EPS, low percentage of bound water and high hydrophobicity of sludge are indispensable if sludge dewaterability is function efficiently (Li et al., 2020b; Yu et al., 2016). Numerous studies have verified that putting TCS in the sludge strongly affected the content of EPS, percentage of bound water and hydrophobicity of sludge, so there is great potential in subsequent sludge conditioning.

Firstly, TCS effectively increased the hydrophobicity of EPS protein. The hydrophilicity of EPS protein leads to strong bonds between water and protein, which can be detrimental to effective discharge of sludge water (Li et al., 2020b; Wei et al., 2018). In more depth, TCS prominently affected the forms and contents of the corresponding functional groups (i.e. C=O and N-H) of EPS protein (Fig. 8). It also changed the composition ratio of the related secondary structure. The increased proportions of α-helix, β-sheet and random coil, as well as the decreased value of α-helix / (β-Sheet + Random coil) under the addition of TCS indicated that the loose structure of protein was fulfilled. Furthermore, the hydrophobicity of the protein was improved (see Section 3.5.2).

Secondly, the content of EPS was significantly reduced by adding TCS. Sludge EPS is a key factor that restricts the amelioration of sludge dewaterability. The high concentrations of EPS would hold lots of bound water (which is difficult to remove), inducing poor efficiency of sludge dewatering (Ai et al., 2021; Chen et al., 2021). Interestingly, energy uncoupling efficaciously reduced the content of macromolecular organic matters (i.e. protein and polysaccharide) of EPS during residual sludge reduction. TCS successfully converted the macromolecular organic matter into small molecular organic matter (i.e. fulvic acid-like and humic acid-like substances), and ultimately worsened the water-holding capacity of EPS.

Thirdly, TCS addition indeed restructured the distribution of free and bound water, and proliferated the amounts of free water (which is easily removed from sludge flocs). The percentage of bound water limits the behavior of sludge dewatering. The explanation for this may its attachment to the sludge surface even despite the high-pressure dewatering process. Consequently, reducing the percentage of bound water before sludge conditioning instructively contributes much to further enhancing sludge dewaterability (Li et al., 2020a). Regarding the tremendous amount of bound water in EPS protein, further decreased protein content, increased hydrophobicity and destroyed protein structure were observed under the

addition of TCS, resulting in less bound water.

Fourthly, sludge bacteria cells would not be destroyed after the addition of TCS. Intracellular organic matters which have strong hydrophilicity were released to the liquid phase after the destruction of sludge cells. The release of corresponding organics may capture the sludge cells entrapped in the network floc structure through hydrogen bonds combining with water molecules, leading to the disintegration of large flocs. As a result, the sludge dewatering process would be limited again (Yu et al., 2018; Zhen et al., 2013). The addition of TCS just decreased the bioactivity of residual sludge and the bacteria cells would not be destroyed, declining the risk of impeding dewatering process by the release of intracellular organic.

4.2 Energy uncoupling deteriorates sludge dewaterability without chemical re-flocculation

Theoretically, the potential of energy uncoupling on sludge conditioning mentioned above plays a constructive role in enhancing sludge dewaterability. The SRF startlingly increased under the addition of TCS without chemical re-flocculation, bringing about the high moisture percentage of filtered sludge as well as an awful dewatering performance. These phenomena mentioned are explained in more detail below.

The channels of water discharge among sludge flocs were blocked under the addition of TCS, which dramatically decreased particle size of sludge and destroyed the completed surface structure of sludge flocs (Sections 3.3.1 and 3.3.3). The loose and block-shaped structure compromised the ability of aggregation among sludge flocs even though the release of bound water increased (Fig. 9). High compressibility of sludge is an essential factor limiting the improvement of sludge dewaterability (Vaxelaire and Cézac, 2004; Wei et al., 2018). The loose and cracked structure of sludge flocs would accelerate the blocking of channels under the high-pressure filtration and sludge fluidity, decreasing the outflow of sludge water. These conclusions also explained the unsatisfactory performance of sludge

dewatering when a small percentage of bound water was used. Additionally, a larger concentration of S-EPS enhanced the glutinousness of sludge water. The elevated attachment between sludge water and flocs limited the efficient outflow of water, leading to the poorer performance of sludge dewatering.

Therefore, the improvement of sludge dewaterability was unattainable under TCS addition alone, even though less EPS content and percentage of bound water and increased hydrophobicity were achieved. It is necessary to put into place a feasible method of chemical re-flocculation conditioning to emphasize the positive effects of TCS addition, and ultimately achieve highly efficient sludge dewaterability.

4.3 Important roles of chemical re-flocculation after energy uncoupling on sludge dewatering

TCS addition combined with chemical re-flocculation effectively meliorated the performance of sludge dewatering compared to traditional chemical flocculation conditioning alone. The SRF and water content of filtered sludge cake decreased significantly, indicating the realization of efficient sludge filtration and sufficient water discharge. The main reasons accounting for improved performance of sludge dewatering are explained below.

Firstly, the concentration of macromolecular organic matter (i.e. proteins and polysaccharides) decreased. Compared to flocculation conditioning alone, the addition of TCS further reduced the content of EPS, especially the decrease of proteins (Fig. 6). The structure of proteins loosened and became hydrophobic with the addition of TCS. Similarly, the fragmentation of sludge flocs resulted in a porosity structure, which could weaken the capacity of storage for inner water held in the pores of sludge. In their research, Wu et al. (2017) proved that the formation of hydrophobic proteins structure caused the decrement of water-holding capacity of EPS proteins, leading to much improved sludge dewaterability. Evidently, adding TCS weakened the negative effects of EPS proteins on sludge dewatering

compared to flocculation conditioning alone.

Secondly, the proportion of easily removed water rose. The percentage of bound water decreased significantly after adding TCS combined re-flocculation conditioning compared to chemical flocculation alone. The decrement of bound water cut down the energy input for separating the bound water in sludge EPS, and further enhanced the efficiency of dewatering equipment to achieve fuel-efficient sludge dewatering.

Thirdly, the channels of sludge water outflow were widened. The discharge of sludge water needs to be executed by means of a water conveyance channel. TCS addition combined with chemical re-flocculation elevated the particle size and aggregation capacity of sludge flocs compared to single flocculation conditioning, leading to a large floc structure being formed. Large pores and wide channels existing between the combined sludge flocs provided the guarantee for the discharge of sludge water, which could be attributed to why TCS addition combined with chemical re-flocculation resulted in superior dewatering performance than chemical flocculation conditioning alone.

Fig. 9.

4.4 Relevance of potential application of energy uncoupling for sludge reduction and dewatering

As a significant method of sludge reduction (Ferrer-Polonio et al., 2017; Tian et al., 2013), energy uncoupling technology enhances sludge dewaterability, simultaneously reducing the large volume and quantity of sludge, triggering positive outcomes in the subsequent treatment and disposal of sludge.

Oxidation methods have been applied widely to enhance the capacity of sludge dewatering in recent years. The sludge bacteria cells would be oxidized and release intracellular organic matters and water to liquid phase after oxidation (Guan et al., 2018). Meanwhile, decreasing the negative effect of intracellular organics on improving

dewaterability needs to employ lots of reagents and leads to the high cost of sludge treatment (Section 4.1). The destruction of sludge bacteria cells also limits the further utilization of sludge on compost and incineration. Compared with the normal Fenton method, sludge still retains abundant bioenergy by using uncouplers, concerning the undestroyed sludge cells (which still have a certain number of organic matters). The combination of uncouplers and flocculants also synchronizes the improvement of lower calorific value of wet sludge and sludge dewatering performance, assisting the subsequent incineration disposal process. The subsequent utilization of sludge could obtain economic benefits which can be an apparent advantage compared with oxidation methods.

Additionally, it is worth noting that uncouplers are typically toxic, which limits the development of energy uncoupling technology by using uncouplers. However, uncouplers (i.e. TCS) were biodegraded quite rapidly through activated sludge (half-life: 6-8 h) (Gatidou et al., 2021). Hence, choosing suitable uncouplers is necessary to develop the technology of energy uncoupling on sludge dewatering in the future. Meanwhile, the toxicity would not be considered if the subsequent utilization of sludge is incineration. The toxicity of residues in filtered water also can be eliminated by oxidation in the disinfection process.

To further intensify the sludge dewaterability, the technology of energy uncoupling can couple with AOP to form a bio-chemical conditioning method holds much promise, exploiting a more environmentally friendly application of uncouplers (eliminate the toxicity of uncouplers). It is imperative to use chemical flocculants to enhance the sludge dewaterability by adding uncouplers. Therefore, the best possible dosages of uncouplers and flocculants need to be established for the sake of sludge dewaterability. Meanwhile, the ability of uncouplers to curtail the dosage of flocculants is still doubtful, so prompt exploration of the relevant issues is needed in the future.

5. Conclusions

We first proposed a novel method of TCS addition coupled with chemical re-flocculation for enhancing sludge dewaterability, and the relevant dewatering mechanism was explained. The following concluding remarks can be made based on what was discussed earlier.

(1) The sludge bacteria cells would not be destroyed under the addition of TCS, decreasing the risk of impeding dewatering process by released intracellular organics.

(2) The addition of TCS and chemical re-flocculation exhibited excellent improvement for sludge dewatering. After adding 0.12 g TCS/g VSS followed by 0.05 g/g VSS $FeCl_3$, the SRF (1.1×10^{12} m/kg) was approximately ten times lower than the control, and the water content in the filtered sludge cake was significantly reduced.

(3) The completed structure of sludge was cracked under the addition of TCS. After flocculation, the sludge flocs combined more tightly than the control which was conditioned by flocculation alone, leading to the formation of wide and abundant channels for water discharge.

(4) The addition of TCS obviously changed the distribution of sludge EPS, leading to the collapse and fragmentation of EPS. The amounts of S-EPS and LB-EPS increased under the addition of TCS, while the content of TB-EPS dropped significantly. Furthermore, the water-holding capacity of extracellular polymeric substances was reduced simultaneously, and the proportion of bound water shrank, enhancing the amount of discharged water in sludge, thus improving the sludge dewaterability.

(5) The forms of EPS proteins did alter with the addition of TCS before chemical flocculation, leading to the stretching and deformation vibrations of corresponding functional groups. The loose structure and high hydrophobicity of EPS protein were obtained under the addition of TCS, and the water combined with EPS proteins can be removed easily under high pressure in the sludge dewatering process.

Credit authorship contribution statement

An Ding: Conceptualization, Methodology, Editing, Supervision. **Wei Lin:** Data curation, Data analyses, Writing. **Renglu Chen:** Data curation, Data analyses. **Huu Hao Ngo:** Conceptualization, Methodology, Editing. **Rourou Zhang:** Data curation, Data analyses. **Xu He:** Conceptualization, Methodology, Writing, Editing. **Jun Nan:** Conceptualization, Methodology, Editing. **Guibai Li:** Conceptualization, Methodology, Supervision. **Jun Ma:** Conceptualization, Methodology, Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was jointly supported by National Natural Science Foundation of China (No. 52070058); State Key Laboratory of Urban Water Resource and Environment (Harbin Institute of Technology) (No. 2021TS17); the Natural Science Foundation of Heilongjiang Province (YQ2020E020); Heilongjiang Touyan Innovation Team Program (HIT-SE-01); Special support from the China Postdoctoral Fund (2018T110303); and special support from the Heilongjiang Postdoctoral Found (LBH-TZ14).

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Table 1. The characteristics of the sludge sample

Parameter	Unit	Value
Water content	%	98.9 ± 0.3
pH		6.8 ± 0.2
MLSS	g/L	11.3 ± 0.5
VSS	g/L	7.06 ± 0.4
Zeta potential	mV	-11.8 ± 0.4
Bound water	g/g DS	25.2 ± 0.6

Table 2. The ratio of extracellular ATP content to total ATP content.

TCS concentration (g/g VSS)	0	0.03	0.06	0.09	0.12
Ratio (‰)	4.9 ± 0.4	2.7 ± 0.5	2.2 ± 0.3	2.5 ± 0.5	2.9 ± 0.6

Table 3. The surface porosity of the sludge sample

TCS concentration (g/g VSS)	With/without Flocculation	Surface porosity (%)
0	without	19.6 ± 3.2
0	with	22.2 ± 2.7
0.12	without	39.1 ± 3.6
0.12	with	43.6 ± 4.1

Table 4. Band assignments for the protein secondary structures of EPS

Wavelength (cm^{-1})	Secondary structures	Samples							
		TCS:0.00 g/g VSS without flocculation		TCS:0.12 g/g VSS without flocculation		TCS:0.00 g/g VSS with flocculation		TCS:0.12 g/g VSS with flocculation	
		area	Proportion of area (%)	area	Proportion of area (%)	area	Proportion of area (%)	area	Proportion of area (%)
1603-1607	Tyrosine side chain	0.06	0.26	0.00	0.00	0.06	0.25	0.00	0.00
1616-1626	Aggregated strands	3.31	15.47	0.52	4.38	3.64	14.97	2.77	15.14
1629-1640	β-Sheet	2.72	12.69	2.51	20.97	2.98	12.26	3.16	17.26
1640-1645	Random coil	2.97	13.89	1.95	16.26	3.42	14.09	3.06	16.72
1649-1650	α-Helix	3.13	14.61	1.95	16.28	3.65	15.04	3.30	18.04
1659-1671	3-Turn helix	5.39	25.20	3.06	25.57	6.16	25.37	2.99	16.33
1678-1691	Antiparallel β-sheet/ aggregated strands	3.83	17.88	1.98	16.54	4.38	18.02	3.02	16.50
	α-Helix /(β-Sheet +Random coil)	0.55		0.44		0.57		0.53	
Sum		21.41	100	11.97	100	24.29	100	18.29	100

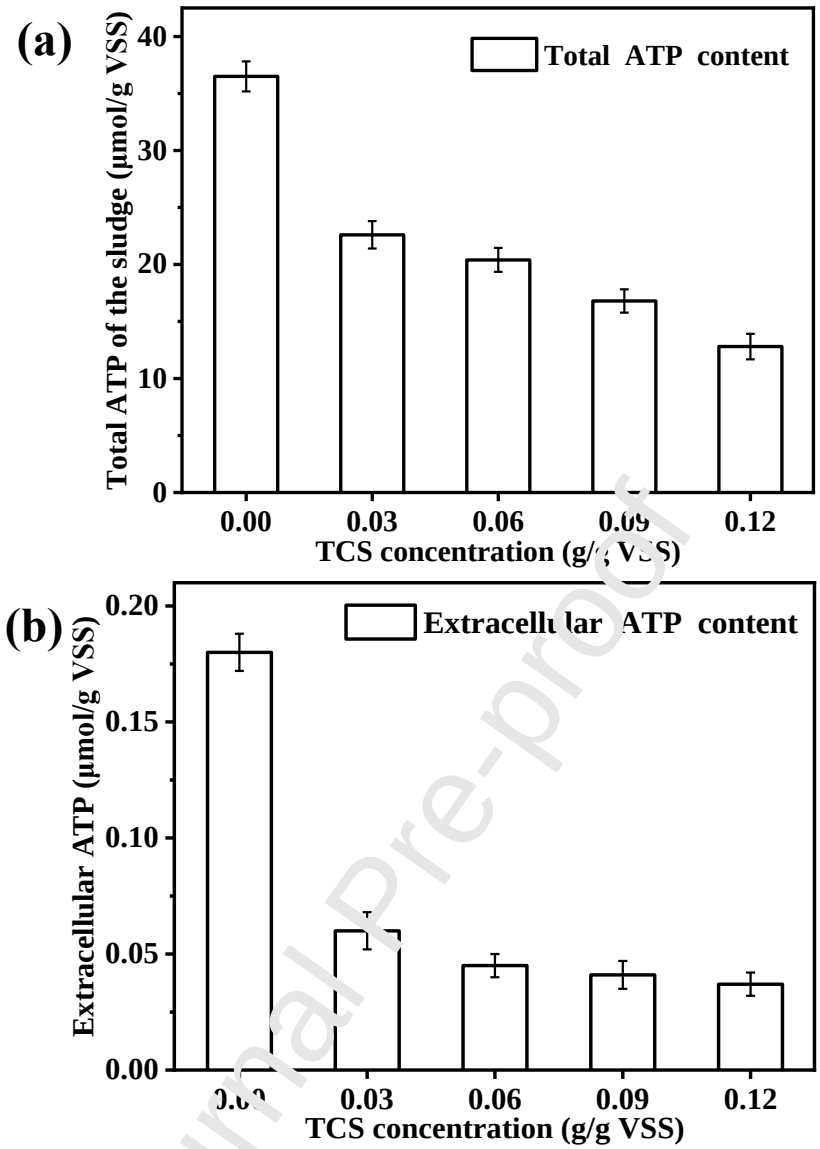


Fig. 1 Effects of TCS addition on (a) total ATP and (b) extracellular ATP contents.

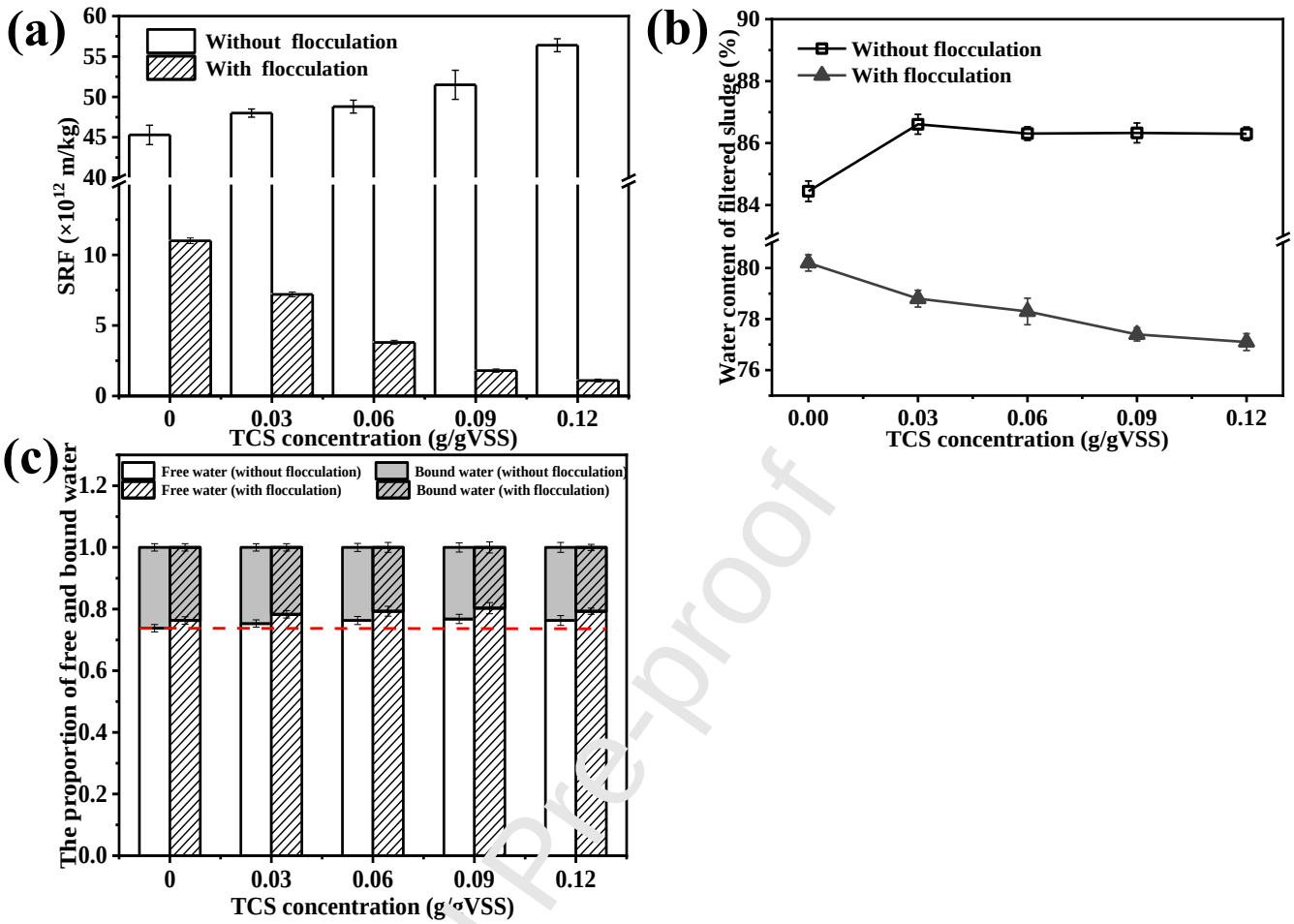


Fig. 2. The capacity of sludge dewatering. (a) SRF; (b) water content of filtered sludge; (c) the proportion of free and bound water (the red line means the proportion of free water of the control sludge sample).

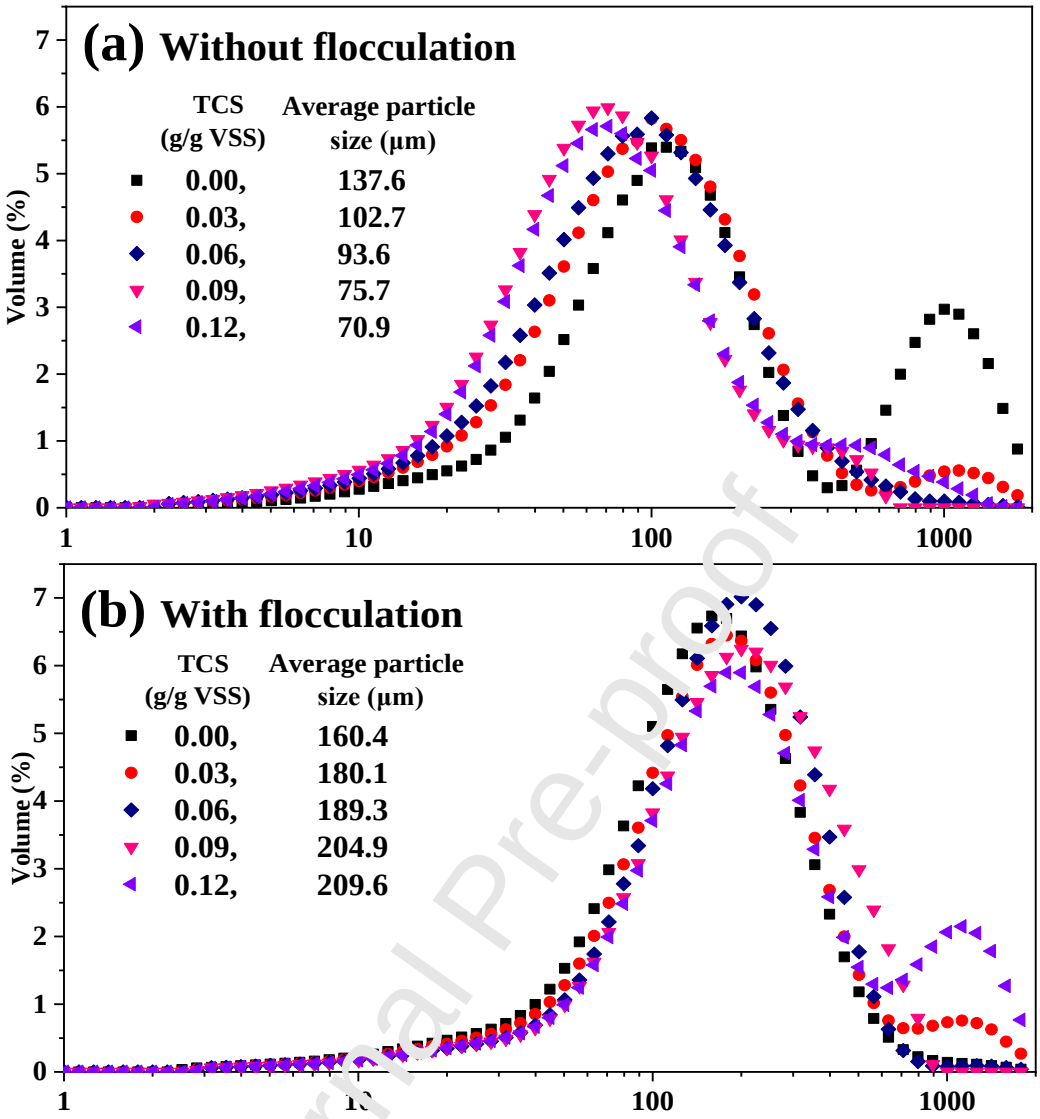


Fig. 3 Particle size of sludge samples.

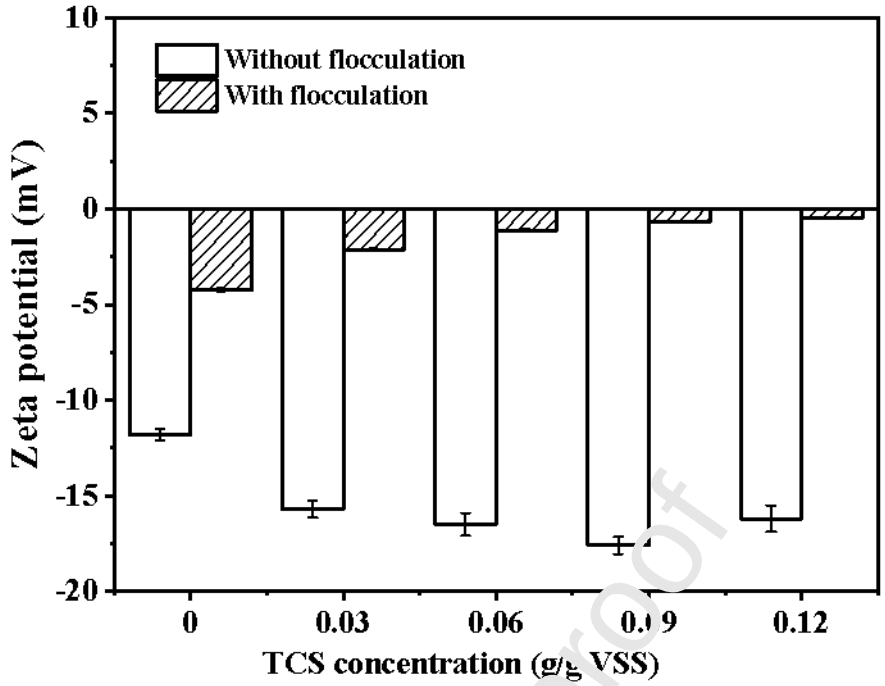


Fig. 4 The zeta potential of sludge samples.

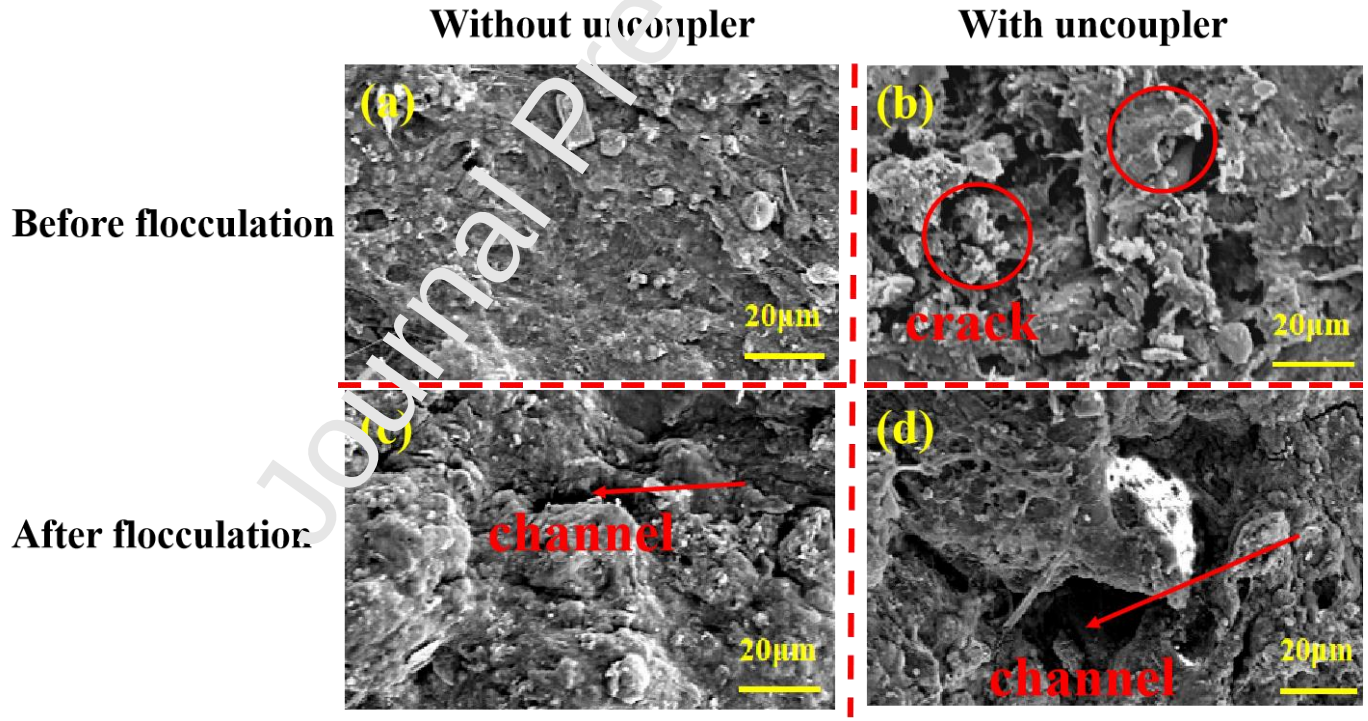


Fig. 5. SEM images of sludge samples (Uncoupler dosage: 0.12 g TCS/g VSS, the crack of sludge sample was circled by red circle, the channel of sludge water discharge was pointed out by red arrows.)

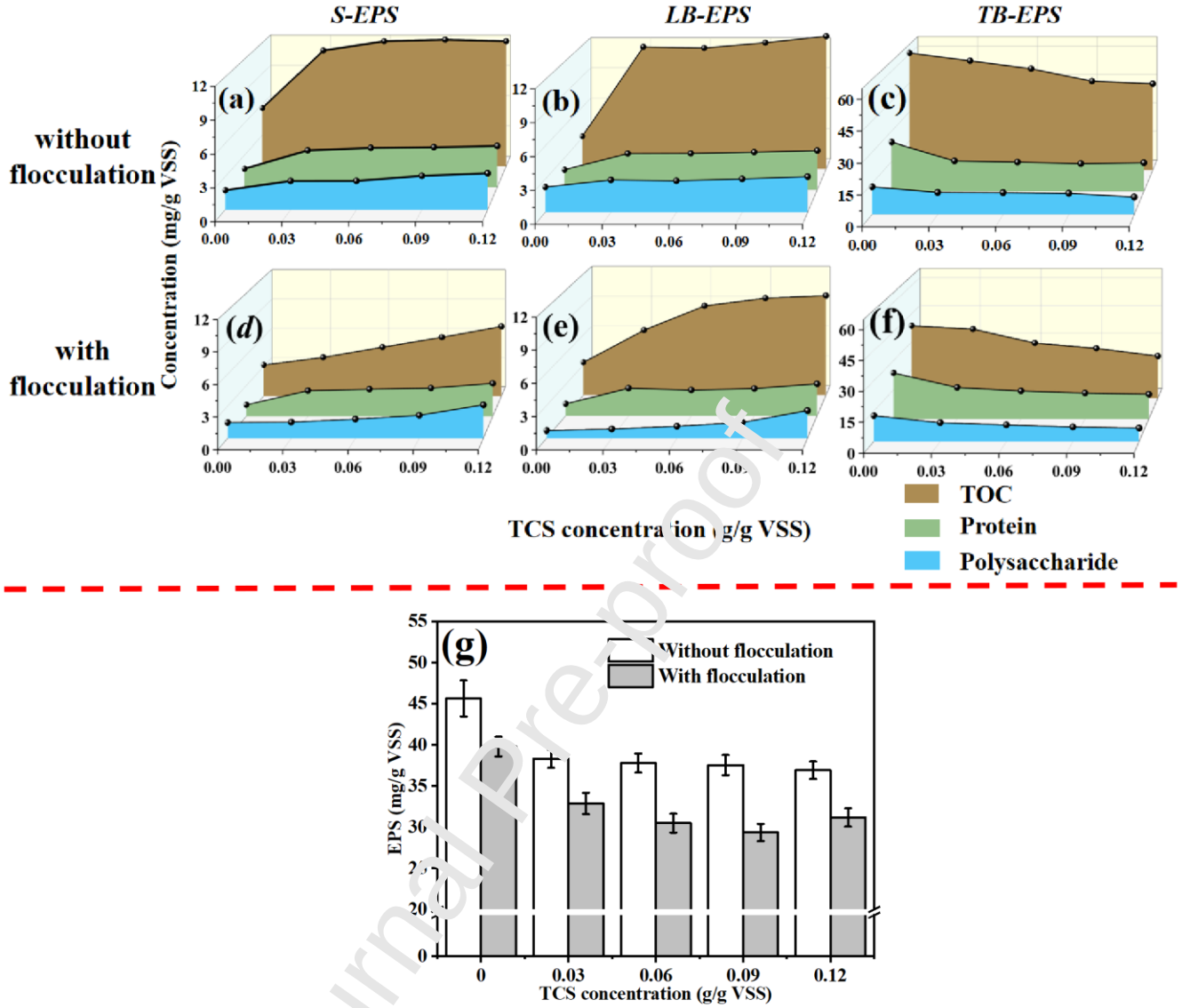


Fig. 6 Effects of TCS addition on the concentrations of EPS.

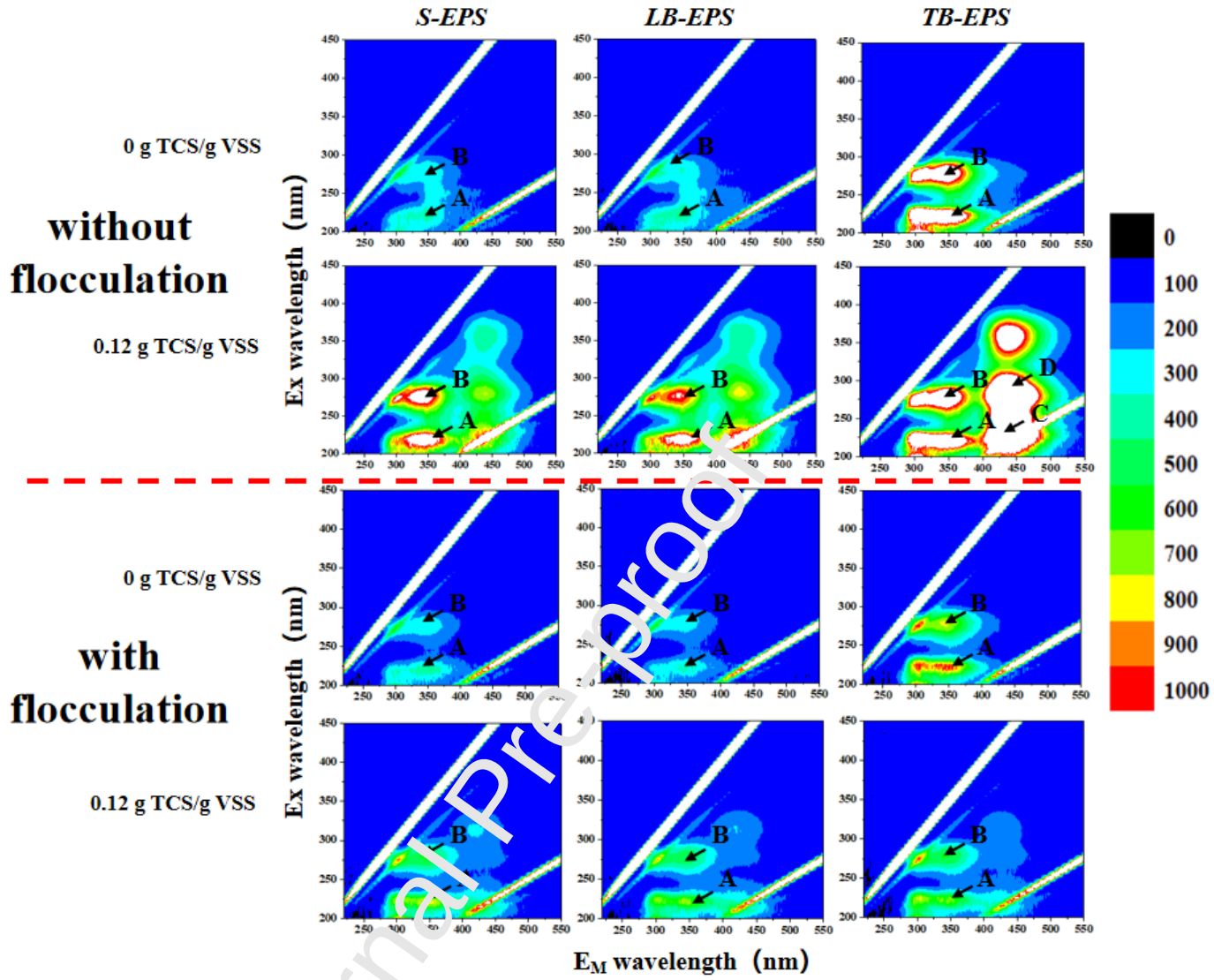


Fig. 7 Effects of TCS addition on the fluorescent organics of EPS.

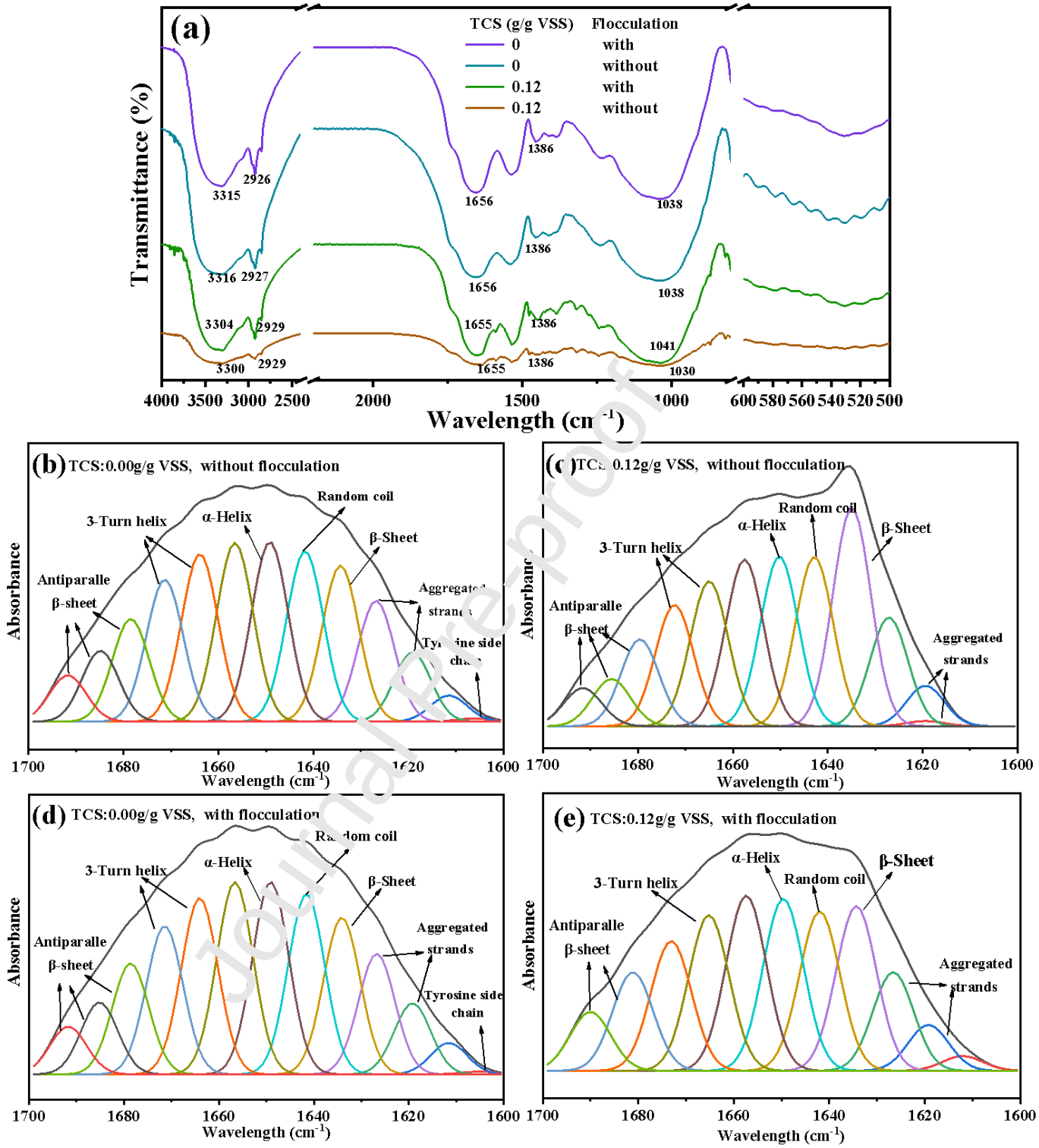


Fig. 8 FTIR spectra and Second derivative resolution enhanced and curve-fitted amide I region (1700~1600cm^{-1}) for proteins of the sludge samples: a) FTIR; b) ~ e) Second derivative resolution enhanced and curve-fitted amide I region (1700~1600cm^{-1}) for proteins.

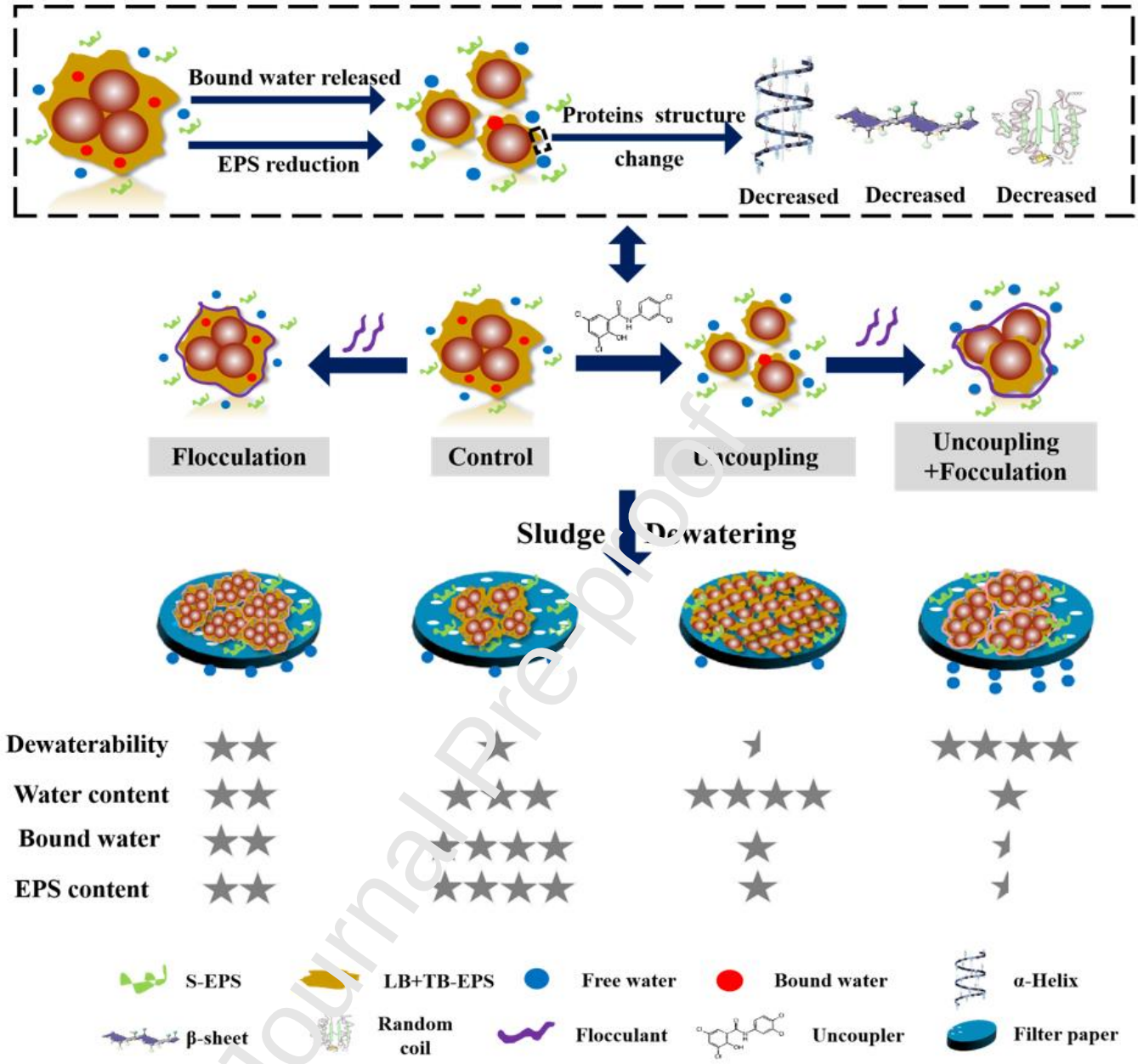
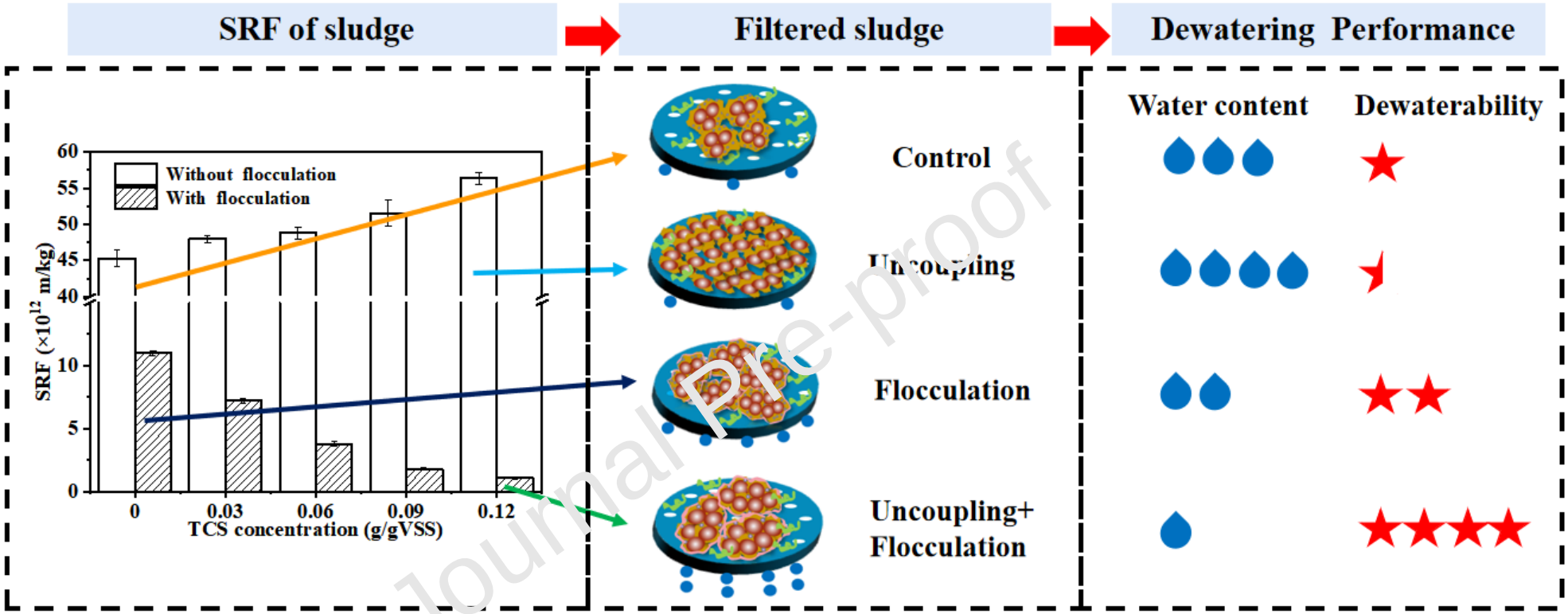


Fig. 9 Mechanism of TCS addition and chemical re-flocculation on sludge dewaterability enhancing.

Graphical abstract



Highlights:

- Energy uncoupling combined with re-flocculation greatly improved sludge dewaterability
- TCS addition cracked sludge flocs and increased sludge roughness and surface porosity
- TCS addition destroyed EPS and promoted transformation of bound water to free water
- TCS improved sludge hydrophobicity by changing secondary structures of EPS protein