

The application of chitosan quaternary ammonium salt to replace polymeric aluminum ferric chloride for sewage sludge dewatering

Minmin Liu^a, Sajid Rashid^a, Wei Wang^b, Huihui Zhang^b, Yawei Zhao^b, Xingmin Fu^b, Nigel Graham^c, Zhaoyang Su^a, Wenzheng Yu^{a*}

^a Key Laboratory of Drinking Water Science and Technology, Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China (wzyu@rcees.ac.cn)

^b Engineering Technology Research Center for Municipal Wastewater Reclamation, R&D Center, Beijing Drainage Group Co., Ltd., Beijing 100124, China

^c Department of Civil and Environmental Engineering, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom (n.graham@imperial.ac.uk)

Abstract

Inorganic coagulants such as poly aluminum ferric chloride (Al/Fe) are applied conventionally to sewage sludge dewatering and can be retained in the sludge cake, causing its conductivity to increase and generate secondary pollution. To reduce these disadvantages, there is a need to develop alternative, more sustainable chemicals as substitutes for conventional inorganic coagulants. In the present investigation, the application of a polymeric chitosan quaternary ammonium salt (CQAS) is explored as a complete, or partial, replacement for Al/Fe in the context of sludge dewatering processes. Laboratory experiments using digested sewage sludge showed that CQAS could effectively substitute for over 80% of the Al/Fe inorganic coagulant in the sludge dewatering process. This substitution resulted in a reduction of sludge cake conductivity by more than 50%. Simulation of sludge dewatering curves and imaging of the sludge surface indicated that the addition of CQAS led to an increase in nanosized pores, and a decrease in the specific resistance of the sludge filter cake as the dosage of Al/Fe decreased to around 30%. The variations of fluorescence emission, quantum yield and carboxylic and amino groups, suggested that the chelating of Al/Fe decreased due to the bridging effects of CQAS. The CQAS had different flocculation bridging effects on various EPS fractions, which varied the amount of protein chelated with Al/Fe in each fraction. This

study provides new information about the benefits of replacing conventional inorganic coagulants with natural organic polymers for sewage sludge dewatering, in terms of reduced sludge cake conductivity and greater dry solids content.

Keywords: Chitosan quaternary ammonium salt; quaternary ammonium polymer coagulant; polymeric aluminum ferric chloride; dual coagulants; sewage sludge; sludge dewatering

1 Introduction

As a product of primary and secondary (biological) wastewater treatment processes, sewage sludge represents a secondary pollutant source that requires careful management and control. This is attributed to its organic content, pronounced odor, and substantial presence of pathogenic organisms, parasitic eggs, organic micro-pollutants and heavy metals (Cao et al., 2021; Wu et al., 2023a). Every year the production of sewage sludge dry solids (ds) is approximately 0.4 million tons in Australia and New Zealand, and approximately 17.8 million tons in the United States (Wu et al., 2020). In 2020, the production was more than 13.0 million tons and 13.3 million tons in the European Union and in China, respectively (Wu et al., 2020). The total sludge production in China has increased significantly with an average annual growth of 13% in the past decade (Yang et al., 2015). Moreover, in 2014, the cost of sludge dewatering was reported to be US\$ 60-90 /ton sludge with a moisture content of 80%, and the cost of sanitary landfill, incineration, building materials and land application was 72-96, 240-360, 240-360 and 240-280 US\$/ton ds in China (Yang et al., 2015). In general, the cost of sludge treatment and disposal has been estimated to account for around 20% of the operating cost of sewage plants in China. The large and growing production of sewage sludge represents a significant challenge in terms of the increasing cost of sludge treatment every year. Therefore, the amount of sludge should be minimized, typically by dewatering processes, in order to facilitate its transportation and reduce the output of hazardous materials from sludge landfills (Wu et al., 2023a) (Xiao et al., 2023a).

The heterogeneous colloidal composition of sludge comprises small particles, suspended stably in water, which are difficult to separate from the aqueous solution without any chemical preconditioning process prior to the application of mechanical pressure (Wang et al., 2018).

Inorganic coagulants such as poly aluminum chloride (PAC) and poly ferric chloride (PFC) are commonly used for improving the dewaterability of sludge. The colloids of the sludge are agglomerated by the PAC and PFC through a combination of charge neutralization, floc formation and polymer bridging effects, to facilitate water separation (Wang et al., 2018). The hydrolyzed cationic species of inorganic coagulants such as $\text{Al}(\text{OH})^{2+}$ and $\text{Fe}(\text{OH})^{2+}$ neutralize the negative ions in the diffusion layer on the surface of the sludge particles, causing the electric double layer to compress and the electrostatic repulsion between the particles to reduce, thus making particles easier to agglomerate (Cao et al., 2021). The subsequent addition of rigid and incompressible flocculant materials as a skeleton can increase the mechanical strength, permeability of the sludge particles and water channels, which promotes the release of adsorbed and bound water, and improve the filtration dewatering performance (Cao et al., 2021). However, the aluminum ions retained in the dewatered cake can lead to adverse environmental effects when disposed to land, such as an increase in conductivity which can stunt plant growth and pollute underground and surface water via rainfall or surface runoff (Xu et al., 2021; Liu et al., 2021). In particular, if the source water is polluted by the aluminum from the underground or surface water, there may be a risk to human health as aluminum has been associated with neurodegenerative diseases such as Alzheimer's disease. Thus, it is desirable to develop metal-free polymer chemicals to substitute the inorganic coagulants for sludge dewatering.

Biological sludges contain complex polymers that are generated by microorganisms through dissolving and absorbing organic matter. A major constituent are extracellular polymeric substances (EPS), which in turn comprise 70%-80% proteins and polysaccharides, and 10%-20% nucleic acids and humic acid (Sam et al., 2022; Cao et al., 2018). Compared with polysaccharides, the proteins play the most significant role in sludge dewatering (Lin et al., 2022). In particular, the content and structure of proteins are the key factors affecting sludge dewaterability. For example, sludge dewaterability could be improved by regulating proteins towards production of a high content of tryptophane and low content of tyrosine (Cao et al., 2021). The proteins extracted from different sludge EPS by cation exchange resin were reported to have a high similarity and many vital molecular functions (Peng et al., 2023). In particular, the predominant molecular function of protein is the cation binding activity arising from the

interaction of free amino groups with cations (Peng et al., 2023). During coagulation the proteins can provide binding sites for Al^{3+} or Fe^{3+} (and their charged hydrolysis species) through a cation exchange reaction (Fuchs and Jupke, 2022), which reduces the effective content of the inorganic coagulant and deteriorates sludge dewatering (Wei et al., 2018; Xu et al., 2018). Therefore, the accessible surface area of proteins in solution should be reduced and then the hydrophobicity and binding free energy would increase, leading to a strong gel-like network of EPS exposing hydrophobic interaction sites (Wu et al., 2021; He et al., 2023). The bridging flocculation of polymers can enable the colloids of sludge aggregates to help coat the surface area of proteins with adjacent material, thereby decreasing their surface area (Delahaije et al., 2017). Thus, if the polymer is applied at the first stage of sludge conditioning, the effective content of inorganic coagulant can be enhanced and result in an improved sludge dewaterability.

Polymers such as polyacrylamide (PAM) and its derivatives have been studied extensively in terms of their selection, design, preparation and optimization for sludge dewatering via bridging flocculation (Zhang et al., 2020). However, the utilization of PAM is typically insufficient to enable a reduction in the dosage of inorganic coagulants, owing to the restricted capacity for bridging flocculation. For anaerobic digested sludge with a capillary suction time (CST) of above 600 s, the percentage of PAM in dry solid sludge can reach 0.8% and the content of inorganic chemicals, such as PAC or PFC, can be around 1% - 3% of dry solids in sludge, which results in the conductivity of the sludge cake increasing to around 20%. In addition, synthetic polymers may contain toxic by-products, such as the acrylamide monomer (a neurotoxin) in PAM, which can cause secondary pollution (Wang et al., 2018). Natural polymers originate from animals and plants, are abundant, renewable and biodegradable. As the derivative of chitin, chitosan occurs in a large number of living organisms, such as shrimps, crabs, tortoises and insects (Kankariya and Chatterjee, 2023). Since chitosan can only be dissolved in acetic acid or hydrochloric acid solutions, many derivatives have been prepared to enable it to have a wide application. In particular, the chitosan quaternary ammonium salt (CQAS) is a kind of cationic coagulation aid with a high molecular weight of around 100-300 kDa, which enables it to have a greater bridging effect in flocculation compared to PAM, with

a molecular weight of approximately 100 kDa. Thus, there is much potential for CQAS to be a good substitute for inorganic coagulants in sludge dewatering.

In this study, CQAS was applied to replace poly aluminum ferric chloride (Al/Fe), partially or completely, for sludge dewatering. The study employed principal component analysis (PCA), and the correlation by linear regression between the indexes of ultraviolet-visible absorbance (UV-Vis) and the fluorescent components extracted from excitation-emission (EEM) fluorescence spectrometry, for various extracellular polymer substances (EPS) components, including soluble EPS (S-EPS), loose-bounded EPS (LB-EPS) and tight-bounded EPS (TB-EPS). Based on these methods, redundancy analysis (RDA) was carried out to obtain an analysis of the relationship between the key indexes and the dosages of Al/Fe and CQAS by combining multiple linear regression and a PCA of the matrix of fitted indexes. Variation partitioning (VPA) was applied to analyze the variations of fluorescent components, which could be explained by at least two of them, because the sludge dewaterability could be impacted by the interactions between protein-like and humic-like substances through charge neutralization and flocculation during conditioning. Moreover, sludge dewatering curves were simulated and the variation of functional groups for dewatered sludge was analyzed. The results, described subsequently, have shown that the CQAS can replace more than 80% of Al/Fe inorganic coagulants for sludge dewatering and decrease the sludge cake conductivity by more than 50%.

2 Materials and methods

2.1 Raw digested sludge (RDS) and reagents

Samples of RDS were obtained from the Gaobeidian reclaimed water plant, of the Beijing drainage group company, China. The principal properties of the RDS are shown in Table 1. The company provided poly aluminum ferric chloride (Al/Fe) solution with an effective constituent mass concentration of 10% and cationic polyacrylamide (PAM), with a cationic degree of 30% and a PAM mass concentration of 0.4%. These reagents are used for sludge dewatering in practice by the company. The CQAS had a 98% degree of substitution, with the MW ranging from 100 to 200 kDa, and was purchased from the Macklin company in China. The viscosity

of a 1% aqueous solution of CQAS at 20 °C was between 10 and 100 mPa • s. The mass concentration of the CQAS solution applied in this study was 5%.

2.2 Conditioning and dewatering of sludge

100 mL of RDS was put into a 250 mL beaker, and 1 mL of the CQAS solution with a mass concentration of 5% was added to the RDS. Then, an electric stirrer (HD2004W, Sile, shanghai) was used for mixing the contents at a speed of 60 rpm for 10 min, followed by the addition and mixing (100 rpm for 10 min) of 0, 0.25, 0.5- or 0.75-mL volumes of Al/Fe solution (10% mass concentration), representing CQAS: Al/Fe ratios of 1:0, 1:0.5, 1:1 or 1:1.5. Separately, 11 mL of PAM solution with a mass concentration of 0.4% was mixed with sludge at 60 rpm for 10 min. In addition, 1.8 mL of Al/Fe solution (mass concentration of 10%) was added to the RDS at 100 rpm for 10 min, and then 11 mL PAM solution was mixed at 60 rpm for 10 min to produce a coagulant ratio of 0: 3.6. Samples of the conditioned sludges were taken for spectral analysis. Lastly, the conditioned sludge was put into a frame filter for dewatering under a pressure of 4 bar for 20 min. The moisture content of the dewatered cake was calculated by the weight loss before and after drying in a drying oven (PH-030A, Yiheng, shanghai) at 105 °C for 2 h.

2.3 The replacement ratio of Al/Fe by CQAS

As shown in Table 2, the dosage of the coagulant chemicals was calculated by their mass ratio in a dry solid of sludge. According to the company, which employed Al/Fe and PAM for the sludge conditioning in practice, the Al/Fe optimum dosage was 3% of the mass fraction of RDS dry solids, which was used as the basis to calculate the replacement ratio of Al/Fe by CQAS. Moreover, the dosage of PAM was 0.73% of the RDS dry solid mass. The dosage of the coagulant was calculated by the mass ratio in a dry solid of sludge based on the equation given in the Supporting Information (SI, Text S1).

2.4 The extraction and measurements of extracellular polymer substances (EPS)

A 50 mL sample of RDS or conditioned sludge was placed in a centrifuge tube and

centrifuged at 3000 rpm for 10 min, and the supernatant liquid was assumed to contain soluble EPS (S-EPS). After the S-EPS was separated, the residual sludge was diluted by NaCl solution with a mass ratio of 0.05% to 50 mL and homogenized with a glass rod and shader (Vortex-Genie 2, scientific industries, USA). The solution was treated in an ultrasonic bath (Ultrasonic Instrument, Kunshan, China) at a frequency of 100 kHz for 10 min, then centrifugated at 5000 rpm for 10 min, and the supernatant liquid was assumed to contain loosely bound EPS (LB-EPS). The supernatant liquid was removed and the residual solids diluted by the addition of 0.05% NaCl solution, then heated at 60 °C for 30 min in a water bath after homogenization. Lastly, it was centrifuged at 5000 rpm for 10 min and the supernatant was assumed to contain tightly bound EPS (TB-EPS).

The three kinds of EPS (S-EPS, LB-EPS and TB-EPS) were diluted 500 times with deionized water (DI) and then filtered by 0.22 µm membrane. The filtrates were examined by UV-vis spectrophotometer (UV-vis, Varian Cary 100, USA) and excitation-emission matrix fluorescence spectrometry (EEM, F-4600, Hitachi, Japan). Specific ultraviolet absorbance at 254 nm [SUVA₂₅₄ (L·mg/C·m)] and 280 nm [SUVA₂₈₀ (L·mg/C·m)] are the absorbance per unit carbon, determined as the absorption coefficient at 254 and 280 nm divided by the total organic carbon (TOC) concentration. The values of SUVA₂₅₄ and SUVA₂₈₀ are associated with the content of aromatic carbon and the content of tryptophan residuals, respectively. Based on the UV-vis spectra of the three types of EPS shown in Figure S2(A-C), the S₂₇₅₋₂₉₅ and S₃₅₀₋₄₀₀ were calculated by measuring the slopes of the UV-vis absorbance curves between 275-295 nm and 350-400 nm (Liu et al., 2022). The fluorescent organic components such as tryptophan-like (C2), tyrosine-like (C1) and humic-like (C3) were extracted from the EEM datasets based on the parallel factor analysis (PARAFAC) modeling.

2.5 The characterization of sludge and data analysis

The capillary suction time (CST, s) of conditioned sludge was measured by commercial CST equipment (304M, Triton, US). After sludge conditioning, droppers were used for taking approximately 0.5 mL quantities of sludge samples of RDS, 0:3.6, 1:0, 1:0.5, 1:1 and 1:1.5, and placing these on white paper. After air drying, the white papers with the sludge samples

were cut down by a scissor, and then put onto a conducting resin for the observation of its surface by field emission scanning electron microscopy (FE-SEM, Hitachi SU8020, Japan) after gold spraying. The zeta potential of sludge was measured by a zetasizer Nano ZS90 (Instruments, Malvern, US) after the sample was diluted 100 times. The dewatered sludge and RDS were dried and ground to a powder, and then their functional groups were characterized by Fourier Transform Infrared Spectroscopy (FTIR, Spectrum Two, PerkinElmer, USA). The spectra were all corrected by removing the background spectrum and normalized. X-ray photoelectron spectroscopy (XPS, Thermo escalab 250XI, USA) was applied to detect the chemical and electronic states of elements such as C, N and O on the sludge surface. The measurement of sludge conductivity is described in the Supporting Information (SI, Text S2). Text S3 showed the methods of graphical representation of fluorescence emission spectra, and fluorescence quantum yield spectra.

Spearman's correlation analysis and principal component analysis (PCA) were performed to compare the relationship and the difference between the variations of various indexes. A redundancy analysis (RDA) was conducted to seek a series of linear combinations of the explanatory variables such as $SUVA_{254}$ and $SUVA_{280}$ to best explain the variation of the response data (dosages of Al/Fe and CQAS). PCA can be used to analyze data without explanatory variables, whereas RDA use both response and explanatory variables. The aim of PCA is to calculate an axis that has maximum variance, but the axes in RDA are not only a linear combination of the response variable, but also of the explanatory variables for modelling a cause-effect relationship. Variance partitioning analysis (VPA) was applied to analyze the part of EEM which could be explained jointly by the tryptophan-like and humic-like components, or tryptophan-like and tyrosine-like components (Yu et al., 2023). The Text S4 presented the graphing methods of fluorescence emission spectra, fluorescence quantum yield spectra and the $R_{adj}^2(\text{Tryptophan-like} \times \text{Tyrosine-like})$ and $R_{adj}^2(\text{Tryptophan-like} \times \text{Humic acid-like})$ analyzed by VPA (Zhu et al., 2020). Sludge cake consolidation was carried out by the dewatering filter under 4 bar constant pressure. The simulation of cake deliquoring curves and related parameters was conducted by the method based on the patent invented by Kang et al. (Xiao et al., 2023b). The data analysis mentioned above were all carried out by R language

(R Development Core Team).

3 Results and Discussion

3.1 Sludge dewatering efficiency and cake conductivity

For the results shown in Figure 1A, after sludge dewatering, around 4 g of sludge cake were dried for the moisture content measurement. As the Al/Fe and CQAS were the only reagents present, the moisture content of the sludge cake reached around 60% for all combinations, which is the requirement of sludge dewatering in practice. When the Al/Fe was used solely as the reagent without substitution, the moisture content of the sludge cake was approximately 60%, and the conductivity of the sludge cake was increased by 20%. In contrast, when the ratio of CQAS to Al/Fe was 1:1, the moisture content and the conductivity of the sludge cake decreased by around 50% and 40%, respectively. Therefore, the substitution of Al/Fe by CQAS achieved both a reduction in the conductivity of the sludge cake and a further increase in dewatering. This represented the optimal ratio, as the dewatering efficiency decreased, and the water content of sludge cake increased, with increasing CQAS content. As shown in Figure 1B, the variations of CST and zeta potential were the same as the degree of sludge dewatering. At a mass ratio of CQAS to Al/Fe of 1:1, the CST was around 20 s and the zeta potential was approximately 0 mV, suggesting the destabilization of the sludge colloids was the result of the reduction of electrical double layer repulsion and enhancement of cake formation. With the dosing of Al/Fe, and their retention in the sludge cake, there was an increase in conductivity (Table S2), and Figure 1C shows that the conductivity of the sludge cake increased by around 20% with the use of Al/Fe only (compared to RDS), and decreased systematically with decreasing Al/Fe dosage. Therefore, the replacement of Al/Fe by CQAS was able to decrease the conductivity of the sludge cake substantially.

3.2 The eigenvalues of EPS types and the correlation between spectral characteristics

From Figure S2, the spectral characteristic indexes ($SUVA_{254}$, $SUVA_{280}$, $S_{275-295}$, $S_{350-400}$ and S_R) were obtained from the UV-vis absorption spectra of various EPS types, and fluorescence components C1 (tyrosine-like component), C2 (tryptophan-like component), C3

(humic-like component) were extracted from EEM datasets of all EPS types. This enabled eigenvalues of various EPS types to be obtained and analysis of the relationship between spectral characteristics. As shown in Figure 2A, there were maximum variances in the C1 and C3 components for the S-EPS. Figure 2B demonstrated that the C1 was significantly positively correlated with C3, and the C3 was negatively correlated with $S_{275-295}$. This suggested that the C1 and C3 might be the humic-like substances with medium molecular weight, which had the maximum variance for the S-EPS. The indexes with maximum variance of TB-EPS comprised $SUVA_{280}$, $SUVA_{254}$, $S_{350-400}$, $S_{275-295}$, C2 and S_R . Moreover, the $SUVA_{280}$, $SUVA_{254}$, $S_{275-295}$ and S_R were greatly positively correlated with C2, illustrating that the protein of TB-EPS was mainly composed of hydrophobic fluorescence protein with low molecular weight. The maximum variance of LB-EPS was linked with $S_{350-400}$, indicating that fine particle aggregation was a significant factor influencing sludge dewaterability. Previous studies indicated that the LB-EPS was significantly correlated with sludge dewatering because the bonding strength between cells would be weakened by LB-EPS resulting in poor sludge dewatering performance (Li and Yang, 2007; Zhang et al., 2019). Therefore, sludge dewatering was greatly impacted by the sludge floc size in this study.

3.3 The emission spectra and quantum yield spectra of various EPS

Fluorophores commonly exist in two ionization states, each of which displays distinct absorption and emission spectra (Xiao et al., 2020). Emission spectroscopy examines the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to a lower energy state. Quantum yield is perhaps the most important characteristic of a fluorophore and is the number of emitted photons relative to the number of absorbed photons (Yu et al., 2021). This is known as Kasha's rule that quantum yield is generally independent of excitation wavelength, and emission spectra are usually independent of the excitation wavelength. Therefore, the emission spectra of molecules can be used in the chemical analysis of substances and quantum yield serves as an important correction factor for determination of other parameters, such as fluorescence quenching. Substances with the largest quantum yields, approaching unity, display the brightest emissions (Yu et al., 2020). In addition, the quantum

yields of fluorescence components could be also affected by the ionization state of the amino and carboxyl groups in tyrosine, tryptophan and humic. Amide groups can act as quenchers through quenching by proton transfer from nearby charged amino groups and electron acceptors such as protonated carboxyl groups. Heavy atoms such as Al^{3+} or Fe^{3+} chelated by the amide and carboxyl groups typically result in lower quantum yields and emission (Poulin et al., 2014). The fluorescence spectra associated with protein conjugation were susceptible to quenching of Al or Fe and protein fluorescence indices were sensitive to the presence of many forms of Al or Fe (Poulin et al., 2014). Moreover, the chelation of Al^{3+} or Fe^{3+} reduces their charge neutralization ability and their floc size. However, the chelation of Al^{3+} or Fe^{3+} can be reduced through flocculation bridging by CQAS due to the decrease of the amino and carboxyl groups exposed to the Al/Fe. Therefore, the emission intensity and quantum yield were positively correlated with the aggregation of components and negatively related with the Al/Fe chelation, which were influenced by the combined effects of flocculation bridging by CQAS and charge neutralization by Al/Fe.

Figure 3D shows that there were various peaks at different excitation wavelengths for quantum yields of S-EPS samples, indicating that there was a different quantum yield for various fluorescence components of S-EPS samples. In contrast, in Figure 3A, most of emission spectra for S-EPS samples almost overlapped, indicating they were not impacted by the chelation of Al^{3+} or Fe^{3+} due to the strong flocculation bridging of CQAS. Moreover, the fluorescence emission intensity and quantum yield of Tryptophan-like $\times$ Tyrosine-like/humic-like for S-EPS of the ratio 1:0, was little greater than those for 0:3.6, indicating that the emission intensity and quantum yield of S-EPS were more greatly increased by the flocculation bridging of CQAS compared with the charge neutralization of Al/Fe.

As shown in Figures 3B and 3E, the emission intensities and quantum yield of the Tryptophan-like $\times$ Tyrosine-like/humic-like components increased, then decreased with increasing mass ratio of CQAS to Al/Fe from 0:3.6 to 1:0, and reached a maximum value at the mass ratio of CQAS to Al/Fe of 1:1. Moreover, the intensity and quantum yield of ratio 1:0 was much lower than that of 1:1. These indicated that compared with S-EPS, the flocculation bridging of CQAS decreased to an extent that LB-EPS was influenced by the synergy between

the flocculation bridging of CQAS and charge neutralization of Al/Fe. From Figures 3C and 3F, the emission intensities and quantum yield of TB-EPS were greater for the samples of 1:1 and 1:0.5 than other samples, suggesting that the synergy between the flocculation bridging of CQAS and charge neutralization of Al/Fe was also the greatest for TB-EPS at the ratios of 1:1 and 1:0.5. In contrast to the LB-EPS, the TB-EPS peaks of emission intensities and quantum yield for Tryptophan-like $\times$ Tyrosine-like/humic-like of the ratio 0:3.6 was greater than those of the 1:1.5. This illustrated that the emission intensity and quantum yield of TB-EPS were more greatly enhanced by the charge neutralization of Al/Fe rather than the flocculation bridging of CQAS. Therefore, in comparison with the LB-EPS and TB-EPS, the CQAS has a strongest flocculation bridging effect with the S-EPS.

3.4 The correlation between spectral characteristic indexes of various EPS and reagent dosages

RDA is a direct gradient analysis that summarizes linear relationships between components of response variables that are explained by a set of variables. This was applied to analyze the correlations between dosages of CQAS or Al/Fe, as the explanatory variables, and the spectral characteristic indexes as response variables. From Figures 4A, 4B and 4C, the distance triplots showed that the line length of red lines was positively correlated with the influence degree of dosages for CQAS or Al/Fe on the indexes. As shown in Figures 4D, 4E and 4F, the cosine of angles between any variables including the dosages for CQAS or Al/Fe and all the indexes were equal to their correlations. Thus, where the angle is lower, the relationship is more positive. From Figures 4D-4F, the angles between moisture content and CQAS or Al/Fe were the same and more than 90°, indicating sludge dewaterability was impacted by both the CQAS and Al/Fe. Moreover, the tryptophan-like component was hydrophobic protein, which was responsible for improving sludge dewaterability (Li and Yang, 2007). However, the C1 (tyrosine-like component) is a kind of fluorescence protein which is more hydrophilic than the C2 (tryptophan-like component) because it contains a larger content of amino and carboxylic groups (Xiao et al., 2020). Therefore, the chelation ability with Al/Fe of C1 was stronger than that of C2.

From Figure 4D, the $SUVA_{280}$ and $SUVA_{254}$ were most negatively correlated with moisture content, and the correlation between them and C2 was more positive compared with them and C1. Moreover, from Figure 4A, their lines were the longest among the other indexes that were negatively related with moisture content. These indicated that most of the protein of S-EPS was not sufficiently chelated with the Al/Fe to enhance the sludge dewaterability significantly. From Figure 4A, the $S_{275-295}$, S_R , $S_{350-400}$ and C1 were most positively correlated with Al/Fe dosage, but were most negatively related with CQAS dosage, and were slightly related with the moisture content. Furthermore, from Figure 4D, the line lengths of $S_{275-295}$, S_R were greater than those of $S_{350-400}$ and C1. These indicated that the charge neutralization of Al/Fe had little effect on the sludge dewatering, but the flocculation bridging of CQAS played an essential role in the deterioration of the protein chelation with Al/Fe.

From Figures 4B and 4E, compared with S-EPS, the $S_{275-295}$ and S_R for LB-EPS were more positively correlated with moisture content and their lines were longer, illustrating that the impact of Al/Fe dosage on the sludge dewaterability was greater than that for S-EPS. Moreover, $SUVA_{280}$, $SUVA_{254}$ and C1 were negatively correlated with dosage of Al/Fe and positively correlated with the moisture content, which was negatively correlated with C2 and $S_{350-400}$. Figure 4B showed that the line length of C1 was the same with that of C2 and they were all much longer than those of $SUVA_{280}$ and $SUVA_{254}$. These indicated that, compared with S-EPS, a larger amount of protein was chelated with Al/Fe for LB-EPS, which had a greater adverse impact on sludge dewaterability due to the lower flocculation bridging of CQAS for LB-EPS. Since the flocculation bridging of CQAS for LB-EPS was less than that for S-EPS, the sludge dewatering was also impacted greatly by Al/Fe dosage. Therefore, the sludge floc size of LB-EPS was determined by the ratio of CQAS to Al/Fe, which could result in the maximum variation of sludge floc size. This was consistent with the PCA analysis.

From Figure 4F, it was evident that C2 and $S_{275-295}$ were negatively correlated with the moisture content, $SUVA_{280}$, and $SUVA_{254}$. Figure 4C showed that compared with LB-EPS, the lines of C2 and $S_{275-295}$ were shorter and the lines of $SUVA_{280}$ and $SUVA_{254}$ were longer. These indicated that the amount of protein chelated with Al/Fe for TB-EPS was larger than that for LB-EPS. Therefore, the flocculation bridging of CQAS for TB-EPS was the lowest for all EPS

types. In addition, the C1 was slightly positively related with moisture content but was greatly positively related with CQAS dosage, indicating that the sludge dewatering was slightly reduced by protein chelation with Al/Fe. Moreover, the C2, $S_{275-295}$, $SUVA_{254}$ and S_R were negatively correlated with the moisture content and the line of S_R was longer than the other lines. Moreover, there was no relationship between moisture content and $S_{350-400}$. These findings illustrated that fine particle aggregation was not correlated with sludge dewaterability. This suggested the sludge dewatering was mainly promoted by the destabilization of the proteins due to their chelation with Al/Fe, rather than the charge neutralization of Al/Fe; this was because of the excess amount of Al/Fe and the weakest flocculation bridging of CQAS.

3.5 The interaction between tryptophan-like and tyrosine-like or humic-like component

Figure 2A showed that the aggregation of components related with $S_{350-400}$ was the key factor influencing sludge dewaterability. Furthermore, from Figure 3, there were some overlaps between tryptophan-like and tyrosine-like or humic-like components in the emission spectra and quantum yield. Therefore, VPA was applied to assess the contribution made by two or three of the fluorescence components for each EPS type (SI, Figure S3). The results showed (SI, Figure S3) that the components of LB-EPS were very similar to the other EPS types. Moreover, the tryptophan-like component emits at a maximum of 320 nm in an environment with a low polarity (Xiao et al., 2019). Since the intensity of 320 nm for LB-EPS was greatest, the tryptophan-like component for LB-EPS was much more hydrophobic than the other EPS. However, since the excitation wavelength of hydrophobic humic acid was greater than the hydrophilic fulvic acid (Xiao et al., 2020), the humic-like component of LB-EPS was more hydrophilic compared with the other EPS, suggesting there was more hydrophobic humic acid removed for LB-EPS. The interactive contribution of EEM for all EPS could be explained only by tryptophan-like $\times$ tyrosine-like and tryptophan-like $\times$ humic-like because the R_{adj}^2 values of tyrosine-like $\times$ humic-like and tryptophan-like $\times$ tyrosine-like $\times$ humic-like were 0 for all EPS samples (SI, Figure S4). Since the R_{adj}^2 (tryptophan-like + tyrosine-like/ humic-like) for S-EPS and TB-EPS were both much greater than that for LB-EPS, the LB-EPS was the key barrier for sludge dewatering.

From Figure 5A, the total of R_{adj}^2 (tryptophan-like + tyrosine-like/ humic-like) increased with increasing CQAS to Al/Fe ratio for S-EPS, suggesting the aggregation of components was mainly improved by flocculation bridging of CQAS. This was consistent with the detection of emission spectra and quantum yield for S-EPS. Moreover, the Al/Fe was the barrier for the aggregation of components because the flocculation bridging of CQAS was enough for destabilization of components and the residual Al/Fe could not affect charge neutralization but improve the stabilization of the components. As shown in Figure 5B, the sum of R_{adj}^2 (tryptophan-like + tyrosine-like/ humic-like) increased with increasing CQAS to Al/Fe ratio, and reached the maximum value at 1:1, then decreased to the minimum value at 1:0.5, followed by the slight increase at 1:0. These indicated that the aggregation of components was mainly improved by flocculation bridging of CQAS and the magnitude of improvement was greatly decreased as the CQAS dosage exceeded the Al/Fe dosage. There was the greatest aggregation of sludge colloids for 1:1, which was consistent with the greatest sludge dewaterability, emission intensity and quantum yield at the ratio of 1:1. Therefore, the flocculation bridging of CQAS in LB-EPS was less than that in S-EPS and the sludge colloids aggregation for LB-EPS was influenced by the synergy between the flocculation bridging of CQAS and charge neutralization of Al/Fe. Furthermore, the total of R_{adj}^2 (tryptophan-like + tyrosine-like/ humic-like) for ratios of 0:3.6 and 1:1.5 were much greater than that of 1:0.5 and 1:0, which suggested that the flocculation bridging of CQAS for TB-EPS was less than that for S-EPS. As shown in Figure 5C, the sum of R_{adj}^2 (tryptophan-like + tyrosine-like/ humic-like) decreased to a minimum value with increasing ratio of CQAS to Al/Fe from 0: 3.6 to 1:1, and then increased gradually from 1:1 to 1:0. This suggested that there was the largest amount of Al/Fe chelated in TB-EPS at 1:1 and the weakest component aggregation. As the TB-EPS is mainly comprised by hydrophobic substances (Guo et al., 2016), the component aggregation did not influence sludge dewaterability. Moreover, the sum of R_{adj}^2 (tryptophan-like + tyrosine-like/ humic-like) for 0:3.6 was greater than that for 1:0, illustrating that the flocculation bridging of CQAS decreased and the Al/Fe was the principal factor affecting the aggregation of sludge colloids.

3.6 The variations of functional groups for sludge cakes

As shown in Figure 6A, the peaks at 2935 cm^{-1} and 2680 cm^{-1} were assigned to aliphatic C-H, and the peaks at 1640 cm^{-1} , 1530 cm^{-1} and 1450 cm^{-1} were attributed to amino groups, which were mainly derived from the protein structure. Since the normalization of spectra was conducted based on the peak value of the C-O group for polysaccharide at 1015 cm^{-1} , as the CQAS to Al/Fe ratio increased, the intensities of peaks related to the protein structure increased and reached the greatest for a ratio of 1:1, then decreased, which was consistent with the sludge dewatering variation. This indicated that the ratio of protein to polysaccharide increased due to the release of polysaccharides to the liquid phase during the filtration. According to previous studies, the protein component in EPS was more important than polysaccharides because the increased binding by the protein content improves the sludge dewatering performance (Higgins and Novak, 1997). There was a strong and positive correlation between increased sludge filterability and the removal of protein and neutrals with low molecular weight (Xiao et al., 2017).

From Figure 6B, the peaks at about 284.7, 286.2, 288 and 289.5 binding energy (eV) were assigned to C-C, C-O, C=O and O-C=O groups, respectively (Liu et al., 2020). Their atomic percent (at. %) decreased gradually with increasing eV, and the HO-C=O group (-COOH) only appeared as the Al/Fe was added without CQAS, indicating there were -COOH groups available for chelating Al^{3+} or Fe^{3+} . Figure 6C shows that the peaks at around 400 and 402 eV were derived from the groups of NH_2 and the NH_2 chelated with metal ion ($\text{NH}_2\text{-Metal}$), respectively. There was a $\text{NH}_2\text{-Metal}$ group only for the sample with a ratio of 0:3.6. These suggested that compared with other samples, the chelating ability of Al^{3+} or Fe^{3+} by the NH_2 and -COOH groups of the protein was greater for the sample with the dosage of 3.6% Al/Fe. Figure 6D displayed peaks at around 531.5 and 532.5 eV, which were attributed to the C-O group and the OH group of $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$ or SiO_2 . The peak at 532.7 eV for RDS was mainly derived from SiO_2 because the content of SiO_2 was much larger than other compounds (SI, Table S2). As the CQAS to Al/Fe ratio was 1:1, the peak at 532.7 eV increased from 35.1% to 43.5%, while the peak increased to 80% for the dosage of 3.6% Al/Fe. This indicated that the amount of $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$ generated for the ratio 0:3.6 sample was larger than that for the ratio 1:1 sample due to the greater dosage of Al/Fe. These all illustrated that the amount

of Al^{3+} and Fe^{3+} chelated by HO-C=O and $-\text{NH}_2$ groups of protein decreased after the replacement of Al/Fe with CQAS. As shown in Figure S5, the peaks of Al 2p and Fe 2p for all samples were similar, indicating that the groups of flocs formed by Al and Fe were the same as the compounds of Al and Fe contained in the RDS.

3.7 The simulation of cake dewatering curves and the surface images of cakes

As shown in Figure 7A and Table 3, the irreducible saturation (S_∞), which is the volume ratio of liquid in the cake to the voids, and specific resistance (SRF) were the lowest for the ratio 1:1 sample. This indicated that as the flow of the liquid ceases, the amount of filtrate retained in the cake voids was less than other samples, and the dewatering forces could more easily overcome the surface tension forces retaining fluid in the cake. Moreover, the porosity, capillary number and permeability of the sludge cake increased to a maximum for the 1:1 sample, illustrating that the fluid would more easily flow through its voids because the magnitude of the permeability is determined by the degree of openness of the cake, which is related to the porosity and capillary pores present in its internal structure. The complexity of the internal pore structures was shown in the SEM images. From observation of Figures 7B-7F, the number of nanosized pores increased, and then decreased, with increasing ratio of CQAS to Al/Fe, and the pore numbers appeared to be greatest for the 1:1 ratio sample compared with other ratios. The threshold pressure (P_b) is a way of expressing the minimum pressure difference applied across a cake to effect any dewatering (Wu et al., 2023b). The results (Table 3) show that the P_b of the sample with a ratio of 1:1 was the lowest, suggesting that the pressure required to drive the filtrate across the cake was much lower than for the other samples. The size of the sludge particles has a major effect on the compaction, where greater forces are required to form cakes with the same moisture content but smaller particle size. The average particle size was the greatest for the 1:1 ratio sample and this was consistent with the results of the PCA analysis, which indicated that the sludge dewaterability was impacted significantly by sludge floc size. This also suggested that the imposed mechanical forces were more appreciable compared with the interparticle repulsive forces and the permeability of the filter cake was the greatest.

3.8 The mechanism of the Al/Fe replacement with CQAS for improving dewaterability

As summarized in Figure 8, if the Al/Fe was added as the only pretreatment chemical for RDS dewatering, its dosage would need to be very great because the -NH_2 and -COOH groups of protein chelate Al^{3+} or Fe^{3+} during coagulation to reduce their effective dosages. In contrast, if the CQAS acts as the pretreatment chemical first, the CQAS attached to one particle can subsequently attach to vacant sites on an approaching particle to achieve bridging flocculation. This in turn implies that the available particle surface area of proteins would be completely or partly covered such that there would be a decrease in the amount of -NH_2 and -COOH groups available to chelate metal ions, thereby increasing the effective dose of Al^{3+} and Fe^{3+} . Moreover, the flocculation bridging of CQAS decreases, while the amount of protein chelated with Al/Fe increases, from the outer S-EPS layer to the inner TB-EPS layer.

The effective content of Al^{3+} and Fe^{3+} can act to neutralize protein charge, described as adsorption destabilization, thereby reducing the extent of double layer repulsive interactions, and hence decreasing the potential energy barrier of proteins to coagulation. Moreover, Al^{3+} and Fe^{3+} are strongly hydrated to form complexes of the type $\text{Al}(\text{H}_2\text{O})_6^{3+}$ and $\text{Fe}(\text{H}_2\text{O})_6^{3+}$, which tend to hydrolyze and give rise to a series of hydroxylated species. For a hydrolyzing salt to be an effective coagulant, it should be used at a concentration less than the solubility of its hydroxide at the solution pH. Therefore, at the optimum ratio of CQAS to Al/Fe, with the adsorption of suitable species at protein surfaces, a coagulant bridge spanning adjacent proteins is formed, which promotes protein destabilization (Xiao et al., 2023c). Furthermore, with precipitating coagulants, the physical presence of the protein precipitate can greatly enhance the kinetics of the coagulation reaction by physically entrapping protein or other materials within the expanded aggregate structure, via sweep coagulation, to improve protein aggregation. Thus, the hydrophobicity and particle size of proteins increased by adsorption destabilization and sweep coagulation, resulting in the enhanced aromatic content of protein. As a result, the sludge dewatering efficiency was increased, while the cake conductivity decreased, after the substitution of Al/Fe by CQAS.

4 Conclusion

The partial replacement of Al/Fe coagulant by CQAS substantially enhanced sludge dewatering, reducing the moisture content of the sludge cake from 60% to 50%. An optimum mass ratio of CQAS to Al/Fe was found to be 1:1, in terms of the highest solids content and the lowest CST. Through numerical analysis by CPA and RDA, the content of aromatic protein was identified as the key property determining the sludge dewaterability, and was related to the CQAS dosage. The combination of fluorescent protein-like and humic-like components of LB-EPS was closely related to the extent of sludge dewatering. The simulation of cake dewatering and SEM results showed that the sludge porosity, and especially the number of capillary pores, was positively related to the extent to which the dewatering forces (applied pressure) can overcome the surface tension force retaining fluid in the cake. The fluorescence emission intensity, quantum yield and the XPS results indicated that the bridging flocculation of CQAS decreased the amount of -NH₂ and -COOH groups of proteins with metal ion chelating properties, thereby increasing the effective dose of Al³⁺ and Fe³⁺. In addition, the cost of Al/Fe before substitution was calculated to be almost 1.5 times the total cost of Al/Fe and CQAS after substitution (SI, Table S3). Therefore, this study has demonstrated an effective and cost-effective method to enhance sewage sludge dewatering, and achieve a substantial reduction in cake conductivity, by the partial replacement of a conventional inorganic coagulant with a natural polymer, thereby increasing the sustainability of sewage sludge disposal. Moreover, this study also indicated that the dosing of metal-free flocculant before the inorganic coagulant could enhance the cost-effectiveness and sustainability of the combined coagulant treatment.

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.

Acknowledgments

This work was also supported by the National Key Research and Development Program of China (2019YFC1906503), the National Natural Science Foundation of China (Grants

52200027).

Author contributions

M. L. and W.Y. have contributed to the design of the study and the critical revision of the article.

M. L., S. R., W. W., H. Z., Y. Z., and X. F. did the experiments, analyzed the data, prepared figures and drafted the article.

M. L., N.G. and W.Y. finished the final version of the manuscript.

Appendix A. Supplementary data

The Supporting Information contains 4 sections of Texts, 3 Tables and 5 Figures that present the following: Calculation of the coagulant dosage (Text S1); Measurement of sludge cake conductivity (Text S2); Graphical representation of fluorescence emission spectra, and fluorescence quantum yield spectra (Text S3); The contributions of Tryptophan-like and Tyrosine-like or Humic acid-like analyzed by VPA (Text S4); VPA calculation (Table S1); The mass contents of elements and compounds for RDS, and samples of conditioned sludge with CQAS to Al/Fe mass ratios of 0:3.6 and 1:1 sample (Table S2); The cost of CQAS and Al/Fe before and after substitution (Table S3); VPA conceptual diagram (Figure S1); The specific UV-visible absorbance (SUVA) spectra of S-EPS, LB-EPS and TB-EPS for various samples and the fluorescence components extracted from all EPS layers (Figure S2); The fluorescence components extracted from the EEM data sets of samples for S-EPS, LB-EPS and TB-EPS using PARAFAC (Figure S3); Venn diagrams of the variation partitioning of EEM data sets of samples for S-EPS, LB-EPS and TB-EPS explained by tryptophan-like, tyrosine-like and humic-like components (Figure S4); XPS spectra of Al 2p and Fe 2p for RS and the sludge at the CQAS to Al/Fe ratios of 0:3.6 and 1:1 (Figure S5).

References

- Cao, B., Zhang, T., Zhang, W. and Wang, D. 2021. Enhanced technology based for sewage sludge deep dewatering: A critical review. *Water Res.* 189.
- Cao, B., Zhang, W., Du, Y., Wang, R., Usher, S.P., Scales, P.J. and Wang, D. 2018.

Compartmentalization of extracellular polymeric substances (EPS) solubilization and cake microstructure in relation to wastewater sludge dewatering behavior assisted by horizontal electric field: Effect of operating conditions. *Water Res.* 130, 363-375.

Delahaije, R.J.B.M., Hilgers, R.J., Wierenga, P.A. and Gruppen, H. 2017. Relative contributions of charge and surface coverage on pH-induced flocculation of protein-stabilized emulsions. *COLLOID SURFACE A* 521, 153-160.

Fuchs, T. and Jupke, A. 2022. Comparison of the impact of anion and cation selection onto cation exchange chromatography of model proteins. *J CHROMATOGR A* 1673.

Guo, X., Wang, X. and Liu, J. 2016. Composition analysis of fractions of extracellular polymeric substances from an activated sludge culture and identification of dominant forces affecting microbial aggregation. *Sci. Rep* 6.

He, S., Feng, L., Zhao, W., Li, J., Zhao, Q. and Wei, L. 2023. Composition and molecular structure analysis of hydrophilic/hydrophobic extracellular polymeric substances (EPS) with impacts on sludge dewaterability. *Chem. Eng. J* 462.

Higgins, M.J. and Novak, J.T. 1997. Characterization of exocellular protein and its role in bioflocculation. *J Environ. Eng.-Asce* 123(5), 479-485.

Kankariya, Y. and Chatterjee, B. 2023. Biomedical Application of Chitosan and Chitosan Derivatives: A Comprehensive Review. *CURR PHARM DESIGN* 29(17), 1311-1325.

Li, X.Y. and Yang, S.F. 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Res.* 41(5), 1022-1030.

Lin, W., Chen, R., Liu, X., Ngo, H.H., Nan, J., Li, G., Ma, J., He, X. and Ding, A. 2022. Deep mechanism of enhanced dewaterability of residual sludge by Na^+ : Comprehensive analyses of intermolecular forces, hydrophilicity and water-holding capacity of EPS. *Chem. Eng. J* 450.

Liu, C.G., Han, Q., Chen, Y., Zhu, S.Y., Su, T., Qu, Z., Gao, Y.D., Li, T., Huo, Y. and Huo, M.X. 2021. Resource Recycling of Mn-Rich Sludge: Effective Separation of Impure Fe/Al and Recovery of High-Purity Hausmannite. *Acs Omega* 6(11), 7351-7359.

Liu, M., Zhao, Z., Lu, Q. and Yu, W. 2022. Release of dissolved organic carbon from

biochar and formation of humic-like component during photoreaction: Effects of Ca^{2+} and pH. *Water Res.* 219.

Liu, M., Zhao, Z. and Yu, W. 2020. Citric acid modified wood membranes for efficient adsorption of tetracycline: Effect of alkali pretreatment concentration and adsorption mechanism. *Chem. Eng. J* 393.

Peng, S., Wang, Z., Yu, P., Liao, G., Liu, R., Wang, D. and Zhang, W. 2023. Aggregation and construction mechanisms of microbial extracellular polymeric substances with the presence of different multivalent cations: Molecular dynamic simulation and experimental verification. *Water Res.* 232.

Poulin, B.A., Ryan, J.N. and Aiken, G.R. 2014. Effects of Iron on Optical Properties of Dissolved Organic Matter. *Environ. Sci. Technol.* 48(17), 10098-10106.

Sam, S.B., Ward, B.J., Niederdorfer, R., Morgenroth, E. and Strande, L. 2022. Elucidating the role of extracellular polymeric substances (EPS) in dewaterability of fecal sludge from onsite sanitation systems, and changes during anaerobic storage. *Water Res.* 222.

Wang, Q., Zhang, W., Yang, Z., Xu, Q., Yang, P. and Wang, D. 2018. Enhancement of anaerobic digestion sludge dewatering performance using in-situ crystallization in combination with cationic organic polymers flocculation. *Water Res.* 146, 19-29.

Wei, H., Ren, J., Li, A. and Yang, H. 2018. Sludge dewaterability of a starch-based flocculant and its combined usage with ferric chloride. *Chem. Eng. J* 349, 737-747.

Wu, B., Dai, X. and Chai, X. 2020. Critical review on dewatering of sewage sludge: Influential mechanism, conditioning technologies and implications to sludge re-utilizations. *Water Res.* 180.

Wu, B., Li, H., Zhou, K., Yu, N., Xu, Q., Chai, X. and Dai, X. 2023a. Crystallization-driven evolution of water occurrence states with implications on dewaterability improvement of waste-activated sludge. *Water Res.* 244.

Wu, B., Wang, H., Dai, X. and Chai, X. 2021. Influential mechanism of water occurrence states of waste-activated sludge: specifically focusing on the roles of EPS micro-spatial distribution and cation-dominated interfacial properties. *Water Res.* 202.

Wu, B., Yang, D., Yu, N., Li, H., Ye, W. and Dai, X. 2023b. A quantitative theory integrating

solid surface hydrophilicity and pore structure features for non-phase-change drying of sewage sludge through gradient increase of ultrahigh filtration pressure. *Water Res.* 247.

Xiao, J., He, D., Ye, Y., Yang, B., Duan, A. and Wang, D. 2023a. Recent progress in persulfate to improve waste activated sludge treatment: Principles, challenges and perspectives. *Chem. Eng. J.* 469.

Xiao, K., Chen, Y., Jiang, X., Yang, Q., Seow, W.Y., Zhu, W. and Zhou, Y. 2017. Variations in physical, chemical and biological properties in relation to sludge dewaterability under Fe (II) - Oxone conditioning. *Water Res.* 109, 13-23.

Xiao, K., Han, B., Sun, J., Tan, J., Yu, J., Liang, S., Shen, Y. and Huang, X. 2019. Stokes Shift and Specific Fluorescence as Potential Indicators of Organic Matter Hydrophobicity and Molecular Weight in Membrane Bioreactors. *Environ. Sci. Technol.* 53(15), 8985-8993.

Xiao, K., Xu, R. and Lai, Z. 2023b. A method for detecting the properties of sludge, p. 9.

Xiao, K., Yu, J., Wang, S., Du, J., Tan, J., Xue, K., Wang, Y. and Huang, X. 2020. Relationship between fluorescence excitation-emission matrix properties and the relative degree of DOM hydrophobicity in wastewater treatment effluents. *Chemosphere* 254.

Xiao, T., Wang, H., Wang, X., Wu, H., Yuan, S., Dai, X. and Dong, B. 2023c. New strategy of drinking water sludge as conditioner to enhance waste activated sludge dewaterability: Collaborative disposal. *Water Res.* 233.

Xu, Q., Wang, Q., Zhang, W., Yang, P., Du, Y. and Wang, D. 2018. Highly effective enhancement of waste activated sludge dewaterability by altering proteins properties using methanol solution coupled with inorganic coagulants. *Water Res.* 138, 181-191.

Xu, Y., Wu, Y., Zhang, X. and Chen, G. 2021. Effects of freeze-thaw and chemical preconditioning on the consolidation properties and microstructure of landfill sludge. *Water Res.* 200.

Yang, G., Zhang, G. and Wang, H. 2015. Current state of sludge production, management, treatment and disposal in China. *Water Res.* 78, 60-73.

Yu, J., Xiao, K., Xu, H., Li, Y., Xue, Q., Xue, W., Zhang, A., Wen, X., Xu, G. and Huang, X.

2023. Spectroscopic fingerprints profiling the polysaccharide/protein/humic architecture of stratified extracellular polymeric substances (EPS) in activated sludge. *Water Research* 235.
- Yu, J., Xiao, K., Xu, H., Qi, T., Li, Y., Tan, J., Wen, X. and Huang, X. 2021. Spectroscopic sensing of membrane fouling potential in a long-term running anaerobic membrane bioreactor. *Chem. Eng. J* 426.
- Yu, J., Xiao, K., Xue, W., Shen, Y.-x., Tan, J., Liang, S., Wang, Y. and Huang, X. 2020. Excitation-emission matrix (EEM) fluorescence spectroscopy for characterization of organic matter in membrane bioreactors: Principles, methods and applications. *Front. Environ. Sci.* 14(2).
- Zhang, D., Wang, Y., Gao, H., Fan, X., Guo, Y., Wang, H. and Zheng, H. 2019. Variations in macro and micro physicochemical properties of activated sludge under a moderate oxidation-in situ coagulation conditioning: Relationship between molecular structure and dewaterability. *Water Res.* 155, 245-254.
- Zhang, W., Cheng, H., Peng, S., Li, D., Gao, H. and Wang, D. 2020. Performance and mechanisms of wastewater sludge conditioning With slag-based hydrotalcite-like minerals (Ca/Mg/Al-LDH). *Water Res.* 169.
- Zhu, Y., Xiao, K., Zhou, Y., Yu, W., Tao, S., Le, C., Lu, D., Yu, Z., Liang, S., Hu, J., Hou, H., Liu, B. and Yang, J. 2020. Profiling of amino acids and their interactions with proteinaceous compounds for sewage sludge dewatering by Fenton oxidation treatment. *Water Res.* 175.

Table1. Principal properties of RDS

Moisture content (%)	Specific resistance of sludge (SRF) (10^{12} m/kg)	pH	Total suspended solid (g/L)	CST (s)	TOC (mg/g SS)
94.0±0.05	6.11 ± 0.14	7.8 ± 0.05	6.00±0.05	596.2±11.9	51.16±0.47

Table 2. The dosage, mass ratio of CQAS and Al/Fe and the replacement ratio of Al/Fe

The mass ratio of CQAS to Al/Fe	1:0	1:0.5	1:1	1:1.5	0:3.6
The dosage of CQAS (mL/100 mL sludge)	1	1	1	1	0
The mass fraction of CQAS in dry solid of sludge (%)	0.83	0.83	0.83	0.83	0
The dosage of Al/Fe (mL/100 mL sludge)	0	0.25	0.5	0.75	1.8
The mass fraction of Al/Fe in dry solid of sludge (%)	0	0.42	0.83	1.25	3
The replacement ratio of Al/Fe by CQAS (%)	100	86.1	72.2	58.3	0

Table 3. The properties of filter cakes

The ratio of CHA to PAFC	Irreducible saturation S_{∞}	Porosity ε	Thickness of filter cake L(m)	Capillary number N_{cap}	Average particle diameter x (m)	Threshold pressure P_b (Pa)	SRF (m/kg)	Permeability k (m ⁻²)
0:3.6	0.186	0.939	0.0096	0.0222	4.23×10^{-7}	50501.0	7.78×10^{11}	1.41×10^{-14}
1:1.5	0.174	0.942	0.0094	0.0596	6.51×10^{-7}	31244.9	6.17×10^{11}	1.87×10^{-14}
1:1	0.157	0.946	0.0083	4.6197	5.01×10^{-6}	3777.0	6.13×10^{11}	2.01×10^{-14}
1:0.5	0.209	0.932	0.0074	0.0072	2.40×10^{-7}	100166.1	8.23×10^{11}	1.20×10^{-14}
1:0	0.228	0.908	0.0071	0.0039	2.42×10^{-7}	138779.0	8.32×10^{11}	8.70×10^{-15}

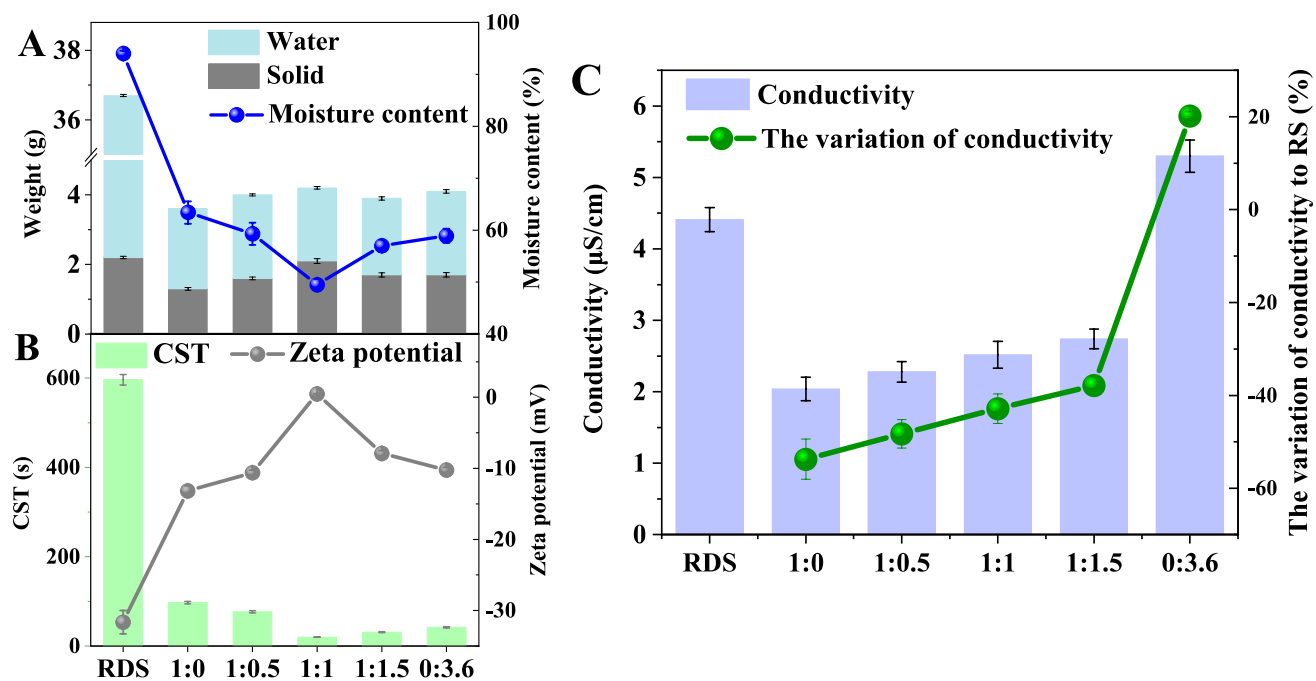
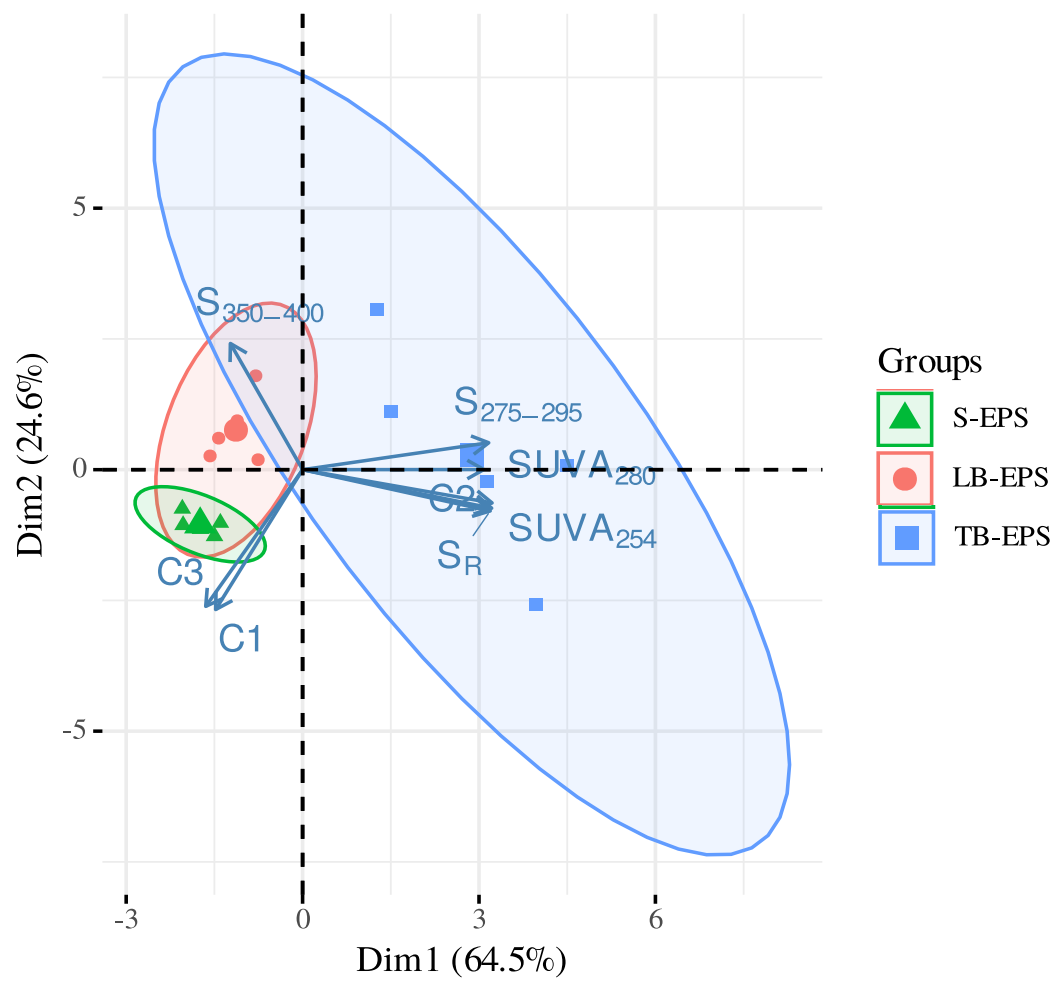


Figure 1. (A) The weights of water and solid, and moisture content for dewatered cakes, (B) the CST and zeta potential, and (C) the conductivity for RDS and the conditioned sludge, at various ratios of CQAS to Al/Fe.

A PCA - Biplot



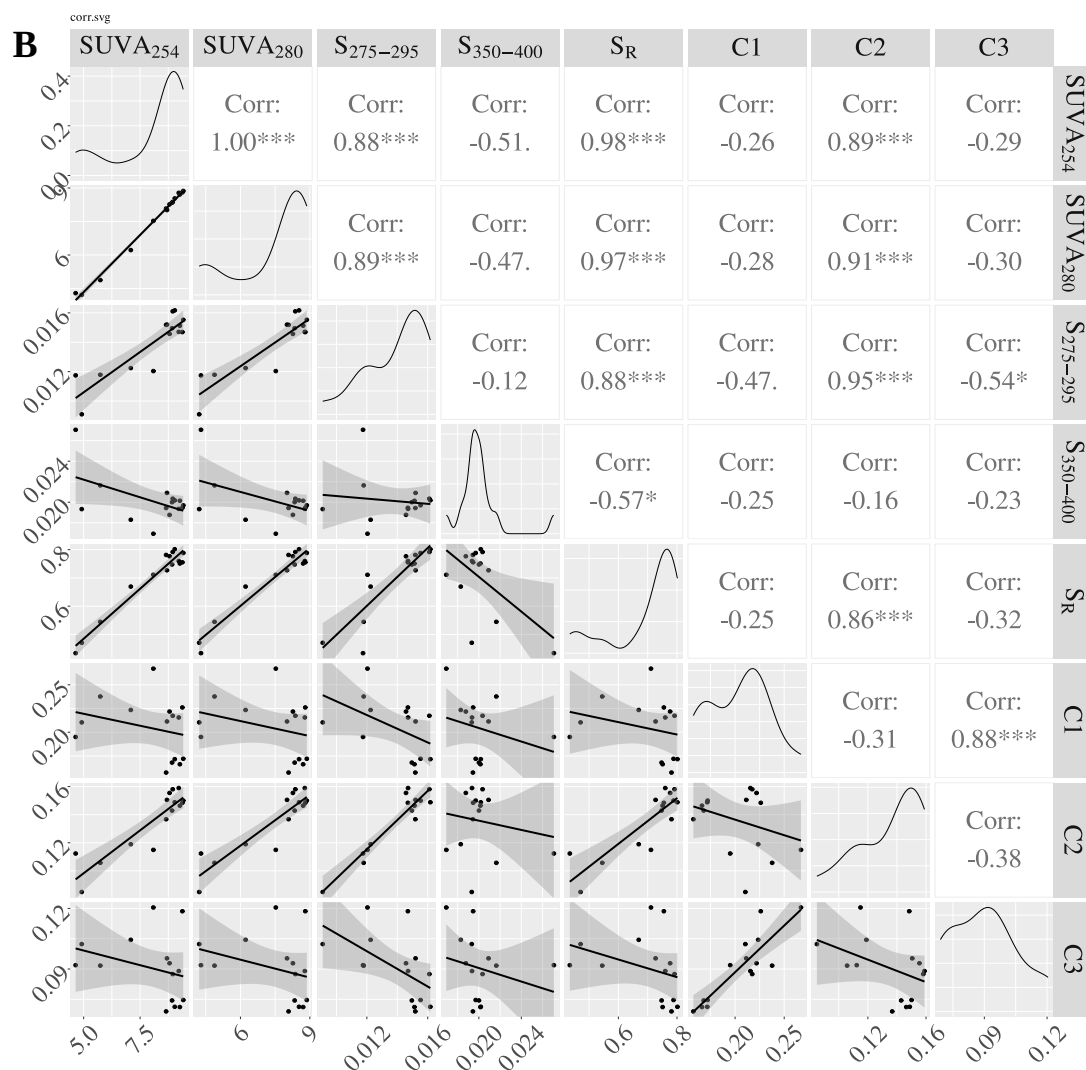


Figure 2. (A) PCA, and (B) the relationships among the indexes and data scatter diagram (below the diagonal line), for the conditioned sludge at various ratios of CQAS to Al/Fe.

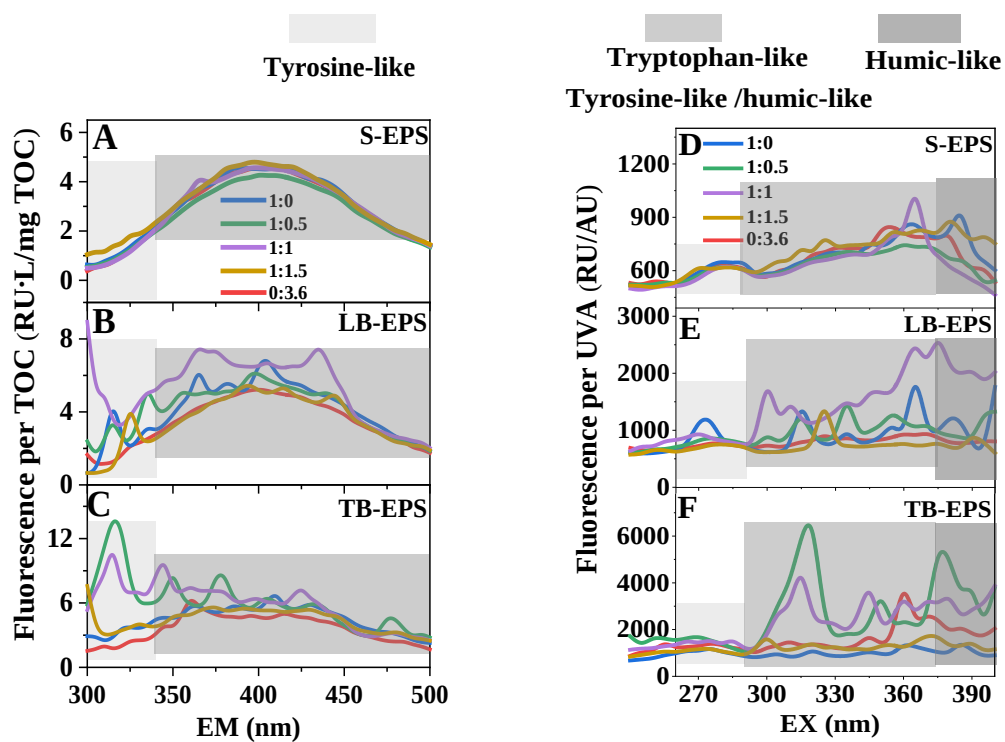


Figure 3. Fluorescence emission spectra for (A) S-EPS, (B) LB-EPS and (C) TB-EPS, and Fluorescence quantum yield spectra for (D) S-EPS, (E) LB-EPS and (F) TB-EPS.

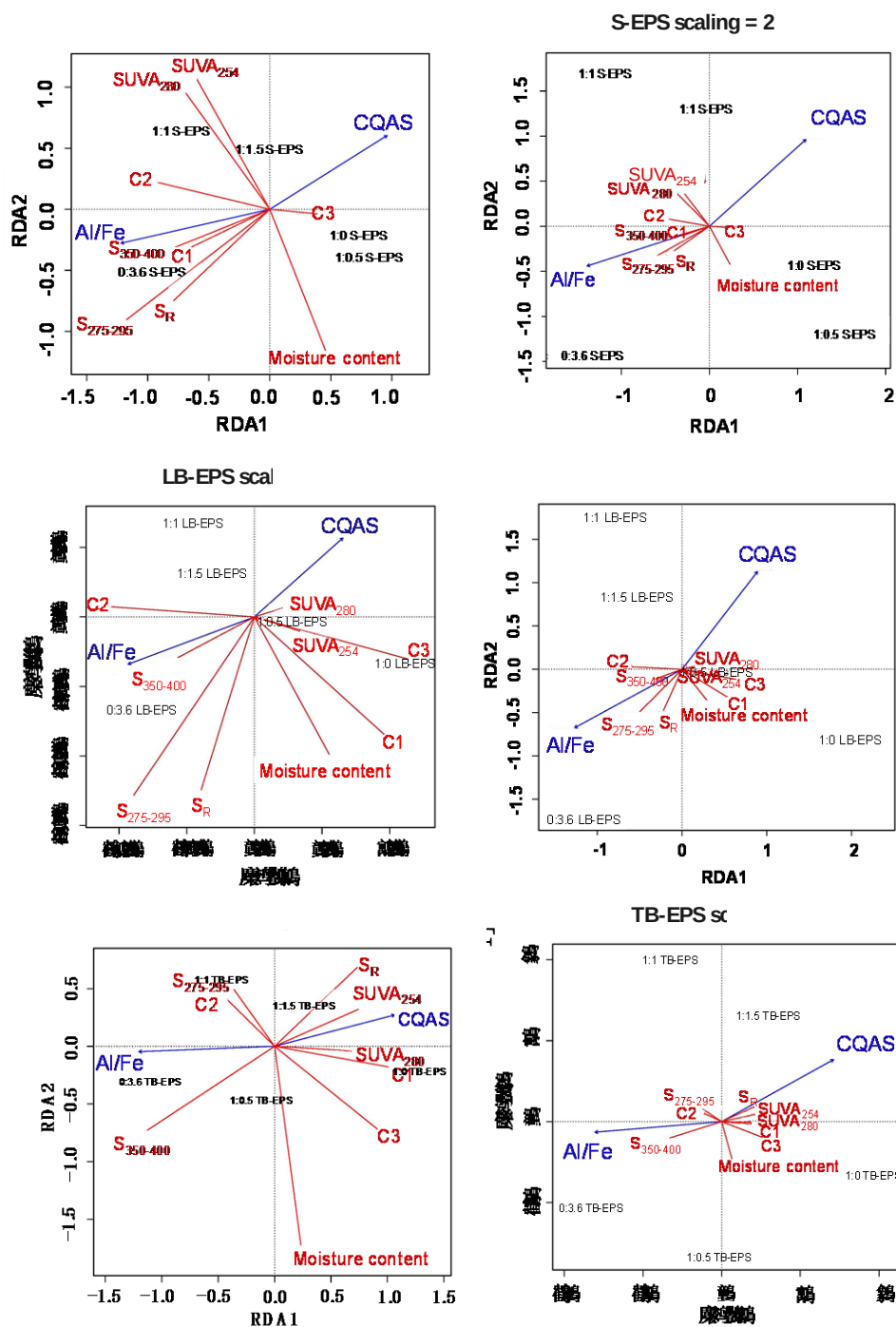


Figure 4. The RDA analysis of samples and indexes of (A, B) S-EPS, (C, D) LB-EPS and (E, F) TB-EPS.

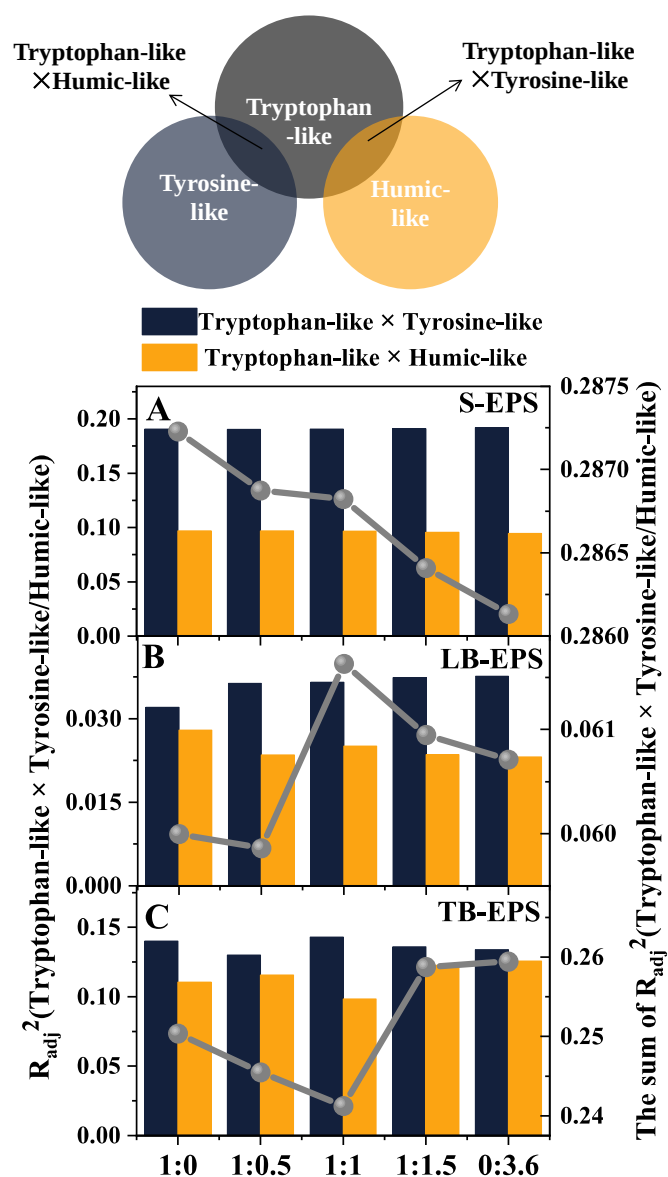


Figure 5. The $R_{adj}^2(\text{Tryptophan-like} \times \text{Tyrosine-like})$ and $R_{adj}^2(\text{Tryptophan-like} \times \text{Humic acid-like})$ by VPA and their sum for (A) S-EPS, (B) LB-EPS and (C) TB-EPS of conditioned sludge at various ratios of CQAS to Al/Fe.

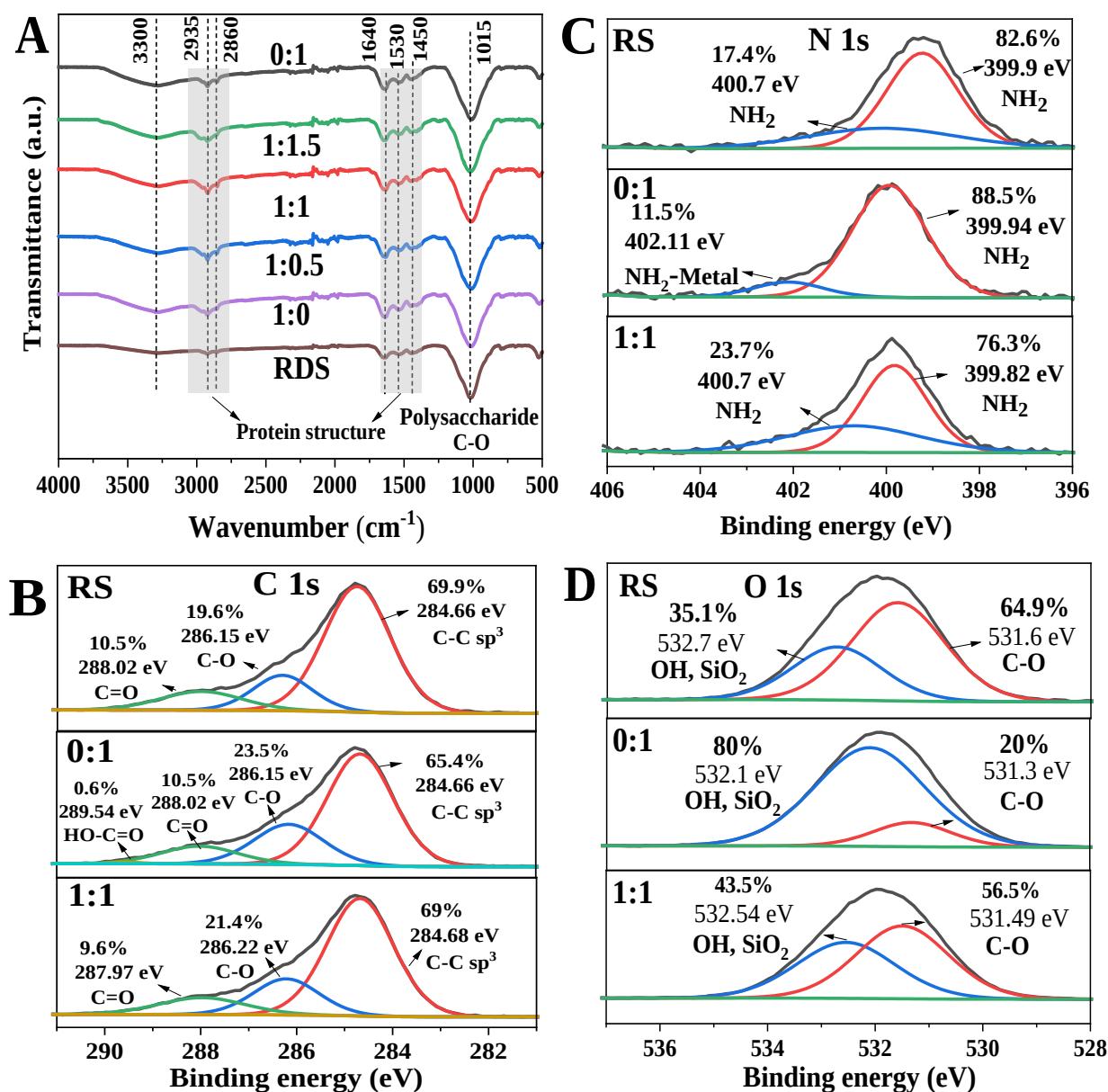


Figure 6. (A) The FTIR spectra for sludge cakes by different conditioning processes; the XPS spectra of (B) C 1s; (C) N 1s and (D) O 1s for the sludge cakes of RS, and the CQAS to Al/Fe ratios equal to 0:3.6 and 1:1. The % values of various groups were denoted with the eV values.

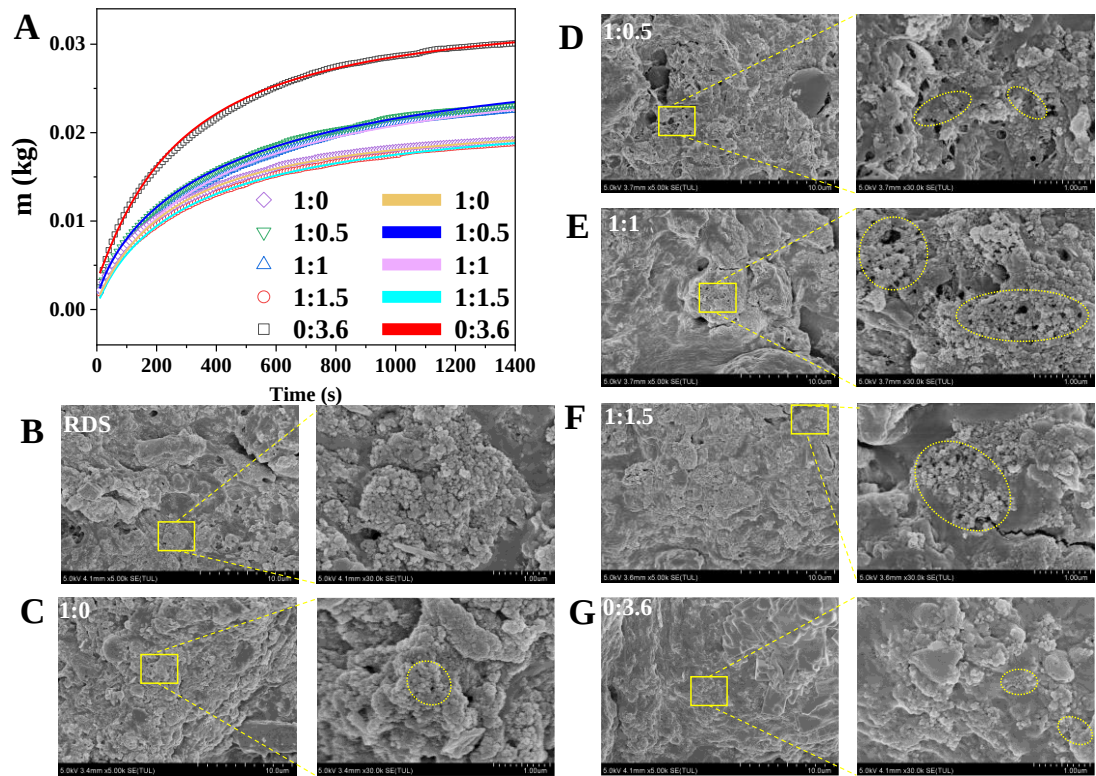


Figure 7. (A) The variation and modeling of filtrate mass for the sludge by different conditioning processes; the SEM images of samples for (B) RDS; (C) 1:0; (D) 1:0.5; (E) 1:1; (F) 1:1.5 and (G) 0:3.6. Higher resolution images are denoted by the yellow rectangles on the original images, and the nanosized pores are indicated by the yellow circles.

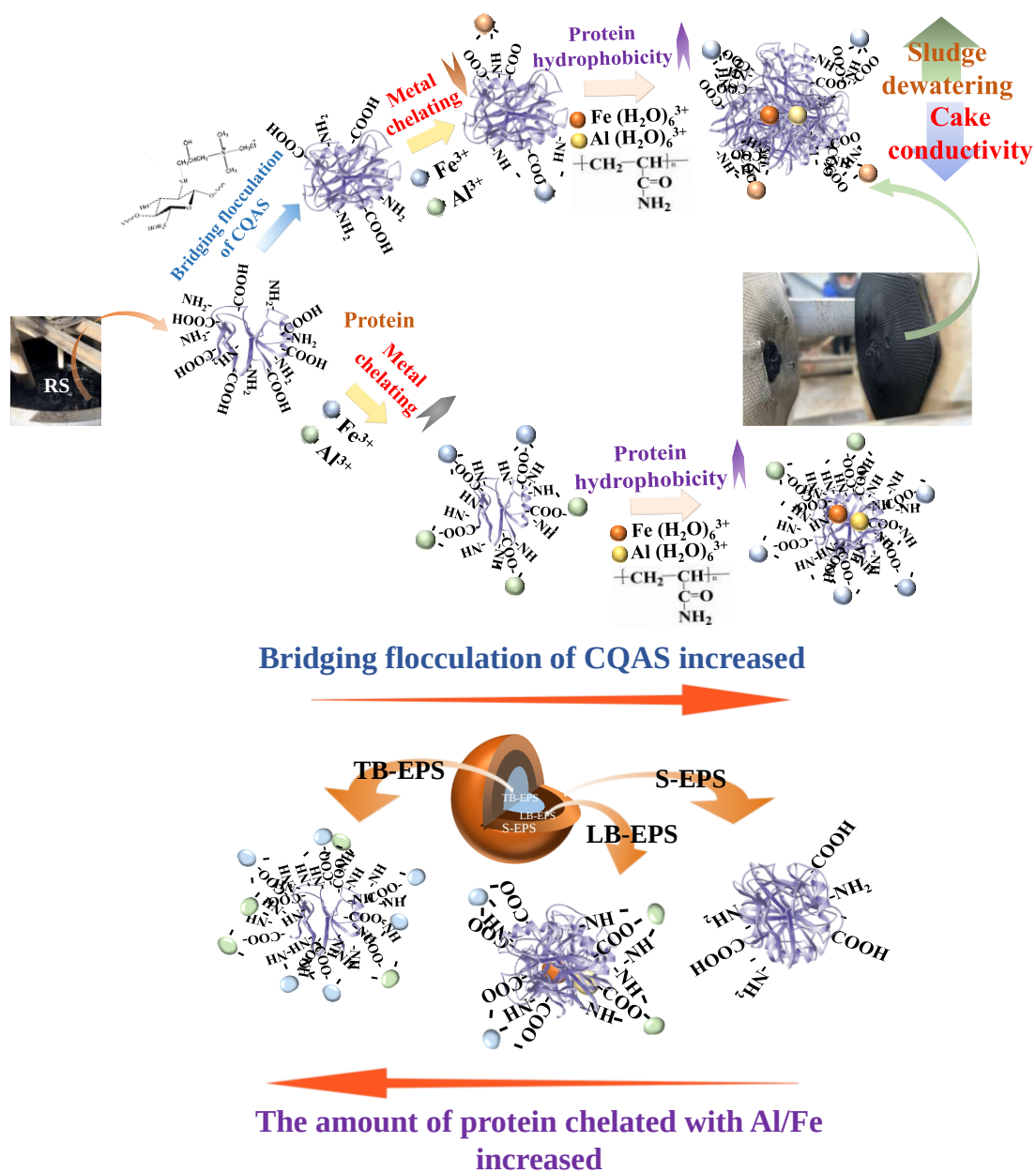


Figure 8. The mechanism of enhanced sludge dewatering by the replacement of Al/Fe with CQAS.