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ORIGINAL PAPER

Distribution Characteristics of Extracellular Polymeric Substance Extracted from Dewatered Sludge Treated with Enzymes and Thermal Pressure

Weiyun Wang[1,2] · Lingyun Wang[1,2] · Xuemei Gao[1,2] · Tianhua Yang[1,2] · Rundong Li[1,2]

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Abstract Extracellular polymeric substance (EPS), a high molecular weight biological polymer that can bind to a large amount of organic matter and moisture, plays a vital role in the process of sludge formation and structural stability, affecting sedimentation and dewatering performance of sludge. The aim of this study was to observe the changes in the key components of EPS extracted from mechanical dewatered sludge before and after enzyme and thermal pressure treatment. The results showed that the soluble protein content increased by 160 and 110% and the polysaccharide content increased by 180 and 200% after adding 0.03 g/g TSS neutral protease and alpha-amylase, respectively. The effect of compound enzyme treatment was better than that of single enzyme treatment. Furthermore, three-dimensional fluorescence spectroscopy indicated the presence of tryptophan and aromatic-like proteins, and Fourier transform infrared spectroscopy showed that the types of functional groups exhibited a sharp decrease in the loosely bound-EPS layer after thermal pressure treatment.

Keywords Dewatered sludge · Enzyme · Thermal pressure treatment · Fluorescence spectroscopy · Infrared spectroscopy

✉ Weiyun Wang
cathywwy@sau.edu.cn

[1] College of Energy and Environment, Shenyang Aerospace University, Shenyang 110136, China

[2] Key Laboratory of Clean Energy, Shenyang 110136, Liaoning Province, China

Introduction

Mechanical dewatered sewage sludge, an inevitable product of sewage treatment, has the characteristics of complex composition and high contents of water, organic matter, heavy metals and pathogenic microorganisms. Although the problem of high moisture content, which is the bottleneck of sludge disposal, can be alleviated by different methods of sludge dewatering, high contents of organic substances and highly hydrophilic floc particles result in poor dewatering properties of excess sludge [1]. As traditional sludge treatment cannot achieve effective reduction and resource utilization, sewage sludge is a potential threat to the environment [2].

It has been reported that sludge is composed of a large amount of microorganism embedded in the polymer network structure of EPS [3]. EPS is mainly derived from intracellular secretion, cell autolysis, hydrolysis of macromolecules and wastewater adsorption, accounting for almost 50~80% of the total organic matter in the activated sludge [4]. It is well established in literature that EPS has an important effect in wastewater treatment systems [5]. The characteristics of EPS include negative charge and gel-like matrix [6], and microorganisms can survive for a longer duration in the presence of EPS forming a stable microbial colony in the flocculent structure [7, 8]. The major constituents of EPS are polysaccharide (PS) and proteins (PN), which account for 70~80% of its contents [9–11]. It has been reported that most of the bound water combines with EPS in the sludge [12], and the PS and PN in the EPS significantly contribute to improving the ability of sludge floc to combine with water [13]. Higgins and Novak targeted extracellular proteins to study their relationship with the settling and dewatering properties of activated sludge and observed a direct correlation between the bound

EPS protein content and the sludge settling properties [14]. Sunil S. Adav et al. used situ fluorescent staining and confocal laser scanning microscopy to probe the contributions of individual components in EPS on structural stability of sludge, and draw a conclusion that the β-polysaccharides were expected to form the backbone of a network-like outer layer with embedded proteins, lipids, α-polysaccharides, and cells to support the mechanical stability of granules [15].

Depending on the degree of integration of the organic sludge with the cell phase, EPS in the sludge can be classified into soluble EPS (SEPS), loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS) [16, 17]. Many studies have shown that the content of TB-EPS is higher than that of SEPS and LB-EPS in activated sludge, and that PN is the dominant component of EPS [18]. The quantity and quality of EPS extraction is not only related to the methods employed, but also to the sludge types [19]. As the products of different stages of sewage sludge treatment, significant differences exist between mechanical dewatered sludge and activated sludge. To the best of our knowledge, previous studies on the distribution of EPS have mainly focused on excess sludge and activated sludge, whereas research on EPS distribution in mechanical dewatered sludge is limited [20]. In this study, belt filter dewatered sludge was used for the extraction of different fractions of EPS. By comparing the PN and PS fractions extracted from different layers of EPS, the effect of the experimental process on the composition of the sludge was analyzed.

An enzyme is an efficient biological catalyst derived from the secretion of living cells. Under very mild conditions, enzymes can catalyze a variety of biochemical reactions and promote the metabolism of organisms. Compared with physical and chemical pretreatment methods, enzymes can not only accelerate the destruction of the sludge floc structure, but also can promote the dissolution of large particulate organic matter and hydrolysis of soluble macromolecular organic matter, thus improving sludge digestion and dewatering performance. In general, extracellular enzymes can be divided into two groups: free-state enzymes and cell-binding enzymes [21]. Studies have shown that the content of soluble enzymes was extremely low in sludge [22], and the enzyme activity was almost absent because the enzymes were mostly inaccessible, combined, or embedded in the sludge matrix rather than being available in the sludge solution [23]. Thus, in this paper extracellular enzymes, namely, alpha-amylase and neutral protease, were added to the sludge either as a single enzyme or compound enzyme to investigate the effects of different dosage and types of enzymes on sludge pretreatment.

Thermal press dewatering is a sludge deep dewatering process in which heat and pressure are applied simultaneously to a thin layer of mechanical dewatered sludge to separate the bound water from the liquid form of sludge. In the present study, the typical organic components such as PN and PS in the EPS were analyzed to determine the characteristics of different EPS fractions. Furthermore, three-dimensional fluorescence spectroscopy (3D-EEM) and Fourier transform infrared (FT-IR) spectroscopy were combined to analyze the key components and organic functional groups of EPS, and the law of material transformation was employed to explore the mechanism of enzyme and thermal pressure treatment in the process of sludge pretreatment from a new angle.

Materials and Methods

Sludge Samples

Raw sludge samples were collected from the belt filter of a municipal wastewater treatment plant of Dalian (China), which has a treatment capacity of 80,000 m^3/d. All the samples were stored at 4 °C and tested within 1 week after collection. The characteristics of the sludge samples are shown in Table 1.

Treatment of Sludge with Bio-enzyme

A total of 30 g of mechanical dewatered sludge sample were added into 250 ml Erlenmeyer flask. The initial moisture content of the raw sludge sample was adjusted to 90% with deionized water, and the sample was rapidly stirred with a magnetic stirrer. To determine the optimal enzyme dosage, the enzymes, neutral protease and alpha-amylase (g/g total suspended solid (TSS)), were added to the samples at different doses. The compound enzyme comprised protease and amylase at a ratio of 1:3. In a 100 ml centrifuge tube, 1 g (dry weight) of the sludge sample was mixed with 0.03 g/g TSS of the enzyme and incubated in a water bath oscillator at 50 °C and 150 rpm for 4 h. All the experiments were performed in triplicate.

Treatment of Sludge with Enzyme-Thermal Pressure

After enzyme treatment, the sludge sample was subjected to thermal pressure treatment. Figure 1 shows the laboratory setup of thermal press dewatering [24]. The sludge sample was wrapped in a polyester fabric material and placed on the mobile plate that was controlled by the operating lever. The temperature and time control knobs were located on the right control panel. The pressure gauge could be controlled

Table 1 Characteristics of the sludge samples

Water contents (%)	pH	TSS (%)	Ash (%)	VSS (%)
85	7.2	15	3.09	11.91

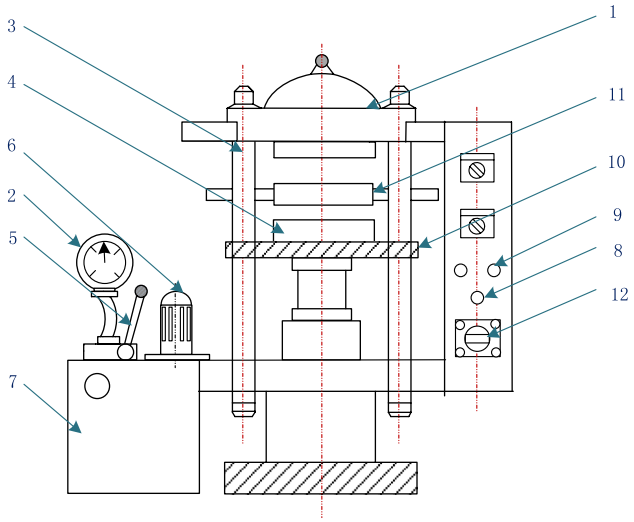

Fig. 1 Laboratory setup of thermal press dewatering. *1* upper engine base; *2* pressure gauge; *3* column shaft; *4* lower plate; *5* operating lever; *6* hydraulic oil pump; *7* cylinder; *8* power switch; *9* temperature control knob; *10* lifting plate; *11* mobile plate; *12* time-control knob

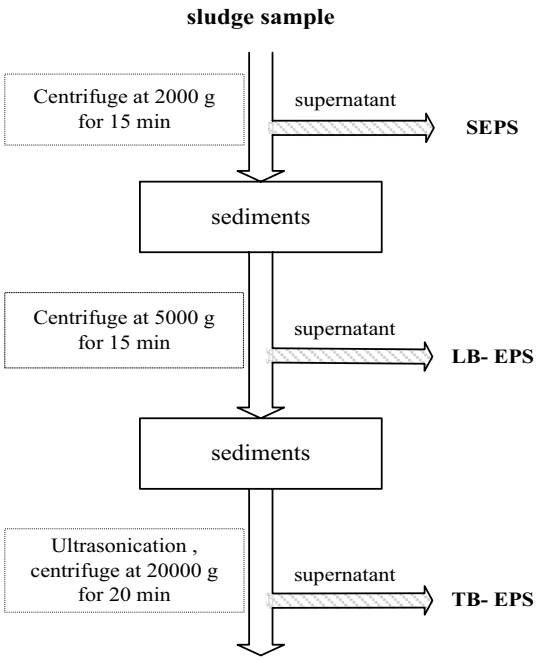

Fig. 2 Extraction process of different EPS fractions

by pressure regulator on the left side of the equipment. Three parallel samples were employed in each treatment condition.

EPS Extraction

First, EPS extraction, the volume of the sludge samples (1 g dry weight) was adjusted to 40 ml using deionized water. Subsequently, centrifugation and ultrasonic methods were used for multi-level extraction of EPS from the sludge samples to analyze the main components of EPS. The sludge samples were centrifuged in a 100 ml centrifuge tube at 2000 *g* for 15 min, and the supernatant was filtered to obtain SEPS. The sediment in the tube was then re-suspended in 40 ml of buffer solution (consisting of Na_3PO_4, NaH_2PO_4, NaCl and KCl at a molar ratio of 2:4:9:1; pH 7) and centrifuged at 5000 *g* for 15 min. The supernatant obtained was the LB-EPS fraction. Then, the residual sediments left in the centrifuge tube was re-suspended in 40 ml of buffer solution, subjected to ultrasonic treatment at 250 W for 20 min and centrifuged at 20,000 *g* for 20 min to collect the TB-EPS. The EPS extraction process is shown in Fig. 2 [24].

Analysis of EPS Components

Colorimetric Analysis

The PS content in the EPS was measured by anthrone sulfuric acid method with glucose as the standard, while the PN content in the EPS was evaluated by Lowry method using bovine serum albumin (BSA) as the standard. The DNA content in the EPS was determined by diphenylamine colorimetric method with calf thymus DNA as the standard.

3D-EEM Analysis

3D-EEM spectra were obtained by using an F-4500 fluorescence spectrophotometer (Hitachi, Japan). The excitation spectrum ranged from 200 to 400 nm at 10 nm increments, while the emission spectrum ranged from 280 to 500 nm at 10 nm increments. The slit width of both the excitation and emission wavelength was 5 nm and the scanning speed was 1200 nm/min.

FT-IR Spectroscopic Analysis

FT-IR spectroscopic analysis was employed to examine the organic functional groups in the EPS extracted from the sludge samples. First, the EPS filtrate was lyophilized to powder in a vacuum freeze drier. Then, 1 mg of lyophilized powder was added to 100 mg of potassium bromide (FT-IR grade), and the preparation was analyzed using an FT-IR spectrometer (Tensor27, BRUKER OPTICS, Germany). The spectral range selected was 4000–400 cm^{-1}.

Results and Discussion

Colorimetric Analysis

Figure 3 indicates that with the increase in the dosage of enzymes, the contents of PN and PS significantly increased. Compared with the blank group, the contents of PN and PS increased by 160 and 180% with the addition of 0.03 g/g TSS of neutral protease, while they increased by 110 and 200% with the addition of 0.03 g/g TSS of alpha-amylase, respectively. Moreover, the dissolution rates of these three components initially increased rapidly and then gradually decreased, and the total content of EPS progressively stabilized at an enzyme dose of about 0.03 g/g TSS, thus indirectly demonstrating that the organic matter in the sludge was slowly converted from solid to soluble liquid state during enzyme treatment. Meanwhile, it was also found that the sludge particles became smaller and uniform following enzyme treatment, which is consistent with the study [24] that revealed the disruption of sludge floc structure, and change in the structure and function of sludge.

Figure 4 illustrates that the efficiency of compound enzyme (protease/alpha-amylase at a ratio of 1:3) was higher than that of the single enzyme. The reason for this observation may be the highly efficient and specific catalytic action of the enzymes, proteases specifically hydrolyze PN, while amylases target PS hydrolysis. Moreover, as the PN and PS in the EPS could often form PN–PN, PN-PS, and PS–PS, they could also be dissolved out during both protease and amylase hydrolysis. As a result, the contents of soluble PN and PS could significantly increase when the two enzymes were used in combination.

The variation in the contents of different EPS components during the process of enzyme-thermal pressure treatment is shown in Fig. 5. The content of dissolved organic matter (DOM) in the EPS decreased. For instance, the total soluble PN and PS contents were reduced by 38 and 53%, respectively, at 5 MPa and 75 °C, and decreased by 47 and 58%, at 7 MPa and 75 °C after protease-thermal pressure treatment. The reduction rate of the EPS exhibited the following trend: LB-EPS > TB-EPS > SEPS. A possible reason for this trend could be the disruption of the sludge floc structure under the conditions of thermal pressure and the subsequent release of the organic matter bound in the sludge into the liquid state, resulting in higher content of SEPS than LB-EPS and TB-EPS; Meanwhile, under the combined action of thermal pressure and enzymes, the macromolecular organic matter in the EPS was decomposed into small molecules or poorly soluble substances, which were difficult to detect by colorimetric method. As a result, the total content of DOM decreased. When the pressure was increased from 5 to 7 MPa at 75 °C, the content

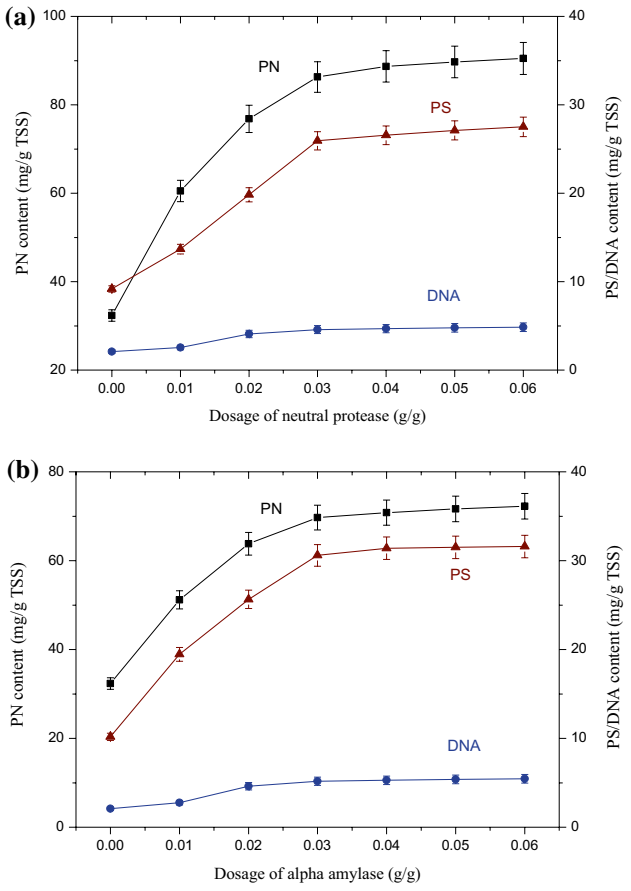

Fig. 3 Effect of enzyme dosage on the contents of different EPS components: neutral protease (**a**) and alpha-amylase (**b**). The PN, PS and DNA contents indicated are the total amount of the respective component obtained with different enzyme dosages

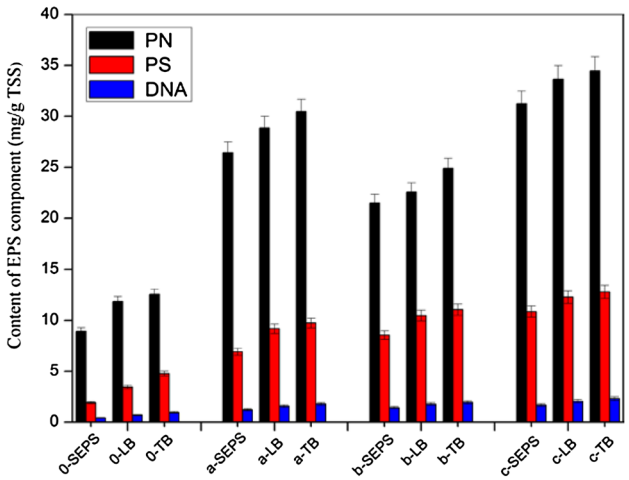

Fig. 4 Effects of different enzymes (dosage = 0.03 g/g TSS) on the contents of EPS components. Blank group (0), protease (**a**), alpha-amylase (**b**), and compound enzyme (**c**)

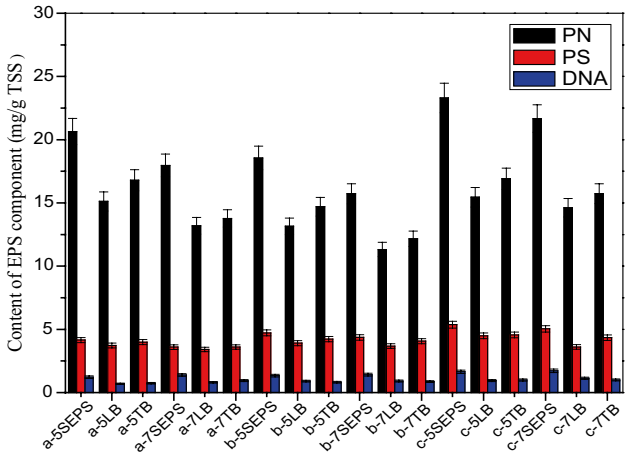

Fig. 5 Effects of thermal pressure on the contents of EPS components after enzyme (enzyme dosage = 0.03 g/g TSS) treatment. Protease (**a**), alpha-amylase (**b**), and compound enzyme (**c**). Thermal pressure 5 MPa (5), 7 MPa (7) at 75 °C

of DOM in the EPS was partially reduced and the moisture content of the sludge decreased by an average of about 10% (the moisture content of sludge in 5 MPa-75 °C and 7 MPa-75 °C was 77 and 75%, respectively).

In addition, a part of the microbial cells may have ruptured, releasing the intracellular nucleic acid. However, the results showed that the nucleic acid content was decreased, when compared with that noted in the blank group as well as the contents of LB-EPS and TB-EPS, which may be owing to the decomposition of nucleic acid under the conditions of applied enzyme and thermal pressure [25].

FT-IR Spectroscopic Analysis

FT-IR spectroscopy was used to detect the functional groups and PN structure of EPS in the sludge so as to determine the role of the EPS components during enzyme treatment. The main functional groups of the EPS were located in the following regions of the FT-IR spectra: 1000 ~ 600 cm^{-1} (fingerprint region), 1200 ~ 1000 cm^{-1} (nucleic acids and carbohydrates), 1300 ~ 1220 cm^{-1} (amide III), 1500 ~ 1300 cm^{-1} (carboxylic group and hydrocarbon-like compounds such as lipids), near 1550 cm^{1} (amide II), and 1700 ~ 1600 cm^{-1} (amide I). As the amide, carboxyl, and carbohydrate bands occur in the range of 1000 ~ 1800 cm^{-1}, this region was further analyzed [26].

As shown in Fig. 6a, the SEPS extracted from the raw sludge exhibited five main absorption bands in the FT-IR spectrum. The strong absorption band of carbohydrate (1038 cm^{-1}) can be attributed to the C–O and C–O–C stretching vibration [27], while the weak absorption band of amide III (1256 cm^{-1}) can be mainly attributed to C=O stretching vibration [28]. Furthermore, the band of

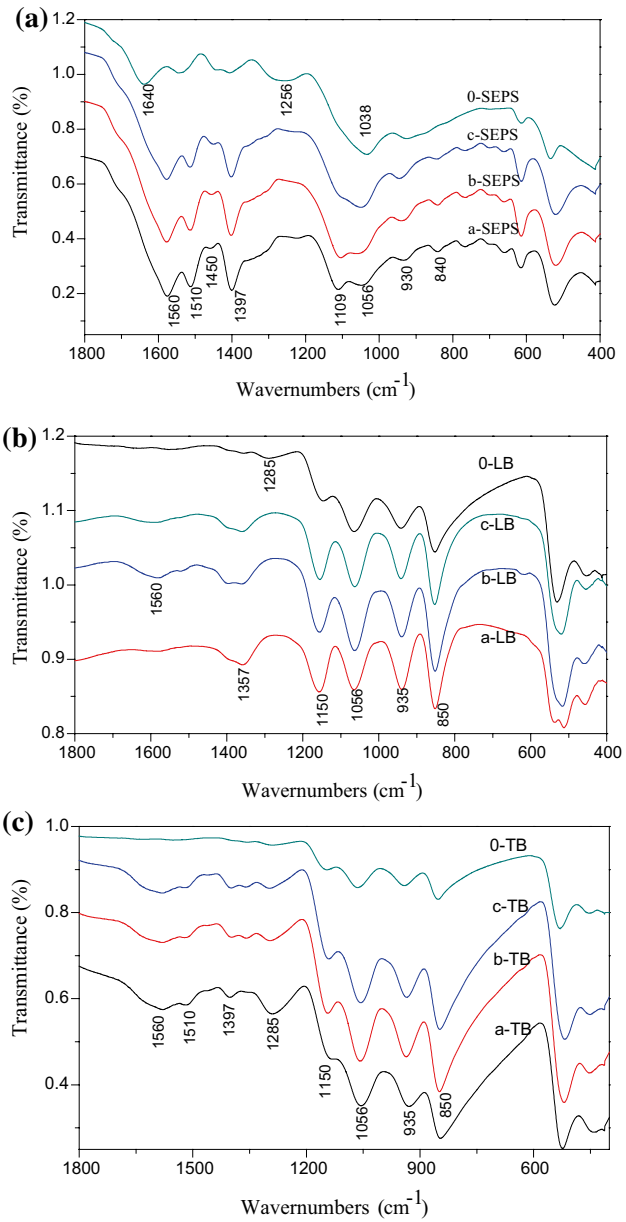

Fig. 6 FT-IR spectra of the EPS fractions extracted from raw sludge (0), neutral protease treated sludge (**a**), alpha-amylase treated sludge (**b**), and compound enzyme treated sludge (**c**)

carboxylic peak (1397 cm^{-1}) corresponded to the symmetric stretching vibration of the deprotonated carboxyl functional group of uronic acids [29], the band located in the region of 1510 cm^{-1} can be assigned to the aromatic ring vibration in phenol owing to the presence of tyrosine [30] and the band of amide I (1640 cm^{-1}) can be mainly attributed to the stretch of C=O in the amide groups [28].

After subjecting SEPS to enzyme treatment, the original absorption peak of carbohydrates in the FT-IR spectra shifted to the left and two new absorption peaks appeared at 1056 and 1109 cm^{-1}, suggesting the existence of

carbohydrates and extracellular nucleic acids [31], which was consistent with the results of colorimetric analysis. In addition, the absorption peaks of amides I and III disappeared, and a new absorption peak of amide II appeared, indicating that the structure and species of the PN had changed during the process of enzyme treatment.

Figure 6b shows the FT-IR spectrum of LB-EPS extracted from raw sludge, indicating three main absorption peaks (1056, 1150 (carbohydrates), and 1285 cm^{-1} (amide III)). After enzyme treatment, the absorption intensity of carbohydrates (1056, 1150 cm^{-1}) exhibited an obvious increase, suggesting that more carbohydrates were released from the sludge EPS. Meanwhile, the weak peak of amide III disappeared and a substituted absorption peak appeared at 1357 cm^{-1}, which may be caused by the stretching vibration of C–N, demonstrating the presence of aryl amine [32, 33]. In addition, a new absorption band at 1560 $cm^{-1}$was noted following alpha-amylase treatment.

Figure 6c illustrates the FT-IR spectrum of TB-EPS extracted from raw sludge, indicating two main absorption peaks for carbohydrates (1056 and 1150 cm^{-1}). However, following the enzyme treatment, some new functional groups were noted. For instance, strong peaks of amide III (1285 cm^{-1}) and amide II (1560 cm^{-1}), as well as weak peaks of carboxylates (1397 cm^{-1}), aromatic amino acids (1357 cm^{-1}), and tyrosine (1510 cm^{-1}) were observed, demonstrating that the types and number of functional groups in the TB-EPS were significantly increased following enzyme treatment of the sludge.

As shown in Fig. 7a, the main functional groups in the SEPS were as follows: carbohydrates (1056 and 1109 cm^{-1}), carboxylic groups (1396 cm^{-1}), aromatic ring vibration (1510 cm^{-1}), and amide II (1560 cm^{-1}). It must be noted that the main types of organic functional groups did not significantly change in the SEPS before and after thermal pressure treatment, and a slight increase in the intensity of the absorption peak near 1056 cm^{-1} occurred, which may be owing to the release of carbohydrates or nucleic acids. In contrast, the types and intensity of organic functional groups significantly changed in the LB-EPS after thermal pressure treatment. Although the absorption band at 1056 cm^{-1} did not change, the band at 1560 cm^{-1} disappeared. Furthermore, when the pressure was increased to 7 MPa, the absorption band located at 1357 cm^{-1} disappeared. With regard to the TB-EPS, the functional group changes were moderate, and the types of organic functional groups were much more than those noted in the LB-EPS. In addition, the absorption band at 1560 and 1510 cm^{-1} disappeared, and under the pressure of 7 MPa, the intensity of the absorption bands obviously decreased and those at 1056 and 1150 cm^{-1} slightly increased.

Recent studies revealed that LB-EPS plays a decisive role in sedimentation, flocculation, and dewatering performance of the sludge despite its low content of each component, when compared with that in TB-EPS. In contrast, the impact of TB-EPS is relatively weak. In this study, after the enzyme-thermal pressure treatment, the content of soluble organic matter in LB-EPS significantly changed, and the water content of the sludge decreased by an average of about 10%, similar to that noted in previous studies.

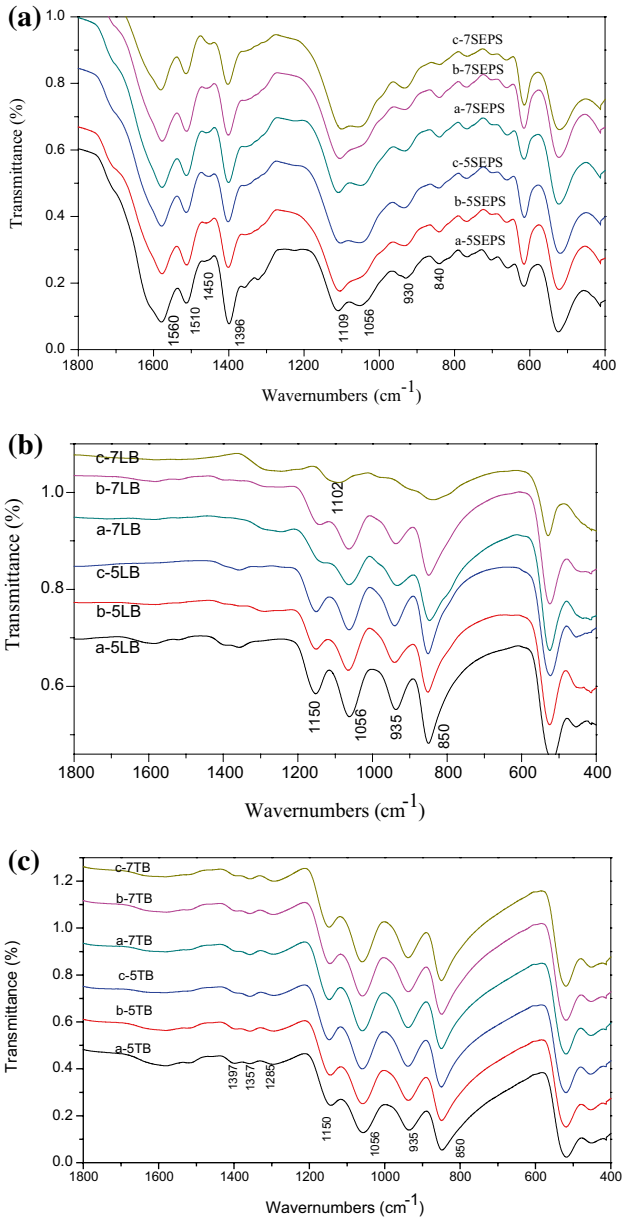

Fig. 7 FT-IR spectra of the EPS fractions extracted from neutral protease treated sludge (**a**), alpha-amylase treated sludge (**b**), and compound enzyme treated sludge (**c**) in 5 MPa (5) and 7 MPa (7) at 75 °C

Fluorescence Spectroscopic Analysis

The DOM in the sludge is mainly composed of humic-acid-like materials, fulvic-acid-like materials, aromatic-protein-like substances, and other types of fluorescent substances [20]. The principle of EEM spectra is that a specific wavelength of ultraviolet irradiation emits different wavelengths of fluorescence, with the fluorescence

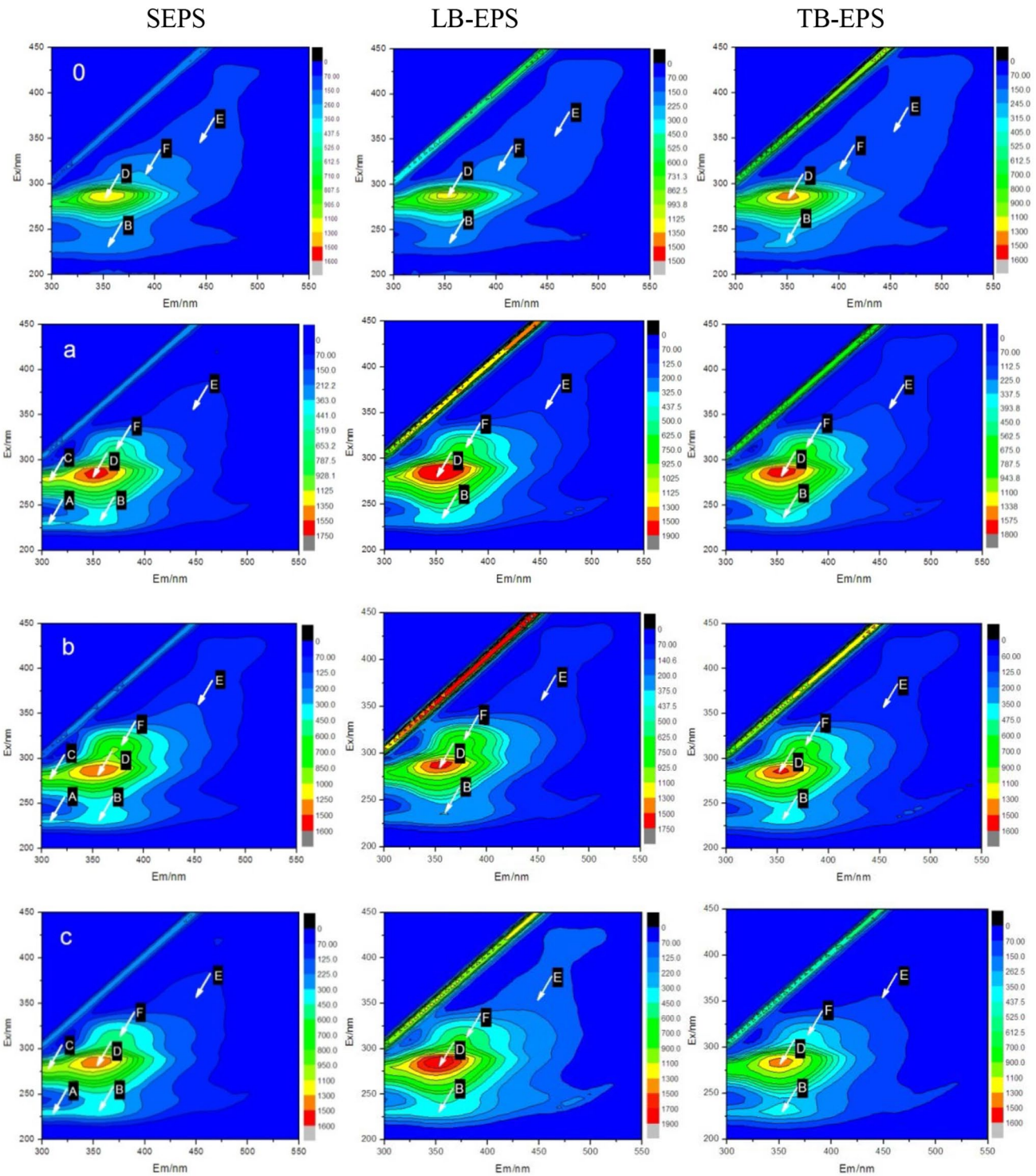

Fig. 8 Fluorescence EEMs for the EPS fractions extracted from raw sludge (0), neutral protease treated sludge (**a**), alpha-amylase treated sludge (**b**), and compound enzyme treated sludge (**c**)

intensity being proportional to the concentration of the luminescent material [34]. Thus, 3D-EEM spectra can be used to detect the characteristics of EPS extracted from sludge.

The EEM spectra of the EPS before and after enzyme treatment (dosage = 0.03 g/g TSS) are shown in Fig. 8. Figure 8(0) illustrates four fluorescence peaks: one strong peak D and three weak peaks B, E, and F for SEPS, LB-EPS, and TB-EPS extracted from raw sludge. Peak D, located at an excitation/emission wavelength (Ex/Em) of 280/350 nm, can be attributed to tryptophan-like proteins [35]. Peaks E and F at Ex/Em of 360/450 and 310/375 nm, respectively, correspond to humic-acid-like materials [36], and peak B at Ex/Em of 230/350 nm can be attributed to aromatic-protein-like substances [37]. It must be noted that the main chemical compositions of the TB-EPS were very similar to those of SEPS and LB-EPS, with both tryptophan-like proteins and aromatic-protein-like substances being the most abundant components, particularly in the TB-EPS, Moreover, humic acid was the most widely distributed substance in the fluorescence spectra. After enzyme treatment, the intensities of peaks B, D, and F were significantly enhanced for all the EPS samples, implying the release of soluble organic matter such as aromatic-protein-like substances, tryptophan-like proteins, humic-acid-like materials in the sludge, as well as indicating that the sludge floc structure was disrupted. The detailed fluorescence spectral parameters, including the position and intensity of the main fluorescence peaks, for all the samples are shown in Table 2.

When compared with alpha-amylase-treated samples, soluble PN, especially tryptophan substances, were eluted from the sludge after pretreatment with protease. In contrast, humic acid was released in large amounts from samples treated with alpha-amylase. These results were consistent with the specificity of the enzyme catalysis. Compound enzyme treatment exhibited the characteristics of both alpha-amylase and protease treatments, especially with regard to the release of bound organic matter. Meanwhile, the SEPS samples presented two new fluorescence peaks C and A at Ex/Em of 340/447 and 225/310 nm, respectively, following enzyme treatment, suggesting the formation of new aromatic-protein-like substances that may be tyrosine. However, no similar fluorescence peaks appeared for the LB-EPS and TB-EPS samples, they may have been obscured by other high-intensity protein peaks or have aggregated together with other carbohydrates [38]. In addition, the concentrations of humic-acid-like materials were found to be independent of the substrate [39], which suggested that the dissolution rate of humic was lower than that of PN, resulting in a relatively low concentration. However, the intensity of peak E for the LB-EPS slightly increased after treatment with compound enzyme, which indirectly confirmed that the humic-acid-like materials mainly occurred in SEPS and LB-EPS [40].

Figure 9 illustrates the 3D-EEM spectra for EPS obtained after enzyme-thermal pressure treatment. Table 3 shows the fluorescence spectral parameters for all the EPS samples. The EEM spectra revealed three fluorescent substances, namely, aromatic-protein-like substances (peak B, 230/350 nm; peak C, 280/310 nm), tryptophan-like proteins (peak D, 280/350 nm), and humic-acid-like materials (peak E, 360/450 nm; peak F, 310/370 nm). The fluorescence intensity of humic-acid-like materials (peaks E and F) obviously decreased for all the LB-EPS and TB-EPS samples. However, following protease-thermal pressure treatment, the fluorescence intensity of tryptophan-like proteins significantly increased for the SEPS samples at a pressure of 7 MPa, indicating the release of bound organic matter, similar to the findings of colorimetric analysis. Moreover, the fluorescence intensity of aromatic-protein-like substances and tryptophan-like proteins significantly decreased for other EPS samples, showing that the content of soluble PN in the EPS was reduced, which may be owing to the combined action of protease and thermal pressure as well as the gradual degradation of PN into small molecules or conversion into insoluble organic matter in the EPS.

After combined treatment of alpha-amylase (or compound enzyme) and thermal pressure, it must be noted that when the pressure was increased to 7 MPa at 75 °C, the fluorescence intensities of the aromatic-protein-like substances and tryptophan-like proteins increased for almost all the SEPS and TB-EPS samples, while that of

Table 2 Fluorescence spectral parameters for all the EPS samples after enzymes treatment

	SEPS				LB-EPS				TB-EPS			
	Peak B	Peak D	Peak E	Peak F	Peak B	Peak D	Peak E	Peak F	Peak B	Peak D	Peak E	Peak F
$\lambda_{ex/em}$	225/350	280/350	360/450	310/375	225/350	280/350	360/450	310/375	225/350	280/350	360/450	310/375
0	226	1193	110	240	183	1160	120	176	258	1300	130	154
a	408	1665	89	522	424	1889	96	680	410	1725	95	621
b	370	1460	90	863	379	1596	93	820	408	1551	84	712
c	377	1465	96	540	442	1900	140	620	415	1400	85	400

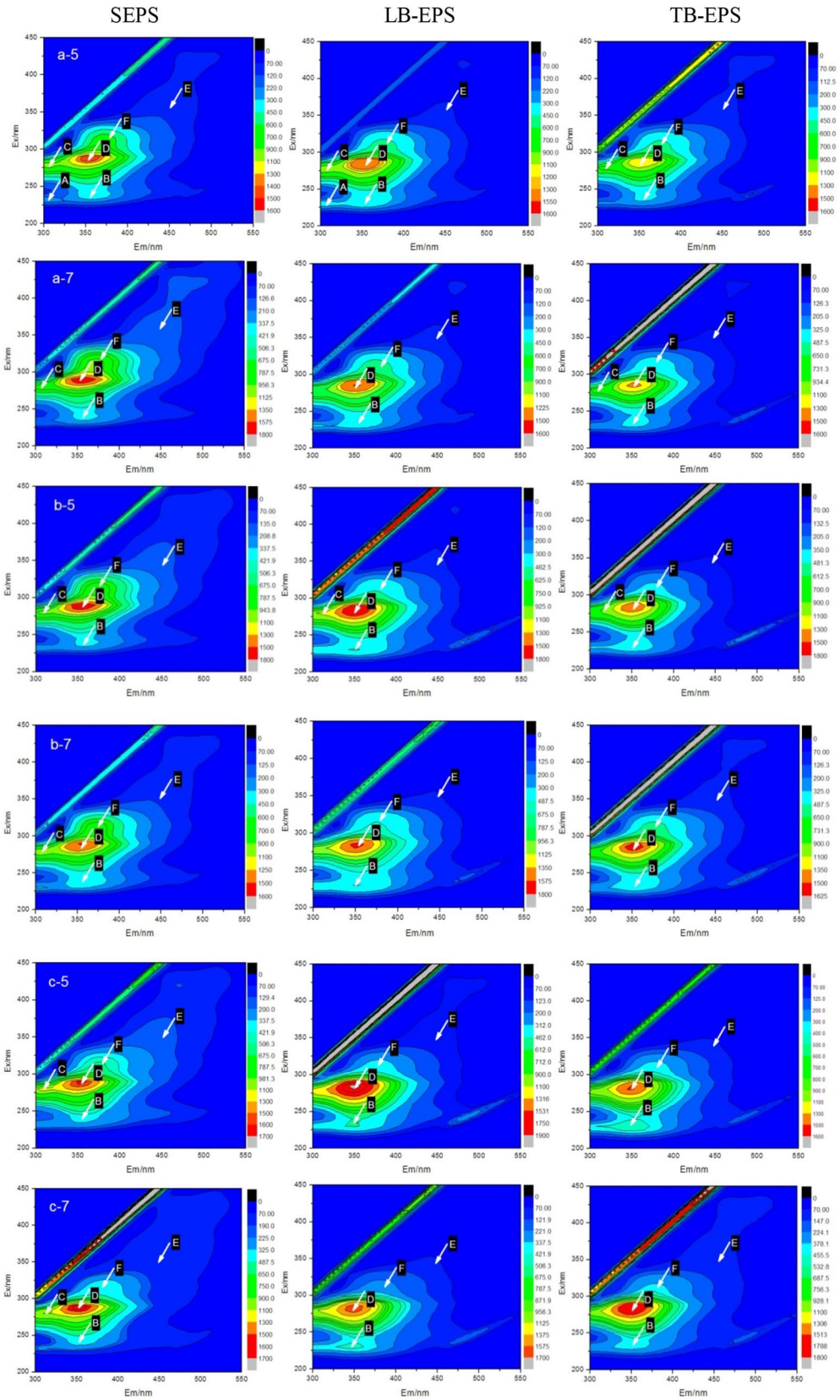

Fig. 9 Fluorescence EEMs for all the EPS fractions extracted from neutral protease treated sludge (**a**), alpha-amylase treated sludge (**b**), and compound enzyme treated sludge (**c**) after enzyme-thermal pressure treatment in 5 MPa (5) and 7 MPa (7) at 75 °C

Table 3 Fluorescence spectral parameters for all the EPS samples obtained after thermal pressure treatment

	SEPS				LB-EPS				TB-EPS			
	Peak B	Peak D	Peak E	Peak F	Peak B	Peak D	Peak E	Peak F	Peak B	Peak D	Peak E	Peak F
$\lambda_{ex/em}$	225/350	280/350	360/450	310/375	225/350	280/350	360/450	310/375	225/350	280/350	360/450	310/375
a-5	352	1463	112	668	379	1520	76	491	327	1288	85	472
a-7	362	1780	172	910	382	1440	78	515	363	1355	76	409
b-5	350	1690	130	881	449	1715	71	417	413	1409	72	380
b-7	360	1490	113	754	450	1621	71	429	428	1601	82	557
c-5	338	1617	150	514	462	1848	94	366	489	1503	73	340
c-7	354	1654	156	413	454	1632	85	270	445	1764	95	396

tryptophan decreased for the LB-EPS samples, which may be related to partial removal of bound water from the sludge. And LB-EPS may have a more significantly effect on the dewatering properties of sludge than SEPS and TB-EPS. This hypothesis was consistent with the conclusion of previous research. X.Y. Li and S.F. Yang demonstrated that the LB-EPS had a negative effect on bioflocculation and sludge-water separation. The parameters for the performance of sludge-water separation were much more closely correlated with the amount of LB-EPS than with the amount of TB-EPS [41].

Conclusion

1. After adding 0.03 g/g TSS neutral protease and alpha-amylase, respectively, the soluble PN content increased by 160 and 110% and the PS content increased by 180 and 200%. Sludge floc could be effectively disrupted, the structure and species of the PN and PS had changed during the process of enzyme treatment.
2. Thermal pressure treatment caused obvious decrease in the content of soluble organic matter. FT-IR spectroscopy results revealed that the types and quantities of organic functional groups decreased significantly, especially in the LB-EPS and TB-EPS, which plays a decisive role in dewatering performance of sludge.
3. 3D-EEM analysis indicated that tryptophan-like proteins, humic-acid-like materials, and aromatic-protein-like substances were the most active fluorescent substances.
4. Protease could promote the dissolution of protein-like substances, while amylase could promote the dissolution of humic-acid-like materials in the EPS.

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