

Characterization of the interactions between tetracycline antibiotics and microbial extracellular polymeric substances with spectroscopic approaches

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Abstract The antibiotics have attracted global attentions for their impact on aquatic ecosystem. The knowledge about the fate of antibiotics encountering extracellular polymeric substances (EPS) is, however, limited. In this study, we investigated the interacting mechanisms of tetracycline (TC) to EPS extracted from aerobic activated sludge. The contributions of the main components of EPS, extracellular proteins, and polysaccharides were evaluated using bovine serum albumin and alginate sodium, respectively. Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, and nuclear magnetic resonance indicated that hydroxyl, carboxyl, and amino groups were the domain chemical groups involved in the interaction between TC and EPS, and the binding of TC onto EPS changed the structure of these chemical groups, thus causing shifts in their UV–visible absorption spectra. In addition, we found that extracellular proteins, rather than polysaccharides, were the major active contents involved in the interaction. Three-dimensional excitation–emission matrix fluorescence spectroscopy showed that the fluorophores in EPS were clearly quenched by TC and the static quenching process was observed, implying the complex formation of TC and EPS. Furthermore, thermodynamic analysis indicated that the binding of TC with EPS is spontaneous and dominated by electrostatic forces.

Keywords Tetracycline · Extracellular polymeric substances (EPS) · Fluorescence quenching · X-ray photoelectron spectroscopy (XPS) · Three-dimensional excitation–emission matrix fluorescence spectroscopy

Introduction

Tetracycline antibiotics are extensively used in veterinary therapeutics and as feed additives to promote animal growth (Erşan et al. 2013). However, only a small amount of the antibiotics given to livestock could be absorbed and metabolized; a considerable percentage, about 30–90 %, is consequently released into the environment (Sarmah et al. 2006). According to the recent research, residues of antibiotics have been frequently detected in surface water, underground water, and drinking water (Sponza and Çelebi 2012). Antibiotics are of concern due to their potential genotoxic effects, disruption of aquatic ecosystems, promotion of antibiotic resistance, complications for water reuse, and even increased human health risks. Hence, the issue of antibiotics and their removal from water resources is an urgent research subject.

Biodegradation is considered a potentially promising technology for antibiotic removal because of its efficiency, low cost, and ease of use. Perez et al. (2005) reported that three low concentrations (20 µg/L) of sulfonamides were removed via biodegradation in an activated sludge process. Li and Zhang (2010) examined 11 target antibiotics, and two sulfonamides were predominantly removed by biodegradation in both freshwater and saline sewage systems. These studies, however, mainly focused on the removal of antibiotics in wastewater treatment plants. Extracellular polymeric substances (EPS) excreted by microbial cells, which are widespread in aquatic systems, are mainly composed of polysaccharides and proteins (Sheng et al. 2008). As metabolic products that accumulate on the surface of microbial cells, EPS provide binding

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sites for nutrients from the environment and promote the transformation of metal ions and organic compounds (Wang et al. 2012; D'Abzac et al. 2010; Ozturk and Aslim 2008; D'Abzac et al. 2012). Yang et al. (2012) found that the sorption affinity of sulfonamides to microbes was enhanced by EPS. Zhang et al. (2012) showed that EPS facilitated the degradation of quantum dots. EPS could protect the bacterial community against chemicals that establish interactions with the polymer matrix, by impeding access of the chemical to the bacterial cells (Henriques and Love 2007). However, the information on the interaction mechanism of EPS and antibiotics is not yet well understood.

This work aimed to gain a comprehensive view of the interaction between antibiotics and EPS. Tetracycline was selected as the research objective because of its wide application, high water solubility, and residual toxicity. Bovine serum albumin (BSA) and alginate sodium (AS) were selected as model protein and polysaccharide, respectively, to investigate the specific contributions of the dominant components in EPS (Herzberg et al. 2009). Experiments were conducted to explore the structural changes of EPS through ultraviolet–visible (UV–vis) absorption spectrometry and three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy. The variation of the functional groups during the interaction was studied by using Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and nuclear magnetic resonance (NMR).

Materials and methods

Reagents

All solutions were prepared from reagent-grade chemicals in ultrapure water. Tetracycline hydrochloride, sodium alginate, and BSA were of analytical grade and purchased from Aladdin Industrial Corporation. Other reagents were commercially available and obtained from Beijing Chemical Reagent Factory, China.

EPS extraction and analysis

The test EPS were extracted from activated sludge which was cultivated in a lab scale sequencing batch reactor fed with synthetic wastewater. The operational conditions of the reactor were described by Shi et al. (2011).

The EPS of sludge samples were extracted using the cation exchange resin technique described by Sheng and Yu (2006). Activated sludge (30 ml) was centrifuged at 3,000 rpm for 15 min at room temperature, and the supernatant was decanted. Then, the sludge pellets were resuspended to original volume with phosphate buffer (2 mmol Na_3PO_4 , 4 mmol NaH_2PO_4 , 9 mmol NaCl, 1 mmol KCl, pH=7.4), and the

solution was transferred to an extraction beaker, followed by addition of the cation exchange resin at a dosage of 80 g/g MLSS. Suspensions were stirred for 1 h by a magnetic stirring apparatus and then centrifuged at 8,000 rpm for 1 h. Finally, the supernatants were filtered through 0.22 μm nylon membranes and were lyophilized to get crude EPS.

The polysaccharide content in the EPS was measured using the anthrone method with glucose as the standard (Loewus 1952). The proteins and humic substances were determined using the Lowry procedure (Frolund et al. 1996) with bovine serum albumin and humic acid, respectively, as the standard. The concentration of proteins, polysaccharides, and humic substances in 100 mg EPS was 40.75 ± 3.17 , 18.87 ± 1.27 , and 2.15 ± 1.03 mg, respectively.

Sample

Prior to characterization, crude EPS and tetracycline were dissolved to the desired concentrations with 0.2 mol L^{-1} phosphate buffer solution (pH=7.4). Different volumes of tetracycline solution were added and mixed with the EPS solution in each tube, and then, phosphate buffer solution was added to maintain the total volume to 25 ml. The final concentration of EPS was 100 mg/L, and the concentrations of tetracycline were 0, 10, 20, 30, 40, and 50 $\mu\text{mol L}^{-1}$. Afterwards, the mixed solution was put into an oscillator and balanced for 1 h at room temperature before UV–visible absorption spectral analysis. The rest of the solutions were lyophilized for FTIR, XPS, and ^1H NMR analysis.

The sample preparation for fluorescence measurements was similar to that of UV–visible spectra. The final concentrations of tetracycline were from 0 to 50 $\mu\text{mol L}^{-1}$, and the temperatures of samples were maintained at either 298 or 308 K (Chi and Liu 2010).

To further determine the role of proteins and polysaccharides, bovine serum (BSA) and sodium alginate were used as the model proteins and polysaccharides, respectively, to provide additional information about the interacting mechanisms between tetracycline and EPS. The experiments were conducted with the same process with that of tetracycline and EPS.

Analytical methods

UV–visible absorption spectra

UV–visible absorption spectra were measured on a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan) with scanning wavelength from 200 to 700 nm at 0.1-nm increments.

Fluorescence measurements

All fluorescence spectra were recorded on an F-4600 fluorophotometer (Hitachi, Japan) and measured at 298 and

308 K. The temperature of the samples was maintained in a water bath thermostat.

Three-dimensional EEM fluorescence spectra were produced with excitation and emission wavelength ranges of 200–450 nm (5 nm slit) and 220–550 nm (5 nm slit), respectively, both at 5-nm increments. The voltage of the photomultiplier tube was 500 V for high light detection.

The fluorescence emission spectra were collected from 290 to 500 nm with 278-nm excitation wavelength, and the excitation and emission slit widths were set at 5.0 nm.

FTIR spectroscopy

FTIR spectra were recorded using FTIR (Aratar, Thermo-Nico-Let, USA). Each spectrum, an average of 256 scans from 4,000 to 400 cm^{-1} , was collected with a resolution of 2 cm^{-1} , and the ordinate was expressed as transmittance. FTIR spectra were measured on KBr pellets prepared by pressing mixtures of 100 mg chromatographic grade KBr and 1 mg dry powdered sample under a vacuum to avoid moisture uptake.

XPS analysis

XPS data were obtained using XPS (Thermo ESCALAB 250, USA) with an Al K α X-ray source ($h\nu=1,486.6$ eV). The X-ray source was run at a reduced power of 150 W, and beam spot was 500 μm during each measurement. All binding energies were referenced to the neutral C1s peak at 284.6 eV to compensate for surface charging effects. A broad survey scan (20.0 eV) was conducted for major element component analysis, and a high resolution scan (70.0 eV pass energy) was used for component speciation.

^1H NMR spectroscopy

^1H NMR spectra were recorded on a Bruker Avance 400 spectrometer at room temperature operating at 400 MHz, equipped with a 5-mm inverse probe with z-gradient coil. Chemical shifts were reported in δ relative to TMS.

Results

Tetracycline binding with EPS

In this work, UV spectroscopy was applied for preliminary analysis of the interaction between tetracycline and EPS. As shown in Fig. 1, the peak at 356 nm was the characteristic of tetracycline, and the band around 270 nm appeared due to phenyl group of tetracycline and protein in EPS (Mandal et al. 2009). A shoulder observed around 245–255 nm is commonly attributed to $\pi-\pi^*$ electron transitions in aromatic and polyaromatic compounds in most conjugated molecules including

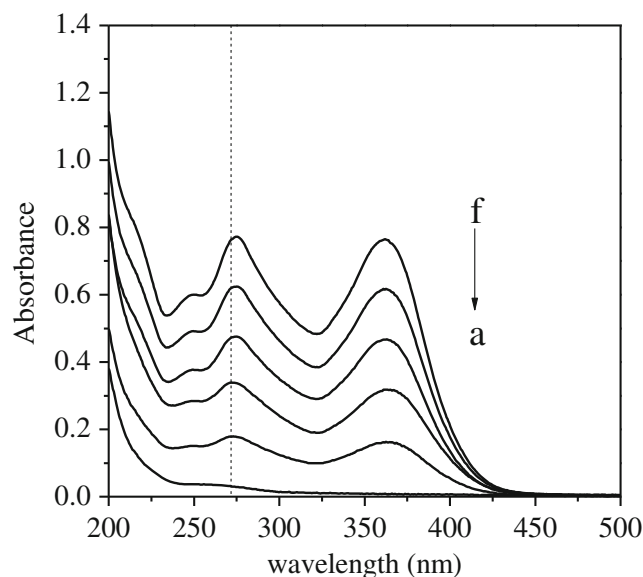


Fig. 1 UV-visible absorbance spectra of EPS in the presence of tetracycline at different concentrations. From *a* to *f*, tetracycline concentration is 0, 10, 20, 30, 40, and 50 $\mu\text{mol L}^{-1}$

tetracycline (Jia et al. 2007). The end absorption at 200 nm reflected the framework conformation of the protein in EPS (Yang et al. 2009). With gradual addition of tetracycline to EPS solution, the intensity of the peak at 356 nm decreased about 8–10 % (data not shown) in comparison of the absorbance curves of pure tetracycline at corresponding concentrations. Moreover, the peak around 270 nm was slightly shifted from 272 to 276 nm, indicating that the interaction between tetracycline and EPS changes the energy of bond orbital in phenyl group and decreases the hydrophobicity of the micro-environment of the aromatic amino acid residues (Wu et al. 2007).

Probing the main functional groups in EPS responsible for tetracycline binding

FTIR spectra in the range of 400–4,000 cm^{-1} were taken to obtain the information about possible variation of the functional groups in EPS. As shown in Fig. 2a, the infrared spectrum of EPS exhibits a strong absorbance at 3,327 cm^{-1} assigned to O–H and N–H stretching vibrations of hydroxyl and amine groups, while the band at 2,975 cm^{-1} represents C–H stretching vibrations (Wang et al. 2011b; Caroni et al. 2012). The major functional groups of protein are also found: the C=O stretching vibration (amide I) at 1,672 cm^{-1} , the N–H deformation vibration (amide II) at 1,561 cm^{-1} , the C–N stretching vibration at 1,441 cm^{-1} , and the C–H bending vibration at 1,362 cm^{-1} (Sun et al. 2009). The polysaccharide region is also displayed at 1,076 and 1,142 cm^{-1} corresponding to C–O stretching vibrations of alcohol groups.

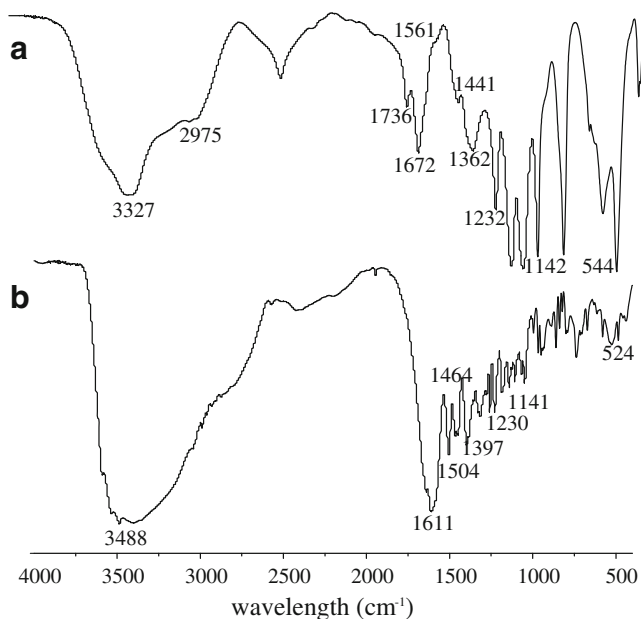


Fig. 2 FTIR spectra of *a* pristine EPS and *b* tetracycline-treated EPS

After EPS reacted with tetracycline, the spectrum exhibited some changes (Fig. 2b). The sharp peak at $3,327\text{ cm}^{-1}$ became a broad one at $3,488\text{ cm}^{-1}$ and the band intensity increased, indicating that the bands of N–H or O–H in EPS were affected by tetracycline. This change may be explained by inductive effects (Gómez-Hortigüela et al. 2013). When electron-withdrawing groups of tetracycline bond with hydrogen atoms of N–H or O–H in EPS, they withdraw electron density from oxygen or nitrogen atoms, which lessen the dipole and increase the vibrational energies. However, it is difficult to distinguish which group causes the shifts for the overlapping signals of hydroxyl and amine groups. The signals of the C=O stretching vibration (amide I) at the $1,672\text{ cm}^{-1}$ and N–H deformation vibration (amide II) at $1,561\text{ cm}^{-1}$ were moderately shifted to $1,611$ and $1,504\text{ cm}^{-1}$, indicating an interaction involving the proteins in EPS.

As the local chemical environment determines the positions of elemental peaks (Omoike and Chorover 2004), X-ray photoelectron high-resolution scans of C1s, O1s, and N1s were expected to obtain the corresponding functional groups. Figure 3 clearly shows the presence of four C (1s) peaks (Fig. 3a), two O (1s) peaks (Fig. 3c), and one N (1s) peak (Fig. 3e) in virgin EPS. The assignment and quantification of these XPS spectral bands are listed in Table S1. The C1s peak was resolved into four component peaks. The peak at 284.3 eV is attributed to C–(C,H) from lipids or amino acid side chains. The peak at 285.1 eV, which is associated with C–(O,N) of alcohol, amine, or ether amide (Yuan et al. 2010), presents the largest percentage in the spectral band (39.46 %). The C=O or O–C–O (287.7 eV), as in carboxylate, carbonyl, amide, acetal, or hemiacetal, respectively, also accounts for a

large part of about 21.54 %. In contrast, the peak at 289.2 eV, which is the contribution of carboxyl or ester group, is much lower (5.94 %).

The O1s peak at 531.8 eV (48.20 %) is attributed to the alcohols, hemiacetal, or acetal groups. The second O1s peak at 530.5 eV (51.80 %) is mainly attributed to the O double bonded to C (O=C), as in carboxylate, carbonyl, ester, or amide (Sun et al. 2011). The high-resolution N1s XPS spectra of EPS exhibit two peaks: 399.2 eV assigned to the nonprotonated nitrogen atoms in the forms of amine or amide and 401.4 eV assigned to the protonated nitrogen (Yuan et al. 2010).

The high-resolution scans of C1s, O1s, and N1s after the interaction of EPS and tetracycline were shown in subpanels b, d, and f of Fig. 3, respectively. A comparison between the EPS XPS data before and after the reaction with tetracycline indicates that the changes of the binding energies mainly reflect at C1s and O1s. With the binding energies of C1s at 287.7, 285.1 eV transformed into 288 and 286.1 eV, and a slight rise was observed at the binding energy of C–(C,H) from 284.3 to 284.5 eV. These shifts indicate that the electron distribution in EPS was disturbed, as the increases in the electronic density around C in C–(O,N) and C=O and decreases around C in C–(C,H). The O1s peaks at 531.8 and 530.5 eV were shifted to 532.4 and 531.3 eV, respectively. These shifts may be explained as decreases in the electronic density toward oxygen atom by electron cloud transferring to carbon or hydrogen atoms, which is in agreement with the results of FTIR analysis.

The ^1H NMR spectrum in Fig. 4(a) displayed multitudinous signals for EPS depicting their complex and heterogeneous nature. The broad peaks in the ^1H chemical shift range of 3.0–0.5 ppm imply saturated primary, secondary, and tertiary hydrogen of alkyl group. The signals at 5.0–3.0 ppm are indicative of hydrogen in hydroxyl which is due to the presence of polysaccharides in EPS. The aromatic ring structure was also concluded by convergence of signals in the region of 7.1–8.3 ppm (De Sousa et al. 2008).

Figure 4(b) showed the ^1H NMR spectrum of EPS in the presence of tetracycline. The signals of hydrogen in tetracycline changed obviously in comparison of the spectrum of pristine EPS (Fig. 4(a)) and that of tetracycline (Fig. 4(c)). The peaks of hydrogen in aromatic rings shifts to 6.59–7.46 ppm, indicating some electrostatic repulsions may be close to the hydrogen and enhance the electron density (Gómez-Hortigüela et al. 2013). On the contrary, the peaks on behalf of hydrogen in alkyl group moved to low field resulting from the decrease of electron density around hydrogen. It is notable that the O–H peaks at about 4.0 ppm were not detected after the interaction. This phenomenon may result from the decrease of electron density around oxygen atoms and the increase around hydrogen atoms in hydroxyl groups (the results in FTIR analysis).

Fig. 3 XPS spectra of pristine EPS (**a, c, e**) and tetracycline-treated EPS (**b, d, f**)

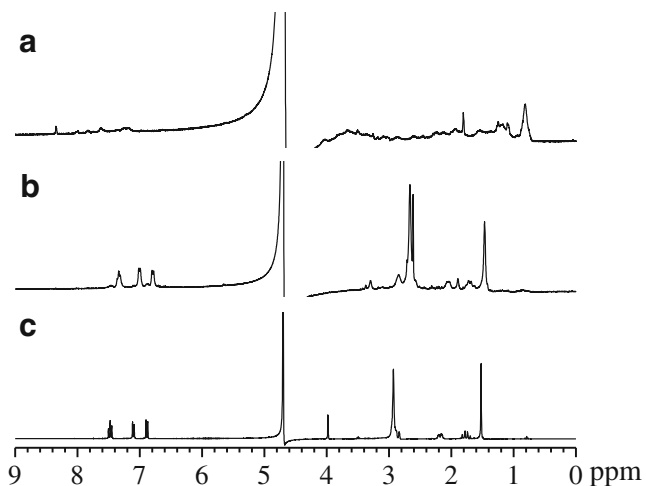
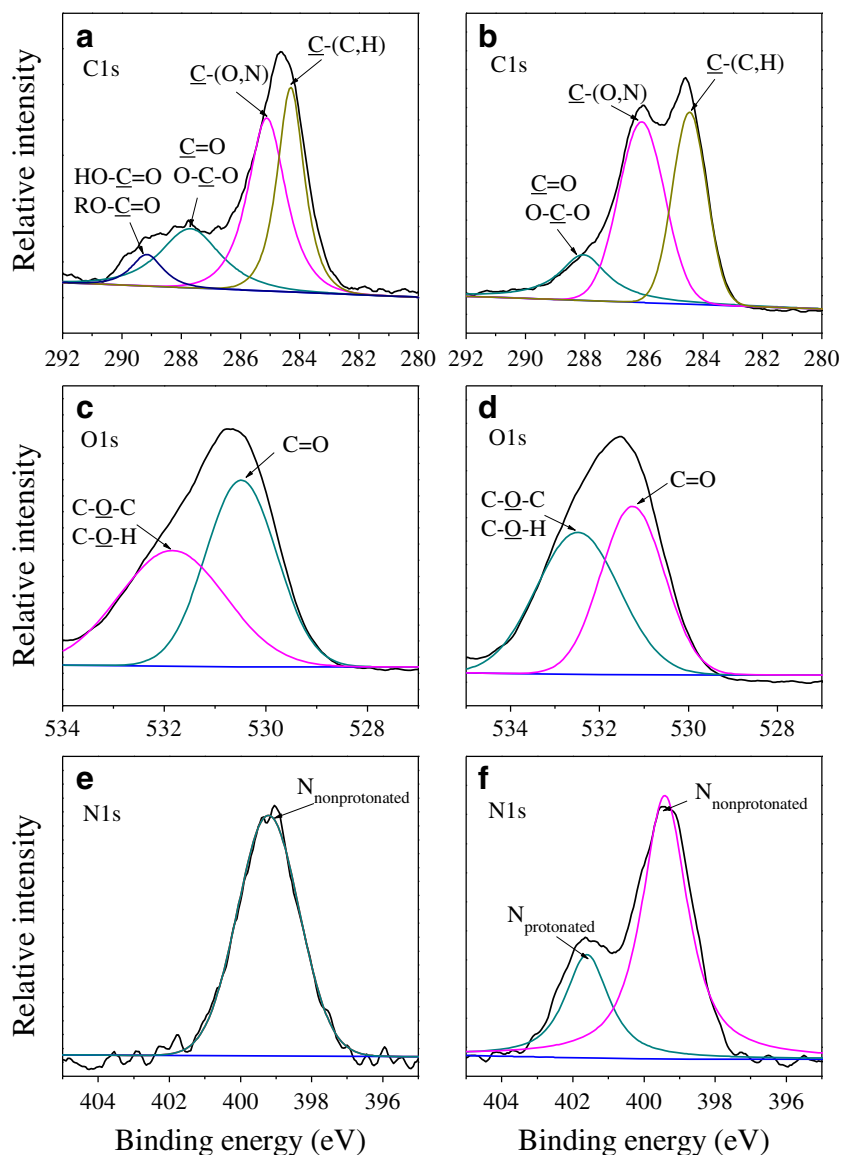


Fig. 4 ^1H NMR spectrum of (*a*) pristine EPS, (*b*) tetracycline-treated EPS, and (*c*) tetracycline

Identification of the dominant active constituents in EPS

To obtain further insight into the influence mechanism of EPS on tetracycline, EPS composition was analyzed and quantified. EPS mainly comprise proteins and polysaccharides, and protein concentration was more than twofold higher than that of the polysaccharides in this study. BSA and AS were selected as model protein and polysaccharide to ascertain the main active components in EPS (Herzberg et al. 2009). Therefore, the interactions between tetracycline and model substances were analyzed by FTIR, XPS, and ^1H NMR. In BSA test, the peaks at $1,655$ and $1,542\text{ cm}^{-1}$ were shifted to $1,610$ and $1,505\text{ cm}^{-1}$ in the FTIR analysis (Fig. S1b), and the O–H signals were not detected in the ^1H NMR spectrum (Fig. S3b), which were in good agreement with the results of EPS analysis. Moreover, no similar results were observed in alginate

sodium test. This implies that proteins were the dominant active constituents in the EPS during the interaction.

Quantification of the thermodynamics of the tetracycline binding with EPS

In this study, three-dimensional EEM fluorescence spectroscopy was applied for characterizing the role of the proteins in EPS by addition of tetracycline. Figure 5 showed the fluorescence spectrogram of EPS mixed with gradient tetracycline concentrations at 298 and 308 K. Three peaks were observed and the fluorophores in EPS were indentified on the basis of their peak positions. The main peaks were located at 235 nm excitation and 295–305 nm emission (peak A), at 285 nm excitation and 355–360 nm emission (peak B), and at 355–360 nm excitation and 440 nm emission (peak C). In general, peaks at shorter excitation wavelengths (<250 nm) and short emission wavelengths (<350 nm) such as peak A have been described as protein-like peaks, which are related to simple aromatic protein such as tyrosine (Chen et al. 2003). Peak B

had been reported by other literatures and was described as aromatic amino acid tryptophan (Sheng and Yu 2006; Métivier et al. 2013). Besides, peak C showed a similar signal to that observed in natural dissolved organic matter and was identified as humic-like fluorophore (Lu et al. 2013). Compared with proteins and humic-like substances, the fluorescence intensity of polysaccharides in EEM spectrum could be neglected (Her et al. 2003). Thus, the fluorescence signals of the EPS were mainly attributed to proteins and humic-like substances.

Fluorescence quenching of EPS by addition of tetracycline was manifested broadly throughout the EEM, and clearly at peak A and peak B, attributed to protein. Although peak C showed quenching in the presence of tetracycline, the relative quenching effect was slight to be negligible compared with the other two peaks (Sheng et al. 2008; Métivier et al. 2013). The quenching data of proteins were analyzed according to modified Stern–Volmer equation and Van't Hoff equation (list in ESI) (Chi et al. 2010; Pan et al. 2010a), and the parameters were listed in Table 1. The value of quenching constant K_a at

Fig. 5 EEM fluorescence spectra of pristine EPS (a, d), EPS in the presence of $2.0 \times 10^{-5} \text{ mol L}^{-1}$ tetracycline (b, e), and EPS in the presence of $4.0 \times 10^{-5} \text{ mol L}^{-1}$ tetracycline (c, f). a–c Measured at 298 K. d–f Measured at 308 K

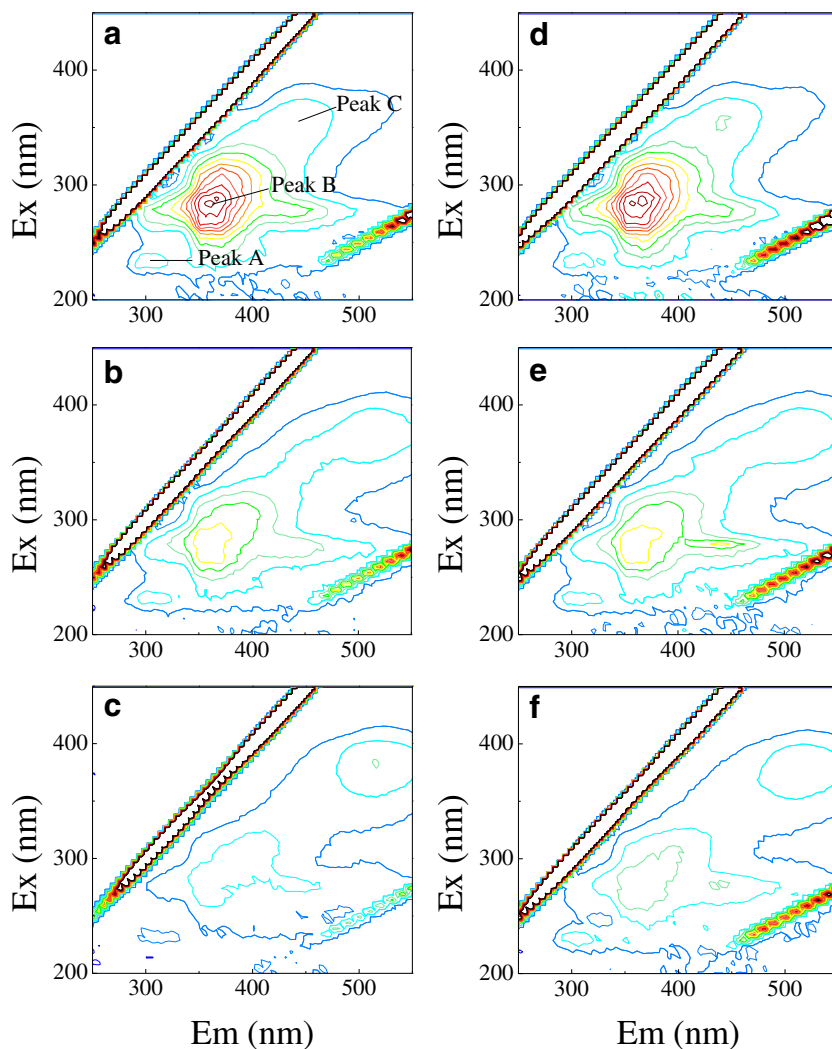


Table 1 Parameters of Stern–Volmer equation and Van't Hoff equation for the interaction of EPS with tetracycline at different temperature

<i>T</i> (K)	K_a^a (10^5 mol L ⁻¹)	ΔH (kJ mol ⁻¹)	ΔS (J mol ⁻¹ K ⁻¹)	ΔG (kJ mol ⁻¹)	K_b^b (10^5 mol L ⁻¹)	n^c
298	1.44	-22.66	22.72	-29.43	1.54	1.05
308	1.07		22.69	-29.65	5.04	1.16

^a The fluorescence quenching constant

^b Binding constant

^c The number of binding sites

298 and 308 K was calculated to be 1.44×10^5 ($R^2=0.9883$) and 1.07×10^5 ($R^2=0.9938$) mol L⁻¹. The value of binding constant K_b is more than 10^5 L mol⁻¹, indicating that strong interaction exists between EPS and tetracycline (Pan et al. 2010a). The number of binding sites n approximately equals 1, which can be concluded that there is one binding site in EPS for tetracycline during the interaction. The free-energy change (ΔG) is negative, implying that the interaction process is spontaneous. Besides, the negative ΔH and positive ΔS indicated that electrostatic forces play the main role during the interaction. Slight shifts were observed for the peak positions of the samples at various tetracycline concentrations. Compared to original EPS, the location of peak B in the presence of tetracycline was blue shifted by 5 nm at both temperatures, indicating the changes in the conformations of proteins in EPS.

Discussion

EPS were excreted by microorganism and widely distributed in aquatic environment. Furthermore, EPS were tightly bound to the biological flocs, and they can provide increased accommodation of the antibiotic substances with the flocs (Yang et al. 2012; Su and Yu 2005). Hence, it was momentous to explore the interaction mechanism of EPS in addition of antibiotics. Identification of dominant active constituents in EPS is important to understand the interaction mechanism and the fate of antibiotics in aquatic system.

Fluorescence quenching is the decrease of the fluorescence signal intensity from a fluorophore induced by a variety of molecular interactions with quencher molecule (Hu et al. 2006). The different mechanisms of quenching are usually classified as either dynamic quenching or static quenching according to their differing dependence on temperature and viscosity (Pan et al. 2010b). Moreover, dynamic quenching and static quenching are caused by diffusion and ground-state complex formation, respectively (Zhang et al. 2008). In this experiment, obvious quenching was observed at the region of peak A and peak B, which were identified as proteins in EPS. The quenching of EPS by tetracycline can be analyzed by the Stern–Volmer equation (list in ESI) and the quenching rate constant K_q at 298 and 308 K was 1.08×10^{13} ($R^2=0.9104$)

and 1.13×10^{13} ($R^2=0.9505$) L mol⁻¹ s⁻¹ (Table S2), respectively. Both values were greater than 2.0×10^{10} L mol⁻¹ s⁻¹, which is regarded as the limiting diffusion rate constant (Zhao et al. 2011). Furthermore, it is observed that the higher temperature results in the lower quenching constant K_b , implying that the quenching mechanism of EPS by tetracycline might be not a dynamic quenching process but a static quenching process (Wang et al. 2011a). These results are consistent with the phenomenon found in BSA test in addition of tetracycline (Table S3). The quenching types of BSA by some quenchers, such as acid yellow (Pan et al. 2010b), CdTe quantum dots (Zhao et al. 2009), nanoAg (Liu et al. 2009), and naphthol (Wu et al. 2007), were all explored to be static quenching due to the formation of a nonluminescent ground state complex between the fluorophore and quencher. Moreover, the effect of tetracycline on protein-like substances was extensively studied by other researchers. Anand et al. (2011) discovered the mechanism of fluorescence quenching induced by tetracycline on human serum albumin, bovine serum albumin, and lysozyme as representative proteins. Additionally, the gradual decrease in fluorescence intensity can be ascribed to static quenching which takes place by tetracycline with the proteins. To further confirm the mechanism of EPS fluorescence quenching by tetracycline, the UV–visible absorption spectra of EPS and EPS–TC complex at various TC dosages were measured. As shown in Fig S5, the absorbance spectra of EPS were quiet different from that of EPS–TC complex which subtracted the spectra of TC at the same dosage. It is well accepted that there should be no difference in absorbance spectra if the quenching was attributed to dynamic quenching (Xu et al. 2013; Tian et al. 2010). In this study, however, the spectra of EPS–TC complex showed obvious differences with that of EPS, implying that the fluorescence quenching of EPS by TC should be a static quenching process with the formation of complex compound (Xu et al. 2013).

The functional groups of EPS and tetracycline were important factors, which could be closely related to the special chemical properties. As shown in ¹H NMR analysis, the increase of electron density around hydrogen atoms in aromatic rings may explain the variation of bond orbital energy in phenyl group from the UV analysis. No ring fault was observed during the whole process via FTIR analysis. It indicates that electron distribution of aromatic rings might be affected

by external groups with no bond breaking. Jiao et al. (2008) noted that the naphthol ring of tetracycline remains intact during photolysis, which is consistent with our results. The changes of amino group were also observed in FTIR and XPS tests. The decrease of dipole and increase of vibrational energies could make amino group unstable. Furthermore, several studies have reported that *N*-methyl and amino group are the first response functional groups in tetracycline degradation due to the low bond energy of N–C (Delépée et al. 2000; Chen et al. 2011). According to FTIR, XPS, and ¹H NMR spectra, the hydroxyl groups are involved in the reaction, especially hydroxyl groups in tetracycline (as shown in ¹H NMR). Khunjar et al. (2011) found the hydroxylation occurred adjacent to hydroxyl group on the ring of 17 α -ethinylestradiol. As one of the most important functional groups in antibiotics, –OH group is considered as an active group for antibiotic activity (Schlunzen et al. 2001). In addition, it has been reported that 9-hydroxytetracycline has a larger antibiotic activity in comparison to that of tetracycline because of more hydroxyl groups (Dalmázio et al. 2007), and the effects on hydroxyl groups may damage or weaken the antibiotic activity of tetracycline (Fan and He 2011), which could cut down the inhibitory action of tetracycline on microorganism. In addition, the stripping of hydroxyl has been observed during the degradation of tetracycline in other papers (Chen et al. 2008; Dalmázio et al. 2007; Schlunzen et al. 2001).

Despite the low concentrations at which antibiotics chemicals have been detected, serious concerns exist about the negative impacts of antibiotics on aquatic ecosystem and human health. Even though there are many studies about the toxicity of antibiotics to microorganism, little was known about the fate of antibiotics when they would encounter EPS from microbial sources before directly interacting with bacteria. In this study, the reaction mechanisms between EPS and tetracycline were explored. The results of FTIR, XPS, and ¹H NMR analysis showed that the proteins were identified as the dominant active constituents in the reaction of EPS with tetracycline. Tetracycline was found to form a complex with the proteins in EPS through electrostatic forces. Moreover, hydroxyl groups were obviously altered during the reaction, which would reduce the biotoxicity of antibiotics and weaken the inhibitory effect on microorganism. This work would be significant for the transport and transformation of antibiotics in aquatic system and be helpful for the biological treatment of antibiotics in the future.

Conclusions

In this study, the reaction mechanisms between EPS and tetracycline, which were typical representatives of antibiotics,

were explored and the proteins were identified as the active constituents in EPS during the reaction.

The quenching mechanism of EPS on tetracycline was a static quenching process, suggesting the formation of complex. There is one binding site during the interaction, and the process is spontaneous in which electrostatic forces play a major role.

The changes of hydroxyl, carboxyl, and amino were observed in the interaction, especially hydroxyl groups in tetracycline. It would damage or weaken the activity of antibiotics and affect the biotransformation and fate of antibiotics in aquatic environment.

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