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Interaction mechanism between stratified extracellular polymeric substances (EPS) from activated sludge and claforan in domestic wastewater

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ABSTRACT

Extracellular polymeric substances (EPS) play a crucial role in claforan bio-adsorption using activated sludge, but the interaction mechanism between claforan and EPS remains unclear. Fluorescence spectroscopy technology was employed to illuminate the mechanism in this study. Two protein-like fluorescence peaks (peak A with Ex/Em = 225 nm/337 nm and peak B with Ex/Em = 280/335 nm) and humic acid-like (peak C with Ex/Em = 350/434) were identified in loosely bound extracellular polymeric substances (LB-EPS). Fluorescence of the peaks A and B for LB-EPS were markedly quenched by claforan at all temperatures whereas fluorescence of peak C for LB-EPS was quenched only at 288 K. Peak A and Peak B interaction with Claforan fits Stern-Volmer equation and Hill model. log K_b was 7.17 and 5.05, respectively, and the number of binding sites (n) was 1.56 and 1.19. log K_b and n for peak C were not able to fit the equation at 298 K. The fluorescence quenching of interaction between peak A and peak B and Claforan was governed a static process, whereas the interaction between peak C and Claforan may be both static and dynamic quenching. The molecular binding mechanisms obtained in this study will add fundamental knowledge of understanding the roles of EPS from activated sludge in claforan bio-adsorption in domestic wastewater.

Keywords: Extracellular polymeric substances; Claforan; Fluorescence quenching; Domestic wastewater

1. Introduction

Pharmaceuticals and personal care products (PPCPs) which contain diverse organic groups, such as antibiotics, hormones, antimicrobial agents, synthetic musks, etc., have raised significant concerns in recently years [1]. China is the world's third largest country of personal care product after the United States and Japan, and the presence of PPCPs in many urban sewage treatment plants, urban water environments and rivers [2]. The occurrence of PPCPs reported in wastewater treatment plants (WWTPs) indicates they cannot be completely removed through wastewater treatment process and then are released into aquatic environment [3–5]. PPCPs can also enter the environment through manufacturing plants, thus directly or indirectly threaten human health. At present in the ecological and health aspects, PPCPs are most concerned about steroid antibiotics.

Extracellular polymeric substances (EPS) are present both outside of the cells and in the interior of microbial aggregates, and account for a main component in microbial aggregates. EPS can influence the properties and functions of microbial aggregates in biological wastewater treatment systems, and specifically EPS are involved in biofilm formation and stability [6]. It was reported that EPS were a main component in biofilms, and proportion of EPS varied from 50% to 80% (w/w) of total biofilms weight [7–9]. In recent years, the effects of EPS on properties and functions of microbial aggregates in biological wastewater treatments are paid much attention, and the EPS are proved to exhibit important roles in mass transfer [10]. And certain studies have suggested that compositions and properties of EPS rather than quantity, had greater influences on some functions

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of microbial aggregates [11]. Different components exhibit their own specific roles, largely determining properties and functions of EPS and microbial aggregates. Specifically, the main roles of extracellular proteins in microbial aggregates were multivalent cations and organic molecules binding, as well as in catalysis and degradation [12]. Furthermore, amino acid compositions and secondary structures of proteins in EPS significantly contributed to hydrophobic interactions and further to high aggregation activity of microbial aggregates [13,14]. So far, there has been little research on the interaction between EPS and Claforan. The aim of the present study was to investigate the binding capacity and binding mode of EPS from aerobic activated sludge for Claforan using fluorescence spectroscopy.

2. Material and methods

Activated sludge was collected from an aeration tank in the Central Municipal Wastewater Treatment Plant, Wenzhou, China. All solutions were prepared from reagent-grade chemicals in ultra pure water. EPS is prevalent in a ctivated sludge flocs. In the actual experimental operation, the EPS extraction methods are not the same. At present, the common extraction methods include: physical extraction and chemical extraction [15]. Hierarchical extraction of EPS using centrifugal/ultrasonic method [16]. The supernatant from the low-speed centrifugation was Slime. The loosely bound extracellular polymeric substances (LB-EPS) and tightly bound extracellular polymeric substances(TB-EPS) were dissolved in the buffer liquid, and the corresponding buffer was Na_3PO_4 (2×10^{-3} mol/L), NaH_2PO_4 (4×10 mol/L), NaCl (9×10 mol/L) and KCl (1×10 mol/L) in a certain ratio (pH=7). Before use, adjust the conductivity of this buffer to the same conductivity as the sludge.

All of three-dimensional excitation-emission matrix (3DEEM) fluorescence spectra were measured using a Spectrofluorophotometer (RF-5301PC, Shimadzu, Japan), and measured at 278, 288, 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 (Ex) and emission (Em) wavelength ranges of 200–450 nm (2 nm slit) and 250–600 nm (2 nm slit), respectively, both at 5-nm increments. Using ultra pure water as a blank contrast and the fluorescence spectral data are processed by Matlab software R2017b .

3. Results and discussion

3.1. The 3DEEM fluorescence of EPS

EPS from activated sludge can be divided into three types, including Slime, LB-EPS and TB-EPS. The three-dimensional fluorescence characteristics shown in Fig. 1. Two fluorescence peaks were identified from the 3DEEM fluorescence spectrum (Fig. 1a), indicating two groups of fluorophores were present in the Slime. The fluorescence of peak A (E_x about 225 nm, E_m about 337 nm) and C (E_x about 350 nm, E_m about 434 nm) was originated from the protein-like substances (peak A) and humic-like acid (peak C). Three fluorescence peaks were identified from the 3DEEM fluorescence spectrum (Fig. 1b and Fig. 1c), indicating three groups of fluorophores were present in the LB-EPS and TB-EPS. The fluorescence of peak A (E_x about 225 nm, E_m about 337 nm), peak B (E_x about 280 nm, E_m about 335 nm) and C (E_x about 350 nm, E_m about 434 nm) was originated from the protein-like substances (peak A and peak B) [17] and humic-like acid (peak C). This is similar to that of Pan, and other fluorescent components such as fulvic-like acid and nucleic acid were not detected in the present study [18].

The fluorescence intensity of peak A is not significantly different among the three EPS. The fluorescence intensity of peak B is larger in LB-EPS than in TB-EPS. The order of the fluorescence intensity in EPS of peak C : Slime>LB-EPS>TB-EPS. Total amount and the contents of proteins and polysaccharides of slime, LB-EPS and TB-EPS in the samples are significantly differentiated in their chemical composition.

3.2. Fluorescence quenching of LB-EPS by claforan

Fluorescence at peaks A and B was clearly quenched by claforan in Fig. 2a and Fig. 2b, indicating that claforan reacted with the protein-like substances of fluorophore in LB-EPS at four different temperatures. Fluorescence intensity at peak A and peak B decreased with increasing claforan concentration, suggesting that increased the claforan concentration is helpful to the occurrence of fluorescence

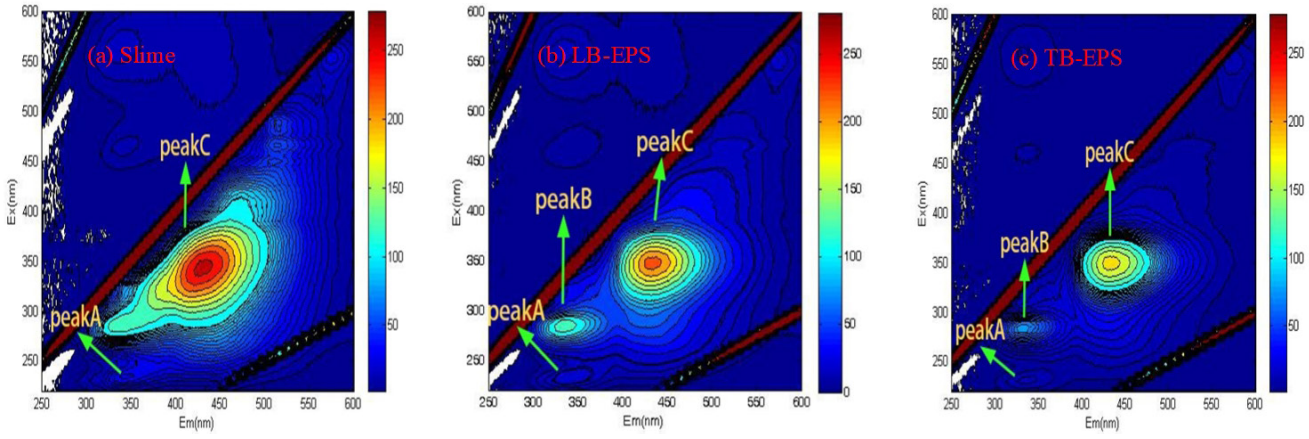

Fig. 1. The exemplified 3DEEM fluorescence spectrum of three different EPS at 298 K.

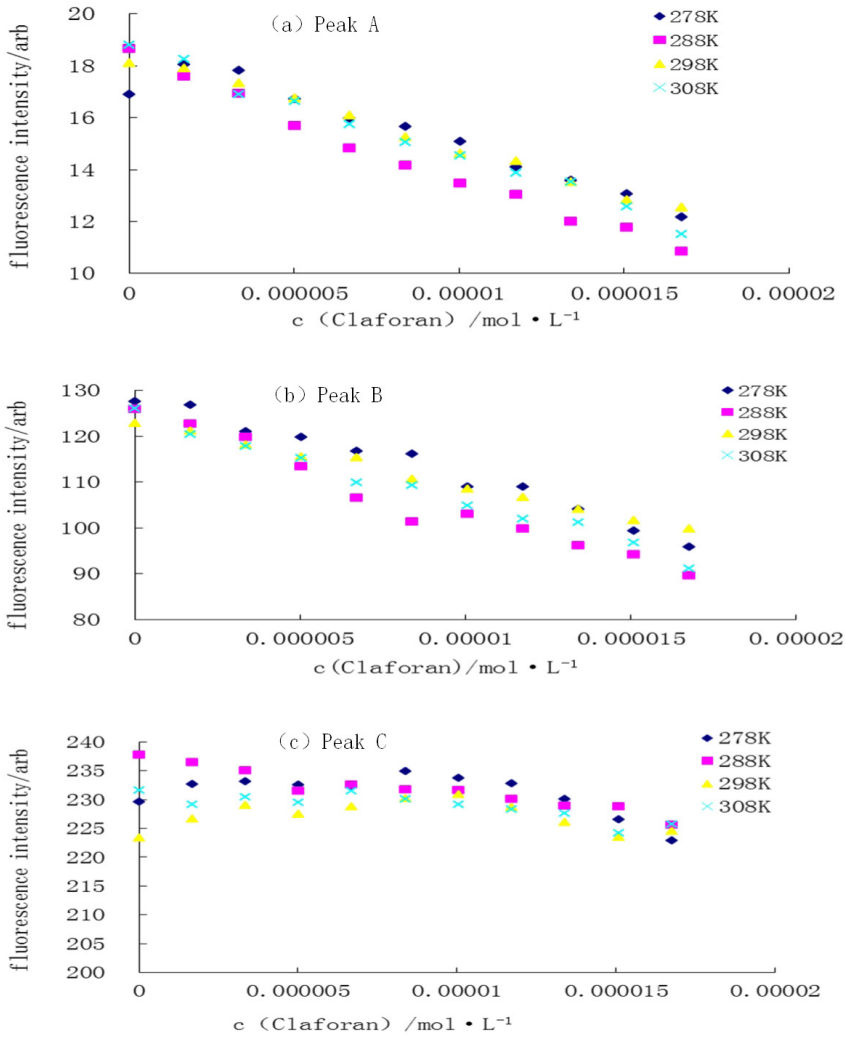

Fig. 2. The fluorescence intensity of peaks varied with increasing Claforan concentration at various temperatures.

quenching reaction. Claforan only reacted with the peak C at 288 K, and the fluorescence intensity did not change obviously at other temperatures.

3.3. Fluorescence quenching mechanism

The 3DEEM fluorescence spectrum of LB-EPS before and after quenching by Claforan are shown in Figs. 3a and 3b. From the figure, it can be seen that the change of the addition of Claforan, the fluorescence peak B and the peak C have obvious quenching effect, and the quenching effect of the peak A is not obvious.

Fig. 4 shows that the fluorescence peak intensity of peak A and peak B decreases with the increase of Claforan concentration, but the fluorescence peak of peak C does not change. This indicates that the interaction between peak A, peak B and Claforan in EPS is strong, and the interaction between peak C and Claforan is independent of Claforan concentration.

Fluorescence quenching is a sensitive and fast method to quantify the interactions between a fluorescent organic contaminant and a quencher. Generally, the fluorescence quenching process can be divided into two types, static quenching process and dynamic quenching process. The dynamic quenching is attributed to the collision between fluorophore and quencher at excited state whereas the static quenching is due to the formation of a complex between fluorophore and quencher with the external forces. Dynamic fluorescence quenching is resulted from molecular collision, not the real binding, and thus it complicates the binding data interpretation [19]. When the temperature increases, the number of effective collisions in the dynamic quenching process increases, thereby strengthening the dynamic quenching. Elevated temperature will reduce the stability of complexes during static quenching.

3.3.1. Stern-volmer equation

In order to calculate the quenching rate constants and reveal the underlying mechanism for quenching, the fluorescence quenching data were modeled with Stern–Volmer equations [18]. The potential mechanism of quenching and

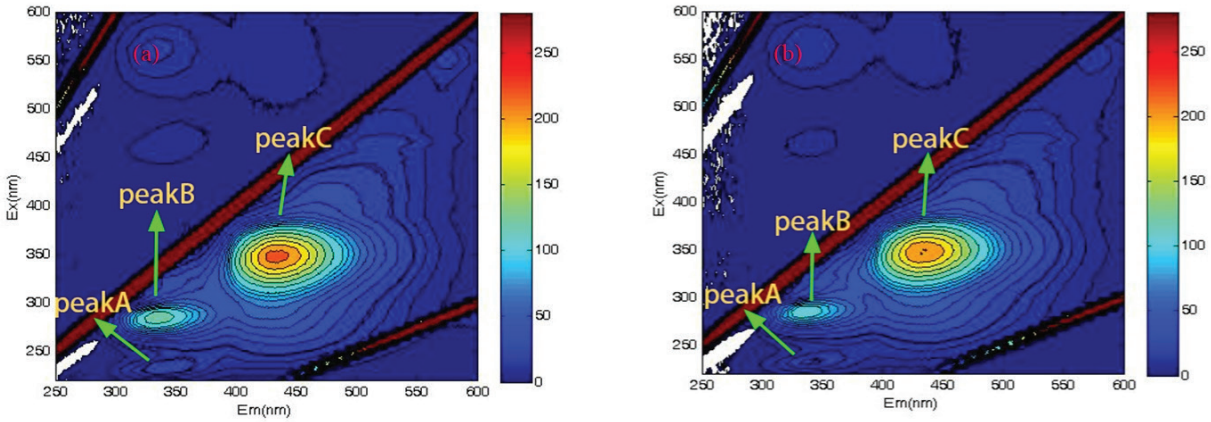

Fig. 3. (a) 3DEEM fluorescence spectrum of LB-EPS in the absence of 0.00001 mol/L Claforan at 298 K; (b) 3DEEM fluorescence spectrum of LB-EPS in the presence of 0.00001 mol/L Claforan at 298 K.

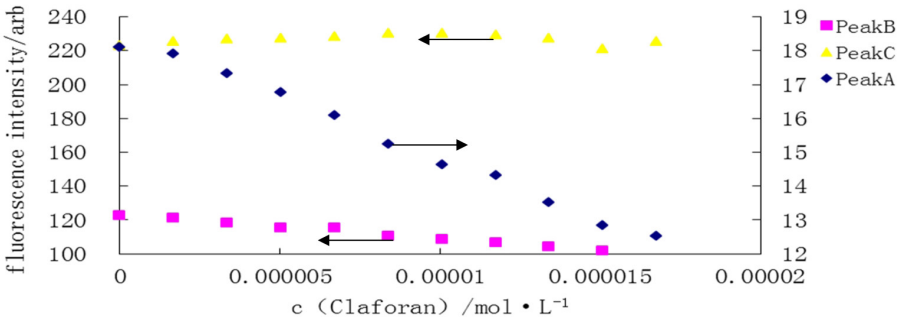

Fig. 4. The fluorescence intensity of peak A , peak B and peak C of LB-EPS with increasing Claforan concentration at 298 K.

quenching rate constant can be fitted using the Stern-Volmer equation [20].

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + K_q \tau_0 [Q] \quad (1)$$

where F, K_q, τ_0, K_{sv} and $[Q]$ are the fluorescence intensities at certain concentrations of antibiotics, quenching rate constant, average lifetime (10^{-8} s) in the absence of quenche, Sterne-Volmer quenching constant and the concentration of antibiotics, respectively.

Based on the linear regression analysis in Table 1, the K_q for peak A and peak B were 2.81×10^{12} (R^2 = 0.9594) and 1.41×10^{12} (R^2 = 0.9904) L/mol/s. The results suggested that the protein like substances played an important role in quenching process. The fluorescent proteins were mainly attributed to tryptophan-like and tyrosine-like components in proteins [20]. Xu et al. research shows that the quenching constant of STZ to proteins was higher than that to humics, suggesting that proteins might be dominant fractions to interact with the LB-EPS [21]. Peak A and peak B of K_q were higher than the maximum diffusion collision quenching rate constant of quenchers with biomacro molecules of 2.0×10^{10} L/mol/s. The results suggested that the quenching of fluorescence intensities might be ascribed to the formation of EPS-antibiotics complexes (static quenching), rather than dynamic quenching. The higher quenching of proteins induced by antibiotics was attributed to the electronic structural changes in protein-like substances due to the formation of EPS-antibiotics complex compound [22,23]. Poor linear relationship between F_0/F and $[Q]$ was observed for peak C of LB-EPS. One possible reason is that the dynamic quenching and static fluorescence quenching occur simultaneously.

Table 1
Stern-Volmer quenching constants for the interaction of Claforan with LB-EPS at 298K

Peak	K_{sv}(KD)(L·mol^{-1})	K_q (L/mol/s)	R^2
Peak A	28120	2.81×10^{12}	0.9594
Peak B	14094	1.41×10^{12}	0.9904
Peak C	12.516	12.5×10^{9}	3×10^{-5}

3.3.2. Perrin equation

To further confirmed that Peak A and Peak B were statically quenched, used the Perrin equation to verify. Take peak B as an example.

$$In\left(\frac{F_0}{F}\right) = K_p[Q] \quad (2)$$

where F_0 and F are fluorescence intensities of the fluorophores in absence and presence of antibiotics, respectively; Q is the concentration of antibiotics (mmol/L), K_p is Perrin static quenching constant (L·mol^{-1}).

According to the Perrin equation, when the peaks B and Claforan interact with each other, 298 K and 288 K, R^2, respectively, 0.9925 and 0.9703, the linear relationship is better. As the temperature increases, the static quenching constant decreases, as shown in Table 2. It can be further determined that the peak B and Claforan interactions are static quenching.

3.3.3. Binding constant and binding-site number

In the static quenching process, the binding constant (K_b) and the binding-site number (n) can be calculated using the modified Hill equation.

$$log\left[\frac{F_0 - F}{F}\right] = \log K_b + n\log[Q] \quad (3)$$

where F_0 and F are fluorescence intensities of the fluorophores in absence and presence of antibiotics, respectively; Q is the concentration of antibiotics (mmol/L), K_b is binding constant, n is the binding-site Number.

Claforan and peak A, peak B binding-site number are 1.5645 and 1.189, respectively. The log K_b are 7.1673 and 5.0516, respectively. From the peak A and the peak B of R^2 is greater than 0.97 can be drawn. When the interaction between Claforan and LB-EPS, their binding constants and binding-site numbers can be well calculated by the Hill equation. The binding-site numbers and binding constants of peak C cannot be calculated by Hill equation.

The log K_b of the fluorescence peaks A and B decreases as the temperature increases. As shown in Table 3, indicating that Claforan and fluorescent substances A and B (both protein-like) binding capacity decreases with increasing temperature. The binding-site number of the fluorescent substance B also decreases with increasing temperature, indicating that the fluorescent substance B can bind more Claforan at low temperatures. When the temperatures are 278 K, 288 K, 298 K, 308 K, the fluorescence peak A and B binding-site numbers are about 1, indicating that LB-EPS has more than one adsorption site for Claforan.

Table 2
The values of K_{sv} and K_p of Perrin equation for the interaction of Claforan with LB-EPS at 288 K and 298 K

T/K	K_{sv}/L·mol^{-1}	K_p/L·mol^{-1}
288	23765 (R^2=0.9750)	20092 (R^2=0.9703)
298	14094 (R^2=0.9904)	12703 (R^2=0.9925)

3.3.4. Calculation of thermodynamic parameters

Fluorescence quenching experiment methods according to different temperatures, the fluorescence intensity of Claforan and LB-EPS is measured at different temperatures (278, 288, 298, 308 K). Through the calculation of thermodynamic parameters, you can determine the quencher and protein between the force, including hydrogen bonds, van der Waals force, electrostatic attraction and hydrophobic force. The binding-site number of peak A is calculated as 2.72, 1.08, 1.56, 1.20 (n>1) and it is shown that the number of adsorption sites of Claforan by LB-EPS is more than one. The binding-site number of peak B is greater than 1, indicating that the number of adsorption sites of Claforan by LB-EPS is more than one. However, at the temperature of 308 K, its binding-site number is 0.90 (n≈1). The binding constant decreases with the increase of temperature, indicating that the binding force is weakened.

The thermodynamics formula is as follows:

$$\ln\frac{K_2}{K_1} = \frac{\Delta H}{R}\left(\frac{1}{T_1} - \frac{1}{T2}\right) \quad (4)$$

$$\Delta G = \Delta H - T\Delta S = -RT\ln K \quad (5)$$

where K, T, ΔS , ΔG, ΔH are the binding constants of claforan at different temperatures, different temperatures (K), entropy (J·K^{-1}·mol^{-1}), Gipps free energy (KJ·mol^{-1}), enthalpy (KJ·mol^{-1}), respectively.

According to the calculation of ΔH and ΔS, the calculation results are shown in Table 3. ΔG<0, indicating that the interaction between Claforan and fluorescent substances A and B (protein-like) is spontaneous. ΔS>0, indicating that the binding of Claforan to fluorescent substances A and B is an exothermic reaction. ΔH>0, ΔS>0, it can be judged that the main force between Claforan and the fluorescent substances A and B is hydrophobic force or electrostatic attraction.

Table 3
The binding constants (log K_b), binding site number (n) and thermodynamic parameters for the interaction of Claforan with LB-EPS at various temperatures

Peak	T(K)	n	log K_b	R^2	ΔG (KJ·mol^{-1})	ΔH (KJ·mol^{-1})	ΔS (J·K^{-1}·mol^{-1})
Peak A	278	2.72	12.66	0.9481	–67411.10	281.90	243.50
	288	1.08	4.99	0.9924			
	298	1.56	7.17	0.9933			
	308	1.23	5.50	0.9784			
Peak B	278	1.55	6.97	0.9365	–37084.62	282.94	134.42
	288	1.20	5.38	0.9715			
	298	1.19	5.05	0.9857			
	308	0.90	3.82	0.9755			

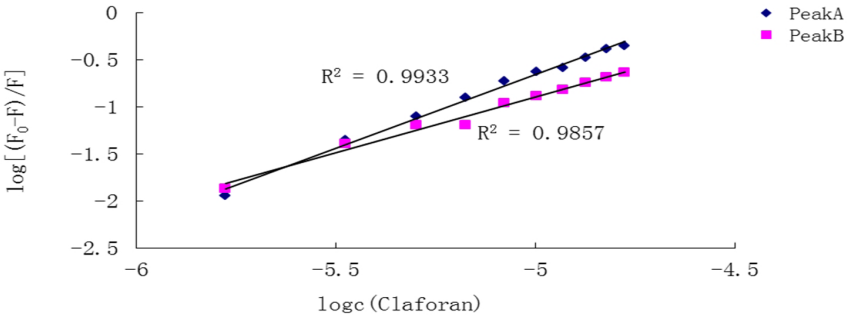

Fig. 5. Modified hill curves for the interaction of Claforan with LB-EPS at 298 K.

4. Conclusions

Extracellular polymeric substances (EPS) play a crucial role in claforan bio-adsorption in domestic wastewater. The fluorescence characteristics of different EPS are different. The fluorescence peaks A and B of LB-EPS can be quenched by Claforan, and the effect of peak C by Claforan quenching is not obvious. Fluorescence peaks A and B interact with Claforan for static quenching. The interaction between the fluorescence peak C and Claforan may be both dynamic and static quenching. The Stern-Volmer equation and the Hill model can well describe the interaction between the fluorescent groups A and B in LB-EPS with Claforan at 278 K, 288 K, 298 K, 308 K. With the increase of temperature, binding-site number gradually decreased, indicate that the temperature on the binding site have a certain impact. The interaction between the fluorescent group C and claforan in LB-EPS can't be fitted by the Stern-Volmer equation and the Hill model. Furthermore, binding of claforan to LB-EPS was spontaneous and exothermic. Electrostatic force and hydrophobic interaction forces play a crucial role in binding of claforan to EPS. Our study clearly demonstrated that fluorescence quenching could be successfully used to provide a better understanding of the chemical heterogeneity associated with Claforan-binding sites within EPS.

Acknowledgments

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