



A novel biosorbent for dye removal: Extracellular polymeric substance (EPS) of *Proteus mirabilis* TJ-1

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ABSTRACT

This paper deals with the extracellular polymeric substance (EPS) of *Proteus mirabilis* TJ-1 used as a novel biosorbent to remove dye from aqueous solution in batch systems. As a widely used and hazardous dye, basic blue 54 (BB54) was chosen as the model dye to examine the adsorption performance of the EPS. The effects of pH, initial dye concentration, contact time and temperature on the sorption of BB54 to the EPS were examined. At various initial dye concentrations (50–400 mg/L), the batch sorption equilibrium can be obtained in only 5 min. Kinetic studies suggested that the sorption followed the internal transport mechanism. According to the Langmuir model, the maximum BB54 uptake of 2.005 g/g was obtained. Chemical analysis of the EPS indicated the presence of protein (30.9%, w/w) and acid polysaccharide (63.1%, w/w). Scanning electron microscopy (SEM) images showed that the EPS with a crystal-linear structure was whole enwrapped by adsorbed dye molecules. FTIR spectrum result revealed the presence of adsorbing groups such as carboxyl, hydroxyl and amino groups in the EPS. High-molecular weight of the EPS with more binding-sites and stronger van der Waals forces together with its specific construct leads to the excellent performance of dye adsorption. The EPS shows potential board application as a biosorbent for both environmental protection and dye recovery.

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1. Introduction

Dyes have been extensively used in industries, such as textile, paper, printing, cosmetics, plastics and rubber, for the coloration of products [1,2]. They usually have a synthetic origin and are based on complex aromatic structures which make them stable and difficult to be biodegraded [1]. Annually, over 7×10^5 tons of dyes are produced worldwide, and 10–15% of them are discharged by the textile industry [3]. A small quantity of dyes can color large water bodies, which not only affects aesthetic merit but also reduces light penetration and photosynthesis. Moreover, many dyes are toxic in nature with suspected carcinogenic and mutagenic effects that affect aquatic biota and also human beings [4,5]. Therefore, the decolorization of dye-containing effluents is considered compulsory prior to discharge by the environmental regulations in most of the countries [3,6].

Since biodegradation are not so efficient in treating dye-containing wastewater, various physical and chemical methods have been used, including sorption, ozonolysis, precipitation, etc. [6,7]. Although ozonolysis and precipitation are efficient in dye removal, there are some limitations of these processes, such as high-running cost, low-removal efficiency, and labor-intensive operation [8]. Currently, the most widely used and effective physical method in industry is sorption with activated carbon, but operation costs are expensive for its regeneration after dye removal [9]. Therefore, developing cost-effective sorbents becomes fairly attractive for the treatment of dye-containing wastewater.

In recent years, biosorption has been considered as a promising technology for the removal of dyes from industrial effluents and natural waters [9]. Biosorption can be defined as the uptake of contaminants, via various physicochemical mechanisms including ion exchange, sorption, complexation, chelation, microprecipitation, etc., by biological materials [10]. Some biomaterials have been reported to remove dyes, including agricultural byproducts like rice husk, bark and orange peel [11–13] and microbiological materials such as algae, fungi and bacteria [14,15]. Their low cost for dye removal attracts people to exploit more biomaterials

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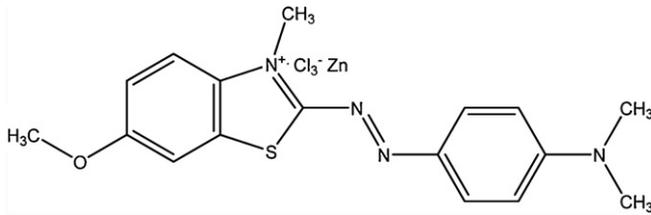


Fig. 1. Chemical structure of BB54.

to alternate traditional processes. Compared with the traditional sorbents, however, most of the present biomaterials need longer time to reach equilibrium and show lower sorption capacity.

Extracellular polymeric substance (EPS) is a microorganism-produced macromolecule and abundant in source [16]. Some EPSs have been reported to be used as biosorbents for heavy metals. Jang et al. [17] found that the biofilm capacity for removal of the heavy metals (Cu, Pb and Ni) in wastewater changed with the ratio of carbohydrate to protein in EPS. Guibaud et al. [18] reported that EPS exhibited a great ability to complex Pb and Ni. The EPS produced by anaerobic sludge under sulfate-reducing bacteria was effective in removing Cd^{2+} from aqueous solution [19]. Accordingly, it appears interesting to study EPSs which can be used as a cost-effective biosorbent for dye removal. To our best knowledge, EPS used as biosorbent for dye removal has never been reported previously.

In the present study, the EPS of *Proteus mirabilis* TJ-1 screened out from activated sludge of municipal wastewater treatment plant was extracted and used as a biosorbent for dye removal in laboratory batch systems. As a widely used and hazardous dye, basic blue 54 (BB54) was chosen as the model dye to examine the adsorption performance of the EPS. The effects of pH, initial dye concentration, contact time and temperature on the sorption of BB54 to the EPS were investigated, and kinetics and isotherm models were used to fit the experimental data. The major components, structure and functional groups of the EPS were obtained by chemical analysis, scanning electron microscopy (SEM) and Fourier-transform infrared (FTIR) spectrometry, respectively, which were used to elucidate the sorption mechanism of the EPS.

2. Materials and methods

2.1. Microorganism culture and EPS preparation

P. mirabilis TJ-1 (GenBank accession No. EF091150) is a sorbent-producing microorganism screened out from the mixed activated sludge of four wastewater treatment plants (Quyang, Anting,

Dongqu and Tongjixinchun in Shanghai, China) by the State Key Laboratory of Pollution Control and Resource Reuse (SKL), Tongji University of China [20]. A 150-mL flask containing 50 mL production medium was inoculated with 1.0 mL pre-culture of strain TJ-1 and incubated at 25 °C in a rotary shaker at 130 rpm for 48 h [20]. The fermentation broth obtained was centrifuged ($4000 \times g$, 30 min) to separate the cells. The cell-free culture supernatant was the liquid EPS and preserved at 4 °C in fridge for further use.

2.2. Preparation of dye solution

BB54 was obtained from Shanghai Jiaye Dyestuff Co., Ltd. (China), and its chemical structure is shown in Fig. 1. Stock solution of concentration 400 mg/L was prepared by dissolving an accurate quantity of dye in distilled water. Other experimental solutions were obtained by diluting the stock solution before being used. A calibration curve was drawn by measuring the absorbances of various dye concentrations at the maximum absorption wavelength 602 nm by SHIMADZU spectrophotometer UV 1700.

2.3. Dye removal by EPS

Biosorption experiments were carried out in a rotary shaker using 150-mL flasks containing 20-mL dye solutions with different initial dye concentrations and pH at 130 rpm and room temperature (25 °C). The pH of the solution was previously adjusted with HCl (1%, w/w) or NaOH (1%, w/w). After adding EPS, the flasks were sealed to prevent change in concentration of the solution during the experiments. After shaking the flasks for predetermined time intervals, the samples were taken out from the flasks and the dye solutions were separated from the sorbent by centrifugation at $4000 \times g$ for 20 min. Dye concentrations in the supernatant solutions were estimated by measuring the absorbance at 602 nm with SHIMADZU spectrophotometer UV 1700 and computed from the calibration curve. The amount of BB54 adsorbed by the EPS was calculated using the following equation [21]:

$$q_t = \frac{(C_0 - C_t)V}{W} \quad (1)$$

where q_t (mg/g) is the amount of adsorbed dye by EPS at time t (min); C_0 (mg/L) and C_t (mg/L) are the liquid phase concentrations of the dye at time 0 (min) and t (min), respectively; V (mL) the volume of the solution, and W (g) the weight of the EPS. The experiments were conducted in triplicate and the negative controls (with no sorbent) were simultaneously carried out to ensure that sorption was caused only by EPS.

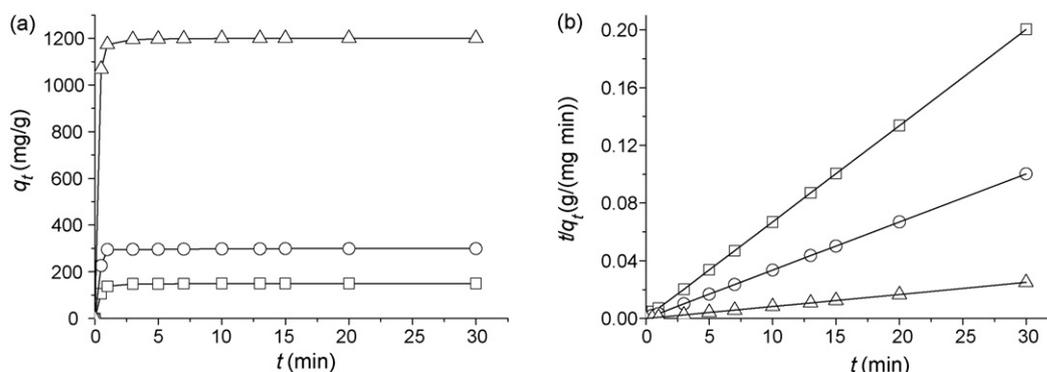


Fig. 2. (a and b) Effect of the initial dye concentration on the sorption capacity of the EPS at pH 12.0 and 25 °C; BB54 Concentration: (□) 50 mg/L, (○) 100 mg/L and (Δ) 400 mg/L.

Table 1

Pseudo-second-order equation parameters for different initial dye concentrations in the biosorption system of BB54 to the EPS

C_0 (mg/L)	$q_{e,exp}$ (mg/g)	k_2 (g/(mg min))	$q_{e,cal}$ (mg/g)	R^2
50	149.7	0.08978	149.3	1.000
100	299.4	0.05445	303.0	1.000
400	1200.2	0.03200	1250.0	1.000

2.4. Biosorption kinetics of the EPS

The pseudo-second-order kinetic model was used to elucidate the biosorption process. The linear equation of the model is expressed as [3]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (2)$$

where k_2 is the rate constant of pseudo-second-order sorption, q_e and q_t being amounts of BB54 adsorbed at equilibrium and time t , respectively.

2.5. Isotherm models to fit batch experiment data

Both Langmuir and Freundlich models were used to fit the isotherm experimental data. The linear form of the Langmuir isotherm equation is shown as follows [3]:

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max} K_L} \right) \frac{1}{C_e} \quad (3)$$

where C_e is the equilibrium dye concentration in the solution (mg/L), q_{max} the monolayer sorption capacity of the biosorbent (mg/L), and K_L is the Langmuir constant (L/mg) and is related to the free energy of biosorption.

The linear form of the Freundlich isotherm equation is given as follows [3]:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (4)$$

where K_F (L/mg)^{1/n} and n are Freundlich sorption isotherm constants being indicative of the extent of the biosorption and the degree of nonlinearity between solution concentration and biosorption, respectively.

2.6. Physical and chemical characterization of EPS

1.33 g of the purified EPS, whose molecular weight (MW) is 1.2×10^5 Da, could be recovered from 1.0 L of fermentation broth of

P. mirabilis TJ-1 [20]. Chemical analyses of the EPS were conducted to identify the components. The protein content was measured by the Bradford method with bovine serum albumin (BSA) as the standard [22]. The total sugar content was determined by the phenol–sulfuric acid method using glucose as the standard solution [23]. The neutral sugar was determined by the anthrone reaction [23]. The uronic acid was measured using the carbazole–sulfuric acid method [23]. Amino sugars were determined according to the Elson–Morgan method with glucose amine as the standard solution [23]. SEM images of the EPS before and after adsorption of BB54 were obtained using Philips XL 30 ESEM. The infrared spectrum of the EPS (as KBr disks) was recorded at room temperature in the wave number range of 4000–400 cm⁻¹ with a FTIR spectrophotometer Nicolet Nexus 670.

3. Results and discussion

3.1. Effect of pH

pH of the solution is one of the primary parameters controlling the sorption process due to its impact on both the surface binding-sites of the sorbent and the ionization process of the dye molecule [24]. In the present biosorption system, the residual BB54 concentration in the aqueous solution decreased with the increase in pH, and became constant at a pH greater than 12 (figure not shown). At lower pH, the surface charge might get positively charged, thus making (H⁺) ions compete effectively with dye cations causing a decrease in the amount of BB54 adsorbed by the EPS [3]. At higher pH, the EPS might get negatively charged, which enhanced the sorption of the positively charged dye cations through electrostatic forces of attraction [3]. This adsorption feature of the EPS is different from those obtained from dried *Cephalosporium aphidicola* cells [1] and acid-treated biomass of brown seaweed *Laminaria* sp. [2] but consistent with those obtained from *Azadirachta indica* leaf powder and *Posidonia oceanica* (L.) fibres [3,24].

3.2. Effects of initial dye concentration and contact time

The effect of initial dye concentration on the sorption capacity of the EPS at various contact times is presented in Fig. 2(a). In order to predict the kinetics of the present sorption process, the pseudo-second-order model was used (Fig. 2(b)). The kinetic data obtained are shown in Table 1. The sorption of BB54 to the EPS was rapid for any initial dye concentration in the first 3 min and attained the equilibrium within 5 min. Then the adsorption rate decreased with the time due to the continuous decrease in the concentration

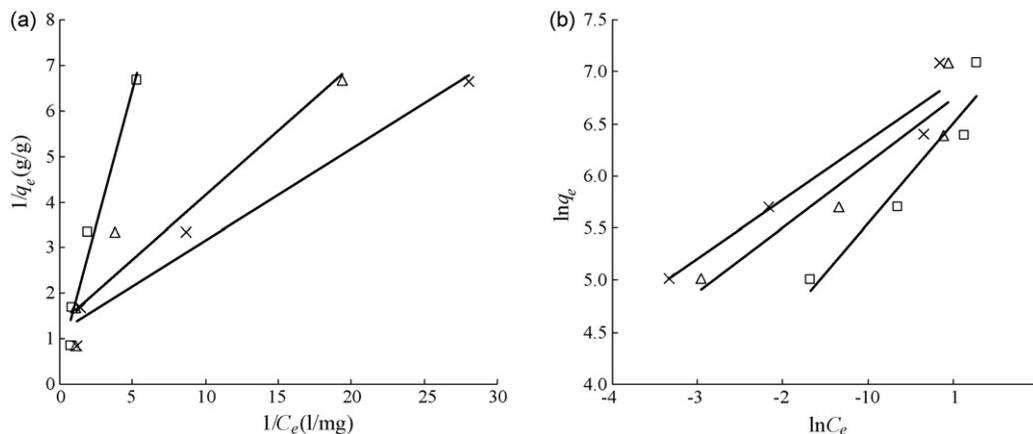


Fig. 3. Langmuir plots (a) and Freundlich plots (b) for the sorption of BB54 to the EPS at various temperatures: (□) 25 °C, (Δ) 35 °C and (×) 45 °C.

Table 2
Langmuir and Freundlich model parameters under different temperature conditions in the biosorption system of BB54 to the EPS

T (°C)	Langmuir model			Freundlich model		
	q_{\max} (g/g)	K_L (L/mg)	R^2	K_F (L/mg) ^{1/n}	n	R^2
25	2.005	0.4194	0.9646	668.7	1.043	0.9133
35	0.7482	4.739	0.9243	842.5	1.627	0.8837
45	0.8701	5.721	0.9714	993.0	1.764	0.9282

driving force. On the other hand, its sorption capacity increased with the increasing initial dye concentration. As we can see from Table 1, raising the dye concentration from 50 to 400 mg/L allowed the EPS to increase its sorption capacity from 149 to 1200 mg/g. The correlation coefficients (R^2) for the pseudo-second-order model are equal to 1 for all investigated initial dye concentrations and the predicted values ($q_{e,cal}$) are fairly satisfactory compared to the experimental ones ($q_{e,exp}$), which indicates that the sorption process of BB54 to the EPS follows the intraparticle diffusion model [25].

3.3. Biosorption isotherms

The effect of temperature on the sorption capacity of BB54 to the EPS was investigated. Langmuir plots and Freundlich plots obtained from the isothermal data are shown in Fig. 3(a) and (b), respectively. The equilibrium sorption capacity of BB54 was found to decrease at higher temperature, indicating that the sorption of the EPS to the dye was favored at room temperatures. The phenomenon may be due to the denaturing of the EPS caused by the higher temperature [18,26]. It then suggests that the sorption mechanism associated with the removal of BB54 by the EPS involved a chemical sorption process. The Langmuir and Freundlich parameters for the sorption of BB54 to the EPS are listed in Table 2. These data indicate that both of the isotherm models described the experimental data well, meaning that the surface of the EPS is made up of homogeneous and heterogeneous biosorption patches [27].

It is interesting to compare the sorption features of dyes between the EPS and various sorbents reported in the literature (Table 3). For *Corynebacterium glutamicum* [28] and acid-treated biomass of brown seaweed *Laminaria* sp. [2], the time taken to reach equilibrium were 2 and 3 h, respectively, and the sorption capacities were 419 and 101.5 mg/g, respectively. The sorption equilibrium of *P. oceanica* (L.) fibres to methylene blue was attained in 10 min, but the sorption capacity was only 5.56 mg/g [3]. Rice husk showed a good sorption capacity to safranin, while the time taken to reach equilibrium was about 6 h. Used as the sorbent of methylene blue, the

activated carbon from coconut husk showed the maximum monolayer adsorption capacity of 434.78 mg/g. Compared to the above sorbents, the EPS shows higher sorption rate and bigger sorption capacity for dye removal.

3.4. Analysis of the sorption mechanism

Chemical analysis of the EPS indicated that it contained protein (30.9%, w/w) and acid polysaccharide (63.1%, w/w). The purified biopolymer was hydrolyzed with trifluoroacetic acid to determine the content in various sugars, and was found to be a mixture of many saccharides including neutral sugar, glucuronic acid and amino sugar (approximate weight ratio of 8.2:5.3:1). The EPS therefore is a natural organic macromolecule (1.2×10^5 Da) containing protein and acid polysaccharide [20].

SEM observations were carried out to elucidate the surface morphology of the EPS before and after adsorption of BB54. As it can be seen from Fig. 4(a), the EPS shows a crystal-linear structure, and its length is about 4.0 μm . After adsorption of BB54 (Fig. 4(b)), the EPS wrapped by the dye is like a well-knit net and separate from water, which reveals the excellent sorption performance of the EPS. To better understand the nature of the functional groups responsible for the biosorption process, the FTIR spectrum of the EPS is presented in Fig. 5. The EPS displays a broad stretching intense peak at around 3400 cm^{-1} characteristic for hydroxyl and amino groups. Further, the asymmetrical stretching peak was noticed at 1700 cm^{-1} , suggesting the presence of carboxyl groups. The absorption peaks around $1000\text{--}1100$ and 980 cm^{-1} are known to be characteristic for all sugar derivatives.

The sorption with a high-molecular weight sorbent involves more binding-sites, stronger van der Waals forces than in the case of the sorption with a low-molecular weight sorbent [16]. For a sorbent, the linear structure can assure more binding-sites to be functional and adsorbing more dye molecules [17,19]. Carboxyl, hydroxyl and amino groups are the preferred groups for most sorption processes [18,26]. The EPS possesses all the characteristics favoring the sorption process, which makes it a strong sorbent agent.

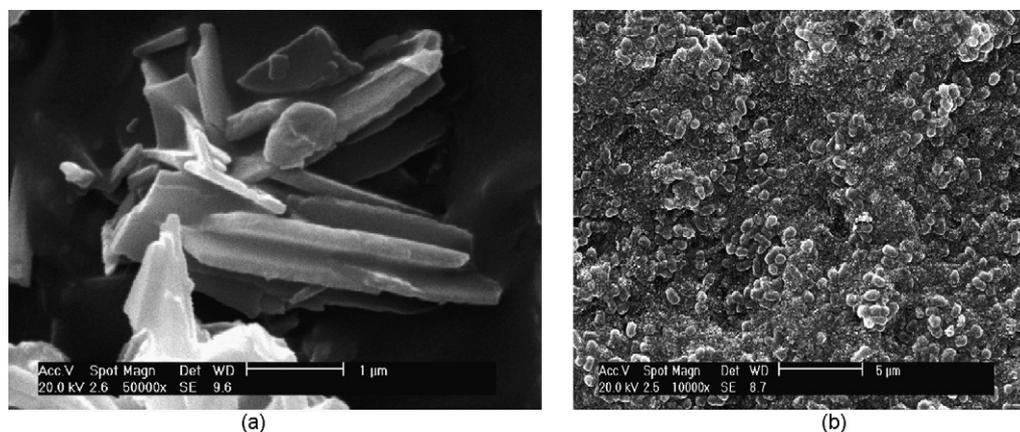
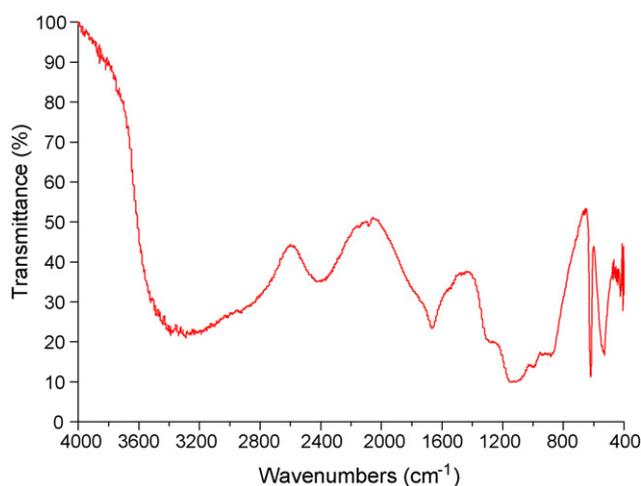


Fig. 4. SEM images of the EPS before (a) and after (b) adsorption of BB54.

Table 3

Comparison of the sorption features of dyes between the EPS and various sorbents reported in the literature

Biosorbent	Dye	q_{\max} (mg/g)	Equilibrium time (min)	Reference
<i>Corynebacterium glutamicum</i>	Reactive Black 5	419	120	[28]
<i>Cephalosporium aphidicola</i> cells	Acid Red 57	109.41	40	[1]
Biomass of <i>Laminaria</i> sp.	Reactive Black 5	101.5	180	[2]
<i>Posidonia oceanica</i> (L.) fibres	Methylene blue	5.56	10	[3]
Green alga <i>Chlorella vulgaris</i>	Remazol Black B	419.5	120	[15]
<i>Azadirachta indica</i> leaf powder	Congo Red	41.2	300	[24]
Orange peel	Congo Red	22.44	90	[11]
Rice husk	Safranine	1119	360	[12]
Cotton	Safranine	838	360	[12]
Bark	Safranine	875	360	[12]
Hair	Methylene blue	158	360	[12]
Coal	Methylene blue	250	360	[12]
Activated carbon from coconut husk	Methylene blue	434.78	120	[29]
EPS	Basic Blue 54	2005	5	This work

**Fig. 5.** FTIR spectrum of the EPS.

4. Conclusions

The EPS of *P. mirabilis* TJ-1 was demonstrated to be an effective biosorbent to remove BB54 from aqueous solution. The experimental results showed that its sorption to the dye could attain equilibrium in only 5 min at pH 12.0 and room temperature. Increasing the initial dye concentration favored the increase of sorption capacity of the EPS to the dye. Kinetic studies suggested that the adsorption of BB54 by the biosorbent followed the internal transport mechanism. Well-fitted straight lines obtained for the Langmuir and Freundlich adsorption isotherm models indicated a feasible and spontaneous adsorption. The EPS is a natural organic macromolecule (1.2×10^5 Da) containing protein (30.9%, w/w) and acid polysaccharide (63.1%, w/w). SEM images indicate that the EPS with a crystal-linear structure can effectively adsorb the dye with more binding-sites from the aqueous solution. FTIR analysis revealed the presence of carboxyl, hydroxyl and amino groups in the EPS, which have been approved to be the preferred groups for most sorption processes. High-molecular weight of the EPS together with the above features may explain the excellent sorption performance of the EPS.

The EPS shows potential board application as a biosorbent for both environmental protection and dye recovery. Further study on its sorption to other dyes is in progress in order to widen its application.

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