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Application of Extracellular Polymeric Substances Extracted From Wastewater Sludge for Reactive Dyes Removal

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Abstract

The aim of this study was to investigate the adsorption of three commercial reactive dyes using extracellular polymeric substances (EPS) extracted from the waste sludge of a beer wastewater treatment plant in Hanoi, Vietnam. EPS was extracted from sludge by HCHO-NaOH method and characterized by the measurement of kaolin flocculation activity, dry weight, chemical composition and functional groups. Adsorption of dyes on EPS was conducted by Jartest at different pH, reaction time and EPS dosage. The EPS was composed of 25% of sludge weight. The FTIR analysis showed the present of amine and carboxyl group in the EPS structure. The removal efficiencies of reactive dyes were high at pH below 6, the contact time was from 30 to 60 min and EPS dosage of 200 – 250 mg/L. At optimum condition, the removal efficiency of 85%, 99% and 99% were obtained for Reactive Yellow 176 (RY 176), Reactive Blue 21 (RB 21) and Reactive Red 241 (RR 241), respectively. The adsorption process could be described by both Langmuir and Fruendlich isothermal equations. The maximum dye adsorption capacity for RY 176, RB 21 and RR 241 was 0.50 g/g, 0.72 g/g and 0.95 g/g, respectively. This study revealed that the EPS in wastewater sludge can be utilized as an effective adsorbent for dyes removal, thereby, enhancing the value of sludge in wastewater treatment.

Highlights

- Extracellular polymeric substances (EPS) was directly extracted from wasted bio-sludge and used as an environmentally-friendly adsorbent.
- The extracted EPS by HCHO-NaOH method was successful in removal of commercial reactive dyes.]
- High adsorption capacity of dyes on EPS was obtained at pH less than 6.

1. Introduction

Textile dyeing industries produce large amounts of wastewater with high pollutant concentration, which are difficult to remove. Dyes are the main pollutant component in textile wastewater with complex structure, large molecular weight and low biodegradability (Kiran et al. 2006; Forgacs et al. 2004; Rai et al. 2005). As low biodegradable substances, dyes are able to accumulate in the food chain, create carcinogens, hinder the penetration of light in the water environment and thus, and affect the development of aquatic systems (Rai et al. 2005; Bae and Freeman 2007).

Technologies such as coagulation, biological treatment, adsorption on activated carbon and advanced oxidation processes (AOPs) have been used to treat textile wastewater. Coagulation techniques are cost-effective but are limited to highly soluble dyes such as azo and reactive dyes (Hai et al. 2007). Biological techniques require long hydraulic retention time and specific bacterial strains to degrade dyes, therefore their applicability at industrial scale could be limited (Zee et al. 2005; Barragan et al. 2007). AOPs (such as Fenton, TiO_2 catalyst) can remove dyes completely but those methods are high cost and difficult to operate (Bandala et al. 2008; Ghorai et al. 2007).

Activated carbon adsorption is widely used in practical as it is easy to control and high effective. Due to the high cost of activated carbon, many studies have been conducted on low-cost adsorbents such as pear seeds, poplar sawdust, rice bran, walnut shell powder, mango leaf powder etc. (Igwegbe et al. 2020; Tezcan et al. 2019; Hong and Wang 2017; Uddin and Nasar 2020; Uddin et al. 2017). EPS which is synthesized by bacterial is also considering as an environmentally friendly and low-cost adsorbent. The applications of EPS in dyes removal have been investigated by several studies (Tao et al. 2021; Harshitha et al. 2021; Gao et al. 2011, Zhang et al. 2009; Deng et al. 2005; Gong et al. 2008). Those studies showed that the EPS synthesis by specific bacterial strains during fermentation have maximum dyes adsorption capacity varied from hundred to several hundred mg per g of EPS and these capacity were comparable to commercial activated carbon (Tao et al. 2021; Harshitha et al. 2021; Gao et al. 2011; Zhang et al. 2009; Deng et al. 2005; Gong et al. 2008).

EPS play an important role in protecting microbial cells from harsh environmental conditions and flocs forming. Therefore, it is not only created by the process of culturing (fermentation) of specific microorganism but also present in the microbial population in biological wastewater treatment systems. Recent studies showed that the biopolymers content in wasted sludge was relatively high (10 - 30%) by appropriate extraction methods (Hoang et al. 2016). Although EPS content in wastewater sludge was substantial high, little information has been reported on the ability of using EPS extracted from wastewater sludge for commercial dyes removal.

In order to reuse the valuable component of wastewater sludge, this study is aimed to extract EPS directly from wastewater sludge and use for commercial reactive dyes removal. Factors affecting dyes removal (pH, reaction time and biopolymer dosage) and adsorption isotherm were also investigated.

2. Materials And Methods

2.1 Materials

Sewage sludge: The sludge was taken from the aerobic tank of the wastewater treatment plant of Hanoi beer factory (Me Linh district, Hanoi, Vietnam). The sludge was concentrated to a concentration of 20 g MLSS/L and stored at -4^{o}C for further experiments.

Dyes: The dyes used in the study were reactive dyes, namely RY 176, RB 21 and RR 241. The dyes were obtained from Dong Xuan textile and garment factory, Hanoi, Vietnam. The dyes were prepared at concentrations of 80 mg/L. The maximum absorption wavelengths of RY 176, RB 21 and RR 241 were 418 nm, 626 nm and 541 nm, respectively.

2.2 Methods

Separation of extracellular polymers:

Chemical method: The HCHO-NaOH method was used to separate EPS from sludge. Prior to EPS extraction, the sludge was washed twice with distilled water and concentrated to a concentration of 20 g/L.

0.42ml of formaldehyde (HCHO) was added to 70 ml of sludge and incubated at 4^oC for 1 hour. Then, 3 ml NaOH 10M was added to the sludge mixture and kept in at 4^oC for 3h. The reaction mixture was then centrifuged at 4^oC for 15 min, 4000 x g to extract EPS from biomass (D'Abzac et al. 2010).

Centrifuge method: The process of separating EPS by centrifuge method is similar to the process of separating EPS by HCHO-NaOH, but omitting the additional step of HCHO and NaOH.

Factors affecting dye removal efficiency by EPS

The effects of pH, reaction time and EPS dosage on dye removal efficiency of crude EPS were performed separately on Jartest equipment (SJ-10, Yhana). To evaluate the effect of pH on the dye treatment efficiency, the pH was varied from 2 to 10 by adding NaOH (0.1 M) or H_2SO_4 (0.1 M). The reaction time was in the range of 5 - 360 minutes to determine adsorption equilibrium. The effect of EPS dosage was examined with the EPS volume from 0.5 to 10 mL.

The test procedure of Jartest was described as follows: 500 mL of the dye solution (80 mg/L) was stirred at 120 rpm. 50 mg/L of Al^{3+} and crude EPS were added and pH was adjusted. Then, the stirring speed was reduced to 60 rpm for dye treatment. Samples were settled for 30 minutes, the supernatant was filtered through a filter paper of 0.45 µm and the remaining dye concentration was measured. The dye removal efficiency was calculated according to the following formula (2.1):

$$H = 100 \times \frac{C_0 - C_1}{C_0} \quad (2.1)$$

where

H - Efficiency (%);

C_0 – Dyes concentration of sample without addition of EPS (mg/L);

C_1 – Dyes concentration of sample with addition of EPS (mg/L).

Adsorption Isotherms

Langmuir and Freundlich models were used to describe the equilibrium adsorption equilibrium by EPS. The linear form of the Langmuir isothermal equation is presented below:

$$\frac{1}{q} = \frac{1}{q_e} + \frac{1}{q_e K_L} C_e \qquad (2.2)$$

The linear form of Freundlich's model is presented as follows:

$$\log(q) = \log(K_f) + \frac{1}{n}\log(C_e) \qquad (2.3)$$

In particular, q and q_e are the adsorption capacity and maximum adsorption capacity of dye on EPS, respectively (mg/g EPS); C_e is the residual dye concentration in the liquid phase (mg/L); K_L is the Langmuir constant (L/mg) related to the free energy of adsorption; K_f and n are Freundlich constants. From equations (2.2) and (2.3), q_e and coefficients such as K_L, K_f, n can be determined.

Analytical methods

Purification of EPS from sludge: The sludge mixture after HCHO-NaOH extraction method was centrifuged at 4000 × g at 4°C (Rotina 420R, Hettich Zentrifugen) for 15 min and the EPS containing liquid phase was collected (the solid phase was removed). The EPS containing liquid phase was purified by precipitation in cold ethanol with a volume ratio of ethanol and sample as 2:1 and stored at -20°C overnight. Sample after precipitation was centrifuged at 4000 × g at 4°C for 15 minutes in order to obtain purified EPS. The EPS after purification was used to analyze dry weight, protein, polysaccharide, nucleic acid and chemical functional group composition.

Determination of EPS concentration: The EPS concentrations were determined by weight method after drying the samples to constant weights at 105^{o}C.

Determination of protein, polysaccharide and nucleic acid concentrations: The EPS collected after purification (by precipitation in cold ethanol) was dissolved in distilled water to the original volume. This solution was used to analyze proteins, polysaccharides and nucleic acids. Protein was analyzed by Lowry method with a standard solution of BSA (Bovine Serum Albumin) (DuBois et al. 1956). Carbohydrate was determined by using phenol - sulfuric acid method with glucose as the standard solution (Lowry et al. 1951). Nucleic acids were analyzed by using diphenylamine method (Dell'Anno et al. 1998).

Measurement of turbidity: The supernatant after Jartest was used to measure the turbidity of the solution. The Hana turbidity meter (Model: HI 93703C) with a standard solution of Fomazin was used for the measurement.

Determination of functional groups in EPS: Functional groups of EPS were determined by using Infrared Spectroscopy (IR) spectrum. 0.1 - 0.2 mg of the dried purified EPS was mixed and milled with 100 mg of KBr. Then, samples were pressed with a force of 5 tons to create pellets. Samples were further placed on infrared spectrum window to determine compositions of functional groups.

Measure kaolin flocculation activity (FA): Procedure for measuring kaolin flocculation activity of EPS was adopted from Bezawada et al., 2013. FA was measured at EPS dosage of 10 mg/L for both crude and purified EPS.

Determination of dye concentrations: Concentrations of RY 176, RB 21 and RR 241 dyes were measured by photometric method (UVD-3200, Labomed Inc.) at the wavelength of 418 nm, 626nm and 541 nm, respectively.

3. Results And Discussion

3.1 Properties of EPS extracted by HCHO-NaOH method

Chemical composition of extracted EPS

Concentrations and compositions of EPS extracted by the HCHO-NaOH method (compared with the centrifuge method) are shown in Table 1. EPS is an important component contributing to the formation of flocs of activated sludge in wastewater treatment systems (Pan et al. 2010). Concentrations of the extracted EPS depends on the employed extraction methods. According to results obtained in Table 1, concentration of EPS extracted by HCHO-NaOH method was higher than that by centrifuge method by a factor of 215.4 (5816 compared to 27 mg/L).

Table 1. Concentrations and compositions of EPS

Method	Concentration (mg EPS.L^{-1})	Protein ($mg.L^{-1}$)	Polysaccharide ($mg.L^{-1}$)	Nucleic acid ($\mu g.L^{-1}$)
Centrifugation	27	89.0	11.4	9.3
HCHO-NaOH	5816	1762.2	439.8	420.3

Physical methods such as centrifugation and heat have been applied to determine EPS concentrations in biomass in various studies (Liu et al. 2002; Laspidou et al. 2002). However only EPS extracted by those methods mostly are soluble forms or weakly bound to microbial cells (Liu et al. 2002; Laspidou et al. 2002). In the aeration tank, microorganisms are significantly impacted by the turbulence of air, consequently EPS which is weakly bound to microbial cell likely to be released into the water and only EPS strongly bound remained. Therefore, concentration of EPS extracted by centrifugation was not high. In contrast, both loosely bound EPS and capsular EPS can be effectively extracted by chemical methods due to the reaction of chemical agents with the bonding between EPS and microbial cells. Concentration of EPS extracted by HCHO-NaOH method was high may be due to the fact that chemicals as NaOH is not only able to separate high molecular weight polymer molecules outside the cell but also can break down the cell wall and separate large molecular organic compounds within microbial cells such as DNA and intracellular polysaccharides.

Nucleic acid is an indicator for cell lysis. The high concentration of nucleic acid in EPS indicates that a large amount of EPS is from high molecular polymer in the interior of the microbial cells.

Determination of functional groups of EPS

Functional groups in EPS extracted from bio sludge were analyzed by IR method. IR spectrums were shown in Figure 1. The IR spectrum of the EPS obtained showed that the peaks occurred between 400 and 3500 cm^{-1}. The presence of NH_2 group was confirmed at the peak of 1638 cm^{-1} (Coates 2000; Wu and Ye 2007), whereas the presence of carboxyl (C=O) and O-C (carboxylic acid group and their derivatives) was demonstrated at the peak of 1100 cm^{-1} (We and Ye 2007).

Functional groups such as NH_2, C = O and O-C in EPS have been considered to be capable of binding and adsorbing organic substances and play an important role in the flocculation ability of EPS (We and Ye 2007). It has been reported that the C = O and NH_2 groups of EPS were involved in the adsorption of dyes (He et al. 2020).

Most of the functional groups present in the EPS are negatively charged such as C=O, O-C, and only the NH_2 group is a positively charged group. This explains why EPS exhibits low activity for negatively charged particle systems such as kaolin or suspended solids in water without the presence of multivalent cations.

Flocculation activity of EPS

Flocculation with kaolin solution is one parameter to measure the activity of EPS as it is the polymeric substances. Figure 2 shows the kaolin flocculation activity of both crude and purified EPS extracted by centrifugation and HCHO-NaOH method. The results showed that the flocculation activity of EPS extracted by HCHO-NaOH method was much higher than by centrifugation method. Flocculation activity of purified and crude EPS extracted by HCHO-NaOH method was 96 and 90%, respectively, whereas flocculation capacity of purified and crude EPS extracted by centrifugation was only 37% and 73%, respectively. High flocculation activity of EPS indicated that using HCHO and NaOH in extraction process did not affect much on quality of EPS.

As showed in Table 2, kaolin flocculation activity of EPS extracted from wastewater sludge was comparable to EPS synthesized by EPS producing strains in synthetic and wastewater medium. These results confirmed that HCHO-NaOH is a potential method to separate a high amount of EPS from sludge (25% by weight), which capable to use for treating wastewater. The ability of EPS in dye removal is presented in the next sections.

Table. 2. Comparison of concentration and flocculation capacity of EPS biosynthesis by bacterial strains

BFs producing bacterium	FA (%)	EPS (mg/l)	Growth medium	References
K. mobillis	94.6	5.2	Dairy wastewater	Wang et al. 2007
Klebsiella sp. N10	86.5	34	Synthetic	Yang et al. 2012
Serratia ficaria	96,1	0,4	Synthetic	Gong et al. 2008
Serratia sp.1	79.1	3.5	Wastewater sludge	Bezawada et al. 2013
Serratia sp.1	70.4	6.9	Wastewater sludge	More et al. 2012
EPS extracted by HCHO-NaOH	96	10	Wastewater sludge of beer factory	This study

3.2 Effect of factors on dye treatment performance of EPS

The capacity of crude EPS extracted by HCHO-NaOH method in treatment of reactive dyes (RY 176, RB 21 and RR 241) was studied. The three reactive dyes used in the study are highly soluble, and difficult to be removed by conventional coagulant such as PAC and alum.

Effects of pH

pH is one of the main parameters controlling the adsorption process due to its effect on binding surface of the adsorbent and the ionization of dye molecules (Bhattacharyya et al. 2004). Effects of pH on dye treatment efficiencies (RY 176, RB 21 and RR 241) by EPS extracted by HCHO-NaOH method are shown in Figure 3. The results showed that adsorption efficiencies were highly dependent on pH. At neutral to alkaline pH (7, 8, 10), dye removal efficiencies were low for RY 176 and RR 241 (in the range of 2.7 - 7.2% and 0.4 - 2.9%, respectively). In contrast, at pH = 7 and 8, high removal efficiencies were recorded for RB 21 (65.7 and 39.8%). Highest removal efficiencies of all dyes were achieved at pH = 2 (87.9% for RY 176, 99.1% for RB 21 and 70.8% for RR 241).

pH is one of the environmental determinants of the dissociation of functional groups in the structure of dyes and ions chemistry of the active centers on the surface of adsorbent. Therefore, the optimal pH depends on both the nature of the adsorbent and the chemical structure of the dye. High removal of reactive dye in acidic condition was reported in many studies (Igwegbe et al 2020; Tezcan et al. 2019; Hong and Wang 2017; Vijayaraghavan and Yun, 2007). Onthe other hand, optimum pH for methylene blue removal was at neutral (Tao et al. 2021; Uddin and Nasar 2020; Uddin et al. 2017; Tan et al., 2008).

EPS is separated from aerobic sludge with main components are polysaccharide and protein with carboxyl, hydroxyl and amino functional groups. The functional group of EPS plays a big role with the adsorption process. With a pH of approximately 7, only amino functional groups are chemically positive (protonate), carboxyl functional groups are negative (deprotonate). At lower pH, both these functional groups are positively charged (Benjamin and Lawler 2013). On the other hand, all three dyes contain a negatively charged sulfonate groups (Fig. 4). Therefore, in an acidic environment, the surface of the positive adsorbent enhances the adsorption of negatively charged dyes. This may be the reason dye adsorption efficiency increases rapidly when the pH is reduced to an acidic environment. This result also shows that the amino function group does not play an important role in the adsorption of dyes RY 176 and RR 241 because at pH> 7, the treatment effect is almost negligible.

Effects of contact time

Effects of contact time on dye removal efficiencies are shown in Figure 5. The results showed that in the first 5 minutes, removal efficiencies of all dyes rapidly increased, followed by gradual increases up to the end of the experiment. Removal efficiency of RB 21 dye reached 85%, whereas 65 and 36% was removal efficiencies of RY 176 and RR 241 dye, respectively. Fast increase in the first 5 min could be due to the high concentration gradient of dyes between liquid phase and solid phase that leads to high adsorption rate. After 60 minutes, insignificant increases in efficiencies could be due to low remaining concentrations of the dyes.

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Table 3. Adsorption capacity of extracted EPS in comparison with other studies

Adsorbent	Dyes	*Adsorption capacity* (mg/g)	pH	Adsorbent dosage (mg/L)	Initial dye concentration (mg/L)	References
Klebsiella oxytoca	Methylene Blue	145	6-8	600	200	Tao et al. 2021
Lysinibacillus sp. SS1	Malachite Green	178.6	6	400	100	Harshitha et al. 2021
Chlorella vulgaris	Remazol Black B	420	2	1000	800	Zümriye and Sevilay, 2005
Cephalosporium aphidicola	Acid Red 57	109	1	400	150	Kiran et al. 2006
Corynebacterium glutamicum	Reactive Black 5	419	1	2500	500	Vijayaraghavan and Yun, 2007
Brow seaweed Laminaria sp.	Reactive Black 5	102	1	2500	50 - 1000	Vijayaraghavan and Yun, 2008
Posidonia oceanica (L.) fibres	Methylene blue	5.56	6-9	2	50	Ncibi et al., 2007
Dacryodes edulis Seeds activated carbon	Congo red (CR) and Vat yellow 4 (VY4)	106.5 (VY4) 115.98 (CR)	2	1000 - 2000	100	Igwegbe et al. 2020
Pyrolysis poplar sawdust	Disperse Orange 30	0.09	2	3000	50	Tezcan et al. 2019
Modified rice bran	Reactive blue 4	151.3	2	-	500	Hong and Wang 2017
Walnut shell powder	Methylene Blue	36.6	8	2000	50 - 200	Uddin and Nasar 2020
Mango leaf powder	Methylene Blue	156	7-10	3000	350	Uddin et al. 2017
Activated carbon from coconut husk	Methylene blue	435	~7	1000	50 - 500	Tan et al. 2008
EPS extracted from wastewater sludge	RB 21	720	4	290	80	this study
	RY 176	502	4	200	80	
	RR 241	952	4	350	80	

3.3 Adsorption isotherms of EPS

The Freundlich and Langmuir adsorption models were used to describe the adsorption of dyes on EPS. Figure 7 demonstrates the curve of the Langmuir and the Freundlich isotherm. The correlation coefficient

(R^2) was 0.935 to 0.996 for the Langmuir model and 0.920 to 0.992 for the Freundlich model. The dye adsorption process on EPS was consistent with both the Langmuir and the Freundlich model. It indicated that the dyes were monolayer adsorbed on EPS in the condition of heterogeneous material surface.

The maximum adsorption capacity (q_e) of EPS for RB 21, RY 176 and RR 241 were 720, 520 and 952 mg/g, which were higher than reported by other studies. As summarized in Table 3, EPS and biomass containing EPS had adsorption capacity from 100 – 400 mg/L (Tao et al. 2021; Harshitha et al. 2021; Zümriye and Sevilay,; 2005, Kiran et al. 2006) and other low-cost adsorbent exhibited lower adsorption capacity (below 150 mg/L) (Vijayaraghavan and Yun, 2008,;Ncibi et al., 2007; Igwegbe et al 2020; Tezcan et al. 2019; Hong and Wang 2017; Uddin and Nasar 2020; Uddin et al. 2017) . Activated carbon from coconut husk had adsorption capacity of 435 mg/g (Tan et al., 2008) .

It is well known that EPS compose of protein and polysaccharide and has several functional group for adsorption of dyes and other organic compound. However, in biomass, the EPS is tightly attached with microbial cells and most of the functional groups are occupied by linking with cell membrane, therefore, they exhibit low adsorption capacity. By addition of NaOH and HCHO, EPS can be detached from the cell, free the functional groups and thereafter exhibited high adsorption capacity.

4. Conclusions

EPS extracted from wastewater sludge by HCHO-NaOH method exhibited significantly high adsorption capacity to other low-cost adsorbents and EPS synthesized by specific strains. Complete removal (85% – 99 %) was archived in acidic condition with EPS dosage from 200 – 350 mg/L. The adsorption of the reactive dyes RB 21, RY 176 and RR 241 on EPS could be described by Langmuir and Fruendlich isothermal equations. The maximum dye adsorption capacity for RB 21, RY 176 and RR 241 was 0.72 g/g, 0.5 g/g and 0.95 g/g, respectively. The result indicated that EPS of wastewater sludge could be reused as an alternative adsorbent for reactive dye removal.

Declarations

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Availability of data and material: The data in this study are available from the corresponding author on reasonable request.

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Figures

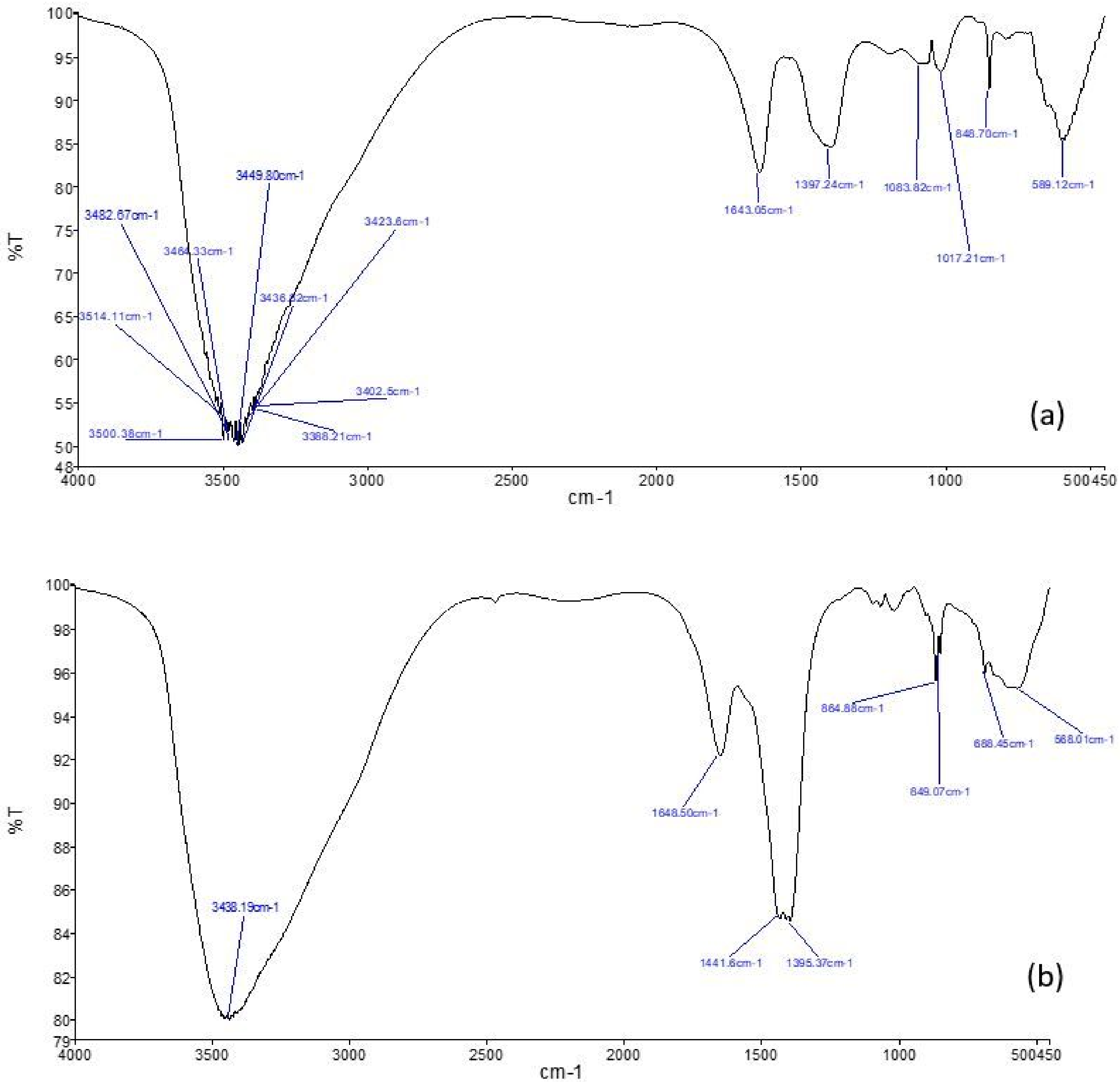


Figure 1

IR spectrum of EPS extracted by (a) Centrifuge method and (b) NaOH – HCHO method

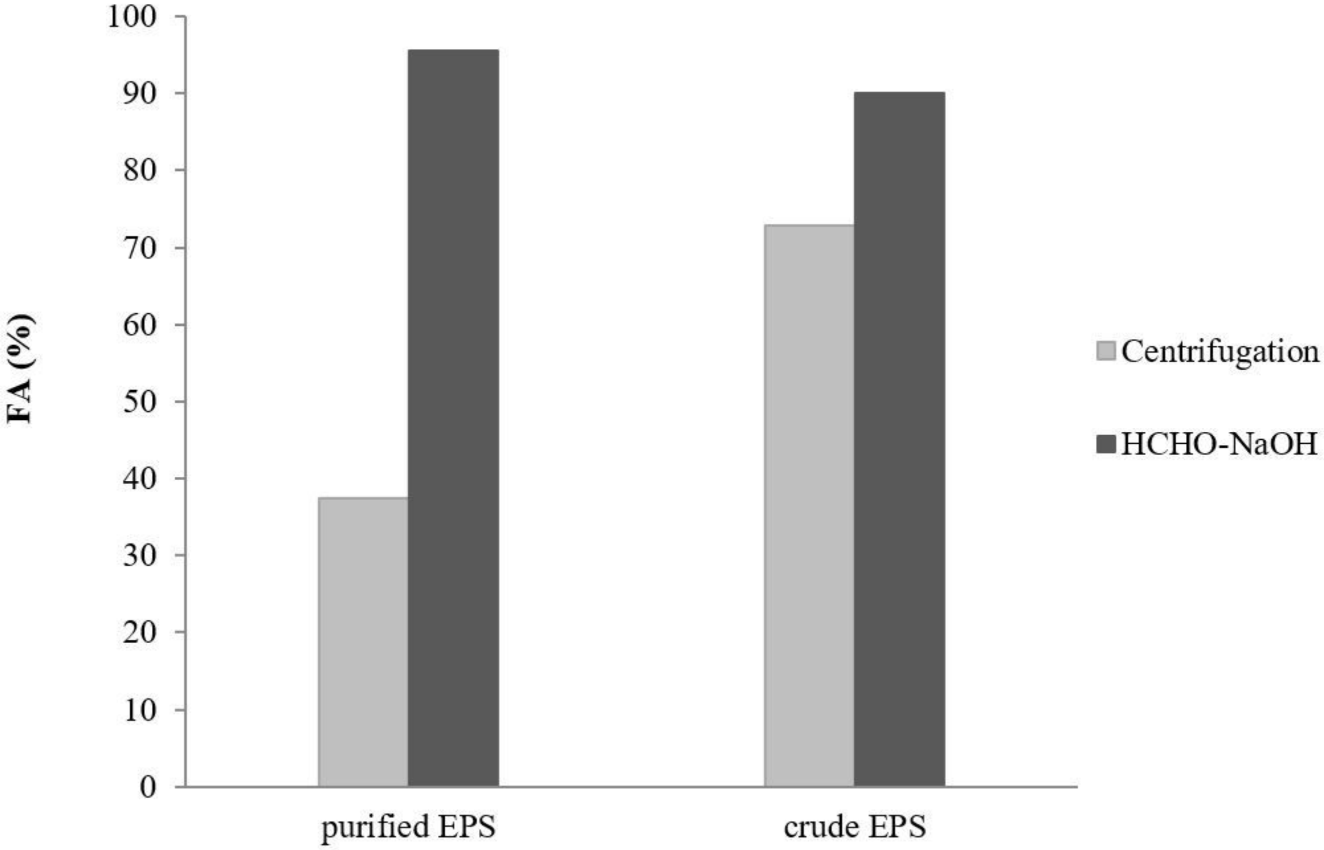


Figure 2

Flocculation capacity of crude and purified EPS extracted by centrifugation and HCHO-NaOH method

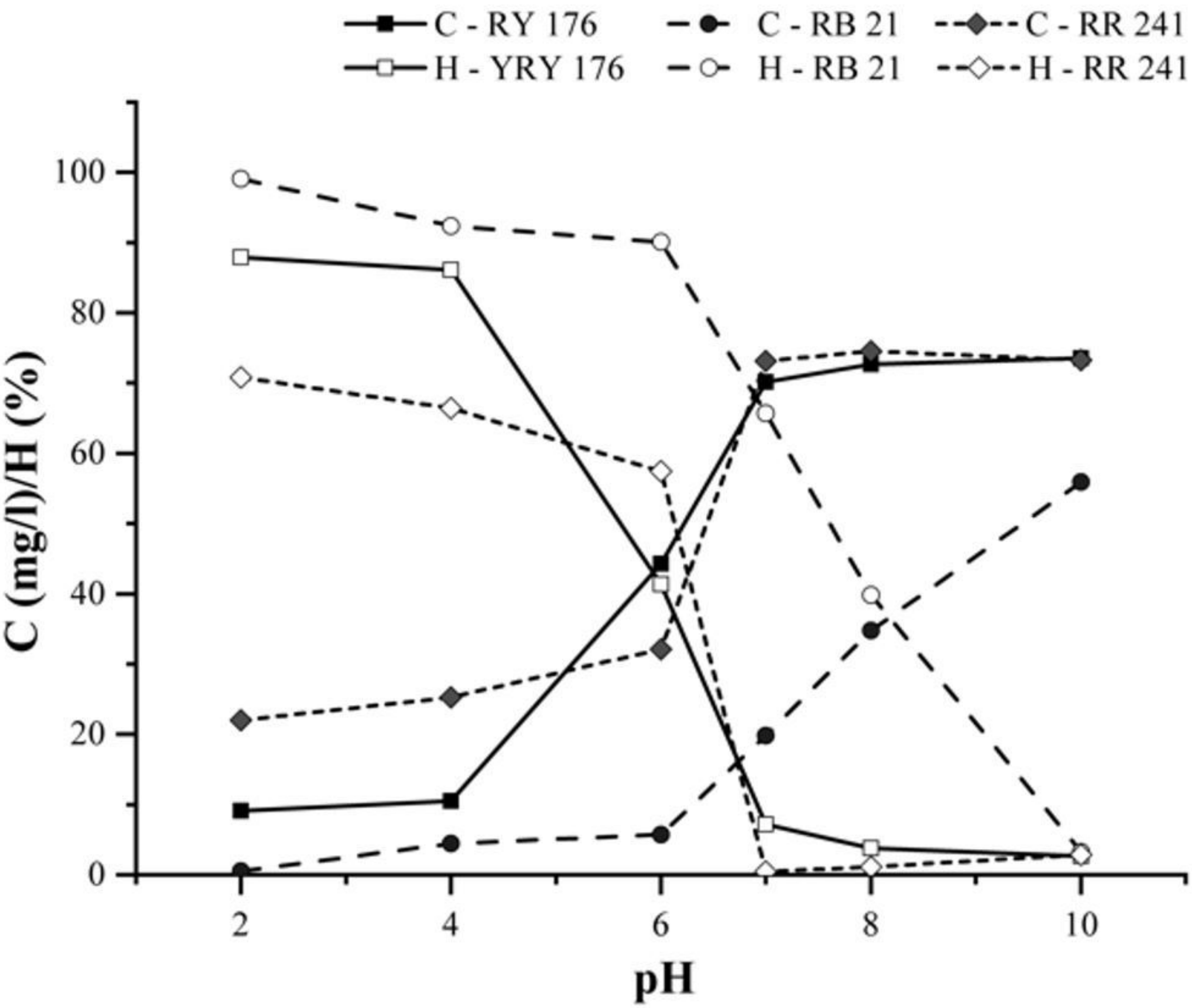
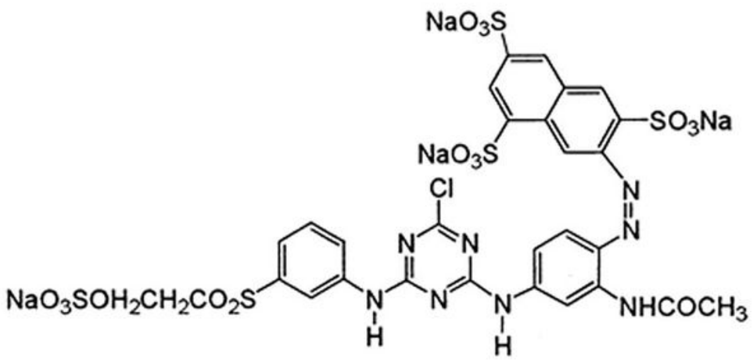
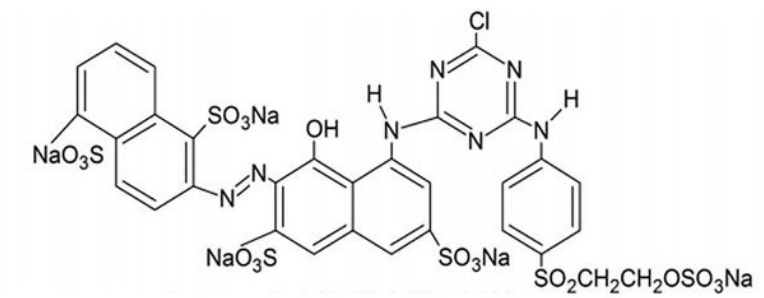


Figure 3

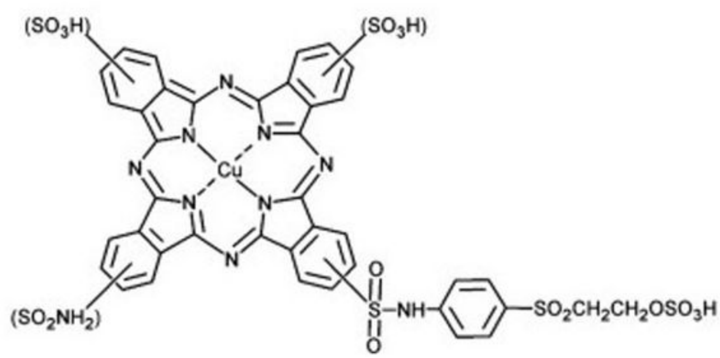
Effects of pH on dye treatment efficiencies by EPS extracted by HCHO-NaOH method



RY 176



RR 241



RB 21

Figure 4

Structure of the reactive dyes

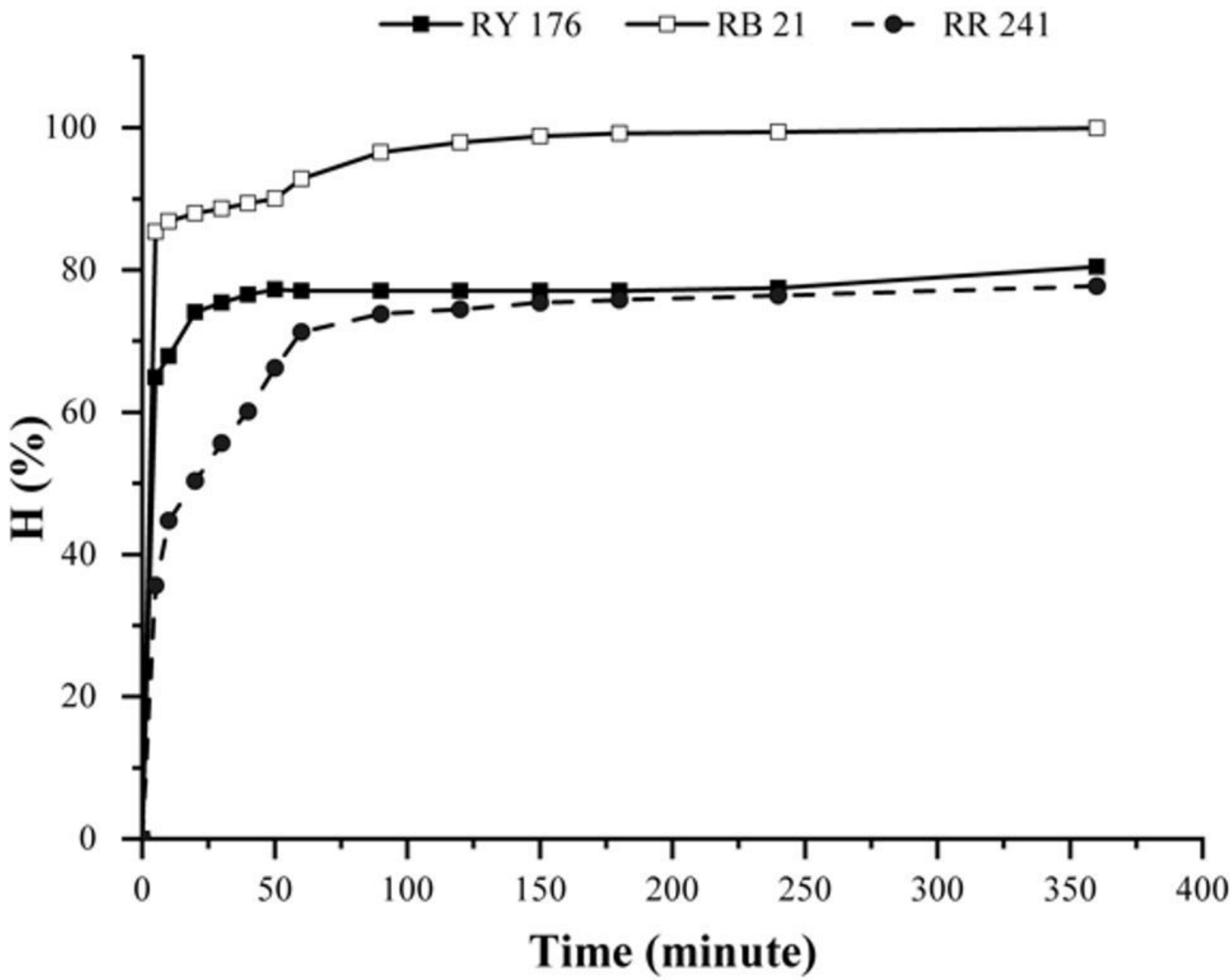


Figure 5

Effects of contact time on dye treatment efficiencies by EPS extracted by HCHO-NaOH method

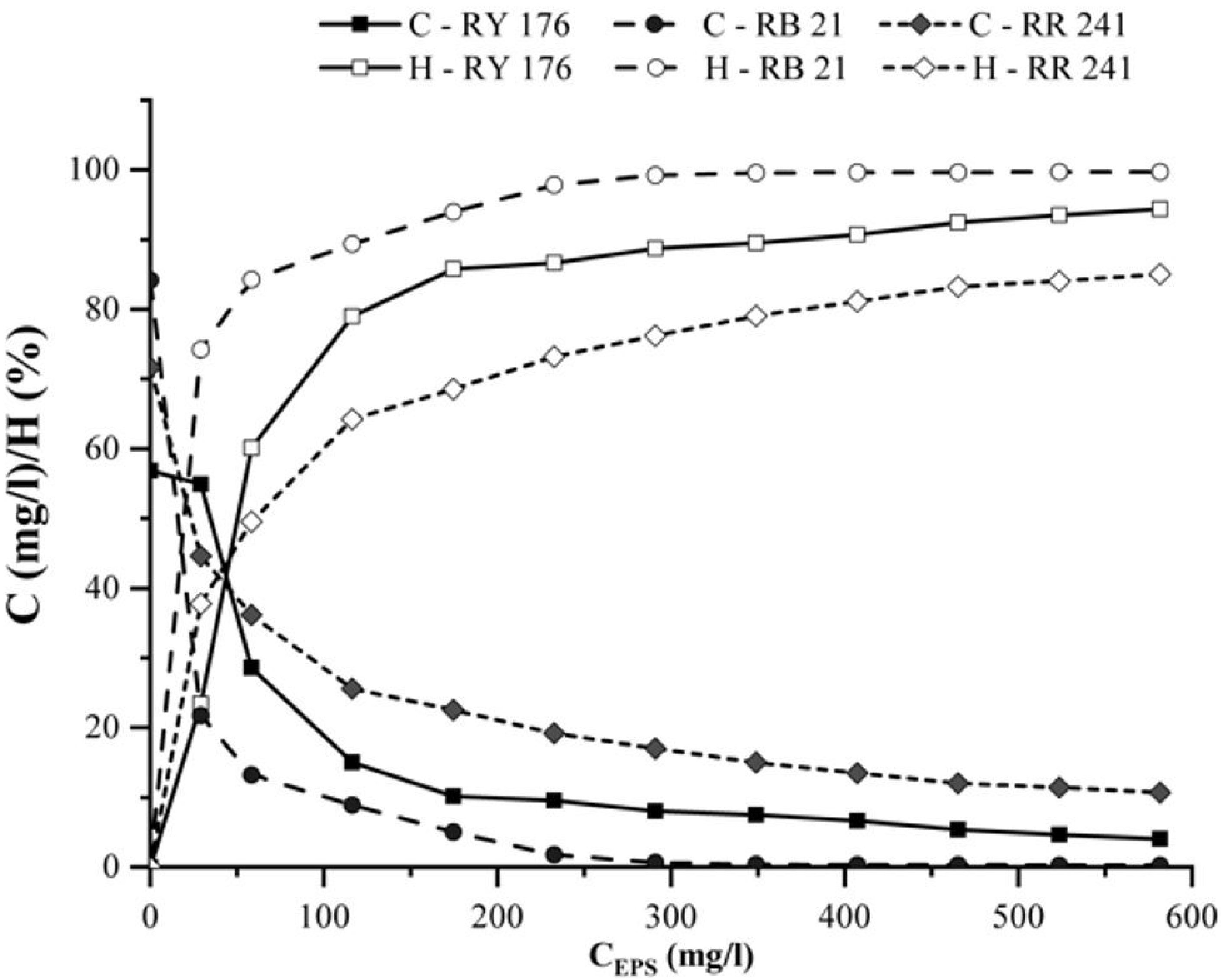


Figure 6

Correlation between EPS concentrations and treatment efficiencies of RY 176, RB 21 and RR 241.

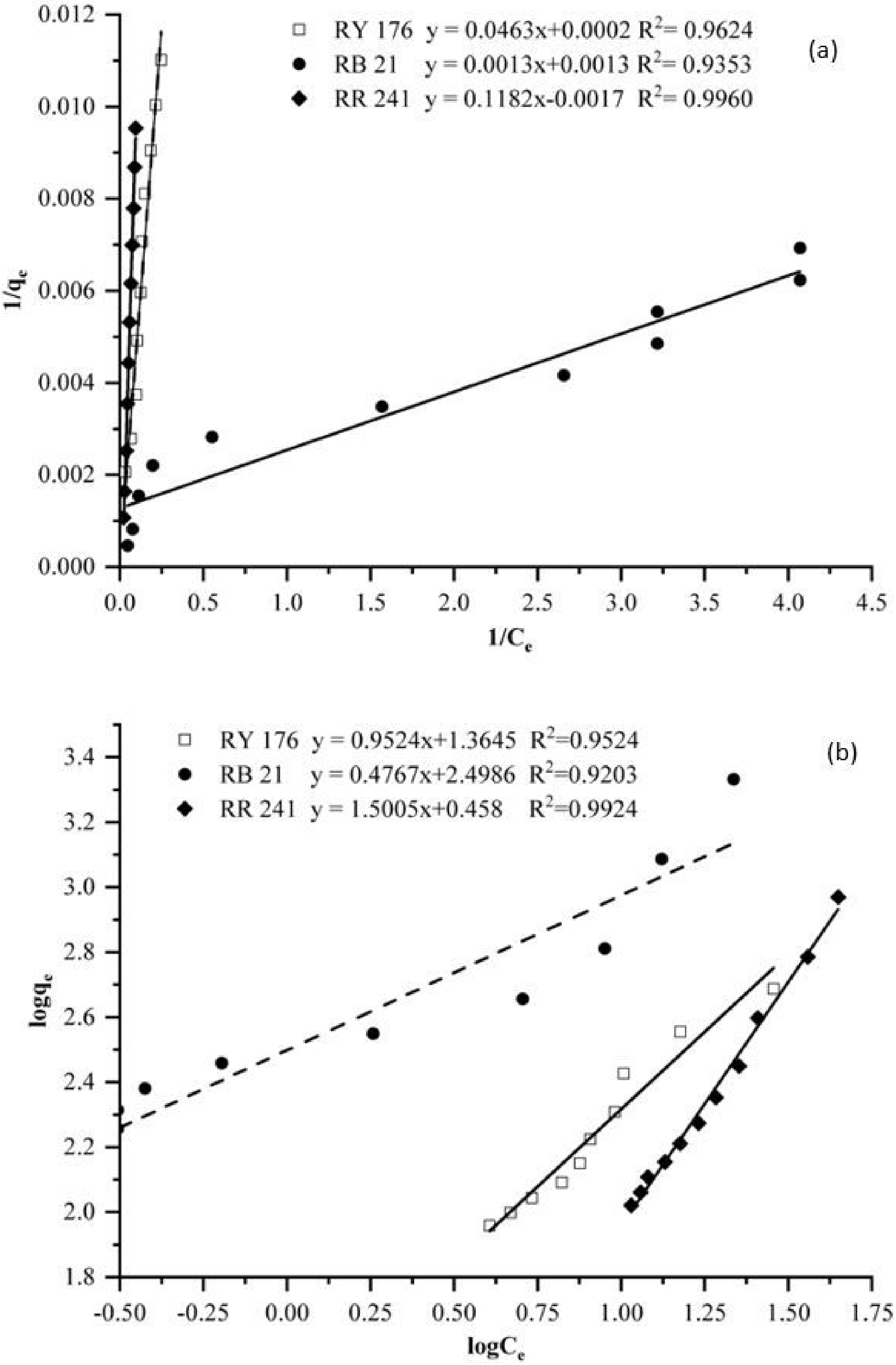


Figure 7

Adsorption isotherm of EPS for three dyes according to Langmuir equation (a) and Freundlich equation (b)