Study on degradation of reactive brilliant red X-3B by three-dimensional biofilm electrode reactor

Shentan Liu 1,2,3,4 and Hongpu Xue 1,2

1College of Geology and Environment, Xi’an University of Science and Technology, Xi’an 710054, Shaanxi, China
2Shaanxi Provincial Key Laboratory of Geological Support for Coal Green Exploitation, Xi’an 710054, China
3 School of Environment, Tsinghua University, Beijing 100084, China
4Email: liushentan@seu.edu.cn

Abstract. The treatment of azo dyes by three-dimensional biofilm electrode reactor (3D-BER) has the characteristics of fast decolorization, high mineralization efficiency and less sludge production and has broad application prospects. The 3D-BER was constructed by filling granular activated carbon (GAC) as a third bipolar electrode in the cathode chamber of conventional BER, and it greatly improved the degradation property of reactive brilliant red (RBR) X-3B. The effects of dye concentration, operating voltage, hydraulic retention time (HRT) and nitrate content on the stability of the experiment were investigated. The results reveal that azo dyes were first decomposed into aromatic amines such as aniline at the cathode, however, the amine substance was difficult to be biodegraded in the anaerobic cathode area, and then the decomposition products of aromatic amines reached the anode region and were further metabolized. Moreover, the water samples were analysed by FTIR and UV-Vis analysis. It was noted that the double bond of the azo dye was broken and degraded into aromatic amines, which were further effectively degraded.

1. Introduction
In recent years, the sewage released from the printing and dyeing textile industry contains a large amount of organic matter, such as azo dyes, sulfurized dyes and organic surfactants, etc. [1] Azo dyes are widely used in the printing and dyeing textile industry due to their excellent color and strong dyeing [2], such as leather, rubber, plastic, food and paper coloring [3]. Azo dyes not only cause serious harm to the environment, but the azo bonds can cause cancer under mutagenic conditions, so the removal of azo dyes from wastewater should not be delayed [4].

There are numerous ways to treat dye wastewater, such as physical, chemical and biological methods. The coupling of electrochemical method and microbial technology has formed a unique biofilm electrode reactor (BER), and then some porous medium was filled between anode and cathode to make a novel three-dimensional biofilm electrode reactor (3D-BER) [5]. Fillers should be selected from materials with large specific surface area, such as activated carbon, zeolite, sulfur and iron [6]. Recently, 3D-BER has been verified to have superiority in treatment of azo dye wastewater. Cao et al. [7] found that microbial degradation of azo dyes first broke the double bond to form substances such as aniline and benzodiazepine, which finally degraded into small molecules, and the factors affecting dye removal rate consisted of voltage, salinity and temperature. Liu et al. [8] used direct electrical
stimulation to enhance biodegradation of RBR X-3B in BERs and found dye decolorization was the result of both the biosorption and degradation, but the degradation processes associated with the reductive cleavage of azo bonds were dominant.

In this experiment, activated carbon fiber/Ti mesh (ACF/Ti) was used as the anode, activated carbon fiber/stainless steel wire mesh (ACF/SSM) as the cathode, and granular activated carbon (GAC) was selected as the filler to construct a novel 3D-BER for azo dye removal. The effects of dye concentration, voltage, hydraulic retention time and nitrate nitrogen on the experiment were studied. The research results may be used to promote the use of 3D-BER in engineering applications.

2. Materials and methods

2.1. Experimental device

![Figure 1. Schematic diagram of three-dimensional biofilm electrode reactor structure.](image)

As shown in Figure 1, 3D-BER consists of three parts: water inlet system, reaction system and power supply. From bottom to top, the reaction chamber has four components: water distributor (5 cm), ACF/SSM cathode (ACF: height of 0.5 cm; SSM: 0.29 mm thickness, mesh hole of 1.83 mm, 12-mesh), filler layer (thickness of 15 cm), and ACF/Ti anode (ACF: height of 0.5 cm; Ti: 0.5 mm thickness, mesh hole of 2 mm, 10-mesh). The electrode distance was 20 cm. The particle size of GAC was 3-5 mm and the specific surface area was 500-900 m²/g.

2.2. Experimental method

Anaerobic sludge obtained from sewage treatment plant was inoculated into the 3D-BER and the culture liquid was added [3]. After the inoculation, energized culture was set. The flow rate was set to 3.47 mL/min, and the voltage was slowly increased from 0.5 V to 2.0 V to maintain stability. Finally, RBR X-3B was gradually added from 25 mg/L to 1000 mg/L for domestication, and each concentration was run for 6 days.

2.3. Determination methods

The water samples were collected, centrifuged at 8000 rpm for 15 minutes, and then filtered with a 0.45 μm microporous membrane. The samples were scanned by UV 9100 B PC ultraviolet-visible scanning spectrometer and Nicolet IR200 FT-IR spectrometer type Fourier transform infrared spectrometer.

3. Results and discussion

3.1. Analysis of influencing factors

3.1.1. Dye concentration. The concentration of dye entering the 3D-BER directly affects the quality of the effluent. As shown in Figure 2(a), when dye concentration was 25 mg/L, the decolorization rate
reached a maximum of 97.7%. When dye concentration increased to 1000 mg/L, the decolorization rate dropped to 93.4%. The results showed that the decolorization effect was inhibited to some extent by excessive dye concentration, but the decolorization rate remained above 90%. Yang et al. [9] used BER to degrade sulfadiazine and found that when the concentration of influent water increased successively, the removal rate showed a downward trend.

3.1.2. Voltage. As shown in Figure 2(b), when no voltage was applied, the decolorization rate only maintained at 71.9%, and the concentration range of RBR X-3B in effluent was 126.4~140.2 mg/L. When the voltage was continuously increased to 0.5 V, 1.0 V, 1.5 V and 2.0 V, the decolorization rate was 76.9%, 90.5%, 91.8% and 94.6%, and dye concentration in effluent was 105.2~121.5 mg/L, 42.4~47.7 mg/L, 35.3~43.4 mg/L and 22.9~28.0 mg/L respectively. However, when the voltage reached 2.5 V, the decolorization rate decreased to 92.0% and dye concentration in effluent was 33.1~44.0 mg/L. Therefore, in order to obtain optimal decolorization effect, it was extremely important to choose an appropriate voltage level. Hao et al. [10] removed antibiotics by BERs, and the results showed that the effluent removal rate increased with the increase of voltage, but too high voltage caused a decline in removal rate. The current density was much larger at higher operating voltages, and it would cause some microorganisms to inactivate, thereby reducing the decolorization reaction of the dye.

Figure 2. Variation of the factors affecting the decolorization rate of reactive brilliant red X-3B in the reactor.

3.1.3. Hydraulic retention time. As shown in Figure 2(c), when the hydraulic retention time was initially set at 6 h, the decolorization rate was observed to be 35.3%, and the concentration of effluent dye was 316.5~334.8 mg/L. As the HRT increased, the average decolorization rate also increased continuously. When the HRT was 48 h, the decolorization rate reached 97.8%, but the curve of 24 h-48 h was close stable. Therefore, the appropriate HRT should be selected in 3D-BER. From the perspective of energy consumption, 24 h was considered as the optimal HRT. Zhao et al. [11] treated sewage by 3D-BER and found that longer HRT could facilitate denitrification process by denitrifying bacteria and result in reducing the accumulation of nitrite. However, under the 3D-BER continuous
operation mode, excessively long HRT under relatively poor organic carbon source conditions could lead to long-term of endogenous respiration phase of heterotrophic bacteria and lower microbial metabolic rate.

3.1.4. Nitrate nitrogen. As shown in Figure 2(d), when nitrate nitrogen was not added to the influent, the decolorization rate can be observed at 91.7%, and the effluent dye concentration range was 35.63–48.06 mg/L. When the nitrate nitrogen was higher than 1.5 mM, it can be seen that the decolorization rate gradually deteriorated. When the concentration of nitrate nitrogen reached 7.5 mM, the decolorization rate was only 43.1%, and the concentration of effluent dye was 281.8–303.6 mg/L. In conclusion, the concentration range of nitrate nitrogen in influent should be controlled within 0–1.5 mM. Cirik et al. [12] found that nitrate could affect the decolorization effect of azo dyes by anaerobic/aerobic sequential batch activated sludge process. When there was no nitrate nitrogen, the decolorization rate of Remazol Brilliant Violet 5R was 93%. Yet as 113 mg/L nitrate nitrogen was added, the decolorization rate dropped to 63%.

3.2. Comparison with other systems
Electrocoagulation is currently a traditional dye wastewater treatment technology, and the hydroxide produced by electrocoagulation can effectively degrade refractory azo dyes. Electrocoagulation has the advantages of fast decolorization and high mineralization efficiency. Hashim [13] and Abdulhadi [14] studied the influences of pH, current density and electrode spacing on dye removal by electrocoagulation and achieved ideal removal effects. Nevertheless, electrocoagulation technology also has some shortcomings: the electrode needs to be replaced regularly; the solution must maintain a certain conductivity; the power consumption was relatively large. Facts have proved that 3D-BER can well avoid these shortcomings and increase experimental credibility during the treatment of dye wastewater.

3.3. Characterization analysis
3.3.1. Fourier infrared spectroscopy (FTIR) analysis. When the initial dye concentration was 400 mg/L, the FTIR curves of inlet water, cathode region outlet water and anode region outlet water shown in Figure 3 were represented by curves a, b and c, respectively. The position and morphology of unknown substances in the water samples on the spectrum were analysed by FTIR spectroscopy. By comparing curves a and b, we found that the absorption peaks showed a downward trend at the positions of 3429 cm\(^{-1}\), 721 cm\(^{-1}\), 620 cm\(^{-1}\), 561 cm\(^{-1}\) and 482 cm\(^{-1}\). However, at the positions of 2930 cm\(^{-1}\) and 2850 cm\(^{-1}\), the absorption peak showed an upward trend. Compared with curve b, the absorption peaks of curve c increased at 3429 cm\(^{-1}\), 2930 cm\(^{-1}\), 2850 cm\(^{-1}\), 1635 cm\(^{-1}\), 1402 cm\(^{-1}\), 721 cm\(^{-1}\), 620 cm\(^{-1}\), 561 cm\(^{-1}\) and 482 cm\(^{-1}\). But at the absorption peak of 1089 cm\(^{-1}\), the distribution of FTIR remains unchanged along the way.

![Figure 3. FTIR comparison of water inflow, cathode water outflow and anode water outflow in 3D-BER.](image-url)
According to the above analysis, azo dye RBR X-3B underwent a reduction reaction after passed through the cathode area under the action of low potential and H₂, decomposing into aromatic amines. Then part of the decomposition products was reduced alcohol, ketone, carboxylic acid etc., while accumulating a large amount of saturated hydrocarbon-CH₂, etc. After flowing to the anode area, the solution contained a certain concentration of Cl⁻ due to the increase in potential. Under the action of electrolysis, a small amount of Cl₂ could be generated. Then, degradation products were further oxidized, thereby generating more hydroxyl groups, ketones, carboxylic acids and saturated hydrocarbons.

3.3.2. UV-Vis spectroscopy (UV-Vis) analysis. It can be seen from (a) and (b) in Figure 4 that the initial dye concentration was 100 mg/L and 200 mg/L, the changes in absorbance along the way were basically similar.

Among them, the ultraviolet-visible absorption spectrum of Influent (curve a) has obvious absorption peaks at wavelengths of 231 nm, 285 nm, and 330 nm, representing structures of the benzene ring, triazine ring, and naphthalene ring respectively. There were obvious absorption peaks at 512 nm and 540 nm, which represented 8-naphthol-3, 6-disulfonic acid large conjugate color systems.

Compared with Influent, cathode effluent (curve b) had significantly lower absorption peaks at 512 nm and 540 nm, while the absorption peaks at range of 248-280 nm or 245-282 nm increased. The reason for this phenomenon was that the cleavage reduction reaction of the azo double bond in the cathode area of the bottom ACF/SSM, and the unsaturated large conjugated chromophore of RBR X-3B was destroyed to produce some aromatic amines and further degradation products such as small molecular organics.

![Figure 4](image)

Figure 4. UV-Vis comparison chart of water inflow (curve a), cathode effluent (curve b), GAC filler (curve c) and anode effluent (curve d) in 3D-BER.

Curve c compared to curve b, the absorbance at 512 nm and 540 nm showed a decreasing trend, while the absorbance at 258~320 nm or 262~312 nm showed a slight increase. The results indicated that the remaining RBR X-3B was further degraded in the GAC filter layer.

Curve d compared to curve c, absorbance at 200~256 nm or 200~230 nm, 332~457 nm or 325~435 nm decreased. However, the absorption peak at 257~331 nm or 231~324 nm raised. The reason was that the intermediate products was further oxidized to form an acid or aldehyde, etc., causing the absorption peak to shift.

3.4. Mechanism analysis
The mechanism of degradation of azo dyes in 3D-BER was the combination of electrochemical action and biological action.
As shown in Figure 5, the electrons produced in the cathode area and the H₂ produced by the electrolysis water were accepted by the microorganisms as electron donors. Eventually, not only the azo dye molecule was degraded into aromatic anilines, but also the decolorization was completed. In the anode area, microorganisms directly transferred electrons to anode or using O₂ produced by electrolysis of water as electron acceptors to catalyze the oxidation of intermediate aromatic amine substances. The individual equations were as follows [15-16].

\[
\begin{align*}
2H_2O + 2e^- & = 2H_2 + 2OH^- \quad (1) \\
H_2O & = 0.5O_2 + 4H^+ + 2e^- \quad (2) \\
0.5O_2 + H_2O + 2e^- & = 2OH^- \quad (3) \\
C_6H_5-N=N-C_{13}H_{21}Cl_4N_3Na_2O_7S_2 + H_2 & \rightarrow C_6H_5-NH_2 + C_{13}H_7Cl_2N_5Na_2O_7S_2 \quad (4)
\end{align*}
\]

In summary, discharged water still had some organic matter that had not been degraded in the entire system of the 3D-BER. Besides, some microbial bacteria were also discharged from the system. Of course, an aerobic reactor or aeration device can be added in the experiment to further degrade the unreacted substances into H₂O and CO₂.

4. Conclusions
By constructing a 3D-BER, the influencing factors of the experiment and the characterization of the water sample were analyzed. The specific conclusions are as follows.

1) When the influent dye concentration was increased from 25 mg/L to 1000 mg/L, the decolorization rate of 3D-BER was not greatly reduced. Even when the concentration of influent RBR X-3B was 1000 mg/L, the decolorization rate was still as high as 93.4%. When the voltage was 1.0~2.0 V and the influent RBR X-3B was 500 mg/L, the decolorization rate was higher (90.5%~94.6%). Therefore, suitable operating voltage for 3D-BER should be 1.0~1.5 V. With the extension of HRT, the decolorization rate of the 3D-BER gradually increased. Taking into account comprehensively, the most suitable HRT was 24 h. When the concentration of nitrate nitrogen in the influent was not higher than 1.5 mM, the decolorization effect of 3D-BER was almost unaffected, and the decolorization rate was higher than 90%.

2) Through FTIR and UV-Vis analysis, we can see that the azo double bonds of RBR X-3B broke in the cathode area of the 3D-BER and dye was decomposed into anilines and cyclic organic compounds, accompanied by a large number of chromophores being destroyed. In the anode area of the reactor, these intermediates were further oxidized into small molecules.

Acknowledgments
This study was supported by the National Natural Science Foundation of China (Grant No. 21806128), the Natural Science Basic Research Plan in Shaanxi Province of China (Grant No. 2019JQ-300) and the China Postdoctoral Science Foundation (Grant No. 2019M653701).
Reference

[1] Yuling Zhu, Jiali Xu, Xiwei Cao, et al. 2018 Characterization of functional microbial communities involved in different transformation stages in a full-scale printing and dyeing wastewater treatment plant *Biochemical Engineering Journal* **137** 162-171

[2] Suvidha Shinde, Nagaiyan Sekar 2019 Synthesis, spectroscopic characteristics, dyeing performance and TD-DFT study of quinolone based red emitting acid azo dyes *Dyes and Pigments* **168** 12-27

[3] Shentan Liu, Xiaojuan Feng, Feng Gu, et al. 2017 Sequential reduction/oxidation of azo dyes in a three-dimensional biofilm electrode reactor *Chemosphere* **186** 287-294

[4] Mohamed Berradi, Rachid Hissou, Mohammed Khudhair, Mohammed Assouag, Omar Cherkaoui, Abderrahim El Bachiri, Ahmed El Harfi 2019 Textile finishing dyes and their impact on aquatic environs *Heliyon* **5**(11) e02711

[5] Shuai Zhang, Hai-Liang Song, Xiao-Li Yang, Ke-Yun Yang, Xiao-Yang Wang 2016 Effect of electrical stimulation on the fate of sulfamethoxazole and tetracycline with their corresponding resistance genes in three-dimensional biofilm-electrode reactors *Chemosphere* **164** 113-119

[6] Lei Feng, Xu-yun Li, Li-hong Gan, Juan Xu 2018 Synergistic effects of electricity and biofilm on Rhodamine B (RhB) degradation in three-dimensional biofilm electrode reactors (3D-BERs) *Electrochimica Acta* **290**(10) 165-175

[7] Xian Cao, Hui Wang, Shuai Zhang, Osamu Nishimura, Xianning Li 2018 Azo dye degradation pathway and bacterial community structure in biofilm electrode reactors *Chemosphere* **208** 219-225

[8] Shentan Liu, Hailing Song, Size Wei, et al. 2015 Effect of direct electrical stimulation on decolorization and degradation of azo dye reactive brilliant red X-3B in biofilm-electrode reactors *Biochemical Engineering Journal* **93** 294-302

[9] Xiao-Li Yang, Shuai Zhang, Hua Li, et al. 2018 Effects of voltage on sulfadiazine degradation and the response of sul genes and microbial communities in biofilm-electrode reactors *Ecotoxicology and Environmental Safety* **151** 272-278

[10] Ruixia Hao, Sumei Li, Jianbing Li, et al. 2013 Denitrification of simulated municipal wastewater treatment plant effluent using a three-dimensional biofilm-electrode reactor: Operating performance and bacterial community *Bioresour Technol* **143** 178-186

[11] Yingxin Zhao, Chuaping Feng, Qinghong Wang, et al. 2011 Nitrate removal from groundwater by cooperating heterotrophic with autotrophic denitrification in a biofilm-electrode reactor *Journal of Hazardous Materials* **192**(3) 1033-1039

[12] Cirik K, Kitis M, Cinar O 2013 Effect of nitrate on anaerobic azo dye reduction *Bioproc Biosyst Eng* **36**(1) 69-79

[13] Khalid S Hashim, Ameer H Hussein, Salah L Zubaidi, et al. 2019 Effect of initial pH value on the removal of reactive black dye from water by electrocoagulation (EC) method *Journal of Physics: Conference Series* **1294**(3) p.072017

[14] B A Abdulhadi, P Kot, K S Hashim, A Shaw and R Al Khaddar 2019 Influence of current density and electrodes spacing on reactive red 120 dye removal from dyed water using electrocoagulation/electroflotation (EC/EF) process *Materials Science and Engineering* **584**(1) p.012035

[15] Minghua Zhou, Wenjing Fu, Heyan Gu, Lecheng Lei 2007 Nitrate removal from groundwater by a novel three-dimensional electrode biofilm reactor *Electrochimica Acta* **52**(19) 6052-6059

[16] Siyuan Zhai, Min Ji, Yingxin Zhao, Xiao Su 2020 Shift of bacterial community and denitrification functional genes in biofilm electrode reactor in response to high salinity *Environmental Research* **184** p.109007