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# Azo dye degradation pathway and bacterial community structure in biofilm electrode reactors

Xian Cao [a, b], Hui Wang [a], Shuai Zhang [a], Osamu Nishimura [b], Xianning Li [a, *]

[a] School of Energy and Environment, Southeast University, Nanjing, Jiangsu, 210096, China
[b] Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, Aoba Aramaki 6-6-06, Sendai 980-8579, Japan

## HIGHLIGHTS

- The removal efficiency of azo dye was improved in biofilm electrode reactor (BER).
- The process of degradation of azo dye was sequential degradation.
- The azo dye X-3B degraded into lower-molecular-weight products in BER.
- The relative abundances of the microbial community were affected by environment.

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## ABSTRACT

In this study, the degradation pathway of the azo dye X-3B was explored in biofilm electrode reactors (BERs). The X-3B and chemical oxygen demand (COD) removal efficiencies were evaluated under different voltages, salinities, and temperatures. The removal efficiencies increased with increasing voltage. Additionally, the BER achieved maximum X-3B removal efficiencies of 66.26% and 75.27% at a NaCl concentration of 0.33 g $L^{-1}$ and temperature of 32 °C, respectively; it achieved a COD removal efficiency of 75.64% at a NaCl concentration of 0.330 g $L^{-1}$. Fourier transform infrared spectrometry and gas chromatography–mass spectrometry analysis indicated that the X-3B biodegradation process first involved the interruption of the conjugated double-bond, resulting in aniline, benzodiazepine substance, triazine, and naphthalene ring formation. These compounds were further degraded into lower-molecular-weight products. From this, the degradation pathway of the azo dye X-3B was proposed in BERs. The relative abundances of the microbial community at the phylum and genus levels were affected by temperature, the presence of electrons, and an anaerobic environment in the BERs. To achieve better removal efficiencies, further studies on the functions of the microorganisms are needed.



## 1. Introduction

Traditional anaerobic biological treatments are widely used in wastewater treatment systems due to their low cost, low environmental impact, and relatively simple requirements (Kong et al., 2018; Qin et al., 2017; Qin et al., 2018). However, both the creation of new types of contaminants due to industrial development and the increased efforts to improve environmental quality require the development of novel methods to enhance the efficiency of anaerobic biological treatment. Bioelectrochemical systems, which combine microorganisms and electrochemistry, have been explored to treat refractory organic wastewater, such as nitro aromatics (Liang et al., 2013), azo dyes (Cui et al., 2016), and antibiotics (Song et al., 2017). Of these, biofilm electrode reactors (BERs) rely on a moderate electrical current supply at the anode to provide electrons for the cathodic reduction of refractory substances to increase removal efficiency (Cao et al., 2017a). A number of studies have reported the treatment of wastewater with BERs. For example, Xia et al. (2016) used a BER to degrade Fe(II)EDTA–NO and found that the bioelectrochemical process significantly enhanced the reduction of Fe(II)EDTA–NO. Meanwhile, Hao et al. (2013) used a BER to remove nitrate from simulated municipal wastewater treatment plant effluent and achieved a nitrate removal rate of 98.3% with a carbon-to-nitrogen ratio of 3.0 and a hydraulic

* Corresponding author.
*E-mail addresses:* cao.xian.c6@tohoku.ac.jp (X. Cao), lwcq306@163.com (H. Wang), zhangshuai198702@163.com (S. Zhang), osamu.nishimura.d2@tohoku.ac.jp (O. Nishimura), lxnseu@163.com (X. Li).

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residence time of 7 h. Furthermore, Rao et al. (2009) used a three-dimensional BER to remove the chemical oxygen demand (COD) from landfill leachate and found that about 60–64% of COD was removed within 1 h, which was between five and seven times greater than that in the control group. Finally, Zhang et al. (2016) used a BER to treat sulfamethoxazole and tetracycline and achieved removal rates of 88–93% and 89–95%, respectively.

Azo dyes, which contain one or more azo bonds (–N=N–), are considered refractory organics that can cause a variety of environmental problems without appropriate treatment. Although physical technologies, such as flocculation and coagulation, are most commonly used for their treatment, these processes require large quantities of chemicals that can cause secondary pollution. Meanwhile, advanced oxidation processes have high removal rates but also high operating costs, and their removal efficiency changes depending on wastewater composition. By contrast, anaerobic biological treatment is considered an economical method with a comparatively low treatment effect. Therefore, BERs are a promising and environmentally friendly method for improving the removal rate of azo dyes in wastewater treatment.

In our previous study, we constructed BERs with various cathodes and anodes to investigate the influence of these structures on the removal efficiency of the azo dye X-3B. However, the X-3B degradation pathway and microbial community structure in the BERs were unclear. In particular, the degradation of X-3B was incomplete. Because some of the intermediate products may be toxic, a better understanding of the microbial community structure and composition would help increase the performance of azo dye treatment. Therefore, we systematically investigated the effects of several parameters, such as voltage, salinity, and temperature. Based on the results of Fourier transform infrared spectrometry (FTIR) and gas chromatography–mass spectrometry (GC-MS), we proposed a degradation pathway of X-3B in BERs. Finally, we analyzed the microbial communities involved in X-3B biodegradation and bioelectricity generation using 16 S ribosomal RNA gene pyrosequencing.

## 2. Methods and materials

### 2.1. System construction and operation

The BER reactors used in this study were constructed from polycarbonate plastic, as described previously (Cao et al., 2017a). All reactors contained 500 mL of anaerobic sludge from the East City Municipal Wastewater Treatment Plant of Nanjing, China. The BERs contained 200 mg $L^{-1}$ of X-3B and a nutrient solution with the following chemical composition (per liter): 400 mg glucose, 134 mg $NH_4Cl$, 330 mg NaCl, 18 mg $Na_2HPO_4$, 33 mg $NaH_2PO_4$, 15 mg $MgSO_4 \cdot 7H_2O$, 340 mg $NaHCO_3$, 2.2 mg $MnSO_4 \cdot H_2O$, 2 mg $ZnSO_4 \cdot 7H_2O$, 1 mg $FeSO_4$, 15 mg $CaCl_2$, 0.24 mg $CoCl_2 \cdot 6H_2O$, and 1.17 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Zhang et al., 2016; Fang et al., 2017).

The experiment consisted of two parts. First, to investigate the influence of voltage on X-3B removal efficiency, the experimental group of BERs with different voltages (0.2, 0.4, 0.6, 0.8, and 1 V) were constructed. Meanwhile, the control group of BERs which had the same setup with the experimental group except not connected with external voltage were also constructed. At that time, the salinity was 0.66 mg $L^{-1}$, and the temperature was 28 °C. Second, to investigate the effects of salinity and temperature on X-3B removal efficiency, BERs with different salinities (0, 0.165, 0.330, 0.660, and 3.300 mg $L^{-1}$) and temperatures (18, 24, 28, 32, and 35 °C) were constructed. At that time, the voltage was 1 V and salinity was 0.330 mg $L^{-1}$.

### 2.2. Chemical analysis

The intermediate products of X-3B were detected with GC-MS (Thermo Fisher Scientific, USA) using a DB-5MASS capillary column (inner diameter: 0.25 mm, length: 30 m). High-purity helium was employed as the carrier gas at a flow rate of 1 mL/min. The temperature program was as follows: the gasification compartment temperature was first set to 60 °C for 0.5 min; it was then increased linearly to 235 °C at a rate of 25 °C/min; after holding at 235 °C for 2 min, it was further increased linearly to 250 °C at a rate of 2 °C/min and then held at 250 °C for 5 min; finally, the temperature was increased linearly to 280 °C at a rate of 15 °C/min and held for 5 min. The temperatures of the ion source and interface were 230 and 280 °C, respectively. Separated components were analyzed with reference to the NIST MS Search 2.0 mass spectral library database. Analysis using diphenyl-p-phenylenediamine was based on the GC-MS selective ion monitoring method using the same temperature program described above. The areas of the peaks at 93, 121, and 136 m/z were used to generate a standard curve (Fang et al., 2013, 2016).

The microbial community structures were analyzed using high-throughput pyrosequencing and a clone library. DNA was extracted from the anode biomass in the soil microbial fuel cells at the end of the experiment. The V4–V5 region of the bacterial 16 S ribosomal RNA gene was amplified via PCR (95 °C for 5 min, followed by 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min) using the primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) and an eight-nucleotide barcode sequence unique to each sample. PCR was performed in triplicate with a 20-μL reaction volume containing 4 μL 5 × FastPfu Buffer, 2 μL 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL FastPfu polymerase, and 10 ng template DNA (Wang et al., 2017).

## 3. Results

### 3.1. X-3b removal efficiency at different voltages

We assessed the influence of voltage on X-3B removal efficiency. Fig. 1 presents the average X-3B removal efficiencies and the current in the experimental and control groups at different external voltages. The average X-3B removal efficiency in the experimental group increased gradually with increasing voltage, whereas that in the control group remained essentially constant. For example, at

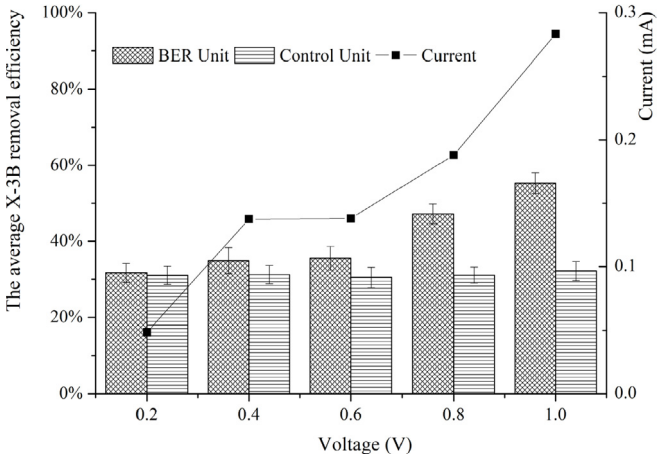


**Fig. 1.** The average X-3B removal efficiencies and the current at different external voltages.