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Comparative study of antiestrogenic activity of two dyes after Fenton oxidation and biological degradation

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ABSTRACT

In present study, two methods (Fenton oxidation and biological degradation) were used to degrade azo dye (Reactive Black 5, RB5) and anthraquinone dye (Remazol Brilliant Blue R, RBBR). The changes of antiestrogenic activities of these two dyes through two degradation methods were detected using the yeast two-hybrid assay method. Fluorescence spectroscopy together with gas chromatography-mass spectrometry (GC-MS) method was performed to analyze the metabolites of RB5 and RBBR after Fenton oxidation and biological degradation. Results indicated that by Fenton oxidation, the decolorization of RB5 and RBBR were 99.31% and 96.62%, respectively, which were much higher than that by biological degradation. Dissolved organic carbon (DOC) reduction rates of RB5 and RBBR after Fenton oxidation were also much higher than that after biological degradation. By Fenton oxidation, the antiestrogenic activities of RB5 and RBBR all decreased below detection limit after degradation, while by biological degradation all of them increased significantly after degradation. Fluorescence spectroscopy analysis and GC-MS analysis confirmed the degradation effects of RB5 and RBBR by these two degradation methods. In addition, fluorescence spectroscopy analysis revealed that the metabolites humic acid-like substances might contribute to the increasing of antiestrogenic activity of RB5 and RBBR after biological degradation.

1. Introduction

Azo and anthraquinone dyes are two of the most common groups of dyes used widely in printing, textile, cosmetics, food and other industries (Dai et al., 2016; Liu et al., 2017). The wastewater including dyes and their byproducts discharged from these industries have great threat to human health and aquatic ecosystem, which were confirmed to be toxic (Jung et al., 2004; Xie et al., 2018; Kamila et al., 2018). Therefore, unutilized dyes and their metabolites during production process need to be treated before discharging to the environment.

Various methods were used to degrade azo and anthraquinone dyes, such as physico-chemical methods (adsorption (Jiang et al., 2014), ozone (Fanchiang and Tseng, 2009), Fenton oxidation (Punzi et al., 2015), photo-Fenton oxidation (Punzi et al., 2015)), and biological methods (fungus (Sen et al., 2016) or bacteria (Cui et al., 2014)). Physico-chemical methods could achieve high efficiency, but these methods are infeasible with high cost of supplying ozone, or electrical power, generation of secondary pollutants and disposal crisis (Sudha et al., 2018; Vatandoostarani et al., 2017). Biological method, compared to physico-chemical methods, is considered to be high efficiency, low-cost and eco-friendly (He et al., 2018; Krishnamoorthy et al., 2018; Maiti et al., 2017). However, lots of studies mainly focused on the efficiency, advantages and disadvantages of different treatment methods. Few studies illustrated the toxicity of the metabolites generated during treatment by these methods, and the comparison of their toxicities. Besides, for different dyes, the changes of their biotoxicity during the treatment processes by different methods have not been reported in previous studies.

During the treatment process, a lot of traditional indicators were used to evaluate the degradation effects, including decolorization rate, TOC removal rate, COD removal rate (Liao et al., 2013; Olukanni et al., 2010). However, these traditional indicators could not reflect the

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degradation effects comprehensively. In recent years, more attention has been paid to the toxic effect caused by dyes and their metabolites (Rawat et al., 2016). Previous studies reported that dyes during degradation process exhibited various toxicities, such as phytotoxicity (Chen et al., 2017), acute toxicity (Novotny et al., 2006), genotoxicity (Salas-Veizaga et al., 2013) and cytotoxicity (Haq et al., 2018), especially antiestrogenic activity (Bazin et al., 2012; Jung et al., 2004). Chemicals with antiestrogenic activity could interfere with the function of the endocrine system of animals, and then affect their reproduction and development (Macgregor and Jordan, 1998; Orlando and Guillette, 2007). Recent years, antiestrogenic activity test——the yeast two-hybrid assay method, was widely used to evaluate the treat of wastewater, due to its sensitivity, credibility, and maneuverability (Garcia-Reyero et al., 2001; Wu et al., 2010). For example, Qianyuan Wu et al. (2009) used the yeast two-hybrid assay method to evaluate the effects of chlorination on antiestrogenic activities in biologically treated wastewater successfully. Moreover, our previous studies also have reviewed the metabolites that generated after biodegradation of dyes, and have detected the increased antiestrogenic activities during printing and dyeing wastewater hydrolysis acidification treatment process (Liu et al., 2016).

In present study, two methods (Fenton oxidation and biological degradation) were used to degrade two typical types of dyes RB5 and RBBR, respectively. Despite of the detection of traditional indicators, such as decolorization rate, DOC reduction rate, the antiestrogenic activity of dyes degraded by two methods was detected by the yeast two-hybrid assay method. In addition, fluorescence spectroscopy and GC-MS methods were performed to analyze the metabolites generated by two degradation methods. This study aimed to evaluate and compare the degradation performance on dyes of Fenton oxidation and biological degradation more comprehensively.

2. Materials and methods

2.1. Dyes, chemicals and culture medium

Azo dye Reactive Black 5 (RB5) and anthraquinone dye Remazol Brilliant Blue R (RBBR) were purchased from Sigma Aldrich, USA. The chemical structures of the two dyes were shown in Fig. 1. $FeSO_4{\cdot}7H_2O$ and H_2O_2 30% were used for Fenton oxidation experiments. All of the other chemicals used in this study were of analytical grade. The microbial consortium FF (whose dominant flora was *Gamma-proteobacteria*), which was preserved in our lab, was grown at 35 ℃ in conventional culture medium consisted of beef extract 5 g/L, peptone 10 g/L and NaCl 5 g/L.

2.2. Bacterial consortium FF

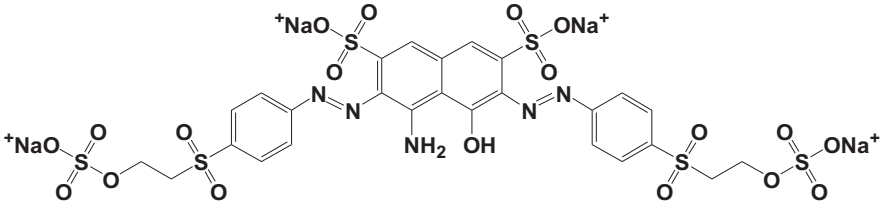
The bacterial consortium FF was isolated from the dyeing wastewater treatment system by enrichment culture technique in our lab (Xie et al., 2013). This bacterial consortium FF had high reduction potential of various dyes. The bacterial consortium FF was identified based on the morphological, biochemical as well as by 16S rRNA based molecular approach. Twenty-one bacterial isolates were isolated from it, and then identified by 16S rDNA genes analysis, and the mainly functional advantages consortium in FF were *Gamma-proteobacteria* including *Klebsiella* and *Proteus* genus. The obtained sequences have been deposited into the NCBI GenBank with accession number JX133917, JX133918, and JX827698-JX827716.

2.3. Fenton oxidation experiments

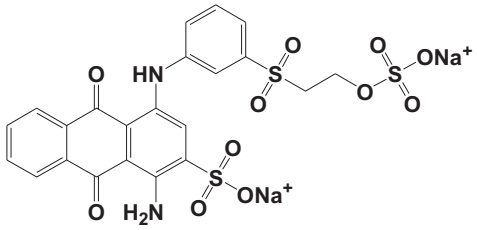
Fenton oxidation experiments were performed in 1000 mL glass beakers containing 500 mL dye solution at concentration of 150 mg/L of dye. The solution was acidified to pH 3 using 1 mol/L H_2SO_4. The appropriate amount of $Fe_2SO_4{\cdot}7H_2O$ was added and the reaction was started when the H_2O_2 was added. In order to achieve the best decolorization effect, for Fenton oxidation of RB5, the concentrations of $Fe_2SO_4{\cdot}7H_2O$ and H_2O_2 were 1 mmol/L and 0.6 mmol/L, respectively. While for Fenton oxidation of RBBR, the concentrations of $Fe_2SO_4{\cdot}7H_2O$ and H_2O_2 were 10 mmol/L and 6 mmol/L, respectively. A magnetic stirrer was used to provide stirring (50 rpm). The reaction time was all 50 min for Fenton oxidation of two dyes. After degradation, samples were withdrawn to measure decolorization rate and DOC reduction rate. The experiments were performed in triplicates.

2.4. Decolorization experiments by bacterial consortium FF

The bacterial consortium used for decolorization experiments was conducted in batch culture for three days, and then inoculated (10% v/v) in erlenmeyer flasks (250 mL each) containing 100 mL sterile culture medium of dye (150 mg/L). The Erlenmeyer flasks were incubated at



(a)



(b)

Fig. 1. The chemical structures of RB5 (a) and RBBR (b).