Chemosphere 158 (2016) 30–36

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Azo dye decolorization by a halotolerant exoelectrogenic decolorizer isolated from marine sediment

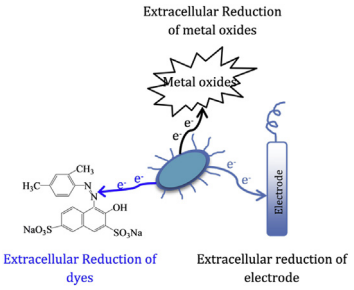
Fangcheng Xu [a, *], Zhiyi Mou [a], Jiya Geng [a], Xiaobo Zhang [b], Chen-zhong Li [c]

[a] Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China
[b] College of Life Sciences, Zhejiang University, Hangzhou 310058, China
[c] Nanobioengineering/Bioelectronics Lab, Department of Biomedical Engineering, Florida International University, Miami, FL, USA

HIGHLIGHTS

- Marine exoelectrogenic bacterium EP1 could decolorize a toxic azo dye under high salt concentrations (up to 20% NaCl).
- New mechanisms of decolorization were found: degradation and bioflocculation.
- Azo dye degradation by EP1 was most probably an extracellular process.
- Proposed the concept of Halophilic/Halotolerant Exoelectrogenic Decolorizer for decolorization under high salt conditions.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form
19 March 2016
Accepted 11 May 2016
Available online 27 May 2016

Handling Editor: Chang-Ping Yu

Keywords:
Exoelectrogenic bacteria
Marine exoelectrogenic bacteria
Shewanella marisflavi EP1
Azo dye decolorization
Flocculation

ABSTRACT

Based on both capabilities of extracellular electron transfer and high salt tolerance, marine exoelectrogenic bacteria have the potential to serve as halotolerant/halophilic exoelectrogenic decolorizers (HEDs) for textile wastewater treatment. However, research in this area is still rare. In this study, we employed *Shewanella marisflavi* EP1 for this purpose. The results showed that EP1 could decolorize Xylidine Ponceau 2R (XP2R) under high NaCl concentrations up to 20%. Two different mechanisms were involved: degradation and bioflocculation. XP2R was decolorized by degradation in the range of 0–7.4% NaCl, by bioaugmented flocculation in 10–20% NaCl; and the range of 7.4–10% NaCl was the transition period from degradation to flocculation. Considering the property of flocculation by strain EP1, it is reasonable that XP2R was hard to penetrate into EP1 cells, thus it was an extracellular process of decolorization. The overall results further suggested that like EP1, marine exoelectrogenic bacteria might serve as a category of functional microbes (i.e., HEDs) for textile wastewater treatment.

1. Introduction

The textile industry is one of the most chemically intensive industries and the major polluter of surface water, especially in developing countries (Verma et al., 2012). This industry consumes a huge quantity of valuable fresh water and generates a large

* Corresponding author.
E-mail address: fcxu@xmu.edu.cn (F. Xu).

http://dx.doi.org/10.1016/j.chemosphere.2016.05.033
0045-6535/

quantity of complex organic wastewater, which contains not only various unused dyes but also bases and inorganic salts. According to the results of the First National General Survey of Pollution Sources of China, the textile industry was the second largest COD (chemical oxygen demand) contributor and the third largest wastewater generator in all industries (Lin, 2008). In South Asia, textile industry even contributed up to 35% of the total industrial wastewater in this area (Bank, 2005; Verma et al., 2012). To our knowledge, high salts and highly polar dyes are two critical factors in textile wastewater treatment by microbial methods.

Various inorganic salts are added as accessory ingredients to improve dyeing performance. The dyeing baths of direct dyes normally bear 0.25–2% of mineral salts while those of reactive dyes contain 3–10% NaCl (Hessel et al., 2007). In the reclaimed and reused wastewaters, the overall concentration of salts is even higher. Due to the shortage of fresh water, membrane separation technologies, such as nanofiltration and reverse osmosis, which are mainly used to obtain high-quality water, have been widely used for wastewater reclamation and reuse (Nataraj et al., 2009; Lu et al., 2010). In such filtration processes, almost all of dyes and inorganic salts are retained. After several iterations of recycling, the concentrated wastewater bears high levels of COD and salts. Many microbes are hard to grow under high salt conditions, and high performance is impossible if microbes grow slowly and the biomass in the reactor is low. Although there are a number of papers published on decolorization by microbes, most decolorizers are usually enriched or isolated from freshwater environments (Xu et al., 2005; Tan et al., 2009). Up to date, only a few halotolerant or halophilic bacteria are identified (Guo et al., 2008; Amoozegar et al., 2011). There is an obvious need to develop new processes of biological decolorization under high salt conditions (Khalid et al., 2012).

Besides the high salt issue, how to decolorize highly polar dyes under high salt conditions is a big problem. The dyes in highly saline textile wastewater are usually highly polar due to auxiliary groups such as hydroxyl, amino and sulfonic. Actually they are made intentionally so that they are soluble in high ionic strength solution. It is generally supposed that highly polar groups are hard to penetrate into the cell, thus are not easy to be utilized by microbes. Marine bacteria are usually halotolerant and even halophilic owing to the particular habitats. However, whether they could decompose dyes outside the cells is uncertain. The reduction reaction is generally the first and also the key rate-limiting step in dye decolorization (Pearce et al., 2003). Based on both capabilities of extracellular electron transfer and high salt tolerance, marine exoelectrogenic bacteria seem to be a category of natively HEDs for textile wastewater treatment, but research in this area is still rare.

Exoelectrogenic microbes, possessing specific extracellular electron transfer pathways, are capable to transfer electrons generated from substrate catabolism to terminals outside the cells or vice versa. Since most of them are found within the Proteobacteria phylum of the Bacteria domain, they are usually termed as exoelectrogenic bacteria (Logan, 2009), sometimes also called electrochemically active bacteria (Chang et al., 2006) and electricigens (Lovley, 2006). Obviously, these nomenclatures are based on their characteristic functionality, and the specific extracellular terminals reveal their possible application. As far as we know, there are mainly two kinds of terminals on which extensive studies have been carried out in the past years. One is the metal oxides and the other is the anode in microbial fuel cells (MFCs) (Lovley, 2012). Thus dyes may be the third category of extracellular electron acceptors from the above statement. Also, most of today's exoelectrogenic bacteria are isolated from terrestrial environments, while relatively fewer strains were from marine or benthic sediments. Sulfonic azo dyes are widely used in textile industry and the reduction of azo bond(s) (–N=N–) could be detected relatively easily by UV–Vis as well as HPLC (Khalid et al., 2010). Consequently, in this study we mainly investigated the decolorization capabilities of sulfonic azo dyes by a marine exoelectrogenic bacterium.

2. Materials and methods

2.1. Dyes, strain and culture media

Xylidine Ponceau 2R (XP2R, C.I. 16150) and 2, 4-Xylidine that is an intermediate of XP2R, were purchased from TCI (Shanghai) Development Co., Ltd, China. *Shewanella marisflavi* EP1 was isolated from marine sediment in Xiamen, China, after inoculated in the anode chamber of an MFC device for 2 months (Huang et al., 2010). EP1 was deposited in the China Center for Type Culture Collection (CCTCC) under accession number of CCTCC M 209016. A synthetic medium was used to culture EP1 cells, including 50 mg L^{-1} dye, 20 mM lactate, 50 mM fumarate, 990 mL M6 solution and 10 mL trace element solution (Bond and Lovley, 2003). The M6 solution consisted of (g L^{-1}): 19.89 NaCl, 0.745 KCl, 0.35 NaH_2PO_4, 0.44 Na_2HPO_4, 0.188 $MgSO_4$ and 2.0 NH_4Cl. Yeast extract and peptone were avoided as nitrogen sources, since these compounds may contain flavin-type substances (Masuda et al., 2010), which can serve as exogenous shuttles for decolorization. NaCl concentration was changed into 0–30% to investigate its effect on decolorization. Besides XP2R, other azo dyes such as Congo Red, Direct Yellow 50 and Acid Brilliant Scarlet GR were used to verify whether the same mechanisms existed.

2.2. Decolorization experiments

Exoelectrogenic bacterium EP1 is a facultative species. At first, it was cultivated under aerobic and anaerobic conditions respectively, and then tested for decolorization under anaerobic conditions. The results showed that EP1 grew much faster under aerobic than anaerobic conditions, but the decolorization rate remained almost the same (data not shown). Therefore, EP1 cells were first cultivated in several 500-mL flasks until the late exponential stage (OD_{600} about 1.0), and then dispensed into serial anaerobic pressure tubes (Bellco Glass, Inc.) with the same volume. After supplementation with azo dye, these bottles were sparged with high-pure nitrogen gas for 2 min to remove dissolved oxygen, and then clipped with thick butyl-rubber stoppers. All decolorization experiments were performed under static conditions at 35 °C, pH7.0.

2.3. Decolorization analysis

Decolorization of XP2R was monitored by a UV–Vis scanning spectrophotometer (UV-1900). Samples were withdrawn using a syringe from anaerobic bottles or tubes at scheduled times, and then centrifuged to remove cells. Percentage of decolorization was calculated according to absorbance decrease at the specific wavelength of 507 nm, using formula as: decolorization efficiency $= (A - B)/A \times 100$, where A is the initial absorbance and B is the observed absorbance at intervals.

2.4. HPLC analysis of XP2R and its products

Theoretically, if XP2R is decomposed by reduction of azo bond, 2, 4- Xylidine will be produced as an intermediate product. According to this rule, an HPLC (Agilent 1200) equipped with an ODS2 column (Thermo Scientific Hypersil, 4.6 mm × 250 mm) was used to detect XP2R and 2, 4- Xylidine. The mobile phase included: a solution containing 5 mM ammonium acetate and 0.06% (v v^{-1}) triethylamine used as eluent A, pure acetonitrile as eluent B, pH 8.0. The flow rate of the mobile phase was 1.0 mL min^{-1}. The elution