International Biodeterioration & Biodegradation 115 (2016) 250–256

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Bioelectrochemical reduction of an azo dye by a *Shewanella oneidensis* MR-1 formed biocathode

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ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form
5 September 2016
Accepted 5 September 2016

Keywords:
AO7 reduction
Biocathode
Bioelectrochemical systems
Shewanella oneidensis MR-1

ABSTRACT

Presently there is great interest to develop pure culture cathodes in bioelectrochemical systems (BES) for achieving decolorization/reduction of azo dyes. In this study, we investigated the decolorization of a model azo dye, acid orange 7 (AO7) in a biocathode inoculated with a model electrogenic microorganism *Shewanella oneidensis* MR-1 (MR-1). The decolorization efficiency of AO7 reached 95.8 ± 4.6%, 86.4 ± 2.0%, and 77.8 ± 1.0% in 46 h in the biocathode fed with lactate, in the biocathode without lactate, and in the abiotic cathode respectively. Thus, enhanced decolorization of AO7 occurred in the biocathode, compared to the abiotic cathode, regardless of the presence of lactate. To further investigate the AO7 reduction mechanism and electron transfer between the electrode and *S. oneidensis*, the cathode potential was controlled such that hydrogen was not produced. In the absence of lactate, the AO7 decolorization efficiency (78.5 ± 0.8%) and sulfanilic acid (SA) production efficiency (70.9 ± 1.5%) in the biocathode were higher than the AO7 decolorization efficiency (63.7 ± 3.6%) and the SA production (56.1 ± 1.9%) efficiency in the abiotic cathode. This suggests that the reduction of AO7, with electrode as the sole electron donor, was enhanced by electron transfer between the cathode and the *S. oneidensis* formed biofilm on the cathode surface. This study demonstrated that *S. oneidensis* enhanced the capture of electrons from the cathode electrode for the reduction of the organic pollutant AO7.

1. Introduction

Azo dyes are compounds with aromatic structures characterized by containing one or more azo linkages (-N=N-) and they are widely used in the textile, leather, cosmetics, and food industries (Elisangela et al., 2009; Tony et al., 2009). Azo dyes can be water or oil soluble, and the presence of a sulfonated group results in the compounds being highly water-soluble. Consequently, azo dyes containing these groups are easily mobilized into water systems during production and applications (Tan et al., 2005). Environmental contamination by these azo dyes even at very low concentrations can cause water streams to become highly colored and give rise to undesirable aesthetic effects (Pandey et al., 2007; Lončar et al., 2012; Singh et al., 2015).

Azo dyes are generally persistent under aerobic conditions (Tan et al., 2013). However, under anaerobic conditions azo dyes can be reduced and decolorized, with the breakdown of azo bonds producing products that are easily mineralized (Tan et al., 2005; Wang et al., 2011). This anaerobic decolorization is a cost efficient process; however, it is usually very slow and requires an organic co-substrate to provide electrons for the reduction (dos Santos et al., 2005). The addition of the co-substrate adds to the costs and unwanted methane production since the dosage typically far exceeds the stoichiometric requirements (Van der Zee and Villaverde, 2005). Thus, treatment of wastewater containing azo dyes is a

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http://dx.doi.org/10.1016/j.ibiod.2016.09.005
0964-8305/

challenging issue that requires improvement. Acid orange 7 (AO7) is an azo dye that is widely used in the textile, food and cosmetics industries, thus it is a significant component of wastewaters produced by these manufacturers that requires removal (Mu et al., 2009).

Recently, wastewater treatment capabilities of bio-electrochemical systems (BES) have being extensively explored for degradation of a number of organic and inorganic contaminates (Wang et al., 2015). Contaminants such as nitroaromatics (Wang et al., 2011; Liang et al., 2014; Feng et al., 2015), azo dyes (Liu et al., 2015), and halogenated aromatics (Liang et al., 2013; Kong et al., 2014a,b) can be reductively eliminated under anaerobic conditions with acclimated biocathode communities. However, the biological reduction mechanisms of the pollutants at the biocathode are unclear and such details are required for optimization of the treatment processes. Bacterial reduction of azo dyes has been investigated using various pure cultures under cathodic conditions while adding specific carbon sources. Mostly these pure cultures are of the *Shewanella* genus and include *Shewanella decolorationis* S12 (Hong et al., 2007; Hong and Gu, 2010; Yang et al., 2015), *Shewanella* sp. J18143 (Pearce et al., 2006), *S. oneidensis* (Pearce et al., 2006) as well as *Pseudomonas* sp. WYZ-2 (Wang et al., 2014). However, mostly these studies focused on the performance of the azo dye removal and the BES conditions rather than on determining details of the cathode electron transfer mechanisms for the reduction of the dye.

S. oneidensis exhibits very diverse respiratory capabilities. In addition to aerobic respiration it can conduct anaerobic respiration using a range of electron acceptors that include fumarate (Rosenbaum et al., 2011), nitrobenzene (Cai et al., 2012), Cr(VI) (Belchik et al., 2011), Fe(III) citrate (Rosenbaum et al., 2012), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Perreault et al., 2012) as well as azo dyes such as Naphthol Green B (Xiao et al., 2012). As a consequence of these diverse respiratory capabilities, *Shewanella* species are often used in electrogenic studies for the reduction of organic pollutants in BES (Rosenbaum et al., 2012).

In this study, a dual chamber BES was used for the removal of azo dye AO7 at a biocathode in the presence of *S. oneidensis*. Removal efficiency of AO7 was compared between biocathodes in the presence and absence of the co-substrate lactate, as well as between the biocathode and the abiotic cathode. This work supports the feasibility of AO7 reduction mediated by the cathodic biofilm of *S. oneidensis*. To our best knowledge, this is the first report to demonstrate that *S. oneidensis* MR-1 enhances cathodic electron capture for the reduction of the organic pollutant AO7.

2. Materials and methods

2.1. Anode and cathode inoculum

The anodic microbial community was enriched from domestic wastewater that was used to inoculate to the BES as previously described (Wang et al., 2011). Sodium acetate was added to the anodic medium to ensure excess electron donor. A pure cultures of *S. oneidensis* MR-1 was used to form biofilms in the cathodic chamber. *S. oneidensis* from glycerol stocks was firstly incubated in Luria-Bertani (LB) medium at 30 °C, shaken at 150 rpm overnight. One loop of bacterial suspension was then streaked onto a LB plate and incubated overnight at 30 °C. From the plate, one pink colony was transferred to liquid LB medium and incubated at 30 °C, shaken at 150 rpm overnight. This suspension was used as the BES cathode inoculum. The cathode medium consisted of a nutrient solution that included Wolf's vitamins and trace elements (Wang et al., 2011). Additionally, when included in the cathode medium batch tests, lactate was added at 10 mM and AO7 was added at 0.28 mM in the presence of lactate and 0.147 mM in the absence of lactate. The cathodic medium was prepared by boiling and purging with N_2 gas (99.9% purity) for 20 min before autoclaving at 121 °C for 30min.

2.2. BES reactor set up

Dual chamber polycarbonate BES reactors were constructed and assembled, using graphite fiber brush and carbon cloth as the anode and cathode materials respectively, as previously described (Liang et al., 2013). The working volume of each chamber was 85 mL. The carbon cloth was immersed in 1 M hydrochloric acid for 24 h and then in deionized water for another 24 h before use. The assembled reactors, excluding the carbon cloth, were covered with aluminum foil and autoclaved at 121 °C for 30 min. Following that, the carbon cloth was assembled into the BES in sterile conditions before starting the batch experiments. An external power of 0.3 V was applied to assist the AO7 reduction in the abiotic and biotic cathodes, unless otherwise mentioned.

All the BES reactors had the same anode conditions. For one set of reactors the cathode was inoculated with *S. oneidensis* while in another set there was no inoculum and the cathode was abiotic. The cathode inoculum was centrifuged at 5000 rpm for 3 min, the supernatant was discarded, and then the pellet was dissolved in sterile cathode medium before transferring it to the cathode chamber. This was incubated under anaerobic conditions for 48 h to allow biofilm to develop on the cathode. Subsequently, the cathode liquid was removed and the cathode chamber was replenished with another batch of freshly prepared MR-1 inoculum and cathode medium. This procedure was repeated six times to achieve adequate biofilm development on the cathode surface (Liang et al., 2013).

Two BES reactors with well-developed cathode biofilms were operated for three repeated batch experiments under particular conditions with AO7 as the electron acceptor. For each batch experiment fresh media was placed in the anode and cathode chambers and the BES reactions were monitored. Each batch experiment lasted approximately 60–80 h and was terminated when the current had nearly stopped decreasing (closed circuit mode). For each reactor, the initial batch experiments were operated in closed circuit mode, and then further batch tests were performed with the two reactors switched to opened circuit, thus operated without external electron supply. Another two BES reactors were operated as abiotic cathodes for three cycles initially with closed circuit, and then as opened circuit mode. A summary of the overall batch test experiment design is provided in the results. All the BES batch experiments were performed at room temperature (26 ± 2 °C).

2.3. Chemicals analysis

During the batch experiments samples taken from the cathode were immediately filtered through a 0.22 μm filter, and AO7 concentrations were measured using UV-vis spectrophotometer (Cary 50, Varian Inc., Australia) at 485 nm (Mu et al., 2009) over the wavelength range of 200–750 nm. The reduced product sulfanilic acid (SA) of AO7 was quantified by HPLC. The reverse-phase HPLC system (model e2695, Waters Co., Milford, MA) was equipped with a Symmetry C18 column (5 μm; 5 × 250 mm, Waters Co., USA) and a UV detector was used for SA measurement at 254 nm as described previously (Mu et al., 2009).

2.4. Cyclic voltammetry analysis and SEM detection

The cyclic voltammetry (CV) analysis was conducted using an