



Short Communication

Electrode as sole electrons donor for enhancing decolorization of azo dye by an isolated *Pseudomonas* sp. WYZ-2



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HIGHLIGHTS

- *Pseudomonas* sp. WYZ-2 was isolated from biocathode in bioelectrochemical system.
- WYZ-2 accelerated the electrons transfer taking electrode as sole electrons donor.
- WYZ-2 modified electrode played a catalytic role for azo dye decolorization.

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ABSTRACT

Pseudomonas sp. WYZ-2 was isolated from a biocathode which accelerating azo dye decolorization. When the electrode was polarized at -0.8 V (vs. SCE), WYZ-2 could exist on electrode, because the current of working electrode stabilized at -0.35 mA from -0.13 mA after inoculation. Moreover, cyclic voltammetry scanned an unidentified redox-active molecule which involved in the electron charge transfer potentially. On azo dye decolorization experiments by WYZ-2 modified electrode, electrochemical tests also indicated that the catalytic ability of WYZ-2 modified electrode was improved because charge transfer resistance decreased to 255Ω from 720Ω , azo dye reduction potential was shifted to -0.78 V from -0.89 V, and the maximum decolorization efficiency of azo dye was increased to 93.4% from 53.2%, comparing with unmodified electrode. Although numerous studies on azo dye decolorization employed biological agents, electrochemical activity bacteria accelerate the decolorization process using electrode as sole electron source has seldom been reported.

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1. Introduction

In microbial fuel cells or microbial electrolysis cells, the anode microorganisms catalyzed the oxidation of organics to produce electrons and then transferred the electrons to abio-cathode which made by noble metal catalyst (Logan et al., 2006). Biocathode was also used as elegant catalyst for an alternative duo to low-cost and increasing the sustainability. In recent years, biocathode was developed into a wider application range, such as generating CH_4 from CO_2 and refractory pollutant removal (Cheng et al., 2009;

Wang et al., 2011). Differently from noble metal catalyst, the biocathode were formed by electroactive microbes, which promoted electron transfer from electrode to electron acceptor in previous studies (Erable et al., 2010). This electrochemical activity biofilm was known to catalyze reduction of electron acceptors such as CO_2 and nitrate (and whatever other electron acceptors already published) by electrode as electron donor.

From electrochemical activity biofilm, the pure bacterium was isolated and displayed electrochemical function with directly enhancing electron transfer (Carbajosa et al., 2010; Parot et al., 2011). It not only played an important role in oxygen reduction (microbial fuel cells), but also used in dechlorination, denitrification and more pollution removal using electrode as electron donor (Strycharz et al., 2008; Su et al., 2012). In this study, a novel attempt is carried out for the decolorization of azo dye wastewater

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using the isolated electroactive bacteria, when the electrode is polarized at low potential as a sole electrons donor. The targeted azo bonds could accept electrons lead to decolorize in low potential. Although numerous decolorization studies of biological agents were reported, most of them insisted on using the electrons from organics oxidation. Differently to previous studies, this study focused on using electrons from electrode, due to the lack of electrons donor in dye industrial wastewater. The electrode as additional electron donor can provide more electrons to enhance decolorization efficiency. Exploiting electroactive bacteria is conducted to further understand the electron transfer pathway and to use these microbes in bioenergy and bioremediation.

2. Methods

2.1. Isolation and 16S rDNA molecular identification

The pure strain WYZ-2 was obtained from a biocathode carbon brush. The carbon fiber with biofilm was placed in a 30 mL of 50 mM phosphate buffer solution (PBS) and oscillated for 10 min. The cell suspension was prepared by serial dilutions, inoculated on solid beef extract–peptone medium and incubated for 3 days at 35 °C. The bacterial colonies were picked up and subsequently inoculated in liquid beef extract–peptone medium for culture. This procedure was repeated several times until the pure strain was obtained. All operations were performed under sterile conditions. Total genomic DNA of the bacteria was extracted using DNA Mini Kit (Watson Biotechnologies Inc., China). The 16S rDNA gene was amplified, and after sequencing, MEGA3.1 software was used for phylogenetic analysis (Luo et al., 2013).

2.2. Three-electrode system set-up

The three-electrode system was contained in a 100 mL glass cell, while a potentiostat (WMPG1000K8, Wonatech, Korea) was used to study the WYZ-2 electrochemical activity by chronoamperometry (CA). With saturated calomel electrode (SCE) as the reference electrode, the platinum electrode as a counter electrode, and three same graphite plates (GP, 2 cm × 3 cm) as a working electrode was used. 50 mM PBS was as the reaction solution during the WYZ-2 electrochemical growth and modified electrode formation. The acid black 1 (AB1) was targeted pollution of azo dye wastewater, initial concentration 100 mg/L.

2.3. WYZ-2 modified electrode operation

50 mL WYZ-2 bacterial culture (beef extract peptone, 24 h, 35 °C) was centrifuged and washed 3 times by 50 mM PBS, before dissolved in 5 mL of 50 mM PBS as inoculum. Working electrode potential was polarized at -0.8 V (vs. SCE, all the potentials were vs. SCE in this work). This potential was chosen because that low potential was conducive to provide electrons. The cathode potential could reach -0.8 V, and even lower than -1 V in the previous studies (Jeremiase et al., 2010; Fu et al., 2013).

2.4. Analysis measurements

Cyclic voltammetry (CV) was performed from -0.4 to -1.2 V with a scan rate of 1 mV/s. Electrochemical impedance spectroscopy (EIS) method was used to characterize the charge transfer resistance (R_{ct}) of working electrode, frequency range was taken from 100 to 10 MHz using a 5 mV sine wave and the obtained data was simulated by ZsimpWin3.10 software in room temperature. Azo dye AB1 concentration was measured by spectrophotometry

and its maximum absorption wavelength was 618 nm. The azo dye decolorization efficiency (DE) was evaluated as follows:

$$DE (\%) = (1 - C_t/C_0)100\%$$

where, C_0 is the initial concentration of AB1, and C_t is the concentration at reaction time (t).

3. Results and discussion

3.1. Isolation and molecular identification of *Pseudomonas* sp. WYZ-2

An isolated bacterium WYZ-2 was from a biocathode in the bioelectrochemical system, which treated azo dye wastewater by cathodic reduction (Wang et al., 2013). The 16S rDNA gene sequence (GenBank accession number KF623283) of WYZ-2 was compared with the GenBank database by the BLAST program and the phylogenetic analysis revealed that the isolated WYZ-2 related to *Pseudomonas* (Supplementary Fig. 1). The nearest neighbor of *Pseudomonas nitroreducens* had a sequence similarity of 98%, generally, the sequence similarity of the 16S rDNA gene was more than 97%, which could be identified to belong to *Pseudomonas*, designated the isolate as *Pseudomonas* sp. WYZ-2.

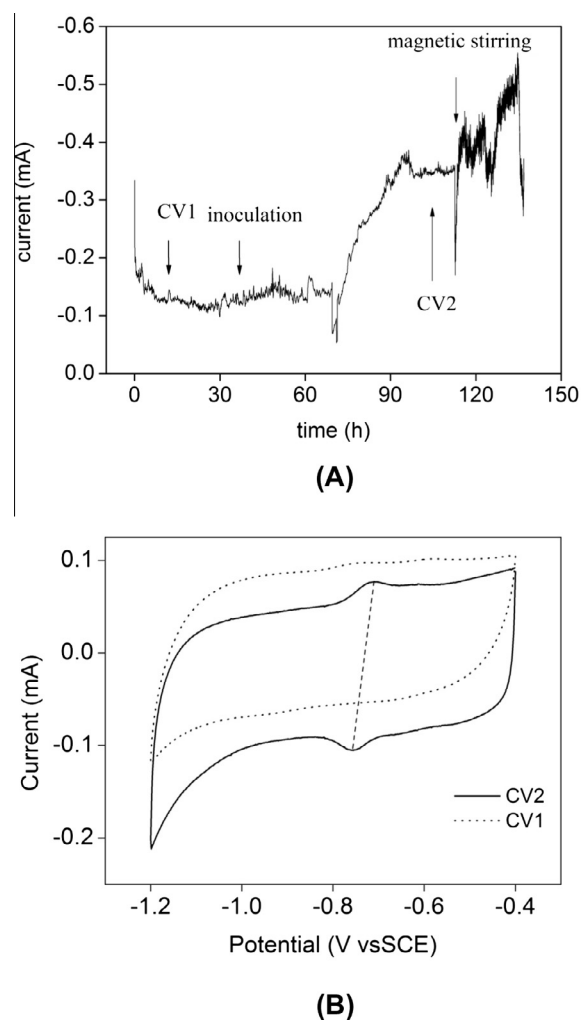


Fig. 1. (A) Working electrode current variation before and after WYZ-2 inoculation by CA. (B) CV record on working electrode before and after WYZ-2 inoculation (scan rate 1 mV/s).

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