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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 (Jeremiasse 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 bio-electrochemical 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).

3.2. WYZ-2 modified electrode formation

In the absence of organics or other electron donors, the electrode was served as a sole electron donor in the 3-electrode system. As seen in Fig. 1(A), when the reactor was startup, the current was stable at about -0.13 mA, no obvious current change was detected during the first 36 h. At the 36 h time point, 5 mL of washed WYZ-2 suspension was added to the reactor as inoculum and the working electrode current had significant improvement from 75 h afterward with a maximal value of -0.38 mA, subsequently reached a stable state of -0.35 mA. Comparing with the recorded current before WYZ-2 inoculation, the current increased 3 times. In order to decrease mass transfer limitations and maximize the working electrode current, magnetic stirring was used in the reactor at the 112 h time point and the current showed a remarkable increase. In previous study (Raghavulu et al., 2011), *Pseudomonas aeruginosa* showed an ability of electrons transfer from substrate oxidation to electrode. In contrast, apparently, the increasing working current demonstrated that *Pseudomonas* sp. WYZ-2 could accelerate to transfer electron from electrode surface, where the electrons were released from electrode and absorbed by bacteria or further arrived at the electron acceptor.

CV measurement was conducted for checking working electrode during WYZ-2 electrochemical grown. Interestingly, one pair of redox peaks was observed with -0.74 V midpoint redox potential in working electrode (CV2 curve) in the recorded CV (Fig. 1B) after inoculating WYZ-2. On the other hand, no redox peaks were detected (CV1 curve) in the unmodified electrode (control) which indicated this redox active molecule was produced by WYZ-2. In the previous studies, some redox molecules were studied from *Dehalococcoides* spp., *Geobacter lovleyi* (Aulenta et al., 2009), which revealed a redox-active molecule with -0.64 V (vs. SCE) potential in the extracellular electron transfer. In addition, some researchers suggested that *Pseudomonas* produced electron shuttles to promote electrode-microbe electron transfer (Pham et al., 2008). But, in this work, no redox peaks was present in the supernatant of the cultured solution. These findings suggest that this redox molecule located bacterial or adsorbed to electrode surface, were not released into the culture solution. Clearly, this compound needs further identification and functional investigation in the further study. Taken as a whole, whether current development in CA or redox active molecule detection in CV, all the results demonstrated that the WYZ-2 could exist on electrode surfaces and displayed an important role for accelerating electron transfer between electrodes and electron accepters.

3.3. Electrochemical decolorization of azo dye AB1

In order to check the ability of electrochemical decolorization by WYZ-2 modified electrode, EIS and CV were conducted at the beginning of every decolorization experiments. As seen from Fig. 2(A), EIS Nyquist plots revealed a great change on the semicircle which corresponded with R_{ct} of electrode. By simulating an equivalent circuit, R_{ct} of WYZ-2 modified electrode decreased to 255Ω comparing with 720Ω of unmodified electrode (control). Moreover, after electrode sterilization, R_{ct} was returned and even higher than control. Obviously, dead WYZ-2 lost the ability of electron transfer and even hindered it. The decreasing of R_{ct} showed that electrode released electron more easily and was benefit to electrochemical reaction, especially for azo bonds breaking.

An interesting change was also revealed in the CV results (Fig. 2B). The azo dye AB1 had two reduction peaks and the left peak (arrow marked) had changed largely in the peak current and peak potential. The peak current increased to -6.97 mA from -4.48 mA and peak potential shifted to -0.78 V from -0.89 V,

Fig. 2. Electrochemical detection in AB1 decolorization experiments. (A) EIS results (B) CV results.

compared to cleaned electrode. According to the results, WYZ-2 had a catalytic ability to decrease the potential of reduction AB1 and increase the reaction current.

Of course, it is not enough for the evaluating of WYZ-2 decolorization ability by EIS and CV. In order to further check the efficiency of decolorization by WYZ-2 modified electrode, the decolorization experiments were conducted by CA method with -1.0 V polarized potential. In Fig. 3, UV spectral profiles revealed AB1 had a prominent absorbance at 618 nm. With time

Fig. 3. UV spectral profiles in AB1 decolorization.

went on, the OD₆₁₈ decreased obviously until the DE achieved at 93.2% in 72 h. Meanwhile, although the control cleaned electrode could catalyze the AB1, the DE was just 53.2% in 72 h. The decreasing of color intensity in 72 h: WYZ-2 modified electrode sample became almost colorless, while high color intensity was obvious in the cleaned electrode sample (Supplementary Fig. 2).

Based on the above considerations, WYZ-2 can improve the electrode surface activity with electrode as sole electron donor. Being characteristics of biocatalyst, low-cost, sustainable will make it has widespread application, especially for the treatment of azo dye industrial wastewater. Moreover, when artificial bio-electrode is used at a low potential, it will provide more electrons and then efficiently accelerate the decolorization process comparing with unmodified electrode. Comparing with the traditional decolorization by anaerobic degradation, abundant electrons were needed from organics in the wastewater, which may not be suitable for azo dye wastewater where the electrons source was insufficient.

4. Conclusions

This work demonstrated that WYZ-2 could exist on electrodes at a low potential taking the electrode as a sole electrons donor without artificial redox mediators. Comparing with the control electrode, the WYZ-2 modified electrode showed a higher current density due to WYZ-2 accelerating electron transfer by a redox-active molecule which played an important role on the electrode surface presumably. Furthermore, the electro-catalytic azo dye decolorization was enhanced by WYZ-2 modified electrode. Based on this, the isolated *Pseudomonas* sp. WYZ-2 had a beneficial potential for bioremediation and conducted to uncover more microbe potential in electrochemical activity research field.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2013.11.001>.

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