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Deciphering characteristics of bicyclic aromatics – mediators for reductive decolorization and bioelectricity generation

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HIGHLIGHTS

- Disclose new decolorized mediators to dye removal and bioelectricity generation.
- Unveil mediating characteristics of bicyclic aromatics 1A2N, 4A1N to MFCs.
- Suggest models of mediator-assisted MFCs for wastewater decolorization.

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ABSTRACT

This first-attempt study quantitatively assessed electron-mediating characteristics of bicyclic aromatics – 1-amino-2-naphthol, 4-amino-1-naphthol (i.e., decolorized intermediates of azo dyes – orange I and II) for color removal and power generation in MFCs. According to cyclic-voltammetric profiles, the presence of reduction and oxidation peak potentials clearly suggested a crucial role of these intermediates as electron-shuttling mediators. Shake-flask cultures also showed that appropriate accumulation of 1A2N, 4A1N apparently enhanced color-removal efficiencies of bacterial decolorization. This study clearly suggested that suitable supplementation of electrochemically active electron shuttle(s) to dye-bearing MFCs is a promising strategy to stimulate reductive decolorization and bioelectricity generation.

1. Introduction

As one of biomass-based renewable energy, microbial fuel cells (MFCs) could simultaneously expedite bioelectricity generation and pollutant degradation during wastewater treatment (Du et al., 2007; Rabaey and Verstraete, 2005; Logan, 2009). In particular, MFCs can be implemented for dye remediation during bioelectricity generation (Solanki et al., 2013). In fact, two mechanisms (Schröder, 2007; Fricke et al., 2008) have been identified for electron transfer from bacteria to electrodes: direct electron transfer (DET) and mediated electron transfer (MET). In fact, DET takes place via a physical contact of the bacterial cell membrane or a membrane organelle with the anode, without diffusional redox species being involved in the electron transfer from anodic cells to the electrode. In contrast, MET usually requires redox mediator(s) to act as an electron shuttle between bacteria and an electron acceptor (Schröder, 2007; Fricke et al., 2008).

Recently, Lovley (2008) mentioned mechanisms of microbial conversion of organics to bioelectricity. Chen et al. (2011b, 2013a) explored characteristics of simultaneous bioelectricity generation and dye decolorization (SBG&DD) using bacterial decolorizers (e.g., *Klebsiella pneumonia* ZMd31, *Proteus hauseri* ZMd44, *Aeromonas hydrophila* NIU01 and mixed consortia), revealing that reductive decolorization and bioelectricity generation are competitive to each other. That is, electrons released from oxidation of co-metabolic organic matter via electron transport chain of bacterial decolorizer(s) through respiratory complexes could be used for either bioelectricity generation or color removal (Chen et al., 2011b). In addition, Chen et al. (2013a) and Zhang et al. (2010) proposed that decolorized intermediates could be generated to act as electron-shuttling mediator(s) in dye-bearing MFCs. As a matter of fact, 2- and 4-aminophenol, dihydroxyl benzene, diaminobenzene were found to be feasible redox mediators to shuttle electron transfer in MFCs (Chen et al., 2013a). However, these –OH and/or $–NH_2$ group(s) containing mono-benzene ring-based compounds can be used as electron-mediating chemicals, but they are not decolorized intermediates of textile dye(s) popularly used in

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industry. Moreover, as Watanabe et al. (2009) indicated, ESs can be used to increase the efficiencies of MFCs, but are still generally costly. Thus, "mediator-less MFCs" (i.e., no exogenous ES(s) added) applied to dye-bearing wastewater treatment are considered to be more economically practical for MFCs of sustainable processes. Recently, Sun et al. (2013) used anthraquinone-2,6-disulphonic disodium salt, riboflavin and humic acid as redox mediators to air–cathode MFCs. Prior studies (Chen et al., 2011b, 2013a; Zhang et al., 2010) proposed that decolorized intermediate(s) might be electron-shuttling mediators to enhance performance of color removal and bioelectricity-generation. To verify this concept, 1A2N and 4A1N (i.e., decolorized intermediates of Orange I (OI), Orange II (OII)) were intentionally used herein to test this postulated hypothesis. In fact, both reductive decolorization and bioelectricity generation of OI, OII-containing wastewater could be "autocatalytically" accelerated when color removal was triggered to be taking place.

In addition, according to Watanabe et al. (2009), deciphering how chemical structure of intermediates affects MFCs to stimulate simultaneous power generation and reductive decolorization would be of great significance to textile dye-treating MFC applications. Prior studies (Chen et al., 2013a,b) also mentioned that –OH and $–NH_2$-containing compounds (e.g., 2-aminohpenol) could significantly enhance performance of bioelectricity generation and color removal. As a matter of fact, previous findings (Chen et al., 2013a; Zhang et al., 2010) revealed that decolorized intermediates of reactive blue 160-RBu160 (e.g., phenyl methadiamine which is amino group-containing) could significantly enhance the performance of reductive decolorization. Regarding this stimulating phenomena, Zhang et al. (2010) disclosed that mono-benzene ring-associated compounds (MBRACs) (e.g., 2-aminophenol (2AP), 1,2-diaminobenzene (12DB), 1,4-diaminobenzene (14DB), benzene-1,2-diol (B12D)) were found to be feasible electron-shuttling mediators. For example, regarding the electron-mediating mechanism of 2AP (Chen et al., 2013a,b), as hydroxyl group is present on the benzene ring of 2AP, the molecules can be in stable resonance to form active radicals, then favorably oxidized to form *o*-quinoniminium ion and *o*-quinonimine as electron-shuttling mediators for electricity generation. In addition, due to acidic characteristics of hydroxyl groups of phenols, the acidic hydrogen can be dissociated to obtain phenolate anions, and then phenolate anions will be favorably oxidized to form active radicals (Chen et al., 2013a,b). Apparently, these steps of free radical formation are vital to form electron-mediating shuttles for bioelectricity generation. The formation of such compounds (e.g., B12D) could significantly reduce internal resistance (i.e., kinetic and diffusion resistance) of MFC to augment power generation as well as color removal. Here, compared to MBRACs, this feasibility study tended to explore characteristics of bicyclic aromatics (e.g., 1-amino-2-naphthol (1A2N), 4-amino-1-naphthol (4A1N)) as model mediating compound(s) to stimulate color removal and power production in MFCs. In addition, suggestions associated to chemical structure effects upon electron-shuttling mediators would be proposed herein for practical applications to MFC-assisted dye bioremediation.

2. Experimental section

2.1. Dye decolorization and bioelectricity generation

To have comparative analysis with prior studies, the model azo dye reactive blue 160 (RBu 160), reactive black 5 (RBk5) and reactive red 141 (RR141) (all purchased from Everlight Chemical Ltd., Taipei, Taiwan) were used to demonstrate color bioremoval capability of bacterial decolorizer(s) (e.g., *P. hauseri* ZMd44) (Zhang et al., 2010). Cell broth harvested from MFC was used as inoculated microbial cells of batch cultures (0.2× LB, 30 °C, 125 rpm, 200 mg L^{-1} RBu160, RBk5 and RR141 with supplementation of 2 mg L^{-1} 1A2N and 4A1N) to evaluate characteristics of dye decolorization (Chen et al., 2013b). Specific growth rate (SGR), and specific decolorization rate (SDR) were determined via time-course profiles of microbial growth and dye decolorization as described elsewhere (Zhang et al., 2010). Due to solubility limitation (max. at ca. 120 mg L^{-1}) and toxicity potency of 1A2N and 4A1N, 40 mg L^{-1} was intentionally selected for feasibility testing as electron mediators (cell growth resisted at ca. 75 mg L^{-1}; data not shown) for ZMd31, ZMd44 and NIU01-seeded MFCs. The reason why this study randomly selected different microbes-seeded MFCs for inspection is to guarantee the symmetric characteristics of ESs for electron-mediating. In addition, nanowire-generating bacteria (e.g., *Shewanella* spp.) were intentionally not used for study to have nearly identical bioelectricity-generating bases of microbes for comparison.

2.2. Cyclic voltammetric determination

Cyclic voltammetry of candidate mediators (e.g., 1A2N, 4A1N) was performed using an electrochemical workstation (Jiehan 5600, Taiwan) at 10 mV s^{-1} scan rate. The working, counter, and reference electrodes were a glassy carbon electrode (0.07 cm^2), platinum electrode (6.08 cm^2), and a Hg/Hg_2Cl_2 electrode filled with saturated $KCl_{(aq)}$, respectively. The glassy carbon electrode (GCE, ID = 3 mm; model CHI104, CH Instruments Inc., USA) was successively polished with 0.05 μm alumina polish and then rinsed with 0.5 M H_2SO_4 and deionized water before use. The experiments were performed in phosphate buffer solutions (PBS; pH = 7.0) at 0.1 M and the solutions were purged with nitrogen for 15 min prior to analysis. The scanning rate was 10 mV s^{-1} over the range from 0.4 to −0.6 V. The redox potentials recorded as Hg/Hg_2Cl_2 reference electrode were corrected by 0.241 V (i.e., E_0 of Hg/Hg_2Cl_2) to the standard hydrogen electrode (SHE).

2.3. Electrochemical measurements

(a) Electrochemical impedance spectroscopy (EIS) (HIOKI 3522-50, Japan) measurement was implemented on steady-state open circuit potential distributed with an amplitude of 10 mV. The frequency range was 10^4–5×10^{-3} Hz. Collected data were analyzed using the software for Nyquist plot (Zview 2.6b, Jiehan Tech.). (b) Power generation measurement: Cell voltage was automatically measured (set at one data point per minute) using a data acquisition system (DAS 5020; Jiehan Technology Corporation) through external resistance R_{out} = 1 KΩ. Note that a relatively high resistance (1000 Ω) was intentionally used in order to compare with prior results. The power densities (P) and current densities (I) of MFCs were determined using linear sweep voltammetry (LSV) measurement and the corresponding voltages were recorded using a multimeter. Note that all MFCs were operated in model of membrane-less single chamber at 25 °C.

3. Results and discussion

3.1. Reductive decolorization analysis

As prior findings (Chen et al., 2013a,b) indicated, capabilities of bioelectricity generation in MFCs could be evaluated via bacterial decolorization tests since both metabolic functions are electron-transfer associated. In addition, as bioelectricity generation and reductive decolorization were competitive to each other