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Deciphering electron-shuttling characteristics of thionine-based textile dyes in microbial fuel cells

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ABSTRACT

Prior studies indicated that –OH and/or $–NH_2$ substituent containing auxochrome compounds (*e.g.*, 2-aminophenol and 1-amino-2-naphthol) could act as electron shuttles (ESs) to stimulate wastewater decolorization and bioelectricity generation in microbial fuel cells (MFCs). This study provided first-attempt to disclose how and why thionine-associated textile dyes (*i.e.*, azure A and azure C) could also own such redox-mediating capabilities in MFCs. Due to the presence of iminium part as mediating group, $–N(CH_3)_2$ or $–N(CH_3)H$ substituent could effectively mediate electron transport compared to $–NH_2$ substituent for bioelectricity generation in MFCs. For dye-laden wastewater treatment, the presence of electron-mediating textile dyes (*e.g.*, thionine, azure A and azure C) in MFCs is promising to stimulate biodegradation of organics and bioelectricity generation. With such ESs as stimulants, using MFC as operation strategy would be cost-effective for wastewater treatment as oxidation of organic pollutants could be automatically accelerated.

1. Introduction

As treatment of wastewater containing myriads of textile dyes through conventional methods still own many disadvantages [1], remediation alternatives of reducing operation cost and promoting process efficiency gradually became more attractive than ever [2]. Moreover, global warming and spiraling energy prices have significantly affected international economy and national security [3]. Therefore, exploring renewable green energy for sustainable development is inevitably of great importance to reduce the dependence on imported fossil fuels. As a matter of fact, industrial wastewater usually contains a variety of organic matter and apparently it could be utilized as a fuel source for energy recycling.

In fact, among all renewable green energy, biomass-based energy would be more environment-friendly. For example, microbial fuel cells (MFCs) are novel bioelectrical devices that can directly transform chemical energy from oxidation of organic matter to bioelectricity via a series of electrochemical reactions catalyzed by microbes [4,5]. For augmentation of bioelectricity-generating capability of MFCs, one of the most intriguing alternatives prevailing in recent years was exogenous supplementation of electron shuttles (ESs) with low toxicity potency. As a matter of fact, ESs (or redox mediators) are organic molecules that can reversibly be oxidized and reduced to serve as electron carriers among multiple redox reactions [6]. With ESs, electron flux in MFCs can be augmented to achieve higher bioelectricity-generating capability. In particular, due to chemical structure effect as mentioned previously [7], the presence of functional groups in the proximity of redox mediators directly affected the performance of bioelectricity generation as well as reductive decolorization. For example, the stronger electron-withdrawing group (*e.g.*, $–SO_3Na$) near azo bond(s) could assist higher decolorization performance (*e.g.*, methyl orange) taking place. In addition, as $–SO_3Na$ showed better capability to pull electron(s) towards azo bond(s) than –COONa, it significantly decreased electron density near azo bond and therefore facilitated color removal [7]. However, prior study [8] also pointed out that dye intermediates with different functional substituents resulted in large difference in biotoxicity potency as well electron-shuttling characteristics. For instance, hydroxyl (–OH) and amino ($–NH_2$)-group bearing aromatics (*e.g.*, 2-aminophenol (2-AP), benzene-1,2-diaminobenzene (b12d), 1,2-diaminobenzene (12db); [9]) were all found to be promising redox mediators due to low toxicity potency to bioelectricity-generating bacteria [10,11]. These all implied that electron-shuttling capabilities and biotoxicity potency of candidate mediators were strongly due to chemical structures. This study selected non-azo textile dyes with different substituents (*e.g.*, $–NH_2$, $–NH(CH_3)$, $–N(CH_3)_2$) as models for comparative analysis upon their redox-mediating capabilities.

Thus, this study extended to explore whether non-azo textile dyes or derived intermediates can also act as redox mediators in MFCs. In fact, Rahimnejad et al. [12] and Park and Zeikus [13] mentioned

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that both methylene blue and neutral red could act as redox mediators to enhance power-generating capabilities of *Escherichia coli* and *Actinobacillus succinogenes* bearing MFCs. Moreover, thionine is a strongly staining metachromatic dye widely used for biological staining [14] and is a well-known electron-shuttling mediator for bioelectricity generation [15,16]. Although thionine-related compounds chemically own capabilities to mediate electron transport, whether they are also biochemically-feasible to enhance bioelectricity production of electrochemically active biofilm in MFCs is still remained open to be discussed [17]. For example, azure A is a phenothiazine dye that is chemically formed by oxidation of methylene blue. It can be used in making azure eosine stains for blood smear staining [18]. This work chose thionine, azure A and azure C as candidate mediators of textile dyes to disclose the mysteries for comparative study. Of course, exogenous supplementation of textile dyes to wastewater during practical treatment will not be allowed. However, if such textile dyes (*e.g.*, thionine, azure A or azure C) were present in dye-bearing wastewater, using MFC as operation strategy seemed to be more promising color removal due to the effect of mediator stimulation. In addition, as textile dye(s) may play as electron mediating shuttles, fed-batch or continuous modes of MFC operation will be more appropriate than batch mode for cost-effective biodegradation of textile dye(s).

2. Experimental section

2.1. MFC construction

Membrane-free air cathode single-chamber MFCs using seed strains *Proteus hauseri* ZMd44, *Klebsiella pneumoniae* ZMd31 and *Aeromonas hydrophila* NIU01 were constructed in cylindrical tubes made by polymethyl methacrylate (PMMA) (cell sizing ID = 54 mm, L = 95 mm) with the operating volume of *ca.* 220 mL (refer to prior study – J. Taiwan Inst. Chem. Eng. 41 (2010) 682–688 for schematic configuration). Porous carbon cloth (CeTech^{TM}) (without waterproofing or catalyst) with a projected area of *ca.* 22.9 cm^2 (*i.e.*, $\pi \times 2.7^2$) on one side were used as anode electrodes. The air cathode sized almost identical to the anode consisted of a polytetrafluoroethylene (PTFE) diffusion layer (CeTech^{TM}) on the air-facing side.

2.2. Inhibition inspection via respirometry

Toxicity effects of aminophenol isomers on cellular respiratory activity associated to microbial cells were inspected individually using automated Columbus Micro-Oxymax Respirometer equipped with CO_2 sensors. The measurement relied on the circulation of air through closed-system testing bottles whereas the liquid in bottles remain static (*i.e.*, mobile liquid-flowing gas respirometer) [19]. As cumulative amount of CO_2 present in blank bottle was nearly negligible (approximately 1–2 mg) for 5 day testing, different time courses of CO_2 production were simply due to the absence or presence of existing toxicant(s) in cultures. For respirometric experiments, a loopful of *P. hauseri* ZMd44 seed taken from an isolated colony on an LB-streak plate was precultured in 50-mL Bacto LB culture, Miller (Luria–Bertani) (per liter; 10 g Bacto tryptone, 5 g Bacto yeast extract, 10 g sodium chloride) for 12 h (*i.e.*, late exponential growth phase) at 30 °C, 125 rpm using a water bath shaker (SHINKWANG, SKW-12). Then, 0.5 mL precultured broth was inoculated into 50 mL bottles containing fresh 1× LB broth and test aminophenol isomers at different concentrations for respirometric experiments at 30 °C, 110 rpm. The calibration of respirometer was conducted by using standard $CO_{2(g)}$ at 0.5% prior to experiments.

2.3. Cyclic voltammetric determination

Cyclic voltammetry of candidate mediators (*e.g.*, thionine, malachite green) was performed using an electrochemical workstation (Jiehan 5600, Taiwan) at 10 mV/s 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 prior to use. 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 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).

2.4. Electrochemical measurements

(a) For comparative analysis upon prior studies, electrochemical impedance spectroscopy (EIS) (HIOKI 3522-50, Japan) measurement was conducted on steady-state open circuit potential distributed with an amplitude of 10 mV at the frequency range of 10^4 to 5×10^{-3} Hz [20]. Collected data were analyzed using the software for Nyquist plot (Zview 2.6b, Jiehan Tech.). (b) Regarding stable power generation measurement, cell voltage was automatically measured (set 1 data point per minute) using a data acquisition system (DAS 5020; Jiehan Technology Corporation) through external resistance R_{out} = 1.0 $k\Omega$ for comparison with prior results [20]. 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. Cyclic voltammetric evaluation

To determine whether model dyes (*i.e.*, thionine, azure C and azure A) own capabilities to enhance bioelectricity generation in MFCs, cyclic voltammograms (CVs) of three compounds were implemented for feasibility study in 0.1 M phosphate-buffered solution (PBS) at neutral pH (Fig. 1). Cyclic voltammetric profiles clearly showed that three chemicals could perform such redox-mediating characteristics as electron shuttles (ESs) (Fig. 1).

Aware that ESs can mediate electron transfer in quasi-reversibility for augmentation of electron flux in fuel cells. According to cyclic voltammetry, the forward scan could produce specific current peaks

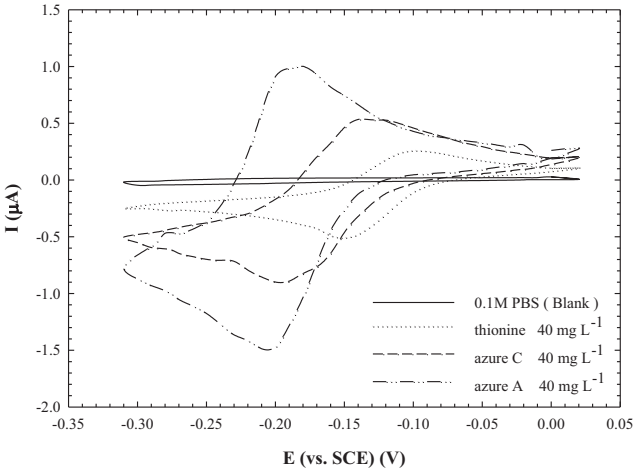

Fig. 1. Cyclic voltammograms for the redox processes of thionine, azure C and azure A in 0.1 M phosphate-buffered solution (PBS) at pH 7.0 (scan rate = 10 mV/s).