



## Exploring redox-mediating characteristics of textile dye-bearing microbial fuel cells: thionin and malachite green



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### HIGHLIGHTS

- Disclose textile dyes as mediators to dye removal and bioelectricity generation.
- Unveil mediating characteristics of thionin and malachite green to MFCs.
- Suggest stimulating strategy for MFCs-based wastewater decolorization.

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### ABSTRACT

Prior studies indicated that biodecolorized intermediates of azo dyes could act as electron shuttles to stimulate wastewater decolorization and bioelectricity generation (WD&BG) in microbial fuel cells (MFCs). This study tended to explore whether non-azo textile dyes (i.e., thionin and malachite green) could also own such redox-mediating capabilities for WD&BG. Prior findings mentioned that  $-OH$  and/or  $-NH_2$  substitute-containing auxochrome compounds (e.g., 2-aminophenol and 1,2-dihydroxybenzene) could effectively mediate electron transport in MFCs for simultaneous WD&BG. This work clearly suggested that the presence of electron-mediating textile dyes (e.g., thionin and malachite green (MG)) in MFCs is promising to stimulate color removal and bioelectricity generation. That is, using MFCs as operation strategy for wastewater biodecolorization is economically promising in industrial applications due to autocatalytic acceleration of electron-flux for WD&BG in MFCs.

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### 1. Introduction

As one of biomass-based renewable energy, microbial fuel cells (MFCs) could simultaneously expedite wastewater bioremediation (e.g., reductive decolorization) and bioelectricity generation (Karra et al., 2013; Zhuang et al., 2012). Although dye biodecolorization and bioelectricity generation are competitive to each other, color removal efficiency still significantly enhanced via exogenous regulation of electron flux in MFCs. As a matter of fact, considering electron-transfer phenomena in MFCs, electrochemical characteristics of microorganisms (Logan and Regan, 2006) inevitably influenced the performance of bioelectricity production through at least three mechanisms: electron shuttling cell-secreting mediators (e.g., phenazine, quinones), membrane-bound redox proteins (e.g., cytochromes as mobile electron carriers), and conductive pili (or nanowires) (e.g., wired communities of *Geobacter sulfurreducens*,

*Shewanella oneidensis*) (Logan, 2008). However, in this work only stimulation of electron-shuttling mediators could be manipulated exogenously from the engineering perspectives. If such mediators were originated from the pollutant species as shown herein, this stimulating approach would be much promising for practical application.

Although most of textile dyes used in industry are azo dyes (>60% of world total), non azo dyes are still present in diverse textile dye-bearing wastewater. Apparently, simultaneous degradation of multiple species of textile dyes via simple and ecofriendly methods is inevitably required for industrial applications. In fact, for sustainable dye bioremediation, reductive decolorization coupled with MFCs to enhance electron-transporting capabilities was mentioned to be technically promising (Du et al., 2007; Solanki et al., 2013). Chen et al. (2010b, 2012) also revealed that electricity production could be improved with facilitation of decolorized intermediates of azo dyes (e.g., phenyl methadamine, 2-aminophenol) as electron shuttles to augment electron flux for wastewater decolorization and bioelectricity generation (WD&BG) in MFCs.

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Watanabe et al. (2009) also suggested anthraquinone, phenazine, viologen, naphthoquinone and cobalamin as basic skeletons of electron shuttles; however, these compounds were evidently chemical structure-complicated and diverse, unfortunately not structure-associated to each other (Sun et al., 2013). Moreover, Rau et al. (2002) disclosed the effect of different quinoid electron shuttles (i.e., –OH substitute containing compounds) on anaerobic dye reduction. Prior studies (e.g., Chen et al., 2013a) also revealed that such decolorized intermediates (i.e., aromatic amines- amino-group(s) containing aromatics) were also electrochemically active redox mediators for dye decolorization. In fact, proposed mechanisms also explained why –OH and/or –NH<sub>2</sub> substitute(s) could own electron-mediating capabilities for reversible electron transport. For instance, disubstituted auxochromes containing hydroxyl and/or amino group(s) (e.g., 1,2-diaminobenzene, catechol) (Chen et al., 2013b) were found to be redox mediators for WD&BG with perspectives of chemical structure.

This first-attempt study extended to explore whether non-azo textile dyes or derived intermediates can also act as redox mediators in MFCs. For example, thionin is a strongly staining metachromatic dye widely used for biological staining (Gong et al., 2008) and is well-known electron mediator for bioelectricity generation (Rahimnejad et al., 2012; Ho et al., 2011). Cyclic voltametric data confirmed that thionin improved redox potential in acclimatized biofilm as compared to mediator-absent cells. These indicated that thionin has significant capability to enhance bioelectricity production from electrochemically active biofilm in MFCs (Lin et al., 2014). With thionin as a model mediator for comparison, this work chose a cationic triphenylmethane dye- malachite green (MG) which is popularly widely used in aquaculture industry to prevent infection. Biodecolorization of MG by various microorganisms (e.g., bacterium – *Kurthia* sp.; *Kocuria rosa*, *Sphingomonas* sp.; *Pseudomonas pseudomallei*, *Citrobacter* sp.; yeast – *Saccharomyces cerevisiae*; fungus – *Ischnoderma resinorum*, *Penicillium pinophilum*) were mentioned (Chen et al., 2009; Jasinska et al., 2012; Kalyani et al., 2009). In addition, some enzymes (e.g., laccase, MG reductase, NADH-DCPI reductase) from myriads of microbes have been extensively explored in terms of MG-decolorizing capabilities (Jadhav et al., 2007). Chen et al. (2010c) also disclosed biodegradation mechanisms of MG and methyl violet B. Moreover, color removal and bioelectricity generation could both be associated with electron transport. This study revealed that MG could play as an electron-mediating shuttle to facilitate electron-transfer capabilities, suggesting that the existence of MG could accelerate rates of power generation as well as dye decolorization. Stimulating effects of dye-mediator (e.g., MG and thionin) would be automatically triggered, suggesting that using MFC as remediation strategy could be more technically appropriate for wastewater decolorization. However, due to non-quasi reversibility of MG such mediating capabilities would be gradually attenuated during batch-mode wastewater decolorization in MFCs.

## 2. Experimental section

### 2.1. MFC construction

Membrane-free air cathode single-chamber MFCs using seeding 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. Porous carbon cloth (CeTech™) (without waterproofing or catalyst) with a projected area of ca. 22.9 cm<sup>2</sup> (i.e.,  $\pi \times 2.7^2$ ) on one side were used as anode electrodes. The air cathode sized almost iden-

tical to the anode consisted of a polytetrafluoroethylene (PTFE) diffusion layer (CeTech™) on the air-facing side.

### 2.2. Cyclic voltammetric determination

Cyclic voltammetry of candidate mediators (e.g., thionin, malachite green) was performed using an electrochemical workstation (Jiehan 5600, Taiwan) at 10 mV s<sup>-1</sup> scan rate. The working, counter, and reference electrodes were a glassy carbon electrode (0.07 cm<sup>2</sup>), platinum electrode (6.08 cm<sup>2</sup>), and a Hg/Hg<sub>2</sub>Cl<sub>2</sub> electrode filled with saturated KCl<sub>(aq)</sub>, 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<sub>2</sub>SO<sub>4</sub> 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<sup>-1</sup> over the range from 0.4 to –0.6 V. The redox potentials recorded as Hg/Hg<sub>2</sub>Cl<sub>2</sub> reference electrode were corrected by 0.241 V (i.e., E<sub>0</sub> of Hg/Hg<sub>2</sub>Cl<sub>2</sub>) to the standard hydrogen electrode (SHE).

### 2.3. Electrochemical measurements

(a) 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<sup>4</sup>–5 × 10<sup>-3</sup> Hz. Collected data were analyzed using the software for Nyquist plot (Zview 2.6b, Jiehan Tech.). (b) Regarding 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<sub>out</sub> = 1 KΩ for comparison with prior results (Chen et al., 2010a). 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 the mode of membrane-less single chamber at 25 °C.

### 2.4. Bioelectro-kinetics analysis – Tafel plot

Tafel plot was employed to obtain kinetic parameters for MFC model (Raman and Lan, 2012) via Tafel equation  $\ln\left(\frac{i}{i_0}\right) = (\beta) \frac{F\eta}{RT}$ , where *i*<sub>0</sub> is exchange current density, *i* is the electrode current density (mA m<sup>-2</sup>), β is the electron transfer coefficient or oxidative Tafel slope, R is the ideal gas constant (8.31 J mol<sup>-1</sup> K<sup>-1</sup>), F is the Faraday constant (96,456 C mol<sup>-1</sup> e<sup>-</sup>), T is the absolute temperature (K) and finally η is the activation over potential. The Tafel equation provides a relationship between the current and the over potential during the oxidation or reduction reaction of an electrode, where anode was kept as the working electrode, Ag/AgCl as the reference electrode, and the cathode as the counter electrode (Mohan et al., 2012). The ZMd44-seeded MFC supplemented with fresh MG (40 mg L<sup>-1</sup>) and diazo dye RBU160 (200 mg L<sup>-1</sup>) were tested by scan range from –0.65 V to 0.05 V which are open circuit voltage (OCV)-dependent.

## 3. Results and discussion

### 3.1. Cyclic voltammetric evaluation

To determine whether non-azo dyes- thionin and MG own capability to enhance bioelectricity generation in MFCs, cyclic voltammograms (CVs) of both compounds were conducted for feasibility study (Figs. 1A and 1B). As shown in Fig. 1A, cyclic voltammetric profiles indicated that thionin and fresh MG could

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