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Interactive influences of decolorized metabolites on electron-transfer characteristics of microbial fuel cells

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ABSTRACT

This study clearly uncovered how and why decolorized metabolites (DM) or intermediates could stimulate bioelectricity-generating capabilities in microbial fuel cells (MFCs) due to synergistic interactions. It also disclosed mysteries behind the reversible stabilities of electron shuttles (ESs) of such biodegraded intermediates in practical MFCs. Electrochemical impedance spectra indicated that DM of reactive green 19 (RG19), reactive blue 160 (RBu160) and malachite green (MG) could significantly stimulate bioelectricity-generating capabilities in MFCs due to significant reduction of internal resistance. With autocatalysis of DM, apparently feedback stimulation of decontaminated species directly controlled the performance of MFC-based bioremediation.

1. Introduction

As known, wastewater treatment via bioremediation is usually considered as one of top-priority alternative(s) due to its environmental friendliness [1]. In particular, for cradle-to-cradle sustainable development, bioelectrochemical systems (BESs) are proposed as bioelectricity-generating microbes bearing electrochemical factories through oxidation of organic matter on anodic biofilm. There are two types of widely studied BESs, microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) exhibiting different reactions on the cathode [2]. MFC could be powered by electrochemically active bacteria (EAB) through organics pollutant degradation by simultaneous wastewater bioremediation and energy recycling [3]. MFCs as renewable means can convert chemically-bound energy into biomass-based electricity generation through EAB or exoelectrogenic microorganisms [4]. EAB at least conducted three mechanisms to implement bioelectricity generation: direct electron transport via membrane bound proteins (e.g., cytochromes), conductive nano-wires generated, indirect shuttles via redox mediators (e.g., riboflavin) [5]. Due to external circuit to direct electron transfer in the fuel cell chamber as a driving force, pollutant degradation could be effectively stimulated [6,7]. However, due to limited cellular capabilities for bioelectricity generation, several methods (e.g., sophorolipid addition, cofactor manipulation) [8,9] were proposed. Recently, Yong et al. [10] used genetically-modified (GM) microbes to overcome such limitations and to augment synthesis of electron shuttles. Without dispute, optimal exogenous or endogenous production of redox mediators would be crucial to electron transport (ET) phenomena in MFCs. In particular, using MFC as mode of operation for reductive decolorization of azo dye(s) seemed to be electrochemically favorable because of sufficient electron transfer for color removal. As known, azo linkage(s) (—N=N—) in azo dyes are electron-withdrawing bonding(s), they are designed to be recalcitrant toward aerobic biodegradation for long term uses in daily life. Inevitably, significant amount of residual dyes could be discharged in associated industrial effluents to impact the environment. That is, azo dyes should be reductively decomposed or decolorized via anaerobic biodegradation. Due to this, electrochemical characteristics of microorganisms [11] would significantly influence the performance of color removal in MFC-assisted wastewater (WW) decolorization. Therefore, to promote ET capability for reductive decolorization, exogenous supplementation of electron shuttles (ESs) at appropriate dye/ES ratio might be technically feasible [12]. In addition, as bacterial decolorization was conducted via reduction, MFCs coupled with effective decolorized metabolites (DM) orintermediate(s) would favorably autocatalyze the

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reaction of color removal. As aforementioned, prior findings [13,14] quantitatively proposed novel assessment upon ET phenomena of MFCs, revealing that DM of reactive blue 160 (RBu160) and reactive green 19 (RG19) synergistically expressed electron-shuttling characteristics to enhance bioelectricity generation and dye decolorization (BG&DD). For example, as Chen et al. [15] and Hsueh et al. [16] proposed, phenyl methadiamine (a diamino groups containing compound, $C_7H_{10}N_2$) might be the major DM of RBu160 to stimulate BG&DD. As Hsueh et al. [16] mentioned, DMs of reactive green 19 (RG19) (e.g., organic sulfides: 3-methylthiopropionic acid or 4-methylthiobutanol) were crucial ESs to enhance significant reduction of textile dyes in MFCs. However, detailed mechanisms behind this stimulation upon BG&DD (e.g., synergistic or antagonistic interactions among DM) were still remained open to be clarified. Here, with supplementation of DMs of RG19 and RBu160 to electrochemically active bacterium *Shewanella* sp. WLP72-inoculated MFCs, stimulating effects upon ET capabilities in MFCs were quantitatively revealed. This study also showed that DMs of non-azo textile dye malachite green (MG) also acted as redox mediators. The novelty of this study was to provide first-attempt mechanisms for such enhancement of electron-transfer capabilities. This work uncovered how and why DM of RG19, RBu160 and MG could be the promising ESs to simulate ET efficiency in MFCs not clarified previously [13,14]. Using DM-supplemented MFC as operation strategy for WW biodegradation would be promising due to significant biostimulation of ESs for contaminant biodegradation. To the best of our knowledge, this study provided first-attempt mechanisms or in-depth understanding for ET enhancement of DM, uncovering mysteries of MFC-assisted bioremediation with autocatalysis of decolorized intermediates for operation optimization.

2. Materials and methods

2.1. Chemicals, bacterial strains and culture conditions

The model azo dyes-reactive blue160 (RBu160), reactive green 19 (RG19) and non-azo dyes malachite green (MG) (purchased from Everlight Chemical Ltd., Taipei, Taiwan) were used to color removal. Marine-based *Shewanella* sp. WLP72, freshwater-originated *Proteus hauseri* ZMd44, and *Enterobacter cancerogenus* BYm30 isolated from northeast Taiwan were used for study. DM of MG was obtained via decolorization of mixed consortia form Fushan Botanical Garden and Nature Center, Taiwan Forestry Research Institute. Bacteria were cultured in Luria-Bertani (LB) broth medium with tap water. Decolorization experiments of decolorizers were carried out as follows: first, one loopful of colony of streak plates was taken for 12 h preculture in 50-mL LB broth at 30 °C, 125 rpm using a water-bath shaker and 1% (v/v) pre-cultured broth was then used for dye-bearing cultures. DM of RBu160, RG19 and MG were harvested at the time of residual dye concentration less than 5% decolorized by inoculations from ZMd44-inoculated, BYm30-inoculated and mixed culture-inoculated MFCs, respectively [14,17]. For industrial situations, dyes were reductively degraded to gradually accumulate/generate DM without external supplementation. However, to have precise system evaluation, exogenous augmentation of DM harvested at specific conditions was implemented for better understanding to such ET phenomena in MFCs.

2.2. Microbial cultures and microbial fuel cells (MFCs)

The DC-MFC construction and inoculation had been described elsewhere [18]. Seed bacteria of DC-MFCs were inoculated after the cells achieved the stationary phase. DC-MFCs used in the study comprised of two cuboid chambers made by transparent polyacrylic plastic (i.e., $L \times W \times H = 11 \times 9 \times 2$ cm). The working volume for each chamber is ca. 200 mL and two compartments were separated by proton exchange membrane (Nafion 211; American Du Pont Inc.). To remove any residues remained on the experimental materials, the electrodes and membrane were pre-treated prior to use. The pre-cultured cells were centrifuged and re-suspended with 200 mL sterilized medium. Potassium hexacynoferrate (III) together with 0.5 M KH_2PO_4 buffer was used as an electron acceptor in the cathode compartment.

Approximately 5 mL concentrated O/N cultured biomass was well-mixed with 0.2× LB (i.e., 20% LB plus 8 g L^{-1} NaCl) in MFCs for acclimatization. For serial acclimation, every 72 h ca. 4 mL cell broth was replaced by impulse injection of fresh sterile 8.8× LB medium (i.e., maintained at 0.2× LB). Then, output voltage of MFC was continuously monitored to ensure whether acclimation was indeed successfully achieved (ca. 220.98 ± 5.36 mV at 15–20 days at 1 KΩ). As aforementioned in Section 1, DM contained mixtures of many ESs (e.g., organic sulfides, aromatic amines). Thus, concentrations of DM were indirectly defined by the volumes of 200 mg L^{-1} RG19, RBu160 and MG decolorized due to lack of quantitative analyses on such ESs concentrations. Note that all of DM species were confirmed to be under threshold levels of toxicity to WLP72 prior to experiments.

2.3. Electrochemical measurements

(a) Electrochemical impedance spectroscopy (EIS) (HIOKI 3522-50, Japan) measurement was carried out via steady-state open circuit potential distributed with amplitude of 10 mV. The frequency range was 10^4 to 5×10^{-2} Hz. Data was collected and analyzed using the software for Nyquist plot (Zview 2.6b, Jiehan Tech.) [19]. (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 [15,16]. 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. The power density (P) and current density (I) were calculated by the formulae $P = V^2/(A \times R)$ and $I = V/(A \times R)$, respectively, where V is the voltage across the external resistor, R is the resistance of each external resistor, and A is the surface area of the anode. All MFCs were operated at 25 °C. To reveal statistical significance for data reproducibility, power-generating curves were performed in duplicate.

2.4. Cyclic voltammetry measurements

Cyclic voltammetry of different model intermediates was performed using an electrochemical workstation (Jiehan 5600, Taiwan) at 1 mV s^{-1} scan rate. The working, counter, and reference electrodes were, respectively, a glassy carbon electrode (0.07 cm^2), platinum electrode (6.08 cm^2), and a Hg/Hg_2Cl_2 electrode filled with saturated $KCl_{(aq)}$. 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 (pH 7.0) at 0.1 M and the solutions were purged with nitrogen for 15 min prior to analysis. The scanning rate was 1 mV s^{-1} over the range from 0.4 to −0.6 V [20]. 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).