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Revealing Characteristics of Color Removal and Electricity Generation Using Indigenous Azo Dye Decolorizer *Proteus hauseri*

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ABSTRACT: This first-attempt study explored simultaneous dye decolorization and bioelectricity generation of indigenous *Proteus hauseri* ZMd44 for dye-bearing wastewater treatment. Additions of diazo dye C.I. reactive blue 160 (RBu160) could stimulate dye decolorization and bioelectricity generation of ZMd44 in single chamber microbial fuel cells (MFCs). However, high-level additions of RBu160 repressed capabilities of power production in MFC due to competition of electrons used for reductive decolorization. Decolorized intermediates of RBu160- phenyl methanediamine and 5-sulfoanthranilic acid as electron shuttles might mediate electron transport for current generation in MFC.

INTRODUCTION

As known, the reductive biodecolorization of azo dyes is strongly related to bacterial capability of electron transfer (e.g., reduced coenzyme NADH as electron donor; Chen, 2002). Meanwhile, microbial fuel cells (MFCs) can use naturally-occurring microbes as renewable electrochemically active biocatalysts to recover sustainable energy from the oxidation of organic matter and simultaneously facilitate bioelectricity generation during wastewater treatment (Du et al., 2007; Sun et al., 2009). Here, the feasibility study tended to recover renewable energy as additional values from dye-bearing wastewater treatment. We also attempted to uncover whether this electron-donating capability of bacterial decolorizers is also associated with cell membrane-bound electron transfer chain for bioelectricity production in MFCs. The finding also indicated that supplementations of reactive blue 160 (RBu160) simultaneously stimulated color removal and bioelectricity generation of *P. hauseri*. To the best of our knowledge, there are no reports indicating the feasibility of simultaneous bioelectricity generation and dye decolorization for *P. hauseri* as indicated herein.

MATERIALS AND METHODS

MFC Construction. Single chamber air cathode, mediator-less, membrane-less MFCs (ML-MFCs; Han et al., 2010) were constructed in cylindrical tubes made by transparent polymethyl methacrylate (PMMA) (cell sizing OD=59 mm, ID=54 mm, electrode spacing L=90 mm) with working volume of ca. 200 mL. The anode electrode was originally constructed from activated carbon cloth (without catalyst) (CeTech^{TM}, Taiwan) while a hydrophobic carbon cloth (CeTech^{TM}, Taiwan) was used as the matrix of the cathode (a projected area of ca. 22.9 cm^2 (i.e., $\pi \times 2.7^2$)). The air cathode was almost identical to the

anode in size and consisted of a polytetrafluorethylene (PTFE) diffusion layer (CeTech^{TM}, Taiwan) on the air-facing side. Prior to construction for MFC, 3.0% H_2O_2 was completely filled in the chamber for 1 day for complete sterilization (Chen et al., 2009). H_2O_2 was completely rinsed and washed out by sterile deionized-and-distilled water after 1 day static incubation prior to study. For cell propagation in MFCs, cell culture of strain ZMd44 was first carried out as described elsewhere (Zhang et al., 2010). Then, cultured cells were sterilely added into MFCs at 30°C for cell propagation in MFC and cell immobilization onto the anode for approx. 20 days.

MFC Operations. (a) *Acclimation step*: Approximately 100 mL cultured broth was well-mixed with ca. 100 mL fresh LB medium in MFCs for acclimation. One half of a fresh medium was added to replace the old broth in MFC every week. To ensure immobilized cells well entrapped on anode in MFC, the output voltage of MFC was continuously monitored using external resistance of 1 KΩ. When no escalation of voltage emerged and reproducible output voltage profiles were revealed after new medium was added, MFC was considered as successfully acclimated. This steady-state and reproducible power generation of MFC (ca. 80±5 mV) was stably achieved after ca. 20 days. (b) *Experimental step*: In MFC, 100h was selected as the time interval for study observation to ensure no other limiting factors restrict cell metabolic activity during operation. For simultaneous dye decolorization and bioelectricity generation of *P. hauseri*, fed-batch mode of MFC operation with impulse injection of RBu160 at different levels was carried out. That is, 5.0 mL of 8.8x LB broth laden with appropriate concentrations of RBu160 (e.g., final concentrations of 450, 900, 1350, 560 mg L^{-1}) was serially added into the ML-MFC for comparative analysis.

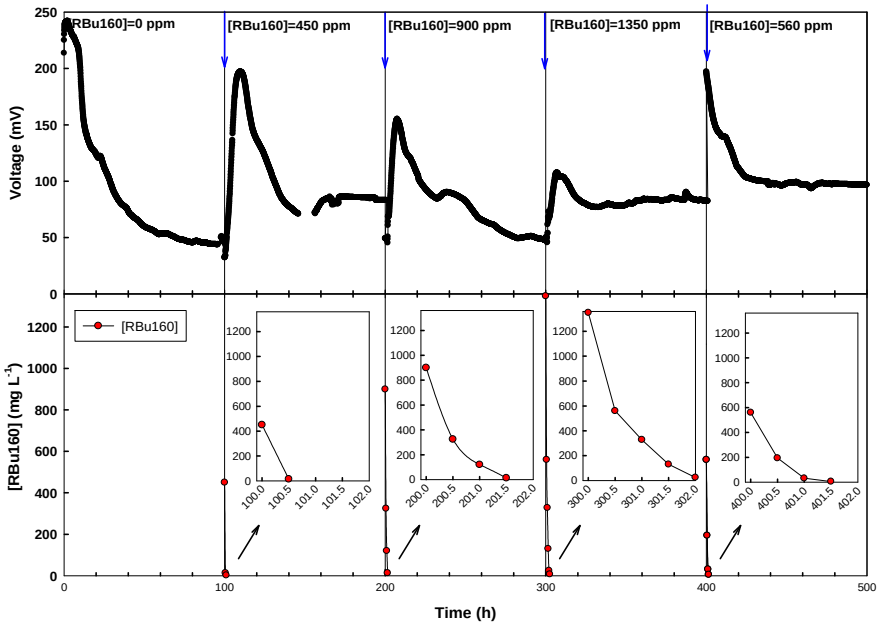


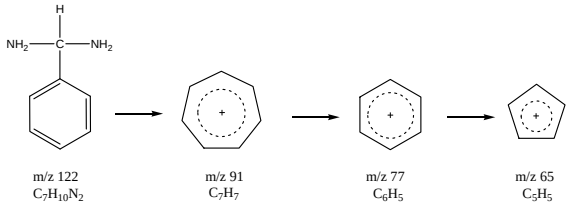
FIGURE 1. Serial decolorization of RBu160 and current generation curves of simultaneous color removal and bioelectricity generation of *Proteus hauseri* ZMd44 in MFC (external resistance = 1 KΩ).

RESULTS AND DISCUSSION

Effects of RBu169 on Bioelectricity Generation. This first-reported comparison on performance of bioelectricity generation in MFC at various concentrations of RBu160 (i.e., 0, 450, 900, 1350, 560 mg L^{-1}; Figure 1) showed that impulse supplementation of RBu160 considerably repressed the peak output voltages of bioelectricity production in MFCs. The maximum cell-voltage gradually decreased as supplementations of RBu160 progressively increased (ca. 245, 197, 152, 109 mV for RBu160= 0, 450, 900, 1350 ppm, respectively; Figure 1). The decreased peak output voltage at high loading of RBu160 might suggest that reductive decolorization and bioelectricity generation of *P. hauseri* are competitive to each other as both reactions all utilized electrons released from bacterial oxidation of organic matter. In addition, possibly due to accumulation of decolorized intermediate(s) of RBu160 as mediators, the steady-state (SS) cell voltages were stabilized at slightly higher levels than the SS-voltage in RBu160-free MFC.

According to mechanisms of electron transfer in MFCs, the major driving mechanism of bioelectricity generation seemed to be the electron flow through a conductive biofilm matrix containing cytochromes or other membrane-associated electron carriers, but not the direct electron transfer via outer-surface redox protein (i.e., the lag phase to generate electricity was not significantly observed in this mediator-less ML-MFC). When the biofilm matrix was involved in electron transport, the ratio of saturated/unsaturated fatty acids of cellular membrane may also influence capabilities of bioelectricity generation of *Proteus hauseri* ZMd44 as compared to a different species in the *Proteus* genus (e.g., *Proteus vulgaris*; Choi et al., 2003).

Simultaneous Dye Decolorization and Current Generation. There are several points to explain simultaneous color removal and power generation of ZMd44 taking place in MFCs. First, Liu et al. (2009) mentioned that azo dye may play a role as an electron acceptor in cathode. However, azo dye RBu160 apparently could not play as an electron acceptor in our air-cathode MFC systems, since oxygen was inevitably the favorable electron acceptor. Second, Chen (2006) and Chen et al. (2009b) mentioned that impulse additions of C.I. reactive red 141 significantly enhanced cell growth of biodecolorizers *Pseudomonas luteola* and *Aeromonas hydrophila* due to stimulating utilization to LB growth media. After additions of RBu160, similar growth-stimulating phenomena likely taking place in *P. hauseri* may also increase the rate of oxidative phosphorylation to accelerate electron transport in the respiratory chain of immobilized cells on the anodic biofilm for bioelectricity production in MFC. Third, decolorized products of textile dye and dye itself (e.g., methyl blue; Mohan et al. (2008)) may play a role as redox mediator in our MFC. Moreover, extracellular metabolites of ZMd44 cells can promote bioelectricity production by mediating electron transport (Pham et al., 2008; Zhang et al., 2008). Compared to extracellular metabolites of ZMd44 of 12 h culture at dye-free LB culture, fragmentation analysis for GC/MS spectrum on extracellular products or intermediates of ZMd44 in MFC (data not shown) indicated that the appearance of signal peak of m/z 122 suggested that $C_7H_{10}N_2$ (i.e., phenyl methanediamine) was the major decolorized intermediate of RBu160 as the typical fragment of a highly stable $C_7H_7^+$ (tropylium ion; m/z 91) was significantly



observed (i.e.,) and derived m/z peaks of 77 ($C_6H_5^+$) and 65 ($C_5H_5^+$) were also found. Therefore, the decolorized intermediates phenyl methanediamine (PM) or/and 5-sulfoanthranilic acid (5-SA) might enhance the performance of bioelectricity generation. Regarding possible mechanisms taking place in MFC during dye decolorization, there are at least three proposed mechanisms to elucidate electron transfer between bacteria and the anode of the MFC: (a) membrane-bound redox proteins (e.g., cytochromes) to transfer electrons to the anodic biofilm of MFC; (b) cell-secreting redox mediators (e.g., phenazine, quinones) as electron shuttles; and (c) nanowires (or conductive pili) for exocellular transport of electrons. For strain ZMd44, decolorized intermediates of RBu160 (i.e., PM and 5-SA) might play a role of external mediators for electron transfer, when they were coupled with NADH oxidation (Roller et al., 1984; Park and Zeikus, 2000). In addition, strain ZMd44 used outer-membrane associated redox proteins (e.g., cytochromes $Cytochrome\,C\,(Fe^{3+}) + e^- \rightarrow Cytochrome\,C(Fe^{2+})\;\; E_0^{'} = 0.254V$) of the respiratory chain to mediate electron transport to the anode for current generation.

CONCLUSIONS

High-level additions of diazo dye RBu160 repressed capabilities of bioelectricity generation of ZMd44 in MFC likely due to the competition of reductive decolorization. This first study reported the capability of simultaneous color removal and bioelectricity generation of *Proteus hauseri*.

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