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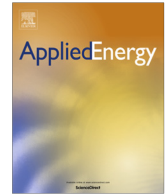

Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode

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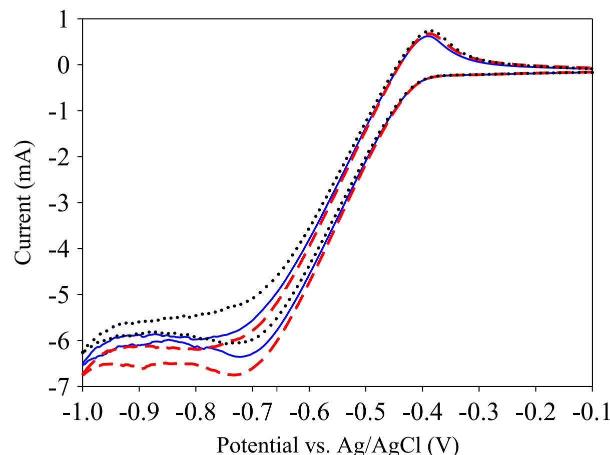
HIGHLIGHTS

- A laccase-producing fungus on cathode of MFC was used to enhance degradation of azo dye.
- Laccase-producing fungal cathodes performed better than laccase-free control cathodes.
- A maximum power density of 13.38 mW/m^2 and an >90% decolorization of acid orange 7 were obtained.
- Growing a fungal culture with continuous laccase production improved MFC's electricity generation.

GRAPHICAL ABSTRACT



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ABSTRACT

Wood-degrading white-rot fungi produce many extracellular enzymes, including the multi-copper oxidative enzyme laccase (EC 1.10.3.2). Laccase uses atmospheric oxygen as the electron acceptor to catalyze a one-electron oxidation reaction of phenolic compounds and therefore has the potential to simultaneously act as a cathode catalyst in a microbial fuel cell (MFC) and degrade azo dye pollutants. In this study, the laccase-producing white-rot fungus *Ganoderma lucidum* BCRC 36123 was planted on the cathode surface of a single-chamber MFC to degrade the azo dye acid orange 7 (AO7) synergistically with an anaerobic microbial community in the anode chamber. In a batch culture, the fungus used AO7 as the sole carbon source and produced laccase continuously, reaching a maximum activity of 20.3 ± 0.3 U/L on day 19 with a 77% decolorization of the dye (50 mg/L). During MFC operations, AO7 in the anolyte diffused across a layer of polyvinyl alcohol-hydrogel that separated the cathode membrane from the anode chamber, and served as a carbon source to support the growth of, and production of laccase by, the fungal mycelium that was planted on the cathode. In such MFCs, laccase-producing fungal cathodes outperformed laccase-free controls, yielding a maximum open-circuit voltage of 821 mV, a closed-circuit voltage of 394 mV with an external resistance of 1000 Ω, a maximum power density of 13.38 mW/m^2, a maximum current density of 33 mA/m^2, and a >90% decolorization of AO7. This study demonstrates the feasibility of

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growing a white-rot fungal culture with continuous laccase production on the cathode of MFCs to improve their electricity generation and azo dye removal efficiency.

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1. Introduction

Azo dyes are a group of poorly biodegradable synthetic colorants that are often found in waste water that is produced by the textile industry [1]. These compounds are highly resistant to degradation by aerobic bacteria but can be easily reduced by anaerobic bacteria to form aromatic amines, which are a group of carcinogens that are stable under anaerobic conditions and must be returned to aerobic conditions before they can be further degraded and mineralized [2,3]. Therefore, a combination of aerobic and anaerobic treatments have been suggested to be required for the effective mineralization of these pollutants [4].

Pollutants in waste water from various sources can be removed by a microbial fuel cell (MFC), in which microorganisms break down organic compounds and convert their chemical energy into electrical energy [5,6]. An MFC typically consists of an anaerobic anode chamber and an aerobic cathode chamber that are separated by a proton exchange membrane. In the anode chamber is a microbial community that often comprises a large number of both fermenting and respiring bacteria that contribute to the versatility of MFCs in degrading pollutants. When waste water enters the anode chamber, fermenting bacteria firstly convert large organic molecules into smaller fermentation products, such as lactate, which are then oxidized by anaerobically respiring bacteria to produce CO_2, protons, and electrons. In the absence of a suitable electron acceptor in the anolyte, electrons pass to the anode interface and are transferred through an external wire to the cathode, where they join oxygen molecules (O_2) and protons to form water molecules, thus completing a circuit [7].

Azo dyes can be degraded in either the anode or cathode chamber of an MFC. When introduced into the catholyte, azo dyes can act as electron acceptors and be decolorized in reductive cathode reactions. In most cases, these reactions transform azo dyes into less colorful compounds, such as aromatic amines and hydrazines, but fail to degrade and mineralize them completely [8]. The dye-reducing reactions proceed better under anaerobic conditions as oxygen competes for electrons from the cathode, and the rate of the reaction depends heavily on the pH of the catholyte [9], the structure of the dye molecules [10], and the use of catalysts such as noble metals. Ding et al. demonstrated that photocatalysis with visible light on a rutile-modified cathode greatly increases the rate of dye reduction [11].

Azo dyes can be introduced into the anolyte, as in an MFC that forms the first part of a two-stage system in which azo dye-containing waste water firstly enters the anode chamber, where dye molecules are reduced by anaerobic bacteria to form aromatic amines, which were then transferred to the second stage, an aerobic bioreactor, for further degradation into smaller compounds by aerobic microorganisms [4,8]. Although these two-in-one systems can effectively remove dye, they are structurally complex and expensive to construct and operate.

In theory, azo dyes can be completely degraded in a single MFC if they are firstly introduced into the anode chamber for reductive transformation by anaerobic bacteria and then transferred to the cathode chamber for aerobic degradation by a second group of aerobic microbes. However, in most MFCs, the anode and cathode chambers are separated by a proton exchange membrane, which prevents movement of azo dyes and their transformed products between the chambers. Furthermore, growing aerobic microbes in the cathode chamber inevitably reduces the level of dissolved oxygen there, and so reduces the cathode potential and the power output of the MFC.

This study involves testing the feasibility of growing a laccase-secreting white-rot fungus on the cathode surface of a single-chamber MFC, in which the proton exchange membrane was replaced with a layer of polyvinyl alcohol-hydrogel (PVA-H), allowing the pollutant acid orange 7 (AO7, an azo dye) to diffuse from the anode chamber to the cathode. White-rot fungi are the only group of organisms that can completely degrade azo dyes. Laccase is one of the enzymes that they produce when they degrade lignin, which refers to a group of highly heterogeneous aromatic polymers that are abundant in the natural habitats of white-rot fungi, and is also used by these fungi to degrade azo dyes and many other aromatic compounds [12]. Additionally, laccase has been used as an enzyme catalyst on the cathode of many MFCs, replacing noble metals in catalyzing the reduction of oxygen [13]. In the single-chamber configuration, the MFCs herein have no cathode chamber and their cathodes are exposed directly to the air to increase oxygen availability [14,15]. Our results demonstrate that such fungal MFCs outperformed control MFCs that are equipped with abiotic or enzyme-free cathodes in both the decolorization of dye and the generation of electrical power.

2. Materials and methods

2.1. Microbes

Anaerobic sludge was collected from an oil-cracking wastewater treatment plant of Nan-Ya Plastics Co., Ltd. (at the Six Naphtha Cracking Industry Site at Mailiao in Yunlin County). The sludge microbial community was acclimated for an extended period in the laboratory and used for the degradation of aromatic compounds; it tested positive for AO7 decolorization. The fungal strain that was planted on the cathode was obtained from a local mushroom grower and was identical to the *Ganoderma lucidum* BCRC 36123 strain from the Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan, ROC. The fungal strain was maintained on potato dextrose agar (PDA) plates.

2.2. Fungal culture on cathode

Mycelium of *Ganoderma lucidum* BCRC 36123 was inoculated onto a 2 mm-thick layer of potato dextrose agar (PDA) medium and incubated to cover fully the surface of the medium. A 1.4 cm × 1.4 cm square of the mycelium-covered medium was then cut from the culture plate to be placed on a cathode.

2.3. MFC setup and operation

Fig. 1 schematically depicts the single-chamber MFC. The anode chamber was a 350 cm^3 container at the bottom of which was placed a 70 cm^2 carbon felt anode. The anolyte (of which each liter contained 1.75 g K_2HPO_4, 2.145 g KH_2PO_4, 10 mg NH_4Cl, 100 mg $MgCl_2{\cdot}6H_2O$, 45 mg $CaCl_2$, 1 mg $FeCl_3{\cdot}6H_2O$, 0.25 mg $CuCl_2{\cdot}2H_2O$, 0.25 mg $CoCl_2{\cdot}6H_2O$, 1 mg $ZnCl_2$, 1 mg $MnCl_2{\cdot}4H_2O$, 0.1 mg $Na_2MoO_4{\cdot}2H_2O$, and 0.02 mg $NiCl_2{\cdot}6H_2O$) was inoculated with 403.29 mg/L of sludge. The air cathode was made from a 6 cm

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