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Original Research

Preliminary Study of Electricity Generation and Sulfate Removal Performance in a Novel Air-Cathode Microbial Fuel Cell (AC-MFC) Using Laccase-Producing Yeast as a Biocatalyst

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Abstract

Fungi produce various types of extracellular enzymes, including the copper-containing oxidative enzyme laccase. This enzyme uses gaseous oxygen (O_2) as an electron acceptor to catalyze oxidation of phenolic compounds, and therefore it can act as a cathode biocatalyst in a microbial fuel cell (MFC). In this study, a new model of the air-cathode microbial fuel cell (AC-MFC) was constructed. For its design, the laccase-producing yeast *Galactomyces reessii* cultured in potato dextrose agar was grown in the cathode chamber, and an anaerobic microbial community was maintained in the anode chamber in order to carry out sulfate removal and, simultaneously, generate electricity. Results showed that the cathode with *G. reessii* outperformed the cathode with sterile gel (negative control), yielding the maximum open circuit voltage of 550.65±14.92 mV, the maximum power density of 0.35±0.01 mW/m^3, the maximum current density of 225.69±17.25 mA/m^3 and sulfate removal of 73.29±1.31%. This study demonstrated the feasibility of using a yeast culture for continuous laccase production in the cathode chamber of the AC-MFC in order to improve their electricity generation and sulfate removal.

Keywords: laccase, microbial fuel cell, electrical power, wastewater treatment, rubber

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Introduction

Microbial fuel cells (MFCs) can directly convert chemical energy contained in organic and inorganic materials into electrical energy by using bacteria capable of transferring electrons to an anodic electrode. This technology has been successfully demonstrated in the area of environmental biotechnology for treating wastewater and simultaneously generating electricity [12, 25]. To obtain high electric power outputs, precious metals such as white gold and platinum (Pt) have been used for a cathodic electrode in the MFC design, which tends to limit its practical applications.

White-rot fungi produce and secrete laccase, a multi-copper oxidoreductase enzyme, in the environment to return nutrients from the plant material to the soil via lignin degradation. Laccase plays a role in transporting electrons from the phenolic compound and aromatic amine to electron acceptors such as oxygen (O_2). Accordingly, laccase has the potential to perform as a cathodic biocatalyst that transfers electrons to gaseous oxygen [9, 17]. Several researchers immobilized fungal enzymes on the electrode surface, and they found that fungal laccases from the white-rot fungi such as *Ganodium lucidum*, *Trametes versicolor*, and *Pleurotus ostreatus* enhanced the electric output [2, 5, 8, 13, 23]. The significant disadvantage of their MFCs, however, is that the enzyme extracted from the fungal sources needs to be purified. The purification of the enzyme is costly and involves additional handling and maintenance during the MFC operation, as the enzyme can be denatured to lose its activity under normal environmental conditions [13]. A previous study has addressed the problem by using whole fungal cells (rather than using the pure enzyme), and this approach outperformed the laccase-free controls and yielded high power outputs [8].

Various types of sulfate-contaminated wastewater are generated in the production processes of sugar, alcohol, pharmaceutical products, monosodium glutamate and natural rubber sheet. The engineered anaerobic treatment and natural anaerobic processes for sulfate-containing wastewater generally result in the generation of hydrogen sulfide (H_2S) [3, 15], a toxic pollutant present in domestic wastewater, sludge discharged from anaerobic bioreactors and industrial off-gases. Because of its adverse characteristics, including being a health hazard and having objectionable odor and corrosive properties, the removal of H_2S from wastewater is necessary [10]. The conventional biological treatment of sulfate-contaminated sewage is a two-stage process. The first stage is the reduction of sulfate to sulfide by sulfate-reducing bacteria; then the reduction of sulfide to sulfur by sulfide-oxidizing bacteria [22]. This treatment system has several drawbacks, such as the large land requirement for the treatment units and a high cost of unit operation [21]. The study for sulfate-removal with simultaneous bioenergy recovery showed that the biological treatment with sulfate-reducing bacteria (SRB) in a dual chamber MFC can be an attractive technique [14].

The major factors currently limiting practical applications of MFCs in wastewater treatment are their high construction, operation and maintenance costs. In the present work, the new type of AC-MFC was designed and constructed using a biocatalyst in place of the expensive platinum-based cathode. Because the laccase-producing yeast *Galactomyces reessii* continuously secretes laccase (biocatalyst) in the cathode chamber, the laccase-based AC-MFC can be applied cost-effectively to the wastewater treatment areas in the future. In this paper, the laccase-based AC-MFC was examined in terms of electricity generation and sulfate removal from synthetic rubber wastewater.

Experimental

Microbes

Anaerobic sludge was collected from a rubber wastewater treatment plant in Phatthalung Province, Thailand. A microbial community in mud was enriched and maintained in nutrient broth (NB) in a laboratory, and used in generating electricity and removing sulfate. The laccase-producing yeast *G. reessii* [4] was obtained from the Department of Biotechnology, Thaksin University, Thailand. *G. reessii* was maintained on potato dextrose agar (PDA).

Gel-Cathode Preparation

A plug of potato dextrose agar (PDA) containing 7-day-old *G. reessii* was cut to a 1.0 x 1.0 cm piece that was inoculated into 100 mL of potato dextrose broth (PDB). The PDB was incubated at 30ºC for 7 days with shaking at 150 rpm. An aliquot (500 µL) of the incubated broth (OD = 0.5 at 600 nm) was inoculated into 5.0 mL of PDA.

Microbial Fuel Cell Design and Operation

A schematic of a new model of the AC-MFC is presented in Fig. 1. The AC-MFC consisted of an anode chamber (25.0 mL) and cathode chamber (6.0). The anode chamber was open to the atmosphere. The anode (2.0 cm^2) and cathode (2.0 cm^2) were made of a plain carbon cloth (CC). The anode chamber was filled with 22.5 mL of anolyte containing 500 mg/L sulfate. One liter of the anolyte solution (pH 7) contained 5.24 g of KH_2PO_4, 10.71 g of K_2HPO_4 and 0.74 g of Na_2SO_4. The sulfate concentration was measured by the turbidimetric method described in the standard methods [1]. Initially, 2.5 mL of a microbial community and nutrient broth (1% v/v) were added as co-substrate to the anolyte, modified from Lai et al. [8] and Miran et al. [14].

In the treatment experiment, the cathode chamber was inserted into the anode chamber. The laccase-

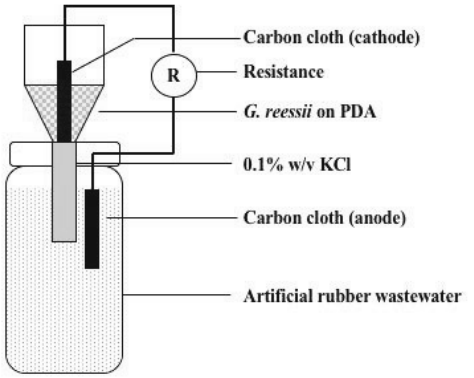

Fig. 1. Diagram of an air-cathode microbial fuel cell (AC-MFC).

based cathode was prepared by covering the plain CC (2 cm^2) with 7-day old *G. reessii* culture. The Pt-coated (0.3 mg/cm^2) CC with 5.0 mL of sterile PDB served as the positive control and the plain CC with 5.0 mL of sterile PDB served as the negative control. The cathode and anode were connected with a stainless wire (0.1 cm diameter, 5.0 cm length). The salt bridge gel was prepared according to previous studies [19, 20] with minor modifications, i.e., 1.0 g/L of KCl and 10.0 g/L of agarose were used. The gel was sterilized at 121ºC for 15 mins. The bottom of the cathode chamber was filled with 500 µL of KCl gel (1% w/v).

Electrochemical Analysis

The electrochemical properties were monitored by the method modified from the previous studies [8]. The laccase-based MFC (Lac-MFC), Pt-based MFC (Pt-MFC), and laccase-free MFC (Gel-MFC) were set up. The open circuit voltage (OCV) was determined within one day of operation. The internal resistance was calculated from the polarization curve produced at the external resistance of 150-2,150 Ω s according to Wu et al. [24].

The current and power were calculated as follows:

$$I = V/R \quad (1)$$

$$P = IV \quad (2)$$

…where I, V and R are the current (A), voltage (V) and resistance (Ω), respectively, and P is the power (W). The current density (CD) and power density (PD) were defined based on the anode volume, V_A (m^3):

$$CD = I/V_A \quad (3)$$

$$PD = P/V_A \quad (4)$$

…where CD and PD are the current density (A/m^3 or mA/m^3) and power density (W/m^3 or mW/m^3), respectively.

Sulfate Removal

The turbidimetric method was used for monitoring the sulfate removal from each round of operation. Briefly, 10.0 mL of treated anolyte (sample) was filtered through filter paper (Whatman No.1), then 4.0 mL of buffer solution (30.0 g/L of $MgCl_2$, 5.0 g/L of CH_3COONa, and 20.0 mL/L of acetic acid) and 0.003 g of $BaCl_2$ were added to the sample. After stirring, the absorbance of the mixtures was measured using a spectrophotometer at 420 nm. The sulfate removal was calculated by:

$$\text{Sulfate removal (\%)} = [(A-B)/A]\times 100 \quad (5)$$

…where A and B are initial and final absorbance, respectively.

Results and Discussion

Electricity Generation Characteristics of AC-MFC

Fig. 2 shows the OCV produced by the Lac-, Pt- and Gel-MFCs. The maximum OCV of the Lac-MFC is 550.65±14.92 mV (n = 3) observed at 420 mins, while the maximum OCV of the Pt-MFC (positive control) and the Gel-MFC (negative control) are 664.65±30.00 mV (n = 3) at 580 mins and 193.68±7.46 mV (n = 3) at 200 mins, respectively.

The closed circuit voltage (CCV) was determined in the range of the external resistances between 150 and 2150 Ω. The electrochemical properties of the Lac-MFC and Pt-MFC are shown in Table 1. Note that the output voltage of the Gel-MFC (negative control) could not be detected. The internal resistance of the Lac-MFC determined from the polarization curve (Fig. 3) at maximum power density is 330 Ω. The CD of the Lac-MFC increased with decreasing external resistance, yielding a maximum CD of 225.69±17.25 mA/m^3. The PD of the Lac-MFC increased from 0.08±0.01 to 0.24±0.01 mW/m^3. On the other hand, the internal resistance of the Pt-MFC is 1002 Ω, maximum CD is 249.51±17.53 mA/m^3, and PD increased from 0.23±0.03 to 0.35±0.01 mW/m^3.

In a study with the fungal laccase of *Trametes versicolor* entrapped on the surface of the air-cathode, Kacem et al. [6] obtained the maximum PD of 15.72 mW/m^3 after 24 days of its operation. Applications of this system, however, are limited by maintenance costs. Lai et al. [7] developed an AC-MFC using the edible white fungus *Ganoderma lucidum* that produces laccase. *G. lucidum* grown in PDA was placed on the carbon cloth cathode. They obtained the maximum CD and PD of 633.75 mA/m^3 and 224.98 mW/m^3, respectively. *G. lucidum* was also used by Lin et al. [11] as a biocathode in dual or two chambers MFC (tMFC). They observed the maximum OCV of 420 mV

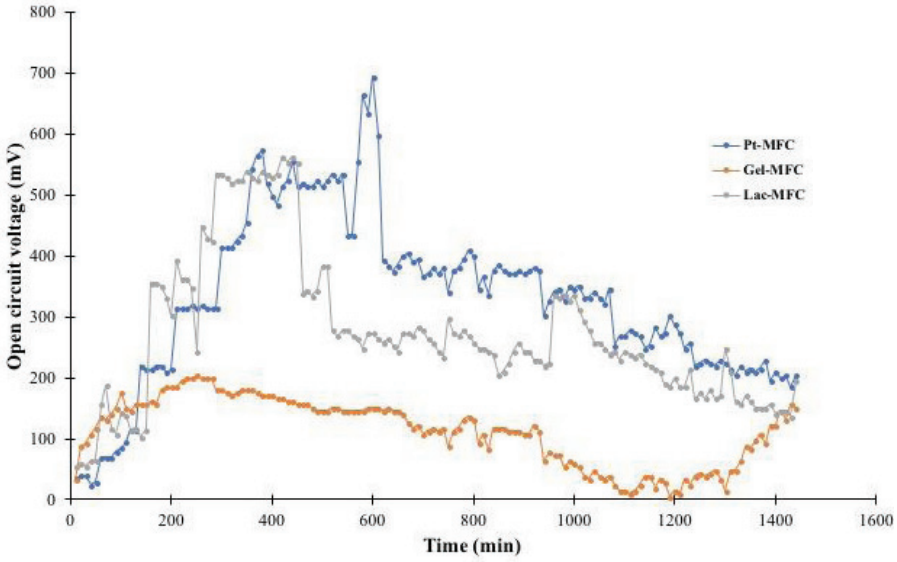

Fig. 2. OCV of three different cathodes in AC-MFC; artificial rubber wastewater was used as anolyte.

Table 1. Resistance, output voltage, current density (CD) and power density (PD) during the AC-MFC operation (n = 3).

Resistance (Ω)	Pt-MFC			Lac-MFC		
	Voltage (mV)	CD (mA/m^3)	PD (mW/m^3)	Voltage (mV)	CD (mA/m^3)	PD (mW/m^3)
150	0.94±0.07	249.51±17.53	0.23±0.03	0.85±0.06	225.69±17.25	0.19±0.03
270	1.30±0.01	191.93±2.15	0.25±0.01	1.26±0.05	186.05±6.95	0.23±0.02
330	1.49±0.01	180.82±1.33	0.27±0.01	1.41±0.01	170.63±1.62	0.24±0.01
1002	2.96±0.06	118.08±2.20	0.35±0.01	1.89±0.10	75.39±3.83	0.14±0.01
2150	4.07±0.14	75.71±2.68	0.31±0.02	2.13±0.15	39.68±2.74	0.08±0.01

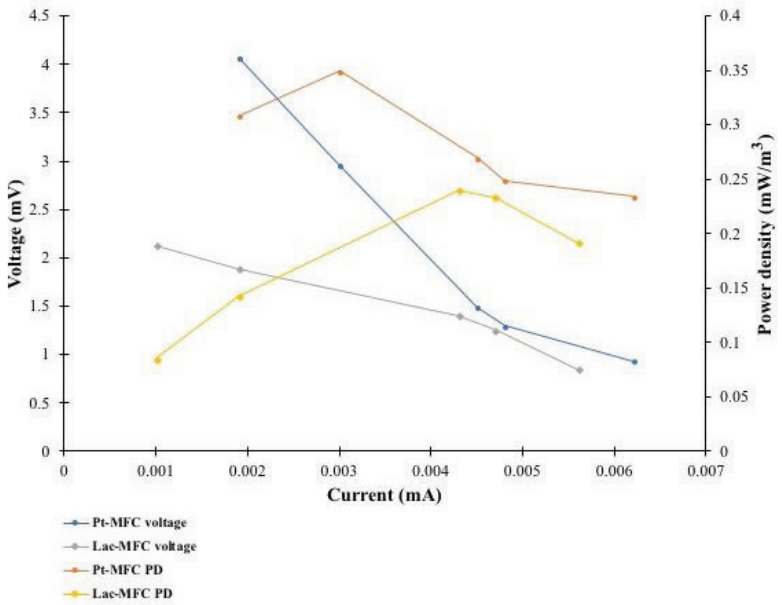

Fig. 3. Polarization curve and power density (PD) current curve of AC-MFC.

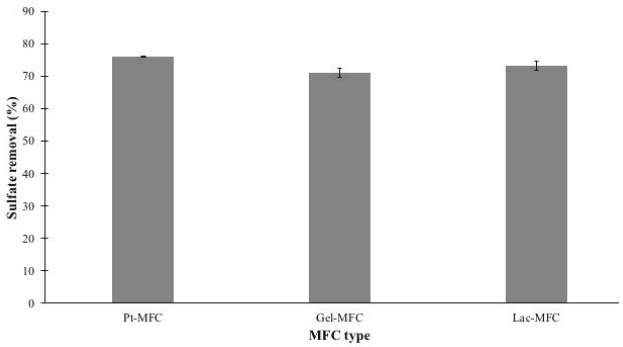

Fig. 4. Sulfate removal (%) of AC-MFC when 500 mg/L sulfate-contaminated artificial rubber wastewater.

after 20 days of its run. Maximum PD and CD were 66.67 mW/m^3 and 333.33 mA/m^3, respectively. Note that the anode and cathode chambers were separated by a PVA hydrogel elastomer (polyvinyl alcohol) membrane.

Sulfate Removal Efficiency

In this study, synthetic rubber wastewater containing 500 mg/L sulfate was used. After 1 day of AC-MFC operation, sulfate removal by the Lac-MFC was 73.29±1.31% (n = 3) while removal by the Pt-MFC (positive control) and Gel-MFC (negative control) were 76.17±0.23% (n = 3) and 71.13±1.43% (n = 3), respectively (Fig. 4). Miran et al. [14] treated synthetic wastewater using the tMFC by adding copper to stimulate the biological reactions. They obtained sulfate removal of 70% after running the tMFC for 2 days. Seo et al. [18] used the AC-MFC to remove sulfate from artificial wastewater containing 200 g/L sulfate and obtained 17.6% sulfate removal after 10 days of operation.

Conclusions

The AC-MFC's performance can be enhanced by using fungal enzyme laccase for the air cathode as a biocatalyst. Nonetheless, the use of commercial laccase limits the MFC applications owing to its high costs. In this study, laccase-producing yeast *G. reessii* was used to catalyze the reduction of O_2 on the surface of the air-cathode. *G. reessii* grown on the CC cathode improved the MFC's usability, as this MFC model successfully removed sulfate from artificial rubber wastewater in a short period of time while generating electricity.

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Conflict of Interest

The authors declare no conflict of interest.

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