

*Short Communication*

# Improved Performance of a Novel-Model Laccase Based Microbial Fuel Cell (LB-MFC) with Edible Mushroom as a Whole-Cell Biocatalyst

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## Abstract

Edible mushroom laccase is a fascinating extracellular enzyme owing to its simple operation. This research examined (a) the electrochemical properties of a single-chamber microbial fuel cell with two edible mushrooms (*Pleurotus pulmonarius* and *Coprinus fimetarrius*), and (b) the laccase activity of edible mushrooms grown in spent mushrooms as a low-cost substrate. The results revealed that the single-chamber MFC with *P. pulmonarius* achieved a maximum open-circuit voltage of  $940.00 \pm 10.00$  mV, a maximum current density of  $97.14 \pm 2.86$  mA/m<sup>2</sup>, and a PD of  $13.22 \pm 0.78$  mW/m<sup>2</sup>. When *P. pulmonarius* was cultured in a sawdust substrate without chemical addition, laccase activity of  $155.17 \pm 0.10$  U/mL was obtained. There has been no previous report of electricity generation from a membrane-less single-chamber MFC using *P. pulmonarius* mycelium cultured on sawdust substrate as a whole-cell cathode catalyst.

**Keywords:** laccase, edible mushroom, whole-cell catalyst, microbial fuel cell, wastewater treatment

## Introduction

Electrical energy is critical in the area of healthy development. Conventional fossil fuel resources such as natural gas, petroleum, and coal have met this energy demand. Because of the global insufficiency of fossil fuels, renewable energy sources appear to be a better alternative [1]. Various renewable energy sources, such as solar, hydropower, wind, geothermal, and biomass, have been studied in terms of energy generation [2].

A microbial fuel cell (MFC) is a green device that utilizes microbial metabolism to convert chemical energy in organic matter to electrical energy (Slate et al. 2019). However, due to the high cost of its components, such as a platinum-coated electrode, this innovation still has some limitations. To solve this problem, some researchers used a bio-cathode with laccase [3-4].

Laccase, also known as bezenediol: oxygen oxidoreductase (EC 1.10.3.2), is a blue multicopper oxidase found in plants, insects, fungi, and bacteria. It has the ability to catalyze the oxidation of both phenolic and non-phenolic compounds [5-6]. The fungal laccase is well known among microbial laccases for a variety of field applications as well as fiber property improvement,

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bioremediation, dye decolorization, and biosynthesis [7-10].

Edible mushrooms contain various types of extracellular enzymes, along with oxidoreductases like laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase [11]. Edible mushrooms such as *Pleurotus eryngii*, *Pleurotus djamor*, *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Auricularia polytricha* [12-13], *Volvariella volvacea* [14], *Coprinus fimetarrius* [15], *Termitomyces* sp. [16] and others have been reported in secreting of extracellular laccase.

In this study, a novel laccase-based cathode microbial fuel cell (LB-MFC) was developed and used to generate electrical energy using two different laccase secreting edible mushrooms, *P. pulmonarius* and *C. fimetarrius*.

## Experimental

### Edible Mushroom

Two laccase secreting edible mushrooms *P. pulmonarius* and *C. fimetarrius* was enriched in sterilized sucrose-yeast (SY) broth, which contained 10% (w/v) sucrose and 0.1% (w/v) Brewer's yeast extract [17] and incubated at 30°C.

### Electrode Preparation

The graphite plate (0.0014 m<sup>2</sup>) was soaked in 100 mL 6% (w/v) H<sub>2</sub>O<sub>2</sub> solution for 60 mins at 50°C, then the 100 mL of 98% (w/v) H<sub>2</sub>SO<sub>4</sub> solution was slowly added and incubated for 50°C for 60 mins. The incubated graphite was washed three times with deionized water and soaked overnight. The graphite was dried at 50°C for 24 hours and heated in the microwave for 5 minutes, modified from [18].

### Domestic Wastewater Preparation

The domestic wastewater was prepared using glucose as a carbon source modified from Tumta et al. (2020). The composition of domestic wastewater includes 6.0 g/L C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, 0.03 g/L CaCl<sub>2</sub>, 0.08 g/L MgCl<sub>2</sub> · 6H<sub>2</sub>O, 0.05 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.11 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.08 g/L (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> · 6H<sub>2</sub>O and 0.01 g/L NaHCO<sub>3</sub> without trace element adding. The wastewater was sterilized before being used.

### Novel MFC Design and Operation

A schematic of novel LB-MFC is shown in Fig.1. The polypropylene plastic chamber with 1,500 mL volume was used as an MFC chamber. The graphite plates (0.0014 m<sup>2</sup>) were used as an electrode. The 48 hr-old exoelectrogen (1 x 10<sup>8</sup> cell/mL) was prepared according to Chaijak et al. [19]. The 10 mL of edible mushrooms *P. pulmonarius* and *C. fimetarrius* in SY media were

inoculated in sawdust and incubated at 30°C for 7 days in dark conditions for growing fungal mycelium, then they were placed on the surface of a cathode electrode for catalyzing the electricity generation. The 10 mL of sterilized water was added into the spent mushroom every 48 hr for keeping a moisture content. The electrodes were connected to the external circuit using copper wire. The 250 g of sterilized volcanic rock was used as a supporter between electrodes.

For operation, the 50 mL of exoelectrogen (1 x 10<sup>8</sup> cell/mL) was inoculated in the MFC chamber, then the 450 mL of sterilized domestic wastewater was added. The opened-circuit voltage (OCV) was collected every 10 mins. At the stationary phase (approximately 500 mV of OCV), the closed-circuit voltage (CCV) was measured at 1,000 Ω external resistance. The current (I), power (P), current density (CD), power density (PD), and internal resistance (R<sub>int</sub>) were calculated according to Ohm's law.

### Laccase Activity

The 1 g of spent mushroom on the cathode surface was collected every 24 hr for 7 days from LB-MFC, it was soaked in 10 mL sodium acetate buffer. The solution was centrifuged at 5,000 rpm for 5 mins for the separation of microbial cells and sawdust from the enzyme. The supernatant was collected and used for laccase activity determination according to Lin et al. [4]. Briefly, the 0.5 mL of the crude enzyme was mixed with 0.2 mL of 0.02 M ABTS (2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) and 2.3 mL sodium acetate buffer. The laccase activity was monitored using UV-Vis spectrophotometry at 420 nm. The enzyme unit (U) is defined as the amount of enzyme transforming 1 μmol of the substrate (ABTS) per minute.

## Results and Discussion

The OCV of LB-MFC was collected every 10 mins for 570 mins, the LB-MFC01 is the laccase-based MFC with *P. pulmonarius* on the cathode and the LB-MFC02 is laccase-based MFC with *C. fimetarrius* on the cathode. The control is the single-chamber MFC without edible mushrooms. The LB-MFC01 produced an OCV of 940.00±5.00 mV with 69.68% higher than the LB-MFC02 and 98.89% higher than the control (Fig. 1). At the stationary phase, the external resistance of 1,000 Ω was connected between two electrodes. The LB-MFC01 provided the CCV of 136.00±4.00 mV whereas the LB-MFC02 generated the CCV of 29.67±1.53 mV. The maximal CD and PD of 97.14±2.86 mA/m<sup>2</sup> and 13.22±0.78 mW/m<sup>2</sup>. The internal resistance of LB-MFC01 has 5,915.75±203.49 Ω with 31.40% lower than LB-MFC02. The electrochemical properties of LB-MFC was shown in Table 1.

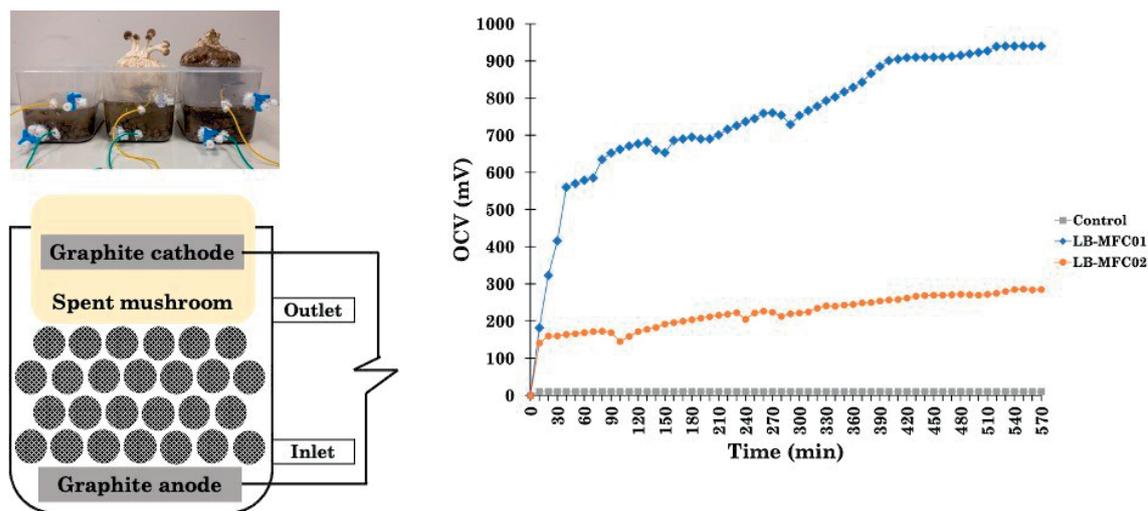


Fig. 1. The diagram of laccase-based cathode microbial fuel cell (LB-MFC), and the opened circuit voltage (OCV).

The laccase activity of edible mushrooms was monitored every 24 hr for 7 days by spectrophotometry at 420 nm of wavelength. The ABTS was used as a substrate for laccase activity. The results found that *P. pulmonarius* has a laccase activity of  $155.17 \pm 0.10$  U/mL whereas *C. fimetarius* has a laccase activity of  $31.00 \pm 1.00$  U/mL. The laccase activity of edible mushrooms is displayed in Fig. 2.

Agricultural waste such as sawdust, rice bran, cornmeal, and soybean meal is commonly used to culture edible mushrooms. According to the previous study, approximately 5 kilograms of spent mushrooms were used to produce each kilogram of fresh mushrooms [20]. The fungal mycelium in the spent mushroom of edible mushroom can continuously release an extracellular enzyme [21]. One of the most well-known white-rot fungi is an edible mushroom, which can produce

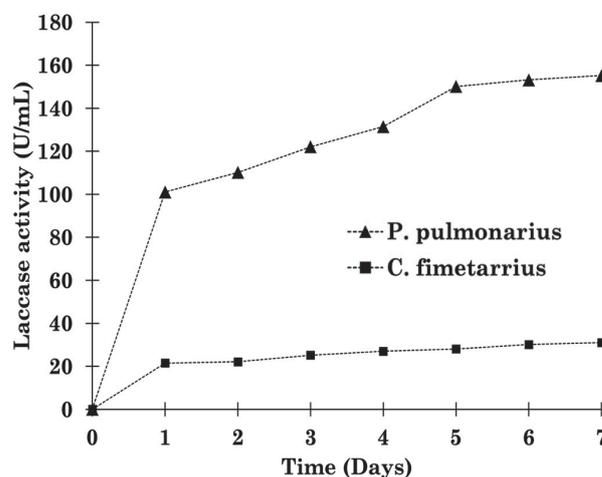


Fig. 2. The laccase activity of edible mushrooms.

Table 1. The electrochemical properties of the LB-MFC with the edible mushroom *P. pulmonarius* (LB-MFC01) and *C. fimetarius* (LB-MFC02).

Electrochemical properties	LB-MFC01	LB-MFC02	Unit
OCV	940.00±5.00	285.00±3.00	mV
CCV*	136.00±4.00	29.67±1.53	mV
Current	0.136±0.000	0.030±0.000	mA
Power	0.019±0.001	0.001±0.000	mW
CD**	97.14±2.86	21.19±1.09	mA/m <sup>2</sup>
PD**	13.22±0.78	0.63±0.06	mW/m <sup>2</sup>
CD***	272.00±8.00	59.33±3.06	mA/m <sup>3</sup>
PD***	37.01±2.18	1.76±0.18	mW/m <sup>3</sup>
Internal resistance	5,915.75±203.49	8,624.04±504.09	Ω

\* CCV at 1,000 Ω of external resistant load

\*\* Based on electrode area

\*\*\* Based on working volume

Table 2. Laccase activity from the spent mushrooms.

Mushroom	Spent mushroom substrate	Laccase activity (U/mL)	Reference
<i>P. pulmonarius</i>	Saw dust	155.17±0.10	This study
<i>C. fimetarius</i>	Saw dust	31.00±1.00	This study
<i>Pleurotus ostreatus</i>	ND	22.00±1.40	[23]
<i>Pleurotus florida</i>	Wheat straw	301.58±2.95	[24]
<i>Pleurotus eryngii</i>	ND	1.33±0.33	[25]
<i>P. ostreatus</i>	ND	0.07±0.00	[26]
<i>P. pulmonarius</i>	ND	0.82±0.01	[27]

a variety of extracellular ligninolytic enzymes such as manganese peroxidase, lignin peroxidase, and laccase [22]. In our study, laccase activity of 155.17±0.10 U/mL and 31.00±1.00 U/mL were obtained from the spent mushroom of *P. pulmonarius* and *C. fimetarius*, respectively. Table 2 shows the laccase activity of the spent mushroom from previous studies.

The MFC is an efficient technology for treating organic matter-containing wastewater while also producing electricity. When the single-chamber MFC with the *P. pulmonarius* as a whole-cell cathode biocatalyst was operated for 570 minutes, the maximal OCV of 940.00±5.00 mV, the maximal CD of 97.14±2.86 mA/m<sup>2</sup> (272.00±8.00 mA/m<sup>3</sup>), and the maximal PD of 13.22±0.78 mW/m<sup>2</sup> (37.01±2.18 mW/m<sup>3</sup>) were achieved. In Lai et al. [28], the laccase producing white-rot fungi was grown on the cathode electrode for enhancing electricity generation. The maximal power density of 13.38 mW/m<sup>2</sup> was reached.

Moreover, Lin et al. [4] demonstrated that the edible mushroom *Ganoderma* sp. laccase can generate electric power with 420 mV of OCV and 0.004 mW/m<sup>2</sup> of PD after 45 days of operation. On the other hand, the mycelium of the edible mushroom *P. eryngii* has grown on the surface of the graphite electrode. The maximum CD of 0.0003 mA/m<sup>2</sup> was obtained [28]. Our previous research found that using Shiitake mushroom (*Lentinula edodes*) as a whole-cell biocatalyst in a single-chamber MFC can generate 473.63 of OCV when 52.66 U/mL of laccase activity was released from mushroom mycelium [29].

### Conclusions

Growing laccase-secreting edible mushrooms on the surface of the cathode electrode in a single-chamber MFC can improve the performance of the MFC's electricity generation from the domestic wastewater. This research adds to our understanding of using edible mushrooms as a whole-cell catalyst for electricity generation in membrane-less MFCs. The novel model demonstrated the effective potential for future growth in bio-electricity generation.

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### Conflict of Interest

The authors declare no conflict of interest.

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