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A NEW REPORT ON USING A LACCASE PRODUCING YEAST FOR MELANOIDIN DEGRADATION AND ELECTRICITY GENERATION BY MICROBIAL FUEL CELL

Chaijak Pimprapa[a,b*], Sinkan Purimprach[a], Wetchapan Patcharida[a]

[a]Microbial Fuel Cell & Bioremediation Laboratory, Faculty of Science, Thaksin University, Phatthalung, Thailand
[b]Microbial Technology for Agriculture, Food and Environment Research Center, Thaksin University, Phatthalung, Thailand

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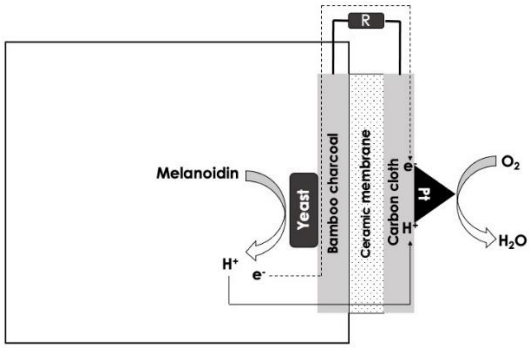
*Corresponding author
pimprapa.c@tsu.ac.th

Graphical abstract



Abstract

Palm oil milled effluent (POME) is one of the most environmental concerned industrial wastewater owing to its complex structure. Melanoidin is a highly stable content in POME that caused the dark color. In this study, the *Galactomyces* sp. rich consortium TM11 with high laccase activity was used to remove a contaminated melanoidin from raw POME. Besides, the single chamber ceramic microbial fuel cell (sCMFC) was developed to eliminate melanoidin and simultaneously generate electrical power. The results indicated that the maximal current density and power density of 215.56±5.09 mA/m^2 and 139.44±6.56 mW/m^2 were reached. Whereas the melanoidin removal of 83.50±2.93% was obtained. This study was the first reported of using laccase producing yeast comsortium to remove melanoidin and generate electrical power.

Keywords: Decolorization, laccase, palm oil milled, biocatalyst, bioremediation

1.0 INTRODUCTION

Palm oil milled effluent (POME) is an effluent discharged from palm oil milling processes which require effective treatment before release into groundwater resource owing to its highly polluting properties [1]. The production value of oil palm in Thailand was approximately 1.46 billion US dollar with a production volume of 16.78 x 10^6 tons [2]. Madaki & Seng displayed about 3.5 tons of POME is generated from each ton of crude palm oil [3]. Hence, Thailand discharge approximately 58.73 x 10^6 tons annually. POME is concerned as the major source of water pollution in producer countries due to the high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) that causes a reduction of aquatic biodiversity and ecosystem [4]

Melanoidin is dark brown to a black colored product of sugar and amino acid produced by non-enzymatic browning reactions called Maillard reaction [5]. It is commonly distributed in food, drink and widely released in huge volume by agriculture-based industries. Melanoidin is concerned from an environmental aspect owing to its structure

complexity, dark color and offensive odor [6]. Moreover, the melanoidin can interact with metal ions and transform to stable complex structure that cause the environmental pollution [7]. It is found in the various wastewater such as molasses wastewater [8], distillery wastewater [9], sugar industrial wastewater [10] and bio-methanated wastewater [11]. The melanoidin has found about 1-10% of total chemical oxygen demand (COD) of agricultural wastewater [12].

Decolorization of agricultural-based wastewater by chemical method and physical method has been accomplished, these processes are required high operating cost that not economically feasible on large scale [13]. Whereas biological methods by using microorganisms and their enzyme have been successfully achieved and can be applied as an industrial scale [14].

Laccase (EC 1.10.3.2) belongs to the multicopper oxidase family. It can catalyze phenolic compound and aromatic amine oxidation with concomitant four-electron reduction of atmospheric oxygen (O_2) to water (H_2O) [15]. Laccase is normally found in plant and fungi, fungal laccase has been interested owing to it is the key enzyme involved in lignocellulose degradation. Moreover, It has been reported in the elimination of various pollutants including POME decolorization [16-17]. Various microbes have been reported in POME decolorization such as *Curvularia clavata*, *Galactomyces reessii* and *Coprinus cinereus* [17-19].

Recently, biological technology like the microbial fuel cell (MFC) has been interested for wastewater treatment owing to its operating condition and using various biodegradable substrates. In MFC, the microbes actively degrade substrate and bio-electricity is generated. It could be used as a power generator in a small device like a biosensor [20]. Moreover, the MFC has been studied in industrial wastewater such as POME [18], swine-farming wastewater [21], municipal wastewater [22] and textile wastewater [23].

In the present study, we developed a novel model single chamber ceramic separator MFC (sCMFC) for the removal of melanoidin from POME. The *Galactomycess* sp. rich consortium (TM11) with laccase activity was used as anode whole-cell biocatalyst to degrade melanoidin and generate bio-electricity. This is the first work evaluating a single chamber CMFC system for melanoidin degradation.

2.0 METHODOLOGY

2.1 Wastewater

The 100 L of POME was collected from an oil palm factory in Trang province, Southern Thailand. It was collected in a sterile container and suddenly kept in an icebox. Then, the POME was transferred to a laboratory at the Faculty of Science, Thaksin University. It was stored at a low temperature of 4 °C to prevent bio-degradation by normal flora. The characteristics of POME used in this experiment is shown in Table 1.

Table 1 The characteristics of POME used in this experiment

Characteristics	Amount
Total Solid (TS) (%)	4.50±0.13
Volatile Solid (VS) (%)	3.12±0.10
Lipid	1.12±0.03
Total volatile fatty acid (TVFA) (mg/L)	1,800±150
Chemical oxygen demand (COD) (mg/L)	4,400±100
Melanoidin (mg/L)	88.00±0.15
C:N ratio	23.15±0.02
pH	5.3±0.1

2.2 Microbe

The *Galactomycess* sp. rich consortium (TM11) was achieved from the Department of Biotechnology, Faculty of Science, Thaksin University. It was maintained in 30% (v/v) glycerol broth. The 10% (v/v) of TM11 (1.0×10^8 cell/mL) was inoculated into the 90% (v/v) potato dextrose broth (PDB, Sigma-Aldrich, United States) and incubated at 30 °C for 5 days.

2.3 Melanoidin Degradation

The 10% (v/v) of 5 days-old TM11 (1.0×10^8 cell/mL) in PDB was inoculated into the 90% (v/v) of sterile POME and raw POME. The mixture of 10% (v/v) of sterile PBD and 90% (v/v) of raw POME was used as a control to determine the melanoidin degradation by POME normal flora.

All reactions were incubated at 30 °C for 7 days. The melanoidin removal (%) was monitored following Azreen *et al*. [24] in Eq. (1), cell density (cell/mL) and laccase activity was monitored every 24 hr. For laccase activity, the solution was fed out and centrifuged at 9,000 rpm for 5 mins to separate the cell pellet. Then the supernatant was used for the determination of laccase activity according to Chaijak *et al*. [18]. One unit (U) of the enzyme is defined as the concentration of the enzyme required to oxidize 1 µmole per minute.

$$\text{Removal (\%)} = (M_{initial} - M_{final}) / M_{initial} \quad (1)$$

Where $M_{initial}$ is the initial concentration of melanoidin and M_{final} is the final concentration of melanoidin.

2.4 CMFC design & Operation

Figure 1 showed the diagram of the novel single chamber CMFC, the acrylic cubic with 100 mL working volume was used as anode chamber. The 30 cm^2 of 0.5 mg/cm^2 platinum-coated Vulcan carbon cloth

(Fuel Cell Store, United States) was used as the air-cathode electrode. The 30 cm^2 of bamboo charcoal was used as an anode electrode. The 1.0 mm diameter copper wire was used to connect between the electrodes. The 1 mm thickness ceramic separator was inserted between the air-cathode and anode.

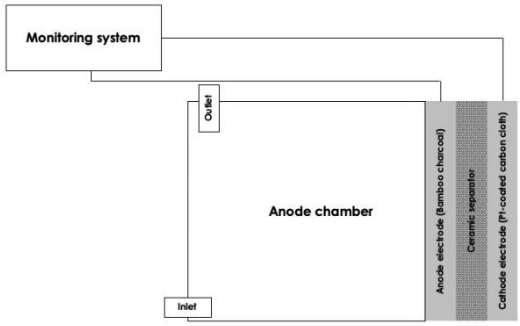


Figure 1 The diagram of the novel single chamber CMFC with whole-cell biocatalyst on the anode surface

The 10% (v/v) of 5 days-old TM11 (1.0 x 10^8 cell/mL) in PDB was inoculated into the 90% (v/v) of raw POME that filled in anode chamber, the CMFC was operated at 30 °C for 5 days. The open-circuit voltage (OCV) was collected every 30 mins. The close circuit voltage (CCV) was determined at 1,000 Ω, and the current (mA), current density (mA/m^3), power (mW), power density (mW/m^3) and internal resistance (Ω) was calculated followed Chaijak *et al*. [25]. The melanoidin removal (%) was monitored.

$$I = V / R \quad (2)$$

$$CD = I/V_{working} \quad (3)$$

$$P = IV \quad (4)$$

$$PD = P / V_{working} \quad (5)$$

$$R_{int} = (V_s \cdot R_L / V_o) - R_L \quad (6)$$

Where I is the current (mA), V is the closed circuit voltage at 1,000 Ω (mV), R is the external resistance (Ω), CD is the current density (mA/m^3), $V_{working}$ is the working volume (m^3), P is the power (mW), PD is the power density (mW/m^3), R_{int} is the internal resistance (Ω), V_s is the opened circuit voltage (mV), V_o is the output voltage (mV), and R_L is the load resistance (Ω).

3.0 RESULTS AND DISCUSSION

3.1 Growth Potential

The growth potential of TM11 in the POME was showed in Figure 2. The results indicated the TM11 in the sterile POME (2.05±0.01 x 10^8 cell/mL) can provide the 21.95% cell density higher than the raw POME (1.60±0.00 x 10^8 cell/mL). Whereas the native microbes of POME have small grown (0.64±0.01 x 10^8 cell/mL).

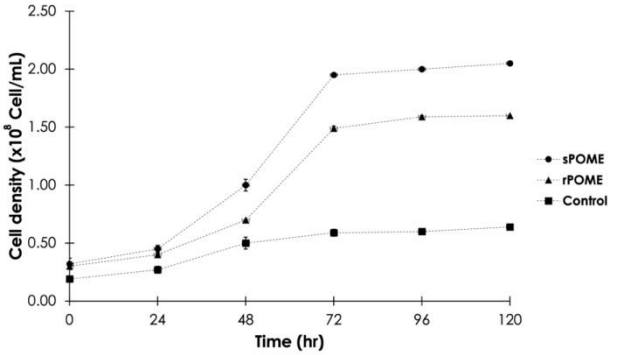


Figure 2 The growth potential of TM11 in the sterile POME (sPOME) and raw POME (rPOME) when were incubated at 30 °C for 5 days. Where the mixture of sterile PDB and raw POME was used as control

3.2 Laccase activity

The ABTS was used as the substrate of laccase. The laccase activity of TM11 consortium was showed in Figure 3. The highest laccase activity of 4.60±0.14 U/mL was found when it was cultured in the sterile POME. Whereas it was achieved 4.35±0.10 U/mL in the raw POME. The native microbe of raw POME showed the laccase activity of only 1.15±0.10 U/mL.

Fungal laccase is found in ascomycetes and basidiomycetes. The yeast produces a true laccase capable of oxidation of phenol and amino-phenol [27]. The ascomycetes yeast *Galactomyces* sp. has been reported in decolorization of POME through laccase function [18].

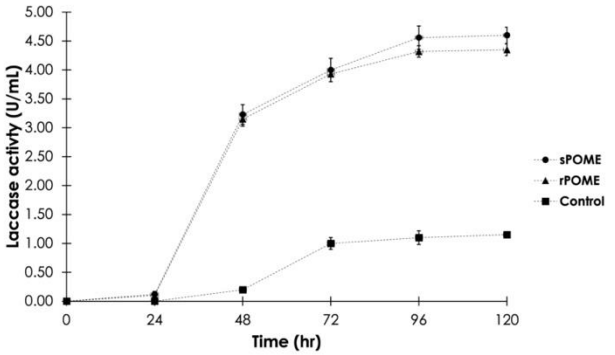


Figure 3 The laccase activity of TM11 when it was cultured in sterile POME (sPOME) and raw POME (rPOME). When native microbe was used as control culture

In a previous study, was accumulated by adding the pure culture of ascomycetes yeast *Galactomyces* sp. into the MFC system, the raw POME was replaced every 5 days for 10 times to ensure it can use the raw POME as a carbon source [18]. The mixed culture TM11 was collected to use for POME treatment. However, the laccase activity of *Galactomyces* sp. rich consortium TM11 and the melanoidin removal potential has been proved yet. The comparison of

laccase activity of *Galactomyces* sp. rich consortium TM11 and other studies were showed in Table 2.

Table 2 Comparison of laccase activity of *Galactomyces* sp. rich consortium TM11 and other studies

Source	Microbe/ Consortium	Laccase activity (U/mL)	Substrate/ Conditions	Reference
MFC system	*Galactomyces* sp. rich consortium TM11	4.35±0.10	Raw POME 30 °C for 5 days	This study
Termite nest	*Galactomyces* sp.	84.78	Malt extract broth at 30 °C for 5 days	[18]
Mushroom fruiting body	*Pleurotus* sp.	122.88	Modified culture broth at room temperature for 19 days	[26]
Euphorbia milii root	*Irpex lacteus*	0.12	Czapek Dox broth at 28 °C for 5 days	[27]
Sea garbage	*Trichoderma asperellum*	0.19	M7 broth at 30 °C for 3 days	[28]

3.3 Melanoidin Degradation

The melanoidin removal was showed in Figure 4. The maximum melanoidin removal of 78.23±2.94% from the sterile POME. Whereas the TM11 can remove 75.94±1.45% melanoidin from raw POME. The native microbe can remove only 5.50±0.23% melanoidin.

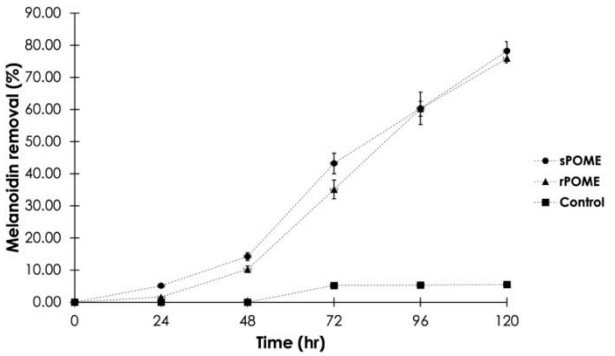


Figure 4 The melanoidin removal (%) of TM11 when it was cultured in sterile POME (sPOME) and raw POME (rPOME). When native microbe was used as control culture

3.4 Electrochemical Properties

The log phase of the cycle was covered between 2,880 to 4,310 min. The maximum OCV of 790±20 mV was achieved between 4,320 to 5,760 min (stationary phase). When the death phase was covered between 5,770 to 7,200 min. The external resistance of 1,000 Ω was connected to studies the other electrochemical properties. The electrochemical properties of single-chamber CMFC was showed in Table 3.

Table 3 The electrochemical properties and melanoidin removal (%) of single-chamber CMFC with whole-cell catalyst growing bamboo charcoal anode

Electrochemical properties	Amount
Open circuit voltage (OCV) (mV)	790±20
Close circuit voltage (CCV)* (mV)	646.67±15.28
Current (mA)	0.65±0.02
Current density (based on electrode area) (mA/m^2)	215.56±5.09
Current density (based on working volume) (mA/m^3)	6,466.67±152.75
Power (mW)	0.42±0.02
Power density (based on electrode area) (mW/m^2)	139.44±6.56
Power density (based on working volume) (mW/m^3)	4,183.33±196.84
Internal resistance (Ω)	222.11±29.09
Melanoidin removal (%)	83.50±2.93

* Study at the external resistance of 1,000 Ω

In this study, the raw POME was treated by CMFC with laccase producing consortium in anode chamber for melanoidin degradation and bio-electricity generation without exogenous media or chemical substance adding. The melanoidin removal, CD and PD of 83.50±2.93%, 215.56±5.09 mA/m^2 and 139.44±6.56 mW/m^2 were achieved. The comparison of melanoidin removal from POME of this study and other studies was shown in Table 4.

Table 4 Comparison of melanoidin removal from POME of this study and other studies

Treatment system	Initial melanoidin (mg/L)	Removal (%)	Power output (mW/m^2)	Reference
CMFC	88.00±0.15	83.50±2.93	139.44±6.56	This study
Anaerobic fermentation	NA	55	None	[13]
Coagulation	87.3	80.93	None	[24]
Aerobic digestion	3,000	60-67	None	[29]

4.0 CONCLUSION

In conclusion, treatment of POME using novel model CMFC with whole-cell biocatalyst has been studied. The result showed that the laccase-producing yeast can digest the contaminated melanoidin from the POME through its metabolism and integrated with air-cathode MFC for electricity generation. It can be concluded that the CMFC does significantly assist the overperformance of the removal of melanoidin removal and simultaneously electricity generation.

Acknowledgement

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