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RESEARCH PAPER

Melanoidin Removal and Electricity Generation of Palm Oil Mill Effluent by Oxidoreductase Producing Consortium with Air-Cathode Microbial Fuel Cell

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Abstract
Melanoidin is the hazardous dark brown byproduct generated during palm oil extraction in the crude palm oil industry. In this study, the laccase-producing consortium W3 (*Bacillus licheniformis and Bacillus subtilis*) was used to degrade melanoidin and decolorize palm oil mill effluent (POME). The microbial fuel cell (MFC) has been applied for enhancing decolorization and generation of electrical energy as a byproduct. The results displayed the maximal melanoidin removal of 95.20±0.10% was gained when the consortium W3 was added into the synthetic wastewater. While the maximal decolorization of 75.10±0.12% and 73.91±0.23% were gained from the sterile POME and raw POME respectively without chemical addition. Moreover, the power output of 2.13±0.05 W/m3 or 0.27±0.01 W/m2 was achieved from the POME-fed MFC with W3. This study gained new knowledge of using the laccase-producing bacterial consortium integrated with MFC for melanoidin removal from the POME and generation of electrical power as an alternative energy source.

Keywords: Decolorization, Electricity generation, Laccase, Melanoidin, Microbial fuel cell

INTRODUCTION

Palm oil is the most rapidly growing and important vegetable oil in the world. Several processes are used in the palm oil mill industry to gain valuable palm oil such as bunch stripping, extraction, and sterilization (Mahlia et al., 2001). The industrial oil extraction processes can generate a significant amount of both solid (oil palm trunk, oil palm frond, empty fruit brunch, palm process fiber, and palm kernel shell) and liquid (palm oil mill effluent; POME) wastes (Dalimin, 1995). Discharge of POME caused a serious negative effect in terms of environmental pollution. Various processes and technology have been used to treat this wastewater including aerobic and anaerobic digestion (Akhbari et al., 2020). Anaerobic digestion has been broadly interested and applied in POME bioremediation owing to its low energy consumption and high performance of organic removal (Akhbari et al., 2019).

Melanoidin is the complex structure compound that formed by various processes such as dehydration, cyclization, rearrangement, retro-aldolization condensation, isomerization and Maillard reaction (Kim & Lee, 2009). In Chandra et al. (2018) have been displayed that the

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melanoidin can caused the dark color in the agricultural wastewater. Both of chemical and physical processes have been applied for melanoidin removal and decolorization. However, these processes are not economically possibility owing to their secondary metabolite and energy consumption (Thanapimmetha et al., 2017). As an alternative process, the microbial degradation have been used for melanoidin degradation and decolorization. In Zhang et al. (2019), the oxidoreductase producing bacterium *Meithermus taiwanensis* WR-220 has been applied in the molasses wastewater for melanoidin removal.

In the steam extraction process of oil palm, the melanoidin is formed in the Maillard reaction of carbohydrates with protein and released of lipid and fatty acid. The previous study has suggested that melanoidin is responsible for the dark brown color of POME (Neoh et al., 2013). Several processes have been used for dark brown color decolorization from POME such as coagulation-flocculation, adsorption, membrane filtration, oxidation, and electrochemical method (Lee et al., 2019). The use of bacterial degradation is a non-complex method, low operating cost, and complied environmental regulations without pollution discharge (Balavinayagamani et al., 2018). Several bacteria including *Bacillus* sp., *Proteus* sp., *Enterobacter* sp., Lactobacillus sp., and *Pediococcus* sp. (Yadav & Chandra, 2012; Tiwari & Gaur, 2014; Krzywonos, 2012), have been reported in use for wastewater decolorization.

Microbial fuel cell (MFC) is a biotechnological technology that converts chemical energy to electrical energy through microbial metabolism. It is developing technology that offers the prospective solution for energy generation by degradation the pollutant in wastewater (Jayashree et al., 2019). In terms of decolorization, the result showed the maximal color removal of 88% was achieved, accompanied by a maximal power output of 23.50 mW/m^2 (Dai et al., 2020). In Tsiakiri et al. (2020), the yeast based MFC has been applied for melanoidin removal and decolorization from the melanoidin wastewater. The results exhibited the 70% of melanoidin degradation was gained after 6 hr of MFC operation. In this study, the laccase-producing consortium (*Bacillus licheniformis* and *Bacillus subtilis*) was used for melanoidin degradation and decolorization in POME. The consortium was applied to the air-cathode MFC for electricity generation during POME decolorization.

MATERIALS & METHODS

Microbe & Wastewater

The POME was collected from the palm oil factory in Phatthalung province, Thailand (Figure 1-6). It was kept in a 30 L sterile plastic container and rapidly transported to the laboratory in the Department of Biology, Thaksin University. The characteristics of POME used in this experiment was shown in Table 1.

Fig. 1. Sampling point of POME.

Table 1. Characteristics of palm oil mill effluent (POME) used in this experiment.

Characteristics	POME	Unit
Chemical oxygen demand (COD)	58,200±800	mg/L
Carbohydrate	27,500±200	mg/L
Protein	4,000±100	mg/L
Fat	6,000±150	mg/L
Melanoidin	88±2	mg/L
pH	4.5±0.1	-

The POME was aliquoted into the 50 mL sterile centrifuge tube and stored at -80 °C until it was being used. The laccase-producing consortium W3 (*Bacillus licheniformis* and *Bacillus subtilis*) was achieved from the Microbial Fuel Cell & Bioremediation Laboratory, Faculty of Science. It was grown in the nutrient broth (Himedia, India).

Synthetic melanoidin

The synthetic melanoidin wastewater was prepared according to Liakos & Lazaridis (2016), the 45.0 g/L glucose (Sigma-Aldrich, USA), 18.8 g/L glycine (Sigma-Aldrich, USA), and 4.2 g/L $NaHCO_3$ (Sigma-Aldrich, USA) were added in 100 mL of deionized water. It was heated in an oven at 95 °C for 7 hr. Then, 100 mL of deionized water was added.

Synthetic wastewater

The synthetic wastewater was prepared according to Li et al. (2021) follows, 0.75 g/L NaNo3, 0.03 g/L $CaCl_2 \cdot 2H_2O$, 0.08 g/L $MgSO_4 \cdot 7H_2O$, 0.08 g/L $K_2HPO_4 \cdot 2H_2O$, 0.18 g/L KH_2PO_4, and 0.03 g/L NaCl and 10% (v/v) synthetic melanoidin. The synthetic wastewater was autoclaved before it was being used.

Melanoidin removal

For melanoidin removal, the 10% (v/v) of 2-days old consortium W3 (1 x 10^8 cell/mL) was inoculated into the synthetic melanoidin wastewater aliquoted in a 250 mL Erlenmeyer flask covered with a rubber stopper for limiting oxygen diffusion from the atmosphere. It was incubated under the static condition at room temperature for 7 days. The 5 mL of sample was collected every 12 hr and centrifuged at 5,000 rpm for 10 mins. The supernatant was used for melanoidin residue determination. The melanoidin removal (Eq. 1) was monitored at 280 nm using UV-Vis spectrophotometry (Shimadzu, Japan).

$$\text{Melanoidin removal (\%)} = \left(\frac{M1 - M2}{M1}\right) \times 100 \quad (1)$$

Where M1 is the absorbance of melanoidin solution before treatment and M2 is the absorbance of melanoidin solution after treatment.

Decolorization

For POME decolorization, the 10% (v/v) of 2-days old consortium W3 (1 x 10^8 cell/mL) was inoculated into the non-pretreated of sterile and raw POME aliquoted in a 250 mL Erlenmeyer flask covered with a rubber stopper. It was incubated under static conditions at room temperature for 5 days. The melanoidin removal was monitored.

MFC operation

The ceramic separator microbial fuel cell (CMFC) used in this experiment was constructed according to the modified design of Chaijak et al. (2019) shown in Figure 2. The MFC chamber

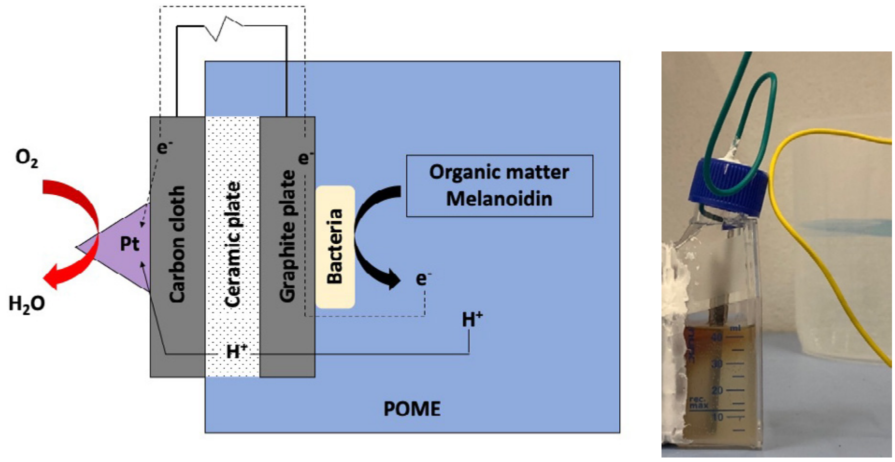

Fig. 2. The schematic of CMFC and CMFC model.

was made from the cell culture bottle with 50 mL of working volume. The 4 cm^2 of microwave-expanded graphite was used as an anodic electrode. The 4 cm^2 of 0.2 mg/cm^2 platinum-coated carbon cloth (Fuel Cell Store, USA) was used as a cathodic electrode. The proton exchange membrane was made from the 4 cm^2 of 0.2 cm thickness ceramic plate. The copper wire was used to connect between electrodes.

For MFC operation, the 5 mL of 2-days old consortium W3 (1 x 10^8 cell/mL) cultured in nutrient broth was added into the MFC chamber. The 45 mL of raw POME was filled and incubated for 2 days to fix the bacteria on an anodic electrode. The anolyte was fed out and then the 50 mL of fresh raw POME was fed in. The opened circuit voltage (OCV) was determined every hour for 5 days. The closed-circuit voltage (CCV) was measured at 1,002 Ω. The current (I), power (P), current density (CD), and power density (PD) were calculated according to Ohm's law.

Where I is the current (A), V is the CCV (V), R is the external resistance (1,000 Ω), CD is the current density (A/m^2 or A/m^3), A is the electrode area (m^2) or working volume (m^3), P is the power (W), PD is the power density (W/m^2 or W/m^3).

The anolyte of CMFC was collected every 24 hr for 5 days of operation. The total COD removal was determined using a High Range Plus COD digestion kit (Hach, Thailand). For melanoidin removal, the anolyte was centrifuged at 5,000 rpm for 10 mins. The supernatant was used for melanoidin removal determination

RESULTS & DISCUSSION

The melanoidin removal (%) by the consortium W3 was monitored every 12 hr. The result was shown in Figure 3. In this study, the maximal melanoidin removal in synthetic wastewater of 95.20±0.10% was gained without chemical addition. In Tripathy et al. (2020), the melanoidin was removed using a multi-oxidant supplemented microwave system. The maximal melanoidin removal of 100% was observed in 20 mins of microwave irradiation at a persulfate dose of 2,500 mg/L.

On the other hand, the melanoidin removal of 99.50% in molasses fermentation wastewater was gained from the submerged nanofiltration (Liu et al., 2013). In Rafigh & Soleymani (2020), the melanoidin has been removed from molasses wastewater using graphene oxide nanosheets.

Moreover, the melanoidin has been removed by using activated charcoal with a maximal adsorption capacity of 12 g/g (Liakos et al., 2016). The study of Arimi et al. (2015) has used anaerobic digestion in combination with coagulation for melanoidin removal from the molasses

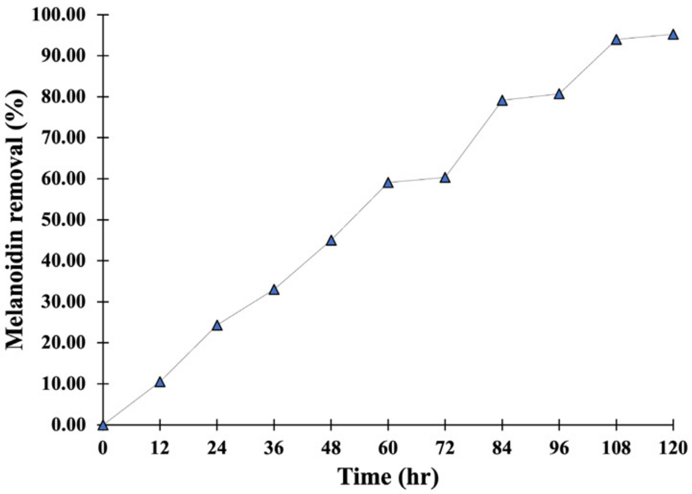

Fig. 3. The melanoidin removal (%) using laccase-producing consortium W3.

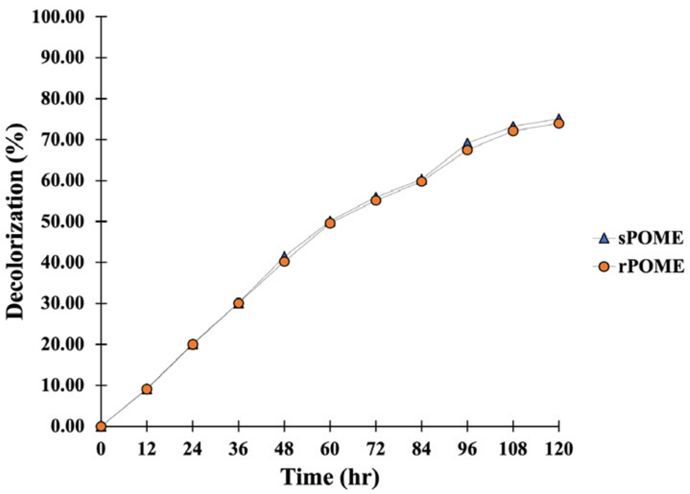

Fig. 4. The decolorization (%) of sterile POME (sPOME) and raw POME (rPOME) using laccase-producing consortium W3.

distillery wastewater. The results showed the maximal melanoidin removal of 48% was achieved when ferric chloride was added to the reaction.

Moreover, the previous study disclosed the laccase enzyme isolated from the fungi *Megaspororia* sp. can be used for melanoidin removal with a maximum efficiency of 48% (Toomsan et al., 2020). Kumar et al. (2022) indicated that the ligninolytic enzyme produced from the *Bacillus albus* can be used as a promising enzyme for melanoidin removal from distillery wastewater. While the bacterium *Leuconostoc mesenteroides* achieved the 40% of melanoidin removal (Bhamare & Kakulte, 2022).

The decolorization (%) of sterile and raw POME by the consortium W3 was monitored every 12 hr. The result was shown in Figure 4. The maximal decolorization of 75.10±0.12% and 73.91±0.23% were gained from the sterile POME and raw POME respectively without chemical and energy requirements. In Kongnoo et al. (2012), the POME has decolorized by using Fenton's process with optimal conditions of 50 mM H_2O_2 and 1 mM Fe^{2+} at pH 3. The maximal color removal of 98.61% was reached. Moreover, the dark color has been removed from the POME using microwave irradiated boiler ash as an adsorbent (Hamzah et al., 2019).

In Jarujareet et al. (2019), bioaugmentation of *Methylobacterium* sp. and *Acinetobacter* sp. PK1

Table 2. Electrochemical properties of ceramic separator microbial fuel cell (CMFC) with laccase-producing consortium W3.

Electrochemical properties	CMFC	Unit
Open circuit voltage (OCV)	882.29±3.06	mV
Close circuit voltage (CCV)	326.67±4.16	mV
Current (I)	0.33±0.00	mA
Current density (CD) *	0.82±0.01	A/m^2
Current density (CD) **	6.52±0.06	A/m^3
Power (P)	0.11±0.00	mW
Power density (PD) *	0.27±0.01	W/m^2
Power density (PD) **	2.13±0.05	W/m^3

* Based on electrode area.

** Based on working volume.

coupled with phytoremediation was used for the decolorization of POME. The color removal efficiency of 52% was gained. On the other hand, *Klebsiella pneumonia* ABZ11 has been used for POME decolorization. The maximal color removal of 81.54% was gained. However, this study cannot provide the power output as a by-product (Abdulsalam et al., 2019).

The OCV of CMFC with laccase-producing consortium W3 was monitored every 1 hr for 5 days. The maximal OCV of 882.29±3.06 mV was obtained. The CCV at 1,002 Ω was measured at the stationary phase. The electrochemical properties of CMFC was displayed in Table 2. In Lee et al. (2019), the power density of 0.34 W/m^2 was gained from the MFC fed with raw POME where the activated carbon has used as an electrode. Furthermore, the consortium of *Saccharomyces cerevisiae, Klebsiella variicola*, and *Pseudomonas aeruginosa* has been applied into the POME-fed MFC chamber for enhancing electricity generation. The maximal power output of 0.10 W/m^2 was generated (Sarmin et al., 2021). In Makhtar & Tajarudin (2020), the membrane-less MFC has been used for electricity generation from the POME. The maximal power generation of 0.02 W/m^2 was detected. On the other hand, the maximal power output of 0.02 W/m^2 was gained where the facultative anaerobic bacteria have been used in an anodic chamber (Amin et al., 2017).

In Lee et al. (2019), the maximal power output of 0.34 W/m^2 has been produced from the

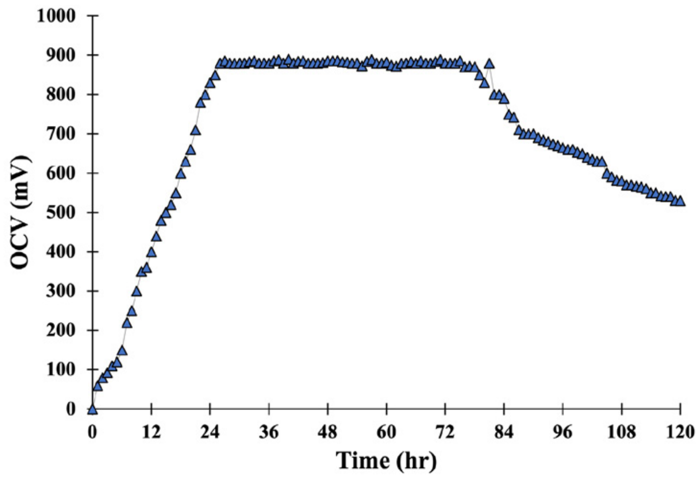

Fig. 5. The opened-circuit voltage (OCV) of CMFC with laccase-producing consortium W3 when the raw POME was used as an anolyte.

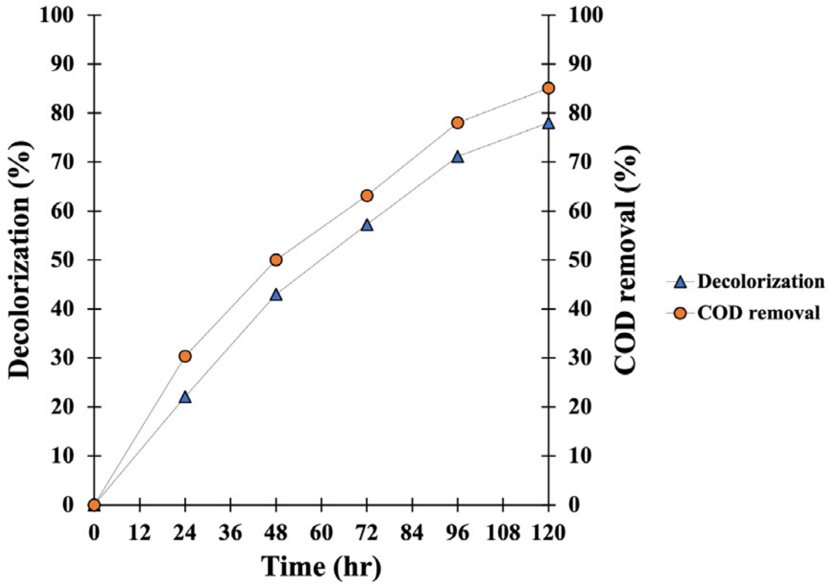

Fig. 6. The decolorization and COD removal of CMFC with laccase-producing consortium W3.

POME-fed MFC with the 91.90% of COD removal. On the other hand, the air-cathode coupling with the adsorption system can be used for COD removal and electricity generation from the POME. The COD removal and power density of 84.80% and 0.72 W/m^3 were gained (Tan et al., 2021). While the report of Makhtar et al. (2020) exposed that the POME-fed membrane-less MFC supplemented with agricultural waste can generate approximately 0.02 W/m^2 of power density.

The decolorization and COD removal potential of CMFC with laccase-producing consortium W3 was determined. The maximal decolorization and COD removal of 78.03±0.33% and 85.10±0.10% were achieved after 5 days of operation at room temperature without chemical adding. In the study of Yap et al. (2021), the *Moringa oleifera* extract was used for POME treatment by anaerobic co-digestion. Whereas the study of Farraji et al. (2021), the zeolite adsorbent can remove the COD of 96.80% and color of 69.90%.

On the other hand, the anaerobic digestion and crystal of magnesium ammonium phosphate have been used for the POME treatment (Ngatiman et al., 2021). In Sailah et al. (2021), electrocoagulation was combined with anaerobic digestion for improving the potential of POME treatment. The results found that the electrocoagulation process at 15V for 30 mins produced the highest level of COD and color removal of 87% and 93% respectively. In Zulfahmi et al. (2021), the water spinach (*Ipomoea aquatica* Forsk) has been used for the POME treatment. The results displayed the COD and color removal of 86.30% and 95.30% were achieved

CONCLUSION

In conclusion, the multi-copper oxidase-producing consortium (*B. licheniformis* and *B. subtilis*) showed the maximal melanoidin removal in synthetic wastewater of 95.20±0.10% after 5 days of incubation under room temperature. Moreover, this consortium can remove the dark color in the raw POME with 73.91±0.23% decolorization. The maximal power output of 0.03 W/m^2 was generated when the W3 consortium was integrated with CMFC. The CMFC with the laccase-producing consortium can reach the decolorization of 78.03±0.33%. This study suggested that the multi-copper oxidase-producing bacterial consortium integrated with CMFC can more efficiently generate electrical energy and melanoidin removal from the untreated POME.

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CONFLICT OF INTEREST

The authors declare that is not any conflict of interest regarding the publication of this manuscript.

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