https://doi.org/10.48048/tis.2023.5490

Performance of Hybrid Process Constructed Wetland-Microbial Fuel Cell for Melanoidin Degradation and Simultaneous Electricity Generation

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Received: 4 August 2022, Revised: 29 August 2022, Accepted: 5 September 2022, Published: 18 March 2023

Abstract

Melanoidin is the main cause of the dark brown color of the palm oil mill effluent (POME) that form under the Maillard reaction. In this study, the constructed wetland integrated with microbial fuel cell (CW-MFC) has been developed for melanoidin removal from the POME and simultaneously electricity generation as a by-product. The macrophyte *Dieffenbachia* sp. has been used as a biocatalyst on the cathode electrode and the oxidoreductase-producing bacterium *Bacillus lichenformis* with laccase and manganese peroxidase activity has been used as an anodic biocatalyst. The maximal melanoidin removal, chemical oxygen demand (COD) removal, enzyme activity, and power output were monitored. The maximal laccase and manganese peroxidase activities of 1.60 ± 0.10 U/mL and 1.45 ± 0.05 U/mL were found during melanoidin degradation. In addition, the maximal melanoidin removal of 93.59 ± 0.10% and 95.12 ± 0.15% were achieved respectively. When the maximal power density of 0.18 ± 0.01 mW/m^3 was generated. This study gained new knowledge about using the CW-MFC system as a biological treatment process of the melanoidin content in the POME and simultaneously generated electrical energy as a by-product.

Keywords: Constructed wetland, Microbial fuel cell, Melanoidin, Palm oil mill effluent, Electricity generation

Introduction

Melanoidin is the dark brown stable (polymer of amino and carbonyl) compound with a high organic load, found in the agricultural wastewater produced through the natural condensation from agricultural-based industries [1-3]. The melanoidin is formed by the Maillard reaction (non-enzymatic process). It also has a high molecular weight that is higher than 5,000 Dalton [4,5]. It is established in various wastewater such as molasses [6], distillery [7], palm oil mill [8], and Baker's yeast effluent [9]. Melanoidin has the potential for antimicrobial activity, antioxidant activity, and cytotoxic activity [10]. Moreover, Rizvi *et al.* [11] indicated that the melanoidin-contaminated soil causes the inhibition of seed germination and let to low yield productivity whereas it can interrupt the sunlight penetration to the water body and accelerate low oxygen concentration owing to low photosynthesis of phytoplankton and aquatic plant.

Both conventional chemical and biological processes have been used previously for melanoidin degradation from effluent [12]. These conventional methods are effective, but they involve a long operating time, produce a huge amount of sludge with toxic by-products, and require a large area for operation [13,14]. Therefore, innovative biotechnological processes have been developed for the alternative method for melanoidin removal from the effluent.

Nowadays, the production of bio-electrical energy and waste treatment by a microbial fuel cell (MFC) is purchased attention [15]. The study by Apollon *et al*. [16] has described that the MFC can generate green electrical energy through the metabolic reaction of an exoelectrogen. Conversely, 2 core problems still exist with the MFC such as the complete utilization of substrate by bacterial catalyst with results in unstable power generation, and low-power generation during the process [17-19]. Hence, it cannot be used on a larger scale like an industrial scale.

Constructed wetland (CW) has been generally applied as an encouraging biotechnological technology for the treatment of various types of effluent owing to its valuable benefits such as easy operation and maintenance, high efficiency, and low operating cost [20]. The CW has been effectively used for a wide range of chemical oxygen demand (COD). It can still act at the COD high as 20,000 mg/L [21,22]. Moreover, the CW system has been found that is used as a standard method for agricultural effluent treatment under European Union (EU) framework [23].

The integrating microbial fuel cell into the constructed wetland to form the constructed wetland – microbial fuel cell (CW-MFC) allows the prospective to obtain bioelectrical energy and a clean environment [24]. The study by Fang *et al.* [25] has indicated that the use of the CW-MFC system can enhance pollutant removal in the constructed wetland and power generation of microbial fuel cells. The CW-MFC has been applied in various effluent treatments including decolorization of effluents such as dyestuff wastewater [26], textile wastewater [27], Congo red contaminated wastewater [28], and azo dye wastewater [29]. No previous study has reported using the CW-MFC for melanoidin removal from agricultural wastewater.

Palm oil mill effluent (POME) is the effluent generated during the palm oil streaming process, which contains a huge amount of nutrients and other harmful chemical compounds including melanoidin. It has been found to enclose high COD as high as 100,000 mg/L, which risks the environment. The previous work has shown the degrading of melanoidin in the POME causes color removal [30,31]. Various processes have been applied for the POME decolorization such as bio-adsorption [32], biodegradation [33], photocatalytic remediation [34], and anaerobic digestion [35]. However, no previous method can both melanoidin removal and generating of electricity as an alternative renewable power.

This work aimed to investigate melanoidin degradation from the POME using the CW-MFC and generating electrical power during the treatment process.

Materials and methods

Microbe

The bacterium *Bacillus lichenformis* with the oxidoreductase activity was gained from the Microbial Fuel Cell & Bioremediation Laboratory, Faculty of Science, Thaksin University. It was used as a whole cell of an anodic electrode of the CW-MFC. The *B. lichenformis* was maintained on nutrient agar (HiMedia, India) and kept at 4 °C until it was used.

Glucose/glycine - melanoidin synthesis

The glucose/glycine melanoidin was synthesized according to Onyango *et al.* [36], the 0.45 g/L of laboratory-grade glucose (Himedia, India), 0.19 g/L of glycine (Sigma-Aldrich, United States), and 0.04 g/L of sodium carbonate (Sigma-Aldrich, United States) was added into 100 mL of deionized water and heated at 95 °C for 7 h, then cooled down at room temperature. The 100 mL of deionized water was added. The glucose/glycine melanoidin was preserved at 0 °C until it was used.

Synthetic melanoidin effluent

The synthetic effluent was prepared according to the modified method of Chormey *et al.* [37]. The synthetic wastewater contained 0.25 g/L $NaHCO_3$, 0.02 g/L KH_2PO_4, 0.02 $MgSO_4$, 0.02 $CaCl_2$ and 0.08 mg/L $(NH_4)_2SO_4$. All chemicals were dissolved in the deionized water and sterilized at 121 °C for 15 min under pressure. The 10 % (v/v) of stock synthetic glucose/glycine melanoidin was added into the sterilized synthetic effluent for synthetic melanoidin effluent preparation. The solution was kept at 0 °C until it was used.

MFC construction

The CW-MFC chamber was made from the 1 L polyethylene terephthalate (PET) plastic chamber. The 25 cm^2 (0.0025 m^2) graphite plate was used as a CW-MFC electrode, they were activated using microwave radiation according to the modified study by Kim *et al.* [38]. The graphite plate was submerged in 10 mL of H_2O_2 (30 wt%) and stirred for 60 min. The 100 mL of H_2SO_4 (98 wt%) was gently added and stirred for 100 min at 40 °C. The graphite plate was washed with deionized water 5 times and submerged overnight at 60 °C in the oven to ensure the chemicals were removed from the electrode surface. The graphite plate was irradiated with microwave radiation for 60 min to activate the electrode. The copper (Cu) wire was used for linking the electrode.

The 250 g of sterile volcanic rock, sand, and soil were placed on top of an anodic electrode. The cathodic electrode was inserted into the soil layer. Then, the macrophyte *Dieffenbachia* sp. was placed on the soil layer (**Figure 1**).

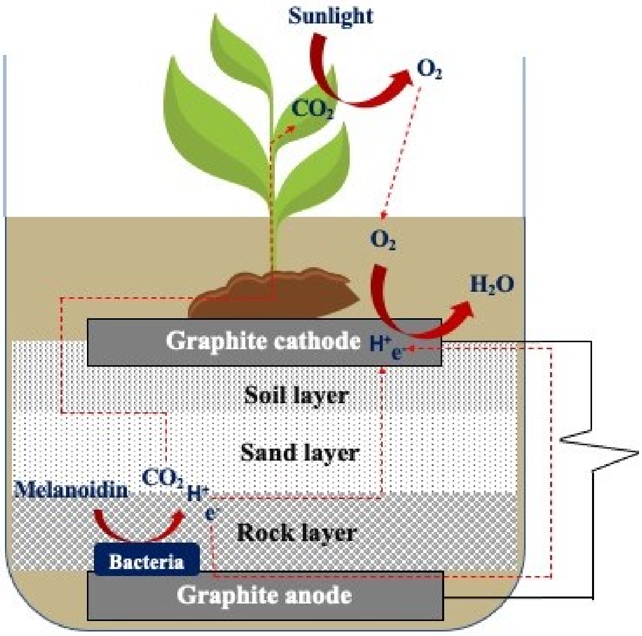

Figure 1 The constructed wetland-microbial fuel cell model used in this experiment.

MFC operation

The 100 mL of oxidoreductase-producing bacterium *B. lichenformis* (1×10^8 cell/mL) was added to the CW-MFC chamber. The 900 mL of synthetic glucose/glycine melanoidin effluent was added. The seed was accumulated for 7 days for immobilizing the active bacterium on the anodic electrode surface. Then, the effluent was fed out and replaced with 1,000 mL of fresh effluent. The open-circuit voltage (OCV) was monitored every hour for 48 h. The close-circuit voltage (CCV) was determined for electrochemical properties calculation. The oxidoreductase activities (laccase and manganese peroxidase) and melanoidin removal were monitored every 6 hr for 5 days.

The MFC fed-out solution was centrifuged at 5,000 rpm for 10 min under 4 °C to preserve the crude enzyme under mild conditions. For laccase activity, the 0.25 mL of the crude enzyme was mixed with the 0.25 mL of 10 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma-Aldrich, United States) and 0.74 mL of sodium acetate buffer (pH 4.5). All reactions were incubated for 5 min at room temperature and the enzyme activity was monitored at 420 nm using UV-Vis spectrophotometry (Shimazu, Japan) [39].

For manganese peroxidase activity, the 0.25 mL of supernatant was mixed with 10 μL of 10 mM H_2O_2 (Sigma-Aldrich, United States), 30 μL of 20 mM $MnSO_4$ (Sigma-Aldrich, United States), 0.1 mL of 1.5 mM 3-methyl-2-benzothiazoline hydrazine (Sigma-Aldrich, United States), 0.3 mL of 6.6 mM 3-(dimethylamino) benzoic acid (Sigma-Aldrich, United States) and 1.46 mL of 100 mM succinate-lactate buffer (pH 4.5). The reactions were incubated at room temperature for 5 min and determined at 590 nm [40].

For melanoidin removal, the 2.5 mL of supernatant was determined at 280 nm using UV-Vis spectrophotometry. The melanoidin removal was calculated as follows:

$$\text{Melanoidin removal (\%)} = [(A_{before} - A_{after}) / A_{before}]\times100 \quad (1)$$

where A_{before} is the absorbance of the synthetic effluent before being fed in the CW-MFC system and A_{after} is the absorbance of the synthetic effluent after being fed in the CW-MFC system.

POME

The POME (initial 2 g/L melanoidin) was collected from the Univanich Palm Oil Public Company Limited wastewater treatment pond, Phatthalung province, Southern Thailand. It was preserved at 0 °C until it was used. For the CW-MFC operation, 1000 mL of raw POME was added into the CW-MFC chamber with the active bacterial consortium on the cathodic electrode surface. The CCV was determined every 24 hr for 5 days for power output calculation and the fresh raw POME was replaced every 5 days for 15 days to ensure the system can produce the electrical energy from the raw POME.

Wastewater analysis

The removal of melanoidin and chemical oxygen demand (COD) were monitored every 24 hr for 5 days. For COD removal, the processed POME was centrifuged at 12,000 rpm for 10 min, then the supernatant was separated. The HR Plus COD kit (Hach, United States) was used. The 0.2 mL of supernatant and 0.2 mL of deionized water were added to the kit vial. All vials were heated in the DRB200 reactor for 2 hr and gently mixed. The vials were cooled down at room temperature and measured using the colorimetric method at 620 nm. The COD removal was calculated.

Results and discussion

Glucose/glycine - melanoidin effluent

The maximal OCV output of 880 ± 15 mV was generated from the CW-MFC with macrophyte *Dieffenbachia* sp. on the cathode and the oxidoreductase-producing bacteria *B. lichenformis* on the anode. The stationary phase was covered between hr-24 to hr-66. The OCV of the CW-MFC with the glucose/glycine-melanoidin effluent was shown in **Figure 2**. In addition, the maximal power output of 0.11 ± 0.00 mW/m^3 was gained between hr-48 to hr-66 (**Figure 3**).

The polarization curve was plotted to obtain the maximal power output generated from the glucose/glycine - melanoidin effluent using the CW-MFC. The maximal current density and power density of 0.50 ± 0.01 mA/m^3 and 0.11 ± 0.00 mW/m^3 were achieved (**Figure 4**).

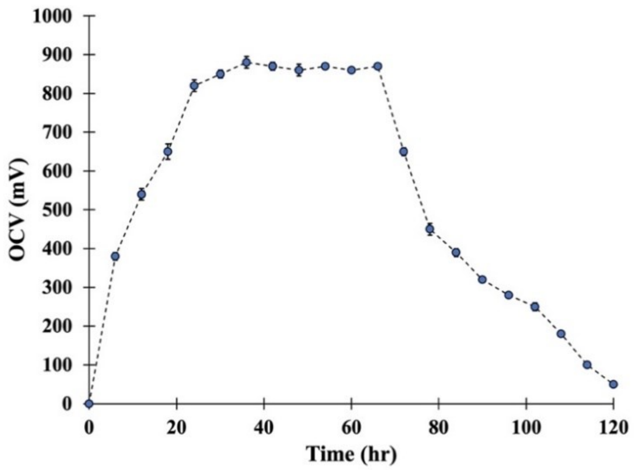

Figure 2 The open-circuit voltage (OCV) generated from the CW-MFC.

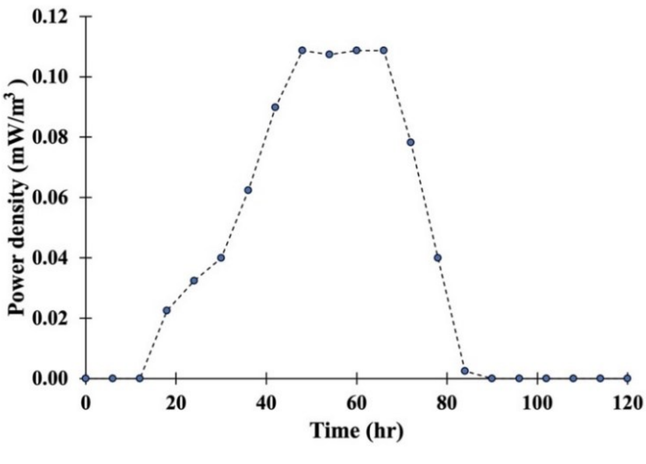

Figure 3 The power output generated from the CW-MFC.

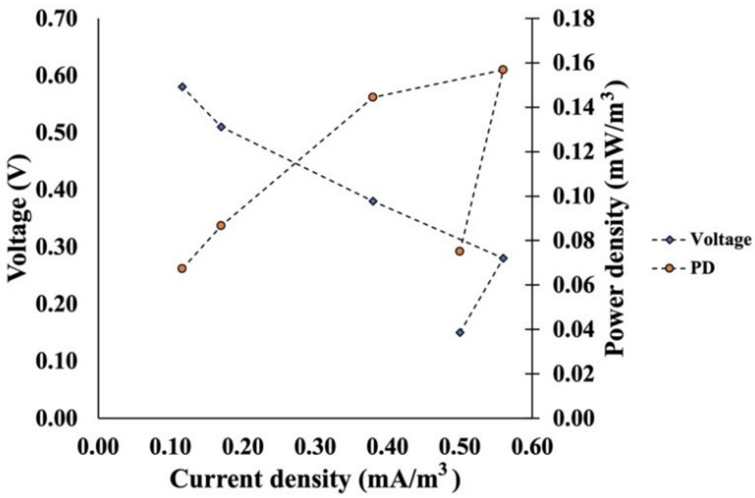

Figure 4 The polarization curve of the CW-MFC.

Moreover, the glucose/glycine-melanoidin removal (initial melanoidin concentration of 2.9 g/L), laccase activity, and manganese peroxidase activity were monitored during the electricity generating process. The result found that the maximal melanoidin removal of 91.35 ± 0.05 % (2.6 g/L melanoidin) was gained from 5 days of operation (**Figure 5**). The maximal laccase and manganese peroxidase activities of 1.60 ± 0.10 and 1.45±0.05 U/mL were expressed at hr-66 of operation time (**Figure 6**).

In Thu and Michele [41], the catalytic wet air oxidation process was used to remove glucose/glycine melanoidin from the sugarcane molasses distillery effluent. However, the two-step reaction with the 3 % of high-value metal platinum (Pt) has been required for the removal process. On the other hand, the multi-oxidant supplemented microwave system was used for the glucose/glycine melanoidin from the wastewater. The melanoidin per H_2O_2 ratio of 2.5/3 mg/L has been required. The 60 % melanoidin removal was gained. The calculated costs were ranging from 5.11 - 33.92 USD/kg of melanoidin removal [42]. In terms of microbial biodegradation, the lactic bacteria *Lactobacillus plantarum*, *Lactobacillus casei* and *Pediococcus parvulus* have been applied for glucose/glycine melanoidin removal from the sugar beet molasses vinasse. The removal of 25.14 % was gained at noncontrolled pH. However, the temperature had to control at 30 °C.

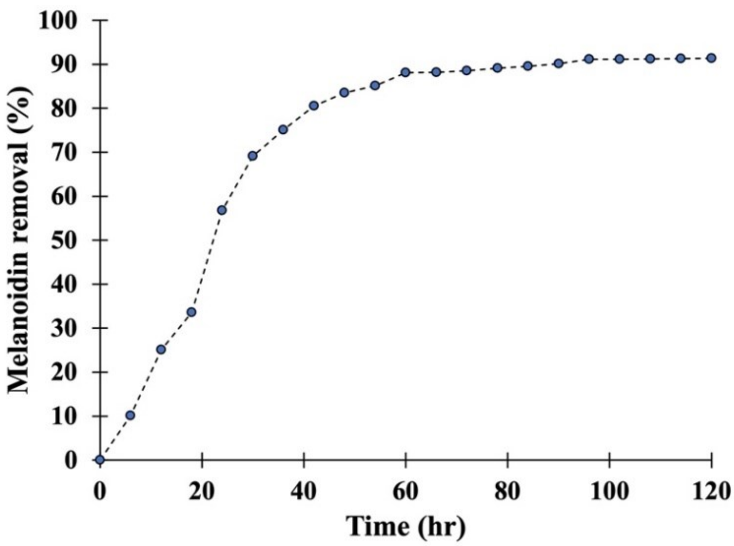

Figure 5 The melanoidin removal (%) gained from the CW-MFC.

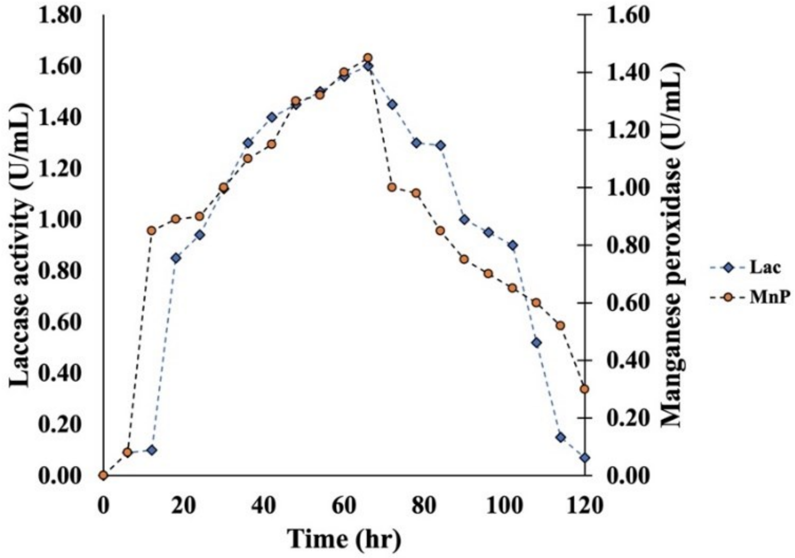

Figure 6 The enzyme activities (laccase and manganese peroxidase) produced from the active bacterium in the CW-MFC.

The oxidoreductase-producing microbes with laccase and manganese peroxidase activities have a high potential for melanoidin removal from wastewater. A previous study has shown that microbial laccase and manganese peroxidase coupling with membrane filtration technique resulted in 90 % of melanoidin removal [43].

In the study of Tsiakiri *et al.* [44], the laccase-producing yeast Saccharomyces cerevisiae was trapped in the silica-alumina support was achieved the 100% of melanoidin removal after 48 hr of operation. However, electric power or other alternative energy was not generated. On the other hand, the melanoidin removal from the sugarcane-molasses wastewater was developed using the oxidoreductase-producing bacterial consortium as a whole-cell biocatalyst. The melanoidin removal of 81 % was achieved through the supplement of 1 % of glucose and 0.2 % of peptone [45].

Moreover, the *Galactomyces* sp. rich consortium with laccase activity has been applied in the laboratory scale-single chamber microbial fuel cell for melanoidin removal from the POME. The 83.50 % of removal potential has been achieved within 3 days of operation [46]. On the other hand, the manganese peroxidase yeast was used for melanoidin removal from the wastewater. The melanoidin removal of 60 % was gained within 2 - 5 days when the glucose and yeast extract has been added into the system as an exogenous medium for active yeast [47].

POME

Various technologies have been applied for dark brown color pigment removal from the POME. In this study, the CW-MFC with the cooperation of phytoremediation and microbial remediation was developed for gaining electrical energy production and melanoidin removal. The maximal power output of 0.18 ± 0.01 mW/m^3 was achieved at the stationary phase without exogenous nutrient addition and chemical condition adjustment. The wastewater composition was analyzed to monitor the COD and melanoidin removal efficiencies. The maximal COD and melanoidin removal of 95.12 ± 0.15 and 93.59 ± 0.10 % (1.87 g/L melanoidin) were obtained when the CW-MFC was operated on for 5 days under room temperature.

On the other hand, the activated sludge was used for melanoidin removal from the POME. 63.45 % of pigment removal has been found when the active bacteria were accumulated for 31 days and supplemented with yeast extract and diary waste [48]. Moreover, the dark color of the POME has been treated using microwave-irradiated abundant palm oil mill boiler ash as a biosorbent. The removal efficiency of 92.31 % was achieved at pH 2, 7.5 g/ 100 mL POME, and 5 hr of contact time [49]. In addition, the electrocoagulation process has been used for the POME treatment. The maximal COD and dark color pigment removal of 56 and 65 % were obtained under 14 voltage and contact time of 3 hr without energy generation [50].

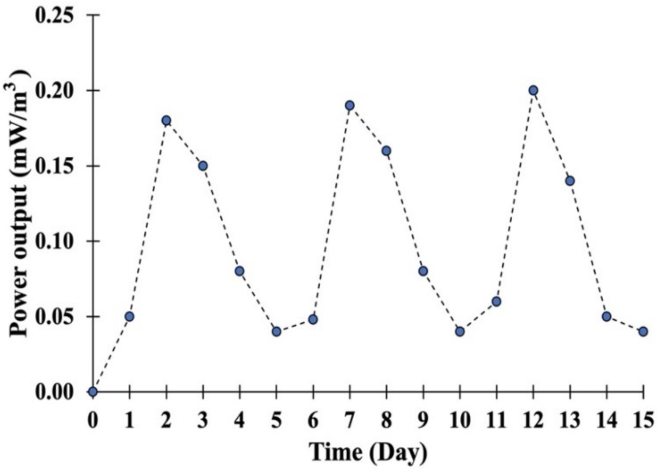

Figure 7 The power output generated from the CW-MFC with the raw POME.

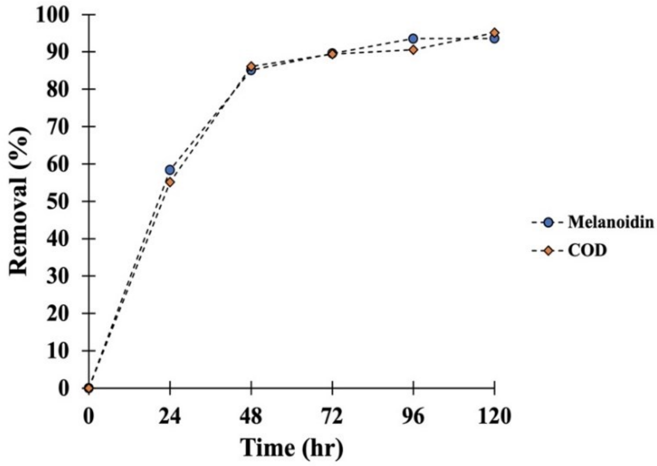

Figure 8 The melanoidin removal (%) and COD removal (%) from the raw POME using the CW-MFC.

Table 1 Comparison of the performance of the CW-MFC with macrophyte *Dieffenbachia* sp. with other work.

System	Wastewater	Melanoidin removal (%)	Power/Chemical supply	By-product	Reference
CW-MFC	Palm oil mill	93.59 ± 0.10	None	Electrical energy	This study
Multi-oxidant coupled with microwave system	Synthetic	60.00	Chemical agent/ Electrical energy	None	[42]
Fenton reaction	Synthetic	88.40	Chemical agent/ Electrical energy	None	[51]
Adsorption	Distillery	84.00	Chemical agent/ Electrical energy	None	[7]
Ozonation	Distillery	63.00	Chemical agent/ Electrical energy	None	[52]
Adsorption	Synthetic	92.00	Chemical agent/ Electrical energy	None	[53]
MFC	Palm oil mill	95.20	Chemical agent	Electrical energy	[54]
Biodegradation	Molasses	74.32	Chemical agent	None	[55]

Conclusions

The CW-MFC system with native macrophyte *Dieffenbachia* sp. planted on the cathode as a plant-biocatalyst was found to be an attractive idea for melanoidin removal and COD reduction from the POME. When the oxidoreductase-producing bacterium *B. lichenformis* were used an anodic biocatalyst. The monitoring of laccase and manganese activities suggested that these enzymes were involved in melanoidin removal from the wastewater. The maximal melanoidin removal and COD removal of 93.59 ± 0.10 % and 95.12 ± 0.15 % were obtained where the CW-MFC was operated for 5 days without energy supply. When the maximal power output of 0.18 ± 0.01 mW/m^3 was produced as a by-product. However, the CW-MFC needed to further develop the use of this process on a higher scale and continuously fed to evaluate its feasibility for the industrial scale.

Acknowledgments

This work was supported by Thailand Science Research and Inno Office of National Higher Education Science Research and Innovation Policy Council, Thaksin University (research project grant) Fiscal Year 2022.

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