

Enhancing Electricity Generation Using Macroalgae *Spirogyra* sp. Based on Microbial Fuel Cell for Palm Oil Mill Effluent Treatment

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Abstract: This study aims to enhance the electricity generation of the PMFC integrated with the macroalgae *Spirogyra* sp. as a whole-cell biocatalyst. In the experiment, macroalgae *Spirogyra* sp. were planted on the cathodic electrode surface of the PMFC. The electrochemical properties, such as open circuit voltage (OCV), current density (CD), and power density (PD), were then evaluated. Subsequently, the removal of melanoidin was studied. The maximal OCV, CD, and PD were 524.55 ± 44.32 mV, 0.60 ± 0.10 mA/m³, and 0.16 ± 0.02 mW/m³, respectively. Furthermore, the maximum melanoidin removal reached $81.40 \pm 1.64\%$ after 48 hr of operation. No previous studies have shown the use of the macroalgae *Spirogyra* sp. as a whole-cell biocatalyst. This study has contributed new knowledge regarding the utilization of macroalgae *Spirogyra* sp. as a whole-cell biocatalyst for electricity generation in the PMFC, where POME has been used as a substrate.

Keywords: biocathode; electricity generation; macroalgae; palm oil mill effluent.

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1. Introduction

Palm oil mill effluent (POME) has emerged as a predominant source of pollution from palm oil mills upon its release into the environment. This effluent adversely impacts water quality, rendering it unsuitable for direct discharge into the environment [1]. POME is characterized by its substantial solids, oil, grease content, and elevated organic levels [2]. Additionally, it harbors various heavy metals in noteworthy concentrations [3]. Furthermore, throughout the biological treatment process, detrimental gases such as methane (CH₄), sulfur dioxide (SO₂), and ammonia (NH₃) are emitted into the atmosphere within the ponding system series [4]. The untreated release of POME poses a substantial threat to aquatic diversity, leading to physiological disorders in fish populations [3]. Consequently, it is crucial to implement effective treatment measures to mitigate these impacts before discharging the effluent into the environment. This proactive approach is essential for preventing adverse effects on human health and environmental pollution [4,5]. Employing suitable methods or technologies for POME treatment is a promising strategy to minimize organic compounds and ensure compliance with standard discharge limits.

Melanoidins result from the Maillard reaction, a chemical process that involves the interaction between polysaccharides and amino acids. It exhibits distinctive traits, including a dark brown color, elevated molecular mass, and intricate composition, encompassing both

aromatic and heterocyclic constituents [6]. These dark brown molecules have been found in various wastewater, including POME [7].

In recent years, the microbial fuel cell (MFC) technology has garnered considerable interest for its capacity to harness energy during wastewater treatment. The prospect of employing MFCs in industrial settings is particularly appealing, given their ability to transform organic wastes into energy. This contributes to reducing waste disposal expenses and addresses energy requirements, making it a compelling solution [8]. Photosynthetic microbial fuel cells (PMFCs) represent an eco-friendly class of fuel cells utilized to generate renewable bioelectricity. These cells operate efficiently and rely on solar energy to achieve substantial power output. Depending on the specific organism employed, PMFCs can leverage the photosynthetic cell machinery found in plants, bacteria, or algae. Among these, PMFCs assisted by algae offer notable advantages characterized by rapid growth, space efficiency, the production of free nascent oxygen, and the generation of value-added products, including bioelectricity [9].

The primary objective of our study is to integrate a photosynthetic microbial fuel cell with a macroalgae-based biocathode alongside a bacterial consortium capable of degrading melanoidin to address the dual goals of decolorizing palm oil mill effluent and enhancing electricity generation. This innovative approach aims to synergistically leverage the photosynthetic and microbial capabilities to achieve sustainable and efficient industrial wastewater treatment while concurrently harnessing the generated electricity for potential applications.

2. Materials and Methods

2.1. Macroalgae and bacterial consortium.

The macroalgae *Spirogyra* sp. (Figure 1) was obtained from the Department of Biology, Faculty of Science and Digital Innovation, Thaksin University. It was cultivated in a modified Waris medium (0.99 mM KNO₃, 0.08 mM MgSO₄ x 7H₂O, 0.15 mM (NH₄)₂HPO₄, and 0.35 mM CaSO₄ x 2H₂O) under LED cool light conditions with a photoperiod of 15:9 hr light/dark [10]. The cultivation conditions were maintained to ensure optimal growth.



Figure 1. The macroalgae *Spirogyra* sp. was used in this experiment.

The bacterial consortium for melanoidin degradation (S5) was obtained from our previous study [7]. It was cultured in nutrient broth (containing 5 g/L peptone, 5 g/L NaCl, and 3 g/L beef extract) at room temperature, with agitation at 150 rpm on an orbital shaker.

2.2. POME preparation.

The POME was obtained from a palm oil extraction factory in Phatthalung province, Thailand. The wastewater was centrifugated at 10,000 rpm for 10 min to remove sediment and then sterilized at 121°C for 15 min to prevent bacterial contamination. The pretreated POME was stored at -25°C to preserve its qualities until it was used in the experiment.

2.3. PMFC operation and calculation.

Microwave-treated expandable graphite felts with a surface area of 10 cm² were employed as both the anodic and cathodic electrodes [11]. Copper (Cu) wire was used to connect the anodic and cathodic electrodes. A plastic box with a working volume of 1,000 mL served as the MFC chamber. A 500 g of sterile sand was utilized as the MFC separator (Figure 2). For the anodic seed, 100 mL of consortium S5 cultured in nutrient broth (1.0×10^6 cells/mL) was employed. The anolyte consisted of 800 mL of sterile POME. Additionally, 100 mL of the macroalgae *Spirogyra* sp. was planted on the cathodic electrode to produce oxygen gas, which serves as the final electron acceptor for the oxidation reaction (modified from Arun *et al.* [12]). The control treatment used the sterile graphite felt without the macroalgae. The melanoidin removal was monitored using UV-Vis spectrophotometry every 6 hours for 2 days. The residue melanoidin was observed at 540 nm.

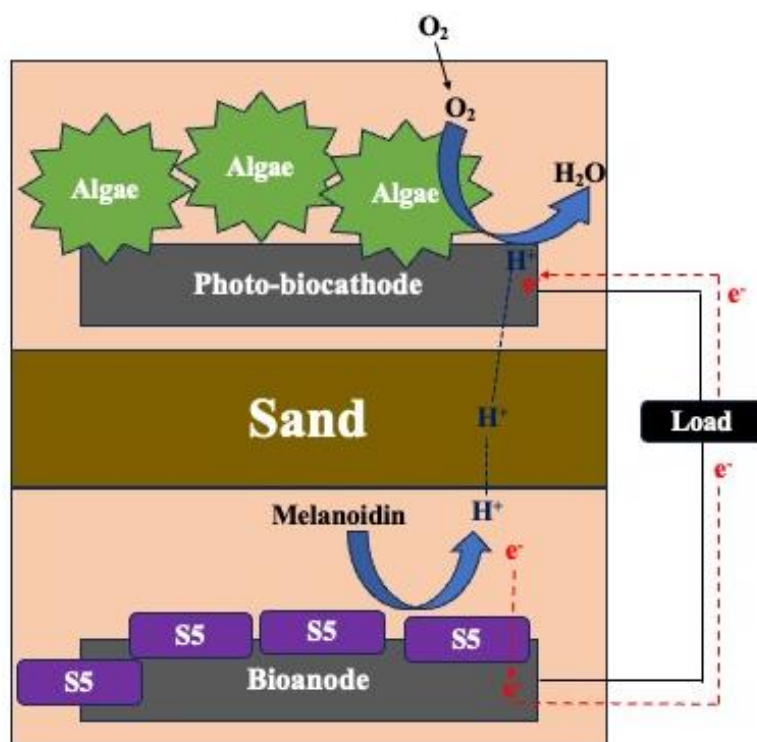


Figure 2. The diagram of PMFC is used in this experiment.

The open circuit voltage (OCV) was monitored every 1 hour for 2 days. The close circuit voltage (CCV) was measured under 300 – 1,000 Ω for use in polarization curve plotting. The electricity properties were calculated as Equations (1) – (4).

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

$$CD=I/A \quad (2)$$

$$P=IV \quad (3)$$

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

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

3. Results and Discussion

The PMFC has been conceptualized, where the MFC incorporates a light source at its electrode, maintaining microbial contact with at least one electrode [13]. Within a PMFC, the synergy between light and microbes plays a collaborative role in elevating cell voltage, leading to increased electricity generation [13-15]. Diverse microorganisms have found application in PMFCs, particularly those with photosynthetic capabilities, serving as anodic microbes. This category typically includes cyanobacteria, the blue-green algae that derive energy through photosynthesis [16]. Conversely, microalgae operate at the cathodes, contributing to bioelectricity generation and producing O_2 , biofuels, carbohydrates, proteins, and carotenoids [17-18].

In our investigation, the membrane-less PMFC integrated with macroalgae *Spirogyra* sp. exhibited a maximal OCV of 524.55 ± 44.32 mV during a 48-hour operation at room temperature. In contrast, the control group demonstrated a significantly lower OCV of only 49.0 ± 7.55 mV (Figure 3). The PMFC integrated with macroalgae *Spirogyra* sp. achieved a maximum current density (CD) and power density (PD) of 0.60 ± 0.10 mA/ m^3 and 0.16 ± 0.02 mW/ m^3 , respectively (Figure 4).

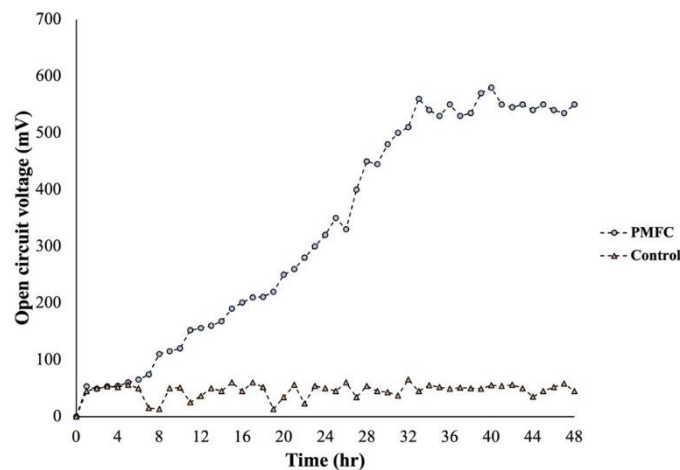


Figure 3. The OCV of the PMFC integrated with macroalgae *Spirogyra* sp.

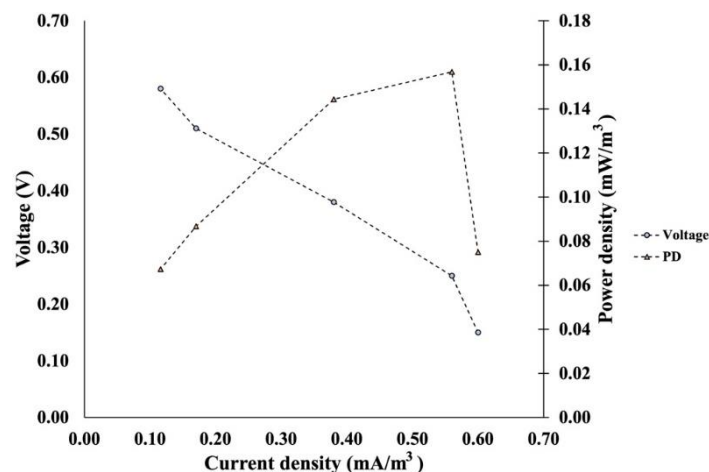


Figure 4. The polarization curve of the PMFC integrated with macroalgae *Spirogyra* sp.

Conversely, the dual-chamber PMFC integrated with microalgae *Chlamydomonas reinhardtii* demonstrated a maximum CD and PD of 39 A/m³ and 15.21 W/m³, respectively. This performance was attained through the utilization of a chemical anolyte enriched with trace metal elements during an 8-day operation [19]. The performance of PMFC integrated with algae is shown in Table 1. No prior research has presented findings on the utilization of PMFC for both melanoidin removal from POME and electricity generation.

Table 1. The electricity generation of PMFC is integrated with algae.

MFC type	Algal strain	Maximal OCV	Maximal power density	Substrate	References
Membrane-less	<i>Spirogyra</i> sp.	524.55±44.32 mV	0.16±0.02 mW/m ³	POME	This study
Dual chamber	<i>Chlorella vulgaris</i>	400 mV	400 mW/m ³	Synthetic medium	[20]
Dual chamber	<i>Chlorella vulgaris</i>	-	558.22 mW/m ³	Synthetic medium	[21]
Dual chamber	<i>Scenedesmus abundans</i>	745.96 mV	838.68 mW/m ²	Pharmaceutical wastewater	[22]
Dual chamber	<i>Chlorella</i> sp.	852.10 mV	-	Domestic wastewater	[23]

Regarding melanoidin removal, the membrane-less PMFC integrated with macroalgae *Spirogyra* sp. demonstrated the highest melanoidin removal efficiency of 81.40±1.64% (Figure 5). This achievement occurred over a 48-hour operational period at room temperature without adding any exogenous medium. In the study by Kheti *et al.*, melanoidin from the Maillard reaction in synthetic wastewater was treated using manganese peroxidase-producing bacteria *Pseudomonas violacea*. The highest melanoidin removal of 83.68% was achieved during a 92-hr operation at 37°C [24]. On the other hand, melanoidin from spent wash effluent underwent treatment by the bacterial strain *Bacillus* sp. for a 144-hour (6-day) operational period. The maximum melanoidin removal achieved was 84% [25].

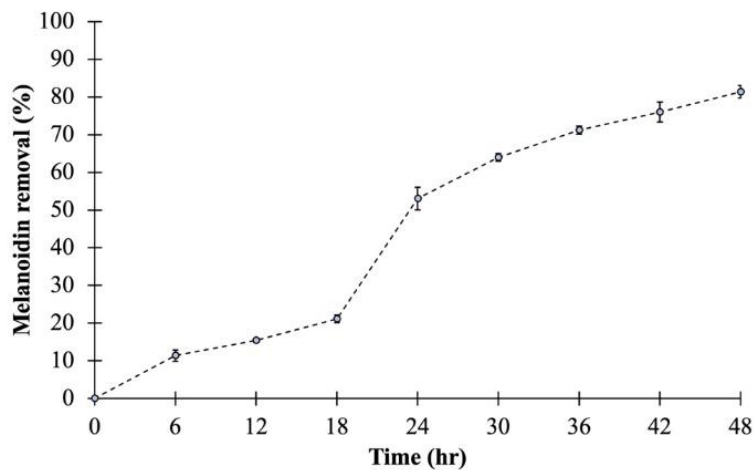


Figure 5. The melanoidin removal (%) of the PMFC integrated with macroalgae *Spirogyra* sp.

4. Conclusions

In summary, the integration of PMFC with *Spirogyra* sp. macroalgae as a biocathode has demonstrated superior electricity generation compared to the control group (sterile graphite felt). Furthermore, this innovative PMFC configuration has proven effective in producing a dark brown color agent, melanoidin, from Palm Oil Mill Effluent (POME) within a 48-hour timeframe. This research contributes novel insights into utilizing macroalgae as a whole-cell biocatalyst on the cathode electrode of PMFCs, showcasing its potential to enhance electricity generation in POME while concurrently removing melanoidin from wastewater.

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Conflicts of Interest

The authors declare no conflict of interest.

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