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UTILIZING MELANOIDIN CONSUMING MICROALGAE FOR ELECTRICITY GENERATION AND WASTEWATER TREATMENT VIA PHOTOSYNTHETIC MICROBIAL FUEL CELL

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ABSTRACT

Aim of the study
In our investigation, we used freshwater microalgae *Chlorella* sp. BF01 to degrade the melanoidin in the palm oil mill effluent, and generate electricity in the photosynthetic microbial fuel cell (PMFC).

Material and methods
The freshwater microalgae *Chlorella* sp. were used in a PMFC consortium for the degradation of melanoidin and electricity generation. The removal (%), electrochemical properties, and biomass recovery were monitored without adding an exogenous medium.

Results and conclusions
In this study, lipid-producing microalgae were employed as whole-cell biocatalysts in a PMFC using palm oil mill effluent as a substrate. The maximum melanoidin removal of 79.54 ± 0.45% was gained. The maximum power density reached was 3.98 ± 0.10 W/m^3. The paper presents research findings that pave the way for a practical implementation of this innovative approach on an industrial scale.

Keywords: palm oil mill effluent, decolorization, bioremediation, phycoremediation, *Chlorella* sp.

INTRODUCTION

The expansion of the oil palm agribusiness has led to the generation of significant quantities of liquid waste known as palm oil mill effluent (POME) (Waidi et al., 2021). It is projected that approximately 2.5 to 3.8 tons of palm oil mill effluent (POME) are generated for every ton of crude palm oil produced through industrial processing (Cheng et al., 2019). At present, the worldwide production of crude palm oil stands at 71.47 million tons, resulting in the generation of approximately 178.68 to 268.01 million tons of POME. Unfortunately, a significant portion of this waste is discharged into the environment without proper treatment. The presence of POME in the environment has diverse adverse effects on aquatic life, water quality, groundwater, soil, and human health (Jasni et al., 2020; Zulfahmi et al., 2021). POME exhibits a dense brownish viscous texture, an unpleasant odour, and a significant colloidal suspension, establishing it as one of the prominent hazardous pollutants (Syahin et al., 2020). The presence of POME poses adverse effects on water quality, the environment, and soil.

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Melanoidins, ranging in colour from dark brown to black, are natural condensation products resulting from non-enzymatic browning reactions known as Maillard reactions (Chandra et al., 2009). The Maillard reaction is a form of non-enzymatic browning that occurs between amino compounds and carbohydrates (Liang et al., 2009). Some study proposed a hypothesis suggesting that melanoidin may be present in anaerobically digested POME as a result of the presence of amino acids and carbohydrate compounds in the latter. Since melanoidin is a contributor to the coloration of POME, it is essential to address its presence before discharging the effluent into rivers or seawater (Zahrim et al., 2009).

Various strategies have been devised for the remediation of contaminants from POME, employing a range of physicochemical and biological treatment processes. In Chan et al. (2012), the POME was treated using anaerobic-aerobic bioreactor. This process achieved high chemical oxygen demand (COD) more than 99%. On the other hand, the POME was treated using the bacterial-fungal consortium integrated with aerobic bioreactor. The maximal COD removal of 91.06% was gained (Bala et al., 2018).

Algae-based treatment is employed for effluents originating from diverse sources, including agricultural, industrial, and domestic wastewater (Krishnamoorthy and Manickam, 2021). This process involves the cultivation of algae, leading to the production of microalgal biomass. Subsequently, this biomass undergoes conversion into valuable products, including bioenergy products, pharmaceuticals, food supplements, pigments, etc. These end products find practical applications in various fields such as animal feeding, medicine, food colourings, and fertilizer production (Kurniawan et al., 2022; Rodriguez-Rangel et al., 2022). Furthermore, certain studies have found that microalgae can serve dual purposes in both wastewater treatment and electricity generation within the context of photosynthetic microbial fuel cells (PMFC) (Greenman et al., 2019). This study aims to evaluate the potential of freshwater microalgae *Chlorella* sp. BF01 for melanoidin removal from the POME, and simultaneously for electricity generation.

MATERIAL AND METHODS

Microalgae strain and culture condition

The freshwater microalgae *Chlorella* sp. BF01 (Figure 1) was isolated from a betta fish farm located in Nonthaburi province, Central Thailand. The microalgae were preserved in BG11 medium (Sigma-Aldrich, United States) under a light intensity of 90 µmol/m^2/s, with a photoperiod cycle of 24:0 h light/dark provided by a 6500 K cool LED array.

Melanoidin synthesis

The melanoidin was prepared according to Thipraksa et al. (2022). Briefly, the stock melanoidin (29,160 mgCOD/L) contains 450 mg/L of laboratory-grade glucose, 188 mg/L of laboratory-grade glycine, and 42 mg/L of sodium bicarbonate ($NaHCO_3$). The stock melanoidin was prepared using reverse osmosis water (RO water), and the solution was heated at 95 ºC for 7 h and then cooled down to room temperature. The stock melanoidin was preserved at 4 ºC for further study.

Artificial POME preparation

The artificial POME contains 255.5 mg/L sodium bicarbonate, 21.70 mg/L potassium dihydrogen phosphate, 15.60 mg/L magnesium sulphate, 2.5 mg/L calcium chloride, and 0.10 ammonium sulphate. The solution was sterilized at 121 ºC for 15 min. The 10% (v/v) of stock melanoidin was filtrated though sterile filter paper and added before used.

Melanoidin removal

The 10% (v/v) freshwater microalgae *Chlorella* sp. BF01 (0.1 g/L dried weight biomass) was added to 90% (v/v) artificial POME. The microalgae were cultured under a light intensity of 90 µmol/m^2/s with a photoperiod cycle of 12:12 hours of light/dark to establish mixotrophic conditions for microalgae growth. Microalgae growth was monitored using UV-Vis spectrophotometry at 680 nm (Kawashima et al., 2009) every 24 h for 7 days. Microalgae biomass concentration was calculated from OD_{680}, where OD_{680} is defined as 0.19 g/L dried cell weight. The glycine-glucose melanoidin was monitored at 450 nm every 24 h for 7 days (Leung and Gue, 2006). Briefly, samples were collected and centrifuged at 12,000 rpm

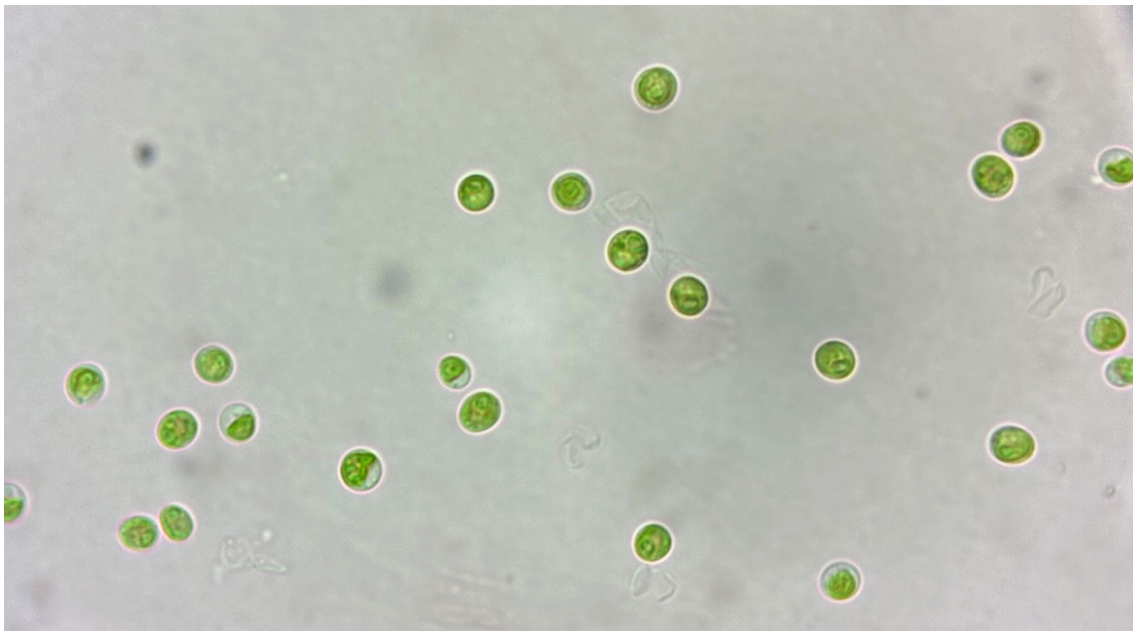

Fig. 1. The freshwater microalgae *Chlorella* sp. used in this experiment (source: Authors' own elaboration)

for 5 min, and the OD_{450} was measured. The melanoidin removal was calculated as follows, where $A_{initial}$ is the initial absorbance value at 450 nm and A_{final} is the final absorbance value at 450 nm. The residue melanoidin (mgCOD/L) was calculated using standard curve of melanoidin.

Melanoidin removal (%) = [(Ainitial – Afinal) / Ainitial] x 100 (1)

Oil extraction and oil content

The Bling and Dryer method was used for crude oil extraction from microalgae. The microalgae cell was dried at 60ºC until achieving the absolute dried biomass. A gram of biomass was added to and mixed with 12.5 mL n-hexane and soaked for 6 h under room temperature at 150 rpm in rotary shaker. The oil content was calculated as follows (Cheah et al., 2018):

Oil content (%) = [Crude oil (mg) / Sample biomass (mg)] x 100 (2)

GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) was employed to analyse the fatty acid profile. A 1 µL aliquot of the oil was introduced into a gas chromatograph equipped with a DB-Wax column (60 m x 250 µm x 0.25 µm). The injector temperature was maintained at 250 °C, with a purge flow rate of 50 mL/min for 2 minutes. The column temperature started at 50°C for 1 min, followed by a ramp-up of 25°C/min to 200°C, and then a gradual increase of 3°C/min to 230 C, held for 18 min. The total runtime for the analysis was 35 min (Martins and van Boekel, 2003; Aravind et al., 2021).

POME treatment

The POME was collected from the crude palm oil extract factory located in Phatthalung province, Southern Thailand. The POME was centrifuged at 10,000 rpm for 10 min to remove sediment, while the supernatant was collected and sterilized at 121ºC for 15 min to avoid microbe contamination.

The 10% (v/v) freshwater microalgae *Chlorella* sp. BF01 (0.1 g/L dried weight biomass) was added to 90% (v/v) sterile POME and incubated under a light intensity of 90 µmol/m^2/s with a photoperiod cycle of 12:12 hours light/dark. The melanoidin removal, microalgae growth and oil content were monitored every 24 h for 7 days.

PMFC operation

The single-chamber PMFC was constructed from an acrylic box with a 1,000 mL working volume. Aluminum plates served as electrodes and 1 mm diameter copper wire was utilized for linking between the electrodes. A 500 mL sterile sand bed functioned as a proton separator between the electrodes. During operation, 900 mL of non-sterile POME was employed as an anolyte with the POME normal flora serving as an anodic whole-cell biocatalyst for electricity generation. Additionally, a cathodic whole-cell biocatalyst consisted of 100 mL of freshwater microalgae *Chlorella* sp. BF01 (0.1 g/L dried weight biomass). The control was the PMFC without cathodic biocatalyst. The electrical properties, such as open-circuit voltage (OCV) and closed-circuit voltage (CCV), were measured to calculate the current density (CD) and power density (PD). A polarization curve was then constructed. The PMFC was operated for 21 days.

Statistical analysis

The average and the standard deviation (mean ± SD) were analysed using IBM SPSS Statistics software version 29.0.10 (IBM, United States).

RESULTS AND DISCUSSION

Growth potential, Melanoidin removal and oil content

In our study, we achieved the maximum cell concentration of 0.093 ± 0.07 g/L (equivalent to 93.29 ± 6.54 mg/L), and a melanoidin removal of 76.77 ± 3.47% (Figure 2). This was accomplished through the cultivation of the freshwater microalgae *Chlorella* sp. BF01 in artificial POME with an initial melanoidin concentration of 2,916 mgCOD/L for the duration of 168 h (7 days). The experiment was carried out under room temperature and without exogenous supplement or commercial gas adding. Furthermore, we obtained the maximum oil content of 22.33 ± 0.67% (equivalent to 3.19% oil/day) from *Chlorella* sp. BF01 when cultured in artificial POME for 7 days (Figure 2).

On the other hand, the melanoidin content in the artificial vinasse digestate has been observed to inhibit the survival rate of *Chlorella pyrenoidosa*. In this study, the zeolite was introduced to the microalgae growth conditions to enhance the specific growth rate. Furthermore, the maximum melanoidin removal of 70% was achieved where with the operation lasting 15 days (Budianto et al., 2023). In the study of Ali et al. (2023), the coagulation-flocculation method and microalgae-bioremediation were employed to treat melanoidin in digested distillery wastewater. Specifically, 3 g/L of ferric chloride and 6 mg/L of polyaluminum chloride were added. The study reported the maximum melanoidin removal of 85%.

Fatty acid profile and HPLC analysis

In our study, we observed the production of hexadecanoic acid methyl ester or methyl palmitate, a saturated fatty acid (Figure 3) from the melanoidin compound in artificial POME through the action of freshwater microalgae *Chlorella* sp. BF01.

In the study of Palanisamy et al. (2020), a 30% (v/v) concentration of POME was utilized for cultivating freshwater microalgae and producing fatty acids. The study reported the maximum dried biomass of 1.56 g/L with the corresponding 39.1% oil content. The GC-MS analysis identified various fatty acids, including linoleic acid, oleic acid, palmitic acid, stearic acid, elaidic acid, and α-linoleic acid. Notably, while fatty acid composition was analysed, melanoidin removal was not investigated in this study. Conversely, the research observed a saturated fatty acid content ranging from 17% to 54.9% in the biomass of freshwater microalgae, specifically *Chlorella vulgaris* and *Chlorella sorokiniana*-I. These microalgae were cultivated in ethanol-spent water contaminated with melanoidin, a by-product discharged from the ethanol industry (Kookal et al., 2023).

POME treatment

In our investigation, the freshwater microalgae *Chlorella* sp. BF01 was cultured in POME under room temperature conditions and without the addition of exogenous chemicals for the duration of 7 days. The result shows the maximum growth rate of 0.097 ± 0.033 g/L (equivalent to 97.21 ± 3.34 mg/L). During this period, the microalgae achieved a maximal melanoidin removal of 79.54±0.45% and oil content of 22.73 ± 0.55% (equivalent to 3.24% of oil/day) (Figure 4).

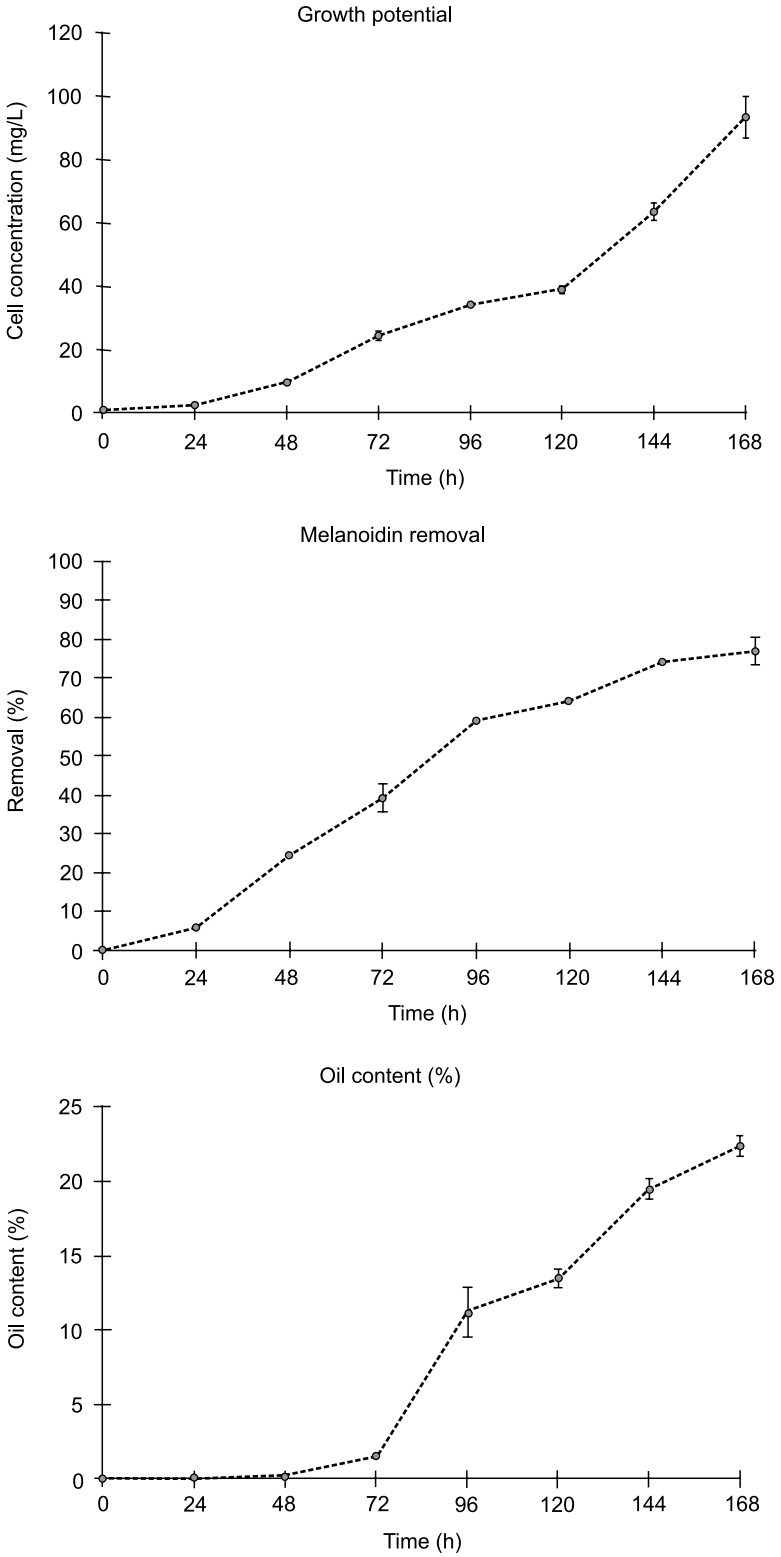

Fig. 2. The growth potential, melanoidin removal, and oil content of microalgae in artificial POME (source: Authors' own elaboration)

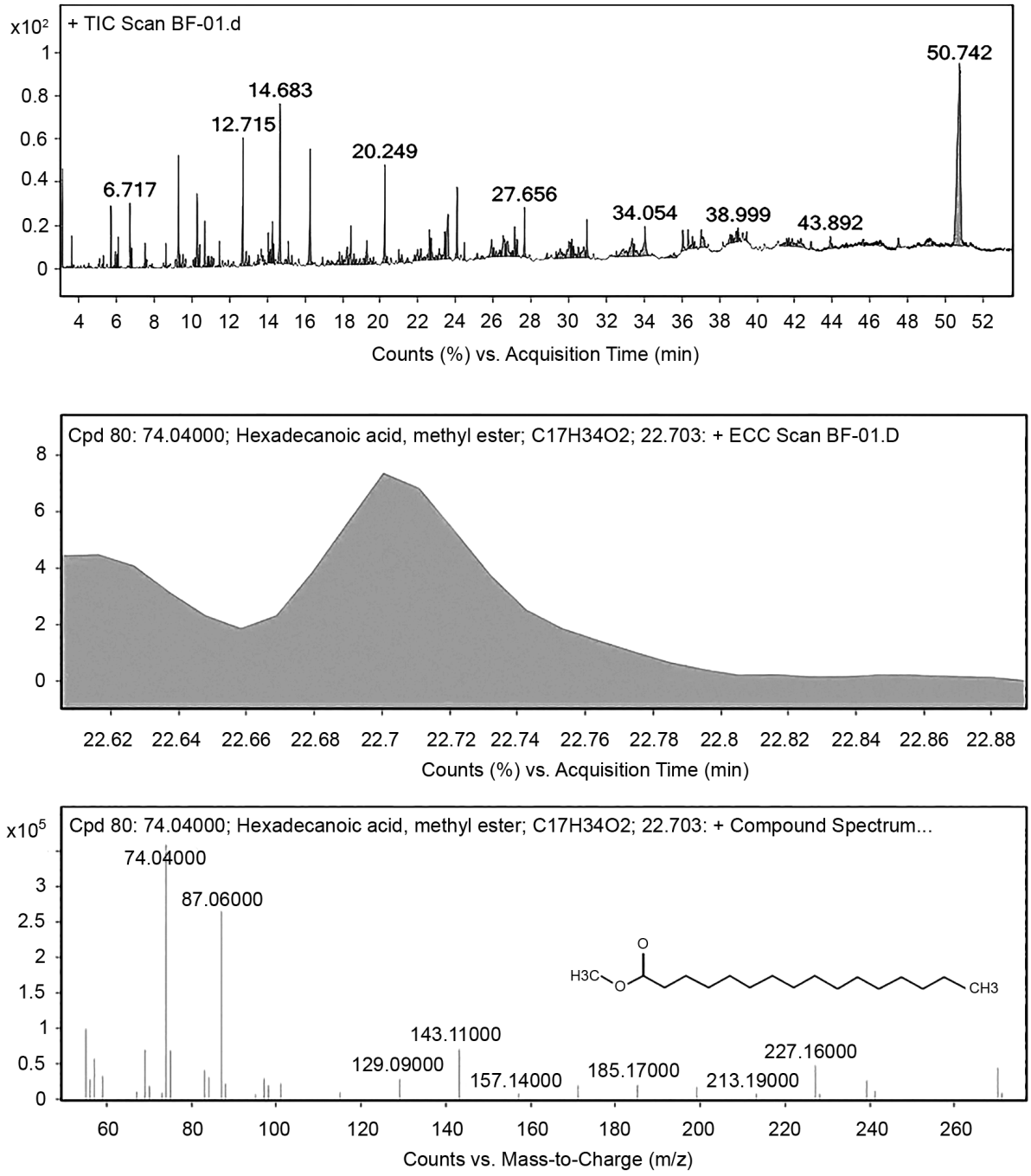

Fig. 3. The fatty acid profile of microalgae in artificial POME (source: Authors' own elaboration)

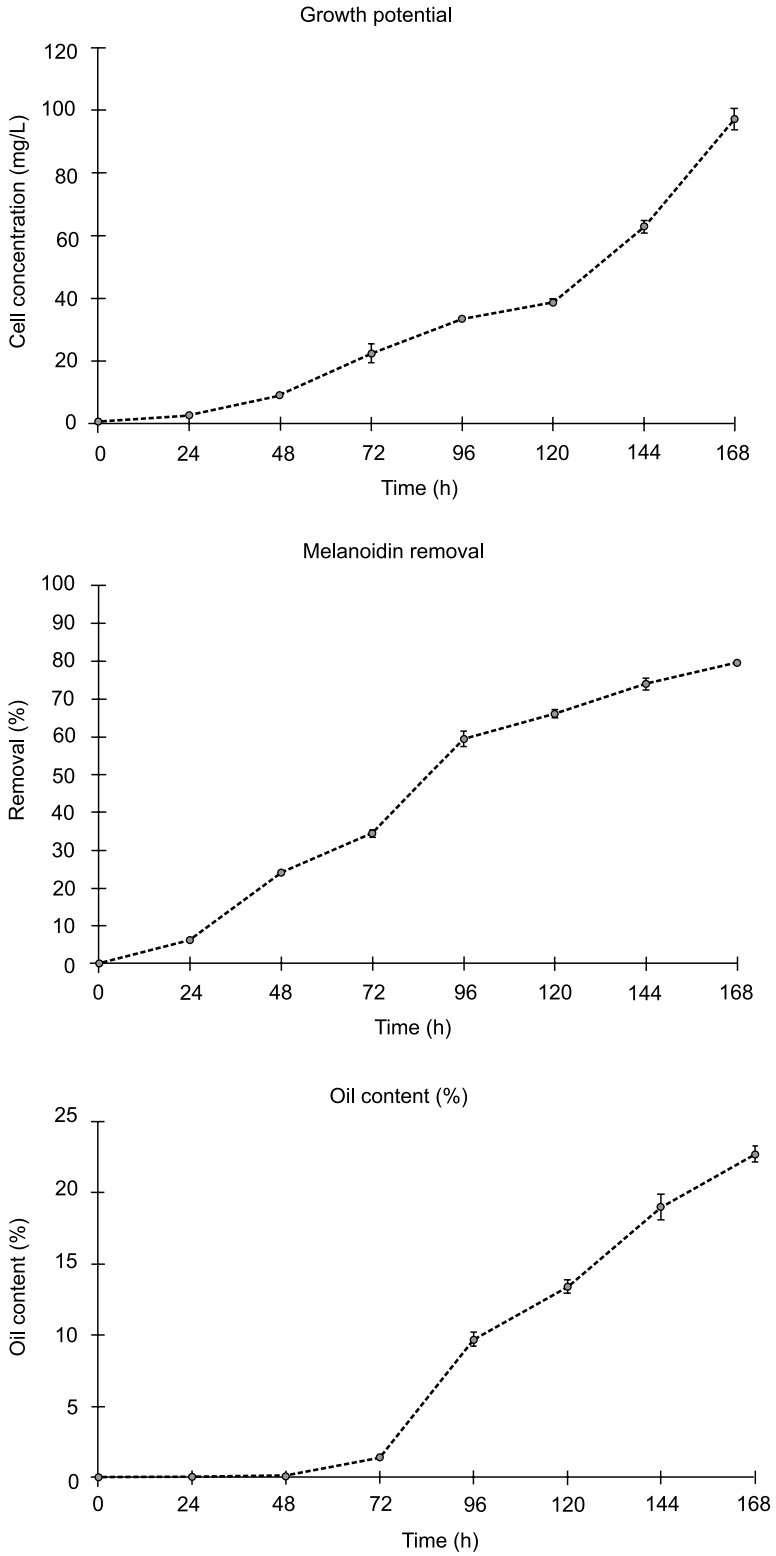

Fig. 4. Growth potential, melanoidin removal and oil content of microalgae *Chlorella* sp. BF01, cultured in POME (source: Authors' own elaboration)

In the study by Low et al., the freshwater microalgae *Chlorella pyrenoidosa* demonstrated the ability to thrive in POME, achieving 71% nutrient removal and 68% lipid production (3.4% of oil/day) during a 20-day operation (Low et al. 2021). However, melanoidin removal has not detected. Conversely, *Nannochloropsis oculata* and *Tetraselmis suecica* microalgae were cultured in an alternative medium supplemented with 10% (v/v) POME and cultivated for 16 days in flask cultivation. Notably, *Nannochloropsis oculata* exhibited the maximum oil content of 39.10 ± 0.73%, while *Tetraselmis suecica* demonstrated oil content of 27.0 ± 0.61%. This highlights the potential variability in oil production among different microalgae species when exposed to POME supplementation (Shah et al., 2016).

Electrochemical properties

In the present study, microalgae were employed as a whole-cell biocatalyst to enhance electricity generation in the PMFC. The maximum OCV of 0.40 ± 0.11 V was achieved in the microalgae-based PMFC, whereas the control (without microalgae) yielded only 0.17 ± 0.04 V. Polarization curve of microalgae-based PMFC exhibited the maximum CD and PD of 27.58 ± 0.10 A/m³ and 3.98 ± 0.10 W/m³, respectively (Figure 5). Electrical properties of microalgae-based PMFC were compared in Table 1.

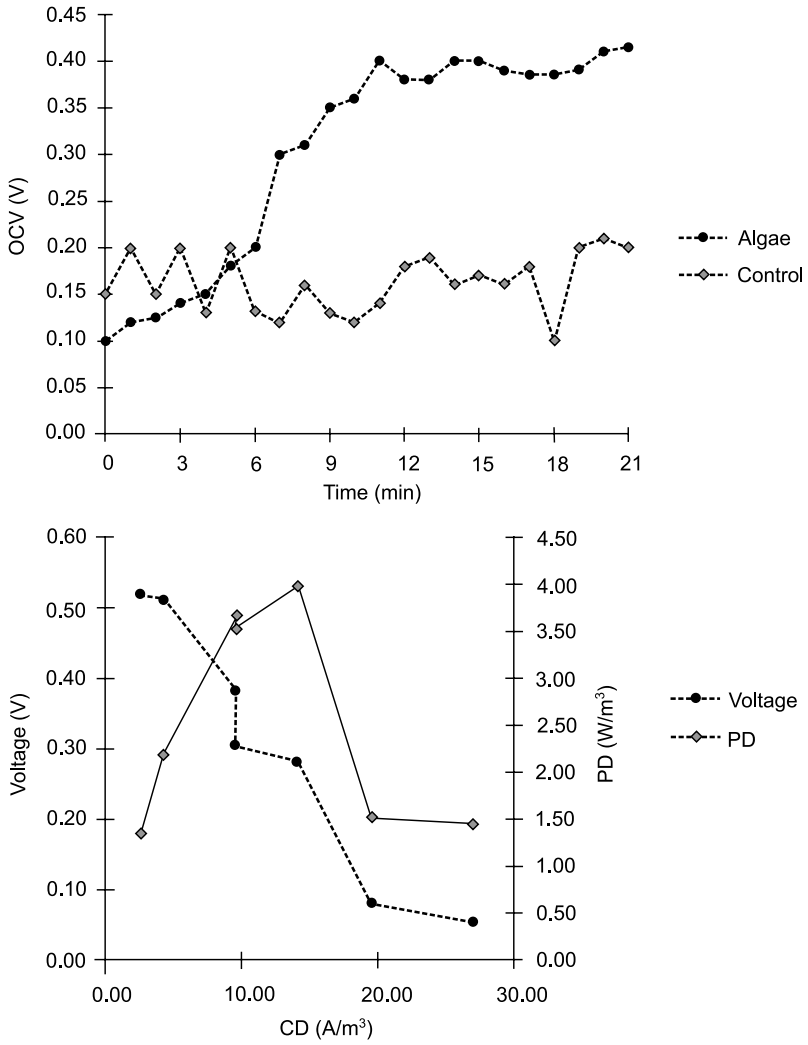

Fig. 5. Electrochemical properties of microalgae-based PMFC (source: Authors' own elaboration)

Table 1. Electrochemical properties of microalgae-based PMFC (source: Authors' own elaboration)

Species	MFC type	PD (W/m^3)	Reference
Chlorella sp. BF01	Single chamber	3.98±0.10	This study
Chlorella vulgaris	Dual chamber	3.72	Zhang et al. (2019)
Scenedesmus acutus	Dual chamber	0.40	Angioni et al. (2018)
Chlorella vulgaris	Multi chamber	1.10	Kakobian and Gude (2015)
Chlorella vulgaris	Dual chamber	2.80	Pei et al. (2018)

In a study by Makhtar and Tajarudin (2020), a membrane-less microbial fuel cell (ML-MFC) was employed to generate electricity from POME. The obtained maximum power density (PD) was 0.02 W/m^2. On the other hand, a high power generation of 0.5 W/m^2 was achieved using a single-chamber microbial fuel cell (MFC) with approximately 90% COD removal efficiency (Sarmin et al., 2021).

CONCLUSIONS

In this study, the whole-cell biocatalyst of *Chlorella* sp. BF01 demonstrated promising potential for electricity generation in the single-chamber PMFC. The maximum CD and PD achieved were 27.58 ± 0.10 A/m^3 and 3.98 ± 0.10 W/m^3, respectively, accompanied by the highest melanoidin removal (79.54 ± 0.45%) and oil content reduction (22.73 ± 0.55%). These findings contribute new insights into the utilization of melanoidin-consuming freshwater microalgae in conjunction with a single-chamber PMFC for electricity generation and the treatment of POME.

ACKNOWLEDGEMENT

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WYKORZYSTANIE MIKROALG ZUŻYWAJĄCYCH MELANOIDYNY W PRODUKCJI ENERGII ELEKTRYCZNEJ ORAZ W OCZYSZCZANIU ŚCIEKÓW ZA POMOCĄ FOTOSYNTETYCZNEGO MIKROBIOLOGICZNEGO OGNIWA PALIWOWEGO

ABSTRAKT

Cel pracy
W badaniach wykorzystano mikroalgi słodkowodne *Chlorella* sp. BF01 do rozkładu melanoidyny w ściekach z tłoczni oleju palmowego oraz do wytwarzania energii elektrycznej w fotosyntetycznym mikrobiologicznym ogniwie paliwowym (PMFC).

Materiał i metody
Mikroalgi słodkowodne *Chlorella* sp. zostały wykorzystane w ogniwie PMFC do degradacji melanoidyny i do wytwarzania energii elektrycznej. Monitorowano procent unieszkodliwiania melatonoidyny, właściwości elektrochemiczne, a także odzysk biomasy bez dodatku pożywki egzogennej.

Wyniki i wnioski
W opisanych badaniach mikroalgi wytwarzające lipidy zostały zastosowane w funkcji biokatalizatorów pełnokomórkowych w ogniwie PMFC, zaś jako substrat użyto ścieków z tłoczni oleju palmowego. Uzyskano maksymalną neutralizację melanoidyny na poziomie 79,54 ± 0,45%. Maksymalna osiągnięta gęstość mocy wyniosła 3,98 ± 0,10 W/m^3. W badaniu zaprezentowano wnioski, które torują drogę do praktycznego wdrożenia tego innowacyjnego podejścia na skalę przemysłową.

Słowa kluczowe: ścieki z rafinerii oleju palmowego, odbarwianie, bioremediacja, phykoremediacja, fitoremediacja, *Chlorella* sp.