

Melanoidin degradation and bioelectricity generation from palm oil mill effluent (POME) using fungal-based microbial fuel cell

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Abstract

Melanoidin is the primary pigment responsible for the dark brown color of various agricultural wastewaters including palm oil mill effluent (POME), presents a significant challenge for degradation due to its antimicrobial properties. This study focused on enriching and selecting a laccase-producing fungal consortium specifically for melanoidin degradation in real POME. Following selection, the consortium community was identified using next-generation sequencing. To simultaneously recover bioelectricity during the degradation process, an upflow microbial fuel cell (MFC) was integrated. The analysis revealed that the consortium TC, predominantly composed of *Candida tropicalis*, followed by *Pichia* sp., *Issatchenkia orientalis*, and *Candida orthopsilosis*, demonstrated the highest laccase activity (5.22 ± 0.07 U/L) and achieved significant melanoidin degradation ($94.70 \pm 0.26\%$). Furthermore, the integrated MFC system yielded a maximum open circuit voltage (OCV) of 0.534 ± 0.031 V and a maximum power density (PD) of 77.84 ± 2.78 mW/m². These findings highlight a novel approach for the concurrent removal of recalcitrant melanoidin from POME and the generation of bioelectricity using a tailored fungal consortium within an MFC system.

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

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Introduction

Renewable energy is increasingly crucial for global development due to its minimal environmental impact. Studies indicate that renewable energy sources now account for approximately 20% of global energy consumption and continue to grow (Jaroenkiekajorn et al., 2021). Biodiesel is a prominent renewable energy option because it produces lower greenhouse gas emissions than petro-diesel, thereby mitigating environmental harm (Silatertruksa and Gheewala, 2012). As a result of growing factors, global biodiesel demand is anticipated to climb to 2.3 million barrels per day by 2040 (Prapasongsa et al., 2017). Global palm oil demand is steadily increasing, driven by both the biodiesel industry and its use as a vegetable oil in the food sector. In 2017, global oil palm production reached approximately 320 million tons with a projected trend of further expansion in the future (Khatun et al., 2017). During crude palm oil production, approximately 2.5 to 7.5 tons of palm oil mill effluent (POME) are discharged per ton of crude palm oil produced (Ahmad et al., 2003). Therefore, the production of 320 million tons of crude palm oil results in the release of approximately 800 million to 2.4 billion tons of POME.

POME is a dark brown, odorous wastewater characterized by a high water content of 95-96%, a total solid of 4-5%, and residue oil of 0.6-0.7%. It also contains suspended insoluble particles (Mohammad et al., 2021). The dark color of POME is primarily attributed to melanoidin, a biopolymer produced by the Maillard reaction (Thipraksa et al., 2022). Several physical-chemical processes, including electrocoagulation, oxidation, dialysis, and the electro-Fenton process have been utilized to remove color from POME (Muddemann et al., 2019). Nevertheless, the substantial operational expenses associated with these methods have led to increased focus on biological treatment alternatives. Tsiakiri et al. (2020) utilized the yeast *Saccharomyces cerevisiae* for the degradation of melanoidin wastewater. Their results demonstrated that 80% melanoidin removal was achieved when the yeast cells were inoculated and cultured for 48 hr. On the other hand, the white rot fungus *Megasperoporia* sp. has been used for melanoidin removal from ethanol industrial wastewater. The results showed that a maximum melanoidin removal of 48% was achieved (Toomsan et al., 2020).

Laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) is a family of multicopper oxidases widely distributed among bacteria, plants, and fungi (Mate and Alcalde, 2017). It is distinguished by their ability to act on a broad range of substrates and effectively degrade persistent environmental pollutants. This is achieved through their capacity to oxidize a variety of phenolic and non-phenolic compounds coupled with the reduction of molecular oxygen to water (Guan et al., 2018). These enzymes offer significant benefits and is employed in a multitude of applications like, bio-sensors, bioremediation, dye decolorization and food industries (Mate and Alcalde, 2017). Previous studies have demonstrated that microbial laccases can effectively remove color from POME (Aka et al., 2021).

Microbial fuel cells (MFC) represent a cutting-edge technology that utilizes microorganisms to convert organic matter into electricity, showcasing significant potential within the domain of sustainable energy solutions (Sonawane et al., 2024). MFCs have demonstrated effectiveness in treating a range of wastewaters, such as swine wastewater (Huang et al., 2024), phenol wastewater (Chen et al., 2024), saline wastewater (Zhao et al., 2024), and cosmetic industrial wastewater (Pugazhendi et al., 2025). No previous study has used an MFC integrated with a laccase-producing fungal consortium for melanoidin removal and simultaneous electrical energy generation as a by-product.

This study aims to select a laccase-producing fungal consortium with melanoidin degradation ability and utilize it for decolorization of palm oil mill effluent and bioelectricity generation in a microbial fuel cell.

Material and Methods

Melanoidin synthesis

Melanoidin was synthesized according to a previous study (Thipraksa et al., 2022). Briefly, 4.50 g of glucose anhydrous, 1.88 g of glycine, and 0.42 g of sodium bicarbonate were dissolved in 100 mL of sterile deionized water (DI). The solution was heated at 95 °C for 7 hr on a hot plate. After cooling, 100 mL of DI water was added. All chemicals were purchased from Himedia company, India.

Sample collection and selection

Soil and decayed plant biomass samples were collected using aseptic techniques and placed in sterile

plastic bags. All samples were then immediately transported to the laboratory in the Faculty of Science and Digital Innovation, Thaksin University, Phatthalung Campus, Thailand. For selection, 1 g of each sample was inoculated into 1% (v/v) melanoidin (3 gCOD/L) in sterile 0.1 M phosphate buffer, pH 7.45 (Sigma-Aldrich, United States).

The samples were incubated at 30 °C for 3 days under static conditions. Subsequently, all samples were transferred into 0.1 M phosphate buffer, pH 7.45 containing 3 gCOD/L melanoidin and incubated for 10 successive transfers to ensure the selected consortium could utilize melanoidin as a carbon source.

Laccase activity

For laccase activity determination, 1 mL of the consortium was inoculated into 9 mL of phosphate buffer containing melanoidin and incubated at 30 °C for 3 days under static conditions. The samples were collected and centrifuged at 12,000 rpm for 10 min using a Microspin 12 High-speed Mini-centrifuge (Biosan Laboratories Inc., United States). The supernatants were collected. Laccase activity was measured using the ABTS assay, following a modified method from a previous study (Muhammad et al., 2024). The reaction volume was 1.0 mL of 100 µM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in 5.0 mM sodium acetate buffer, pH 4.0 (93.047 mg/L sodium acetate and 232.1 mg/L acetic acid). The 10 µL of the crude enzyme was used. The solution was incubated for 1 min. An extinction coefficient of 29,300 M⁻¹cm⁻¹ was used to quantify substrate oxidation. The reaction was monitored at a wavelength of 420 nm.

$$\text{Laccase activity (U/L)} = (\Delta A)(Vt)(Df)(106) / (t)(\epsilon)(d)(V_s) \quad \text{---- (1)}$$

Where ΔA is the final absorbance – initial absorbance, V_t is the total volume (1 mL), D_f is the dilution factor, t is the reaction time (1 min), ϵ is the extinction coefficient (29,300 M⁻¹cm⁻¹), d is the optical path (1 cm), V_s is the crude enzyme volume (0.01 mL) and 10⁶ is the correction factor (µmol/mol).

Melanoidin degradation

The 1 mL of the consortium was inoculated into 9 mL of 0.1 M phosphate buffer, pH 7.45 containing 3 gCOD/L melanoidin and incubated at 30 °C for 3 days under static conditions. The samples were then collected and centrifuged at 12,000 rpm for 10 min.

Melanoidin removal was determined using an SP-UV500 UV-Vis spectrophotometer (PerkinElmer, United States) at wavelengths between 450-470 nm (Chaijak et al., 2024). The melanoidin removal was calculated and the consortium with the highest removal was selected for subsequent experiments.

$$\text{Melanoidin removal (\%)} = [(AB_{\text{before}} - AB_{\text{after}}) / AB_{\text{before}}] \times 100 \quad \text{----- (2)}$$

Where AB_{before} is the initial absorbance value of melanoidin solution and AB_{after} is the final absorbance value of melanoidin solution.

Metagenome analysis

The fungal consortium was analyzed using MacroGen metagenome analysis (MacroGen, South Korea). The ITS region was amplified and sequenced to analyze the fungal community, utilizing the ASV (DADA2) analysis tool with the UNITE_Fungi (VSEARCH) database.

Effect of melanoidin concentration

Melanoidin concentrations ranging from 2, 4, 6, 8, and 10 gCOD/L were used to study the effect of melanoidin concentration in wastewater. The solutions were incubated at 30 °C for 3 days under static conditions. Subsequently, melanoidin removal was determined.

POME and decolorization

The POME was collected from an oil extraction factory located in Phatthalung province, Southern Thailand. The sample was filtered through sterile medical gauze 2-3 times to remove sediment and stored at -25 °C in a freezer (Hitachi, Japan) to preserve wastewater quality until use.

The 1 mL of the consortium was inoculated into 9 mL of POME and incubated at 30 °C for 7 days under static conditions. The sample was collected every 24 hr and centrifuged at 12,000 rpm for 10 min. Melanoidin removal was determined.

MFC setup and electrochemical properties

An upflow microbial fuel cell (upflow MFC) was constructed as depicted in Figure 1. The anodic chamber consisted of a 250 mL plastic beaker, while the cathodic chamber was formed using a plastic funnel. The working volume of the MFC was 100 mL. The 4 cm² copper and aluminum plates were served as the electrodes. A proton exchange membrane (PEM)

was created using 0.1% (w/v) KCl in 1.5% (w/v) agarose gel. For operation, 10 mL of a microbial consortium was inoculated into 90 mL of POME and incubated at 30 °C. The open-circuit voltage (OCV) was monitored every 6 hr for 3 days using a digital multimeter. The closed-circuit voltage (CCV) was measured using different external resistive loads ranging from 300 to 5,000 Ω . Electrochemical properties were calculated using equations (3) - (6).

$$I = V / R \quad \text{--- (3)}$$

$$CD = I / A \quad \text{--- (4)}$$

$$P = I \times V \quad \text{--- (5)}$$

$$PD = P / A \quad \text{--- (6)}$$

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

The upflow MFC was scaled up to working volumes of 100 - 500 mL. The maximal CD and PD were subsequently calculated.

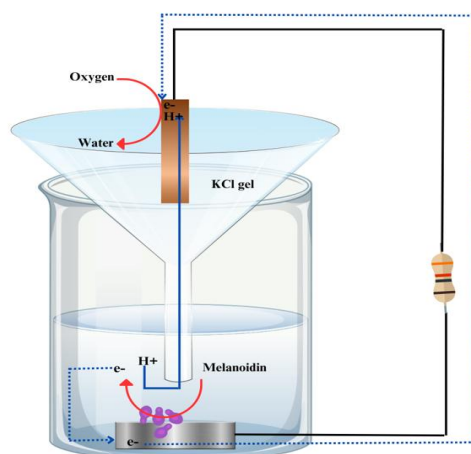


Figure-1. Schematic of the upflow microbial fuel cell (upflow MFC). The anode and cathode chambers were made from a 250 mL plastic beaker and a plastic funnel, respectively.

Results and Discussion

Laccase activity

The crude enzyme was collected from the incubated medium supplemented with melanoidin after removing the cell pellet. The TC fungal consortium exhibited the maximum laccase activity with 5.22 ± 0.07 U/L as shown in Figure 2.

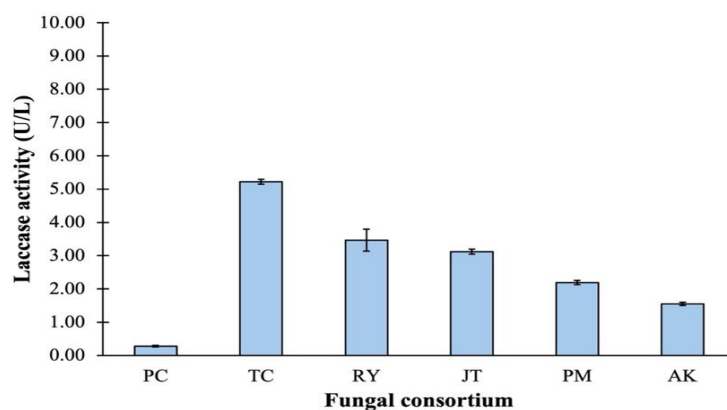


Figure-2. Laccase activity (U/L) of different fungal consortia (PC, TC, RY, JT, PM, and AK). Crude enzymes were collected from melanoidin-supplemented medium after cell removal.

In Liang et al. (2020), laccase was produced by the fungal strain *Pycnoporus* sp. W-9, which was isolated from rose dregs. The maximum laccase activity reached 12.20 U/mL and the enzyme was used for phenol degradation. Moreover, laccase-producing fungi have been isolated from decaying wood, leaf compost, and soil samples collected from forest plantations in India. The results showed that the basidiomycete *Hexagonia hirta* MSF2 exhibited the highest laccase activity, reaching 1944 U/mL (Kandasamy et al., 2016).

On the other hand, the laccase-producing fungus was isolated from the soil sample. The highest laccase production was observed in the fungal strain *Curvularia lunata* MY3 (Hamed et al., 2024). In Eltoukhy et al. (2025), a laccase-producing fungus was isolated from a soil sample. The fungal strain

exhibiting the highest laccase activity was identified as *Paraconiothyrium brasiliense*. Furthermore, this strain demonstrated maximum degradation of bisphenol. Moreover, the laccase-producing endophytic fungus *Trichoderma harzianum* AUMC14897 was isolated from the plant *Opuntia ficus-indica* and used for decolorization of wastewater (Salem et al., 2024).

Melanoidin degradation

Melanoidin degradation was determined from the supernatant using UV-Vis spectrophotometry at 470 nm and the melanoidin removal was calculated. The TC fungal consortium achieved a maximum melanoidin removal of $70.03 \pm 0.93\%$ (Figure 3).

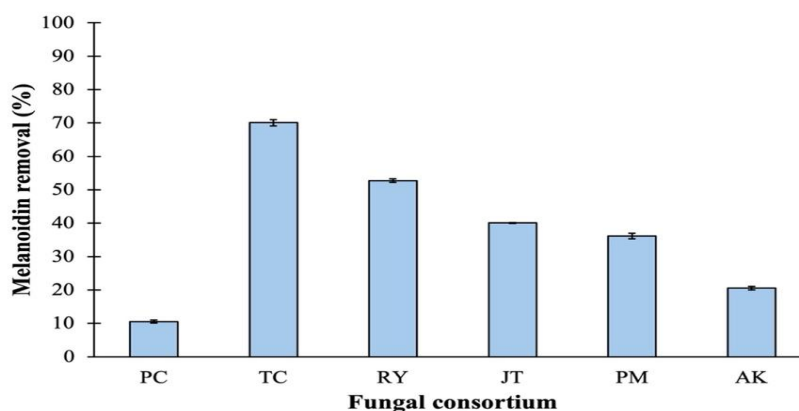


Figure-3. Melanoidin removal (%) by different fungal consortia (PC, TC, RY, JT, PM, and AK). Removal was measured from the supernatant at 470 nm using UV-Vis spectrophotometry.

In Akhtar et al. (2024), the melanoidin pigment formed during the Maillard reaction was removed from ethanol distillery wastewater using iron oxide nanoparticles under the Fenton oxidation process. However, this process still incurs a high operating cost. Furthermore, melanoidin pigment has been removed from molasses wastewater by coagulation-flocculation processes. A maximum melanoidin removal of 60.20% was achieved when ZnO was used as a chemical catalyst (Khalik et al., 2024).

On the other hand, micro-electrolysis combined with bioremediation has been used for melanoidin removal from molasses wastewater. Ligninolytic fungi were used as whole-cell biocatalysts, achieving a maximum melanoidin removal of 97.10% (Chen et al., 2016).

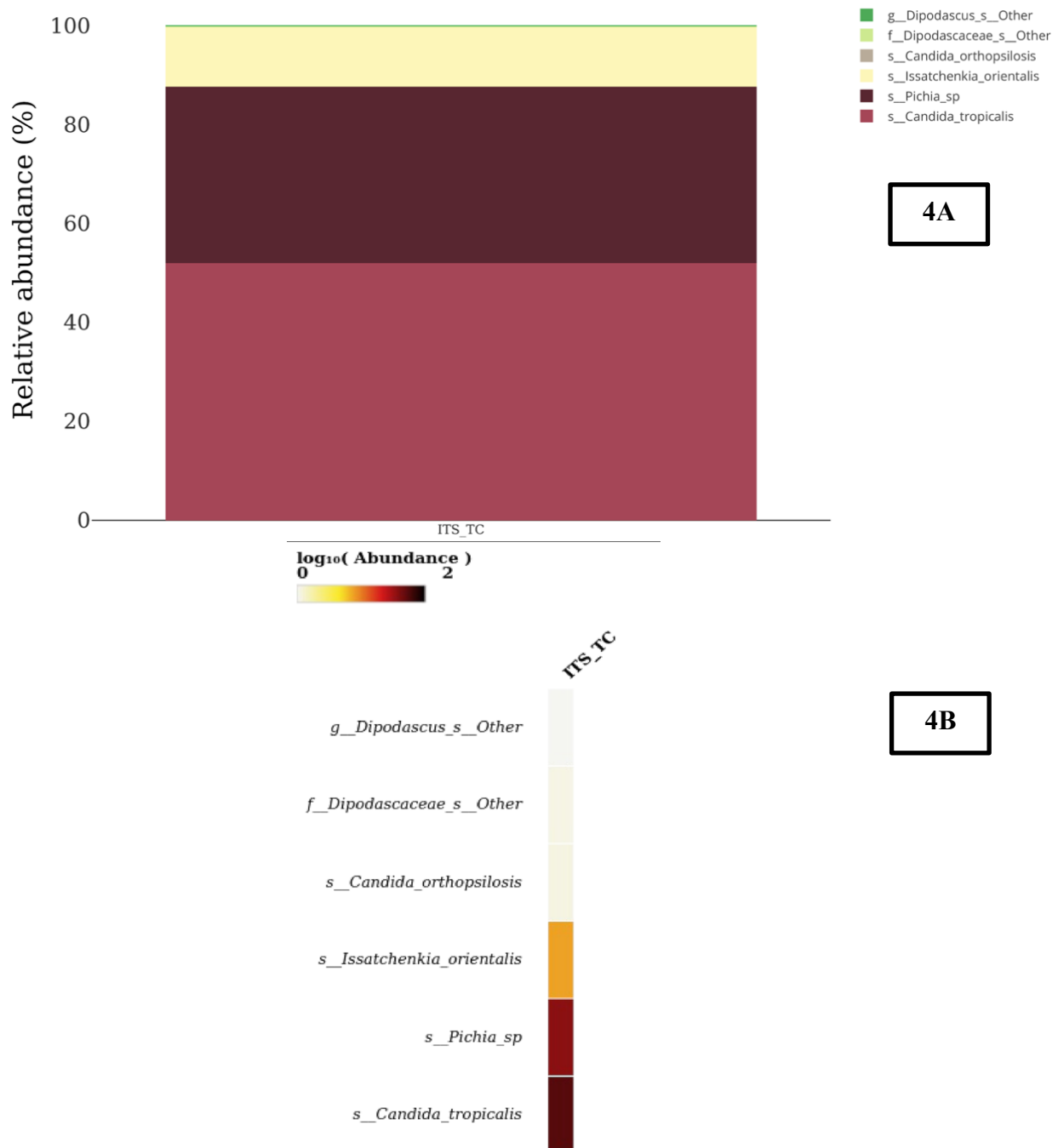
Moreover, biochar derived from *Leucaena leucocephala* plant waste has been used for the removal of melanoidin from ethanol distillery wastewater. Maximum melanoidin removal of 90.00% was achieved when the system was incubated at an initial soluble pH of 2.0, an agitation speed of 100 rpm, and a temperature of 65 °C (Insoongnoen et al., 2020).

Metagenome analysis

A metagenomic study of the TC fungal consortium, targeting the ITS region was performed. The microbial community profile of the TC consortium is illustrated in Figure 4. The diversity index was showed in Table 1.

Table-1. Diversity index of the TC consortium.

| Sample ID | Shannon | Gini-Simpson | PD_whole_tree | ASVs |
|-----------|---------------|----------------|---------------|------|
| ITS_TC | 1.64038044427 | 0.606409675401 | 0.750519217 | 11 |

**Figure-4.** The microbial communities of TC fungal consortium (4A) and the heatmap of species (4B).

The metagenomic analysis of the TC fungal consortium indicated a dominant presence of specific taxonomic groups. The phylum Ascomycota represented 51.98% of the community, with the remaining 48.02% belonging to other phyla. Similarly, the class Saccharomycetes, the order Saccharomycetales, the family Saccharomycetales_fam_Incertae_sedis and the genus *Candida* each constituted 51.98% of their respective taxonomic levels, while the remaining 48.02% consisted of other members.

The data indicated that the TC fungal consortium was primarily composed of *Candida tropicalis*, followed by *Pichia* sp., *Issatchenkia orientalis*, *Candida orthopsilosis* and other species.

On the other hand, a laccase-producing consortium was enriched from a decaying wood sample gathered from a mountain in China. A maximum laccase activity of 66.83 U/L was achieved. This consortium

comprised *Cladosporium bruhmei* SP8, *Aspergillus terreus* HJ4, and *Cladosporium bruhmei* LZ10 (Hu et al., 2025). However, it does not exhibit decolorization properties. In Mendes et al. (2022), the fungi *Candida* and *Yarrowia* spp. isolated from activated sludge of a wastewater treatment plant were used for decolorization of textile effluent. Furthermore, the fungus *Issatchenkia orientalis* isolated from activated sludge has been found to degrade textile azo dyes from wastewater (Jafari et al., 2014).

Effect of melanoidin concentration

Various concentrations of melanoidin solution, ranging between 2 and 10 gCOD/L were used. The maximum melanoidin removal of $75.02 \pm 0.08\%$ was achieved with an initial melanoidin concentration of 2 gCOD/L. The results are shown in Figure 5.

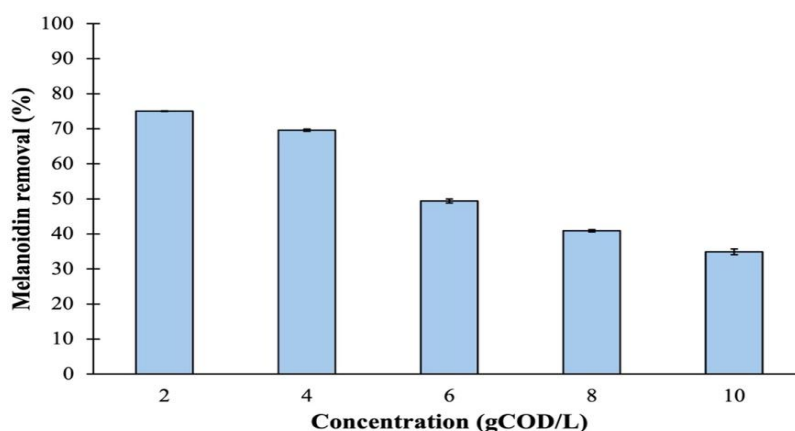


Figure-5. Effect of initial melanoidin concentration (2–10 gCOD/L) on melanoidin removal (%).

Decolorization

Raw POME was used for the study of melanoidin pigment degradation in real wastewater. The initial melanoidin concentration of 3.25 ± 0.05 gCOD/L was measured before use by calculation using a standard curve. Melanoidin degradation was monitored every 24 hr for 7 days of operation. The maximum melanoidin degradation of $94.70 \pm 0.26\%$ was observed (Figure 6).

In contrast, the photocatalytic process using NiCaO as a catalyst effectively degraded melanoidin pigments. However, the high cost of the catalyst remains a significant limitation for large-scale applications (Noorsham et al., 2023). Advanced oxidation processes (AOPs), such as sono-Fenton and photo-

Fenton have been explored for melanoidin removal. Watcharenwong et al. (2023) demonstrated that these processes, enhanced by UV light and ultrasonic waves, promote the breakdown of H_2O_2 into hydroxyl radicals leading to improved decolorization of melanoidin in ethanol wastewater. These methods offer promising results but may require optimization for practical implementation.

Biological approaches, including the use of white-rot fungi like *Ganoderma* sp., have been investigated for decolorization of POME. Rittibud et al. (2024) reported a 47.7% color removal efficiency. However, melanoidin removal was not specifically addressed in their study. This suggests that while fungi can contribute to color removal, their effectiveness in

melanoidin degradation may vary and warrants further investigation.

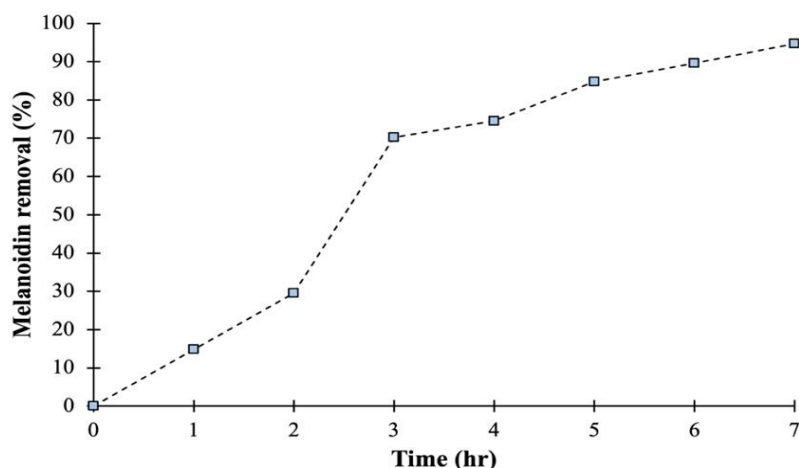


Figure-6. Melanoidin degradation (%) in raw POME (initial concentration 3.25 ± 0.05 gCOD/L) monitored over 7 days.

Electrochemical properties

The dual chamber MFC was used for recovery of the electrical energy from the melanoidin degradation. The OCV was monitored every 6 hr for 3 days. The OCV of the dual chamber MFC was showed in the Figure 7. The maximum OCV of 100 mL working volume MFC was 0.534 ± 0.031 V while the control yielded 0.323 ± 0.028 V. The maximum OCV of 200 mL working volume MFC was 0.564 ± 0.099 V while

the control yielded 0.326 ± 0.030 V. The maximum OCV of 300 mL working volume MFC was 0.411 ± 0.061 V while the control yielded 0.261 ± 0.087 V. The maximum OCV of 400 mL working volume MFC was 0.306 ± 0.002 V while the control yielded 0.141 ± 0.034 V. The maximum OCV of 500 mL working volume MFC was 0.433 ± 0.062 V while the control yielded 0.274 ± 0.018 V. The electrochemical properties of the dual-chamber MFC are shown in Table 2.

Table-2. The electrochemical properties of the dual-chamber MFC using the POME as substrate.

| Working volume (mL) | CCV* (V) | Current (mA) | Power (mW) | CD** (mA/m ²) | PD** (mW/m ²) |
|---------------------|-------------------|-------------------|-------------------|---------------------------|---------------------------|
| 100 | 176.43 ± 3.14 | 0.176 ± 0.003 | 0.031 ± 0.001 | 441.08 ± 7.84 | 77.84 ± 2.78 |
| 200 | 98.90 ± 0.36 | 0.099 ± 0.000 | 0.010 ± 0.000 | 247.25 ± 0.902 | 24.45 ± 0.18 |
| 300 | 66.50 ± 0.50 | 0.067 ± 0.000 | 0.006 ± 0.000 | 166.25 ± 1.250 | 11.06 ± 0.17 |
| 400 | 47.13 ± 2.06 | 0.047 ± 0.002 | 0.002 ± 0.000 | 117.83 ± 5.14 | 5.56 ± 0.49 |
| 500 | 33.20 ± 0.72 | 0.033 ± 0.000 | 0.001 ± 0.000 | 83.00 ± 1.80 | 2.76 ± 0.12 |

* at the external resistance of 1,000 Ω

** at the electrode surface area of 0.0004 m²

In Thipraksa et al. (2022), a maximum PD of 80 mW/m² was achieved using a dual-chamber MFC integrated with a bacterial consortium containing *Citrobacter werkmanii*, *Enterococcus faecalis*, and *Escherichia fergusonii*. This system also resulted in

86.02% melanoidin removal. Nevertheless, prior research has identified *C. werkmanii* as a uropathogenic pathogen carrying drug-resistant genes that could be harmful to human health (Parvez et al., 2020).

On the other hand, the anion-exchange MFC has been used for electricity generation from POME. The results indicated a maximum power PD of 180 mW/m² was achieved alongside a decrease in the chemical oxygen demand (COD) of wastewater. However, no report showed color or melanoidin removal (Nor et al., 2024). In Ng et al. (2024), an activated carbon coated

anode MFC was used for electrical energy recovery from POME treatment. A maximum PD of 504.10 mW was achieved per 1 m³ of wastewater. However, this system requires immobilization of powdered activated carbon on the electrode before use, resulting in high operating costs.

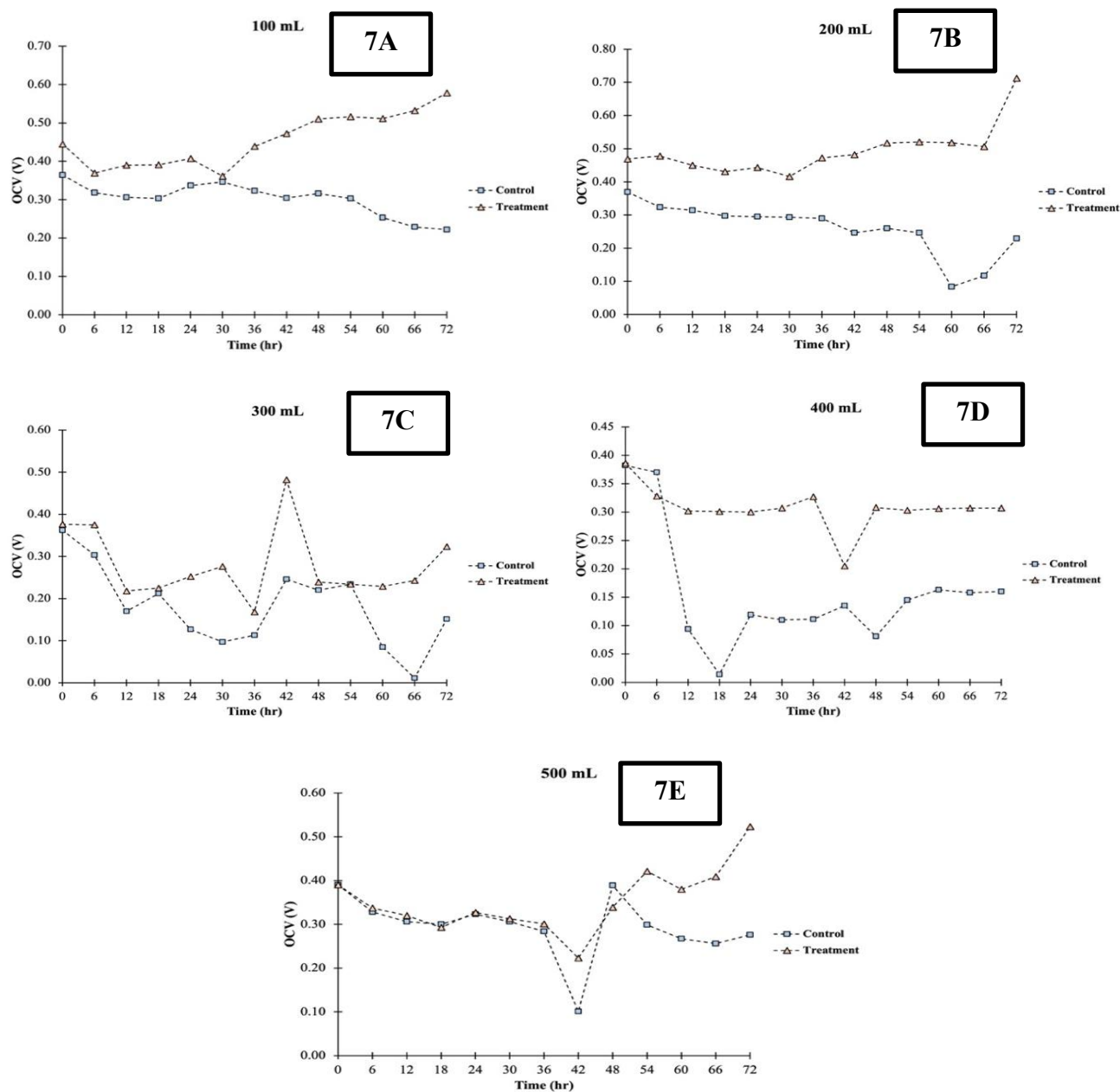


Figure-7. The open circuit voltage (OCV) of a dual-chamber MFC using raw POME (initial concentration 3.25 ± 0.05 gCOD/L) as the substrate at different working volumes: 100 mL (7A), 200 mL (7B), 300 mL (7C), 400 mL (7D), and 500 mL (7E).

Conclusions

This study successfully enriched and selected a laccase-producing fungal consortium (TC) based on its high enzyme activity and melanoidin degradation potential. The consortium TC primarily composed of *Candida tropicalis*, followed by *Pichia* sp., *Issatchenkia orientalis*, and *Candida orthopsilosis*, exhibited the highest laccase activity and melanoidin degradation capabilities. Integration of this consortium with an upflow microbial fuel cell (MFC) demonstrated the feasibility of simultaneous electricity generation and melanoidin removal from palm oil mill effluent (POME). These findings offer novel insights into the dual-function application of fungal consortia for bioremediation and bioenergy recovery. Future research should aim to optimize operational parameters such as retention time, pH, and substrate concentration to maximize performance. Moreover, scaling up the system, assessing long-term stability, and conducting omics-based analyses could enhance understanding of microbial interactions and system efficiency. Integrating this approach with other treatment technologies and performing economic and life cycle assessments are recommended to evaluate its practical and commercial viability.

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Contribution of Authors

Rothjanawan K & Palasai W: Analyzed and interpreted data and manuscript write up.

Yodrach R, Thipraksa J, Michu P & Kongthong A: Conducted the experiment and collected data.

Chaijak P: Conceptualized the experiment, data analysis and manuscript write up.

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