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Melanoidin degradation and electric energy production from palm oil waste using immobilized laccase-producing bacteria

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Abstract

Melanoidin is a high molecular weight pigment that is problematic in agricultural wastewaters like palm oil mill effluent (POME). This study presents a novel approach combining a laccase-producing bacterial consortium primarily *Lactiplantibacillus plantarum*, immobilized on hydrothermally modified granular activated carbon (GAC) for efficient melanoidin degradation and simultaneous electricity generation in a microbial fuel cell (MFC). The hydrothermal modification of GAC enhanced bacterial immobilization and electron transfer, contributing to improved biodegradation performance. Gas chromatography-mass spectrometry (GC-MS) analysis identified a number of key degradation metabolites including silanediol, dimethyl; (1-methylethyl)benzene; limonene; and butylated hydroxytoluene, confirming an effective melanoidin breakdown. The system achieved 81.36 ± 1.07% melanoidin removal with electrochemical characterization that showed a maximum current density of 61.50 ± 1.98 mA/m² and power density of 1.51 ± 0.10 mW/m². These findings demonstrated the synergistic effect of hydrothermally modified GAC and the selected bacterial consortium offering a sustainable and innovative strategy for treating melanoidin-rich wastewater while recovering bioenergy.

Keywords: Biodegradation; biochar; color removal; laccase; melanoidin; microbial fuel cell

1. Introduction

Palm oil (*Elaeis guineensis*) is a crucial economic crop widely cultivated across Asia, Africa, and Latin America, primarily used for cooking in many developing countries. The crude palm oil extraction process, however, has brought up significant environmental concerns, particularly related to water and soil pollution from pesticide residues and nutrient runoff leading to eutrophication [1]. Of these environmental challenges, palm oil mill effluent (POME) stands out as a major pollutant. It is a dark brown wastewater produced during crude palm oil extraction with approximately 2.5 to 3.75 tons discharged per ton of crude palm oil processed [2]. This wastewater primarily consists of 95–96% water, 0.6–0.7% residual oil, and 2–4% suspended solids [3].

A key contributor to dark coloration of POME is melanoidin, a high-molecular-weight polymeric pigment formed via the Maillard reaction during the hydrothermal processing of palm oil [4,5]. Melanoidin is present in various food processing wastewaters, including those from coffee production, molasses distilleries, baker's yeast plants, ethanol distilleries, and palm oil mills [4,6–8]. Untreated melanoidin discharge is potential to disrupt microbial communities in groundwater, leading to ecosystem poisoning [9], and negatively affecting aquatic environments by inhibiting seed germination and reducing photosynthesis through light attenuation [10].

Numerous chemical, physical, and biological methods such as biosorption, electrochemical degradation, oxidation, coagulation, membrane filtration, and the Fenton process have then been applied to remove melanoidin from wastewater [11]. Of these, biodegradation has attracted attention in view of its cost-effectiveness and non-toxic byproduct production. Prior studies have demonstrated melanoidin degradation by organisms such as *Bacillus* sp., *Stenotrophomonas* sp., *Bjerkandera adusta* and *Pseudoduganella violacea* [12–14].

Enzymes from the oxidoreductase family including oxidases, dehydrogenases, peroxidases and oxygenases have broad substrate specificity and catalytic versatility, making them valuable in agriculture, environmental remediation, medicine, and chemical diagnostics [15]. Most oxidoreductases

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require hydrogen peroxide as a co-substrate. Conversely, laccase uniquely uses only molecular oxygen, enhancing its practical applications [16,17]. Microbial laccase has thus gained interest for pigment degradation in wastewater treatment [18].

Microbial fuel cells (MFCs) offer a promising sustainable technology that harnesses microorganisms to convert chemical energy from organic compounds in wastewater directly into electricity, producing clean energy without greenhouse gas emissions [19]. They have been effectively applied to treat various wastewaters, including pharmaceutical [20], industrial [21], baker's yeast effluent [22], tannery [23], and azo dye-containing wastewaters [24].

In this study, we integrated a laccase-producing bacterial consortium to a microbial fuel cell system aimed to simultaneously degrade melanoidin and generate electricity from POME. This approach addresses the dual challenge of wastewater treatment and renewable energy recovery, filling a gap in the sustainable management of melanoidin-rich agricultural effluents.

2. Materials and Methods

2.1. Chemicals and media

All chemicals used in this study were of analytical grade and procured from Himedia (India) and Sigma-Aldrich (USA).

2.2. Melanoidin synthesis

Melanoidin pigment was synthesized by following the method reported by Onyango et al. [25] with minor modifications. A solution was prepared by dissolving 4.50 g of glucose monohydrate and 0.42 g of sodium carbonate in 100 mL of deionized water. The resulting mixture was then incubated in a laboratory water bath (GFL, United Kingdom) at 95°C for 7 hr. Upon the completion of the heating process, the reaction mixture was allowed to cool to room temperature, after which 100 mL of deionized water was added to dilute the solution.

2.3. Bacterial consortium

A laccase-producing bacterial culture (consortium TC_Bac) with a laccase activity of 3.02 ± 0.27 U/L was obtained from Thaksin University, Thailand. This culture was maintained in nutrient broth (5.0 g/L beef extract, 5.0 g/L peptone, 1.5 g/L sodium chloride, and 1.5 g/L yeast extract) at ambient temperature without agitation. The liquid medium was replaced every 48 hr to ensure the viability of the bacterial consortium.

To obtain the cell pellet, the cultured medium was centrifuged at 12,000 rpm for 10 min using a microcentrifuge (Biosan Laboratories Inc., USA). The resulting pellet was then transferred into a 1.5 mL DNA-free microcentrifuge tube. Genomic DNA from the bacteria was isolated using the E.Z.N.A® Bacterial DNA Kit (Omega BIO-TEK, USA).

To analyze the bacterial consortium community composition, next-generation sequencing (NGS) of the 16S rRNA gene was performed. The V3-V4 region was targeted, and Amplicon Sequence Variants (ASVs) were determined by means of the DADA2 analysis tool and the NCBI 16S ribosomal RNA database (Bayesian classifier).

2.4. Melanoidin degradation and metabolites

For melanoidin degradation analysis, the 3 gCOD/L melanoidin solution in phosphate buffer (pH 7.2) was prepared. A total of 10 mL of a 48 hour-old bacterial consortium culture was added to 90 mL of the melanoidin solution and incubated at ambient temperature without agitation for 48 hr.

Melanoidin removal was monitored every 6 hr by collecting the samples of the incubated solution and centrifuging at 12,000 rpm for 10 min using a microcentrifuge to remove the cell pellet. The resulting supernatant was then collected for melanoidin removal analysis.

Melanoidin removal (%R) was assessed by measuring the absorbance at 470 nm with a UV-Vis spectrophotometer (PerkinElmer, USA) as described in reference [26]. The percentage of melanoidin removal was calculated using the following formula:

$$\%R = [(C_0 - C_t)/C_0] \times 100 \quad (1)$$

where C_0 is the initial melanoidin concentration (gCOD/L) and C_t is the final melanoidin concentration (gCOD/L).

The degraded metabolites of melanoidin were analyzed by means of gas chromatography–mass spectrometry (GC-MS) (Agilent Technologies, USA) based upon the procedure described in a previously published method [27].

2.5. POME collection

Raw POME was obtained from a palm oil processing facility situated in Phatthalung Province, Southern Thailand. The sample was filtered through sterile medical gauze to remove sediments. The filtered sample was then stored at -25ºC in a freezer (Hitachi, Japan) until being used.

Table 1 presents the properties of the raw POME utilized in this study.

Table 1. Characteristics of the raw POME

Characteristic	Value	Unit
Total dissolved solid (TDS)	1264±11	mg/L
Chemical oxygen demand (COD)	4450±100	mg/L
Electroconductivity (EC)	2100±25	µS/cm
Melanoidin	2.95±0.10	gCOD/L
pH	7.95±0.50	-

2.6. Melanoidin degradation in POME

The raw POME was sterilized at 12°C for 15 min in an autoclave (Hirayama, Japan) and stored at 4°C until use.

For melanoidin degradation in the raw POME, a total of 10 mL of a 48 hr-old bacterial consortium culture was added to 90 mL of the non-sterile POME (nsPOME) and sterile POME (sPOME) and incubated at ambient temperature without agitation for 48 hr. The melanoidin removal was measured and calculated. The degraded metabolites of both nsPOME and sPOME were analyzed using GC-MS.

2.7. Biochar preparation

The granular activated carbon (GAC) was prepared through a hydrothermal process [28]. Briefly, 5.0 g of GAC was immersed with 100 mL of 1.0 M nitric acid solution in a 500-mL Erlenmeyer flask, which was then covered with aluminum foil. The immersed GAC was heated at 121°C for 60 min in an autoclave and subsequently allowed to cool. The hydrothermally treated GAC was washed 2-3 times until it reached pH 6-7. Subsequently, the hydrothermally treated GAC was dried in a hot air oven (Memmert, Germany) at 80°C for 24 hr and stored in a desiccator (Schott-Duran, Germany) until use.

2.8. Immobilization on GAC

A total of 10 mL aliquot of a 48 hr-old bacterial consortium culture was mixed with 10 g of hydrothermally treated granular activated carbon (GAC), resulting in a loading ratio of 1 mL culture per 1 g GAC. The mixture was incubated at room temperature (~25°C) under static conditions for 48 hr to allow bacterial cells to adhere and immobilize onto the GAC surface.

Following incubation, the immobilized GAC was gently rinsed with sterile phosphate-buffered saline (PBS) to remove any loosely attached cells. Immobilization efficiency was assessed by comparing the bacterial cell density in the initial culture and the supernatant after incubation using optical density measurements at 600 nm (OD_{600}).

2.9. Melanoidin removal

A total of 1 g of immobilized GAC was added to the nsPOME and incubated at ambient temperature without agitation for 48 hr. Meanwhile, non-immobilized GAC was used as a control. Melanoidin removal was measured and calculated.

2.10. MFC

The design of the dual-chamber MFC is depicted in Fig. 1. A 4 cm² copper plate and a 4 cm² aluminum plate served as the cathodic electrode and the anodic electrode, respectively. A 250-mL glass beaker (Duran, Germany) served as the anodic chamber, and a plastic funnel served as the cathodic chamber. A 0.1% (w/v) potassium chloride (KCl) gel was used as the proton exchange membrane.

For operation, a total of 10 g of immobilized GAC and 100 mL of nsPOME were added to the anodic chamber. The open-circuit voltage (OCV) was monitored every 6 hr for 48 hr. The closed-circuit voltage (CCV) was measured at 1,000 Ω. The current (A), power (W), current density (A/m³) and power density (W/m³) were then calculated as follows:

$$I = V / R \quad (2)$$

$$P = I \times V \quad (3)$$

$$CD = I / A \quad (4)$$

$$PD = P / A \quad (5)$$

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 (m^3) or electrode surface area (m^2), CD is the current density (mA/m^3 or mA/m^2) and PD is the power density (W/m^3 or mW/m^3).

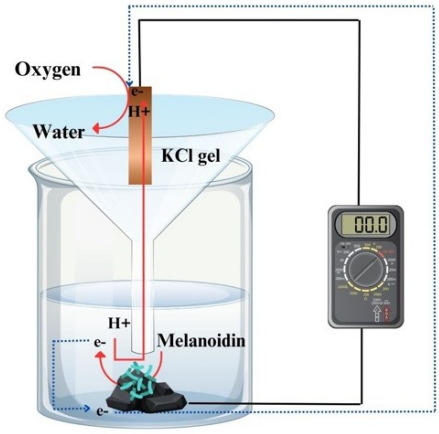


Fig. 1. Design of dual-chamber MFC used in this experiment

The internal resistance (Rint) was determined according to Eq. 6.

$$R_{int} = (V_{ocv} \times R_L / V_{ccv}) - R_L \quad (6)$$

where Rint is the internal resistance (Ω), V_{ocv} in the open circuit voltage (V), V_{ccv} is the close circuit voltage (V), and R_L is the load resistance (Ω).

Furthermore, the polarization curve was constructed, and the coulombic efficiency was calculated using Eq. 7-9.

$$C_{the} = \Delta COD \times V \times [(4 \times F) / 32] \quad (7)$$

$$C_{rec} = I \times t \quad (8)$$

$$CE = (C_{rec} / C_{the}) \times 100 \quad (9)$$

where C_{the} is the theoretical coulombs (C), ΔCOD is the change in COD (g/L), V is the volume of the anolyte (L), F is the Faraday's constant (96485 C/mol), 4 is the number of electrons transferred per mole of oxygen, 32 is the molar mass of oxygen (g/mol), C_{rec} is the total coulombs actually recovered as electrical current (C), I is the current (A), t is the time (second), and CE is the coulombic efficiency (%).

2.11. Statistical analysis

All experiments in this study were conducted in triplicate to ensure reproducibility. Data were presented as the mean ± standard deviation (SD).

3. Results and Discussion

3.1. Bacterial community

The laccase-producing bacterial consortium (consortium TC) was analyzed through metagenomic analysis (Fig. 2) and the diversity index are shown in Table 2. The consortium TC belonged entirely to the phylum Bacillota (100%).

In the class level, the consortium TC comprised 21.27% of Clostridia and 78.73% of Bacilli. In the order level, the consortium consisted primarily of Lactobacillales (78.73%), Eubacteriales (21.21%), and Peptostreptococcales (0.06%).

In the family level, the dominant families were Lactobacillaceae (78.73%), Clostridiaceae (21.21%), and Peptostreptococcaceae (0.06%). In the genus level, the consortium was mainly composed of *Lactiplantibacillus* (59.18%), *Clostridium* (21.21%), *Liquorilactobacillus* (19.56%), and *Paraclostridium* (0.06%).

In the species level, the primary species include *Lactiplantibacillus plantarum* (59.18%), *Clostridium tyrobutyricum* (21.21%), *Liquorilactobacillus nagelii* (19.56%), and *Paraclostridium bifermentans* (0.06%).

Regarding biosafety, *Lactiplantibacillus plantarum* is widely regarded as a generally recognized as safe (GRAS) microorganism and is commonly used in food fermentation and probiotic applications with no known pathogenicity to humans [29]. *Clostridium tyrobutyricum* is an anaerobic bacterium primarily involved in fermentation processes and is generally considered as non-pathogenic. However, some *Clostridium* species can be opportunistic pathogens, so strain-specific safety assessments are recommended [30]. *Liquorilactobacillus nagelii* is commonly isolated from food and fermentation environments and is not associated with human pathogenicity [31]. *Paraclostridium bifermentans* is less commonly studied, but related species in the *Paraclostridium* genus generally are environmental bacteria with the limited reports of human infection, suggesting low risk though caution and further safety evaluation are advised [32].

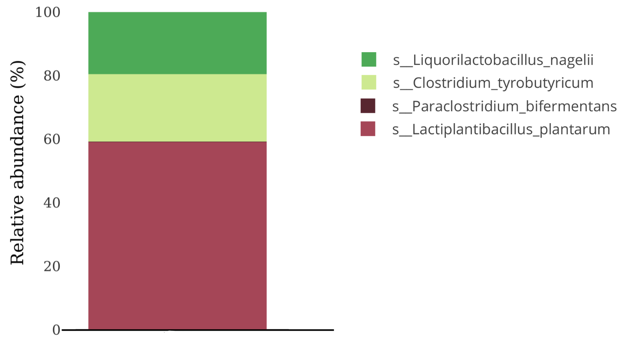


Fig. 2. The bacterial community of consortium TC_Bac

Table 2. The diversity index of consortium TC_Bac

Sample ID	Shannon	Gini-Simpson	PD_Whole_tree	ASVs
16S_TC	2.503847	0.785602087	0.48715254	10

In the melanoidin degradation pathway mediated by laccase-producing bacteria, melanoidin is initially broken down by laccase into aromatic and phenolic radicals. These radicals are then depolymerized into smaller aromatic compounds, which are subsequently transformed into quinones, aldehydes, and organic acids. These intermediate compounds are taken up by bacterial cells and utilized in cellular respiration, producing the end products such as carbon dioxide, short-chain fatty acids, and bacterial biomass [33–36].

On the other hand, the laccase-producing consortium comprises *Pseudomonas*, *Phenylobacterium*, and *Caulobacter*. This community has demonstrated a high potential for lignin degradation and humic substance formation during composting [37]. In Chaudhary et al., the laccase-producing bacterium *Bhargavaea beijingensis* was isolated from a soil sample collected from a pulp and paper mill industry. However, no application of this bacterium was reported [38].

Furthermore, the laccase-producing bacterium *Bacillus subtilis* strain KSK02 has been used for reactive red-120 dye degradation; however, this bacterium requires a co-substrate for the process [39].

In Thipraksa et al., the laccase-producing bacterial consortium S5, primarily comprising *Citrobacter werkmanii*, *Enterococcus faecalis* and *Escherichia fergusonii*, was used for melanoidin degradation in POME and electricity generation [4]. However, *Citrobacter werkmanii* has been reported as an opportunistic human pathogen [40]. This raises concerns about its industrial application.

3.2. Melanoidin degradation and metabolites

The consortium TC was inoculated into a 3 gCOD/L melanoidin solution and incubated at room temperature. Melanoidin removal was monitored every 6 hr for 2 days. The degradation of melanoidin was assessed using UV-Vis spectrophotometry at 470 nm. The results showed a maximum melanoidin removal of 77.55 ± 0.43% (Fig. 3).

The degraded metabolites of the melanoidin solution were analyzed using GC-MS. The analysis identified silanediol, dimethyl as a degraded metabolite. The chromatogram is shown in Fig. 4.

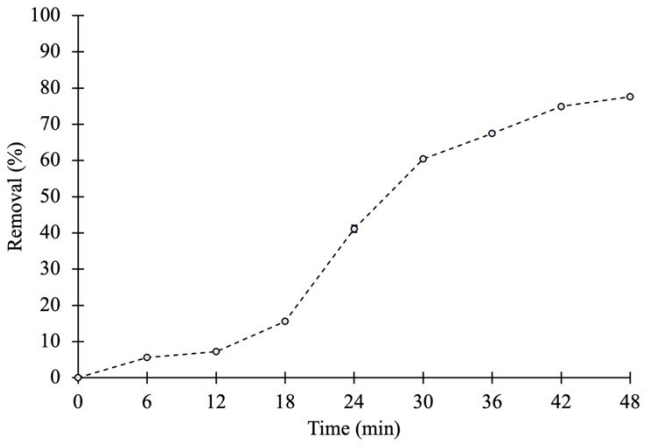


Fig. 3. The melanoidin removal of consortium TC in 3 gCOD/L melanoidin solution

The sPOME and nsPOME were used to evaluate the potential of consortium TC for melanoidin degradation in

POME. A 10% (v/v) inoculum of the consortium was added to the POME and incubated for 48 hr. The melanoidin removal in sPOME is shown in Fig. 5A showing the maximum percentage of 80.02 ± 0.09%. While, the melanoidin removal in nsPOME is shown in Fig. 5B with the maximum percentage of 81.36 ± 1.07%.

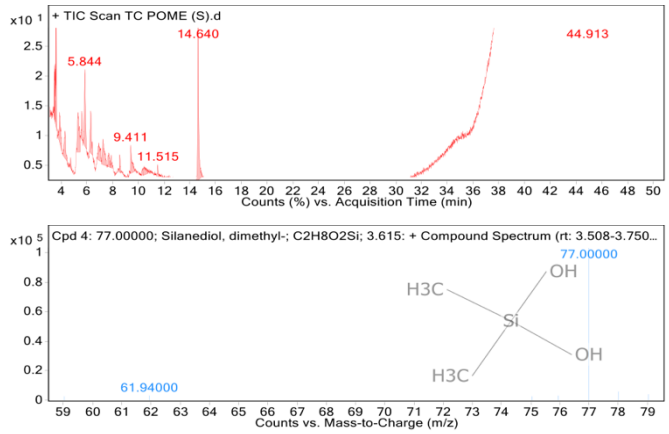


Fig. 4. The chromatogram of degraded metabolite of the melanoidin solution

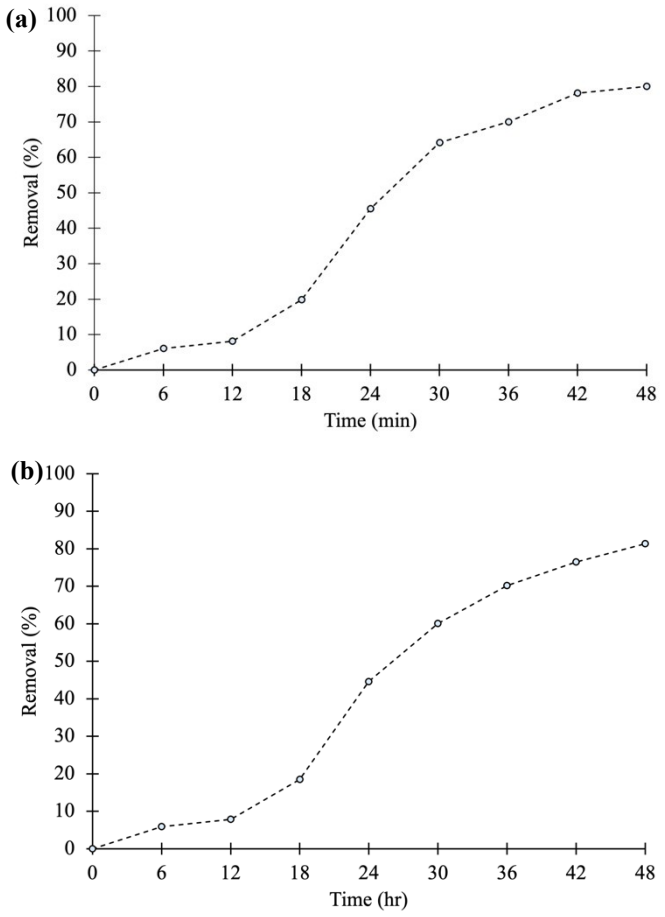


Fig. 5. (a) The melanoidin removal of consortium TC in sPOME and (b) nsPOME (5B)

The degraded metabolites of sPOME are shown in the chromatogram (Fig. 6). The metabolites, however, could not be identified.

The degraded metabolites of nsPOME are also shown in the chromatogram (Fig. 7). Here, a number of degraded metabolites were identified including silanediol, dimethyl; (1-methylethyl)benzene; limonene and butylated hydroxytoluene.

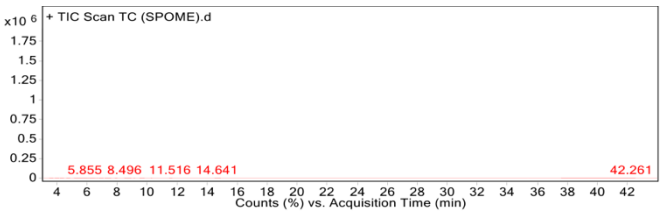


Fig. 6. The chromatogram of degraded metabolite of the sPOME.

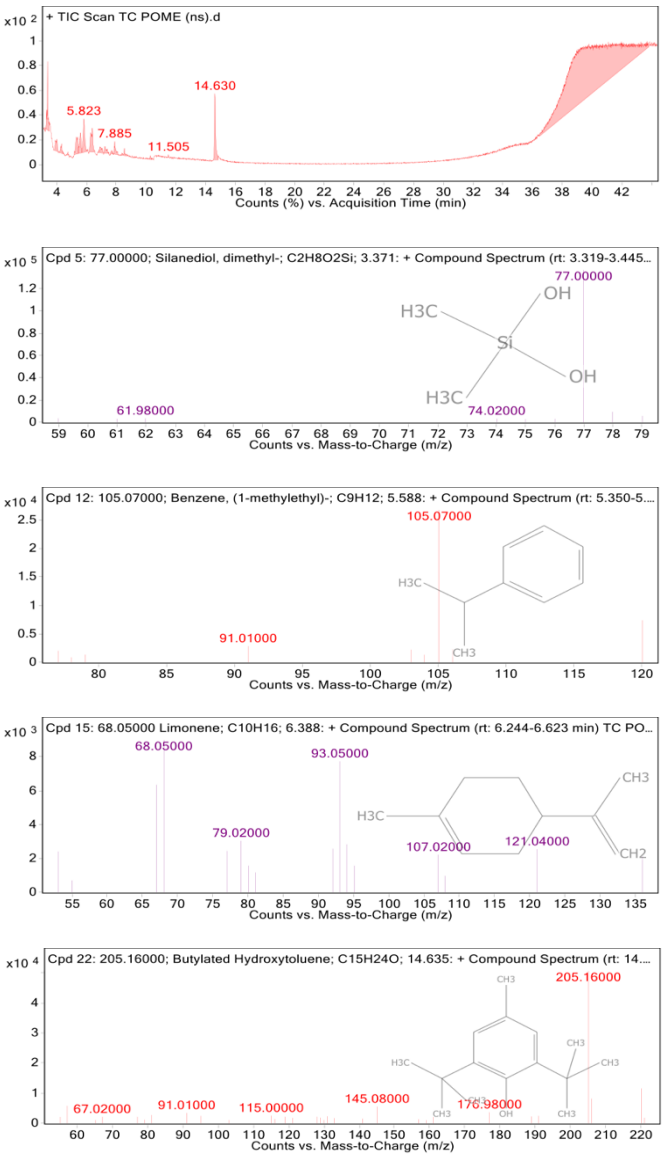


Fig. 7. The chromatogram of degraded metabolite of the nsPOME

The removal of melanoidin from raw POME was investigated using both non-immobilized (control) and immobilized GAC. The reaction was carried out at room temperature for 48 hr under static conditions with the results as shown in Fig. 8. A maximum melanoidin removal of 81.67 ± 0.49% was achieved from immobilized GAC, significantly higher than the 5.95 ± 0.05% removal as observed in the control.

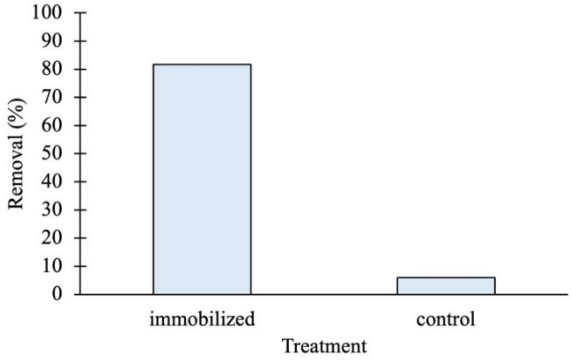


Fig. 8. The melanoidin removal from the raw POME using immobilized GAC and non-immobilized GAC (control)

On the other hand, iron oxide nanoparticles synthesized with green tea extracts were employed for melanoidin removal from ethanol distillery wastewater, achieving a maximum removal of 99.60%. However, the COD removal was only 48.60% [8], and the process incurred a high operational cost.

Yang et al. investigated the treatment of melanoidin-rich Chinese distilled spent grain through solid-state fermentation using *Aspergillus awamori*; the maximum melanoidin removal achieved was 24.4% [41].

Additionally, acid-treated chitin obtained from shrimp waste was applied for melanoidin removal from synthetic wastewater via adsorption with a reported maximum removal capacity of 32.15 mg/g of chitin. However, this method involves significant costs related to chitin preparation [42].

The comparison of color removal in various colored wastewaters using the MFC system is presented in Table 3.

Table 3. The color removal from wastewater using MFC system

Wastewater	MFC type	Removal (%)	Reference
Palm oil mill effluent (POME)	Dual chamber MFC	81.67 ± 0.49	This study
Textile effluent	Single chamber MFC	89.55	[43]
Textile effluent	Dual chamber MFC	72.90	[44]
Azo dye wastewater	Constructed wetland-MFC	85.20	[45]
Azo dye wastewater	Dual chamber MFC	91.26	[46]
Dye containing wastewater	Up-flow constructed wetland-MFC	87.00-92.00	[47]

3.3. Electrochemical properties

Electrochemical properties were analyzed using Ohm's law, with the results as presented in Table 4. The coulombic efficiency (CE) based on melanoidin removal from wastewater was 0.29±0.01% and the internal resistance of this MFC system was 18,072.71 ± 473.13 Ω.

The polarization curve, as shown in Fig. 9, was constructed using various external resistances. The maximum current density (CD) and power density (PD) achieved were 0.29 ± 0.01 A/m² and 0.003 W/m², respectively.

Table 4. The electrochemical properties of the MFC

Electrochemical properties	Value	Unit
OCV	469.00±10.15	mV
CCV*	24.60±0.79	mV
CD**	61.50±1.98	mA/m^2
PD**	1.51±0.10	mW/m^2
CD***	246.00±7.94	mA/m^3
PD***	6.06±0.39	mW/m^3

* at the external resistance of 1,000 Ω.

** based on the electrode surface area.

*** based on the working volume.

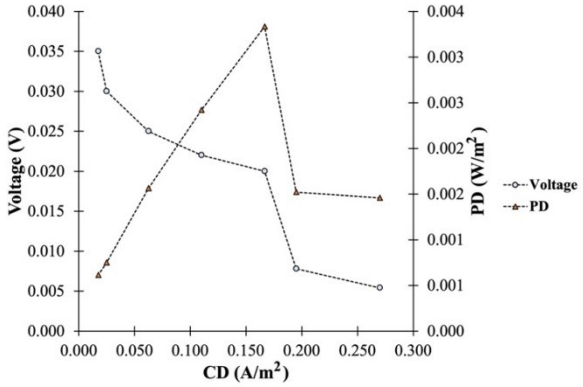


Fig. 9. The polarization curve of the MFC

In Nor et al., an anion-exchange MFC was used for electricity generation from POME substrate, and the maximum PD achieved was 0.18 W/m². Melanoidin removal data, however, was not provided. Furthermore, this system incurs a high operating cost due to the anion-exchange membrane [48].

Furthermore, an air-cathode MFC has been used for POME treatment and electricity generation. A maximum OCV of 189.25 mV was achieved when methylene blue was used as a mediator [49].

4. Conclusion

This study demonstrated that a laccase-producing bacterial consortium immobilized on hydrothermally modified granular activated carbon can effectively degrade melanoidin from POME with *Lactiplantibacillus plantarum* playing a key role in the pigment breakdown. The consortium showed significant melanoidin removal in non-sterile conditions and its application in a microbial fuel cell enabled simultaneous electricity generation, underscoring its potential for integrated bioremediation and energy recovery. These findings suggest a promising and sustainable approach for treating melanoidin-rich wastewaters. Future studies should focus on the evaluation of the long-term stability and reusability of the immobilized consortium to assess its durability and feasibility for continuous operation.

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