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Enhanced bioelectricity recovery and melanoidin degradation in CO_2-capturing microbial fuel cells via biochar-immobilized whole-cell biocatalysts

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Abstract

Melanoidin-rich wastewater from agro-industrial processes, particularly palm oil mill effluent, poses significant environmental problem owing to its recalcitrant nature and high organic load. The present study developed a carbon-capturing microbial fuel cell (MFC) integrated with biochar-immobilized laccase-producing microbial consortia for an integrated system enabling concurrent melanoidin removal, bioelectricity recovery, and carbon fixation under the tested conditions. Empty fruit bunch (EFB) biochar produced at 600 °C (BC600) was selected as an immobilization support due to its superior adsorption capacity and functional surface properties. The immobilized system demonstrated a maximum melanoidin removal efficiency of 73.15±1.10% and significant COD reduction with degradation exhibiting a close association with enhanced laccase activity. In the MFC, enhanced electrochemical performance was observed, with a maximum open circuit voltage of 619.17±10.49 mV, along with current and power densities of 8.29±0.15 A/m^3 and 1.00±0.20 W/m^3, respectively. Coupling with microalgae resulted in the simultaneous fixation of carbon (0.13±0.00 g/L/day). A phytotoxicity assessment confirmed no inhibitory effects on rice seed germination. This finding indicates that the substance is environmentally safe. It is imperative to note that the present study proposes a novel integration of biochar-based immobilization with a carbon-capturing MFC for the concurrent removal of pollutants, energy recovery, and CO_2 mitigation. This sustainable approach offers a promising solution for the treatment of melanoidin-rich wastewater.

Keywords: Bioelectricity recovery; carbon capture; environmental remediation; Sustainable energy; whole-cell biocatalyst

1. Introduction

Maillard reaction is a non-enzymatic browning activity that occurs when the carbonyl group of reducing sugars and the amino groups of proteins react under conditions of elevated temperature [1]. Melanoidin is a dark brown, high-molecular-weight polymer formed as the final product of the Maillard reaction. Its significance is widely recognized in the domains of thermally processed agricultural products and the fermented food industries, where it has been demonstrated to significantly contribute to the coloration of foodstuffs and related by-products [2-3]. From an environmental perspective, melanoidin is considered as a major pollutant due to its high formation potential from polysaccharides and proteins, intense dark coloration, strong ultraviolet light absorption properties, and resistance to biodegradation [4].

As reported in previous studies, the concentration of melanoidin in agricultural waste streams ranges from 2 to 20 g/L [5]. Melanoidin-contaminated wastewater poses significant environmental risks in view of its potential adverse effects on crop growth and ecological health [6]. Furthermore, exposure to melanoidin has been associated with potential adverse health effects on humans, including eye irritation, dermatological reactions, headaches, fever, nausea, and abdominal discomfort [7]. Melanoidin has been detected in various types of agricultural wastewater, such as ethanol distillery effluent [8], molasses wastewater [9], coffee wastewater [10], and palm oil mill effluent (POME) [11].

Palm oil mill effluent (POME) is a dark brownish agricultural wastewater that is characterized by a high organic load. It is estimated that the production of POME amounts to approximately 0.1 ton per ton of crude palm oil (CPO) manufactured. It is imperative to note that, due to its prominent chemical oxygen demand, biological oxygen demand, and melanoidin content, untreated POME poses a considerable risk to aquatic environments [11-12]. Thailand is the world's third-largest producer of crude palm oil (CPO) with an annual

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production of approximately 3.30 million metric tons. Consequently, a substantial volume of POME is generated, estimated at approximately 0.33 million tons per year [13]. The large-scale generation of POME poses significant environmental management challenges, particularly due to its high organic load and potential to cause severe water pollution if improperly treated.

The technologies employed in Melanoidin treatment can be broadly classified into physicochemical and biological approaches. Physicochemical methods employed in this process encompass a range of techniques, including coagulation, flocculation, electrocoagulation, and membrane-based processes, as well as hybrid systems such as membrane bioreactors and bioelectrode-assisted technologies. Despite the efficacy of these methods in the removal of melanoidin, they are frequently associated with high operational costs and substantial chemical consumption. In contrast, biological approaches have attracted increasing attention due to their cost-effectiveness, environmental compatibility, and lower chemical requirements [14]. The utilization of microbial and enzymatic treatments is a prevalent in the degradation and decolorization of melanoidin. It has been observed that microorganisms are capable of removing melanoidin from wastewater through the utilization of enzymatic mechanisms involving enzymes such as manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase [15].

Laccase (EC. 1.10.3.2), also identified as *p*-benzenediol:dioxygen oxidoreductase, has attracted considerable attention for its potential environmental applications due to its catalytic capability, which requires only molecular oxygen as the electron acceptor and produces water as the exclusive by-product [16]. It has been widely applied in environmental applications, primarily in the management of diverse types of effluent, including those from pulp and paper, chemical, agro-industries, pharmaceutical, textile printing, oil, dyeing, and palm oil industries [11,17].

A microbial fuel cell (MFC) is an electrochemical system that is capable of directly converting the chemical energy accumulated in organic substances into electrical energy through the metabolic activity of electrochemically active bacteria, also known as exoelectrogens [18]. It has been documented that MFC has been employed for the treatment of diverse industrial effluents, including those from petrochemical, tannery, brewery, dairy, textile, and agro-industrial wastewaters. Concurrently, the generation of bioelectricity has been observed [19]. As demonstrated in previous studies, the incorporation of raw bamboo biochar into the anode chamber has been shown to significantly enhance bioelectricity generation performance [20]. The objective of this study is to examine the enhancement of electricity generation and melanoidin removal from POME in a carbon-capturing MFC through the application of biochar-immobilized laccase-producing consortium. The novelty of this study does not lie in the introduction of a new individual component but rather in the evaluation of the synergistic integration of established technologies to simultaneously address pollutant removal, energy recovery, and biological carbon utilization in melanoidin-rich wastewater.

2. Materials and Methods

2.1. Chemicals

Potato dextrose broth (PDB) and nutrient broth (NB) were obtained from HiMedia (Mumbai, India). The following substances were purchased from Sigma-Aldrich (Missouri, USA): Glucose, glycine, $NaHCO_3$, KH_3PO_4, $MgSO_4$, $CaCl_2$, $(NH_4)_2SO_4$, $KMnO_4$, and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).

2.2. Melanoidin synthesis

Synthetic melanoidin (3 g COD/L) was prepared in accordance with the method described by Thipraksa et al. [11]. Briefly, glucose (0.450 g/L), glycine (0.188 g/L), and $NaHCO_3$ (0.042 g/L) were dissolved in distilled water and incubated at a temperature of 95°C for 7 hours. Subsequently, the reaction mixture was cooled to room temperature, followed by the addition of 100 mL of distilled water. The prepared stock solution was stored at 0 °C until further use.

2.3. Biochar preparation

Empty fruit bunch (EFB) was obtained from a crude palm oil (CPO) factory in Trang Province, Thailand. The EFB fibers were cut into pieces measuring approximately 1 cm and then subjected to pyrolysis in a fixed-bed reactor under a nitrogen atmosphere at temperatures of 300 (BC3000, 400 (BC400), 500 (BC500), 600 (BC600), and 700 (BC700) °C for a period of 1.5 hours. The biochar obtained was subsequently immersed in potassium hydroxide solution. Finally, the biochar was dried and microwave-activated at 600 W within 10 minutes [21].

2.4. Screening

For the purpose of screening, 0.5 g of activated EFB biochar (with dimensions of 1 cm) was added to 10 mL of melanoidin solution with a COD value of 3 g/L) in a 250 mL Erlenmeyer flask, which was then covered with aluminum foil. The mixture was shaken at 150 rpm for 60 minutes. The suspension was then subjected to a centrifugal process at a speed of 10,000 rpm for 10 minutes. The optical density of the supernatant was observed at 470 nm employing a microplate reader. The melanoidin removal efficiency (%) was calculated according to Eq. (1). The EFB biochar demonstrating the most significant melanoidin removal potential was selected for subsequent experiments, with the raw EFB used as a control.

$$R\% = [(A - B)/A] \times 100 \tag{1}$$

where R% is melanoidin removal, A is the initial absorbance and B is the final absorbance of the supernatant measured at 470 nm, respectively.

2.5. Functional group analysis

The Fourier transform infrared spectroscopy (FTIR) was employed with the objective of examining the surface chemical bonds and functional groups of EFB biochar.

2.6. Mixed consortium preparation

The laccase-producing consortium [22-23] was achieved from the Major of Biotechnology, Faculty of Science and Digital Innovation, Thaksin University.

The laccase-producing bacterial consortium was cultured in nutrient broth (HiMedia, India). Briefly, 1 g of cell pellet was suspended in 100 mL of sterile nutrient broth and incubated in an orbital shaker at 150 rpm for 48 hr at room temperature.

The laccase-producing fungal consortium was prepared as follows: 1 g of fungal cell pellet was suspended in 100 mL of sterile potato dextrose broth (HiMedia, India) and incubated in an orbital shaker at 150 rpm for 48 hours at room temperature.

The mixed consortium was prepared by combining the bacterial seed and fungal seed at a ratio of 1:1. The microbial community composition was analyzed through next-generation sequencing.

The laccase activity of the microbial consortium was evaluated. Briefly, 1 g of immobilized EFB biochar was inoculated into 100 mL of melanoidin solution and incubated with shaking at 150 rpm for a period of 7 days. A 5-mL sample of the solution was collected daily and subjected to a centrifugation at 12,000 rpm for a duration of 10 minutes to obtain the supernatant.

The activity of extracellular laccase activity was determined by means of the ABTS assay and measured at an absorbance wavelength of 420 nm (ε = 36,000 $M^{-1}cm^{-1}$). The reaction mixture (100 μL) consisted of 20 μL of 50 mM ABTS, 20 μL of the supernatant, and 60 μL of 100 mM sodium citrate buffer (pH 4.0) [22-23].

2.7. Synthetic melanoidin removal

Immobilized EFB biochar (1 g) was introduced into 100 mL of melanoidin solution (3 g COD/L) and incubated at room temperature with agitation at 150 rpm for a period of 7 days. Samples (5 mL) were withdrawn daily and centrifuged at 12,000 rpm for a period of 10 minutes. The resulting supernatant was collected and the degree of absorbance at 470 nm was measured using a microplate reader to quantify the residual melanoidin. The efficiency of removal (%) was determined by calculating the relative reduction in absorbance in comparison to the initial concentration.

2.8. Effect of melanoidin concentration

Melanoidin solutions with initial concentrations ranging from 0.5 to 3.0 g COD/L were prepared from a stock solution to evaluate the effect of substrate concentration on melanoidin removal. Immobilized EFB biochar (1 g) was added to 100 mL of each melanoidin solution and the mixture was then incubated at room temperature with shaking at 150 rpm for a period of 7 days. Having completed incubation, a total of 5 mL of samples were subjected to centrifugal process at 12,000 rpm for a duration of 10 minutes. The supernatant was then analyzed at 470 nm by means of a microplate reader to determine residual melanoidin concentration. The efficiency of removal (%) was calculated from the decrease in absorbance compared with the initial value.

2.9. Effect of pH

The impact of initial pH on melanoidin removal was evaluated by utilizing melanoidin solutions (3 g COD/L) that were adjusted to pH values ranging from 3 to 8 prior to incubation. The experiments were subsequently conducted under the aforementioned conditions.

2.10. Effect of temperature

The impact of temperature on melanoidin removal was evaluated by incubating melanoidin solutions (3 g COD/L) at temperatures ranging from 20 to 45 °C under the same experimental conditions as previously described.

2.11. Melanoidin removal in raw POME

Raw palm oil mill effluent (POME) was collected from a palm oil extraction factory located in Trang, southern Thailand. The sample was pretreated by filtration through sterile medical gauze (2–3 cycles) to remove coarse biomass and suspended solids.

The performance of melanoidin removal in raw POME was evaluated through a variety of treatment systems. Briefly, 100 mL of raw POME was subjected to four distinct treatment conditions: untreated control (no addition), EFB biochar alone, free microbial consortium, and immobilized microbial consortium on EFB biochar. All experiments were conducted at room temperature under continuous shaking at 150 rpm for a period of 7 days. Samples were collected posttreatment and analyzed for melanoidin removal efficiency and chemical oxygen demand (COD) reduction. It is pivota; to note that all experiments were performed in triplicate.

2.12. MFC operation

The fabrication of a dual-chamber microbial fuel cell (MFC) was undertaken, with the apparatus being constituted of an acrylic cube with a total working volume of 10 mL (Fig. 1). A layer of Parafilm (Bemis, USA) served as an economical proton exchange membrane (PEM). Both anode and cathode were composed of plain carbon cloth electrodes (Fuel Cell Store, USA) with a thickness of 15 mm and an effective surface area of 2 cm^2. The carbon dioxide produced in the anodic compartment was conveyed to the cathodic chamber through a PVC oxygen tube with a diameter of 4 mm, manufactured by U Smiles, (Thailand).

The cathodic chamber was filled with 9 mL of BG11 medium which had been supplemented with a microalgal culture that had been grown for 7 days (OD_{680} = 1.0). Meanwhile, the anodic chamber contained 10 mL of untreated POME along with 0.1 g of a microbial consortium immobilized on EFB biochar. The MFC system was operated under static conditions at ambient temperature for a duration of 7 days.

Electrochemical performance was evaluated by means of the recording of the open circuit voltage (OCV) at six-hour intervals. Closed circuit voltage (CCV) measurements were conducted using external resistances ranging from 300 to 5,000 Ω. Polarization and power density (PD) curves were subsequently generated based on CCV, current density (CD) and calculated power density. The electrochemical properties were calculated based on Ohm’s law as follows Eq (1-5)

$$I = V/R \tag{2}$$

$$P = I \times V \tag{3}$$

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

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

where I is the current (A), V is the closed-circuit voltage (V), R is the external resistance (Ω), P is the power (W), and A is the working volume (m^3) or electrode surface area (m^2). Furthermore, 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).

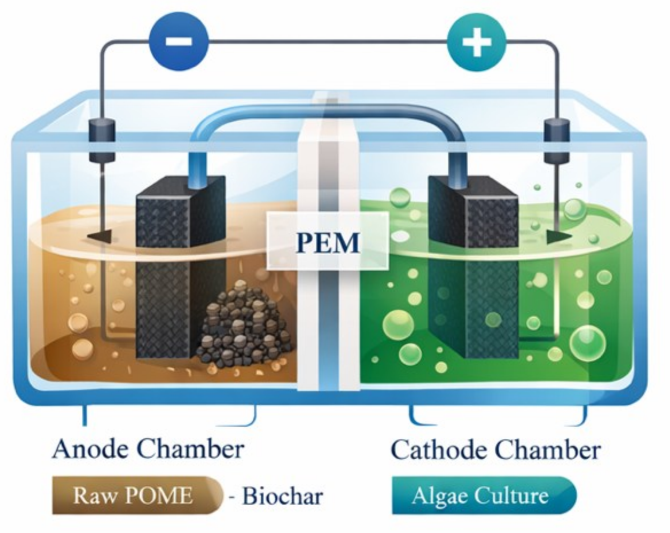


Fig. 1. Dual-chamber MFC employed in this experiment

2.13. Carbon fixation

The calculation of microalgal biomass productivity was undertaken in accordance to Eq. (6):

$$P_m = (Cmt - Cm0)/t \quad (6)$$

where P_m is the microalgal biomass productivity (g/L/day), Cmt is the biomass concentration at time t (g/L), Cm0 is the initial biomass concentration (g/L), and t is the cultivation time (day).

Meanwhile, the calculation of carbon fixation rate (g/L/day) was conducted utilizing Eq. (7) [24]:

$$\text{Carbon fixation rate} = Wdry \times C \times (mCO2/mC) \quad (7)$$

where Wdry is the dry biomass productivity (g/L/day), C is the carbon content of biomass (assumed to be 0.5), mCO2 is the molecular weight of CO_2 (44 g/mol), and mC is the molecular weight of carbon (12 g/mol).

2.14. Phytotoxicity

The phytotoxicity of immobilized EFB biochar following wastewater treatment in the MFC was evaluated in accordance to a previous study [25]. Briefly, 1 g of immobilized EFB biochar was added to 10 mL of sterile distilled water and the mixture was then homogenized. Then, 1 mL of the resulting solution was applied to 20 rice seeds (*Oryza sativa* L.). The experiment was conducted using normal EFB biochar and sterile distilled water as the control. The process of seed germination was evaluated and calculated according to Eq. (8).

$$\text{Germination (\%)} = (A/B) \times 100 \quad (8)$$

where A denotes the final number of seeds emerged and B represents the total number of seeds that have been sown measured at seven days after germination.

3. Results and Discussion

3.1. Biochar

The melanoidin removal efficiency of EFB biochars prepared at different pyrolysis temperatures is demonstrated in Fig. 2. Of the tested materials, BC600 exhibited the highest removal efficiency (21.22±0.95%), followed by BC700 (18.50±0.50%). In contrast, the control (raw EFB) exhibited a negligible removal efficiency of 1.19±0.03%, indicating that pyrolysis temperature significantly influenced the adsorption performance of the biochar.

Conversely, *Raphanus sativus* press-cake has been reported as an effective precursor for biochar production via microwave-assisted carbonization either by direct heating or by chemical activation. The biochar produced demonstrated effective melanoidin removal from synthetic wastewater with adsorption mechanisms primarily attributed to hydrogen bonding interactions between surface functional groups of the biochar and melanoidin molecules [26]. Furthermore, iron-impregnated activated carbon prepared from *Mangifera indica* leaf biomass has been reported to achieve an elevated melanoidin removal efficiency (85.60%) in synthetic wastewater. Nevertheless, this method is associated with relatively high operational costs, which may limit its practical application [27].

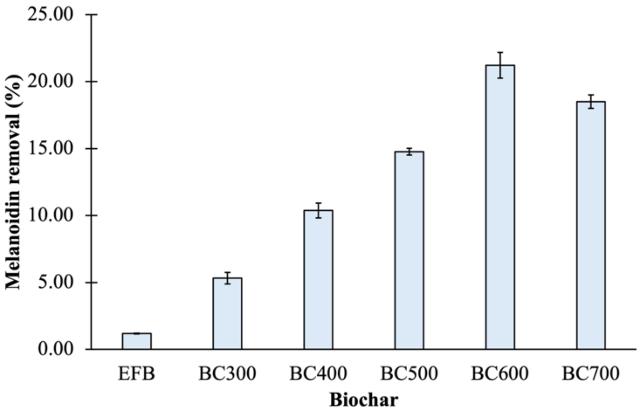


Fig. 2. Melanoidin removal (%) using different EFB biochars: raw EFB (control), EFB biochar prepared at 300 °C (BC300), 400 °C (BC400), 500 °C (BC500), 600 °C (BC600), and 700 °C (BC700)

The FTIR spectrum of the BC600 (Fig. 3) revealed the presence of several characteristic functional groups on the biochar surface. A broad band was observed at approximately 3275 cm^{-1} corresponding to the stretching vibration of hydroxyl (O–H) groups. These groups may originate from phenolic structures or adsorbed moisture on the biochar surface. As well documented, comparable O–H stretching bands at approximately 3400 cm^{-1} are frequently reported in biochar derived from lignocellulosic biomass [28].

The peak detected at 1624 cm^{-1} is attributed to aromatic C=C stretching vibrations, indicating the presence of condensed aromatic structures generated during the carbonization of lignocellulosic components such as lignin. The persistence and strengthening of this band are typical of biochars produced at moderate to high pyrolysis temperatures, reflecting the progressive formation of stable aromatic carbon matrices [29-30]. The enhanced melanoidin removal performance observed for BC600 may be associated with the combined influence of surface functional groups and textural properties generated during the pyrolysis process. FTIR analysis suggested the presence of aromatic carbon structures and residual oxygen-containing groups, which have been postulated to contribute to adsorption and microbial interaction. However, due to the absence of Brunauer–Emmett–Teller (BET) surface area and pore size distribution analyses, the quantitative evaluation of contribution of physical properties such as specific surface area, pore volume, and pore accessibility was not possible. Consequently, the mechanism underlying the superior performance of BC600 should be interpreted with caution, and future studies should include BET and pore structure characterization to establish structure–function relationships.

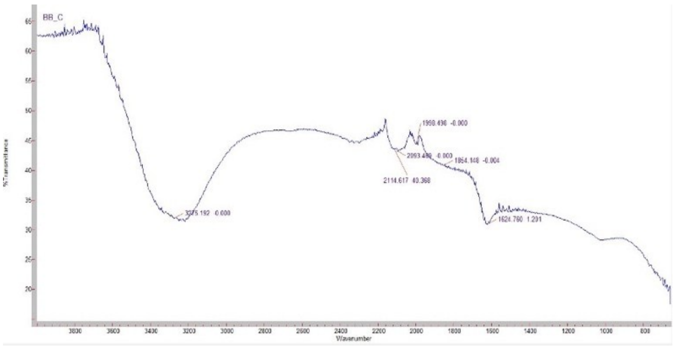
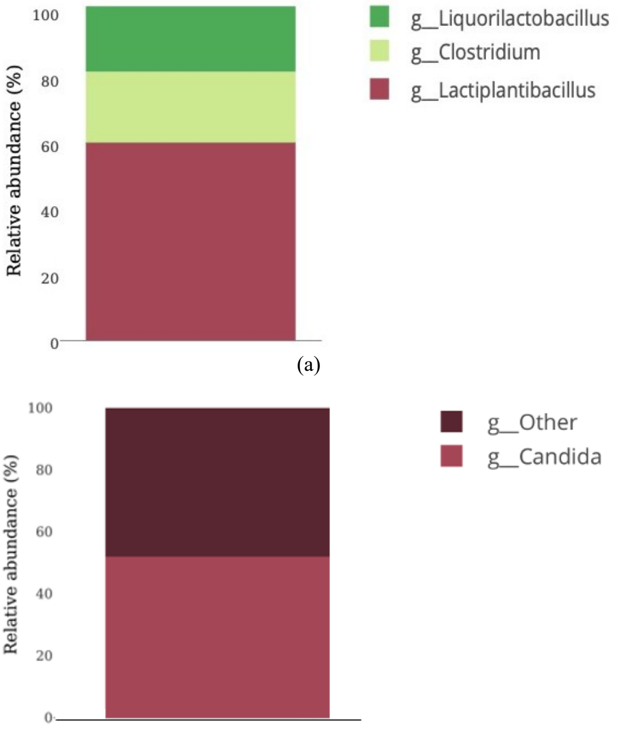


Fig. 3. The FTIR spectrum of the BC600 biochar

3.2. Laccase activity and melanoidin removal

The increase in laccase activity during the incubation period suggests that the NGS analysis revealed that the consortium to consist predominantly of *Liquorilactobacillus*, *Clostridium*, *Lactiplantibacillus* and fungal members including *Candida*. However, the present analysis provides taxonomic information only and does not directly assign metabolic functions to individual taxa. Consequently, laccase production and bioelectrochemical activity observed in this study should be interpreted as collective consortium performance rather than activity attributable to specific microorganisms. It has been previously documented that members of the genera *Lactiplantibacillus* and *Liquorilactobacillus* have demonstrated activity related to multicopper oxidase. Nevertheless, further analyses at the level of individual isolates as well as analyses based on omics technologies are required to validate these functional roles within the current system (Fig. 4)[31-32].

The activity of laccase in the consortium cultured in melanoidin solution was monitored at regular intervals throughout the incubation period. The results demonstrated that the maximum laccase activity (6.89 ± 0.04 U/L) was observed on day 5, followed by day 4 (6.38 ± 0.03 U/L) (Fig. 5).



(a)

(b)

Fig. 4. Microbial communities of the laccase-producing consortium: (A) laccase-producing bacterial community and (B) laccase-producing fungal community

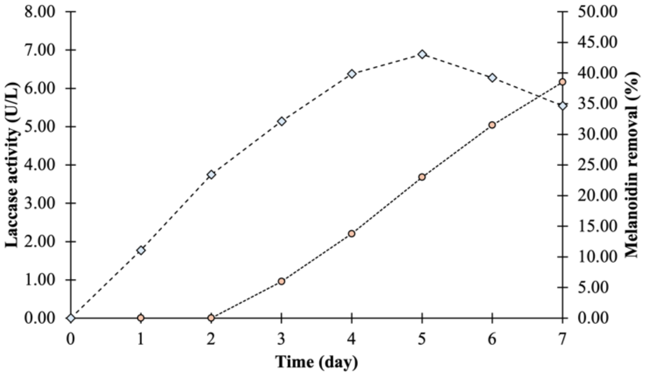


Fig. 5. Laccase activity of the microbial consortium and melanoidin removal (%) of the microbial consortium during 7 days of incubation

The removal of melanoidin increased progressively during the incubation period, which corresponded with the increase in laccase activity produced by the microbial consortium. The highest rate of degradation was observed around day-5, coinciding with the maximum laccase activity. Although laccase activity increased during the incubation period and coincided with enhanced melanoidin removal, the present data do not permit quantitative attribution of removal solely to enzymatic degradation. The observed performance is likely the result of a combination of mechanisms including initial adsorption onto the biochar surface, microbial transformation, and extracellular oxidative activity. It is evident that, due to the absence of desorption and carbon mass balance analyses, it remains unfeasible to definitively differentiate between irreversible degradation of melanoidin and physical retention within the immobilized matrix. It is recommended that future studies integrate desorption testing and degradation product analysis to verify oxidative cleavage pathways.

Tsiakiri et al. [33] conducted a study on laccase and melanoidin removal, determining that multicopper oxidases, such as laccase are responsible for the removal of both natural and synthetic melanoidins in wastewater. Conversely, laccase immobilized on a glass support achieved 68% melanoidin degradation in baker's yeast effluent within 24 hours [34,35,36].

3.3. Effect of melanoidin concentration

The effect of melanoidin concentration on removal efficiency is presented in Fig. 6. The removal efficiency exhibited a gradual decline with increasing initial melanoidin concentration, declining from 77.83±0.76% at 0.5 g COD/L to 42.85±0.13% at 3.0 g COD/L. While this trend may indicate reduced treatment efficacy under the condition of elevated substrate loading, the present data do not allow the confirmation of substrate inhibition, owing to the absence of determined kinetic parameters. The observed reduction may instead reflect multiple interacting factors, including limited accessibility of active enzymatic sites, mass transfer resistance within the immobilized biochar matrix, and possible accumulation of intermediate degradation products. It is recommended that future studies encompass time-course degradation experiments and kinetic model fitting using substrate inhibition models (e.g., Haldane kinetics) to quantitatively verify the governing mechanism.

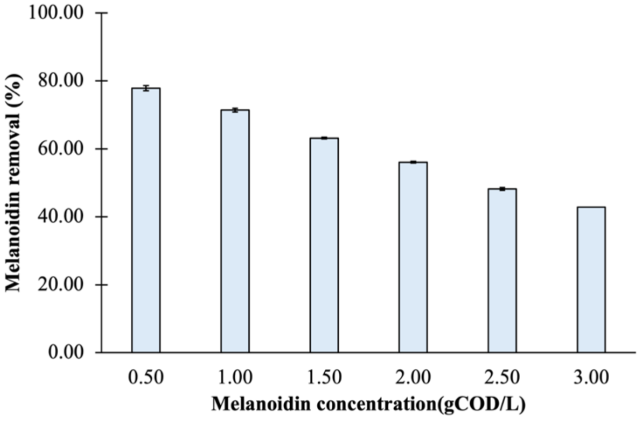


Fig. 6. Effect of melanoidin concentration on removal efficiency of laccase producing microbe immobilized on BC600

3.4. Effect of pH

The effect of pH on melanoidin removal by the immobilized microbial consortium is presented in Fig. 7. The findings demonstrated that melanoidin removal increased under acidic conditions, reaching a maximum value of 48.40±0.53% at a pH of 4. The removal efficiency exhibited a gradual decline with an increase in pH with 42.77±0.25% observed at pH 7, which corresponded to the condition employed in the previous experiment. The lowest removal efficiency was observed at a pH of 8 (34.50±0.50%) In accordance with the findings of Georgiou et al. [34,35,36], the immobilized laccase study revealed that laccase immobilized on a glass support achieved 68% degradation of melanoidin-containing baker's yeast effluent within 24 hours at a pH of 4.5. Conversely, a bacterial consortium capable of producing manganese peroxidase and laccase achieved up to 70% decolorization of Maillard reaction products (melanoidin) at an optimum pH of 8.1 [37].

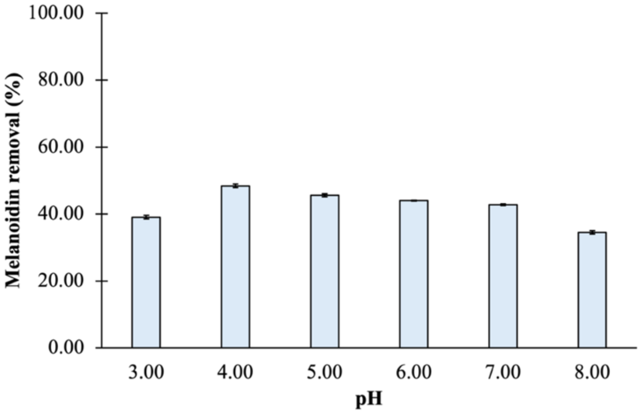


Fig. 7. Effect of pH on removal efficiency of laccase producing microbe immobilized on BC600

3.5. Effect of temperature

Fig. 8 depicts the effect of temperature on melanoidin removal. The removal efficiency exhibited a positive correlation with temperature, reaching a maximum at 35 °C (47.70±0.26%), followed by a slight decrease at higher temperatures.

Conversely, environmental factors including temperature have been demonstrated to have a great influence on the process of microbial degradation. The findings indicated that the degradation (or decolorization) of melanoidin occurs optimally at temperatures between 20 and 37 °C, with deviations from this range having an adverse effect on microbial activity [38].

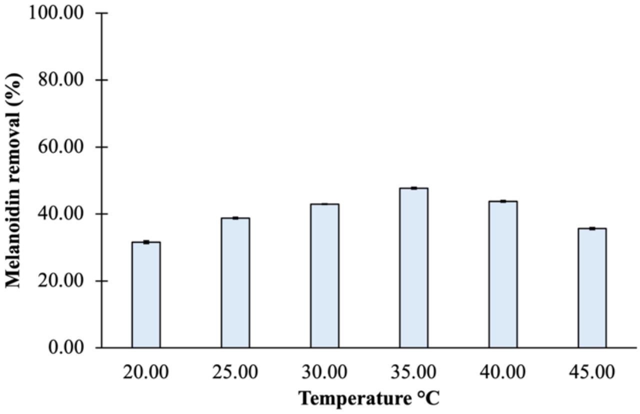


Fig. 8. Effect of temperature on removal efficiency of laccase producing microbe immobilized on BC600

3.6. Melanoidin removal in raw POME

The melanoidin removal efficiency in raw POME under different treatment systems is presented in Table 1. The immobilized microbial consortium exhibited the highest removal efficiency (73.15±1.10%), followed by the free microbial consortium (62.70±0.95%) and EFB biochar alone (38.40±0.80%). The control group demonstrated minimal removal (5.20±0.50%).

Table 1. Removal efficiency in raw POME under different treatment systems

Characteristics	Control	Biochar	Free cell	Immobilized microbial consortium
Melanoidin removal (%)	5.20±0.50	38.40±0.80	62.70±0.95	73.15±1.10
COD removal (%)	3.50±0.10	40.20±1.20	68.60±0.90	84.30±0.85

As posited by Ahmed et al. [39], a study was conducted to examine the removal of coal fly ash melanoidin. The study found that chemically treated coal fly ash could be used as a low-cost adsorbent for the removal of melanoidin from distillery effluent, achieving a maximum removal efficiency of 84% at pH 6 and a contact time of 120 minutes. Moreover, a combination of biological and chemical sequential processes has been employed for the removal of melanoidin and chemical oxygen demand from industrial molasses-based baker's yeast wastewater, resulting in a maximum color removal efficiency of 94.65%. However, this approach still involves high operating costs [40].

3.7. Electrochemical properties and carbon fixation

The OCV of the MFC is illustrated in Fig. 9. The treatment achieved a maximum OCV of 619.17±10.49 mV, which was substantially higher than that of the control (52.00±3.54 mV). The maximum current density (CD) and power density (PD) were 8.29±0.15 A/m^3 and 1.00±0.20 W/m^3, respectively (Fig. 10). The electrochemical properties and carbon fixation rate are presented in Table 2.

Despite the MFC demonstrating promising electrochemical performance in laboratory conditions, the reactor operated at a relatively modest working volume (10 mL). Consequently, the reported current density and power density should be interpreted as proof-of-concept values rather than direct indicators of scale-up performance. It is evident that small-volume MFC systems generally benefit from higher surface-area-to-volume ratios, reduced ion transport distances, lower internal resistance, and improved substrate accessibility. This, in turn, may enhance apparent electrochemical outputs. Consequently, linear extrapolation of current and power densities to larger systems may not be appropriate. It is recommended that future studies undertake an evaluation of the effects of reactor scale-up through the investigation of electrode configuration, reactor geometry, mass transfer behavior, and internal resistance under the conditions of increased operating volumes.

Conversely, textile wastewater was treated using an MFC integrated with gamma radiation–modified bacteria. The findings demonstrated that a maximum OCV of 554.67 mV was achieved, accompanied by 96.42% decolorization [41]. Moreover, an up-flow MFC has been employed for the purpose of electricity generation from dark-colored distillery wastewater. This study explores the application of a continuous up-flow MFC in the treatment of dark brown distillery wastewater, with a focus on the simultaneously generating bioelectricity. The wastewater contains recalcitrant melanoidins. A maximum power density (PD) of 0.10 W/m^2 was achieved [42]. However, no previous studies have reported on the carbon capture potential of metabolites generated during wastewater degradation.

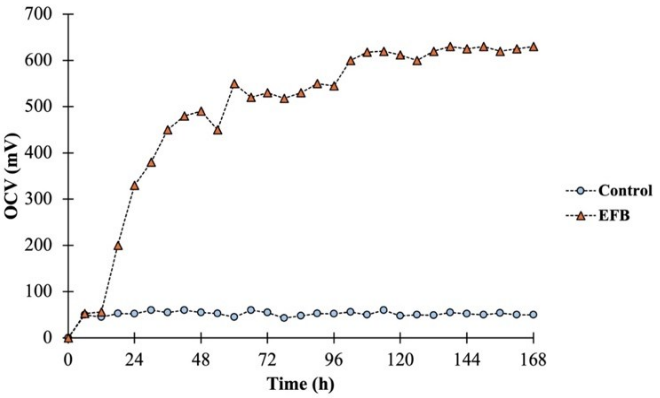


Fig. 9. Open circuit voltage (OCV) of the MFC during the experimental period.

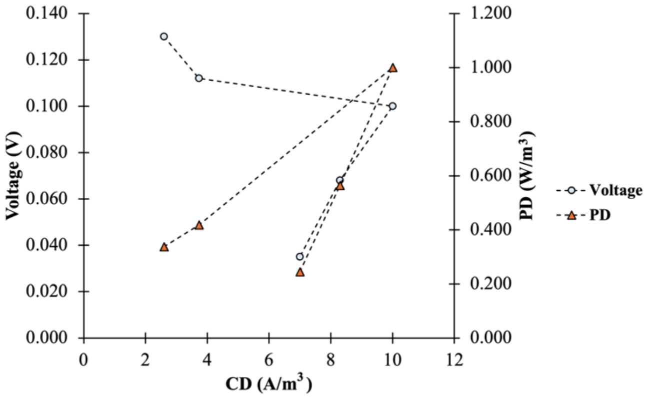


Fig. 10. Polarization curve of the MFC during the experimental period

Table 2. Electrochemical properties and carbon fixation rate of the MFC during the experimental period

Characteristics	Value
OCV (mV)	619.17±10.49
CCV (mV)	130.15±5.20
CD (A/m^3)	8.29±0.15
CD (A/m^2)	0.42±0.01
PD (W/m^3)	1.00±0.20
PD (W/m^2)	0.05±0.01
P_m (g/L/day)	0.08±0.00
Carbon fixation rate (g/L/day)	0.13±0.00

Despite the observation that melanoidin removal, electrochemical performance, and carbon fixation occurred simultaneously in the present system, the current experimental design does not permit direct attribution of electricity generation to melanoidin oxidation alone. As untreated POME contains a variety of biodegradable organic compounds, electron recovery is likely to have originated from overall microbial metabolism in general, rather than exclusively from melanoidin degradation. Similarly, carbon fixation reflects microalgal utilization of available CO_2 in the cathodic chamber and should not be interpreted as direct fixation of melanoidin-derived carbon. Consequently, the observed processes should be regarded as concurrent system outcomes rather than confirmed mechanistic pathways.

3.8. Phytotoxicity

The phytotoxicity of immobilized EFB biochar following MFC treatment was evaluated based on seed germination (see Table 3). The findings demonstrated that the immobilized EFB biochar exhibited no phytotoxic effects in comparison with sterile distilled water and untreated EFB biochar. This finding is consistent with previous studies, which have reported that the absence of phytotoxicity at typical biochar application rates does not inhibit seed germination or early plant development [43]. Furthermore, previous studies have indicated that non-phytotoxic behavior can be observed following wastewater treatment processes [44].

Table 3. Effect of immobilized EFB biochar on rice germination

Treatment	Germination (%)
Control (H_2O)	85.00±5.00
Normal EFB biochar	81.67±2.89
Immobilized EFB biochar	83.33±7.64

4. Conclusion

This present study proposes a novel and an efficient strategy for the removal of melanoidin, bioelectricity generation, and carbon fixation. This strategy involves the integration of a carbon-capturing MFC with biochar-immobilized laccase-producing consortia. It is important to note that this is the first study to couple biochar-based immobilization with a carbon-capturing MFC for an integrated system enabling concurrent melanoidin removal, bioelectricity recovery, and carbon fixation under the tested conditions. The EFB biochar (BC600) provided an effective support for microbial immobilization and adsorption. The system achieved a maximum melanoidin removal of 73.15±1.10% and significant COD reduction with degradation closely associated with laccase activity. In the MFC, enhanced electrochemical performance was observed, with a maximum OCV of 619.17± 0.49 mV. Moreover, the results of phytotoxicity study demonstrated that there were no inhibitory effects on rice seed germination indicating environmental safety. Nevertheless, further validation is required under larger reactor configurations is required before practical performance and scalability can be established.

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