



Decolorization and Bioelectricity Generation from Palm Oil Mill Effluent by a Photosynthetic Bacterial Consortium

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Abstract

Palm oil mill effluent (POME) is the dark brown agricultural wastewater from palm oil extraction factories. It is difficult to decolorize using conventional methods. Melanoidin is a dark-colored polymer formed through the Maillard reaction which is the primary cause of the dark color in POME. This study investigated the potential of a photosynthetic bacterial consortium consisting of Blastochloris sulfoviridis and Lentimicrobium saccharophilum for POME treatment and bioenergy generation. The consortium effectively removed melanoidin content (68.89±0.84%) and color $(60.87 \pm 1.22\%)$ from POME without the addition of chemicals or culture medium. Additionally, a microbial fuel cell (MFC) integrated with the consortium generated a-power output of up to 5.70 ± 1.06 W m⁻³. The degraded metabolites were analyzed by gas chromatography-mass spectrometry (GC-MS) after treatment. The results revealed that melanoidin was converted to 1-ethyl-2methylbenzene, 1,2,4-trimethylbenzene, decamethylcyclopentasiloxane, dodecamethylcyclohexane, butylated hydroxytoluene, and stigmasta-3,5-diene. Following treatment, the cell pellet was recovered and analyzed for valuable by-products. Carotenoid and astaxanthin pigments were extracted with yields of 0.32±0.01 and 0.02±0.00 mg g⁻¹, respectively. These findings demonstrate the versatility of the photosynthetic bacterial consortium, which offers a sustainable solution for POME treatment while simultaneously POME decolorization and producing bioenergy and valuable compounds.

Keywords: biodegradation; color removal; melanoidin; palm oil waste; pigment degradation

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INTRODUCTION

Palm oil is a high-value agricultural product that can be used as a raw material in various products such as daily, food, body care, and cosmetics products (Mutsaers, 2019). It is mainly produced in Equatorial Africa and Southeast Asia, especially Indonesia, Malaysia, Thailand, Colombia, and Nigeria (Mba et al., 2015; Jagaba et al., 2021; Sulaiman et al., 2024). Wastes include palm oil shells, palm oil fiber, empty fruit bunches, palm kernels, and palm oil mill effluent (POME) produced during palm oil manufacture (Abdullah and Sulaim, 2013). The palm oil industry generates approximately 2.5 to 3.0 m³ of dark-brown POME from each ton of crude palm oil, which contains a high chemical oxygen demand (COD) of about 100 g l⁻¹ (Saputera et al., 2021). The discharge of dark-brown POME can cause damage to the ecosystem, especially to water resources and aquatic ecosystems (Abrams et al., 2015). A suitable wastewater treatment

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system can help mitigate the negative effects caused by the pigment of POME on the environment (Rakhmania et al., 2023).

Various processes have been used for POME treatment such as catalytic stream reforming (Valizadeh et al., 2021), anaerobic ponding system (Tamrin and Yaser, 2017), biological treatment (Chan et al., 2012), phytoremediation (Darajeh et al., 2014), microbial fuel cell (MFC) (Baranitharan et al., 2013) and microbial degradation (Ratnasari et al., 2021). Many different microbes are used in microbial degradation. In Said et al. (2021), an anaerobic bacterial consortium consisting of Bacillus strain BCT-71120 tovonensis and Stenotrophomonas rhizophila strain e-p10 was used to treat POME. The highest reduction of COD and total solids was achieved reaching 86% and 80%, respectively.

For color removal, the electrocoagulation process has been used for decolorization and mineralization of raw POME. The results showed that using 14 volts for 3 hours and an electrolyte concentration of 13.41 g l⁻¹ achieved a 56% reduction in COD and a 65% reduction in color (Rakhmania et al., 2023). On the other hand, photodegradation utilizes light for the removal of color and phenolic compounds present in POME. The cyanobacteria Arthrospira platensis was integrated into this system, effectively removing color and phenolic compounds (Nur et al., 2021). Moreover, previous study by researchers demonstrated that the *Citrobacter* sp. rice-based facultative anaerobic bacterial consortium can successfully remove melanoidin and decolorize POME through its extracellular enzyme (Thipraksa et al., 2022). However, this process does not generate any value-added by-products from the bacterial biomass.

Photosynthetic bacteria are a group of bacteria with a broad range of metabolic capacities, including aerobic and anaerobic cellular respiration, fermentation, and nitrogen fixation (Sasikala and Ramana, 1995). It has been used for various applications such as wastewater treatment and value-added substance recovery (Cao et al., 2020). Furthermore, photosynthetic bacteria are effective in removing color, heavy metals, and micropollutants from wastewater (Talaiekhozani and Rezania, 2017).

MFCs are bio-electrochemical technologies that convert organic materials into bioelectricity through microbial activity (Obileke et al., 2021). This technology can utilize various substrates, especially wastewater, providing two primary benefits, including wastewater treatment and electricity generation (Xu et al., 2017). MFCs are devices capable of producing sustainable bioelectricity by degrading organic matter. These devices have garnered significant interest worldwide due to their potential applications in various fields (Priya et al., 2022).

Several studies have reported using MFCs integrated with effective microbial consortia for color removal from wastewater and electricity generation. In Xu et al. (2020), a two-chamber MFC with a sponge anode was utilized for azo dye wastewater treatment. The results showed a maximal color removal of 62.84% and a maximal power output of 2.82 W m⁻³. On the other hand, an H-type MFC integrated with the bacterium Pseudomonas gessardii was used to treat reactive dye wastewater. A maximal power output of 474 mW m⁻² was achieved (Agrahari et al., 2024). No previous study has been reported on using photosynthetic bacteria for POME decolorization and electricity generation in an MFC.

This study comprehensively evaluated the color removal capabilities of a photosynthetic microbial consortium. Next-generation sequencing was employed to analyze the microbial community structure, while gas chromatography-mass spectrometry (GC-MS) was utilized to identify degraded metabolites. Additionally, a two-chamber MFC system was used to monitor bioelectricity generation (Figure 1), and the production of carotenoid and astaxanthin by-products was assessed.

MATERIALS AND METHOD

Photosynthetic consortium

The photosynthetic consortium was gained from the Department of Biotechnology, Faculty of Science and Digital Innovation, Thaksin University, Phatthalung Campus, Thailand. It was preserved in the modified Ormerod medium contains 0.6 g 1^{-1} KH₂PO₄, 0.9 g 1^{-1} K₂HPO₄, 0.2 g 1^{-1} MgSO₄.7H₂O, 0.075 g 1^{-1} CaCl₂.2H₂O, 0.0188 g 1^{-1} FeSO₄.7H₂O, 0.02 g 1^{-1} EDTA, 6 g 1^{-1} malic acid, and 15 µg 1^{-1} biotin (Choi et al., 2022).

A molasses-based medium $(0.25 \text{ g } \text{I}^{-1} \text{ molasses}, 6.4 \text{ g } \text{I}^{-1} \text{ yeast extract}, 0.5 \text{ g } \text{I}^{-1} \text{ K}_2\text{HPO}_4, 0.5 \text{ g } \text{I}^{-1} \text{ K}_2\text{HPO}_4, 0.2 \text{ g } \text{I}^{-1} \text{ MgSO}_4.7\text{H}_2\text{O}$ and 0.05 g $\text{I}^{-1} \text{ CaCl}_2.2\text{H}_2\text{O}$) was used to enrich a photosynthetic consortium for application in wastewater treatment (Saejung and Puensunnern, 2018). Briefly, a 10% (v/v) inoculum of the consortium was added to fresh



Figure 1. The mechanism of the decolorization and bioelectricity generation processes

molasses medium (90% v/v) in a 250 ml erlenmeyer flask. The flask was incubated at 30 °C for 10 days under anoxic conditions with a rubber stopper. An LED lamp with a light intensity of 500 lux was used as the light source for the growth of the photosynthetic consortium.

Synthetic wastewater

The synthetic POME was prepared according to the previous study (Chaijak et al., 2024). Briefly, the melanoidin solution was prepared by mixing 22.5 g l⁻¹ glucose (Himedia, India), 9.4 g l⁻¹ glycine (Himedia, India), and 2.1 g l⁻¹ NaHCO₃ (Himedia, India) with 500 ml of distilled water and heating the mixture at 95 °C for 7 hours. Afterward, 500 ml of distilled water was added to the solution. To prepare synthetic wastewater, a 10% (v/v) melanoidin solution was mixed with a 90% (v/v) phosphate buffer (Himedia, India). The resulting mixture was sterilized at 121 °C for 15 minutes.

For color removal, the 10% (v/v) of the consortium (OD₆₀₀ = 1.0) was added to synthetic POME (90% v/v) in a 250 ml erlenmeyer flask. The flask was incubated at 30 °C for 10 days under anoxic conditions with a rubber stopper. An LED lamp with a light intensity of 500 lux was used as the light source for the growth of the photosynthetic consortium. The samples were collected every 2 days, to track color removal at 450 nm using a UV-Vis spectrophotometer (Shimadzu, Japan).

POME

POME was collected from the wastewater treatment plant of a palm oil extraction factory in Trang Province, Southern Thailand. The sample was obtained from the sedimentation tank following the biogas plant. The sample was preserved at -25 $^{\circ}$ C in a freezer (Haier, China) until further analysis.

For the experiment, the frozen sample was thawed to a liquid state and filtered through sterile gauze 2 to 3 times. The 10 ml of the consortium were inoculated into 90 ml of filtered POME and incubated at 30 °C for 10 days under anoxic conditions with a light intensity of 500 lux. Color removal was monitored at 450 nm using a UV-Vis spectrophotometer. The degraded POME was collected for further analysis.

Degraded metabolite

The degraded melanoidin in the POME was extracted using ethanol as a solvent (Wang et al., 2023). The 2 ml of extracted liquid was used for analysis in the GC-MS. A GC-MS analysis was performed using an Rtx-5MS column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness). Helium was employed as the carrier gas at a flow rate of 1 ml minute⁻¹. The sample injection port temperature was set to 300 °C with a split ratio of 10. The initial temperature was at 80 °C for 2 minutes, followed by a temperature increase to 315 °C at a rate of 5 °C per minute and held for 11 minutes. The ion source and interface temperatures were maintained at 200 and 220 °C, respectively. Data acquisition was conducted in scan mode over a mass-to-charge ratio range of 35 to 1000 with a solvent delay of 3 minutes (Yu et al., 2023).

Next-generation sequencing (NGS)

The bacterial community of the photosynthetic consortium was analyzed using NGS

(BMKGENE, China). The V3-V4 region of the 16S rRNA gene was amplified using specific primers (forward primer, 5'-ACTCCTACGGGAGGCAGCA-3'; reverse primer, 5'- GGACTACHVGGGTWTCTAAT-3') and sequenced. Taxonomic classification was performed using the Bayesian method and the Silva 138 database.

Electricity generation

An aluminum plate (4 cm²) served as the anodic electrode, while a copper-plated electrode (4 cm²) functioned as the cathodic electrode. The MFC chamber consisted of 240 ml glass cell culture bottles. A proton exchange membrane (PEM) was fabricated using a polyvinyl chloride tube (4.5 mm diameter) filled with 0.1% (w/v) KCl (KMLABS, Thailand) in 15 g l⁻¹ agarose gel (Himedia, India). Alligator clips connected the anodic and cathodic electrodes.

For operation, 180 ml of POME was mixed with 20 ml of consortium and placed in the anodic chamber. The cathodic chamber was filled with sterile distilled water and oxygenated using an air pump. The open circuit voltage (OCV) was monitored every 24 hours for 10 days. The closedcircuit voltage (CCV) was measured at an external resistance of 1,000 Ω . The electrochemical properties were calculated using Equation 1, 2, 3, and 4.

$$I = \frac{V}{R}$$
(1)

$$\mathbf{P} = \mathbf{I} \times \mathbf{V} \tag{2}$$

$$CD = \frac{I}{A}$$
(3)

$$PD = \frac{P}{A}$$
(4)

Where, I is the current (A), V is the CCV (V), R is the external resistance (Ω), P is the power (W), CD is the current density (A m⁻³), A is the working volume (m³), and PD is the power density (W m⁻³).

By-products analysis

The treated POME was centrifuged at 12,000 rpm for 10 minutes. The supernatant was discarded, and the pellet was washed with sterile distilled water 2 to 3 times. One gram of wet cells was mixed with 1 ml of methanol-acetone (2:3 v/v) solution and vortexed. The liquid phase was collected, and the extraction process was repeated 3 times. The carotenoid content was

determined by measuring absorbance at 480 and 770 nm using a UV-Vis spectrophotometer. Total carotenoid content was calculated according to Equation 5 (Patthawaro et al., 2020).

Where, A_{480} and A_{770} are the absorbance at 480 and 770 nm, respectively.

For astaxanthin, 1 g of wet cells was mixed with 1 ml of ethanol and vortexed. The liquid phase was collected, and the extraction process was repeated 3 times. The astaxanthin was measured at a wavelength of 478 nm (Khoo et al., 2019) and calculated using Equation 6.

Astaxanthin (mg g⁻¹) =
$$\frac{(OD_{478} - 0.0035)}{81.88}$$
 (6)

RESULTS AND DISCUSSION

Melanoidin removal

A photosynthetic consortium was investigated for its efficacy in degrading melanoidin within a synthetic POME solution. The consortium was introduced to the melanoidin-containing medium, and melanoidin removal was monitored over a 10-day incubation period. The results demonstrated that the consortium effectively degraded melanoidin, achieving a maximum removal rate of 68.89±0.84% (Figure 2). These findings suggest the potential application of this photosynthetic consortium as a promising strategy for treating melanoidin-contaminated wastewater in liquid environments.

Activated carbon (AC) has emerged as a potential adsorbent for the removal of melanoidins from molasses effluent. Powdered activated carbon (PAC) is effective in removing melanoidins, but the resulting melanoidin-PAC immobilized generates a secondary waste product (Liakos and Lazaridis, 2016). To overcome this limitation, Fe-impregnated activated carbon has been investigated as an alternative adsorbent. Studies have demonstrated its effectiveness in removing melanoidins from distillery wastewater achieving a maximum removal efficiency of 85.60% at an adsorbent dose of 62.50 mg l⁻¹. The impregnation of Fe onto AC appears to enhance its adsorption capacity and selectivity for melanoidin removal (Rizvi et al., 2020). A previous study reported that a bacterial consortium dominated by Citrobacter sp. produced extracellular laccase,



Figure 2. Melanoidin removal of photosynthetic bacterial consortium in synthetic POME

which degraded 86.02% of the melanoidin present in POME after 48 hours of incubation (Thipraksa et al., 2022).

Color removal

The photosynthetic bacterial consortium exhibited significant color removal capabilities when inoculated into POME. As monitored by UV-Vis spectrophotometry over 10 days, the consortium achieved a maximum color removal of $60.87\pm1.22\%$ (Figure 3). In contrast, the control treatment using the POME indigenous bacteria demonstrated negligible color removal potential. These findings suggest that the photosynthetic bacterial consortium possesses specific metabolic pathways or enzymes that are particularly effective in degrading the chromophores present



Figure 3. Color removal of photosynthetic bacterial consortium in synthetic POME

in POME. Figure 4 illustrates the relationship between color removal and melanoidin removal.

Electrocoagulation and limestone treatment have emerged as alternative methods for color removal from POME. Electrocoagulation has demonstrated promising results. achieving a maximum color removal of 65.00% under optimized conditions of 14 volts and 3 hours of electrolysis time. However, the requirement for external energy poses a limitation for its practical application (Rakhmania et al., 2023). On the other hand, limestone offers a more energy-efficient approach. Studies have shown that limestone prepared at 800 °C can effectively remove color from POME, reaching a maximum removal of 61.00% at a filtration rate of



Figure 4. The relationship between color removal and melanoidin removal

20 ml minute⁻¹ and a retention time of 317 minutes (Dashti et al., 2021).

Metabolite

The degraded metabolites of the POME were analyzed using the GC-MS method. Methanol was used as the solvent for extracting the degraded metabolites. The chromatogram of the degraded metabolites is shown in Figure 5a.

The degraded metabolites were mainly composed of 1-ethyl-2-methylbenzene (Figure

5b), 1,2,4-trimethylbenzene (Figure 5c), decamethylcyclopentasiloxane (Figure 5d), dodecamethylcyclohexane (Figure 5e), butylated hydroxytoluene (BHT) (Figure 5f), and stigmasta-3,5-diene.

The 1-ethyl-2-methylbenzene is a volatile organic compound (VOC), identified as a significant pollutant in wastewater treatment plants. It is frequently detected in rubber wastewater, petrochemical wastewater, and other industrial effluents (James and Stack, 1997).





Figure 5. Chromatograms of degraded POME

It is the colorless toluene that was used as a solvent in various reactions (Canneaux et al., 2012). While decamethylcyclopentasiloxane has been identified in Canadian wastewater (Silva et al., 2021), there are no reported instances of human toxicity (Lee et al., 2023). Butylated hydroxytoluene has been studied as an antioxidant agent (Yehye et al., 2015) while stigmasta-3,5diene has not been reported in human toxicity.

Microbial community

The bacterial consortium was analyzed using NGS. Phylum-level diversity revealed Proteobacteria (21.90%), Bacteroidota (20.19%), unclassified bacteria (15.38%), Synergistota (9.18%), Firmicutes (8.96%), Spirochaetota (6.79%), Chloroflexi (3.98%), Cloacimonadota (3.83%), Patescibacteria (2.95%), Caldisericota (2.01%), and others (4.83%).

The family level diversity revealed was Xanthobacteraceae composed of (17.68%),unclassified bacteria (15.38%),Lentimicrobiaceae (9.36%), Synergistaceae (9.18%), Spirochaetaceae (5.93%), Bacteroidetes (5.69%),Cloacimonadaceae (3.83%),Christensenellaceae (3.60%), Rhodospirillaceae (2.62%), unclassified SBR1031 (2.41%), and others (24.32%).

The class level diversity revealed Alphaproteobacteria (20.64%), Bacteroidia

(17.87%), unclassified bacteria (15.38%), Synergistia (9.18%), Spirochaetia (5.93%), Clostridia (5.34%), Anaerolineae (3.94%), Cloacimonadia (3.83%), Ignavibacteria (2.15%), Caldisericia (2.01%), and others (13.74%).

The order level diversity revealed Rhizobiales (17.68%), unclassified bacteria (15.38%), Sphingobacteriales (9.39%), Synergistales (9.18%), Bacteroidales (8.39%), Spirochaetales (5.93%), Cloacimonadales (3.83%), Christensenellales (3.60%), SBR1031 (2.76%), Rhodospirillales (2.64%) and others (21.21%).

The genus level diversity revealed unclassified (15.38%),Blastochloris bacteria (8.38%), unclassified Xanthobacteraceae (7.66%),uncultured prokaryote (6.61%), Lactivibrio (6.29%), unclassified Bacteroidetes (5.69%),unclassified Spirochaetaceae (4.76%),unclassified Cloacimonadaceae (3.83%),Christensenellaceae (2.92%), Lentimicrobium (2.87%) and others (35.61%).

The species level diversity (Figure 6) revealed unclassified bacteria (15.38%), Blastochloris sulfoviridis (8.37%), unclassified Xanthobacteraceae (7.66%),uncultured prokaryote (6.71%), unclassified Lactivibrio (6.29%), unclassified Bacteroidetes (5.69%), (3.83%). unclassified Cloacimonadaceae unclassified Pararhodospirillum (2.62%),



Figure 6. The species-level diversity of bacterial consortium

Lentimicrobium saccharophilum (2.57%), unclassified SBR1031 (2.41%), and others (38.46%).

Heras et al. (2020) utilized the nonsulfur anoxygenic phototroph *Blastochloris* sp. for nitrogen-deficient wastewater treatment. Conversely, *Lentimicrobium* sp. has been employed for high-sulfate wastewater treatment (Li et al., 2020). There are no published reports of its application for POME treatment.

Electrochemical properties

The OCV of the dual-chamber MFC was monitored every 24 hours for 10 days.

A maximum OCV of 546.67 \pm 5.77 mV was achieved during operation (Figure 7). Meanwhile, the maximum CD and PD of the dual-chamber MFC integrated with the photosynthetic bacterial consortium were 16.83 \pm 1.61 A m⁻³ and 5.70 \pm 1.06 W m⁻³, respectively. Conversely, the MFC was utilized to generate electricity from POME. A maximum power density of 0.50 W m⁻³ was achieved at a hydraulic retention time of 12 days (Ng et al., 2024). In Albarracin-Arias et al. (2021), the bio-electricity generation was provided by MFCs integrated with pure electrogenic culture *Shewanella* sp. and POME sludge. The results





Figure 7. The OCV of dual chamber MFC integrated with photosynthetic bacterial consortium



found that the maximal power generation was 3.30 W m^{-3} .

By-products

The cell pellet was recovered from the treatment system after the MFC operation. The cells were disrupted by an organic solvent, and then the pigment by-product was determined. The carotenoid content was 0.32 ± 0.01 mg g⁻¹, and the astaxanthin content was 0.02±0.00 mg g⁻¹ (Figure 8). On the other hand, carotenoids were produced using pure photosynthetic bacteria Rhodopseudomonas faecalis PA2 with agricultural wastes such as soybean, coconut, and cassava meal without additional nutrients proposed. The maximal carotenoid production of 0.08 to 96.43 mg g⁻¹ was achieved, but astaxanthin was not found. However, none of these methods produced electricity or other green energy (Patthawaro et al., 2020).

CONCLUSIONS

The photosynthetic bacterial consortium composed of В. sulfoviridis and L. saccharophilum demonstrated significant potential for sustainable wastewater treatment and bioenergy production. This study found that the consortium effectively removed melanoidin content (68.89±0.84%) from synthetic wastewater and color (60.87±1.22%) from POME without the need for additional chemicals or culture medium. Moreover, the integrated MFC system generated a notable maximum power output of 5.70±1.06 W m⁻³. Following the treatment process, valuable by-products were recovered from the cell pellet. Carotenoid and astaxanthin pigments were extracted with yields of 0.32 ± 0.01 and 0.02 ± 0.00 mg g⁻¹, respectively. These findings highlight the versatility of the photosynthetic bacterial consortium, which can contribute to both environmental remediation and bioresource recovery. The consortium could be integrated with other wastewater treatment processes such as anaerobic digestion, to improve overall treatment efficiency and additional recover valuable by-products. Moreover, an economic analysis should be conducted to evaluate the cost-effectiveness of the consortium for wastewater treatment and bioenergy production.

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