

# Enhancing bioelectricity generation through co-cultivation of bacteria consortium and microalgae in photosynthetic microbial fuel cell

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## Abstract

This study investigates the effect of microbial configuration on the electrochemical performance of photosynthetic microbial fuel cells (PMFCs). The PMFC configuration incorporating both bacteria and microalgae exhibited the highest open-circuit voltage (OCV) of  $397.95 \pm 31.53$  mV, significantly higher than that of the OCVs obtained in the sterile control (C1) and the microalgae-only configuration (C2), which were  $32.47 \pm 22.43$  mV and  $284.59 \pm 12.63$  mV, respectively. Furthermore, the PMFC containing only microalgae achieved a current density (CD) of  $20.96 \pm 0.18$  mA/m<sup>2</sup> and a power density (PD) of  $0.40 \pm 0.01$  mW/m<sup>2</sup> under room temperature conditions. Notably, the combined bacteria and microalgae configuration demonstrated a substantial performance improvement, yielding a significantly higher CD of  $49.33 \pm 0.36$  mA/m<sup>2</sup> and PD of  $0.78 \pm 0.01$  mW/m<sup>2</sup> at room temperature. This configuration also achieved a maximum decolorization of  $93.57 \pm 0.10\%$  with a corresponding algal biomass recovery of  $134.90 \pm 2.69$  mg/L. These findings highlighted the critical role of microbial composition in PMFC performance. The combination of bacteria and microalgae yielded superior results compared to other configurations under the investigated conditions.

**Keywords:** Electricity generation; microalgae; microbial fuel cell; palm oil mill effluent; photosynthesis

## 1. Introduction

Microbial fuel cells (MFCs) have emerged as a promising technology for the bioelectrochemical degradation of organic matter in wastewater [1]. This method harnesses the metabolic activity of microorganisms to generate electricity [1]. However, while functioning as bioenergy sources, MFCs produce carbon dioxide (CO<sub>2</sub>) as a byproduct during the oxidation of organic substrates. The growth and activity of microorganisms within an MFC are significantly determined by the characteristics of the anolyte solution and influent substrate [2,3]. In particular, MFCs inoculated with mixed microbial cultures often exhibit superior electricity generation compared to those inoculated with pure cultures [4].

The MFCs are reliant upon the interplay between diverse microorganisms and substrates for electricity generation. The power output of MFCs is significantly determined by the choice of substrate [8]. A detailed exploration of microbial specificity, therefore, offers a two-fold advantage. Firstly, it can minimize the formation of unwanted byproducts by promoting the desired metabolic pathways within the microbial community [9] and secondly, it allows for the selective identification of

microorganisms capable of maximizing power density within the MFC system [10]. Research efforts worldwide are investigating the potential of MFCs as multifunctional devices, capable of synthesizing valuable products alongside electricity generation. These advancements often involve minor adjustments to the composition of the respective electrodes within the MFC [5]. Recent studies have highlighted a key focus on enhancing the power output of MFCs. This factor is pivotal for propelling and limiting the large-scale implementation of MFC technology [6,7].

Photosynthetic microbial fuel cells (PMFCs) represent an advancement over traditional MFCs by incorporating a light source near an electrode that maintains microbial contact [8]. This configuration leverages the combined effects of light and microbial activity to enhance both cell voltage and electricity production [9,10]. PMFCs utilize various photosynthetic microorganisms as anode respiring bacteria. These microorganisms, including cyanobacteria (commonly referred to as blue-green algae), derive energy through photosynthesis and contribute to bioelectricity generation within the MFC [11]. Conversely, microalgae can be employed at the cathode, where they not only contribute to bioelectricity but also produce valuable byproducts such as oxygen, biofuels, carbohydrates, proteins, and carotenoids [12–14].

Algae-assisted PMFCs integrate living microalgae with self-

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sustaining catalyst bacteria and cost-effective electrodes, such as graphite, to achieve bioelectrochemical oxygen production. These systems aim to convert light energy into electricity by harnessing the photosynthetic activity of living phototrophic microorganisms. Compared to conventional MFCs, algae-assisted PMFCs offer several advantages. They, in particular, eliminate the need for external redox mediators due to the synergistic relationship between the living microalgae and electrochemically active bacteria. Through photosynthesis, the microalgae component releases free oxygen, eliminating the requirement for external aeration [15-17].

Traditional wastewater treatment processes often face significant challenges, including high energy consumption and operational costs. Furthermore, the need for multiple treatment units necessitates the optimization of efficiency within each stage [18]. Algal-based PMFCs offer a promising alternative for wastewater treatment by addressing these limitations. PMFCs demonstrate lower energy requirements while simultaneously generating valuable energy outputs [19]. Within the PMFC system, bacteria play a crucial role in organic matter degradation. These bacteria utilize enzymes to break down organic compounds as a source of carbon, effectively reducing the organic content of the wastewater [20].

Palm oil mill effluent (POME) is a complex mixture characterized by the high levels of solids, oil and grease, and elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The significant presence of these pollutants raises serious environmental concerns due to their detrimental impact on various ecosystems [21]. POME typically appears as a deep brown liquid with a pungent odor and a temperature range of 79.85 - 83.85°C. The colloidal composition of POME is approximately 95–96% water, 4–5% total solids, and 0.6–0.7% oil [22]. Several treatment technologies have been explored for POME management, including aerobic-anaerobic reactors, microbial fuel cells (MFCs), microalgae cultivation, photocatalysis, and catalytic steam reforming [23-27]. However, these methods often face limitations associated with the high costs and the need for further optimization to improve their performance [28].

In this study, the microalgae *Chlorella* sp. and an effective bacterial consortium [29] were utilized for the decolorization of POME and enhancement of electricity generation in a membrane-less photosynthetic microbial fuel cell. The production of microalgae biomass was evaluated as well.

## 2. Materials and Methods

### 2.1. Microalgae

The microalgae *Chlorella* sp. was gained from the Department of Biotechnology, Faculty of Science and Digital Innovation, Thaksin University. It was cultured and maintained in the microalgae medium BG11 (Sigma-Aldrich, United States). Fig. 1 visualizes the microalgae cell. For PMFC operation, the microalgae, before being used, was cultured in the BG11 for 7 days.

### 2.2. Bacterial consortium

The *Citrobacter* sp. dominant effective bacterial consortium

(S5) was achieved from our previous research [29]. It was cultured and maintained in the nutrient broth (HiMedia, India). For PMFC operation, the S5 was enriched in the nutrient broth for 2 days before used.

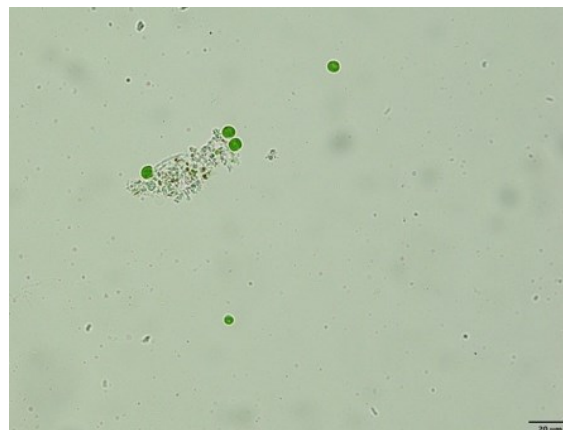


Fig. 1. Microalgae *Chlorella* sp. used in this experiment.

### 2.3. POME

Synthetic POME was employed in this study to avoid sediment disruption between the operation and control, ensuring consistent POME quality. For the synthesis process, 4.5 g/L glucose, 1.88 g/L glycine, and 0.42 g/L were dissolved in deionized water, and incubated at 95°C for 7 hours. The solution was subsequently cooled down at room temperature [30].

### 2.4. PMFC operation

Fig. 2 depicts the PMFC model. A 1 L plastic bottle served as the PMFC chamber with 20 cm<sup>2</sup> graphite plates employed as electrodes, which were connected by copper wires (0.1 cm diameter) and a 500 g sterile sand layer was used as an electron separator. The anolyte consisted of 400 mL synthetic POME. A 50 mL bacterial consortium (OD<sub>600</sub> = 1.0) served as the anodic biocatalyst, degrading organic matter in synthetic POME and generating electrons. Additionally, a 50 mL microalgae solution (OD<sub>540</sub> = 1.0) was utilized as a cathodic biocatalyst to enhance electricity generation. An adjustable resistor was used as the external resistance and a multimeter served as the electrical measurement tool. The electrical energy generated was compared with PMFCs without microbes (C1) and PMFCs with microalgae (C2). The experiment was showed in Fig. 3.

The open circuit voltage (OCV) was measured every 6 hours for 7 days. The closed circuit voltage (CCV) was collected for use in the calculation of the electrochemical properties.

### 2.5. Calculation

The electrochemical properties were calculated according to the Ohm's law that follows Equations (1) – (4):

$$I = V/R \quad (1)$$

$$CD = I/A \quad (2)$$

$$P = IV \tag{3}$$

$$PD = P/A \tag{4}$$

where I is the current (A), V is the CCV (V), R is the external resistance ( $\Omega$ ), CD is the current density ( $A/m^3$ ), A is the working volume ( $m^3$ ), P is the power (W), and PD is the power density ( $W/m^3$ ). Here, the polarization curve was constructed based on the data of CD, PD and voltage.

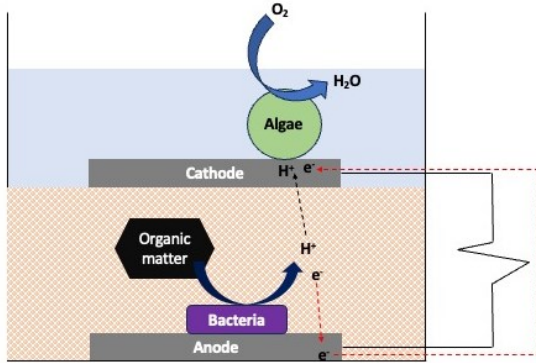


Fig. 2. The PMFC model used in this experiment.

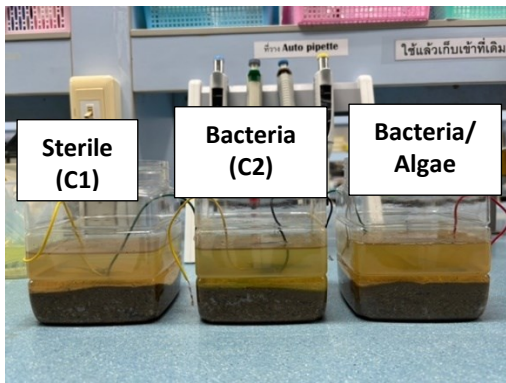


Fig. 3. The PMFC used in this experiment.

### 2.6. Decolorization and biomass recovery

Biomass production was monitored every 6 hour for 7 days using the light absorbance at 680 nm measured by UV-Vis spectrophotometry. The dried biomass of microalgae was calculated based on  $OD_{680}$  data, where  $OD_{680} = 1.0$  corresponded to 0.19 g/L [31]. The color removal (melanoidin removal) was monitored at 540 nm every 6 hour for 7 days [32]. The color removal was calculated as shown in Equation (5) as follows:

$$Removal (\% = [(A - B)/A \times 100] \tag{5}$$

where A refers to the initial absorbance and B is the final absorbance at 540 nm.

## 3. Results and Discussion

### 3.1. Electrochemical properties

The maximal OCV was obtained from PMFC with the bacteria/microalgae reaching  $397.95 \pm 31.53$  mV, while the maximal OCV values for C1 (sterile) and C2 (microalgae) were

$32.47 \pm 22.43$  mV and  $284.59 \pm 12.63$  mV, respectively (Fig. 4). This research findings indicated that the PMFC employing microalgae achieved CD and PD values of  $20.96 \pm 0.18$  mA/m<sup>3</sup> and  $0.40 \pm 0.01$  mW/m<sup>3</sup> respectively under the room temperature conditions (Fig. 5). Furthermore, an enhanced performance was observed with a higher CD of  $49.33 \pm 0.36$  mA/m<sup>3</sup> and PD of  $0.78 \pm 0.01$  mW/m<sup>3</sup> for the PMFC with bacteria/microalgae under room temperature (Fig. 6). The control did not gain the CCV where it was connected with external resistance.

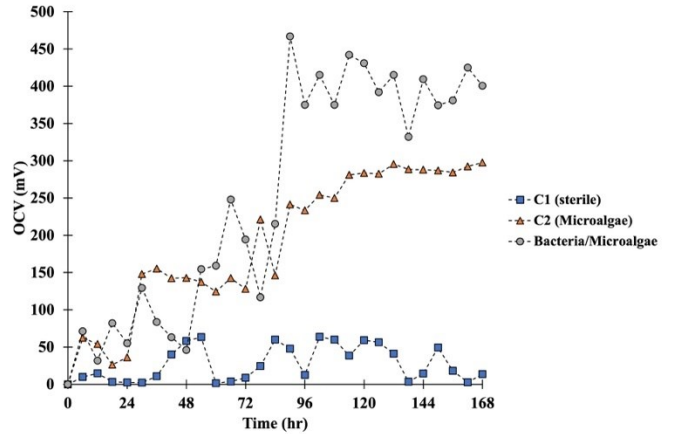


Fig. 4. The open circuit voltage (OCV) produced by PMFC.

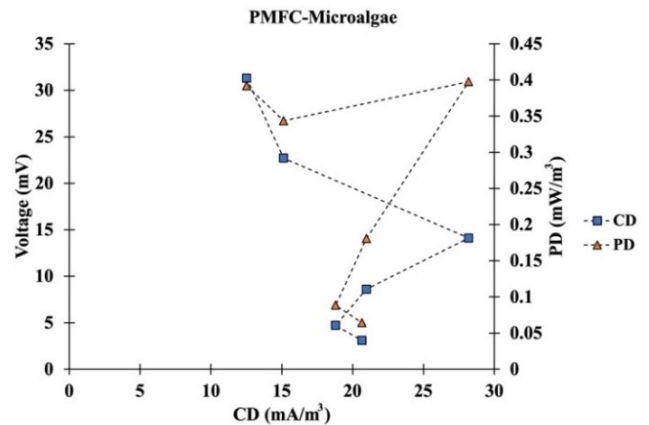


Fig. 5. The polarization curve of PMFC with microalgae

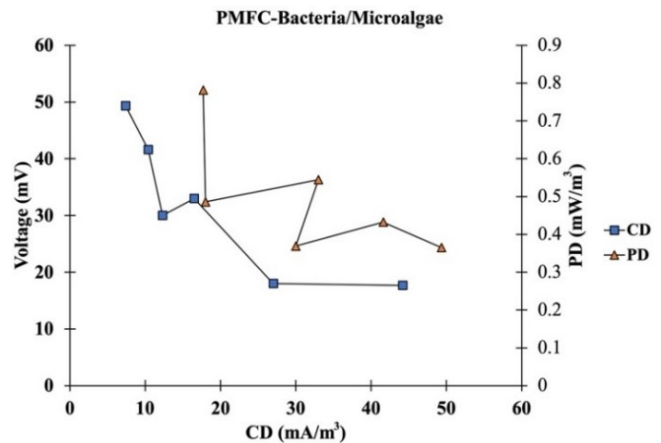


Fig. 6. The polarization curve of PMFC with bacteria/microalgae

The selection of microalgae in an algae-assisted PMFC is a crucial factor that determines both the efficiency of the PMFC

and the biomass yield. This choice plays a pivotal role in determining the quantity of value-added products that can be recovered. Additionally, certain algae, such as diatoms, possess distinctive cell walls composed of silica with nano porous structures. These unique characteristics enable diatoms to effectively adsorb metals, ions, and organic compounds, contributing to the multifunctional capabilities of the PMFC system [13]. Several studies have delved into the realm of PMFC research.

Table 1. Electrochemical properties of the PMFC.

Microalgae	MFC type	PD	Reference
<i>Chlorella</i> sp.	Membrane-less	$0.78 \pm 0.01 \text{ W/m}^3$ $0.20 \pm 0.00 \text{ W/m}^2$	This study
<i>Chlorella vulgaris</i>	Dual chamber	$0.089 \text{ mW/m}^2$	[32]
<i>Synechococcus</i> sp.	Dual chamber	$0.096 \text{ mW/m}^2$	[33]
<i>Chlorella vulgaris</i>	Dual chamber	$1.1 \text{ W/m}^3$	[34]
Mixed microalgae	Membrane-less	$22.19 \text{ mW/m}^2$	[35]

### 3.2. Decolorization and biomass recovery

The maximum color removal efficiency achieved was  $93.57 \pm 0.10\%$  when utilizing a PMFC containing bacteria and microalgae. Notably, this high removal rate was attained at room temperature without the addition of any culture medium. In comparison, the PMFC configurations with only microalgae and the control exhibited significantly lower color removal efficiencies of  $68.57 \pm 0.21\%$  and  $32.14\% \pm 0.10\%$ , respectively (Fig. 7). The by-product of this PMFC process is the biomass of microalgae. The highest biomass production was observed in the PMFC containing bacteria and microalgae, reaching  $134.90 \pm 2.69 \text{ mg/L}$ . In contrast, the PMFC with only microalgae yielded a significantly lower biomass of  $25.84 \pm 3.42 \text{ mg/L}$  (Fig. 8).

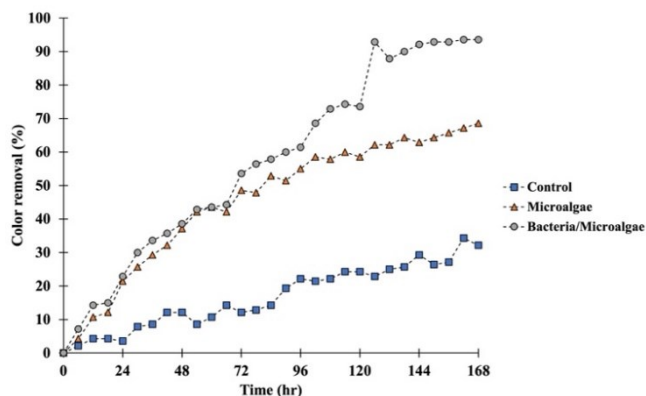


Fig. 7. The color removal (%) of the POME using PMFC

On the other hand, the decolorization of POME was pursued through the electrocoagulation process. Optimal results were achieved with a maximal color removal efficiency of 65%, employing an electrolyte concentration of  $13.41 \text{ g/L}$  [36]. In the study by Nur et al., photodegradation was employed for color removal in POME. The cyanobacterium *Arthrospira platensis* was utilized for biodegradation, resulting in a remarkable maximal color removal efficiency of 94%. This underscores the

effectiveness of utilizing *Arthrospira platensis* in the biodegradation process for achieving substantial color removal in POME [37]. Saidu et al. demonstrated that the freshwater microalga *Chlorella sorokiniana* could remove up to 86% of color from POME. However, biomass recovery was not reported in their study [38].

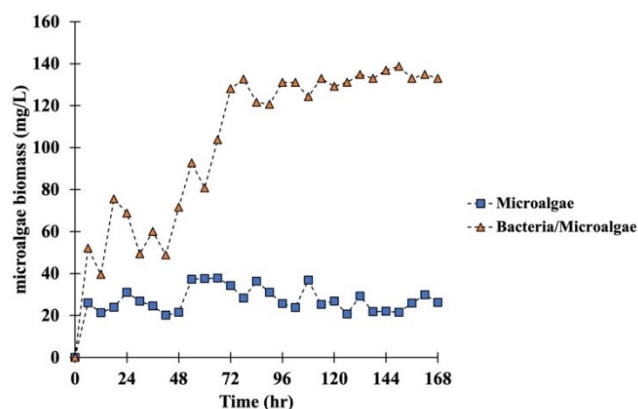


Fig. 8. The microalgae biomass produced by PMFC.

## 4. Conclusion

This study investigated the effect of microbial configuration on photosynthetic microbial fuel cell (PMFC) performance. The PMFC containing both bacteria and microalgae achieved the highest open-circuit voltage (OCV) and significantly outperformed configurations with only bacteria (sterile) or microalgae. Notably, the combined bacteria and microalgae configuration also demonstrated superior current density (CD) and power density (PD) compared to other setups. Additionally, this configuration achieved impressive decolorization alongside algal biomass recovery. These findings highlighted the critical role of microbial communities in PMFCs. For optimal performance under the investigated conditions, incorporating both bacteria and microalgae within the PMFC design appears to be the most promising approach.

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## References

1. N. Habibul, Y. Hu, Y. K. Wang, W. Chen, H. Q. Yu and G. P. Sheng, *Bioelectrochemical chromium (VI) removal in plant-microbial fuel cells*, Environ. Sci. Technol. 50 (2016) 3882-3889.
2. B. E. Logan, *Exoelectrogenic bacteria that power microbial fuel cells*, Nat. Rev. Micro. 7 (2009) 375-381.
3. Y. Sun, J. Wei, P. Liang, and X. Huang, *Microbial community analysis in biocathode microbial fuel cells packed with different materials*, AMB Expres. 2 (2012) 21.
4. D. Xing, Y. Zuo, S. Cheng, J. M. Regan, and B. E. Logan, *Electricity generation by Rhodospseudomonas palustris DX-1*, Environ. Sci. Technol. 42 (2008) 4146-4151.
5. H. Bird, E. S. Heidrich, D. D. Leicester, and P. Theodosiou, *Pilot-scale*

- microbial fuel cells (MFCs): A meta-analysis study to inform full-scale design principles for optimum wastewater treatment*, J. Clean. Produc. 346 (2022) 131227.
6. S. Mohyudin, R. Farooq, F. Jubeen, T. Rasheed, M. Fatima, and F. Sher, *Microbial fuel cells a state-of-the-art technology for wastewater treatment and bioelectricity generation*, Environ. Res. 204 (2022) 112387.
  7. V. Vinayak, M. J. Khan, S. Varjani, G. D. Saratale, R. G. Saratale and S. K. Bhatia, *Microbial fuel cells for remediation of environmental pollutants and value addition: Special focus cells with photocatalytic and photoelectric fuel cells*, J. Biotechnol. 338 (2021) 5-19.
  8. M. J. Khan, N. Singh, S. Mishra, A. Ahirwar, F. Bast, S. Varjani, B. Schoefs, J. Marchand, R. G. Saratale and V. Vinayak, *Impact of light on microalgae photosynthetic microbial fuel cells and removal of pollutants by nanoadsorbent biopolymers: Updates, challenges and innovations*, Chemos. 288 (2022) 132589.
  9. S. M. Sathe, I. Chakraborty, B.K. Dubey and M. M. Ghangrekar, *Microbial fuel cell coupled Fenton oxidation for the cathodic degradation of emerging contaminant from wastewater: Applications and challenges*, Environ. Res. 204 (2022) 112135.
  10. S. V. Ambekar and M. M. Ghangrekar, *Performance evaluation of microbial fuel cell using novel anode design and with low-cost components*, J. Environ. Eng. Sci. 17 (2022) 157-163.
  11. L. Mekuto, A. V. A. Olowolafe, R. Huberts, N. Dyantyi, S. Pandit and P. Nomngongo, *Microalgae as a biocathode and feedstock in anode chamber for a self-sustainable microbial fuel cell technology: a review*, S. Afr. J. Chem. Eng. 31 (2020) 7-16.
  12. A. K. Gupta, K. Seth, K. Maheshwari, P. K. Baroliya, M. Meena, A. Kumar, V. Vinayak and H. Harish, *Biosynthesis and extraction of high-value carotenoid from algae*, Front. Biosci. (Landmark Ed). 26 (2021) 171-190.
  13. M. J. Khan, N. Bawra, A. Verma, V. Kumar, A. Pugazhendhi, K. B. Joshi and V. Vinayak, *Cultivation of diatom Pinnularia saprophila for lipid production: A comparison of methods for harvesting the lipid from the cells*, Bioresour. Technol. 319 (2021) 124129.
  14. K. Seth, A. Kumar, R. P. Rastogi, M. Meena, V. Vinayak, *Bioprospecting of fucoxanthin from diatoms – Challenges and perspectives*, Algal Res. 60 (2021) 102475.
  15. S. B. Velasquez-Orta, T. P. Curtis and B. E. Logan, *Energy from algae using microbial fuel cells*, Biotechnol. Bioeng. 103 (2009) 1068-1076.
  16. S. V. Mohan, G. N. Nikhil, P. Chiranjeevi, C. N. Reddy, M. V. Rohit, A. N. Kumar and O. Sarkar, *Waste biorefinery models towards sustainable circular bioeconomy: Critical review and future perspectives*, Bioresour. Technol. 215 (2016) 2-12.
  17. R. Chandra, G. V. Subhash and S. V. Mohan, *Mixotrophic operation of photo-bioelectrocatalytic fuel cell under anoxygenic microenvironment enhances the light dependent bioelectrogenic activity*, Bioresour. Technol. 109 (2012) 46-56.
  18. Z. Du, H. Li and T. Gu, *A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy*, Biotechnol. Adv. 25 (2007) 464-482.
  19. A. Rhoads, H. Beyenal and Z. Lewandowski, *Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant*, Environ. Sci. Technol. 39 (2005) 4666-4671.
  20. M. Zhou, H. Wang, D. J. Hassett and T. Gu, *Recent advances in microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for wastewater treatment, bioenergy and bioproducts*, J. Chem. Technol. Biotechnol. 88 (2013) 508-518.
  21. S. Mohammad, S. Baidurah, T. Kobayashi, N. Ismail and C. P. Leh, *Palm oil mill effluent treatment processes – A review*, Processes. 9 (2021) 739.
  22. H. Kamyab, S. Chelliapan, C. T. Lee, T. Khademi, A. Kumar, K. K. Yadav, S. Rezaia, S. Kumar and S. S. Ebrahimi, *Improved production of lipid contents by cultivating Chlorella pyrenoidosa in heterogeneous organic substrates*, Clean Technol. Environ. Policy. 21 (2019) 1969-1978.
  23. A. Y. Zahrim, A. Nasimah and N. Hilal, *Pollutants analysis during conventional palm oil mill effluent (POME) ponding system and decolourisation of anaerobically treated POME via calcium lactate-polyacrylamide*, J. Water Process. Eng. 4 (2014) 159-165.
  24. M. A. Islam, M. Rahman, A. Yousof, C. K. Cheng, W. C. Wei, *Performance of Klebsiella oxytoca to generate electricity from POME in microbial fuel cell*, MATEC Web of Conferences. 38 (2016) 03004.
  25. H. Saidu, H. Jamaludin, K. Iwamoto, M. M. Salleh, A. Yahya and S. E. Mohamad, *Low-cost biodiesel production*, Asian J. Appl. Sci. 10 (2017) 57-65.
  26. N. S. M. Aris, S. Ibrahim, B. Arifin and Y. Hawari, *Effect of operating parameters on decolourisation of palm oil mill effluent (POME) using electrocoagulation process*, Pertan. J. Sci. Technol. 25 (2017) 197-206.
  27. F. Gormez, O. Gormez, E. Yabalak and B. Gozmen, *Application of the central composite design to mineralization of olive mill wastewater by the electro/Fell/persulfate oxidation method*, SN Appl. Sci. 2 (2020) 1-11.
  28. Z. H. Mussa, M. R. Othman and M. P. Abdullah, *Electrochemical oxidation of landfill leachate: investigation of operational parameters and kinetics using graphite-PVC composite electrode as anode*, J. Braz. Chem. Soc. 26 (2015) 939-948.
  29. J. Thipraksa, P. Chaijak, P. Michu and M. Lertworapreecha, *Biodegradation and bioelectricity generation of melanoidin in palm oil mill effluent (POME) by laccase-producing bacterial consortium integrated with microbial fuel cell*, Biocatal. Agricul. Biotechnol. 43 (2022) 102444.
  30. J. Thipraksa and P. Chaijak, *Using of oxidoreductase producing consortium with high manganese peroxidase activity for melanoidin degradation and generation from palm oil mill effluent*, Bioint. Res. Appl. Chem. 13 (2023) 268.
  31. W. Y. Cheah, P. L. Show, J. C. Juan, J. S. Chang and T. C. Ling, *Microalgae cultivation in palm oil mill effluent (POME) for lipid production and pollutants removal*, Ener. Cover. Manag. 174 (2018) 430-438.
  32. A. Khandelwal, M. Chhabra and P. Yadav, *Performance evaluation of algae assisted microbial fuel cell under outdoor conditions*, Bioresour. Technol. 310 (2020) 123418.
  33. R. Lakshmidivi, N. N. Gandhi and K. Muthukumar, *Carbon neutral electricity production from municipal solid waste landfill leachate using algae-assisted microbial fuel cell*, Appl Biochem. Biotechnol. 191 (2020) 852-866.
  34. B. Kokabian and V. G. Gude, *Sustainable photosynthetic biocathode in microbial desalination cells*, Chem. Eng. J. 262 (2015) 958-965.
  35. B. Neethu and M. Ghangrekar, *Electricity generation through a photo sediment microbial fuel cell using algae at the cathode*, Water Sci. Technol. 76 (2017) 3269-3277.
  36. R. Rakmania, H. Kamyab, M. A. Yuzir, F. F. Al-Qaim, L. D. A. Purba and F. A. Riyadi, *Application of Box-Behnken design to mineralization and color removal of palm oil mill effluent by electrocoagulation process*, Environ. Sci. Pollut. Res. 30 (2023) 71741-71753.
  37. M. M. A. Nur, G. M. Garcia, P. Boelen and A. G. J. Buma, *Influence of photodegradation on the removal of color and phenolic compounds from palm oil mill effluent by Arthrospira platensis*, J. Appl. Phyco. 33 (2021) 901-915.
  38. H. Saidu, M. Shaza Eva, J. Haryati and Y. Adibah, *Phycoremediation of palm oil mill effluent (POME) by freshwater microalgae*, Adv. Sci. Lett. 24 (2018) 3652-3657.