JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT
Volume 11, Number 1 (October 2023):4961-4967, doi:10.15243/jdmlm.2023.111.4961
ISSN: 2339-076X (p); 2502-2458 (e), www.jdmlm.ub.ac.id

Research Article

Electricity production from palm oil mill effluent (POME) through the integration of a microbial fuel cell and bilirubin oxidase-producing bacteria

Junjira Thipraksa[1], Panisa Michu[2], Pimprapa Chaijak[1*]

[1] Department of Biology, Faculty of Science, Thaksin University, Thailand
[2] Department of Biotechnology, Faculty of Science, Thaksin University, Thailand

[*]corresponding author: chaijak.pimprapa@gmail.com

Article history:
Received 11 April 2023
Revised 21 May 2023
Accepted 27 May 2023

Keywords:
bilirubin oxidase
bioenergy
electricity generation
microbial fuel cell
palm oil mill effluent

Abstract

The microbial fuel cell (MFC) is a device that harnesses microbial metabolism to convert chemical energy into bio-electrical energy. Extensive research has demonstrated its efficacy in both wastewater treatment and power generation applications. This study focused on the integration of a microbial fuel cell (MFC) with a biocathode constructed using the oxidoreductase-producing bacterium *Bacillus* sp. MCO22 and rice straw as a cost-effective substrate. The MFC utilized palm oil mill effluent (POME) as a chemical energy source for electricity generation in the anodic chamber. The ability of the MFC was evaluated by monitoring biochemical oxygen demand (BOD) activity and electrochemical properties. Post-operation, chemical oxygen demand (COD) and color removal were measured. The results revealed that the MFC with the BOD-based cathode achieved a maximum current density and power density of 0.58±0.01 A/m^2 and 0.17±0.00 W/m^2, respectively. Furthermore, it exhibited high COD and color removal rates of 95.10±0.10% and 98.53±0.33%, respectively, without requiring an external power supply. This study presents novel insights into utilizing a BOD-producing bacterium as a whole-cell biocatalyst on the MFC cathodic surface for both electricity generation and agricultural wastewater treatment.

To cite this article: Thipraksa, J., Michu, P. and Chaijak P. 2023. Electricity production from palm oil mill effluent (POME) through the integration of a microbial fuel cell and bilirubin oxidase-producing bacteria. Journal of Degraded and Mining Lands Management 11(1):4961-4967, doi:10.15243/jdmlm.2023.111.4961.

Introduction

Agricultural activities generate agricultural wastewater during the yield harvesting process. Solid-form waste is typically incinerated for energy production (Fareed et al., 2020). While liquid-form waste is treated using various methods such as constructed wetland (Nan et al., 2020), flocculation (Picos-Corrales et al., 2020), nanoparticle absorption (Wang et al., 2018), bio-purification (Gikas et al., 2018), photocatalysis (Becerra et al., 2020) and microbial fuel cell (Pandit et al., 2021).

The microbial fuel cell (MFC) is a scheme that couples microbial metabolism to transform the chemical energy derived from organic matter into electrical energy. It has garnered significant attention as a promising technology for alternative energy production and liquid-waste treatment (Kim et al., 2021). Nevertheless, the performance of MFCs is hindered by the high cost and irreversible nature of the electrode reactions.

Previous studies have indicated that the cathodic electrode, predominantly employing costly metal catalysts such as platinum (Pt) or white gold, constitutes a major portion of the structural expenses (Rismani et al., 2008). According to Mani et al. (2021), the utilization of biocathodes with microbial enzyme catalysts shows promise as a potential alternative to expensive platinum (Pt) metal catalysts for oxygen reduction in MFC. Various oxidoreductases, such as laccase (Lac), lignin peroxidase (LiP), manganese peroxidase (MnP), bilirubin oxidase (BOD), and cellobiose dehydrogenase (CDH), have been identified for their significant potential in enhancing electricity generation in MFCs (Lai et al., 2017; Chaijak et al., 2018; Trifonov et al., 2021; Scheiblbrandner et al., 2022). Evans et al. (2021) established that the implementation of oxidoreductase-based biocathodes can improve the electrical power production of MFCs. Furthermore, Qing et al. (2022) described a 2.39-fold increase in power output achieved by utilizing a BOD-based cathode in MFCs.

Bilirubin oxidase (BOD) is a multi-copper oxidoreductase (MCO) enzyme characterized by the presence of 4 Cu ions (Cu^+ or Cu^{2+}) and its ability to catalyze the reduction of molecular oxygen (O_2) under physical conditions (Santoro et al., 2013). In contrast to other oxidoreductases like laccase (Lac), BOD exhibits exceptional activity and stability even under extreme environmental conditions (Mano, 2012). BOD is naturally produced by a variety of microorganisms, including filamentous fungi, yeast, and bacteria. Sadeghian et al. (2020) conducted a study showcasing the efficient decolorization of congo red and malachite green using BOD derived from the thermophilic bacterium *Thermosediminibacter oceani*.

Palm oil mill effluent (POME) refers to the agricultural liquid waste generated through the oil extraction process, which poses a significant environmental concern owing to its high nutrient and phosphorus content (Low et al., 2021). Numerous techniques have been employed for POME treatment, including chemical coagulation (Mohamad et al., 2021), photocatalysis (Norhan et al., 2021), phytoremediation (Fernando et al., 2021), constructed wetlands (Norhan et al., 2021; Saputera et al., 2021), and MFC (Sarmin et al., 2021).

This study aimed to develop a bilirubin-based biocathode within an MFC setup to enhance electricity generation while simultaneously treating POME, addressing both energy production and wastewater treatment aspects.

Materials and Methods

Melanoidin degrading bacterial consortium

The facultative anaerobic consortium (S5) consisting of *Citrobacter werkmanii*, known for its ability to degrade melanoidin, was obtained from the Faculty of Science, Thaksin University (Thipraksa et al., 2022). The consortium was cultivated in nutrient broth (HiMedia, India) under static conditions at room temperature for 48 hours and subsequently stored at 4 ºC until its utilization in the present experiment.

Cathodic whole-cell catalyst

The bacteria *Bacillus* sp. MCO22 isolated from the biomass-rich soil was achieved by the Department of Biology, Faculty of Science, Thaksin University. It was preserved on the nutrient agar (HiMedia, India), and kept at 4 ºC until it was used in this experiment.

Bilirubin oxidase (BOD) activity

The colony of bacteria *Bacillus* sp. MCO22 was inoculated into the nutrient broth and incubated at room temperature for 48 hr with 150 rpm shaking. The growing cell was determined the concentration using the absorbance at 600 nm in the UV-Vis spectrophotometer (Shimazu, Japan) before being used in the next section. The 10 mL of liquid culture (OD_{600} = 1.0) was re-cultured into the 90 mL of nutrient broth, then the 1 mL of reaction was collected every 6 hr for monitoring the BOD activity. For BOD activity, the incubated medium was centrifuged at 12,000 rpm for 10 mins at 4 ºC to remove the suspended cell. The assay contained the 190 µL of cell-free supernatant and 10 µL of 2.0 mg/mL bilirubin. The reaction was incubated at 30 ºC for 60 mins (Feng et al. 2020). The BOD activity was monitored at 445 nm using UV-Vis spectrophotometry.

Biocathode preparation

The sun-dried rice straw was cut into 1.0 cm size, it was dried at 60 ºC for overnight to remove moisture content. The 10 mL of liquid culture (OD_{600} = 1.0) was added to 100 g of prepared rice straw and incubated at room temperature for 48 hr.

MFC operation

The dual-chamber MFC was assembled according to Chaijak et al. (2018), and 10 cm^2 of carbon cloth (Fuel Cell Store, United States) was used as the electrode. The H^+ exchange membrane was made from a 2 mm thickness ceramic plate. The fermented rice straw with the BOD-producing bacterium was transferred into the cathode chamber for immobilizing the BOD-producing bacteria on the cathodic electrode surface. The sterile rice straw was used as a negative control. The positive control was made from the 0.2 mg/cm^2 platinum (Pt) coated carbon cloth (Figure 1).

The 4 mL of melanoidin-degrading consortium (OD_{600} = 1.0) and the 36 mL of POME were filled into the anodic chamber. The cultures were accumulated at room temperature for 7 days. The anolyte was fed out, then the 40 mL of raw POME was fed in. The half-cell potential was measured using an Ag/AgCl reference electrode (Fuel Cell Store, United States), and the voltage was recorded at 5-min intervals for a duration of 1 hr.

The open-circuit voltage (OCV) was determined on an hourly basis. The closed-circuit voltage (CCV) was measured at resistance values ranging from 50 to 5,000 Ω, and a polarization curve for the microbial fuel cell (MFC) was constructed based on these measurements. The chemical oxygen demand (COD) was quantified utilizing the High Range Plus COD Digestion kit (Hach, India) following the prescribed procedure provided by the manufacturer. Melanoidin removal was evaluated at a wavelength of 280 nm using UV-Vis spectrophotometry.

Results and Discussion

BOD activity

The BOD is one of the members of the multicopper oxidase family that has been revealed to bind a type I (blue copper ion), a paramagnetic type II copper and a diamagnetic type II copper (Sakasegawa et al., 2006). It has been found in various fungi and bacteria such as *Magnaporthe oryzae* (Durand et al., 2012a), *Thermosediminibacter oceani* (Sadeghian et al., 2020), *Bacillus pumilus* (Roucher et al., 2019), and *Ochrobactrum* sp. BF15 (Martini et al., 2021). In this study, the BOD activity of the bacteria *Bacillus* sp. MCO22 is shown in Figure 2. The maximum BOD activity of 2.15±0.02 U/mL was gained where the bacteria *Bacillus* sp. MCO22 was cultured in the liquid medium for 48 hr.The comparison of the BOD activity of bacteria is shown in Table 1.

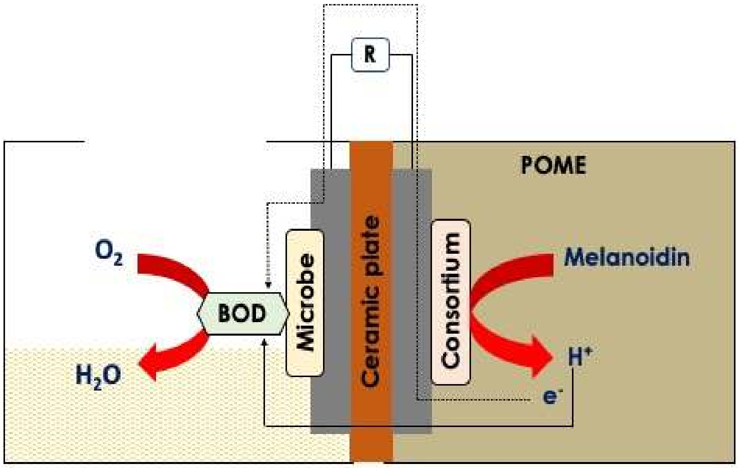

Figure 1. Diagram of MFC with whole-cell biocatalyst.

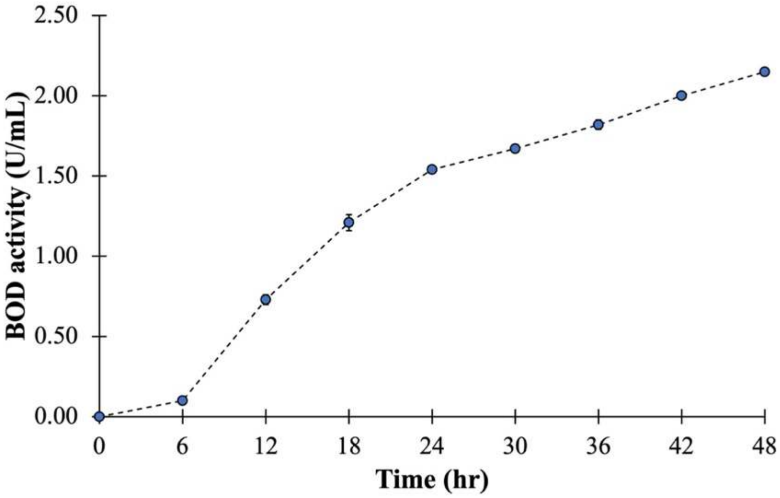

Figure 2. The bilirubin oxidase (BOD) activity of *Bacillus* sp. MCO22.

Table 1. Reviews of bilirubin oxidase activity.

Microbe	Source	BOD activity (U/mL)	Reference
Bacillus sp. MCO22	Biomass-rich soil	2.15±0.02	This study
Myrothecium verrucaria	Laboratory	101	Bayineni et al. (2018)
Thermosediminibacter oceani	Laboratory	0.5	Sadeghian et al. (2020)
Myrothecium verrucaria ITCC-8447	Laboratory	1.54	Agrawal et al. (2020)
Micromonospora sp. MP36	Bark compost	0.09-0.16	Itoh et al. (2021)

Half-cell potential

For cathodic catalysts, two multicopper oxidase enzymes have been used for enhancing electricity generation, like laccase and BOD. They can use a minimum of 4 Cu ions to catalyze the 4 electron reduction of oxygen gas (O_2) to water (H_2O) without the discharge of hydrogen peroxide (H_2O_2) (Solomon et al., 1996). Durand et al. (2012b) showed the BOD-based biocathode of *B. pumilus* can enhance electricity generation in biofuel cells. For this experiment, the half-cell potential of the whole-cell biocatalyst cathode was measured by comparing it with the universal reference electrode (Ag/AgCl electrode). The maximal voltage of 621.20±3.96 mV was achieved from the bilirubin (BOD) based cathodic electrode. While the 639.60±5.77 mV and 154.40±0.55 mV were gained from the positive and negative control, respectively (Figure 3).

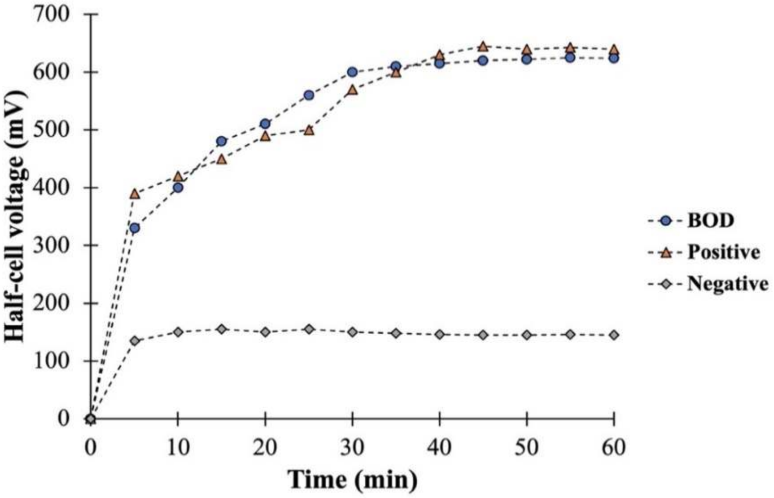

Figure 3. The half-cell potential of bilirubin (BOD) based cathode, platinum-based cathode (positive control), and non-catalyst cathode (negative control).

Milner and Yu (2018) used the aerobic biocathode with oxidoreductase enzyme for enhancing electricity generation. The maximal voltage potential of 400 mV was generated. On the other hand, the biocathode MFC with laccase can provide the maximal half-cell voltage of 249.67 mV (Chaijak et al., 2018).

Electrochemical properties

For electricity generation, the *Bacillus* sp. MCO22 was used as the whole-cell biocatalyst, and the rice straw was used as a cathodic substrate. Figure 4 displays the opened-circuit voltage (OCV) of the bilirubin oxidase (BOD) based MFC compared with the positive control (platinum-based MFC) and negative control (non-catalyst MFC). The maximum OCV of 907.44±2.46 mV was generated from the bilirubin oxidase (BOD) based MFC. Whereas 927.44±2.13 mV and 164.56±2.51 mV were produced from the positive control and negative control. The polarization curve was generated by calculating the closed-circuit voltage (CCV) at various external resistance values ranging from 50 to 5,000 Ω, following Ohm's law. Figure 5 illustrates the achieved maximum current density of 0.58±0.01 A/m^2 and power density of 0.17±0.00 W/m^2. In a study by Pal et al. (2020), the implementation of a biocathode comprising wheat straw degraded by white-rot fungi *Phlebia floridensis* and *Phlebia brevispora* demonstrated significant enhancements in electricity generation within a dual-chamber microbial fuel cell (MFC). A maximum power density of 0.03 W/m^2 was attained. Conversely, laccase-based biocathode MFCs have also been utilized for electricity generation, resulting in a maximum power output of 0.07 W/m^2 (Mani et al., 2018). A comparison of power outputs achieved by the biocathode-based MFCs is presented in Table 2.

COD and melanoidin removal efficiencies

The supernatant was collected from the anolyte after MFC operation and used for the melanoidin and COD removal efficiencies analysis. The maximal COD and melanoidin removal of 95.10±0.10% and 98.53±0.33% were achieved when the MFC system was operated for 48 hr. In the study conducted by Bashir et al. (2019), an optimum power input of 40.21 mA/cm^2 resulted in chemical oxygen demand (COD) and color removal efficiency of 71.30% and 96.80%, respectively. Conversely, the utilization of microalgae *Scenedesmus* sp. and *Chlorella* sp. for palm oil mill effluent (POME) treatment yielded a maximum COD removal efficiency of 48% (Hariz et al., 2020). Furthermore, the electrocoagulation process has been employed for COD removal during POME treatment. Nasrullah et al. (2019) reported an 85% COD removal efficiency achieved by supplying electrical energy for a duration of 50 min.

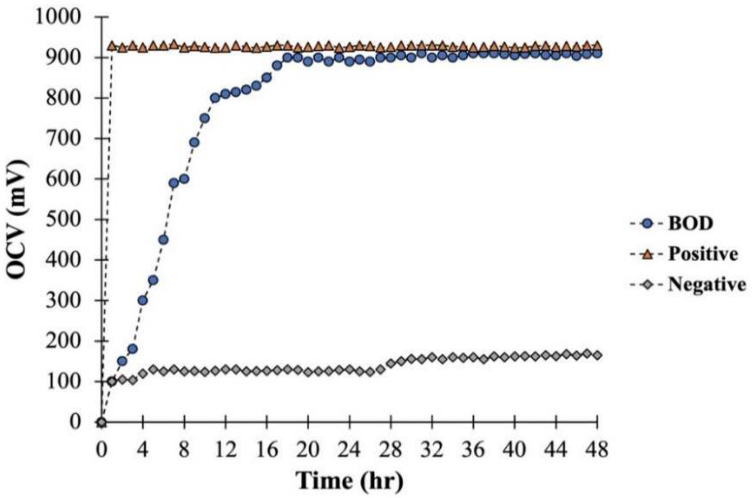

Figure 4. The opened-circuit voltage (OCV) of bilirubin (BOD) based cathode MFC, platinum-based cathode MFC (positive control), and non-catalyst cathode MFC (negative control).

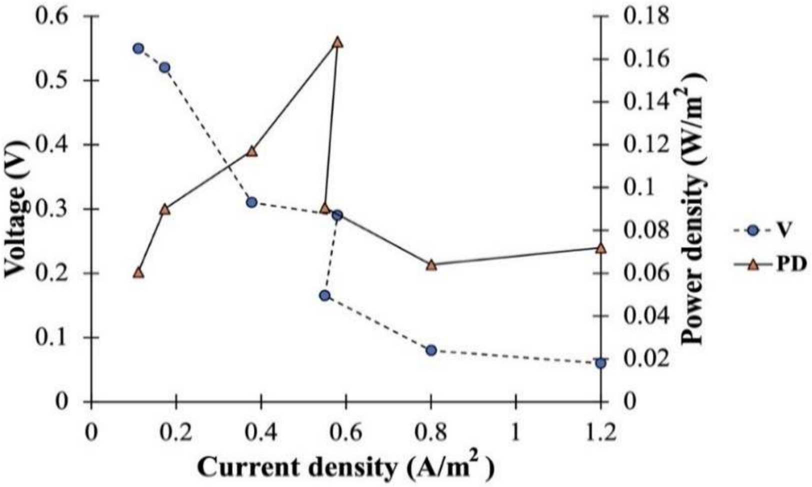

Figure 5. The polarization curve of bilirubin (BOD) based cathode MFC.

Table 2. Reviews of the biocathode-based microbial fuel cell (MFC).

Microbe	Enzyme	Catalyst type	Substrate	Power output (W/m^2)	Reference
Bacillus sp. MCO22	Bilirubin oxidase	Whole-cell	Rice straw	0.17±0.00	This study
Phlebia floridensis and *Phlebia brevispora*	Oxidoreductase	Whole-cell	Wheat straw	0.03	Pal et al. (2020)
Galactomyces reessii	Laccase	Whole-cell	Coconut coir	0.06	Chaijak et al. (2018)
Aspergillus sydowii NYKA 510	Laccase	Whole-cell	Banana peel	0.16	Abdallah et al. (2019)
Myrothecium verrucaria	Bilirubin oxidase	Pure enzyme	NA	0.002	Tan et al. (2020)

Conclusion

- The rice straw material can be used as the biocathode substrate for electricity generation by dual-chamber MFC.
- The bilirubin oxidase-producing bacteria can be used for the whole-cell biocatalyst on the cathodic electrode.
- The electrical energy of 0.17 W/m2 was produced from the MFC system when the POME was used

as a substrate.

- The maximal COD removal and melanoidin removal from the POME of 95.10±0.10% and 98.53±0.33% were gained without power input.

Acknowledgments

This work was supported by Thailand Science Research and Inno Office of National Higher Education Science Research and Innovation Policy Council, Thaksin University (research project grant) Fiscal Year 2022.

References

Abdallah, Y.K., Estevez, A.T., Tantawy, D.E.D.M., Ibraheem, A.M. and Khalil, N. 2019. Employing laccase-producing *Aspergillus sydowii* NYKA 510 as a cathodic biocatalyst in self-sufficient lighting microbial fuel cell. *Journal of Microbiology and Biotechnology* 29:1861-1872, doi:10.4014/jmb.1907.07031.

Agrawal, K., Shakar, J., Kumar, R. and Verma, P. 2020. Insight into multicopper oxidase laccase from *Myrothecium verrucaria* ITTCC-8447: a case study using in silico and experimental analysis. *Journal of Environmental Science and Health, Part B* 55:1048-1060, doi:10.1080/03601234.2020.1812334.

Bashir, M.J.K., Lim, J.H., Amr, S.S.A., Wong, L.P. and Sim, Y.L. 2019. Post treatment of palm oil mill effluent using electro-coagulation-peroxidation (ECP) technique. *Journal of Cleaner Production* 208:716-727, doi:10.1016/j.jclepro.2018.10.073.

Bayineni, V.K., Suresh, S., Sharma, A. and Kadeppagari, R.K. 2018. Improved bilirubin oxidase productivity of *Myrothecium verrucaria* and studies on the enzyme overproduced by the mutant strain in the solid-state fermentation. *The Journal of General and Applied Microbiology* 64:68-75, doi:10.2323/jgam.2017.07.002.

Becerra, D., Barrientos, I., Rodriguez, A., Machuca-Martinez, F. and Ramirez, L. 2020. Treatment of agricultural wastewater with chlorpyrifos by coupling of heterogeneous photocatalysis and anaerobic biological process. *Topics in Catalysis* 63:1261-1271, doi:10.1007/s11244-020-01281-4.

Chaijak, P., Sukkasem, C., Lertworapreecha, M., Boonsawang, P., Wijasika, S. and Sato, C. 2018. Enhancing electricity generation using a laccase-based microbial fuel cell with yeast *Galactomyces reessii* on the cathode. *Journal of Microbiology and Biotechnology* 28:1360-1366, doi:10.4014/jmb.1803.03015.

Durand, F., Gounel, S., Kjaergaard, C.H., Solomon, E.I. and Mano N. 2012a. Bilirubin oxidase from Magnaporthe oryzae: an attractive new enzyme for biotechnological applications. *Applied Microbiology and Biotechnology* 96:1489-1498, doi:10.1007/s00253-012-3926-2.

Durand, F., Kjaergaard, C.H., Suraniti, E., Gounel, S., Hadt, R.G., Solomon, E.I. and Mano, N. 2012b. Bilirubin oxidase from *Bacillus pumilus*: A promising enzyme for the elaboration of efficient cathodes in biofuel cells. *Biosensor & Bioelectronics* 35:140-146, doi:10.1016/j.bios.2012.02.033.

Evans, J.P., Gervasio, D.F. and Pryor, B.M. 2021. A hybrid microbial-enzymatic fuel cell cathode overcomes enzyme inactivation limits in biological fuel cells. *Catalysts* 11:242, doi:10.3390/catal11020242.

Fareed, A., Zaidi, S.B.A., Ahmad, N., Hafeeez, I., Ali, A. and Ahmad, M.F. 2020. Use of agricultural waste ashes in asphalt bunder and mixture: A sustainable solution to waste management. *Construction and Building Materials* 259:120575, doi:10.1016/j.conbuildmat.2020.120575.

Feng, H., Wang, M., Wang, B., Zhang, L., Zhang, F., Xu, J., Tian, Y., Gao, J., Peng, R. and Yao Q. 2020. Heterologous expression and characterization of bilirubin oxidase gene from *Myrothecium verrucaria* in *Arabidopsis thaliana*. *Biotechnology & Biotechnological Equipment* 34:421-429, doi:10.1080/13102818.2020.1766378.

Fernando, J.S.R., Premaratne, M., Dinalankara, D.M.S.D., Perera, G.L.N.J. and Ariyadasa, T.U. 2021. Cultivation of microalgae in palm oil mill effluent (POME) for astaxanthin production and simultaneous phycoremediation. *Journal of Environmental Chemical Engineering* 9:105375, doi:10.1016/j.jece.2021.105375.

Gikas, G.D., Perez-Villanueva, M., Tsioras, M., Alexoudis, C., Perez-Rojas, G., Masis-Mora, M., Lizano-Fallas, V., Rodriguez-Rodriguez, C.E., Vryzas, Z. and Tsihrintzis, V.A. 2018. Low-cost approaches for the removal of terbuthylazine from agricultural wastewater: constructed wetlands and biopurification system. *Chemical Engineering Journal* 335:647-656, doi:10.1016/j.cej.2017.11.031.

Hariz, H.B., Takriff, M.S., Yasin, N.H.M., Ba-Abbad, M.M. and Hakimi, N.I.N.M. 2020. Potential of the microalgae-based integrated wastewater treatment and CO2 fixation system to treat palm oil mill effluent (POME) by indigenous microalgae; *Scenedesmus* sp. and *Chlorella* sp. *Journal of Water Process Engineering* 32:100907, doi:10.1016/j.jwpe.2019.100907.

Itoh, N., Hayashi, Y., Honda, S., Yamamoto, Y., Tanaka, D. and Toda, H. 2021. Construction and characterization of a functional chimeric laccase from metagenomes suitable as a biocatalyst. *AMB express* 11:90, doi:10.1186/s13568-021-01248-y.

Kim, B., Jang, N., Lee, M., Jang, J.K. and Chang, I.S. 2021. Microbial fuel cell driven mineral rich wastewater treatment process for circular economy by creating virtuous cycles. *Bioresource Technology* 320:124254, doi:10.1016/j.biortech.2020.124254.

Lai, C.Y., Wu, C.H., Meng, C.T. and Lin, C.W. 2017. Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode. *Applied Energy* 188:392-398, doi:10.1016/j.apenergy.2016.12.044.

Low, S.S., Bong, K.X., Mubashir, M., Cheng, C.K., Lam, M.K., Lim, J.W., Ho, Y.C., Lee, K.T., Munawaroh, H.S.H. and Show, P.L. 2021. Microalgae cultivation in palm oil mill effluent (POME) treatment and biofuel production. *Sustainability* 13:3247, doi:10.3390/su13063247.

Mani, P., Kumar, V.T.F., Keshavarz, T., Chandra, T.S. and Kyazze, G. 2018. The role of natural laccase redox mediators in simultaneous dye decolorization and power production in microbial fuel cell. *Energies* 11:3455, doi:10.3390/en11123455.

Mani, P., Fidal, V.T., Keshavarz, T., Chandra, T.S. and Kyazze, G. 2021. Laccase immobilization strategies for application as a cathode catalyst in microbial fuel cells for azo dye decolorization. *Frontier Microbioliology* 11:620075, doi:10.3389%2Ffmicb.2020.620075.

Mano, N. 2021. Features and applications of bilirubin oxidases. *Applied Microbiology and Biotechnology* 96:301-307, doi:10.1007/s00253-012-4312-9.

Martini, M.C., Berini, F., Ausec, L., Casciello, C., Vacca, C., Pistorio, M., Lagares, A., Mandic-Mulec, I., Marinelli, F. and Papa, M.F.D. 2021. Identification and characterization of a novel plasmid-encoded laccase-like multicopper oxidase from *Ochrobactrum* sp. BF15 isolated from an on-farm bio-purification system. *Food Technology and Biotechnology* 59: 519-529, doi:10.17113/ftb.59.04.21.7253.

Milner, E.M. and Yu, E.H. 2018. The effect of oxygen mass transfer on aerobic biocathode performance, biofilm growth and distribution in microbial fuel cells. *Fuel Cells* 18:4-12, doi:10.1002/fuce.201700172.

Mohamad, N.A., Hamzah, S., Harun, M.H.C., Ali, A., Rasit, N., Awang, M., Rafizah, W., Rahman, W.A., Azmi, A.A.A.A.R., Habib, A.A.A., Zahid, M.S.A., Mustofa, A.A.F., Latfi, S.A., Aripin, S.M. and Saad R. 2021. Integration of copperas and calcium hydroxide as a chemical coagulant and coagulant aid for efficient treatment of palm oil mill effluent. *Chemosphere* 281:130873, doi:10.1016/j.chemosphere.2021.130873.

Nan, X., Lavrnic, S.A. and Toscano, A. 2020. Potential of constructed wetland treatment system for agricultural wastewater reuse under the EU framework. *Journal of Environmental Management* 275:111219, doi:10.1016/j.jenvman.2020.111219.

Nasrullah, M., Zularisam, A.W., Krishnan, S., Sakinah, M., Singh, L. and Fen Y.W. 2019. High performance electrocoagulation process in treating palm oil mill effluent using high current intensity application. *Chinese Journal of Chemical Engineering* 27:208-217, doi:10.1016/j.cjche.2018.07.021.

Norhan, M.A., Abdullah, S.R.S., Hassan, H.A. and Ismail, N.I. 2021. A constructed wetland system for bio-polishing palm oil mill effluent and its future research opportunities. *Journal of Water Process Engineering* 41:102043, doi:10.1016/j.jwpe.2021.102043.

Pal, M. and Sharma, R.K. 2020. Development of wheat straw based catholyte for power generation in microbial fuel cell. *Biomass and Bioenergy* 138:105591, doi:10.1016/j.biombioe.2020.105591.

Pandit, S., Savia, N., Sonawane, J.M., Sani, A.M., Gupta, P.K., Mathuriya, A.S., Rai, A.K., Jadhav, D.A., Jung, S.P. and Prasad, R. 2021. Agricultural waste and wastewater as feedstock for bioelectricity generation using microbial fuel cells: Recent advances. *Fermentation* 7:169, doi:10.3390/fermentation7030169.

Picos-corrales, L.A., Sarmiento-Sanchez, J.I., Ruelas-Leyva, J.P., Crini, G., Hermosillo-Ochoa, E. and Gutierrez-Montes, J.A. 2020. Environment-friendly approach toward the treatment of raw agricultural wastewater and river water via flocculation using chitosan and bean straw flour as bioflocculants. *ACS Omega* 5:3943-3951, doi:10.1021/acsomega.9b03419.

Qing, S., Wang, L.L., Jiang, L.P., Wu, X. and Zhu, J.J. 2022. Live microalgal cell modified by L-cys/Au@carbon dots/bilirubin oxidase layers for enhanced oxygen reduction in a membrane-less biofuel cell. *SmartMat* 2022:1-13, doi:10.1002/smm2.1100.

Rismani-Yazdi, H., Carver, S.M., Christy, A.D. and Tuovinen, O.H. 2008. Cathodic limitations in microbial fuel cells: An overview. *Journal of Power Sources* 180:683-694, doi:10.1016/j.jpowsour.2008.02.074.

Roucher, A., Roussarie, E., Gauvin, R.M., Rouhana, J., Gounel, S., Stines-Chaumeil, C., Mano, N. and Backov, R. 2019. Bilirubin oxidase-based silica microcellular robust catalyst for online dyes degradation. *Enzyme and Microbiology Technology* 120:77-83, doi:10.1016/j.enzmictec.2018.10.004.

Sadeghian, I., Rezaie, Z., Rahmatadadi, S.S., Hemmati, S. 2020. Biochemical insight into a novel thermo/organo tolerant bilirubin oxidase from *Thermosediminibacter oceani* and its application in dye decolorization. *Process Biochemistry* 88:38-50, doi:10.1016/j.procbio.2019.09.030.

Sakasegawa, S.I., Ishikawa, H., Imamura, S., Sakuraba, H., Goda, S. and Ohshima, T. 2006. Bilirubin oxidase activity of *Bacillus subtilis* CotA. *Applied and Environmental Microbiology* 72:972-975, doi:10.1128%2FAEM.72.1.972-975.2006.

Santoro, C., Babanova, S., Atanassov, P., Li, B., Ieropoulos, I. and Cristiani, P. 2013. High pwer generation by a membraneless single chamber microbial fuel cell (SCMFC) using enzymeatic bilirubin oxidase (Box) air-breathing cathode. *Journal of the Electrochemical Society* 160:720, doi:10.1149/2.058310jes.

Saputera, W.H., Amri, A.F., Daiyan, R. and Sasongko, D. 2021. Photocatalytic technology for palm oil mill effluent (POME) wastewater treatment: Current progress and future perspective. *Materials* 14:2846, doi:10.3390/ma14112846.

Sarmin, S., Tarek, M., Roopan, S.M., Cheng, C.K. and Khan, M.M.R. 2021. Significant improvement of power generation through effective substrate-inoculum interaction mechanism in microbial fuel cell. *Journal of Power Sources* 484:229285, doi:10.1016/j.jpowsour.2020.229285.

Scheiblbrandner, S., Csarman, F. and Ludwig, R. 2022. Cellobiose dehydrogenase in biofuel cells. *Current Opinion in Biotechnology* 73:205-212, doi:10.1016/j.copbio.2021.08.013.

Solomon, E.I., Sundaram, U.M. and Machonkin, T.E. 1996. Multicopper oxidases and oxygenases. *Chemical Reviews* 96:2563-2605, doi:10.1021/cr950046o.

Tan, J.T., Yan, X., Huang, W., Engelbrekt, C., Duus, J.O., Ulstrup, J., Xiao, X. and Zhang, J. 2020. Bilirubin oxidase oriented on novel type three-dimensional biocathodes with reduced graphene aggregation for biocathode. *Biosensors and Bioelectronics* 167:112500, doi:10.1016/j.bios.2020.112500.

Thipraksa, J., Chaijak, P., Michu, P. and Lertworapreecha, M. 2022. Biodegradation and bioelectricity generation of melanoidin in palm oil mill effluent (POME) by laccase-producing bacterial consortium integrated with microbial fuel cell. *Biocatalysis and Agricultural Biotechnology* 43:102444, doi:10.1016/j.bcab.2022.102444.

Trifonov, A., Stemmer, A. and Tel-Vered, R. 2021. Carbon-coated magnetic nanoparticles as a removable protection layer extending the operation lifetime of bilirubin oxidase-based bioelectrode. *Bioelectrochemistry* 137:107640, doi:10.1016/j.bioelechem.2020.107640.

Wang, T., Ai, S., Zhou, Y., Luo, Z., Yang, Y., Zhang, J., Huang, H., Luo, S. and Luo, L. 2018. Adsorption of agricultural wastewater contaminated with antibiotics, pesticides and toxic metals by functionalized magnetic nanoparticles. *Journal of Environmental Chemical Engineering* 6:6468-6478, doi:10.1016/j.jece.2018.10.014.