Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Article
Volume 13, Issue 3, 2023, 268
https://doi.org/10.33263/BRIAC133.268

Using of Oxidoreductase Producing Consortium with High Manganese Peroxidase Activity for Melanoidin Degradation and Electricity Generation from Palm Oil Mill Effluent

Junjira Thipraksa [1], Pimprapa Chaijak [2,3,*]

[1] Department of Biology, Faculty of Science, Thaksin University 93210, Thailand; amptipraksa@gmail.com (J.T.);
[2] Microbial Fuel Cell & Bioremediation Laboratory, Faculty of Science, Thaksin University 93210, Thailand; chaijak.pimprapa@gmail.com (P.C.);
[3] Microbial Technology for Agriculture, Food and Environment Research Center, Thaksin University 93210, Thailand
* Correspondence: chaijak.pimprapa@gmail.com (P.C.);

Scopus Author ID 57195740686
Received: 25.05.2022; Accepted: 22.06.2022; Published: 7.07.2022

Abstract: Melanoidin is the dark high molecular weight formed via the Maillard reaction under high-temperature conditions. Here, the crude enzyme of melanoidin degrading bacterial consortium was extracted and determined the oxidoreductase activities like manganese peroxidase (MnP), laccase, and lignin peroxidase (LiP). The melanoidin degrading ability and electricity generation were measured. Finally, the oxidoreductase-producing consortium was used as a whole-cell biocatalyst in the air-cathode MFC. The result indicated that the MnP activity of 38.10±0.10 U/mL was produced from the consortium. The melanoidin removal of 85.10±2.00% and maximal power output of 475.69±1.13 W/m^3 were achieved. This study gained new knowledge about using oxidoreductase-producing consortium with high MnP activity for melanoidin removal and electricity generation from palm oil mill effluent.

Keywords: decolorization; manganese peroxidase; melanoidin; palm oil mill effluent.

1. Introduction

Palm oil mill effluent (POME) is one of the most interested in emergency water pollution owing to its contaminants [1]. The POME is discharged into the groundwater, which provided water pollution like chemical oxygen demand (COD), grease, oil, and total nitrogen increasing [2]. The POME is characterized by dark-brownish color due to the Maillard reaction product such as melanoidin [3]. Melanoidin is the high molecular weight product formed during the Maillard reaction of carbohydrate and amino acid under high temperatures. It can increase the color of wastewater [4].

Oxidoreductases possess a wide range of substrate specifications and can degrade aromatic compounds such as polycyclic aromatic hydrocarbon and other dyes [5]. Microbial oxidoreductases such as manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase (Lac) gain much attention in various biotechnological applications, including decolorization [6]. Chaijak *et al*. have shown that oxidoreductases such as Lac and MnP produced from the yeast *Galactomyces reessii* achieve high performance for phenol removal and decolorization

of POME [7]. Moreover, some previous study has reported that the microbial lignin peroxidase (LiP) has a high potential for decolorizing industrial wastewater [8].

A microbial fuel cell (MFC) is a biotechnological device that can convert chemical energy in organic and inorganic matter to electrical energy through microbial metabolism under oxygen-limit conditions. The MFC has been used for various types of wastewater decolorization, such as palm oil mill effluent [9], textile wastewater [10], refractory sewage [11], and olive oil mill effluent [12].

In this study, the crude enzyme of a melanoidin-degrading consortium was extracted, and the oxidoreductase activities (MnP, LiP, and Lac) were determined. The potential for melanoidin degradation and electricity generation was determined and compared to commercial enzymes. Finally, the oxidoreductase consortium was used as a whole-cell biocatalyst for electricity generation from the POME via air-cathode MFC.

2. Materials and Methods

The melanoidin-degrading consortium (S5) was selected from the biomass-rich forest soil sample and maintained in the nutrient broth (Sigma-Aldrich, United States). The microbial diversity of consortium S5 is shown in Figure 1.

The crude extracellular enzyme of the bacterial consortium was prepared according to Younes *et al*. [13]. Briefly, the 25 mL of the consortium was inoculated into the 225 mL sterile nutrient broth supplemented with the 10% (v/v) of 29,180 mgCOD/L synthetic melanoidins and incubated at room temperature under static conditions for 48 hr. The crude extract was collected and filtrated through a paper filter (Whatman no.1) 5 times at 4 °C.

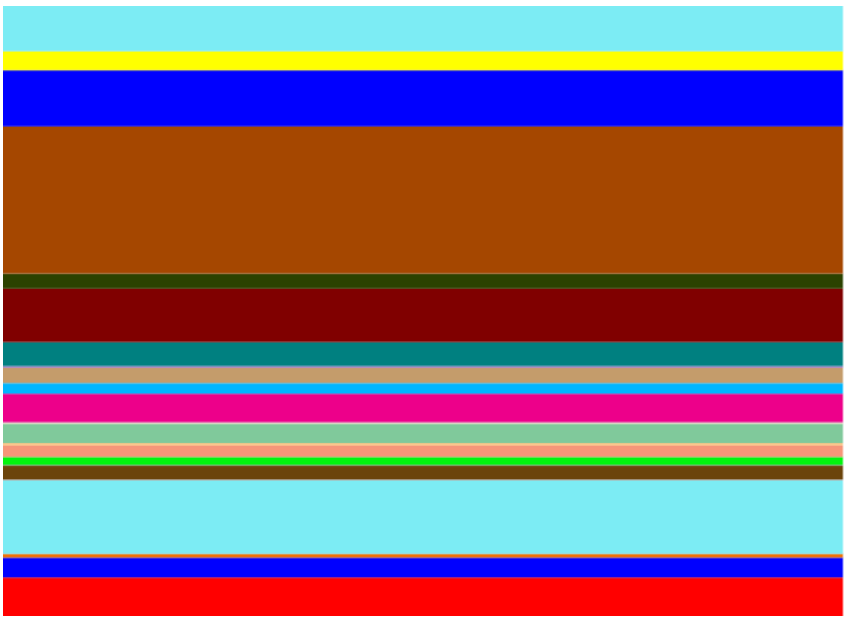
The MnP activity was measured using UV-Vis spectrophotometry. The 250 µL of the crude enzyme, 10 µL of 10 mM H_2O_2, 30 µL of 20 mM $MnSO_4$, 100 µL of 1.5 mM 3-methyl-2-benzothiazolinone hydrazine (MBTH), 300 µL of 6.6 mM 3-(dimethylamino)benzoic acid (DMAB), 1460 µL of 100 mM succinate-lactate buffer (pH 4.5). The enzyme activity was determined at 590 nm [14].

For Lac activity, the reaction contains the 250 µL of the crude enzyme and the 250 µL of 10 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 740 µL of buffer (0.2 M dipotassium hydrogen phosphate and 0.1 M citric acid, pH 4.5). The enzyme activity was monitored at 420 nm [15].

The activity of LiP was studied by determining the H_2O_2-mediated catalytic oxidation of veratryl alcohol. The 250 µL of the crude enzyme, 1 mL of 4 mM veratryl alcohol, 500 µL of 0.2 M H_2O_2, and 1 mL of 100 mM tartrate buffer (pH 3.0) were mixed. Then the reaction was measured at 310 nm [16].

The synthetic melanoidin wastewater was prepared according to Raji *et al*. [17], containing 4.5 g of glucose, 1.88 g of glycine and 0.42 g of sodium bicarbonate, and 100 mL of deionized water. The solution was incubated at 95 °C for 7 hr and added 100 mL of deionized water.

The 10 U/mL of crude oxidoreductase and commercial enzymes (MnP, Lac and LiP) was added to the synthetic POME (255 mg/L $NaHCO_3$, 22 mg/L KH_2PO_4, 16 mg/L $MgSO_4$, 3 mg/L $CaCl_2$, 0.1 mg/L $(NH_4)_2SO_4$, and 10% (v/v) synthetic melanoidin). All reactions were incubated at 30 °C for 2 hr. Then the melanoidin removal was monitored using UV-Vis spectrophotometry at 280 nm [18].



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Bacteria;__Bacteroidetes;__Flavobacteriia;__Flavobacteriales;__Flavobacteriaceae;__Coenonia
Bacteria;__Firmicutes;__Bacilli;__Lactobacillales;__Enterococcaceae;__Enterococcus
Bacteria;__Firmicutes;__Bacilli;__Lactobacillales;__Streptococcaceae;__Lactococcus
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Bacteria;__Firmicutes;__Negativicutes;__Selenomonadales;__Sporomusaceae;__Acetonema
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Bacteria;__Proteobacteria;__Betaproteobacteria;__Neisseriales;__Chromobacteriaceae;__Laribacter
Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfovibrionales;__Desulfovibrionaceae;__Desulfovibrio
Bacteria;__Proteobacteria;__Epsilonproteobacteria;__Campylobacterales;__Arcobacteraceae;__Aliarcobacter
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Bacteria;__Proteobacteria;__Gammaproteobacteria;__Pseudomonadales;__Pseudomonadaceae;__Pseudomonas
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Figure 1. The microbial community of the S5 consortium.

For electricity generation, the 10 U/mL of crude oxidoreductase and commercial enzymes was added into the anode chamber of air-cathode MFC. The 40 mL of synthetic POME was used as an anolyte. The anode electrode was made from the 20 cm^2 of microwave-activated graphite plate. The 20 cm^2 of 0.2 mg/cm^2 platinum on Vulcan was used as a cathode electrode. The open-circuit voltage (OCV) was monitored every 60 mins for 24 hr. The closed-circuit voltage (CCV) was studied at 1-1,000 Ω. The electrochemical properties were calculated according to Ohm's law.

The 10% (v/v) oxidoreductase producing consortium was added into the anode chamber, then the synthetic POME was filled and incubated for 48 hr to immobilize the bacterial biofilm on the electrode surface. The anolyte was fed out, and the fresh synthetic was fed in. The OCV was monitored, and the electrochemical properties were calculated.

3. Results and Discussion

The crude extract was used to determine the MnP, Lac, and LiP activities to characterize oxidoreductase. The results displayed the MnP activity of 38.10±0.10 U/mL, Lac activity of 20.21±0.20 U/mL, and LiP activity of 15.80±0.05 U/mL was observed from the crude extract of the bacterial consortium S5 where it was cultured in the liquid media supplemented with melanoidin. Moreover, maximal melanoidin removal of 85.10±2.00% was achieved from the crude extract, followed by the commercial MnP, Lac, and LiP of 70.00±1.53%, 45.10±2.50%, and 20.30±3.10% respectively (Figure 2).

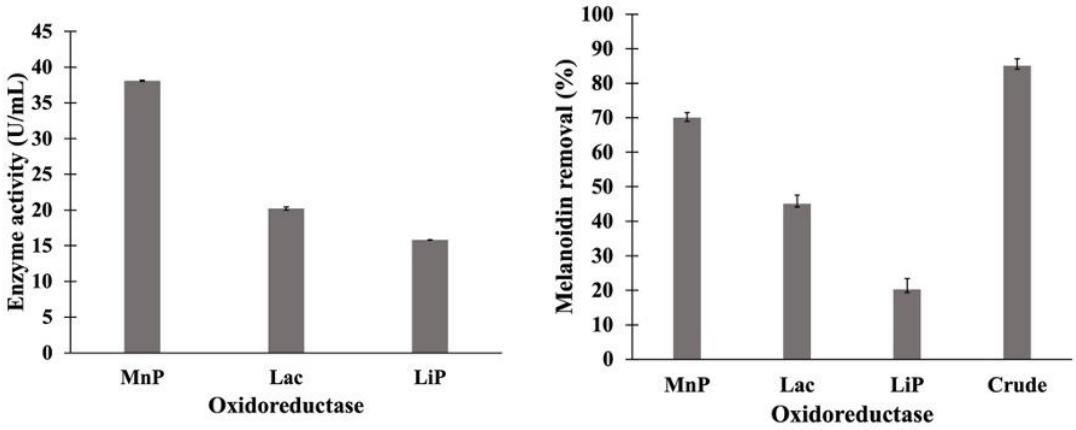

Figure 2. Oxidoreductase activity of consortium S5 and the melanoidin removal of S5 crude enzyme and commercial enzyme (MnP, Lac, and LiP).

In Chandra *et al*. [19] the melanoidin degradation bacterial consortium with MnP and Lac activities of 3.8 U/mL and 2.39 U/mL has shown the high ability of melanoidin degradation from the effluent. The melanoidin removal of 81% was gained where it was cultured and supplemented with 1.0% of glucose and 0.2% of peptone [19]. Additionally, the melanoidin removal of 80% was obtained from the bakery yeast cell with Lac activity immobilized on alumina/silica particles [20]. Toomsan *et al*. exhibited that the white-rot fungi *Megaspororia* sp. with Lac activity have a high melanoidin removal ability. The maximal melanoidin removal of 48% was reached [21]. No previous study has reported using the oxidoreductase-producing consortium with high MnP activity for melanoidin degradation from the POME.

For electricity generation, the crude extract and commercial oxidoreductases (MnP, Lac, and LiP) were used for catalyzing the melanoidin degradation and electrical energy production via air-cathode MFC. The maximal OCV of 950±5 mV was gained from the air-cathode MFC with the crude extract, followed by MnP, Lac, and LiP of 830.10±6.00 mV, 650.10±5.00 mV, and 410.33±8.01 mV respectively. The maximal current density and power density of 300.00±0.25 A/m^3 and 392±1.05 W/m^3 were produced from the air-cathode MFC with MnP. In contrast, the current density and power density of 300.00±0.25 A/m^3 (5.50±0.10 A/m^2) and 517.51±0.33 W/m^3 (9.51±0.13 W/m^2) were produced from the air-cathode MFC with the crude extract. The polarization curve is displayed in Figure 3. Furthermore, the OCV, maximal current density, and power density of air-cathode MFC with oxidoreductase

producing bacterial consortium S5 of 864.17±3.13 mV, 275.00±0.33 A/m^{3,} and 475.69±1.13 W/m^3 were generated (Figure 4).

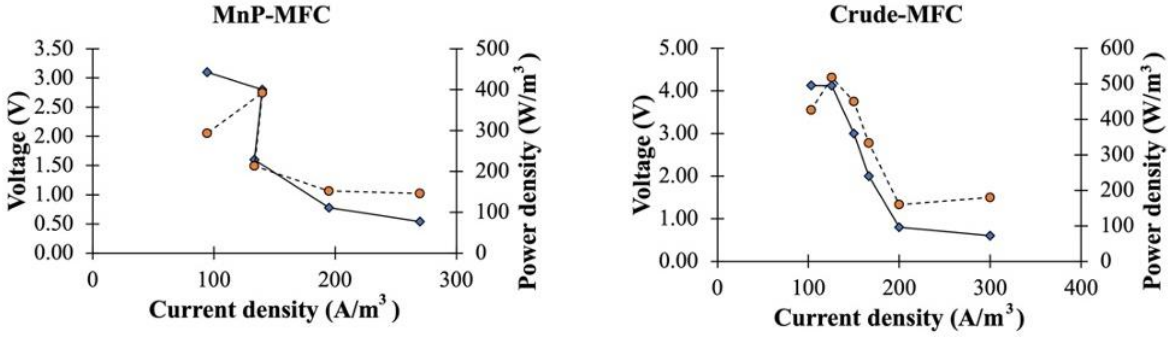

Figure 3. Polarization curve of air-cathode MFC with commercial MnP and crude extract of consortium S5.

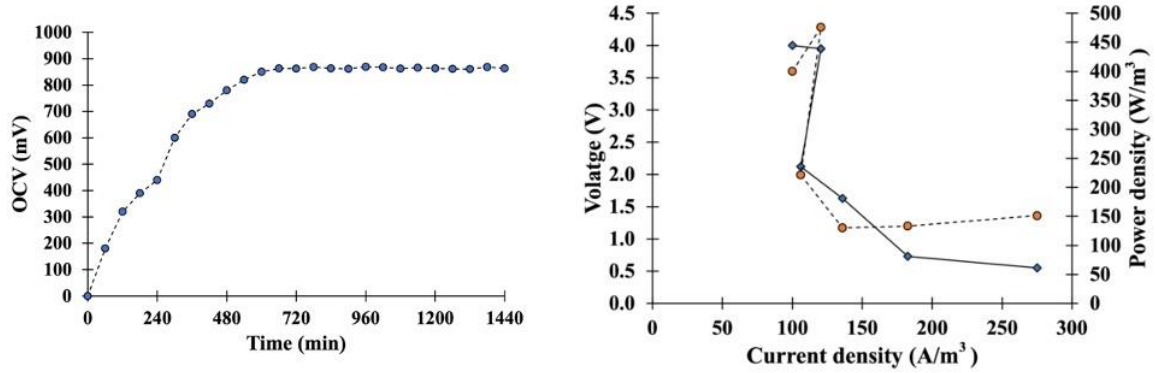

Figure 4. The opened-circuit voltage and polarization curve of the air-cathode with oxidoreductase producing consortium.

On the other hand, the maximal current density and power density of 0.58 A/m^2 and 0.17 W/m^2 were produced from the MFC with melanoidin-distillery wastewater [22]. Marassi *et al*. revealed the air-cathode MFC with *Clostridium butyricum* could generate the maximal power output of 0.21 W/m^2 when the melanoidin contaminated bioethanol wastewater was used as the anolyte [23]. Moreover, the dual-chamber MFC with proteobacteria and archaea *Methanothrix* sp. can produce the maximal power output of 0.017 W/m^2 where the melanoidin-contaminated molasses wastewater was used as the anolyte [24]. The study of Nookwam *et al*. has displayed the MFC with a photosynthetic-cathodic chamber successfully used for wastewater treatment and electricity generation. The maximal power output of 116.9 W/m^3 was produced [25].

4. Conclusions

In conclusion, the oxidoreductase activity of melanoidin degrading bacterial consortium was determined. The result showed that the highest oxidoreductase activity was MnP, followed by Lac and LiP. Moreover, the result indicated that the main biocatalyst for melanoidin degradation was MnP. This study gained new knowledge about using MnP-producing consortium coupling with MFC for melanoidin degradation and electricity generation from the POME.

Funding

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.

Acknowledgments

The author would like to thank Microbial Technology for Agriculture, Food and Environment Research Center, Thaksin University for the laboratory support.

Conflicts of Interest

The authors declare no conflict of interest.

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