In Situ *Trametes versicolor* Laccase Biocathode Performance Assessment in Dual-Chamber Microbial Fuel Cells

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Abstract

White-rot fungi (WRF) synthesize ligninolytic extracellular oxidative enzymes such as laccase (Lcc), which has been described as one of the most interesting types of redox enzymes that can improve microbial fuel cell (MFC) performance. Therefore, and in order to test that performance, WRF *Trametes versicolor* MUM 04.100 was immobilized in nylon sponge and fixed in the MFC cathode chamber, while Lcc activity, bioelectricity production, and organic matter removal were monitored. It was found that current density measured in the MFC supplemented with fungi was 2.1 times higher (42.81 ± 4.91 mA/m^2) than current density obtained in the control MFC (absence of fungus in the cathode chamber, 20.31 ± 4.30 mA/m^2). Maximum Lcc activity (23.08 U/L) and the highest value of organic matter removal (COD) (92%) from domestic wastewater was obtained on the last cycle after biofilm maturation and glycerol pulse. This work evidences that Lcc continuously synthesized by MUM 04.100 immobilized in the biocathode is a promising approach to enhance MFC power performance and wastewater treatment.

Keywords Municipal wastewater · Fungal enzyme immobilization · Glycerol · Bioelectricity

Introduction

Microbial fuel cell (MFC) technology has been receiving much interest because it offers a solution for low potency and decentralized energy production in zero carbon scenarios. By using electrogenic (anodophilic) microorganisms [1], MFC extracts electricity from organic wastes while providing their mineralization, which is a significant asset from a pollution prevention perspective Potter, 1911) [2–5].

The MFC typically comprise an anode compartment and a cathode compartment, separated by a proton-conducting membrane (PEM). In the anode compartment, the electrogenic microorganisms oxidize the organic matter, under anaerobic conditions, releasing protons, and electrons [6, 7]. The electrons migrate through an external circuit, from the anode compartment to the cathode, generating an electric current. The protons cross the membrane toward the cathode. Finally, the electrons and protons combine with the oxygen present at the cathode to form water [8, 9].

Many factors influence the performance of MFC: these can be the nature of the materials used and the distance between them, the substrates used, the microbial inoculum, reactor configuration, ionic strength, internal and external resistance, presence or absence of a proton exchange membrane (PEM), and the properties of the catalysts [10, 11]. However, an important limitation in the production of energy by MFC are the electrodes, for which bacterial adhesion, electron transfer, and oxidation of substrates are factors that directly depend on its performance [12, 13]. The production of electricity by MFC technology is generally described

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according to the microbial composition of the biofilm on the anode, which plays an important role in the decomposition of the substrate. However, rapid microbial growth can easily block the pores of these materials preventing diffusion of the substrate. In contrast, studies related to the importance of biofilm formation are still limited. MFC have great potential for expanding current knowledge on the phylogenetic and functional diversity of complex microbial communities, when combined with molecular approaches, including metagenomics [12–14].

Other important factor that limiting MFC optimization is the cathode kinetics, whereas oxygen is the most practical electron acceptor because of its availability and non-toxicity [15]. Different approaches have been investigated to obtain high electric power outputs. The use of noble metals such as gold (Au), palladium (Pd), platinum (Pt), and Pt-alloy are extensively reported by their high oxygen reduction reaction (ORR) capability on the cathodic electrode surface. However, these metals have application limited due to the fact associated by their high cost and low stability, excluding commercial MFCs application [16]. An alternative by this bottleneck, non-precious, transition metals such and inorganic nanostructured materials (e.g., carbon nanomaterials such as carbon black, activated carbon, graphite, graphene, carbon nanotubes, carbon nanofibers, biomass-derived carbons) have attracted much attention in the application of ORR [9, 10, 16, 17]. However, some cathode materials and their chemical catalyst are considered expensive and harmful to the environment. Sarma et al. [17] reinforce significant advantages associated with biocathodes, mainly due to the use of microorganisms that promote an eco-friendly environment instead of the use of hazardous chemical catalysts. These biocatalysts are considered as interesting candidates to improve cathodic activity and can provide additional environmental benefits, such as biosensors, biotransformation, biosorption, and bioaccumulation [18].

Recently, Zhao et al. [19] evaluated the efficiency of biocathode MFC based on *Corynebacterium vitaeruminis* LZU47-1 to the Cr (VI) reduction. The maximum power density and Cr (VI) removal efficiency were 252.36 mW/m^2 and 98.63%, respectively. Yadav et al. [20], employed *Chlorella vulgaris* at the cathode chamber obtained a maximum power density of 54.48 mW/m^2. Authors highlighted that power generation in MFC depends on the oxygen concentration at the cathode and microalgae emerges as an alternative to provide continuous supply of oxygen in this chamber. To overcome this limitation, some authors devoted their research to create a biocathode based on white-rot fungi (WRF) immobilized in different matrices for continuous laccase (Lcc) synthesis [21, 22].

Laccases enzymes use oxygen as an electron acceptor and oxidize a wide range of organic substrates. Fungal Lccs generally have copper atoms grouped in two sites of the tridimensional structure of the protein: one copper ion is on T1 site (the Lcc redox center), which receives four electrons from the electron donor and three copper ions on T2/T3 site, where oxygen is reduced to water [23]. Savizi et al. [24] conducted a study immobilizing commercial Lcc on a surface-modified graphite electrode cathode MFC. Authors detected a power density of 58.8 mW/m^2 that represented an increase of 65% when compared to the power density obtained with a simple graphite cathode. *Trametes versicolor* in nature produces Lcc by lignin degradation with a high redox potential (780 mV vs. NHE). According to Mani et al. [25], *T. versicolor* Lcc is thermodynamically favorable to oxygen reduction at the cathode chamber.

In a recent study, Lin et al. [22] incorporated into the ceramic MFC air cathode electrode the WRF *P. eryngii*. Maximum power density obtained was 41.3 mW/m^2 that represents 3.52 times that of an MFC without WRF. In addition, WRF cathode enhanced the copper (Cu^{+2}) removal and increased Lcc activity by 1.68-fold-462 U/L. Liu et al. [26] planted *Ganoderma lucidum* on cathode chamber and reinforced the importance of Lcc biocathode to decolorise Acid Orange 7 (AO-7) and power density 223 mW/m^2 generation. Kipf et al. [27] showed that the continuous supply of Lcc-containing supernatant from a *T. versicolor* culture improved MFC performance. Similarly, Lin et al. [28] described better MFC performance when applied *G. lucidum* crude extract for continuous Lcc synthesis in cathode chamber. In this context, the study goal was to design a new MFC with immobilized *T. versicolor* MUM 04.100 for in situ Lcc synthesis and to assess the resulting power performance using voltammetry techniques. The research allows new insights about a cathodic chamber MFC development of an eco-friendly, low-cost, and bio-based solution for wastewater treatment. Thus, this proposal addresses two Sustainable Development Goals proposed by United Nations promoting access to sanitation according to Goal 6 and Goal 7, by affordable and clean energy minimizing the impacts of untreated wastewater discharges.

Materials and Methods

Culture Conditions

The white-rot fungi *Trametes versicolor* MUM 04.100 strain was obtained from Micoteca da Universidade do Minho, the Portuguese Culture Collection of filamentous fungi from University of Minho, Portugal. The organism was cultured on tap water agar-cellulose plates (15 g/L TWA-cellulose, Oxoid Technical Agar No. 3, supplemented with a Whatman grade 4 filter paper strip) and transferred to a fresh medium every month. For fungal immobilization, five 6-mm-diameter plug discs were cut with a sterile condition, from the

periphery of a 7-day-old colony, grown in TWA-cellulose plate and inserted in one 250-mL Erlenmeyer flask containing 50 mL of liquid culture medium (LCM) composed by malt extract 3 g/L, glycerol 10 g/L, yeast extract 3 g/L, peptone 5 g/L, and 2 pieces of 1 cm^3 cubes of nylon sponge (NS) (ScotchBrite 3 MSACompany, Madrid, Spain) pre-treated as described by Rodríguez Couto et al. [29] and incubated at 150 rpm, 30 °C for 7 days. Thereafter, free biomass was removed from the NS supports by filtration under sterile conditions. Scanning electron microscopy (SEM) was used to explore the morphology of the NS support with immobilized cells and the fungal mycelium colonization. Before SEM analysis, all samples were coated with gold and platinum (80/20%). Figure 1(a) depicts the microphotographs obtained by SEM for NS support. Figure 1(b) confirms the immobilization of the MUM 04.100 in the NS supports.

MFC Setup and Operation

The MFC setup (Fig. 2) consisted of two compartments of polymethyl methacrylate with equal dimensions (12 cm × 8 cm × 5 cm). The two chambers were separated by a proton exchange membrane (Nafion Membrane 117, DuPont Co., USA) and closed with silicone rubbers, rubber gaskets, and stainless steel screws. The networking volume used for each chamber was 350 mL. Carbon Toray (QUINTECH, USA) electrodes with 48 cm^2 of surface area (4 cm × 6 cm × 2 cm) were connected through copper wires, to an external resistance of 500 Ω. After the MFC performance assessment (power curve determination at day 36), the external resistance was changed to 1000 Ω. Municipal wastewater with an organic matter concentration, expressed in terms of chemical oxygen demand (COD), of 762 ± 100 mgO_2/L, a conductivity of 790 ± 20 µS/cm and a pH 7.0 ± 0.2, was used as batch anolyte at room temperature (≈ 22 °C). To avoid substrate limitations on bioelectricity production, wastewater was changed whenever the current density decreased to approximately 80%.

Two NS cubes inoculated with MUM 04.100 strain were fixed in the lateral cathode chamber surface with 4 needles, under sterile conditions. The catholyte solution was composed of LCM and was oxygenated using an air pump system with a 0.22-µm pore size filter coupled to it. During the MFC operation, one pulse of glycerol (5 g/L final concentration in LCM) was incorporated to maintain fungal metabolism and, consequently, the synthesis of Lcc for a prolonged fermentation time [30]. Electroactive biofilm was considered at steady state when similar values of power density were obtained along three cycles consecutively [3, 13, 21] A control assay, without the fungal strain MUM 04.100 and consequently the absence of the presence of Lcc in the cathode chamber, was also carried out using the same configuration described. In this control, the cathodic chamber was only filled with sterile LCM as catholyte. Another assay was performed with the fungal strain MUM 04.100 inoculated in the bulk of the cathodic chamber (e.g., without the NS cubes).
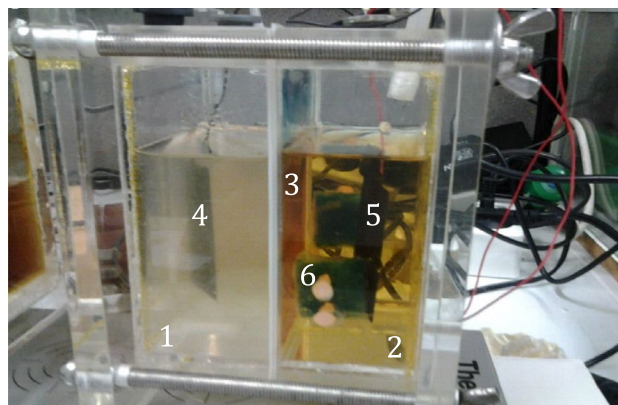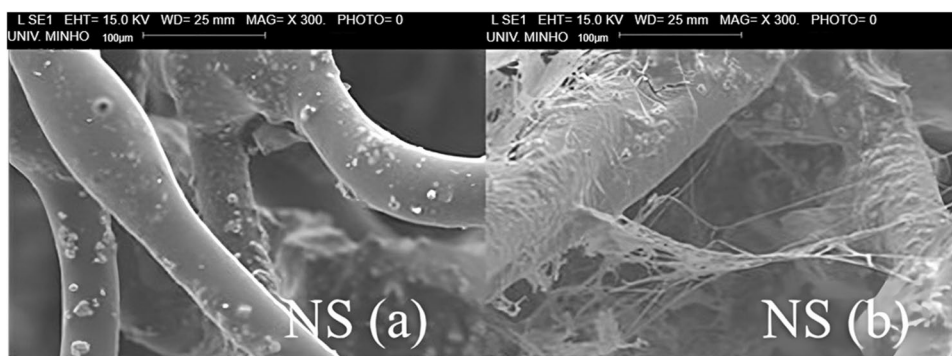

Fig. 2 Schematic representation of the microbial fuel cell (MFC): (1) anodic chamber; (2) cathodic chamber; (3) Nafion 117 membrane; (4 and 5) electrodes; (6) *Trametes versicolor* MUM 04.100 immobilized

Fig. 1 Scanning electron microscope (SEM) images of nylon sponge (NS) support without fungi (**a**) and the NS support colonized by MUM 04.100 (**b**), after 7 days of incubation at 30 °C. Bars 300 µm

Analytical Methods

Bioelectrochemical Analysis

The voltage between anode and cathode electrode was registered every 30 min with a USB-9215A BNC connector datalogger (PXI4, model PCI-6023E, National Instruments™, NI) and a data acquisition software (Labview 6.0) [13]. Following Ohm's law, current density (j) was calculated as $j = i/A$, where i is the electric current and A is the anode surface area. Power density (P) was calculated as the product of current intensity and voltage (V) divided by the surface area of the anode ($P = iV/A$).

A series of resistances between 71 kΩ to 10 Ω were used to determine the polarization (V as a function of j) and power (P as a function of j) curves in the stable phase of power generation (36th day). The internal MFC resistance (R_{int}) was estimated from the polarization curve slope in the region dominated by ohmic losses [4, 7, 13], which corresponds to the maximum power densities. The open circuit voltage (OCV) was measured at infinite resistance.

Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

The biochemical oxygen demand (BOD) was determined using the dilution method with a 5-day incubation period at 20 °C. The chemical oxygen demand (COD) was determined through the digestion method, using dichromate in acidic medium and titration with ammonium ferrous sulfate.

Enzymatic Assays

The laccase activity (EC 1.10.3.2) was assessed from the syringaldazine oxidation by measuring the increase of the absorbance at 525 nm [30]. The same reaction mixtures with boiled supernatant samples were used as a blank. Enzyme activity was expressed as one unit (U) per L, where U is the quantity of the enzyme responsible for changing the absorbance in 0.01 per minute.

Statistical Analysis

The data were analyzed for normality of distribution using the Shapiro–Wilk test and homoscedasticity of variances using the Levene test. As the results were normal and homoscedastic, the differences between the means of the treatments were evaluated by means of analysis of T test, with a significance level of $p < 0.05$ [31].

Results and Discussion

Current Density in Time and Laccase Activity by MUM 04.100

The present study assessed the bioelectricity production in a MFC with MUM 04.100 strain inoculated in NS supports in the cathode chamber. Current density measurements are depicted in Fig. 3. As observed, the assay with MUM 04.100, inoculated in the cathode, took a long time to reach a significant current peak (9 days). This fact is related to the need of first growing the electroactive biofilm on the electrode surface and then promoting its acclimatization with 3 additions of new wastewater until maximum stable current

Fig. 3 Variation of electrical current density along time in the microbial fuel cell (MFC) with *Trametes versicolor* MUM 04.100 immobilized in the cathode chamber (black line) and in the control assay without the presence of the fungus (gray line); vertical lines represent the period when wastewater was replaced in the anodic chamber
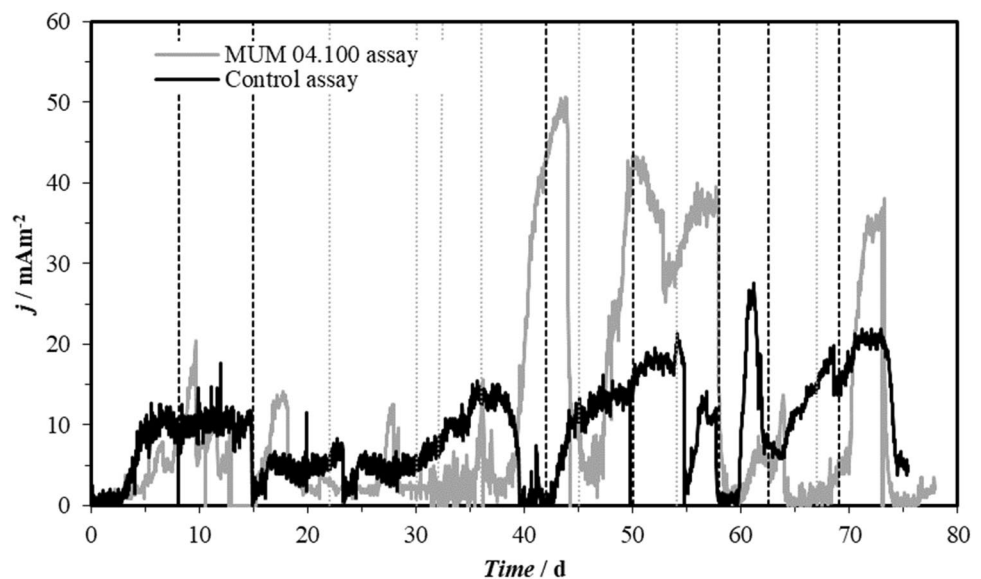
densities were obtained, around 11.8 ± 1.58 mA/m^2 (until the 16th day). After this acclimation time, 3 more cycles (between the days 16 and 36) with addition of wastewater were performed and the maximum current densities achieved were 13.33 ± 0.84 mA/m^2. In a MFC, each cycle corresponds to a replica, since it is difficult to reproduce identical results in different MFCs, for which the acclimatization conditions may influence the results differently. Nevertheless, and after the polarization and power curves carried out at day 36, the external resistance was increased from 500 to 1000 Ω. This adjustment resulted in a rise of current production to a maximum of 50.63 mA/m^2 (12.30 mW/m^2) around day 44. After this period, three more current peaks were obtained after changing the anolyte solution with fresh wastewater. The current peaks achieved average values of 42.81 ± 4.91 mA/m^2, which corresponded to an average power density of 8.91 ± 2.10 mW/m^2. These values are slightly lower to the ones found in literature for a single-chamber MFC with a fungal cathode (13.38 mW/m^2), but they are still within the same range [32].

Regarding the control assay, a similar MFC operated in the same conditions (same anolyte and catholyte solutions), but without the fungal presence in the cathode chamber, the current densities were lower when compared with previous ones. Indeed, maximum current densities where approximately 20.31 ± 4.30 mA/m^2. This control assay showed that the Lcc enzyme, synthesized by the fungus on the cathode compartment, enhanced the current density.

Furthermore, the set of experiments indicated a positive effect on MUM 04.100 Lcc synthesis after the glycerol pulse (Fig. 4), in line with previous studies [33, 34]. The maximum Lcc activity was detected for the pH range between 5.0 and 6.5. This agrees with a previous Lcc experiment, where an electricity increment was reported for a pH 5.5–6.0 [21].

It should be pointed that the MFC, including the same anodic and cathodic conditions and without the immobilization of MUM 04.100 in the NS cubes, revealed a decontrolled growth leading to an unfeasible MFC operation—no current density was observed during the experiment (data not shown). This fact indicates that the immobilization of the fungus controls its growth and stimulates the excretion of the Lcc. Urra et al. [35] emphasize that the immobilization of cells is an effective resource that allows for a continuous production of enzymes during secondary metabolism, which is restricted to physiological and morphological conditions of the fungus. The uncontrolled growth of the mycelium can reduce the efficiency of the process after a short period of time. These results are in agreement with Rubenwolf et al. [36] observations. According to their results, the feasibility of decoupling the electrode lifetime from the Lcc lifetime is a prerequisite for the achievement of a self-regenerating enzymatic MFC, in which enzyme-producing microorganisms are integrated into the electrode to continuously resupply fresh enzymes. As an alternative to the cathode inoculation with enzyme-producing microorganisms. Sané et al. [37] used its crude culture supernatant to supply unpurified Lcc enzymes. According to these authors, this operational strategy decreased time and costs needed for purifying enzymes. Nevertheless, many studies describe that the best performance to improve Lcc production is the microorganism's immobilization on different supports, since the system ensures a higher rate of oxygen transfer, reducing excessive mycelial growth [38].

Fig. 4 Laccase (Lcc) activity in a *Trametes versicolor* MUM 04.100 strain culture immobilized in a cathodic chamber
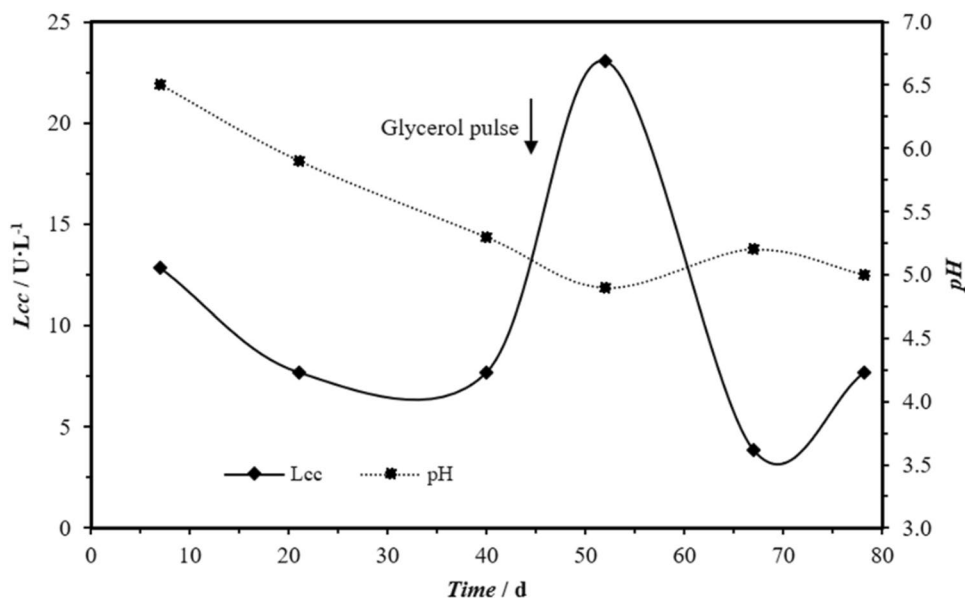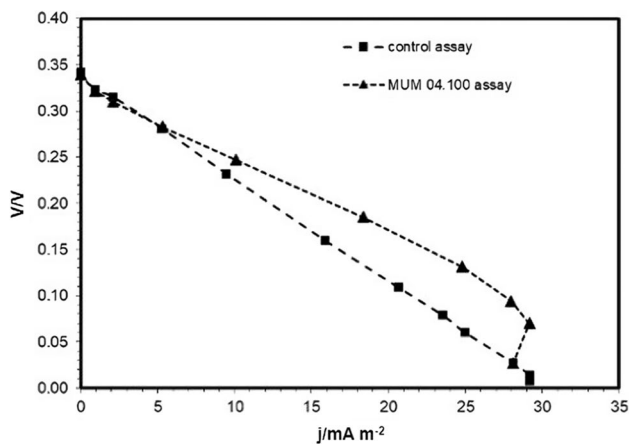
Fig. 5 Polarization curves of the microbial fuel cell (MFC) with *Trametes versicolor* MUM 04.100 immobilized and in the control, assay performed for 36 days
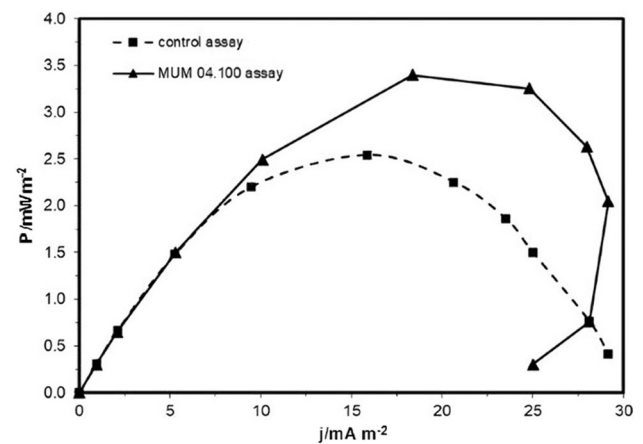

Fig. 6 Power density curves of the microbial fuel cell (MFC) with *Trametes versicolor* MUM 04.100 immobilized and in the control, assay performed for 36 days

Microbial Fuel Cell Performance

The OCV values of the MFC, measured during the stable phase of current production, were 0.339 V and 0.342 V, for the fungal MFC and control MFC, respectively (Fig. 5). The obtained polarization curves showed the relation among voltage and current density, between 71 k and 10 Ω. The shape of the polarization curves confirmed the occurrence of ohmic losses probably generated by membrane resistance, wastewater electrolyte resistance and bacterial metabolism [4, 13, 39–41]. Initial slopes, usually present in polarization curves due to activation losses, were observed in all assays. However, they were more pronounced in the MUM 04.100 MFC. This fact confirms that more time is needed by this MFC to obtain a mature electroactive biofilm (more than 6 days were necessary to obtain a current density similar to the one observed in control MFC). A sudden decrease in the voltage at the end of the polarization curve was observed in the MUM 04.100 MFC, probably due to mass transfer limitations near the electrode surface. The R_{int} values determined in the region dominated by ohmic losses (maximum power densities in polarization curve) were 77 and 110 Ω, for the MUM 04.100 MFC and for the control MFC, respectively. These values were lower than expected, because the distance between the cell electrodes and the membrane was rather high (> 2 cm). A possible explanation might be the cathodic solution used in the present work. MFCs operated with pH buffers revealed an increase of proton transfer and a R_{int} decrease due to proton concentration polarization [41].

The maximum power density in the present work was obtained for the MFC with fungi and was 3.40 mW/m^2 (Fig. 6). This value corresponded to a current density of 18.35 mA/m^2 and was the result of the application of an external resistor equal to 2100 Ω. In this MFC, power

disturbance was observed in the polarization curve at higher current densities. This phenomenon is not well understood, but biofilm acclimation with lower resistance (500 Ω) can be considered as the main cause [40, 41]. This can be overcome by controlling anode potential and thus stabilize the biofilm development. Furthermore, the mass of anode biofilm, the number of EPS, as well as the internal resistance, increase with the increase of the external resistance [41]. After this performance assay, we changed the resistor for a higher resistance (1000 Ω) in both MFC. We did not change it to the resistance where the maximum power density (2200 Ω) was observed, in order to avoid an increase of the internal resistance. Effectively, the current density increased to values higher than the observed in performance assays.

In the control experiment, the maximum power density obtained was 2.54 mW/m^2, equivalent to a current density of 15.87 mA/m^2 (external resistor = 2100 Ω). The lower values reflect the relatively high oxygen reduction overpotential in the biocathode in the absence of a mediator, in this case being the Lcc [42]. Only when comparing the maximum power densities and current densities between the immobilized fungi MFC and the control, it was possible to observe that the Lcc produced by MUM 04.100 enhanced power densities. These results are in agreement with previous current densities observed by Simões et al. [21].

Furthermore, fungal Lcc biocathodes enhancement (Table 1) have been considered, by several recent investigations, as an alternative to common MFCs where the abiotic cathodic chamber is filled with a buffer solution.

As observed, the recovered power densities from MFCs using Lcc to catalyze the O$_2$ reduction into H$_2$O are still much lower when compared to the ones obtained using nitrification/denitrification microbial consortiums in the cathode, suggesting further research of this type of biocathode. In

Table 1 Power density of recent investigations on laccase (Lcc) biocathodes in microbial fuel cells (MFC)

MFC configuration	Biocathode (Lcc)	Anode inoculum	Power density (mW/m^2)	Reference
H-type	Commercial *Trametes versicolor*	*Shewanella oneidensis*	38 ± 1.7	[43]
Two chamber	*Phlebia floridensis* *Phlebia brevispora*	*Pichia fermentans*	33.19 12.90	[44]
Two chamber	*Galactomyces reessii*	Microbial community	59	[45]
Two chamber	*Pleurotus ostreatus*	Real wastewater	180.5	[21]
Single chamber	*Ganoderma lucidum*	Anaerobic sludge from an oil-cracking	13.38	[32]
Two chamber	*Trametes versicolor* MUM 04.100	Real wastewater	12.30	Present study

addition, in nitrifying/denitrifying biocathodes, apart from the bioenergy production, it is possible to obtain an ammonia–nitrogen removal from wastewater, thus reducing the need for post treatments [46]. However, in those biocathodes, the fraction of nitrogen released as N_2O represents a significant environmental threat due to its high global warming potential.

Therefore, in the search for a high-performing and environmentally friendly cathode, MFCs with biocathodes catalyzed by the in situ production of Lcc by *T. versicolor* look like a good strategy. In addition, and looking into the low-power density observed, this indicates that the present MFC configuration can be improved by, for example, reducing the space between both electrodes, and controlling both anolyte and catholyte solution pH, leading to an increase of Lcc production in the cathodic chamber [21, 45]. MFC with the fungus-based biocathodes had longer stable performance than a MFC with only commercial Lcc, due to continuous secretion of the enzyme as reported by Wu et al. [47]. Another important point favoring the use of fungus-based biocathodes is the COD remotion in less time. In our study, efficient COD remotion, current density, and BOD were statistic different as compared by control ($p > 0.05$). This information can provide the possibility to use industrial wastewaters, for example, from pulp and paper industry or agricultural waste containing lignocellulosic material for fungus growth. In addition, the insertion of the Lcc into the cathode compartment of the MFCs has been the subject of several studies [21, 48]. According to Christwardana et al. [49], Lcc is an important catalyst in the oxygen reduction reaction for the cathode electrode, but it has low durability in the system. In this sense, the present study utilizes an innovative approach, since the organism that synthesizes the enzyme is in situ and, in this way, the production of the enzyme occurs continuously, reducing the need to add a new enzymatic solution at the cathode. Such an approach could, for example, address the need for continuous supplementation of the air-breathing Lcc cathode proposed by Kipf et al. [27].

Conclusion

Our findings indicate that *Trametes versicolor* MUM 04.100 Lcc biocathode induces a better MFC performance. Lcc was continuously synthesized in situ and consequently improved the reduction of O_2 to H_2O. The current density detected for this strain was 42.81 ± 4.91 mA/m^2. This performance corresponds to the maximum Lcc activity (23.08 U/L) which occurred after the glycerol pulse. The current density obtained in the control (absence of fungus in the cathode chamber) was lower, 10.3 mA/m^2. This work provides an effective and promising approach to enhance the MFC performance. MFC biocathodic chamber is a sustainable promising cost-effective approach to improve energy performance.

Declarations

References

1. Hoang AT, Nižetić S, Ng KH et al (2022) Microbial fuel cells for bioelectricity production from waste as sustainable prospect of

future energy sector. Chemosphere 287:132285. https://doi.org/10.1016/j.chemosphere.2021.132285

2. Ramya M, Kumar S (2022) A review on recent advancements in bioenergy production using microbial fuel cells. Chemosphere 288:132512. https://doi.org/10.1016/j.chemosphere.2021.132512

3. Ottoni CA, Simões MF, Santos JG, Peixoto L et al (2019) Application of microbial fuel cell technology for vinasse treatment and bioelectricity generation. Biotechnol Lett 41:107–114. https://doi.org/10.1007/s10529-018-2624-2

4. Logan BE, Hamelers B, Rozendal R et al (2006) Microbial fuel cells: methodology and technology. Environ Sci Technol 40:5181–5192. https://doi.org/10.1021/es0605016

5. Potter MC (1911) Electrical effects accompanying the decomposition of organic compounds. Proc R Soc Lond B 84:260–276. https://doi.org/10.1098/rspb.1911.0073

6. Nawaz A, Haq I, Qaisar K, Gunes B et al (2022) Microbial fuel cells: insight into simultaneous wastewater treatment and bioelectricity generation. Process Saf Environ Prot 161:357–373. https://doi.org/10.1016/j.psep.2022.03.039

7. Logan BE, Wallack MJ, Kim K-Y et al (2015) Assessment of microbial fuel cell configurations and power densities. Environ Sci Technol Lett 2:206–214. https://doi.org/10.1021/acs.estlett.5b00180

8. Mohyudin S, Farooq R, Jubeen F et al (2022) Microbial fuel cells a state-of-the-art technology for wastewater treatment and bioelectricity generation. Environ Res 204:112387. https://doi.org/10.1016/j.envres.2021.112387

9. Xu Z, Chen S, Guo S et al (2021) New insights in light-assisted microbial fuel cells for wastewater treatment and power generation: a win-win cooperation. J Power Sources 501:230000. https://doi.org/10.1016/j.jpowsour.2021.230000

10. Yaqoob AA, Ibrahim MNM, Guerrero-Barajas C (2021) Modern trend of anodes in microbial fuel cells (MFCs): an overview. Environ Technol Innov 23:101579. https://doi.org/10.1016/j.eti.2021.101579

11. Gunaseelan K, Jadhav DA, Gajalakshmi S, Pant D (2020) Blending of microbial inocula: an effective strategy for performance enhancement of clayware biophotovoltaics microbial fuel cells. Bioresour Technol 323:124564. https://doi.org/10.1016/j.biortech.2020.124564

12. Lu L, Lobo FL, Xing D, Zj R (2019) Active harvesting enhances energy recovery and function of electroactive microbiomes in microbial fuel cells. Appl Energy 247:492–502. https://doi.org/10.1016/j.apenergy.2019.04.074

13. Peixoto L, Rodrigues AL, Martins G, Nicolau A et al (2013) A flat microbial fuel cell for decentralized wastewater valorization: process performance and optimization potential. Environ Technol 34:1947–1956. https://doi.org/10.1080/09593330.2013.827223

14. Leininger A, Yates MD, Ramirez M, Kjellerup B (2021) Biofilm structure, dynamics, and ecology of an upscaled biocathode wastewater microbial fuel cell. Biotechnol Bioeng 118:1305–1316. https://doi.org/10.1002/bit.27653

15. Nájera MC, Verea L, Lastres O et al (2020) Electricity production in a two chamber microbial fuel cell with bioanodes and biocathodes catalyzed with gold. Fuel Cells 6:762–768. https://doi.org/10.1002/fuce.202000051

16. Peera SG, Maiyalagan T, Liu C et al (2021) A review on carbon and non-precious metal based cathode catalysts in microbial fuel cells. Int J Hydrog Energy 46:3056–3089. https://doi.org/10.1016/j.ijhydene.2020.07.252

17. Sarma PJ, Malakar P, Mohanty K (2023) Self-sustaining bioelectricity generation in plant-based microbial fuel cells (PMFCs) with microalgae-assisted oxygen-reducing biocathode. Biomass Conv Bioref. https://doi.org/10.1007/s13399-023-03848-z

18. Liu Y, Guo S, Wang J, Li C (2022) Fundamental development and research of cathodic compartment in microbial fuel cells: a review. J Environ Chem Eng 10:107918. https://doi.org/10.1016/j.jece.2022.107918

19. Zhao S, Chen Z, Khan A et al (2021) Elevated Cr(VI) reduction in a biocathode microbial fuel cell without acclimatization inversion based on strain *Corynebacterium vitaeruminis* LZU47-1. Int J Hydrog Energy 46:3193–3203. https://doi.org/10.1016/j.ijhydene.2020.05.254

20. Yadav G, Sharma I, Ghangrekar M, Sen R (2020) A live biocathode to enhance power output steered by bacteria-microalgae synergistic metabolism in microbial fuel cell. J Power Sources 449:227560. https://doi.org/10.1016/j.jpowsour.2019.227560

21. Simões MF, Maiorano AE, Santos JG et al (2019) Microbial fuel cell-induced production of fungal laccase to degrade the anthraquinone dye Remazol Brilliant Blue R. Environ Chem Lett 17:1413–1420. https://doi.org/10.1007/s10311-019-00876-y

22. Lin C-W, Lai C-Y, Liu S-H et al (2021) Enhancing bioelectricity generation and removal of copper in microbial fuel cells with a laccase-catalyzed biocathode. J Clean Prod 298:126726. https://doi.org/10.1016/j.jclepro.2021.126726

23. Curran LMCLK, Pham LTM, Sale KL, Simmons BA (2022) Review of advances in the development of laccases for the valorization of lignin to enable the production of lignocellulosic biofuels and bioproducts. Biotech Adv 54:107809. https://doi.org/10.1016/j.biotechadv.2021.107809

24. Savizi ISP, Kariminia H-R, Bakhshian S (2012) Simultaneous decolorization and bioelectricity generation in a dual chamber microbial fuel cell using electropolymerized-enzymatic cathode. Environ Sci Technol 46:6584–6593. https://doi.org/10.1021/es300367h

25. Mani P, Keshavarz T, Chandra TS, Kyazze G (2017) Decolourisation of acid orange 7 in a microbial fuel cell with a laccase-based biocathode: influence of mitigating pH changes in the cathode chamber. Enzyme Microb Technol 96:170–176. https://doi.org/10.1016/j.enzmictec.2016.10.012

26. Liu S-H, Tsai S-L, Guo P-Y, Lin C-W (2020) Inducing laccase activity in white rot fungi using copper ions and improving the efficiency of azo dye treatment with electricity generation using microbial fuel cells. Chemosphere 243:125304. https://doi.org/10.1016/j.chemosphere.2019.125304

27. Kipf E, Sané S, Morse D et al (2018) An air-breathing enzymatic cathode with extended lifetime by continuous laccase supply. Bioresour Technol 264:306–310. https://doi.org/10.1016/j.biortech.2018.04.086

28. Lin CW, Wu C-H, Lin Y-Y et al (2018) Enhancing the performance of microbial fuel cell using a carbon-fiber-brush air cathode with low-cost mushroom *Ganoderma* laccase enzyme. J Taiwan Inst Chem Eng 85:115–120. https://doi.org/10.1016/j.jtice.2017.12.025

29. Rodríguez Couto R, Sanromán MA, Hofer D, Gübitz GM (2004) Stainless steel sponge: a novel carrier for the immobilization of the white-rot fungus *Trametes hirsuta* for decolourization of textile dyes. Bioresour Technol 95:67–72. https://doi.org/10.1016/j.biortech.2003.05.002

30. Ottoni C, Lima L, Santos C, Lima N (2014) Effect of different carbon sources on decolourisation of an industrial textile dye under alkaline-saline conditions. Current Microbiol 68:53–58. https://doi.org/10.1007/s00284-013-0441-3

31. ZAR JH (1996) *Biostatistical analysis*. Third editions, Prentice-Hall International Editions, New Jersey, USA.

32. Lai CY, Wu C-H, Meng C-T, Lin C-W (2017) Decolorization of azo dye and generation of electricity by microbial fuel cell with laccase-producing white-rot fungus on cathode. Appl Energy 188:392–398. https://doi.org/10.1016/j.apenergy.2016.12.044

33. Braham SA, El Siar H, Arana-Peña S et al (2021) Positive effect of glycerol on the stability of immobilized enzymes: is

it a universal fact? Process Biochem 102:108–121. https://doi.org/10.1016/j.procbio.2020.12.015

34. Bhattacharyya A, Ahmed A, Wadhwa R et al (2022) High production of *Trametes cinnabarina* laccase (lac1) by suspended and immobilized cells of recombinant *Pichia pastoris* from crude glycerol. Waste Biomass Valor 13:2149–2168. https://doi.org/10.1007/s12649-021-01661-1

35. Urra J, Sepúlveda L, Contreras E, Palma C (2006) Screening of static culture and comparison of batch and continuous culture for the textile dye biological decolorization by *Phanerochaete chrysosporium*. Braz J Chem Eng 23:281–290. https://doi.org/10.1590/S0104-66322006000300002

36. Rubenwolf S, Sané S, Hussein L et al (2012) Prolongation of electrode lifetime in biofuel cells by periodic enzyme renewal. Appl Microbiol Biotechnol 96:841–849. https://doi.org/10.1007/s00253-012-4374-8

37. Sané S, Jolivalt C, Mittler G et al (2013) Overcoming bottlenecks of enzymatic biofuel cell cathodes: crude fungal culture supernatant can help to extend lifetime and reduce cost. Chemsuschem 6:1209–1215. https://doi.org/10.1002/cssc.201300205

38. Rodríguez Couto S (2009) Dye removal by immobilised fungi. Biotechnol Adv 27:227–235. https://doi.org/10.1016/j.biotechadv.2008.12.001

39. Faria A, Gonçalves L, Peixoto JM et al (2017) Resources recovery in the dairy industry: bioelectricity production using a continuous microbial fuel cell. J Clean Prod 140:971–976. https://doi.org/10.1016/j.jclepro.2016.04.027

40. Logan BE (2012) Essential data and techniques for conducting microbial fuel cell and other types of bioelectrochemical system experiments. Chemsuschem 5:988–994. https://doi.org/10.1002/cssc.201100604

41. Liu T, Yu Y-y, Li D et al (2016) The effect of external resistance on biofilm formation and internal resistance in *Shewanella* inoculated microbial fuel cells. RSC Adv 6:20317–20323. https://doi.org/10.1039/C5RA26125B

42. Schaetzle O, Barrière F, Schroder U (2009) An improved microbial fuel cell with laccase as the oxygen reduction catalyst. Energy Environ Sci 2:96–99. https://doi.org/10.1039/B815331K

43. Mani P, Fidal VT, Keshavarz T et al (2021) Laccase immobilization strategies for application as a cathode catalyst in microbial fuel cells for azo dye decolourization. Front Microbiol 11:620075. https://doi.org/10.3389/fmicb.2020.620075

44. Pal M, Sharma RK (2020) Development of wheat straw based catholyte for power generation in microbial fuel cell. Biomass Bioenergy 138:105591. https://doi.org/10.1016/j.biombioe.2020.105591

45. Chaijak P, Sukkasem C, Lertworapreecha M et al (2018) Enhancing electricity generation using a laccase-based microbial fuel cell with yeast *Galactomyces reessii* on the cathode. J Microbiol Biotechnol 28:1360–1366. https://doi.org/10.4014/jmb.1803.03015

46. Al-Mamun A, Baawain MS, Egger F et al (2017) of a baffled-reactor microbial fuel cell using autotrophic denitrifying bio-cathode for removing nitrogen and recovering electrical energy. Biochem Eng J 120:93–102. https://doi.org/10.1016/j.bej.2016.12.015

47. Wu C, Liu X-W, Li W-W et al (2012) A white-rot fungus is used as a biocathode to improve electricity production of a microbial fuel cell. Appl Energy 98:594–596. https://doi.org/10.1016/j.apenergy.2012.02.058

48. Sarma H, Bhattacharyya PN, Jadhav DA et al (2021) Fungal-mediated electrochemical system: prospects, applications and challenges. Curr Res Microb Sci 2:100041. https://doi.org/10.1016/j.crmicr.2021.100041

49. Christwardana M (2017) Combination of physico-chemical entrapment and crosslinking of low activity laccase-based biocathode on carboxylated carbon nanotube for increasing biofuel cell performance. Enzyme Microb Technol 106:1–10. https://doi.org/10.1016/j.enzmictec.2017.06.012