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Isolation and bioelectrochemical characterization of novel fungal sources with oxidasic activity applied in situ for the cathodic oxygen reduction in microbial fuel cells

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ABSTRACT

Brazilian filamentous fungi *Rhizopus* sp. (SIS-31), *Aspergillus* sp. (SIS-18) and *Penicillium* sp. (SIS-21), sources of oxidases were isolated from Caatinga's soils and applied during the in situ cathodic oxygen reduction in fuel cells. All strains were cultivated in submerged cultures using an optimized saline medium enriched with 10 g L^{-1} of glucose, 3.0 g L^{-1} of peptone and 0.0005 g L^{-1} of $CuSO_4$ as enzyme inducer. Parameters of oxidase activity, glucose consumption and microbial growth were evaluated. In-cell experiments evaluated by chronoamperometry were performed and two different electrode compositions were also compared. Maximum current densities of 125.7, 98.7 and 11.5 μA cm^{-2} were observed before 24 h and coulombic efficiencies of 56.5, 46.5 and 23.8% were obtained for SIS-31, SIS-21 and SIS-18, respectively. Conversely, maximum power outputs of 328.73, 288.80 and 197.77 mW m^{-3} were observed for SIS-18, SIS-21 and SIS-31, respectively. This work provides the primary experimental evidences that fungi isolated from the Caatinga region in Brazil can serve as efficient biocatalysts during the oxygen reduction in air-cathodes to improve electricity generation in MFCs.

1. Introduction

The gradual depletion of fossil fuels and the environmental concerns for their consumption have driven an intensive search for alternative sources for energy production. Biofuel cells (BFC) are considered a promising alternative for clean energy generation and also obey general sustainability requirements [15]. However, the high cost of noble metals such as Au, Pt, Rh and Os, commonly used in coated electrodes as catalysts, is still considered one of the limiting factors for scaled-up applications of microbial fuel cells (MFC) and conventional fuel cells. Even though, abiotic cathodes that use oxygen as electron acceptor are frequently adopted for BFC [21,22]. Enzymes as biocathodes can potentially eliminate limiting factors such as decreased efficiency due to the accumulation of metabolites, work under mild reaction condition such as temperature and pressure. Additionally, due to their high substrate specificity they are able to perform the electron transfer throughout suitable mediated systems and employing co-substrates [3,8]. These types of enzymatic cathodes have been investigated in small scale enzymatic biofuel cells [8]. On the other hand, energy production obtained from the BFC is not yet satisfactory and their performance and power output generation can be affected by a number of factors, such as cellular activity, substrate biotransformation and the inefficient electron transfer from the biocatalysts to the electrodic materials. Studies on enzymes for electron interactions are being mainly focused on copper-containing oxidoreductases (Fig. 1), which can catalyze the direct reduction of oxygen while performing the simultaneous oxidation of many organic compounds such as phenols. Mono- and polyphenol oxidases from fungal species such as *Agaricus bisporus* [28], *Coriolus hirsutus* [8], *Trametes versicolor*, *Coriolopsis gallica* and *Pleurotus ostreatus* [2,33]; plant laccase from *Rhus vernicifera*; and bacterial laccase from *Streptomyces coelicolor* were already studied and applied to these bioelectrodes [30]. Others less electrochemically explored, but highly promising correspond to the fungal bilirubin oxidase (BOD) from *Myrothecium verrucaria* [14,23] and bacterial BOD from *Bacillus pumilus* [5].

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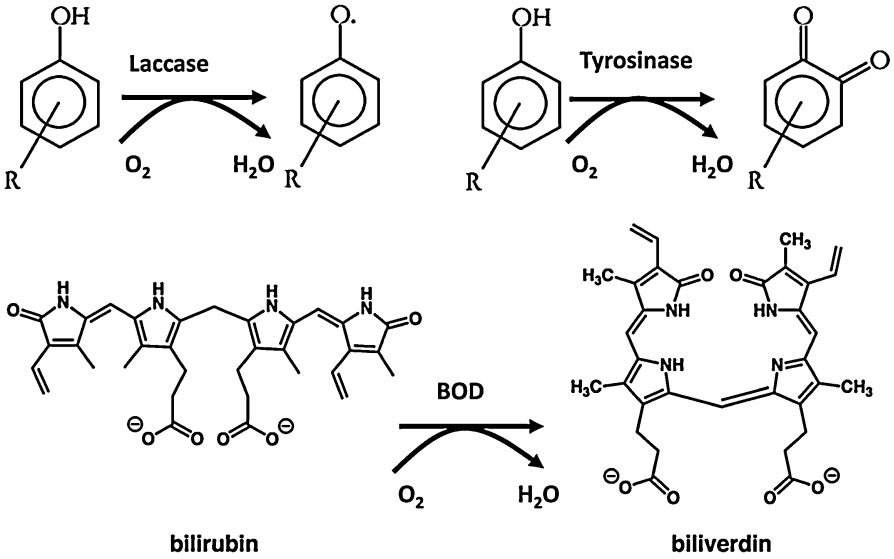

Fig. 1. Reactions catalyzed by multi-copper oxidases applied to biocathodes.

EC 1.10.3.1: Tyrosinase: polyphenol oxidase:

$$2\,\text{catechol} + O_2 \leftrightarrow 2\,1,2\text{-benzoquinone} + 2H_2O \quad (1)$$

EC 1.10.3.1: Laccase: urishinol oxidase

$$4\,\text{benzenediol} + O_2 \leftrightarrow 4\,\text{benzosemiquinone} + 2H_2O \quad (2)$$

EC 1.3.3.5: bilirubin oxidase

$$2\,\text{bilirubin} + O_2 \leftrightarrow 2\,\text{biliverdin} + 2H_2O \quad (3)$$

Laccase (LAC) and tyrosinase (TYR) are able to oxidize phenolic compounds and to reduce simultaneously oxygen into water (Eqs. (1) and (2)). Depending on the microbial source from which these enzymes were extracted, the redox potential of the T_1 site may vary from 430 mV up to 780 mV vs. NHE [24]. Laccase from *T. versicolor* is the most attractive one since redox potential of its T_1 site is ca. 780 mV vs. NHE [30]. Nowadays, the best performances with laccase electrodes are obtained with osmium based polymers as redox mediators [23] Actually these electrodes are able to deliver a current density of 860 $\mu A\,cm^{-2}$ at only −70 mV vs. O_2/H_2O at pH 5.0. Under the same conditions, an identical current density is obtained at −400 mV vs. O_2/H_2O with a platinum wire as catalyst. Nevertheless, performances of laccase from *P. ostreatus* electrodes drop drastically in the presence of chloride ions what constitutes both a major problem and a great challenge for its use in biofuel cells [2]. On the other hand, BOD (Eq. (3)) is naturally capable of catalyzing the oxidation of bilirubin into biliverdin and to simultaneously reduce dioxygen [20]. BOD is very similar to laccase. BOD electrodes are greatly related to the amino-acids sequence around T_1 site of the enzyme [29]. It is clearly reported that the most efficient BOD enzyme comes from *M. verrucaria*. Redox potential of its T_1 site is included between 650 and 750 mV vs. NHE, and its future application in BFC is closely related with the observed thermal stability up to 60 °C [23]. These biocatalysts have been extensively used in cathodes for enzymatic fuel cells and electrochemical biosensors due to their high redox potential, however the almost mandatory use of electron shuttles such as 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and other suitable molecules more recently studied as triphenylmethane dyes has been widely recognized as an effective way to avoid the loss of current during the bioelectrochemical process [1,18,31]. However, the application of such enzymatic fuel bioelectrodes has been limited, specially attributed to the high costs of production and purification and the short half-life time associated with the enzyme inactivation in non-biological environments as the ones commonly found in the surface of electrodic materials of BFC. In this regard, biocathodes inoculated with the fungi for the in situ oxidase production may offer a potential solution [25,34]. Also the in situ secretion of oxidases by the filamentous fungi in air-cathodes might be a more attractive way to achieve sustainable and cost-efficient electricity generation, especially for three main reasons: longer life-time of the biocatalysts since these are being produced under more compatible biological conditions; the use of low cost substrates such as residua or contaminated effluents; and the possibility of concomitant production of other natural occurring electro active molecules as microbial by-products like azaphylones, quinone-like pigments as melanins, terpene as carotenoids, etc. The simultaneous utilization of such molecules could improve even more the coulombic efficiencies by reducing the charge and mass transportation problems previously observed for these systems.

Currently, the Brazilian North and Northeast Network of Filamentous Fungi (RENNORFUN) aims to describe the biodiversity of filamentous fungi in soils from the Caatinga and the Amazon regions of Brazil throughout poly-phasic and molecular taxonomy as well as to demonstrate the applicability in industrial processes of the isolated micro-organisms and their by-products. In this context, this study aimed the isolation and identification of novel fungal species capable to produce biocatalysts with high oxidasic activity that can be applied to the cathodic reduction of oxygen in electrodes for biosensors and BFCs.

2. Methodology

2.1. Fungal strains, media and cultivation conditions

All strains belong to the RENNORFUN Culture Collection from the Catholic University of Pernambuco, Brazil, stocked in slant tubes containing Sabouraud agar solid medium under refrigeration at 4 °C until their use. Initial selection was based on previous in-plate observations associated with pigment production and oxidase or tannase activities, since pigment production can be by-products of the reactions catalyzed by these enzymes [17,27]. Table 1 shows the culture media used for selection of fungal strains with oxidasic activity. Twelve fungal strains were originally chosen: two *Rhizopus* spp.; three *Aspergillus* spp.; three *Penicillium* spp.; two *Eupenicillium* spp. and two *Talaromyces* spp. The fungal cultures were evaluated visually in terms of substrate degradation and color