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Laccase immobilization with Ruthenium complex as catalyst for biocathode application

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Biofuel cell research has attracted the interest of several groups due to its potential technological applications, such as energy source for biomedical devices, microchips, and perhaps as battery for some small portable electronic devices. In this context, several challenges have to be faced in terms of the cathodic half-cell, so as to attain an efficient material for oxygen reduction concerning biocathode applications. Considering the oxygen reduction reaction by biocatalysts, laccase is one of the most employed enzymes in biocathode preparation. Many organic compounds act as substrates for laccase but ABTS is generally preferred for evaluation of mediated electron transfer processes due to its availability, non-toxicity, and relatively low cost. Numerous studies have revealed that certain redox-active compounds can mediate the oxidation of substrates by laccase.[1-3] These mediators are usually compounds that act as an electron shuttle between the oxidized enzyme and another compound. In typical interactions of laccase with a substrate, the catalytic site of the enzyme abstracts electrons from the substrate and releases an oxidized product. In cases where a mediator is present, the mediator can be oxidized by the enzyme and subsequently oxidize another compound that is either a substrate or non-substrate resulting in the formation of oxidized product and regeneration of the mediator.[4]

In this work we investigate the performance of a biocathode based on oxygen reduction by the enzyme laccase, employing ruthenium complex as mediator and ABTS as substrate, focusing on the improvement of the electron transfer process.

The biocathodes were prepared by anchoring the enzymes on a 1 cm^2 carbon cloth (HT1400W, ELAT® GDL BASF) using modified-Nafion membrane[5] in 1:2 ratio to the enzymes[6] and a suspension of 8 mg mL^{-1} redox complex ruthenium (Ru(2,2 bipyridine)$_2$Cl$_2$).The redox polymer used in this study was prepared as described earlier.[7]

Power density measurements were performed in a cell consisting of two compartments. A gas diffusion membrane (ELAT) consisting of 40 % metal in C ($Pt_{0.66}Ru_{0.34}$, E-TEK commercial mixture) hot pressed in a Nafion® NRE-212 membrane was employed as the anode. The cell anodic compartment (10mL) was filled with acetate buffer, pH 4.5 and 100 mmol L^{-1} methanol. The cathodic compartment (10 mL) was filled with acetate buffer, pH 4.5 oxygen saturated. Assays as a function os substrate concentration were also carried out.

Fig. 1 shows the power density curve obtained for the biocathode in presence and absence of oxygen, with the substrate concentration kept in 1 mM. An open circuit voltage of 234 mV and 186 mV were achieved respectively. The maximum power density obtained was about 43 μW cm^{-2} and the maximum current density was 300 μA cm^{-2}, both in the presence of oxygen. This data just confirm the expected catalytic behavior of the enzyme towards the oxygen reduction reaction.

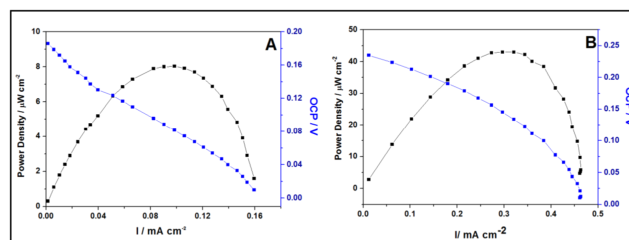

Fig.1. Power density curve obtained for the prepared biocathode in the absence (A) and in the presence of oxygen (B).

In order to evaluate the influence of substrate concentration on the biocathode performance (Fig. 2), power tests were performed in different substrate concentrations (ABTS 0.1 to 1 mM).

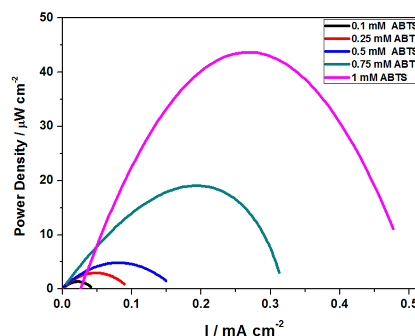

Fig.2. Power density curves as a function of ABTS concentration.

As it can be seeing, the biocathode performance increases almost linearly with the substrate concentration. This behavior confirm the laccase reaction mechanism and shows the importance of correct amount of substrate during the reaction of electron transfer and consequently the oxygen reduction reaction by the enzyme laccase. Higher concentrations were not used because may be not appropriate for some applications, where important considerations include the costs of mediators and the possibilities of creating negative impacts on enzymes.

The results have shown that the enzyme laccase can be effectively immobilized onto carbon platforms using ruthenium complex as mediator and ABTS as substrate. The employed system can also be improved by the use of other mediators species such as osmium complex, porphyrins and ferrocene groups.

Acknowledgements

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