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Optimized electrode arrangement and activation of bioelectrodes activity by carbon nanoparticles for efficient ethanol microfluidic biofuel cells

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HIGHLIGHTS

- Development of the first ethanol microfluidic biofuel cell based on bioelectrodes.
- Device optimized as function of electrode patterns in the microfluidic channel.
- Shorter and wider electrodes delivered higher current and power densities.
- Carbon nanoparticles with higher porosity enhanced bioelectrocatalytic processes.
- The miniature biofuel cell generated maximum power density of 90 μW cm^{-2} at 0.6 V.

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ABSTRACT

This work presents the construction of an ethanol microfluidic biofuel cell based on a biocathode and a bioanode, and operating in a Y-shaped microfluidic channel. At the anode, ethanol was oxidized by alcohol dehydrogenase, whereas at the cathode, the oxygen was reduced by laccase. Fuel and oxidant streams moved in parallel laminar flow without turbulent mixing into a microchannel fabricated using soft lithography methods. The enzymes were immobilized in the presence of reactive species at gold electrode surfaces. Bioelectrocatalytic processes were enhanced by combination of enzymes and carbon nanoparticles, attributed to appropriate electron transport and high amount enzyme loading. The benefit of the nanoparticles with higher surface porosity was explained by the high porous structure that offered a closer proximity to the reactive species and improved diffusion of the substrates within the enzyme films. The microfluidic BFC was optimized as function of electrode patterns, showing that higher current and power densities were achieved for shorter and wider electrodes that allow for thinner boundary layer depletion at the electrodes surface resulting in efficient catalytic consumption of fuel and oxidant. This miniaturized device generated maximum power density of 90 μW cm^{-2} at 0.6 V for a flow rate 16 μL min^{-1}.

1. Introduction

Recent increasing demands on small-scale sources for portable electronics have significantly boosted the interest in miniaturized fuel cells. Microfluidic techniques have been used for miniaturization of these devices based on microfluidic channels of submilliter in height [1]. Combination of microfluidic technologies and biological materials (enzymes) has given rise to the development of microfluidic biofuel cells [2–5]. These devices used enzymes as catalysts to convert chemical energy into electricity [6]. The components of these devices are analogous of the conventional microfluidic fuel cells based on anodic and cathodic compartments [1,7,8]. Fuel and oxidant streams move in parallel laminar flow without turbulent mixing into a microchannel fabricated using soft lithography methods [9]. These devices operate without the need of a separation membrane allowing for different pHs at the anolyte

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and the catholyte for optimal kinetics reaction. The mixing of the flows can only occur through diffusion, restricted to a thin interfacial zone in the center of the channel [1,10].

An important step to construct biofuel cells is the immobilization of enzymes on conductive support to ensure their electrical contact with electrodes and limit their denaturation. Electrodes modified by enzymes are the subject to develop prospective bioelectrodes to deliver high catalytic current density [11]. However, current densities are limited by low coverage of enzymes on the electrodes, low stability and sluggish electron transfer. The use of conductive nanomaterials with remarkable surface properties, like carbon nanotubes or nanoparticles, for bioelectrode modification provides an alternative and frequently used option to increase enzyme loading [12,13]. The similar dimensions of the particles and the enzymes enable nanomaterials to operate as an electrical wire decreasing electron transfer distance between the electrode and the active site of the enzymes [14]. Besides, their proximity can possibly accelerate the biocatalytic process. These materials are attractive for sensing and biosensing applications [15], for efficient bioelectrocatalysis [16] and could have a significant role in the development of biofuel cells. Incorporation of carbon nanoparticles (CNPs) is well-established for electrode surface modification in order to improve electron transfer rate between enzymes and electrode surfaces. One simple method involves encapsulation of carbon nanoparticles in organic [17] or inorganic polymer films [18]. Bioelectrodes based on bilirubin oxidase immobilization have been prepared with phenylsulfonated CNPs by layer-by-layer approach [19–21]. The authors showed that the resulting electrode promoted mediatorless bioelectrocatalytic oxygen reduction with good efficiency, as the current densities increased with the amount of deposited nanomaterial. Biocomposite CNP-laccase biocathodes have been prepared by entrapment of CNPs within enzyme polymer matrix for O_2 bioelectrocatalysis [22]. The improved performance was ascribed to the increased amount of enzyme molecules then electronically wired to the electrode through the CNP relays. Carbon Ketjen black was combined with cuperous oxidase [23] or bilirubin oxidase (BOD) [24] for oxygen reduction, or with glucose dehydrogenase (GDH) for glucose oxidation [25], and its developed surface led to increased current densities. Carbon Vulcan was mixed with multicopper oxidases in Nafion to construct efficient bioelectrodes for ethanol biofuel cell [26] and glucose/O_2 biofuel cells [27,28].

To date a few microfluidic BFCs based on immobilized enzymes electrodes and working from glucose as fuel have been developed. One device, composed of a BOD-adsorbed cathode and GDH-adsorbed anode with enzymes encapsulated in polylysine in the presence of Ketjen black, was reported to generate 30 μW cm^{-2} at 0.29 V in an air-saturated solution at pH 7, with a flow rate 1 mL min^{-1} [29]. The originality of this work was the electrode rather smart configuration in a single flow channel. More recently, a microfluidic BFC working from laccase and glucose oxidase covalently bounded onto single-walled carbon nanotube electrodes [30] showed a robust immobilization technique for microfluidic application. The system delivered a maximum power density of 1.65 μW cm^{-2} at 0.235 V. Higher power density generation of 64 μW cm^{-2} at 0.54 V with a flow rate 70 μL min^{-1} was achieved with a microfluidic device using pyrolyzed photoresist film electrodes [31]. The relevance of this work is the combination of thin and flexible films patterned by means of a cutter plotter to build the device, allowing mass production.

Besides, a microfluidic and microfabricated ethanol biofuel cell was developed based on a bioanode composed of a micromolded carbon ink modified with the enzyme alcohol dehydrogenase and methylene green [32]. The bioanode was sealed under a PDMS flow microchannel powering by hydrodynamic flow of ethanol and NAD^+. When the integrated microfluidic bioanode was assembled with an external platinum cathode, the complete biofuel cell produced a maximum power density of 5 μW cm^{-2} at 0.34 V.

This paper describes the development of an ethanol microfluidic BFC based on a biocathode and a bioanode, operating in a Y-shaped microfluidic channel to generate maximum power density. The dimensions and operating conditions of the microfluidic device were such that fluid flow was pressure driven and characterized by very low Reynolds number. At the anode, ethanol was oxidized by alcohol dehydrogenase, whereas at the cathode, the oxygen was reduced by laccase. Electrochemical characterizations of the device were performed by varying the electrode configuration and the flow rate of the streams through the microchannel. In particular, the work focused on the influence of carbon nanoparticles on the electron transfer in attempt to improve efficiency of the bioelectrocatalytic process. The performances of the devices were evaluated from voltage, current density and delivered power density.

2. Experimental

2.1. Materials

Laccase from *Trametes Versicolor* (20 U mg^{-1} solid), Diaphorase (3–20 U mg^{-1}), Alcohol Dehydrogenase (300 U mg^{-1}), β-nicotinamide adenine dinucleotide sodium salt (NAD^+), 2-methyl-1,4-naphthoquinone (VK3), Aceton, polyethylenimine (PEI) (50% (w/v) in H_2O), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS), Nafion® solution (5 wt%), sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$) and sodium phosphate monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$) were purchased from Sigma–Aldrich and used without further purification. The phosphate buffer was prepared with $Na_2HPO_4{\cdot}2H_2O$ and $NaH_2PO_4{\cdot}H_2O$ (pH 5, 7 or pH 9, 0.1 M). The carbon nanoparticles powder as Super P® and KS6 were purchased from TIMCAL.

2.2. Bioelectrodes preparation

The biocathode to be employed in the electroreduction of oxygen was prepared by adsorption of enzymes and mediators on the surface of the electrodes by drop casting. 333 μL of laccase (15 mg mL^{-1}) and Super P® (15 mg mL^{-1}) in phosphate buffer 0.1 M (pH 5) solution was mixed on a vortex mixer. Sequentially, 100 μL of the solution was mixed with ABTS (5.4 mg mL^{-1}) and 10 μL Nafion®. Then, 6 μL of the preparation was coated onto Au electrode and left to dry at room temperature before keeping in a low humidity environment.

The bioanode to be employed in the oxidation of ethanol was prepared by adsorption of successive coatings separated by a dried step at room temperature. 167 μL of ADH (30 mg mL^{-1}) and KS6 (15 mg mL^{-1}) in phosphate buffer 0.1 M (pH 7) solution was mixed on a vortex mixer and 6 μL of the preparation was pipetted onto the electrode and dried at room temperature. The same procedure was conducted for the immobilization of NAD^+ (30 mg mL^{-1}) and then diaphorase (20 mg mL^{-1}). The last coating on the electrode consisted in pipetting 10 μL of a solution containing VK3 (60 mg mL^{-1}), 190 μL acetone and 10 μL PEI, followed by drying.

2.3. Fabrication of the microfluidic cell

The microfluidic chip was fabricated from a standard soft lithography method described elsewhere [33]. Typically, a glass slide was preliminary cleaned, modified sequentially by three photoresist layers (35 μm Etertec HQ-6100) exposed to UV light through a photomask. The structure was then developed by