Alcohol dehydrogenase as bioanode for methanol and ethanol oxidation in a microfluidic fuel cell

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Abstract. In this work, an enzymatic bioanode was developed using functionalized carbon nanofibers for the immobilization of alcohol dehydrogenase enzyme, using Nafion, tetrabutylammonium bromide and glutaraldehyde onto Toray carbon paper. Two alcoholic fuels, methanol (MeOH) and ethanol (EtOH) were used to demonstrate the oxidation versatility of the electrode created as well as being able to convert energy from these fuels using an air-breathing microfluidic device. Commercial Pt/C (30% E-TEK) onto carbon nanofoam (Marketech Inc) was using as inorganic cathode. Carbon nanofibers were previously treated with nitric acid to produce oxygen-containing functional groups in order to facilitate the enzyme immobilization. A phosphates buffer solution pH 8.86 was used as electrolyte. The use of ethanol as fuel shows a better performance obtaining 8.643 mAc m⁻² and 0.847 V as current density and open circuit voltage respectively, compared to that use methanol as fuel (2.655 mAc m⁻² and 0.567 V).

1. Introduction

Alcohols are commonly used as fuels in microfluidic fuel cells due to easy transport, store and can be obtained from various sources, being the most used the methanol (MeOH) and ethanol (EtOH) [1]. Biological catalysts such as enzymes have been used for the alcohol oxidation reactions, since they have advantages over conventional catalysts, such as high specificity, selectivity and low cost [2].

Alcohol dehydrogenase enzyme carries out the alcohols oxidation to their corresponding aldehyde; ethanol is oxidized into acetaldehyde and methanol into formaldehyde, although with less efficiency. The cofactor of Alcohol dehydrogenase is NAD⁺; both alcohol and NAD⁺ may bind to the active center of the ADH, which contains zinc ions and during enzymatic alcohol oxidation, NAD⁺ is reduced to NADH [3]. Alcohol dehydrogenase enzyme structure is a homo tetramer of polypeptides, with 4 subunits each with its catalytic and co-enzymatic domain. To carry out the reaction requires addition of ethanol and ADH, the presence of NAD⁺, and zinc as cofactor. Zinc fulfills structural and catalytic functions in the enzyme [4]. In the first instance, the zinc atom is bound to the oxygen of the hydroxyl group of alcohol, stabilizing the substrate. A serine unit presents an oxygen atom to the hydrogen of this hydroxyl group, and removes it by transfer of protons. In turn, NAD⁺ removes hydrogen from the carbon attached to the hydroxyl group of ethanol, reducing to NADH [5].

Several supports have been reported, including different nanostructured materials have shown to increase the stability of immobilized enzymes and improve electrical conductivity for applications as biocatalysts in microfluidic fuel cells [6-8]. The creation of electrodes modified with this enzyme, which depends greatly on the enzymatic immobilization used for the bioelectrocatalytic process, where an efficient enzymatic confinement technique may serve to increase power density and stability of the microfluidic device when ethanol or methanol is used as fuel, being able in the future to have applications in the field of medicine and alcoholic beverage industry.
2. Experimental

2.1 Bioelectrode construction.

Carbon nanofibers were treated with HNO$_3$/H$_2$SO$_4$ (1:3) for 3 h at 40 °C in a water-bath sonicator, later was centrifuged and the precipitate was washed with deionized water were made to pH 7.0 and dried at 90°C for 8hrs to obtain carboxylic group functionalized. A catalytic ink was prepared using 1mg alcohol dehydrogenase (EC 1.1.1.1 initial activity ≥300 units/mg protein from Saccharomyces cerevisiae), 4mg of carbon nanofibers functionalized, 5 μL of Nafion solution (Sigma Aldrich), 500μL of 0.1M of buffer phosphate pH= 8.86, and 5 mg of tetrabutylammonium bromide (Sigma Aldrich). The catalytic ink was deposited by dipping on Toray carbon paper (EC-TP1-060T) whose dimensions were of 2.5x30 mm. The schematic representation of the bioanode developed as well as the reactions of alcohol dehydrogenase enzyme for the oxidation of ethanol and methanol is presented in figure 1.

![Figure 1](image1.png)

**Figure 1.** Scheme of bioelectrode arrangement and enzymatic reactions of the enzyme alcohol dehydrogenase.

2.2 Microfluidic fuel cell evaluation.

Schematic representations of the single microfluidic fuel cells used are illustrated in figure 2, whose design was previously reported [9]. The abiotic cathode Pt-based for the hybrid MMFC was prepared using commercial Pt/C (30% E-TEK) supported on carbon nanofoam (2×0.3 cm, Marketech®). Briefly, 120 μL of isopropyl alcohol and 14 μL of Nafion were added per mg of catalyst and mixed for 30 min. The catalytic ink was deposited on the carbon nanofoam surface using the spray technique.

![Figure 2](image2.png)

**Figure 2.** Scheme of air-breathing microfluidic fuel cell used for bioanode evaluation.
The microfluidic fuel cells were evaluated by injecting different fuel for two evaluation, 0.1M of methanol and 0.1M of ethanol in 0.1M buffer solution N2-saturated (4 U. P. D. Praxair) and oxygen from the air as the oxidant within two aqueous streams consisting in 0.1M phosphate buffer (pH 7) and 0.3 M KOH injected on the anodic and the cathodic side, respectively, with a flow rate of 50 µL min⁻¹ for both streams.

3. Results

Scanning electron microscopy technique was used to analyse the morphology of different electrode arrangements, as shown in figure 3, where a) is the naked carbon paper Toray and b) is when deposited on this paper the functionalized carbon nanofibres covering large part of the surface and where placing the catalytic ink q contains the enzyme alcohol dehydrogenase (Figure 3c).

![Image of scanning electron microscopy](image)

**Figure 3.** Scanning electron microscopy images of (a) Toray carbon electrode, (b) functionalized carbon nanofibers onto Toray carbon paper and (c) ADH bioanode on surface b.

![Graph of polarisation and power density](image)

**Figure 4.** Polarisation and power density curves obtained from microfluidic fuel cell evaluated with ethanol or methanol as fuel in 0.1M phosphates buffer pH 8.86 and 0.3M KOH as oxidant. 10 mV s⁻¹ scan rate.

![Graph of chronoamperometry](image)

**Figure 5.** Chronoamperometry obtained from microfluidic fuel cell tested in the presence of 0.1M methanol or ethanol as fuel in 0.1M phosphates buffer pH 8.86 and 0.3M KOH as oxidant. 10 mV s⁻¹ scan rate.

**Table 1.** Performance of microfluidic fuel cell using methanol and ethanol as fuel.

| Fuel   | Current density (mA/cm²) | Potential(V) |
|--------|--------------------------|--------------|
| Methanol | 2.655                    | 0.567        |
| Ethanol  | 8.643                    | 0.847        |
The use of ethanol as fuel shows a better performance obtaining 8.643 mA cm\(^{-2}\) and 0.847 V as current density and open circuit voltage respectively, compared to that use methanol as fuel (2.655 mA cm\(^{-2}\) and 0.567V) according to Figure 3 and table 1, it can be attributed that the enzyme has a greater affinity to the oxidation of ethanol than methanol, because methanol is a fuel difficult to handle in fuel cell technology, due in many aspects to its toxicity. It can also be observed that when there is no presence of fuel of an alcoholic nature, the performance of the cell is very low, indicating the oxidation selectivity of alcohol dehydrogenase enzyme.

Figure 5 shows the stability test by chronoamperometry technique of the air-breathing microfluidic fuel cell using ethanol or methanol as fuel. In the presence of methanol, the device shows a low stability and less current density is produced, decreasing the yield. In contrast, when ethanol is used as fuel, good stability is maintained during the tests. The results could be attributed to enzyme used as anode, whose affinity is greater when ethanol is used as fuel.

4. Conclusions
With the construction of an enzymatic bioanode based on the enzyme alcohol dehydrogenase and the use of functionalized carbon nanofibers, a microfluidic device was evaluated using ethanol and methanol as fuel, according to the developed analyses it was possible to determine that the electrode can oxidize these fuels, taking a greater affinity over ethanol, where a good power density could be obtained by using it. Due to the chemical nature of the methanol involved the immobilized enzyme activity and the stability of the microfluidic fuel cell, being a difficult fuel to handle.

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