Urease enzyme as anodic catalyst in a microfluidic fuel cell

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Abstract. In this research, enzymatic bioanodes based in urease enzyme were developed using graphene oxide as support material on porous electrodes, for application in microfluidic devices that use urea as fuel. Urease is a metalloenzyme that due to its bimetallic site is able to carry out the oxidation of urea with the formation of ammonia and carbonic acid. Graphene oxide was used because of its characteristics that favour the process of enzymatic immobilization. The performance of the microfluidic fuel cell was carried in the absence and in the presence of 0.1 M urea in 0.1M phosphates buffer where can observed an open circuit voltage above 1 V and important power density value when urea is used as fuel (2.82 mW cm⁻²). It was also evaluated by electrochemical techniques the effect of the concentration of urea on the power density generated by the microfluidic device, as well as stability, generating good energy conversion results for a first stage of research, where the objective is the creation of bioelectrodes with urease enzyme immobilized for application in Lab-on-a-Chip devices.

1. Introduction

Microfluidics fuel cells or membraneless fuel cells have attracted attention because of their potential applications because they exploit the co-laminar flow that occurs in the microscale to separate the fuel from the oxidizing species to generate electrical current from a variety of organic liquid fuels and oxidants under laminar flow regimen with low Reynolds number and high Peclet number, eliminating the need for a membrane performing this function, as in conventional fuel cells [1-3]. The anode has been the focus of research in this area, because it is where the oxidation reaction of the fuel occurs.

Among the types of electrodes used as anodes in several devices of energy conversion are those based in enzymes, which act as biochemical transducers that combine an electrochemical process with immobilized enzyme activity [4, 5]. The urease enzyme can be used for the construction of bioanodes due to its ability to carry out the oxidation of urea present in body fluids. Urease is a non-redox metalloenzyme involved in the overall nitrogen cycle in plants, algae, fungi and various microorganisms. Its bimetallic reaction site (Ni-II) catalyzes the rapid hydrolytic decomposition of urea to give ammonia and carbamate, this reaction continues hydrolyzing to form ammonia and carbonic acid as products [6, 7]. The urease activity is strongly dependent on the pH in the range of about 4.5-10.5 pH, with an increasingly slower reaction as the range increases, having an optimum pH between 7.4 pH. Urea use as fuel presents 2 Mcal / kg equivalent to 8.368MJ / kg of energy density, making it attractive for use in microfluidic fuel cells for biomedical applications, where obtaining a good open circuit potential close to the theoretical could be used for the feeding of different biomedical devices [8, 9].
anode: $CO(NH_2)_2 + 6OH^- \rightarrow N_2 + 5H_2O + CO_2 + 6e^- \quad E^0 = -0.746V$

cathode: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad E^0 = +0.401V$

complete reaction: $CO(NH_2)_2 + 3O_2 \rightarrow 2N_2 + 4H_2O + 2CO_2 \quad E^0 = 1.147V$

The nanostructured materials as graphene oxide are considering excellent candidates for enzymatic immobilization due to reduce the diffusion limitations and provide greater surface area improving the electronic transfer in the electrode [10, 11]. The low potency and stability of cells using enzyme electrodes is due to the complexity of enzymes and easy denaturation, since all enzymes have the characteristics of being sensitive to minimum changes in temperature and pH, their active sites can be reached to be disabled at the time of the enzyme-support immobilization, without forgetting that the shelf life and the poor power density of the microfluidic devices depend on the enzymatic stability [12]. Nanostructured supports favor enzymatic immobilization, mass transfer resistance and effective enzyme loading due to their higher surface area than macro-scale supports tend to have excellent biocompatibility, stability and reuse life of the enzyme [13]. Enzymatic immobilization can be performed without modifications on its surface and has a large surface area with abundant functional groups that facilitate the anchoring of the enzyme and the electronic transfer. In this work, graphene oxide was used as a support to immobilize the enzyme urease for the construction of enzyme electrodes for application in microfluidic fuel cells for urea oxidation.

2. Experimental

2.1 Bioelectrode construction.

A catalytic ink was prepared using 1mg urease (EC 3.5.1.5 Type III, powder, 15,000-50,000 units/g solid), 2mg of graphene oxide, 7 μL of Nafion solution (Sigma Aldrich), 500μL of 0.1M of buffer phosphate pH= 7.4, and 8 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.

2.2 Microfluidic fuel cell evaluation.

The microfluidic device used was previously reported for application in enzymatic microfluidic fuel cells using the glucose oxidase [14], alcohol dehydrogenase [15], aldehyde dehydrogenase enzymes [16] immobilized at the bioanode. This device (Figure 1) consisted of two supporting plates made of Poly-(methyl methacrylate) (PMMA) and patterned using a computer numerical control CNC, a home-made silicone elastomer film (Silastic®, Dow Corning, prepared using an Elcometer® Film Applicator with a final thickness of 200 μm) used as both the gasket and cell channel structure.

![Scheme of air-breathing microfluidic fuel cell](image-url)
The electrodes consisted of commercially available carbon nanofoam (Marketech Inc) for cathodes and 10 mm long × 2 mm wide Toray paper (100 µm in thickness) for bioanodes. The device was evaluated at different urea concentration $\text{N}_2$-saturated (4 U.P.D. Praxair) as the fuel and oxygen taken from the air as the oxidant within two aqueous streams that contained a phosphate buffer (pH 7.4) and 0.3 M KOH on the anodic and the cathodic side, respectively with 50 µL min-1 of flow rate for both streams.

3. Results

The performance of the microfluidic fuel cell was carried in the absence and in the presence of 0.1 M urea in 0.1M phosphates buffer (Figure 2) where can observed an open circuit voltage above 1 V and important power density value when urea is used as fuel (2.82 mW cm$^{-2}$). It can also be observed that in absence of urea the power density of the microfluidic device is very low, attributing it to the absence of substrate necessary to carry out the oxidation process at the anode. Further, the microfluidic device was evaluated used 10, 50 and 100 Mm of urea, increasing the current density linearly with the fuel concentration (Figure 3).

**Figure 2.** Polarisation and power density curves obtained from air-breathing microfluidic fuel cell using 0.1M urea in 0.1M phosphates buffer pH 7.4 as fuel and 0.3M KOH as oxidant, at 10 mV s$^{-1}$.  

**Figure 3.** Polarisation and power density curves obtained from air breathing microfluidic fuel cell at different urea concentration in 0.1M buffer phosphates pH 7.4 at10 mV s$^{-1}$ as fuel and 0.3M KOH as oxidant.

**Figure 4.** Chronoamperometry tests of air breathing microfluidic fuel cell evaluated with 0.1M urea in 0.1M phosphates buffer pH 7 as fuel and 0.3M KOH as oxidant at10 mV s$^{-1}$.  


Finally, the stability of the microfluidic device was tested by chronoamperometry technique at 0.1M urea (Figure 4) showing excellent behaviour as function of the time. The results shows that the bioanode based in urease enzyme oxides successfully urea as fuel and could has potential possibilities of use in future biomedical applications.

4. Conclusions

Urea is an attractive fuel because it can be found in different biological fluids, such as blood and urine, having various interesting biomedical applications. The creation of enzymatic electrode based on the urease was important because using graphene oxide could develop a bioanode that can oxidize urea at different concentration that has selectivity and presented good stability when it was evaluated in microfluidic fuel cell obtaining a good open circuit potential higher of 1.0 V close to the theoretical open circuit potential reported.

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