Anode based on alcohol dehydrogenase enzyme and Titanium dioxide nanotubes for photocatalytic microfluidic device.

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Abstract. Alcohol dehydrogenase enzyme (ADH) has been used for the development of bioanodes for the ethanol oxidation reaction. In this work, the immobilization of this enzyme was carried out using titanium dioxide nanotubes for the development of photoelectrodes for an air-breathing microfluidic device. TiO2 nanotubes (TNT) have excellent properties such as pH resistance, superior mechanical strength, good biocompatibility making them great candidates for the process of immobilization of ADH. TNT were fabricated by electrochemical anodic oxidation on Ti foils. Then enzyme alcohol dehydrogenase was immobilized and the electrode was evaluated in the microfluidic fuel cell using an inorganic cathode based on commercial Pt/C, obtaining a good performance when was exposed under ultraviolet light.

1. Introduction

Enzymes are molecules of a protein nature that, thanks to their advantages of biocompatibility and biodegradability, help catalyze many chemical and biochemical reactions carried out in our body [1]. The main and major characteristic of the enzymes is their specificity, reason why each reaction is catalyzed by a specific enzyme of the cell. This specificity of the enzymes depends on the charge, shape and hydrophobic and hydrophilic characteristics of the same. Many times, the same enzyme is able to catalyze the same reaction in parallel to it, which helps to give a more sophisticated regulation of the reaction. Enzymatic catalysts help regulate the function and structures of cells and organisms, and catalyze the synthesis of macromolecules, the transmission of genetic information, and the conversion of chemical energy. The substances on which these enzymes act are substrates, which can be either a small molecule or a macromolecule [2].

Enzymes have many advantages for industrial use; unfortunately many times due to their lack of stability, and their difficult recovery and reuse of the enzyme makes the immobilization process of this is the most viable for its use [3]. The disadvantage of these is that in industrial chemical processes cannot be reusable. That is why the immobilization of enzymes was created, which helps us that in this
type of industrial processes, the enzymes can be more profitable in terms of their use [4]. The immobilized enzymes are those that are physically located in a certain place, retaining their catalytic activity for reuse. Thanks to the process of enzymatic immobilization some of the disadvantages of its use are reduced, as the cost factor due to its great ease of reuse [5].

Alcohol dehydrogenase is a dimeric enzyme system, composed of zinc. It belongs to a family of enzymes that help in the oxidation of alcohol to produce aldehydes and ketones in our body [6]. They are metalloenzymes composed of dimers with subunits of amino acid residues, and each subunit contains two fractions, one that is the catalytic part, and the second that is the union between it and the coenzyme [7].

Titanium dioxide (TiO₂) is a semiconductor material, non-toxic and with a high degree of biocompatibility. TiO₂ is one of the most studied compounds in the science of materials, due to its diverse properties that make it functional for many areas. In 1999, some reports demonstrated the possibility of creating ordered arrays of TiO₂ nanotubes by an electrochemical anodizing process on a titanium sheet [3]. Its high photocatalytic titanium nanotubes (TNT) activity results from its specific surface area, crystallinity and porous structure [7]. Due to its forbidden band, the TNT require irradiation with UV light to generate photo-induced charge carriers (electron-hole pairs) and this feature has been used in several fields such as alternative energy technologies, photocatalysis, solar cells, gas sensors and photo-electrochemical cells.

Using the nanostructured material, better photocatalytic properties can be obtained due to its short electron trajectory, high mass transport rate and remarkable light collection, among others [8]. The self-organized anodic materials of TiO₂ have become of great interest thanks to its nanoscale and controllable geometry, which favors it for electrocatalytic and photocatalytic applications. In this anodizing process, the use of a viscous electrolyte increases the conductivity of the formation of high resolution TiO₂ nanotubes [9]. TiO₂ nanotubes are formed using this anodizing method to have a better arrangement and are oriented perpendicular to the substrate. The formation of these nanotubes is achieved by means of a balance between the electrochemical processes and the chemical dissolution of the substrate in the presence of an electrolyte [10]. The arrangement of TiO₂ nanotubes in this way is used in several applications as they are in sensors, since it facilitates the transfer of electrons, so it serves as an ideal buffer for the immobilization of some biological compounds such as enzymes, DNA, cells, bacteria, antibodies, etc.) [7]. In this investigation, the enzyme alcohol dehydrogenase was immobilized on TNT for application in a microfluidic fuel cell.

2. Experimental

2.1 Titanium dioxide nanotubes synthesis

The nanotubes of TiO₂ will be manufactured by electrochemical anodic oxidation in sheets of Ti. The sheets will be pre-treated with sandpaper, and then immersed in ethanol, placed in an ultrasonic bath, dried under N₂ flow. A solution based on ethylene glycol with a concentration of 0.1M NH₄F (96% purity, Alfa-Aesar) and 2% w/w deionized water as electrolyte was used. Different anodization times will be evaluated with a voltage step of 60 V through a power supply.

2.2 Bioanode construction.

The electrode was constructed using a catalytic ink with alcohol dehydrogenase (EC 1.1.1.1 initial activity ≥300 units/mg protein from Saccharomyces cerevisiae), tetrabutylammonium bromide, Nafion and TNT and the immobilization was carried out by covalent binding. The catalytic ink was deposited by dipping on Toray carbon paper (EC-TP1-060T) whose dimensions were of 2.5x30 mm.
2.3 Cathode construction.

The abiotic cathode Pt-based 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 nanofoil surface using the spray technique.

2.4 Microfluidic fuel cell evaluation.

This microfluidic device 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 [11, 12]. The device was evaluated at different ethanol in 0.1M buffer phosphate pH 8.87 (4 U.P.D. Praxair) as fuel and oxygen taken from the air as the oxidant, respectively with 60 μL min⁻¹ of flow rate for both streams.

3. Results and discussion

The evaluation of the bioanodes developed in a microfluidic fuel cell using ethanol in buffer 0.1M phosphate pH=8.86 was carried out in the presence and absence of ultraviolet light. The power density of the device in the presence of ultraviolet light increased notably, as can be seen in Figure 1.

![Figure 1](image)

**Figure 1.** Schematic representation of the microfluidic device in the presence and absence of UV light using 0.1M ethanol in 0.1M buffer phosphate pH 8.86 as fuel.

Figure 2 shows the evaluation of the potential as a function of the time generated by the microfluidic fuel cell using ethanol as fuel and exposed to ultraviolet light where it can be seen that the devices is stabilized after 3000 seconds reaching 1V.
Figure 2. Evaluation of potential vs time from microfluidic fuel cell in the presence of 0.01M ethanol in 0.1M buffer phosphates pH 8.5 at 10 mV s⁻¹ as fuel and 0.3M KOH.

4. Conclusions
Among the various reported inorganic nanomaterials, titanium dioxide nanotubes are emerging as one of the promising materials for energy conversion due to their microstructure, low cost, high safety and long cycle life. An electrode with the alcohol dehydrogenase enzyme immobilized on titanium dioxide nanotubes could be developed and evaluated in a microfluidic device for oxidation of ethanol, performing tests with ultraviolet light exposure, where the power density could be increased taking advantage of the photocatalytic power of TNT.

Acknowledgements
Authors acknowledges to the Mexican Council for Science and Technology (CONACYT) for financial support through project Laboratorios Nacionales Grant no. LN 293442, and CONACYT-SENER Grant no. 246079.

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