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Enzyme-Based Microfluidic Biofuel Cell to Generate Micropower

A.Zebda¹, C. Innocent¹, L. Renaud², M. Cretin¹,
F. Pichot³, R. Ferrigno² and S. Tingry¹

¹Institut Européen des Membranes

²Institut des Nanotechnologies de Lyon

³Centrale de Technologies en Micro et Nanoélectronique, Université de Montpellier 2
France

1. Introduction

Enzymatic biofuel cells (BFCs) employ enzymes to catalyse chemical reactions, thereby replacing traditional electrocatalysts present in conventional fuel cells. These systems generate electricity under mild conditions through the oxidation of renewable energy sources (Calabrese Barton et al., 2004). At the anode side the fuel is oxidized and the electrons, which are released by the oxidation reaction, are used to reduce the oxidant at the cathode side (Fig. 1).

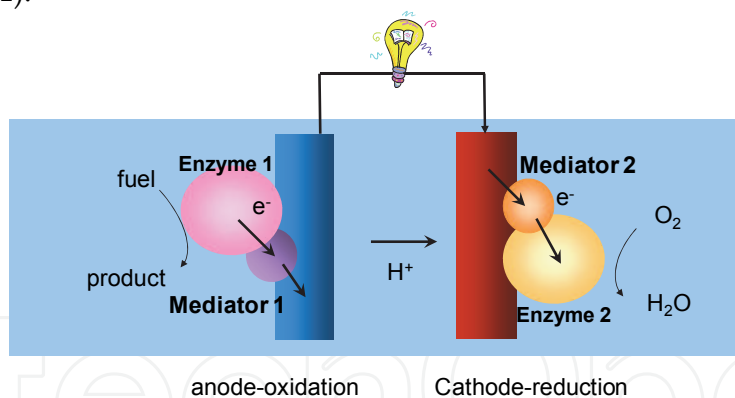


Fig. 1. Enzymatic biofuel cell principle.

Efficient connection is achieved by the use of appropriate redox mediators that are typically dyes or organometallic complexes, responsible for transferring the electrons from the enzymes to the electrode surface. The advantages of biocatalysts are reactant selectivity, activity in physiological conditions at room temperature, and manufacturability, compared to precious metal catalysts. Abundant organic raw materials such as sugars, low aliphatic alcohols, and organic acids can be used as substrates for the oxidation process, and mainly molecular dissolved oxygen acts as the substrate being reduced. The concept of biochemical fuel cell appeared in 1964 with the works of Yahiro and co-workers (Yahiro et al., 1964), which described the construction of a methanol/ O_2 cell. In the nineties, BFCs have come in to prominence with the recent advancements in novel electrode chemistries developed by Katz and Willner (Wilson, 2002), and Heller (Degani et al., 1987). The most studied biofuel

cell operates with glucose as fuel and oxygen as oxidant (Service, 2002). At the anode, glucose is oxidized to gluconolactone by the enzymes glucose oxidase (GOx) or glucose dehydrogenase, and at the cathode, dioxygen is reduced to water by the enzymes laccase or Bilirubin oxidase (BOD), which are multicopper oxidases.

Typical enzymatic fuel cells demonstrate powers in the range of microwatt to milliwatt. However, the tests are often performed under quite different conditions (concentration, temperature, pH, mass transport conditions, *etc.*), which complicates the comparison between different configurations in literature. Compared to conventional fuel cells, BFCs show relatively low power densities and short lifetime related to enzyme stability and electron transfer rate (Bullen et al., 2006). The improvement of the performance requires optimization of the components and solutions are described in literature in terms of catalysts, enzymatic electrode assemblies and design (Davis et al., 2007; Ivanov et al., 2010). Nowadays, the explosive growth of portable, wireless consumer electronics and biomedical devices has boosted the development of new micro power sources able to supply power over long periods of time. Miniature BFCs are considered as promising alternative (Gellett et al., 2010) to power supply in wireless sensor networks (WSN). However, the miniaturization of these devices imposes significant technical challenges based on fabrication techniques, cost, design of the device, and nature of the materials. These devices must provide similar performances to larger biofuel cells in terms of efficiency and power density while using less reagents, space and time consumption.

The number of miniaturized biofuel cells mentioned in literature is mainly restricted to a few devices. These devices include conventional devices that have been miniaturized, as well as micro-devices that use completely novel methods of energy conversion. Therefore, the present chapter presents the recent advances in the miniaturization of BFCs. Miniature conventional BFCs will be first presented but, here, we will focus the discussion on the development of microfluidic enzymatic BFCs, where microfluidics plays a direct and essential role. These micro-devices operate within the framework of a microfluidic chip. They exploit the laminar flow of fluids that limits the convective mixing of fuel and oxidant within a microchannel, eliminating the need for a membrane. As a result, the reaction kinetics can be optimized for both the cathode and anode independently by adjusting the composition of the fuel and oxidant stream. A discussion on the parameters affecting the performances of the microfluidic BFCs is proposed and directed towards interesting theoretical and experimental works. Finally, issues that need to be considered are presented to improve microfluidic device performances for desirable solution in the energy conversion process.

2. Miniature BFCs

In this section we briefly describe conventional BFCs that have been miniaturized. The number of miniaturized BFCs mentioned in literature is mainly restricted to a few devices working from glucose and O₂ that have mostly been designed by reducing the electrode size and cell volume. Different strategies have been used to miniature BFCs design.

Heller and co-workers have successfully demonstrated the efficiency of original miniature membraneless BFCs functioning under physiological conditions. They have developed the first handmade miniature device containing only two components, an anode and a cathode of 7- μ m diameter and 2-cm long carbon fibers, placed in a polycarbonate support. The anode was modified by GOx and the cathode was modified by either laccase or BOD, within

and mediated by redox osmium-based hydrogels (Mao et al., 2003). In 2001, they developed the first miniature membraneless BFC that delivered $140 \mu\text{W cm}^{-2}$ at 0.4V (Chen et al., 2001). This simple device suggested that the goal of a miniature autonomous sensor-transmitter system could be realistic (Bullen et al., 2006; Heller, 2004). After further developments based on the improved redox polymer connecting the reaction centers of enzymes to the electrodes, the devices delivered higher power densities of $431 \mu\text{W cm}^{-2}$ at 0.52 V (Mano et al., 2002) and $440 \mu\text{W cm}^{-2}$ at 0.52 V (Mano et al., 2003), in pH 7.2, 37 °C and 15 mM glucose. The high power density delivered by these devices comes from the cylindrical mass transport at the carbon fibers and the use of efficient redox polymers to transport electron. They also showed that this system produced a power density of $240 \mu\text{W cm}^{-2}$ at 0.52V when implanted in a living organism, near the skin of a grape. Later, by replacing carbon fibers by engineered porous microwires made of oriented carbon nanotubes, the most efficient glucose/ O_2 BFC ever designed was developed (Gao et al., 2010) and delivered high power density of $740 \mu\text{W cm}^{-2}$ at a cell voltage of 0.57 V. The success of the experiment probably results in the increase of the mass transfer of substrates.

Another miniature devices presently lower performance described stacked biofuel cell designs. One work described a stacking structure composed of six cells connected in series on a chip (Nishizawa et al., 2005), composed of GOx anode and polydimethylsiloxane-coated Pt cathode. The performance of the arrayed cells on the chip was $40 \mu\text{W cm}^{-2}$ in air-saturated buffer solution containing 5 mM glucose. Another work reported the development of a miniature BFC with a footprint of 1.4 cm^2 , by adopting the design of stackable proton exchange membrane (PEM) fuel cells (Fischback et al., 2006). This device consisted of an air-breathing cathode and an enzymatic anode composed of crosslinked GOx clusters on the surface of carbon nanotubes. This study demonstrated the important role of buffer solution in determining the performance and stability of miniature BFCs. In buffered fuel solution the initial performance was very high ($371 \mu\text{W cm}^{-2}$), but quickly dropped due to a deactivation of the proton exchange membrane. However, in unbuffered solution, the initial performance was lower ($117 \mu\text{W cm}^{-2}$) due to low pH condition, but its performance was very stable for 10 hours. This work suggested that the use of miniature system and unbuffered fuel solution will be a benefit to practical applications.

Currently, in such miniature devices, current density and delivered power output are mainly limited by the diffusion of fuel to the electrode surface. One interesting innovation to maximize the transport efficiency is to use hydrodynamic flow and to pump the fuel to the electrode.

3. Microfabricated devices

3.1 Advantages of microfluidics

An alternative approach towards the miniaturization of energy conversion devices is the use of microfabrication techniques. Microchemical systems have inherent advantages over macrosystems, including increased rates of mass transfer, low amount of reagents, increased safety as a result of smaller volumes, and coupling of multiple microreactors. Microfluidic techniques are ideal for miniaturization of devices featured with typical scale of channels of submillimeter in height and with laminar flow. Application of microfluidics to fuel cells has been developed rapidly since the years 2000 (Ferrigno et al., 2002; Choban et al., 2004). In such devices, all functions and components related to fluid delivery and removal, reactions sites and electrodes structures are confined to a microfluidic channel. In the channel, as

illustrated in Fig. 2, the flow of streams of fuel (colored pink) and oxidant (colored blue) is kept near-parallel, which ensures minimal diffusional mixing between the streams. The only way that molecules in opposite streams can mix is by molecular diffusion across the interface of the two fluid streams. The lack of convective mixing promotes laminar flow of fluids.

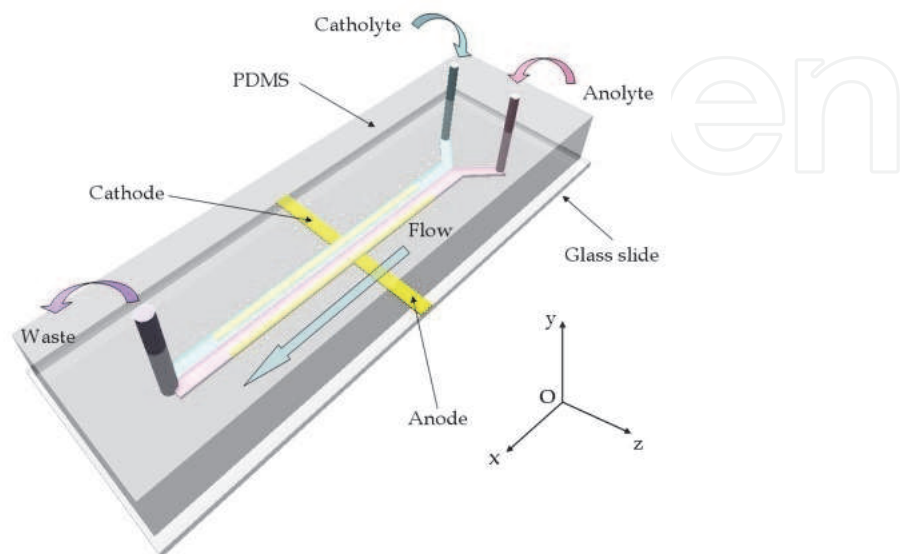


Fig. 2. Laminar flow of streams in a microfluidic channel.

The electrochemical reactions take place at the anode and cathode located within the respective streams, without needing a membrane to minimize the ohmic drop, what maximises the current density. Protons diffuse through the liquid-liquid interface created by the contacting streams of fuel and oxidant. The cathode and the anode are connected to an external circuit. The technique to force the fluid through microchannels is the pressure driven flow, in which the fluid is pumped through the device *via* positive displacement pumps, such as syringe pumps.

As summarized by authors (Luo et al., 2005; Gervais et al., 2006; Sun et al., 2007), the limiting factors in laminar flow-based microfluidic fuel cells that influence the performances are (i) cross-diffusional mixing of fuel and oxidant at the interface between the two streams, and (ii) the formation of depletion boundary layers at the surface of the electrodes as the result of the reaction of fuel and oxidant. Interesting papers have presented theoretical and experimental works to describe how to prevent or reduce these phenomena by concentrating research efforts on designs, electronic and ionic conductivity, and electron-transfer kinetics in microfluidic fuel cells (Lee et al., 2007). The role of flow rate, microchannel geometry, and location of electrodes within microfluidic systems was also studied (Choban et al., 2005; Sun et al., 2007; Amatore et al., 2007; Chen et al. 2007).

Similarly to microfluidic fuel cells, advanced microfabrication techniques can be applied to build components of microfluidic enzymatic BFCs. The number of devices presented to date is limited. The devices have been developed based on both laminar flow within a microchannel and biological enzyme strategies. Indeed, the advantage of the co-laminar flow is to choose the composition of the two oxidant and fuel streams independently for optimum enzymatic activity and stability to improve reaction rates and current density (Zebda et al., 2009a).

3.2 Microfluidic biofuel cell fundamentals

In a microfluidic channel, the relationship between the fluid velocity and the absolute pressure for an incompressible viscous liquid is given by the classical fluid dynamics theory and the well-known Navier-Stokes equation:

$$\frac{\partial \vec{v}}{\partial t} + (\vec{v} \cdot \vec{\nabla}) \vec{v} = -\vec{\nabla} \left(\frac{P}{\rho} \right) + \mu \Delta \vec{v} \quad (1)$$

Where \vec{v} stands for the fluid velocity vector with components (u, v, w) , each expressed for a set of Euler components (x, y, z, t) , P is the absolute pressure, ρ is the relative density, and μ is the kinematic viscosity.

In the case of a microfluidic horizontal straight channel (x -direction), the flow is always laminar under low pressure drop (typically a few bar), leading thus to a unidirectional flow and a uniform absolute pressure in the cross-section. For a fixed pressure drop ΔP between the inlet and the outlet of the channel, Eq. 1 simplifies to:

$$\frac{\partial u}{\partial t} = -\frac{1}{\rho} \frac{\Delta P}{L} + \mu \left(\frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} \right) \quad (2)$$

Where L is the length of the microchannel. When the permanent flow is reached, the time derivative term becomes zero and Eq. 2 simplifies to:

$$\frac{-\Delta P}{\eta L} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} = 0 \quad (3)$$

Where η is the dynamic viscosity (10^{-3} Pa.s for water at 20°C), defined as the product of the dynamic viscosity and the relative density ρ . Due to the very large aspect ratio of the rectangular cross-section of the microchannel, a 2D approach is usually considered that leads to a pseudo infinite-plate flow (except in the borders). The directions along the length and height of the microchannel are indicated as x and y coordinates, respectively (see fig. 2). A typical parabolic rate profile is obtained for pressure driven flow:

$$u(y) = \frac{\Delta P}{2 \cdot \eta \cdot L} \left(\frac{h^2}{4} - y^2 \right) \quad (4)$$

Where h is the height of the microchannel and y is defined as $y=0$ at the middle of the microchannel and $y= \pm h/2$ at the upper and under walls. By considering a rectangular microchannel (with l the width of the microchannel) in Eq. 4, the flow rate, Q , in laminar regime, is deduced and is proportional to the applied pressure (Eq. 5):

$$Q = \frac{\Delta P \cdot l \cdot h^3}{12 \cdot \eta \cdot L} \quad (5)$$

In electrochemical laminar flow systems, the mass transport is achieved by both diffusion and convection transport. In the case of Y-shaped microchannel, the mixing between the two laminar streams occurs by transverse diffusion. Microscale devices are generally characterized by high Péclet number, Pe , ($Pe = U_{av} h / D$, with U_{av} the average velocity of the flow, h the height of the microchannel and D the diffusion coefficient of the molecule). In

this condition, the transverse diffusion is much lower than the convection, and the diffusive mixing of the co-laminar streams is restricted to a thin interfacial width, δ_{mix} , in the center of the channel (Fig. 3) that grows as a function of the downstream position (x) and the flow rate, determined from Eq. 6 (Ismagilov et al., 2000):

$$\delta_{mix} = \left(\frac{(Dhx)}{U_{av}} \right)^{\frac{1}{3}} \quad (6)$$

Where D is the diffusion coefficient for ions of type i and U_{av} is the average flow velocity defined as:

$$U_{av} = \frac{\Delta P \cdot h^2}{12 \cdot \eta \cdot L}$$

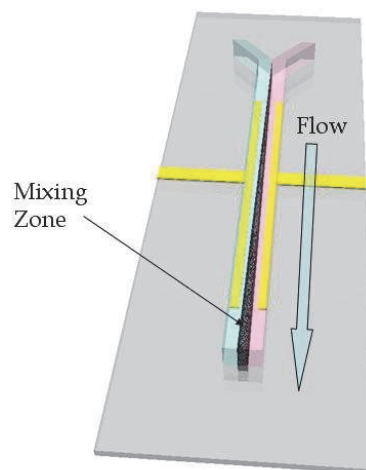


Fig. 3. Schematic of a laminar flow in a microchannel with the formation of the diffusion region during operation of a microfluidic BFC.

For fast electron transfer and in excess of supporting electrolyte, the kinetics of a simple electrochemical redox reaction is controlled by diffusion and convection. The concentration profiles of the chemical species involved in the reaction are determined by solving the convective diffusion equation (Eq. 8):

$$\frac{\partial c_i}{\partial t} + \vec{\nabla} \cdot (-D_i \vec{\nabla} c_i) + \vec{v} \vec{\nabla} c_i + R_i = 0 \quad (8)$$

Where c_i is the concentration of species i , D_i its diffusion coefficient, t the time, \vec{v} the fluid velocity vector (given by Eq. 1) and R_i a term describing the rate of net generation or consumption of species i formed by homogeneous chemical reaction.

In the case of a microfluidic biofuel cell as described in this work, Eq. 8 can be simplified into a 2-dimensionnal cartesian steady state (Eq. 9):

$$-D_i \left(\frac{\partial^2 c_i}{\partial x^2} + \frac{\partial^2 c_i}{\partial y^2} \right) + u(y) \frac{\partial c_i}{\partial x} = 0 \quad (9)$$

The boundary conditions associated are usually: (i) $c_i = c_i^\circ$ (bulk concentration) at the inlet of the microchannel, (ii) $c_i = 0$ at the electrode surface and (iii) no flux at the other walls (no electrochemical reaction).

Those simulations were exploited in order to calculate the diffusive flux at the electrode, defined as:

$$J_{diff}(x) = D \left. \frac{\partial C}{\partial y} \right|_{electrode} \quad (10)$$

And, therefore, the total current is expressed as:

$$I_{tot} = \int_{electrode} n.F.J_{diff}(x)dx = n.F.D. \int_{electrode} \left(\left. \frac{\partial C}{\partial y} \right|_{y=0} \right) dx \quad (11)$$

Where n is the number of electron exchanged, and F is the Faraday constant.

The pumping power $W_{pumping}$, required to sustain steady laminar flow in the microchannel by the syringe pump, is estimated on laminar flow theory (Bazylak et al., 2005) as the pressure drop multiplied by the flow rate:

$$W_{pumping} = \frac{8.\eta.L.Q^2}{l.h^3} \quad (12)$$

One can note that the contributions from inlet and outlet feed tubes are not included, because they are negligible.

The fuel utilization (FU) is estimated by the following Eq. 13, defined as the current output divided by the flux of reactant entering the channel (Bazylak et al., 2005):

$$FU = \frac{I}{n.F.C.Q} \quad (13)$$

The fuel utilization is maximized for the lowest flow rate and decreases with flow rate. Typical fuel utilization for microfluidic fuel cell is $\sim 1\%$ (Hayes et al., 2008).

3.3 Manufacturing technology

3.3.1 Fabrication of the microchannel

Microchannel architecture typically represents a T- or Y-channel configuration. There are mainly two fabrication techniques. The first one utilizes conventional chip-manufacturing techniques of semiconductor industries. Silicon wafers are patterned by lithography step followed by etched step in order to get the desired form of the channel (Moore et al., 2005; Lee et al., 2007). The second technique allows the fabrication of microchannels by rapid prototyping using standard soft lithography procedure to build the channel in poly(dimethylsiloxane) (PDMS) (Duffy et al., 1998). PDMS is relatively inert and compatible with most solvents and electrolytes (Kjeang et al., 2008). Besides it is permeable to gases, which is essential for biofuel cells working from enzymes with oxygen as the cofactor. Typically, a pretreated microscope glass slide or a silicon wafer is coated with a thin layer of photoresist by spin-coating and exposed directly to UV light through a photomask that defines the desired channel structure. Several thick photoresist layers are sequentially laminated on the first layer

to get the desired channel depth, and then exposed to UV light. The structure is then developed by spraying an aqueous solution of sodium carbonate (1% wt) and hardened by a final irradiation. The result is a master with a positive pattern defined by the master. The channel structure is thus obtained by pouring PDMS monomer over the master, followed by curing at 70 °C during 2 h. After cooling, the PDMS slab is peeled off from the master, and holes are punched to provide fluid access (Stephan et al., 2007).

3.3.2 Fabrication of the microelectrodes

Most of the microfluidic devices employ patterned electrodes positioned in parallel on the bottom wall or on sides of the channel (Kjeang et al., 2008). Electrodes, with varying length and wide, are patterned by coating glass slides with conductive materials such as gold, graphite over an adhesive layer (often chromium or titanium) by standard sputtering techniques (Zebda et al., 2010) or by photolithography and sputtering (Moore et al., 2005; Lim et al., 2007; Togo et al., 2008). The inter-electrode gap varies between 0.2 mm and 1.4 mm (Lee et al., 2007; Togo et al., 2008; Zebda et al., 2010).

3.3.3 Fabrication of the microfluidic devices

The microfluidic device is finally obtained by physically clamping the PDMS slab with the glass substrate that accommodated the electrode pattern. This approach works well with elastomer polymer like PDMS. Alternatively, an irreversible seal may be achieved between both parts by oxygen-plasma treating prior to improve the adhesion (Lim et al., 2007; Lee et al., 2007). Alignment of the flow channel over the microelectrodes is often aided by a microscope. As an example, a device, consisted of a Y-shaped channel with two inlets and two outlets, is presented in Fig. 4. The pressure-driven laminar flow required for injection of fuel and oxidant is typically driven by a syringe pump via polyethylene tubing.

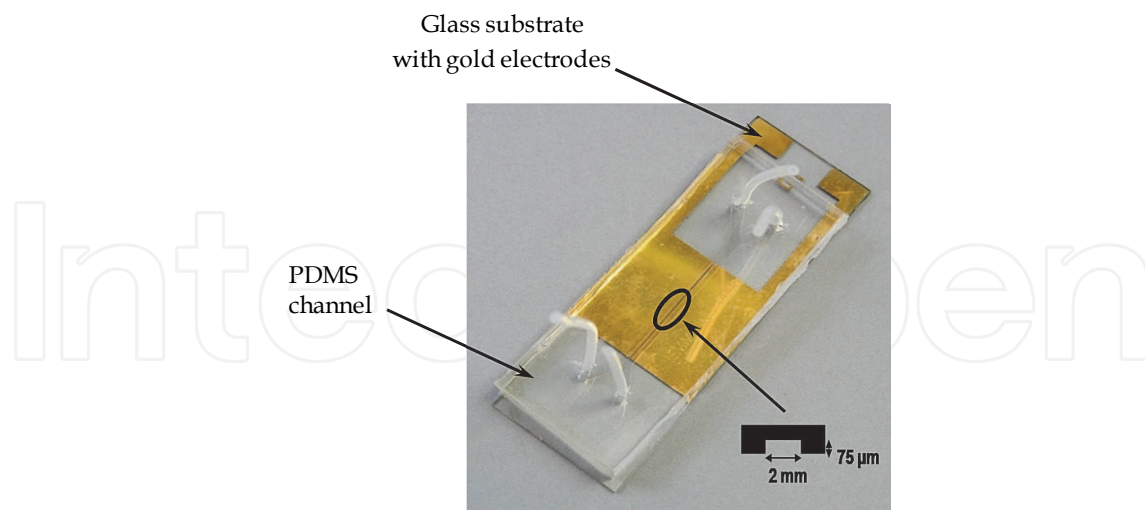


Fig. 4. Schematic microfluidic BFC based on a Y-shaped channel with two inlets and two outlets.

3.4 Performances of microfluidic biofuel cells

This paragraph mainly describes microfluidic BFCs involving mediated monoenzymatic systems, which are capable of only partial oxidation of the fuel. Devices have been

developed either with diffusional enzymes flowing through the microchannel, or with immobilized enzymes on electrode surface. This paragraph also includes preliminary works on devices allowing improvement of fuel utilization by complete oxidation, which have been designed with multienzymatic systems.

The performances of microfluidic BFCs are evaluated from cell voltage and current density. The cell voltage of the biofuel cell reflects both the open circuit voltage (OCV), partially controlled by the formal potential of the two redox mediators and the overpotential losses. The delivered current density reflects the rate of catalytic turnover and transport processes as a function of the surface area of the electrode. The power density is the product of cell voltage and cell current density. As already mentioned, the performances of the microfluidic BFCs are limited (i) by cross-diffusional mixing (δ_{mix}) of fuel and oxidant at the interface between the two streams, (ii) by formation of depletion boundary layers at the surface of the electrodes as the result of the reaction of fuel and oxidant, and (iii) by low concentration and low diffusion coefficient of oxygen. These factors depends on geometric and process parameters such as the microchannel dimensions, the electrode parameters (number of electrodes, electrode surface area, electrode spacing), and operating conditions (electrolyte, flow rate, pH, concentration of species). The influence of these parameters on open circuit voltage, current density and power density, have been evaluated both experimentally and theoretically in literature.

3.4.1 Microfluidic BFCs with soluble enzymes

3.4.1.1 Strategies to limit the cross-diffusional mixing

In order to restrict fuel and oxidant mixing to a thin interfacial width δ_{mix} sufficiently far from the electrodes (see Fig. 3), the flow rate should be increased to an optimal value to provide little to no fuel crossover, while yielding high reactants consumption (Lee et al., 2007), and besides, the electrodes must have sufficient separation distance within the microchannel (Kjeang et al., 2009). Generally, to confirm that the diffusive crossover doesn't contribute to the loss of current, the width of the mixed region δ_{mix} is calculated using Eq. 6. (Zebda et al., 2009b). Another strategy to prevent the direct contact and the reaction between oxidant and fuel was proposed in the case of a microfluidic fuel cell working from formic acid as fuel (Sun et al., 2007). A three-stream laminar flow fuel cell was developed that consisted to introduce a third stream containing only electrolyte solution between fuel and oxidant streams.

3.4.1.2 Strategies to reduce the depletion layer effect

Rapid transport of reactants to the electrodes is essential to provide high power densities. When a heterogeneous reaction occurs at electrode surface, depletion of the reactant results in formation of a depletion zone near the electrodes surface where lower conversion rates occur as the reactant concentration is lower than in the bulk region. The thickness of the depletion layer increases usually in the direction of the convective flow (Fig. 5), thus resulting in the decrease of the current density along the electrode length.

Concentration profiles of reactive species are usually described computationally by resolving the convection-diffusion Eq. 9 and by setting appropriate boundary conditions. Modelling results in targeting optimal electrodes configuration in microchannel, fuel utilization and flow rate. Fig. 6 describes the 2D profile concentration during the operation of a glucose/O₂ biofuel cell based on Y-shaped microfluidic channel of height, h , and with

electrodes length L . As observed, the concentration of the active species decreases near the gold electrode surface that generates a depletion zone gradually increasing. The thickness of the depletion zone is a function of the distance from the inlet edge of the microchannel, and decreases with high flow rates. According to the Fick's law (Eq. 11), it results that the simulated current density decreases sharply because of the concomitant increase of the depletion zone.

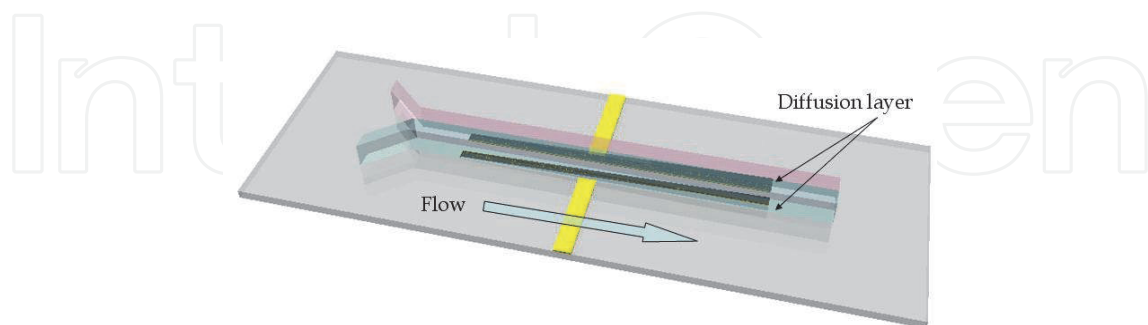


Fig. 5. Schematic of the formation of the depletion zone near the electrodes surface.

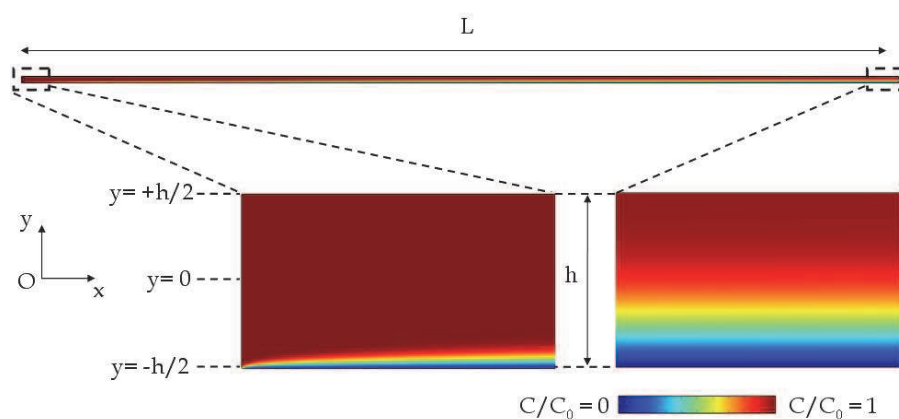


Fig. 6. 2D profile of ABTS concentration during the operation of a glucose/ O_2 biofuel cell based on Y-shaped microfluidic channel ($Q=100 \mu\text{L min}^{-1}$).

Reduction of the thickness of the depletion boundary layer is necessary to increase mass transport since the diffusion distance will be shorter and the diffusional flux will be higher. Several approaches have been developed to control the transport rate of reactants towards the electrode surface and thus the current density. It was demonstrated experimentally that the adjustment of flow rates controls the electrochemical processes that take place at the electrodes and regulate the depletion layer thickness. As observed in Fig. 7, the delivered current densities are influenced by flow rate of streams containing GOx for the anolyte and laccase for the catholyte during the operation of a glucose/ O_2 biofuel cell based on Y-shaped microfluidic channel.

The shape of the voltage-current density curves indicates that the current density is maximal when the voltage is almost zero, due to the consumption of all the fuel instantaneously at the electrode. Maximal current densities increase with flow rate from ~ 0.4 to 0.7 mA cm^{-2} as the impact of mass transport limitations is reduced from the bulk solution to the electrode surface. The maximum current density is thus limited by the diffusion of fuel and oxygen to their respective electrodes. The performance of the biofuel cell was evaluated at the operating flow rate of $1000 \mu\text{L min}^{-1}$. With an oxidant stream under oxygen at pH 3 and a

fuel stream under nitrogen gas at pH 7, the maximum power density delivered by the biofuel cell is $110 \mu\text{W cm}^{-2}$ at 0.3 V (Fig. 7). Moreover, the pumping power to sustain the necessary flow in the microchannel was evaluated, according to Eq. 13, and compared with the delivered power by the cell. By varying the flow rate, it was found that the ratio of the input power to the output power increased from 1.5 % at $100 \mu\text{L min}^{-1}$ to 76 % at $1000 \mu\text{L min}^{-1}$. This experiment pointed out the importance of the flow rate on the power output delivered by the microfluidic BFC.

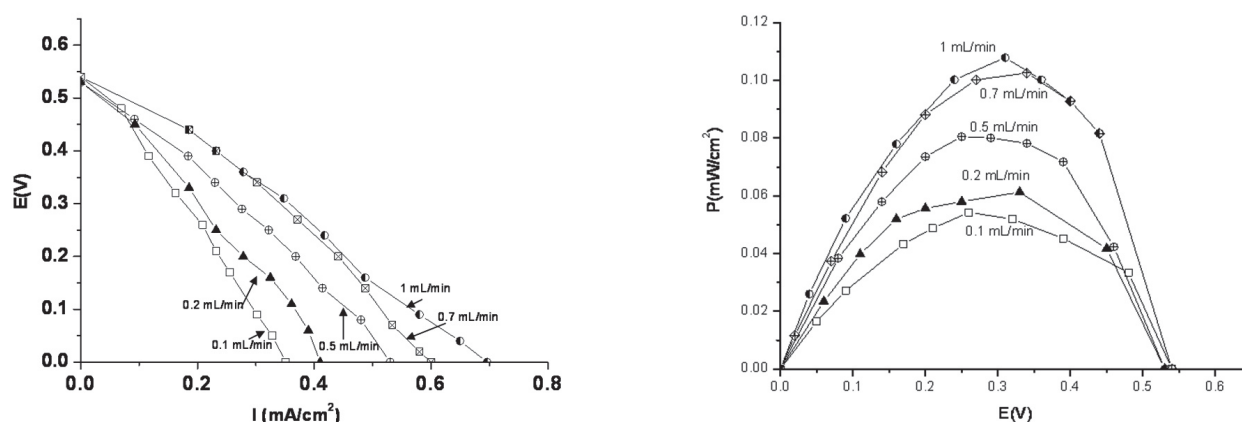


Fig. 7. Voltage-current density and power density-voltage plots generated from a microfluidic glucose/ O_2 biofuel cell at different flow rates. At the anode, glucose is oxidized by GOx in the presence of the redox mediator hexacyanoferrate $\text{Fe}(\text{CN})_6^{3-}$, whereas at the cathode, oxygen is reduced by the laccase in the presence of the redox mediator 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) ABTS.

Another way to reduce depletion layer limitation and to enhance the transport rate of reactants towards the electrode surface lies in optimization of electrode geometry. Detailed experiments and simulations have revealed that current density decreases with increasing length of electrodes in the direction of the convective flow (Lim et al., 2007). To promote uniform current density across the entire electrode assembly, Palmore and co-workers have demonstrated that splitting electrodes into smaller units separated by a gap in a microfluidic cell decreased the diffusion layer and improved the delivered power density by about 25% (Lee et al., 2007). In this work, the microfluidic fuel cell was built from a biocathode operating with laccase and ABTS as mediator to perform oxygen reduction, and from an anode operating with ABTS under N_2 . When operated at pH 4, this microfluidic cell exhibited a maximum power density of $26 \mu\text{W cm}^{-2}$ at the open-circuit voltage 0.4 V with a flow rate of $100 \mu\text{L min}^{-1}$. However, since the geometrical surface area required for the gaps did not contribute to any net current, the overall current density was not improved.

The depletion layer can be also manipulated to overcome mass transfer limitations by the development of original microfluidic configurations. This strategy was pointed out by simulation and experiments in the case of a microfluidic fuel cell working from formic acid (Yoon et al., 2006). These configurations featured, along the electrodes, either multiple periodically located outlets to remove consumed species or multiple periodically located inlets to add fresh reactants in the microfluidic channel. Such devices require controlling the volumetric flow rate through each segment of the fluidic network. For both configurations, mass transfer was enhanced and reactant conversion at the electrodes was increased from 10 to 100 %.

3.4.1.3 Strategies to overcome oxygen limitation

A few studies report the analysis of oxygen limitation. However, the use of dissolved oxygen in enzymatic fuel cells is one of the main limitations of these systems due to low concentration (~ 0.2 mM) and low diffusion coefficient of oxygen ($1.97 \cdot 10^{-5}$ cm² s⁻¹ at 20 °C) (Barton, 2005). Usually, an exponential decay in the availability of oxygen at the cathode is observed along the length of microchannel (Bedekar et al., 2008). As a result, the oxygen flux is very low, limiting the generation of current. In the middle portion of the channel, the oxygen remains unconsumed and can still diffuse to the electrode. However, due to pressure-driven convective flow, the oxygen is still not consumed. To increase the availability of oxygen at the cathode surface, one strategy consists of designing a branched-microchannel configuration with several electrodes that allows periodically full contact of the electrolyte with the electrodes (Bedekar et al., 2008).

Another promising approach developed until now and only for fuel cells (methanol or acid formic/O₂), is to incorporate cathodes that access the surrounding air with higher diffusivity and O₂ concentration. Porous gas diffusion electrodes allow gaseous reactants to pass. Devices were developed either with flow-through porous electrodes able to increase delivered power densities with near complete fuel utilization (Kjeang et al., 2008), or with an air-breathing porous cathode structure (Jayashree et al., 2005). In the latter case, the use of air breathing cathode showed that the rate of oxygen reduction is enhanced with corresponding increase in current densities. However this approach requires precise control of pore size of the electrode to maintain constant rate of air delivery to the cathode.

3.4.2 Microfluidic BFCs with immobilized enzymes

A few works to date present enzyme-based microfluidic BFCs with immobilized enzymes. Enzymes in solution are only stable for a few days, it is why immobilization of active enzymes is of interest in order to improve lifetime (Bullen et al., 2006) and to develop integrated microfluidic BFC designs. Besides, full selectivity of both enzymatic half-cells allows microfluidic BFC operation in a single, combined fuel and oxidant channel with mixed reactants at constant concentrations favourable for stability studies. Additionally, the close proximity of the enzymes with the electrodes reduces ohmic losses because electrons are harvest from reaction sites with lower electrical resistance.

In microfluidic BFCs described in literature, enzymes with their respective redox mediators are immobilized on electrodes surface by encapsulation in polymer film. Currently, Nafion® is commonly used as it possesses surfactant properties interesting to immobilize enzymes in micellar structures when treated by quaternary ammonium salt (Moore et al., 2004). Such matrix provides an optimal enzyme environment where the enzyme retains its activity for greater than 90 days. Based on this strategy, a microchip-based bioanode with alcohol dehydrogenase enzymes immobilized in treated Nafion® associated with an external platinum cathode delivered a power density of 5 μW cm⁻² (Moore et al., 2005). The low value was attributed to the thick coating of polymer, casting by hydrodynamic flow and thus difficult to control in the microchannels.

Another polymer to entrap enzymes is poly-L-lysine. This polymer mixed with an enzyme solution can stand on electrode surface after drying in air. According to this technique, a microfluidic glucose/O₂ BFC was developed (Togo et al., 2007; Togo et al., 2008). The originality of this study concerned the location of the electrodes in a single flow channel. This device has been developed to generate electric power from glucose oxidation with an anode coated by immobilized glucose deshydrogenase and a bilirubin oxidase-adsorbed O₂

cathode (Togo et al., 2008). The device was featured with a specific electrode-arrangement within a microchannel to prevent dissolved O_2 to react at the anode as an interfering substance. A large biocathode (10 times the anode size) was placed strategically upstream of a bioanode to pre-electrolyse O_2 to protect the anode vicinity from interfering oxygen. The maximum cell current was increased by 10% with this cell configuration at pH 7. The experimental results showed the influence of the channel height that should be in the same order of the depletion layer thickness for optimal operating of the device. However, in such design, the composition of fuel and oxidant could not be adjusted independently for optimum enzymatic activity and stability.

In order to choose independently the composition of the two streams, the configuration based on Y-microfluidic single channel is required. This approach has been developed for a microfluidic glucose biofuel cell working from GOx and laccase immobilised on gold electrodes in a poly-L-lysine matrix. The immobilization process was realized by mixing the enzymes and their respective redox mediators in poly-L-lysine solution. After drying, the device was tested with a phosphate buffer pH 7 solution with 50 mM glucose for the anolyte, and with a citrate buffer pH 3 solution saturated with oxygen for the catholyte. Different redox mediators were tested for efficient electron transfer at the anode. Among the mediators hexacyanoferrate ($Fe(CN)_6^{3-}$), ferrocene and 8-hydroxyquinoline-5-sulfonic acid hydrate (HQS), the higher power density ($60 \mu W cm^{-2}$) was obtained at a cell voltage 0.25 V with $Fe(CN)_6^{3-}$ (Fig. 8).

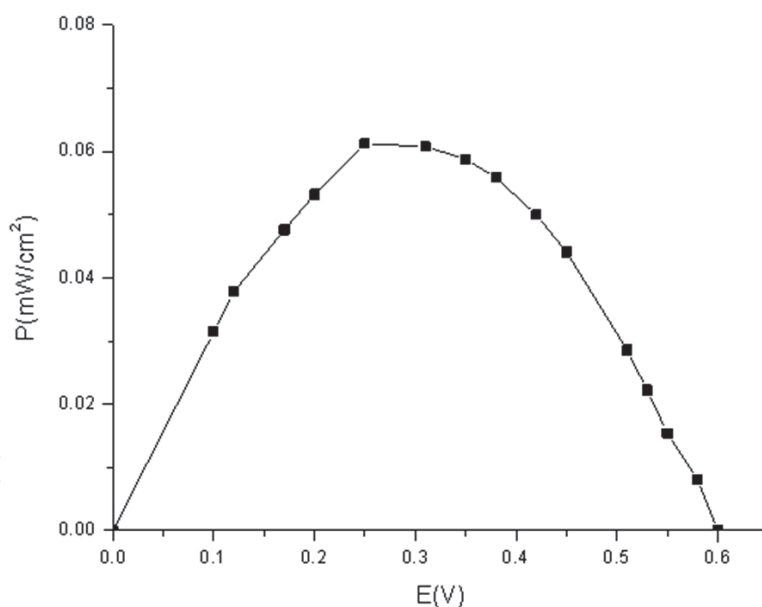


Fig. 8. Experimental power density *vs* voltage plot generated from a microfluidic glucose/ O_2 biofuel cell at 23 °C and under flow rate $300 \mu L min^{-1}$. At the cathode, the couple laccase/ABTS and at the anode the couple GOx/ $Fe(CN)_6^{3-}$ are immobilized in a poly-L-lysine film.

3.4.3 Microfluidic BFCs with immobilized multi-enzyme systems

In general there are numerous combinations of enzymes and mediators that have been employed in biofuel cells but the respective studies typically involve monoenzymatic systems, which are capable of only partial oxidation of the fuel. Improvement of fuel

utilization can be achieved by complete oxidation, which can be realized by introduction of enzyme cascades to increase the overall efficiency of the fuel cell (Nick et al., 2005; Sokic-Lazic et al., 2010; Addo et al., 2010). By this way, the overall performance of the BFC is increased as well.

In a theoretical work described by Kjeang and co-workers, a concept of an enzymatic fuel cell is proved, without presenting experimental system (Kjeang et al., 2006). This work describes how to optimize the structure of a microfluidic enzymatic fuel cell, involving three-step-catalyzed methanol oxidation (Fig. 9). Different enzyme patterning strategies are tested, *e.g.*, spatially distributed- or evenly-mixed enzymes along the electrode surface. The model predicts high fuel utilization at low flow rates, *i.e.*, in the diffusion dominated and mixed mass, transfer conditions. According to the model, the investigated theoretical concept is reaction limited, which means that the system performance can be improved by improving enzyme turnover numbers. This work also demonstrates that the power required for pumping of the fuel is negligible in comparison to predicted power of the fuel cell.

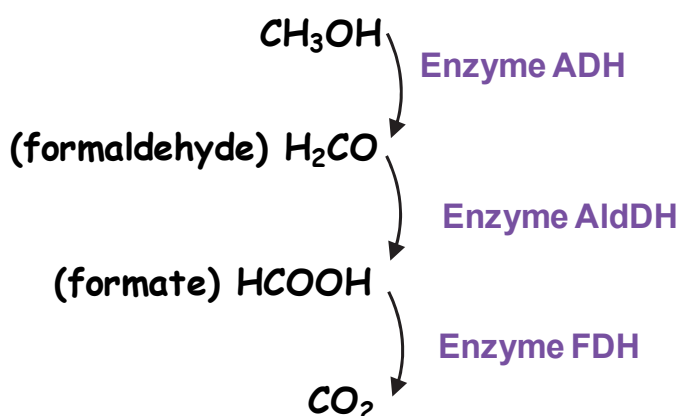


Fig. 9. Schematic of the three-step-catalyzed methanol oxidation by the enzymes alcohol dehydrogenase (ADH), aldehyde dehydrogenase (AldDH) and formate dehydrogenase (FDH).

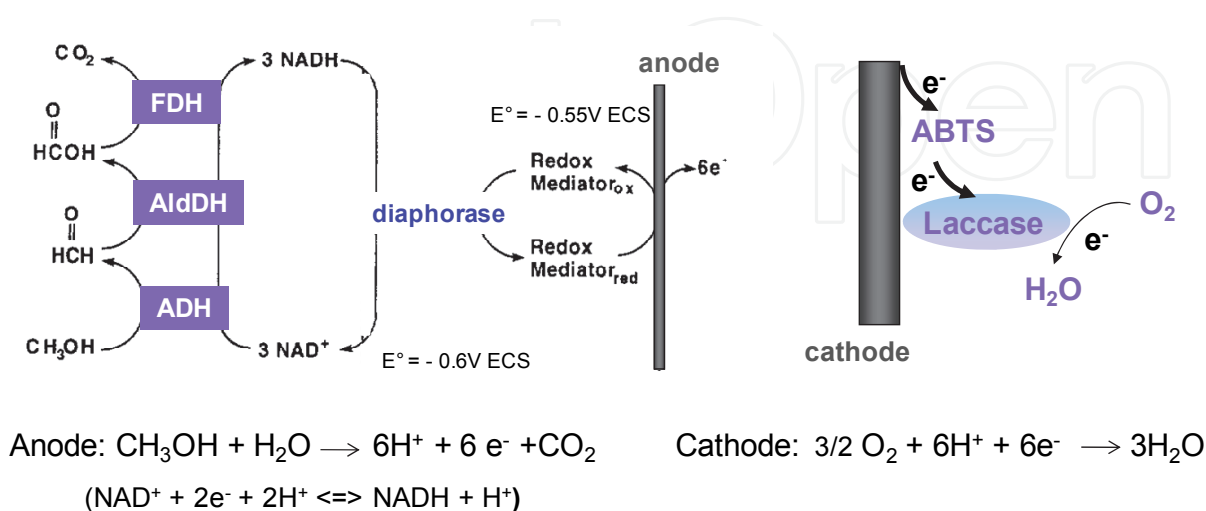


Fig. 10. Electron transfer steps at the biocathode and the bioanode.

Based on this work, we built a methanol/ O_2 microfluidic BFC able to completely oxidize methanol, according to the electron steps described for each electrode in Fig. 10. The enzymes and the mediators were immobilized in poly-L-lysine.

These enzymes are NADH-dependant. The electrochemical connection and regeneration of NADH at the anode is achieved using the enzyme diaphorase and the redox mediator benzylviologen as already described (Palmore et al., 1998). At the anode, a mixture of the three dehydrogenase enzymes was deposited along a gold electrode surface according two configurations (Fig. 11). When enzymes are mixed along the anode, optimal power density is achieved as observed in Fig. 12. The Y-channel device delivers a power density of $70 \mu W cm^{-2}$ at a cell voltage 0.25 V.

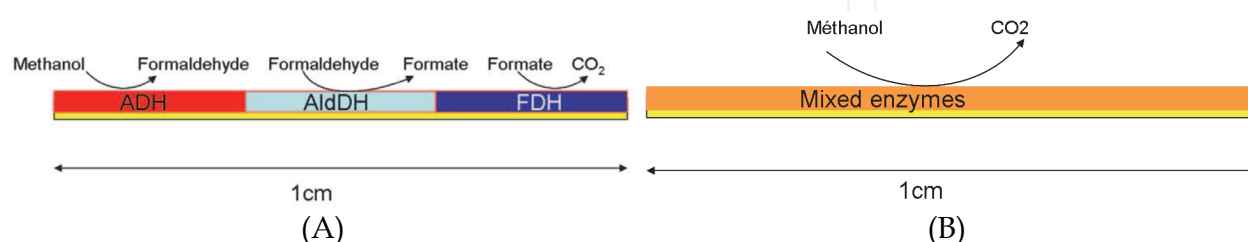


Fig. 11. (A) enzymes are distributed along the anode surface in three distinct, equally sized zones of 10 mm in length, (B) enzymes are mixed randomly along the anode surface of 10 mm in length.

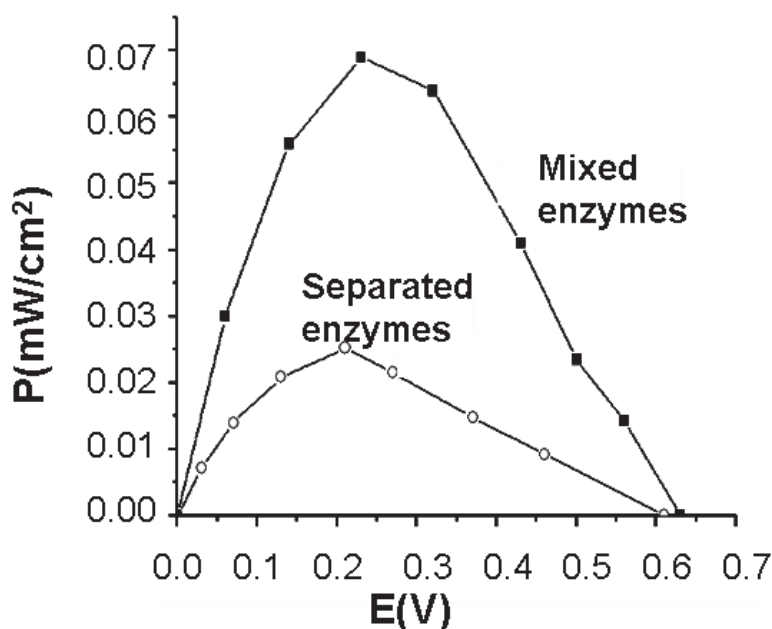


Fig. 12. Power density-voltage plots generated from a microfluidic methanol/ O_2 biofuel cell for 2 cases: separated and mixed enzyme patterns at the anode (1:1:1 molecular basis).

4. Conclusion and perspectives

Microfluidic BFCs could be an effective solution for small power sources applications such as biological sensors, implantable medical devices or portable electronics. However significant research efforts must be made for practical applications. Researches must be

aimed at identifying most robust and active enzymes, more efficient immobilization environment for enzymes and mediators in microfluidic environment, and at increasing enzyme lifetimes.

To deliver higher power densities, the challenges are in the area of energy density and fuel utilization. Microfluidic BFCs designs should integrate advanced immobilization configurations to improve enzyme performance and high surface area electrodes to enhance rates of convective-diffusive reactant transport. More nanofluidic research and development will be needed to demonstrate the real potential of this form of energy conversion system. Besides, the power output of single microfluidic BFC is inadequate for most practical applications. The enlargement of a single cell by increasing the geometrical electrodes area and the microchannel is constrained by fuel/oxidant crossover and higher ohmic losses. In order to produce adequate power, multiple independent cells could be stacked as in typical fuel cells.

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51000 Rijeka, Croatia
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Phone: +86-21-62489820
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