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Performances of Enzymatic Glucose/O₂ Biofuel Cells

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1. Introduction

Nowadays, the development of stable devices capable of converting chemical energy into electrical one both to supply implantable devices and microelectronic apparatus is related in numerous papers (Bullen et al., 2006; Davis & Higson, 2007; Minteer et al., 2007). It is actually of great interest since it could be at the origin of new insight concerning the treatment of illness such as diabetes, deafness or heart disease (Heller, 2004; Katz & Willner, 2003). Besides it could also be a cheap solution to provide energy for microelectromechanical systems (Calabrese Barton et al., 2004) or to treat wastewater (Fishilevich et al., 2009). In the case where at least one of the catalyst used comes from biological resources (enzyme, microorganisms), these devices are called “biofuel cells”. As any fuel cell, a biofuel cell consists of two separated, or not, electrodes, an anode and a cathode. The research topic concerning biofuel cells is very vast and can be divided in three subsections. The first one includes microbial fuel cells which are bio-electrochemical systems that drive a current intensity by mimicking bacterial interactions found in nature. The second one deals with enzymatic biofuel cells which use enzymes as catalysts. In this kind of device the specificity of enzymes leads to the non-separation of each compartment of the cell, allowing to minimize the size of the system (Heller, 2004). The last kind of biofuel cells deals with hybrid biofuel cells that result in the combination of an enzymatic catalyst with an abiotic one. In these applications, it is possible to vary the operating conditions of the cell (pH value, concentration of reactants) so as to increase the power density. This chapter will only be focused on the second and third kind of biofuel cells. For the latter devices, the ideal fuel is obviously glucose which is present in all organic tissues and the oxidant is dioxygen. Nowadays there is a need for improvement in what concerns both the lifetime (in the range of a few days) (Calabrese Barton et al., 2004) and the power density (classically lower than 1 mW cm⁻²) (Neto et al., 2010) of these apparatus. For this reason we describe herein both phenomena affecting stability and power density of biofuel cells and proposed solutions in terms of electrode assembly, catalysts used and design of the cells. Moreover, since the major way to increase enzymatic electrodes lifetime and efficiency is to improve the enzyme connection with the electrode surface, we will have a special look on the different immobilization techniques presently reported in literature. Besides, in the past few years it has noticeably been demonstrated that abiotic catalysts obviously increased the stability of the device (Kerzenmacher et al., 2008) and involved fast substrate conversion kinetic characteristics (Choi et al., 2009). Consequently, we will have a particular glance on new abiotic nanocatalysts and their use in hybrid biofuel cells.
2. Enzymatic electrodes for glucose/O\textsubscript{2} biofuel cells

Except the lack of stability of enzyme molecules due to their proteic nature, one of the major problems encountered with enzymatic electrodes concerns electron transfer between the enzyme and the electrode surface. In the next part we will describe the different electron transfer mechanisms occurring between an enzyme and the electrode as well as the immobilization techniques of the protein.

2.1 Electron transfer between enzyme and electrode

Enzymes are proteins which have high molecular weights. The active sites of these molecules are located in the organic matrix at a depth of several angstroms from the surface. It is thus easy to understand that kinetically fast electron transfer between enzymes and electrodes surface is difficult to obtain because of great insulation of the active centers (Armstrong et al., 1985). Different strategies have been used by the past to make efficient electrical connections between the enzyme and the electrode surface. Corresponding electron transfer mechanisms can be arranged in two different classes: mediated electron transfer (MET) and direct electron transfer (DET).

The major interest in directly transferring electrons between enzymes and electrodes is to reduce the electrode overpotential which is of particular importance for biofuel cells applications. DET is possible as soon as the distance between the active center of the enzyme and the electrode surface is in the order of a tunneling one (Degani & Heller, 1987). Different evidences for DET between enzymes classically used in glucose/O\textsubscript{2} biofuel cells and electrodes have already been given. Actually, laccase (Gupta et al., 2004), bilirubin oxidase (Shleev et al., 2005) and glucose oxidase (Wang et al., 2009) are capable of exhibiting non-negligible catalytic current densities without the presence of a redox mediator.

In the case of MET, a redox molecule acts as a substrate and is able to transfer electrons between the electrode surface and the active center of the enzymatic molecule. Let’s notice that current densities obtained with MET are generally higher that what can be delivered in the case of DET. However, to get efficient MET, the redox mediator must possess some properties which can be deduced from Marcus theory as it was already mentioned by Rusling et al. (Rusling et al., 2008). This theory is used to describe outer sphere electron transfer between an electron donor (D) and an electron acceptor (A) as depicted in Fig. 1.

![Fig. 1. Curves presenting potential energy of reactants (R) and products (P) (ΔG\textsuperscript{0}) as a function of reaction coordinates (RC).](www.intechopen.com)
The rate \( k \) of electron transfer can be described as follows (Eq.1.) by an Arrhenius type law.

\[
k = \Lambda Ke^{-\frac{\Delta G^0}{RT}}
\]

where \( \Lambda \) is the collision frequency, \( K \) is the electronic transmission factor, \( \Delta G^0 \) is the Gibbs free energy, \( R \) is the gas constant and \( T \) the temperature. \( \lambda \) is the reorganization energy (energetic cost associated to the reorganization of both solvent and molecules and necessary to proceed in electronic transfer between the donor and the acceptor). From this relation it can be deduced that to have an efficient electron transfer between enzyme and mediator, it is essential that the redox mediator used presents a highly reversible redox system to minimize \( \lambda \) value. It is also fundamental to minimize the \( \Delta G^0 \) value. Thus it is very important that formal potentials of mediator and enzyme are close. Moreover, since active centers of enzyme are greatly insulated in high molecular weight molecules it is necessary to use small mediator molecules to reduce the distance of electron transfer and to guarantee a high \( k \) value.

2.2 Immobilization of enzymes on electrode surfaces

It is of great interest to develop new non-damaging immobilization techniques of enzyme for the development of stable biofuel cells. In fact, it is very difficult to propose a technique that does not affect the stability of biomolecules. Enzymes are proteins which possess tridimensional structures in which active centers are insulated. To keep the stability of the molecule and to preserve its catalytic efficiency it is necessary not to modify this tridimensional structure and particularly not to affect the environment of the active center. Different combining techniques can be used to immobilize enzymes onto the surface of solid electrodes:

- immobilization into a polymer network
- adsorption on an electrode material
- covalent grafting to an electrode
- immobilization within a membrane.

The first technique consists in immobilizing enzyme in an electropolymerized thin film. It is a very simple technique since it only needs to dip the electrode into a solution containing both monomers and biomolecules. Then the growth of polymer film can be realized by different ways: chronoamperometry (Brunel et al., 2007), chronopotentiometry or cyclic voltammetry (Fei et al., 2007). Different monomers such as pyrrole (Habrioux et al., 2008), aniline (Timur et al., 2004) or phenol (Bartlett et al., 1992) can be electropolymerized. This kind of films can be either conductive or not. The main advantage in using tridimensional conductive films lies in their ability to transfer electrons. Moreover, to increase the number of enzymatic molecules immobilized close to the electrode surface, a first adsorption step of enzymatic molecules can be performed (Merle et al., 2009). Other non-electropolymerized films can be used for enzyme holding. Currently, both chitosan and Nafion® are commonly used (Habrioux et al., 2010; Klotzbach et al., 2008). These two polymers possess surfactant properties interesting to immobilize enzymes in micellar structures (Moore et al., 2004). Moreover, the hydrophobic/hydrophilic property of the polymers can be tuned by modifying the chemical structure of these molecules (Klotzbach et al., 2008; Thomas et al., 2003). It is also possible to simply use retention properties of the Nafion® film for buffering its sulfonic groups (Habrioux et al., 2010). The main problem associated with the use of
these polymers lies in the non-control of the film thickness. One of the most promising immobilization techniques has been proposed by Heller’s group. This approach consists in immobilizing enzymes in an osmium-based redox polymer (Mao et al., 2003) which is able to swell in contact with water. It acts both as an immobilizing network and an electrochemical mediator. The whole structure of the film leads to very fast electron transfer between the active centers of enzymes and the electrode surface. Another smart technique consists in covalent grafting of enzyme to the electrode surface. Thus Merle et al. (Merle et al., 2008) realized the grafting of amino groups on a carbon electrode before coupling these functions with amino-groups of enzymes using glutaraldehyde. This seems to confer a remarkable stability to the resulting electrode. Another well-known approach has been proposed by Willner et al. (Willner et al., 1996) that consisted in the reconstitution of the enzyme after the grafting of its active center on a gold electrode.

2.3 Enzymatic oxidation of glucose
The development of efficient enzymatic electrodes to oxidize glucose is of relevance for the development of implantable glucose/O\textsubscript{2} biofuel cells. Nowadays, enzymes classically used to perform efficient oxidation of glucose to gluconic acid are either glucose oxidase (GOD) or glucose dehydrogenase (GDH). In the next part the properties of these two enzymes will be explained in details.

2.3.1 Glucose oxidation catalyzed by glucose dehydrogenase
Contrary to GOD, GDH is non-sensitive towards oxygen (Zhang et al., 2007). This is an attractive property for its use in glucose oxygen biofuel cells. However, GDH is an NAD-dependant enzyme. It is well-known that the oxidation of glucose catalyzed by GDH is rather limited by oxidation kinetics of NADH into NAD\textsuperscript{+}. Even if the use of modified electrodes allows to reduce the overpotential associated to the oxidation of NADH into NAD\textsuperscript{+} (Delecous-Servat et al., 2001), the stability of the electrodes remains poor. Another solution based on the use of a pyrroloquinoline quinone (PQQ) cofactor proposes to suppress the NAD dependence. Nevertheless, the PQQ cofactor has a limited stability (Wang, 2007).

2.3.2 Glucose oxidation catalyzed by glucose oxidase
2.3.2.1 Properties of the enzyme
GOD is by far the most used anode catalyst in glucose/O\textsubscript{2} biofuel cells. Its molecular weight (155 kDa) and molecular size (60 Å × 52 Å × 77 Å) are high (Alvarez-Icaza et al., 1995). This constitutes a limitation for current densities obtained with a solid electrode since the footprint of the enzyme is great. This enzyme possesses two identical linked FAD (Flavine Adenine Dinucleotide) subunits which are responsible for β-D-glucose oxidation (Zhu et al., 2006) to gluconic acid (two electrons reaction product). The redox potential of FAD-FADH\textsubscript{2} cofactor is ca. -0.36 V vs. Ag/AgCl/KCl(sat.) at a pH value of 7.2 (Stankovich et al., 1978), which is of particular interest for biofuel cells applications since it allows low-potential glucose oxidation. In this reaction, dioxygen is the natural electrons acceptor. Therefore, during the oxidation process, dioxygen is reduced towards hydrogen peroxide. The formation of H\textsubscript{2}O\textsubscript{2} leads to inhibition of the enzyme because it modifies the amino groups in the vicinity of the active center (Kleppe, 1966). The pH value and the temperature have also
an effect on GOD performances. Temperatures higher than 40 °C lead to a drastic decrease of activity (Kenausis et al., 1997). The pH value which optimizes GOD activity greatly depends on the electron acceptor. This value is equal to 5.5 and 7.5 when oxygen (Kenausis et al., 1997) methylene blue (Wilson & Turner, 1992) are used, respectively.

2.3.2.2 Performances of GOD electrodes towards β-D-glucose oxidation

In the case of MET, the use of suitable electrochemical mediators is of importance to increase the rate of electron transfer between the enzyme and the electrode surface since it allows to raise current densities. The second interest lies in the possibility to inhibit the formation of peroxide. Actually, it is just necessary to use a mediator which is able to realize faster electron transfer with GOD than oxygen can do. One of the most efficient systems has been developed by Heller’s group (Mano et al., 2005; Mao et al., 2003). It consists of a tridimensional matrix of an osmium based redox polymer containing GOD. The formal potential of the polymer is -195 mV vs. Ag/AgCl at pH 7.2. The covalent chain composed of thirteen atoms long allows the increase of the electron diffusion coefficient (Mao et al., 2003) by increasing the collision probability between reduced and oxidized forms of the osmium centers. The reticulation with PEGDGE (polyethyleneglycoldiglycydilether) allows the formation of a redox hydrogel capable of swelling in contact with water. It is probable that the matrix structure is responsible for a weak deformation of the protein structure. Such electrodes are able to deliver a catalytic current at potentials as low as -360 mV vs. Ag/AgCl in a physiologic medium containing 15 mM glucose (Mano et al., 2004).

2.4 Enzymatic reduction of oxygen to water

Generally, enzymes used to catalyze the reduction of oxygen into water are either laccase or bilirubin oxidase (BOD). The main property of these enzymes is their ability to directly reduce oxygen to water at potentials higher than what can be observed with platinum based electrodes (Soukharev et al., 2004). These two enzymes are classified in “multicopper oxidases” class and contain four Cu$^{2+}$/Cu$^{+}$ active centers which are commonly categorized in three types: T$_1$, T$_2$ and T$_3$. T$_1$ site is responsible for the oxidation of the electron donor. The trinuclear center composed both of T$_2$ center and two equivalent T$_3$ centers is the place where oxygen reduction occurs (Palmer et al., 2001). The associated mechanism is proposed in Fig. 2.

![Fig. 2. Oxygen reduction catalyzed by “multicopper oxidases”](image-url)
2.4.1 Reduction of oxygen catalyzed by laccase

Laccase is able to oxidize phenolic compounds and to simultaneously reduce oxygen into water. The microorganism from which it is extracted greatly determines the redox potential of the $T_1$ site which can vary from 430 mV vs. NHE up to 780 mV vs. NHE (Palmore & Kim, 1999). Laccase from *Trametes versicolor* is the most attractive one since redox potential of its $T_1$ site is ca. 780 mV vs. NHE (Shleev et al., 2005). Nowadays, the best performances with laccase electrodes are obtained with osmium based polymers as redox mediators (Mano et al., 2006). Actually these electrodes are able to deliver a current density of 860 µA cm$^{-2}$ at only -70 mV vs. O$_2$/H$_2$O at pH 5. In the same conditions, the identical current density is obtained at -400 mV vs. O$_2$/H$_2$O with a platinum wire as catalyst. Nevertheless, performances of laccase (from *Pleurotus Ostreatus*) electrodes drop drastically in the presence of chloride ions (Barton et al., 2002) what constitutes both a major problem and a great challenge for its use in implantable glucose/O$_2$ biofuel cells.

2.4.2 Reduction of oxygen catalyzed by bilirubin oxidase

BOD is naturally capable of catalyzing the oxidation of bilirubin into biliverdin and to simultaneously reduce dioxygen (Shimizu et al., 1999). BOD is very similar to laccase. Performances of BOD electrodes are greatly related to the amino-acids sequence around $T_1$ site of the enzyme (Li et al., 2004). It is clearly reported that the most efficient BOD enzyme comes from *Myrothecium verrucaria*. Redox potential of its $T_1$ site is included between 650 and 750 mV vs. NHE, and the enzyme is thermally stable up to 60 °C (Mano et al., 2002b). It is thus possible to use it at physiological temperature without denaturing the protein. To build efficient BOD electrodes intended in working at physiological pH value, it is judicious to use positively charged mediator molecules since the isoelectric point of BOD is close to pH = 4. Actually, during oxygen reduction reaction, the use of an osmium based redox polymer has lead to performances such as 880 µA cm$^{-2}$ at 0.3 V vs. Ag/AgCl (physiological conditions) at a scan rate of 1 mV s$^{-1}$ (Mano et al., 2002a). Additionally, the redox osmium based hydrogel conferred a very favorable environment to stabilize BOD since 95% of the initial activity of a BOD electrode can be preserved after three weeks storage (Mano et al., 2002a). This remarkable stability probably results in auspicious electrostatic interactions between the swelling matrix and the enzyme. Performances of BOD electrodes are furthermore unaffected in the presence of chloride ions. In fact BOD remains active for chloride concentrations lower than 1 M (Mano et al., 2002a). This property is of major interest for the development of implantable microscale glucose/O$_2$ biofuel cells using BOD as cathode catalysts. The major encountered problem with BOD electrodes is the relative lack of stability of the enzyme in physiological serum. Cupric centers of BOD are indeed capable of binding with one urea oxidation product, oxidation reaction catalyzed by the enzyme (Kang et al., 2004). This phenomenon can nevertheless be limited by spreading a Nafion® film on the catalyst (Kang et al., 2004). It is moreover reported that chemically modified Nafion® is capable of constituting a favorable environment to stabilize BOD (Topcagic & Minteer, 2006). Consequently, it seems of interest to immobilize BOD in Nafion® films. A promising technique for the development of efficient BOD electrodes has already been reported in literature (Habrioux et al., 2010). It consists in firstly adsorbing BOD/ABTS$^2$ (2,2-azinobis-3-ethylbenzothiazoline-5-sulfonic acid) complex on a carbon powder, Vulcan XC 72 R in order to increase both enzyme loading, the stability of the protein and the quality of the percolating network in the whole thickness of the polymer film. Actually, to realize the electrochemical reaction, a triple contact point (between the catalytic system, the electrolyte and the electronic conductor) is required. Once the catalytic
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system is adsorbed, a buffered Nafion® solution is added. The whole system is then immobilized onto a solid carbon electrode (Fig. 3).

Fig. 3. Method used for the preparation of BOD cathodes according to the process described in Ref. (Habrioux et al., 2010)

Previous studies have shown the interest lying in the use of ABTS$^{2-}$ as redox mediator in combination with multicopper oxidases. One of them was carried out by Karnicka et al. who have shown that wiring laccase to glassy carbon through a ABTS$^{2-}$/carbon nanotube system was a very efficient pathway to reduce molecular oxygen into water (Karnicka et al., 2008). The combination of ABTS$^{2-}$ with BOD is also known to exhibit a high electrochemical activity towards oxygen reduction reaction (Tsujimura et al., 2001). These observations are confirmed by electrochemical studies performed on electrodes previously described (Fig. 3). Results are shown in Fig. 4.

Fig. 4. Oxygen reduction reaction catalyzed by BOD/ABTS$^{2-}$/Nafion® electrode in a phosphate buffered solution (pH = 7.4, 0.2 M) at 25 °C. Curves registered at different rotation rates ($\Omega$), in an air-saturated electrolyte at $\Omega =$ 100 rpm (●); $\Omega =$ 200 rpm (●); $\Omega =$ 400 rpm (Δ); $\Omega =$ 600 rpm (□) and in an oxygen saturated electrolyte at $\Omega =$ 600 rpm (○). Scan rate 5 mV s$^{-1}$.

Curves of Fig.4 clearly show the interest of such electrodes that exhibit a catalytic current from potentials as high as -50 mV vs. O$_2$/H$_2$O (0.536 V vs. SCE). Furthermore the half-wave potential is only 100 mV lower than the reversible redox potential of O$_2$/H$_2$O. This value is in good agreement with that reported by Tsujimura et al. (0.49 V vs. Ag/AgCl/KCl(sat.) at pH = 7.0) (Tsujimura et al., 2001). Let’s notice that the half-wave potential value is very close to the redox potential of T$_1$ site of BOD (0.46 V vs. SCE). This has already been explained by the fact that the reaction between ABTS$^{2-}$ and BOD is an uphill one (Tsujimura et al., 2001).
Fig. 4 also shows that electrochemical performances of BOD/ABTS\(^{2-}\)/Nafion\(^{®}\) clearly depend on the amount of oxygen dissolved in the electrolyte. The limiting current is a plateau and increases from 0.56 mA cm\(^{-2}\) in an air saturated electrolyte to 1.61 mA cm\(^{-2}\) in an oxygen saturated (at a rotation rate of 600 rpm). Dependence of limiting current with oxygen concentration in the electrolyte is presented in Fig.5. In this figure, current obtained at 0.2 V vs. SCE is plotted versus oxygen saturation.

![Image](image-url)

**Fig. 5.** Electrochemical activity of BOD/ABTS\(^{2-}\)/Nafion\(^{®}\) electrode: dependence of the current value at 0.2 V vs. SCE with oxygen concentration

The current linearly increases with the oxygen concentration from low values to around 35%. This linearity suggests that the reaction is of a first order with oxygen concentration thereby, the Koutecky–Levich plots can be considered. Assuming that the rate determining step is an enzymatic intramolecular electron transfer step, it is possible to express the current density of a BOD/ABTS\(^{2-}\)/Nafion\(^{®}\) electrode working in an air saturated solution as follows (Schmidt et al., 1999):

\[
\frac{1}{j} = \frac{1}{j^0_L} \frac{\bar{\eta}}{\bar{\alpha}} e^{\frac{\bar{\eta}}{RT}} + \frac{1}{j^\text{diff}_L} + \frac{1}{j^\text{film}_L} + \frac{1}{j^\text{ads}_L} \tag{2}
\]

In Eq.2, \(j^\text{diff}_L\) represents the diffusion limiting current density expressed by Levich equation:

\[
j^\text{diff}_L = \frac{0.2nFD^\frac{1}{2}C_0\sqrt{\Omega}}{\nu} \tag{3}
\]

In Eq.3, \(n\) is the number of electrons exchanged, \(D\) the diffusion coefficient, \(C_0\) is the oxygen concentration, \(\Omega\) is the rotation rate, \(F\) the Faraday constant and \(\nu\) is the kinematic viscosity. Then, \(j^\text{film}_L\) corresponds to the limitation due to oxygen diffusion in the catalytic film and \(j^\text{ads}_L\) is the limiting current density due to oxygen adsorption on the catalytic site. Since these two last contributions to the total current density do not depend on \(\Omega\), it is impossible to separate them. They will be described according to Eq.4.

\[
\frac{1}{j_L} = \frac{1}{j^\text{film}_L} + \frac{1}{j^\text{ads}_L} \tag{4}
\]

In Eq.2, \(\bar{\eta}\) is the overpotential (\(\bar{\eta} = E - E_\text{eq}\)), \(j_0\) the exchange current density, \(\bar{\alpha}\) the transfer coefficient, \(R = 8.31\) J mol\(^{-1}\) K\(^{-1}\), \(F = 96500\) C mol\(^{-1}\) and \(T\) the temperature. \(\bar{\theta}\) and \(\bar{\theta}_c\) are the...
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covering rates of the active sites of the enzyme at $E$ and $E_{eq}$, respectively. We will assume that $\Theta \approx \Theta_c$ for all potential values. From Eq. 2, when $\Omega \to \infty$, the limit of $1/j$ can be expressed as follows:

$$\frac{1}{|j|} = \frac{1}{|j_0|} + \frac{1}{|j_0|e^{\eta/b}} \Theta_c$$

In Eq. 5, when $\eta \to \infty$, $1/j_0 \to 1/j_L$. It is thus possible to determine $j_L$ value by extrapolating and reporting the $1/j$ values as a function of the potential value $E$. Transforming Eq. 5 (Grolleau et al., 2008), it becomes as follows:

$$\eta = E - E_{eq} = -b \left[ \ln \frac{1}{j_L} + \ln \frac{1}{j_k} - \frac{1}{j_L} \right]$$

where $b = RT/\alpha n F$ is the Tafel slope. The plot of the $\eta$ values vs. $ln(j_k/(j_L - j_k))$ (Fig. 6) permits the calculation of $b$ and $j_L$ values.

Fig. 6. Curve obtained from Koutecky-Levich treatment on oxygen reduction reaction catalyzed by BOD/ABTS$^{2-}$/Nafion® system.

Under these experimental conditions, calculated values for both Tafel slope and exchange current density are respectively of 69 mV/decade and 25 µA cm$^{-2}$. The high value obtained for $j_0$ confirms the ability of BOD/ABTS$^{2-}$/Nafion® system to activate molecular oxygen in a physiological type medium. Moreover, it also certifies that the oxygen reduction reaction starts at very high potentials. The reference catalyst classically used to reduce molecular oxygen is platinum. It can be noticed that under similar conditions, the exchange current density is only of 5 µA cm$^{-2}$ when we used platinum nanoparticles as catalyst. This clearly shows the great interest lying in these electrodes to reduce oxygen in glucose/O$_2$ biofuel cells. Nowadays, the major problem encountered with these electrodes is the lack of stability of the redox mediator (ABTS$^{2-}$) (Tsujimura et al., 2001).

3. Abiotic catalysts for glucose/O$_2$ biofuel cells

In this part, a complete description of non-enzymatic catalysts which are used or potentially usable in glucose/O$_2$ biofuel cells systems is given. The major problem in employing abiotic catalyst in such applications lies in their lack of specificity. Consequently, their application in implantable microscale devices is difficult. Nevertheless, they often lead to fast substrate
conversion kinetic characteristics and their stability is incomparably higher than enzymes one. Thus, they can be used as catalysts in biocompatible devices intended in supplying long-term high power densities.

### 3.1 Non-enzymatic oxidation of glucose

#### 3.1.1 Different offered possibilities

A promising approach consists in using metallophthalocyanines to realize glucose oxidation. Particularly, cobalt phthalocyanines seem to exhibit interesting properties (Zagal et al., 2010). Furthermore, reactivity of these electrodes can be modulated by simple modification of the complex structure what is of interest for the development of electrodes. These catalysts could be used for glucose electrooxidation in glucose/O$_2$ biofuel cells but it is not still developed.

The other approach lies in the use of metallic nanomaterials as catalysts. Oxidation of glucose on metallic surfaces has extensively been studied. Among all these investigations, numerous ones have been devoted to the understanding of catalytic effect of platinum on glucose oxidation process (Kokoh et al., 1992a; Kokoh et al., 1992b; Sun et al., 2001). Experiments led to conclude that the major oxidation product is gluconic acid (Kokoh et al., 1992b; Rao & Drake, 1969). Actually, the oxidation process involves dehydrogenation of the anomeric carbon of glucose molecule (Ernst et al., 1979). The major interest in including platinum in the catalyst composition lies in its ability to oxidize glucose at very low potentials (lower than 0.3 V vs. RHE). However, it is also well-known that platinum surfaces are particularly sensitive to poisoning with chemisorbed intermediates (Bae et al., 1990; Bae et al., 1991). To solve this problem, different heavy atoms (Tl, Pb, Bi and W) have been used as adatoms to modify platinum surfaces to raise electrochemical activity of platinum (Park et al., 2006). Other studies relate glucose oxidation on platinum alloys in which the second metal can be Rh, Pd, Au, Pb (Sun et al., 2001), Bi, Ru and Sn (Becerik & Kadirgan, 2001). It appears that the most efficient catalysts are Pt-Pb or Pt-Bi (Becerik & Kadirgan, 2001). However, these catalysts are sensitive to poisoning of the second metal which prevents their use in fuel cells systems. Moreover most of the materials previously cited are toxic. The only one which could be environmentally friendly is gold even if the oxidative stress caused by nanoparticles on living cells is not well-known. Besides, synthesis of alloyed materials allows increasing significantly catalytic activity of pure metals by synergistic effect. This has noticeably been observed with platinum-gold nanoalloys (Möller & Pistorius, 2004).

#### 3.1.2 Oxidation of glucose on gold-platinum nanoparticles

The oxidation of glucose on gold-platinum nanoparticles has been investigated in numerous studies (Habrioux et al., 2007; Sun et al., 2001). Jin and Chen (Jin & Chen, 2007) examined glucose oxidation catalyzed by Pt-Au prepared by a co-reduction of metallic salts. An oxidation peak of glucose was visible at much lower potentials than on gold electrode. Moreover, they showed that both metals favored the dehydrogenation of the glucose molecule. They concluded that the presence of gold prevents platinum from chemisorbed poisonous species. The efficiency of such catalysts towards glucose oxidation is thus not to be any more demonstrated, and greatly depends on the synthesis method used to elaborate the catalytic material.

#### 3.1.2.1 Synthesis of gold-platinum nanoparticles

Various gold-platinum nanoparticles synthesis methods have been already studied: Polyol (Senthil Kumar & Phani, 2009), sol-gel (Devarajan et al., 2005), water-in-oil microemulsion
Performances of Enzymatic Glucose/O$_2$ Biofuel Cells (Habrioux et al., 2007), electrodeposition (El Roustom et al., 2007) and Bönnemann (Atwan et al., 2006). Among all these methods, the water-in-oil microemulsion technique produces particles that exhibit high catalytic activity towards glucose electrooxidation (Habrioux et al., 2007). It consists in mixing two microemulsions, one containing the reducing agent in the aqueous phase and the other containing one or several metallic precursors in the aqueous phase. Collisions of water nanodroplets permit to obtain metallic nanoparticles which can be then cleaned and dispersed onto a carbon support. The choice of the different components of the microemulsions is not unique and influences the physical properties of the obtained nanoparticles. Actually, both surfactant molecules and oil-phase chemical nature have an effect on interfacial tension of the surfactant film that determines water solubility in micelles (Paul & Mitra, 2005). This greatly affects intermicellar exchanges. Moreover, the chemical nature of the reducing agent controls the rate of the nucleation step and subsequently the kinetic of particles formation. In the system described herein, n-heptane is used as oil phase, non-ionic polyethylene glycol-dodecylether as emulsifier molecule and sodium borohydride as reducing agent. The synthesized particles have been dispersed onto Vulcan XC 72 R and then washed several times with acetone, ethanol and water, respectively to remove surfactant from their surface (Habrioux et al., 2009b). The removal of surfactant molecules from all the catalytic sites without modifying structural properties of the catalyst is currently a great challenge (Brimaud et al., 2007). Since electrocatalysis is a surface phenomenon depending on the chemical nature of the surface of the catalyst, on its crystalline structure and on the number of active sites, it is useful to precisely know the physico-chemical properties of the used nanoparticles to understand their electrochemical performances.

3.1.2.2 Electrochemical behaviour of gold-platinum nanoparticles towards glucose electrooxidation

This part aims at showing the importance to realize a correlation between the structural properties of the catalysts and their electrocatalytic activities towards glucose oxidation. The use of nanocatalysts indeed involves a deep structural characterization of the nanoparticles to fully understand the whole of the catalytic process. Therefore, in order to show the presence and the proportion of gold and platinum at the surface of the catalysts, electrochemical investigations have been carried out (Burke et al., 2003). It is indeed possible to quantify surface compositions of the catalysts by using cyclic voltammetry and by calculating the amount of charge associated with both reduction of platinum and gold oxides (Woods, 1971). The charge calculated for pure metals was 493 μC cm$^{-2}$ and 543 μC cm$^{-2}$ for Au and Pt, respectively, for an upper potential value of 250 mV vs. MSE (Habrioux et al., 2007) in a NaOH (0.1 M) solution. The atomic ratio between gold and platinum can be thus determined according to Eq. 7 and Eq. 8 assuming that for all bimetallic compositions, the oxidation takes place only on the first atomic monolayer.

\[
\%_{\text{Au}} = \frac{S_{\text{Au}}}{S_{\text{Au}} + S_{\text{Pt}}} \times 100
\]  

(7)

and

\[
\%_{\text{Pt}} = \frac{S_{\text{Pt}}}{S_{\text{Au}} + S_{\text{Pt}}} \times 100
\]  

(8)

Both voltammograms used and results of the quantification are shown in Fig. 7. Mean diameter of the different nanoparticles weighted to their volume (obtained from
transmission electron microscopy measurements) as well as their mean coherent domain size weighted to the volume of the particles (obtained from X-ray diffraction measurements) are also presented in Fig. 7.

Fig. 7. Voltammograms (after 19 cycles) of gold-platinum nanoparticles recorded at 25 °C in alkaline media (0.1 M NaOH). Scan rate = 20 mV s⁻¹. The surface composition of the used catalyst is given on the right of the corresponding voltammogram.

In Fig. 7 it is noticed that for all compositions, desorption of oxygen species occurs in two peaks. The reduction of the gold surface takes place at -0.38 V vs. MSE whereas the potential for which platinum surface is reduced depends on the amount of gold in the alloy. Indeed, for pure platinum nanoparticles this potential is ca. -0.8 V vs. MSE (reduction of platinum oxides). The potential at which oxygen species desorption occurs, shifts to lower potentials when the atomic ratio of gold increases in the composition of alloys. The deformation of this peak increases with the amount of gold probably because of the formation of more complex platinum oxides. The quantification realized on the different bimetallic compositions, clearly shows a platinum enrichment of nanoparticles surfaces. Desorption of gold oxides is indeed invisible for low gold containing samples (i.e. with gold content lower than 40%). These nanoparticles exhibit a typical core-shell structure composed of a gold core and a platinum shell (Habrioux et al., 2009b), while high gold content samples (i.e. with gold content higher than 80%) possess a surface composition that is close to the nominal one. This results in a purely kinetic effect. Actually, reduction of gold precursor is considerably faster than reduction of platinum cation. Consequently, there is firstly formation of a gold seed on which platinum reduction occurs. So, the natural tendency of these systems is to form core-shell particles. Furthermore, let’s notice that both mean diameter of nanoparticles weighted to their volume and their mean coherent domain size weighted to their volume increase with gold content but ever stay in the nanometer range. That is only the result of differences in reduction kinetics of the particles since the ratio water to surfactant remains constant whatever the synthesized sample. To correlate surface composition with efficiency to
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oxidize glucose for all gold-platinum catalysts compositions, voltammograms were first recorded in alkaline medium. Results are shown in Fig. 8.

Fig. 8. Voltammograms (after 19 cycles) of gold-platinum nanoparticles recorded at 3 °C in alkaline medium (0.1 M NaOH) in the presence of 10 mM glucose. Scan rate = 20 mV s$^{-1}$.

Surface composition of the used catalyst is given on the right of the corresponding voltammogram.

In Fig. 8, different oxidation peaks appear during the oxidation process on gold-platinum nanocatalysts. When platinum content decreases in the bimetallic surface composition, intensity of peak A, located at ca. -0.7 V vs. SCE, diminishes. For pure gold catalyst, this peak is furthermore invisible. It is thus related to the oxidation phenomenon on platinum. It has already been attributed to dehydrogenation of anomeric carbon of glucose molecule (Ernst et al., 1979). Peaks B and C correspond to the direct oxidation of glucose molecule (Habrioux et al., 2007) and are located both in gold and platinum oxides region. In the case of catalysts with nominal compositions such as Au$_{70}$Pt$_{30}$ or Au$_{80}$Pt$_{20}$, the different oxidation peaks located between -0.3 V vs. SCE and 0.4 V vs. SCE are not well-defined. For these catalysts, the presence of platinum at their surface allows a low potential oxidation of glucose molecule, which starts earlier than on pure gold. Moreover, on these catalysts, after the dehydrogenation step, current densities raise rapidly. Furthermore, in the potential region where formation of both gold hydroxides and platinum oxides occurs, current densities are very high (i.e. 12 mA mg$^{-1}$ at 0.2 V vs. SCE). This is the result of a synergistic effect between the two oxidized metals at the bimetallic catalyst surface (Habrioux et al., 2007). Such effect between gold and platinum has already been observed for CO oxidation (Mott et al., 2007).

On these catalysts, during the negative going scan, two oxidation peaks, E and F, are visible. During the reduction of both oxidized gold and platinum clusters, oxygenated species are desorbed from the surface and stay at its vicinity. Subsequently, there is desorption of adsorbed lactone from the electrode surface what implies the formation of both peak E and
peak F (Beden et al., 1996). Fig. 9 shows the reactions involving in the oxidation of glucose on the catalyst surface.

Fig. 9. Oxidation of glucose on gold-platinum catalysts

The remarkable electrocatalytic activity of both Au₈₀Pt₂₀ and Au₇₀Pt₃₀ nanocatalysts towards glucose electrooxidation is probably the result of a suitable surface composition combined with a convenient crystallographic structure. An X-ray diffraction study (Fig. 10) based on Warren’s treatment of defective metals and previously described (Vogel et al., 1998; Vogel et al., 1983) combined with high resolution transmission electron microscopy (HRTEM) measurements allowed to exhibit the peculiar structure of high gold content catalysts (Habrioux et al., 2009b).

Each experimental diffractogram has been fitted with five Pearson VII functions what gives two important parameters: the accurate peak position $b$ ($b = 2\sin\theta/\lambda$) and the integral line width $db$. The value of $db$ is plotted versus $b$ in Fig.10b. As a result of best fits, it can be assumed that line profiles of diffractograms are lorentzian. This implies that all contributions to the integral line width can be added linearly and can be expressed as follows:

$$
db = db_{size} + db_{stacking\ fault} + db_{strain}
$$

(9)
with

$$d_{\text{ave}} = \frac{1}{L_v}$$  \hspace{1cm} (10)

$$d_{\text{stacking fault}} = \frac{\alpha V_{hkl}}{a}$$  \hspace{1cm} (11)

and

$$d_{\text{strain}} = \frac{2\sigma b}{E_{hkl}}$$  \hspace{1cm} (12)

where $L_v$ is the mean coherent domain size weighted to the volume of the particles, $a$ the stacking fault probability, $V_{hkl}$ a parameter depending on the miller indexes, $\sigma$ the mean internal stress and $E_{hkl}$ the young modulus. The fit of Williamson-Hall diagrams with the expression given by Eq.7 leads to the determination of $L_v$, $a$ and $\sigma$ for each catalyst. It has been concluded that for catalysts with nominal compositions of $\text{Au}_{70}\text{Pt}_{30}$ and $\text{Au}_{80}\text{Pt}_{20}$, both $\sigma$ and $a$ values were high (Habrioux et al., 2009b). For $\text{Au}_{80}\text{Pt}_{20}$, these values were indeed of 510 N.mm$^{-2}$ and 8.2%, respectively for $\sigma$ and $a$. In the case of $\text{Au}_{70}\text{Pt}_{30}$, these values were of 490 N.mm$^{-2}$ and 7.4%. HRTEM observations have confirmed the results of the fit since the observed particles present numerous twins and stacking faults, as shown in Fig. 11.

Fig. 11. HRTEM observations of $\text{Au}_{70}\text{Pt}_{30}$ nanoparticle (left image) and Au nanoparticle (right image).

As a result of the high internal mean strain existing in these particles, there is an important strain energy which leads to the formation of twins and stacking faults. Consequently the equilibrium shape of the particles is modified and the interaction between the different surface atoms is changed. Accordingly, the catalytic behaviour of these particles is greatly affected. This can also explain the remarkable activity of these particles towards glucose oxidation both in alkaline medium as shown in Fig. 8, and in physiological type medium, as shown in Fig. 12. Let’s notice that at low potential values, current densities obtained with $\text{Au}_{70}\text{Pt}_{30}$ and Pt catalysts are similar. Competitive adsorption between phosphate species and glucose molecules can be involved to explain this phenomenon. Actually, de Mele et al. (de Mele et al., 1982) showed that phosphate species are capable of creating oxygen-metal bonds with platinum surfaces and thus inhibiting glucose oxidation. This engenders the low current density observed at low potentials on pure platinum. On $\text{Au}_{70}\text{Pt}_{30}$ catalyst, it is possible that modification of 5d band center of platinum due to the presence of gold allows discriminating the adsorption of phosphate species. Furthermore, the oxidation of glucose on high gold content catalysts starts at a very low potential value (i.e. -0.5 V vs. SCE), which
can easily be compared with values observed for catalysts such as Pt-Bi, Pt-Sn (Becerik & Kadirgan, 2001) or Pt-Pd (Becerik et al., 1999).

Fig. 12. Voltammograms (after 19 cycles) of gold-platinum nanoparticles recorded at 37 °C in a phosphate buffered solution (0.1 M pH 7.4) in the presence of 10 mM glucose. Scan rate = 20 mV s⁻¹.

3.2 Oxygen reduction reaction on abiotic catalysts
It is difficult to tailor non-enzymatic catalyst, capable of exhibiting electrochemical performances similar to those shown by laccase or BOD in physiological type media. The major problem with enzymes lies in the natural lack of stability of the proteins. One of the possibilities to tailor new efficient and stable cathode catalysts for glucose/O₂ biofuel cells is to artificially reproduce active centers of enzymes and to stabilize their environment by mimicking the structure of enzymatic proteins and by removing all organic parts responsible for instability of enzymes. The possibility of designing this kind of catalyst has already been discussed (Ma & Balbuena, 2007).

4. Design of glucose/O₂ biofuel cells
The global reaction associated to the glucose/O₂ biofuel cell can be described according to Eq. 13:

\[
C_6H_{12}O_6 + \frac{1}{2}O_2 \rightarrow C_6H_{12}O_6 + H_2O
\] (13)

Gibbs free energy associated to this reaction is \( \Delta G^0 = -251 \text{ kJ mol}^{-1} \). This implies that the theoretical cell voltage is \( E^0 = 1.3 \text{ V} \) (Kerzenmacher et al., 2008). Furthermore, when the cell delivers a current \( j \), the cell voltage \( E(j) \) can be expressed as follows:

\[
E(j) = E_{eq} - \eta_a - \eta_c - Rj
\] (14)

where \( \eta_a \) is the anodic overvoltage, \( \eta_c \) the cathodic one, \( R \) the cell resistance and \( E_{eq} \) the equilibrium cell voltage. In Eq.14, it clearly appears that both values of \( \eta_a \), \( \eta_c \) and \( R \) must be very low in order to increase the cell performances.

Since the development of the first biofuel cell realized by Yahiro et al. (Yahiro et al., 1964) that consisted in a two-compartment anionic membrane cell in which two platinum foils
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were used as conducting supports, numerous progress have been realized in designing devices. Nowadays, four main designs are developed. The first one has been developed by Heller’s group. It simply consists in using two carbon fibers of 7 µm diameter as electrode materials. On these fibers, enzymes are immobilized in a redox osmium based hydrogel capable of immobilizing enzymes. These two electrodes are directly dipped into the electrolyte. In a physiological medium containing 15 mM glucose, the device was primarily able to deliver a power density of 431 µW cm$^{-2}$ at a cell voltage of 0.52 V (Mano et al., 2002c). The device exhibited a high stability, since after one week of continuous working, it was still capable of delivering 227 µW cm$^{-2}$. Based on this study, and by replacing carbon fibers by newly engineered porous microwires comprised of assembled and oriented carbon nanotubes, Mano’s group (Gao et al., 2010) recently made the most efficient glucose/O$_2$ biofuel cell ever designed. It indeed achieved a remarkably high power density of 740 µW cm$^{-2}$ at a cell voltage of 0.57 V. The success of the experiment probably lies in the increase of the mass transfer of substrates. Other promising but presently less performing designs of glucose/O$_2$ biofuel cells have been developed in the recent past years. The first one consists in using a microfluidic channel to build a glucose/O$_2$ biofuel cell. The laminar flow obtained in the channel at low Reynold’s number prevents the electrodes from depolarization phenomena and/or from degradation. The mixing of the reactants indeed occurs only on a very small distance in the middle of the channel. The development of such glucose/O$_2$ biofuel cells seems of great interest for various applications. It is very simple to use abiotic and non-specific materials as catalysts. Moreover, it offers the possibility of working with two different pH values for the catholyte and the anolyte what can be interesting to improve electrochemical performances of each electrode (Zebda et al., 2009a). Nowadays, these devices are capable of delivering 110 µW cm$^{-2}$ for a cell voltage of 0.3 V (Zebda et al., 2009b) by using GOD and laccase as catalysts. Glucose/O$_2$ biofuel cells realized with classical fuel cell stacks have also been carried out (Habrioux et al., 2010). Both the used system and the obtained performances are described in Fig. 13.

Fig. 13. a) Description of the glucose/O$_2$ biofuel cell design, b) Characteristic $E$ vs. $j$ of glucose/O$_2$ cell performed at 20 °C: anode (Au$_{70}$Pt$_{30}$/Vulcan XC 72R, metal loading 40%); cathode (BOD/ABTS/Vulcan XC 72 R system). Test realized in the presence of a phosphate buffered solution (0.2 M; pH 7.4) containing 0.3 M glucose. The cathodic compartment contains an oxygen saturated phosphate buffered solution (pH 7.4; 0.2 M).
Fig. 13 shows that the maximum power density obtained is 170 µW cm$^{-2}$ for a cell voltage of 600 mV. However, let's notice that performances of the biofuel cell rapidly decrease for current densities higher than 300 µA cm$^{-2}$. This is clearly due to a very low ionic exchange rate between the two compartments of the cell since this value is too weak to correspond to mass transfer limitation of glucose molecule. The last design of glucose/O$_2$ biofuel cell developed in the last past years is the concentric device (Habrioux et al., 2008; Habrioux et al., 2009a). It is based on concentric carbon tubes as electrodes and operates at physiological pH. An oxygen saturated solution circulates inside the internal tube composed of porous carbon, which is capable of providing oxygen diffusion. The whole system is immersed in a phosphate buffered solution (pH 7.4, 0.1 M) containing various glucose concentrations. Oxygen consumption occurs at the cathode such that no oxygen diffuses towards the anode. This allows to use in this device both abiotic and enzymatic materials as anode and cathode catalysts, respectively. BOD/ABTS/Vulcan XC 72 R system is immobilized on the internal surface of the inner tube whereas Au-Pt nanocatalysts are immobilized on the internal surface of the outer tube. The surfaces of the cathode and anode were 3.14 and 4.4 cm$^2$, respectively. The system is fully described in Fig. 14.

Different fuel cell tests realized by using various nominal compositions of Au-Pt nanomaterials have been realized. The best performances are obtained with Au$_{70}$Pt$_{30}$ as anodic catalyst. Actually, the maximum power density achieved is approximately of 90 µW cm$^{-2}$ for a cell voltage of 0.45 V. Results are shown in Fig. 15.

Fig. 14. Schematic view of the glucose/O$_2$ biofuel cell system

Fig. 15. Fuel cell performances obtained with Au (▲), Au$_{80}$Pt$_{20}$ (■), Au$_{70}$Pt$_{30}$ (□) and Pt (△) nanoparticles as anode catalysts. These performances were obtained in a phosphate buffered solution (0.2 M, pH 7.4) containing 10 mM glucose at 37 °C. A saturated oxygen solution circulated in the inner tube of the device.
When Au$_{80}$Pt$_{20}$ is used as anode catalyst, the open circuit voltage is lower (i.e. 0.64 V). This is clearly explained by the surface composition of the catalyst which only contains 29 at.% of platinum. In the case of pure platinum, the open circuit voltage is very low due to strong competitions between phosphate species and glucose for adsorption. Such competition also occurs on other Au-Pt catalysts but the presence of gold allows a weaker interaction between phosphate species and the metallic surface. Consequently, higher glucose concentrations were used so as to improve biofuel cell performances. The obtained results are given in Fig. 16.

![Fig. 16. Fuel cell performances obtained with 10 mM glucose (△), 100 mM glucose (●), 300 mM glucose (○) and 700 mM glucose (□), with Au$_{70}$Pt$_{30}$ nanoparticles as anode catalyst. Performances obtained in a phosphate buffered solution (0.2 M, pH 7.4) at 37 °C. A saturated O$_2$ solution circulated in the inner tube.](image)

The data show a strong increase in cell voltage with glucose concentration. The raise observed in cell voltage between 0.1 M and 0.3 M can be attributed to the slow adsorption of phosphate species due to the presence of a higher glucose concentration. The maximum power density was also increased from 90 µW cm$^{-2}$ (for a glucose concentration of 10 mM) up to 190 µW cm$^{-2}$ (for a glucose concentration of 0.7 M). Nevertheless, in all cases, the fuel cell performances are greatly limited by resistance of the cell.

5. Conclusion

In this chapter we clearly show the importance of both electrodes assembly and global design of the cell on power output of the glucose/O$_2$ biofuel cell. Moreover, it seems that a suitable choice of well-characterized nanocatalysts materials can lead both to an increase of the cell performances and to an improvement of their lifetime resulting in the abiotic nature of these materials. The approach, which consists of the utilization of an abiotic anode catalyst and an enzyme for a four electrons reduction, can undoubtedly open new outlooks for biofuel cells applications. This hybrid biofuel cell combines the optimized fuel electrooxidation, as developed in classical fuel cells, with the complete reduction of dioxygen to H$_2$O without H$_2$O$_2$ production. Moreover, a concentric membrane-less design associated with an appropriate immobilization of the catalysts can avoid a costly separator of the cell events. Nevertheless, progresses to develop an efficient cell design are still necessary.
6. References


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This book aspires to be a comprehensive summary of current biofuels issues and thereby contribute to the understanding of this important topic. Readers will find themes including biofuels development efforts, their implications for the food industry, current and future biofuels crops, the successful Brazilian ethanol program, insights of the first, second, third and fourth biofuel generations, advanced biofuel production techniques, related waste treatment, emissions and environmental impacts, water consumption, produced allergens and toxins. Additionally, the biofuel policy discussion is expected to be continuing in the foreseeable future and the reading of the biofuels features dealt with in this book, are recommended for anyone interested in understanding this diverse and developing theme.

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