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# An improved glucose/$O_2$ membrane-less biofuel cell through glucose oxidase purification

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ABSTRACT

A key objective in any bioelectrochemical systems is to improve the current densities and mass transport limitation. Most of the work is focused on increasing the specific surface of the electrodes or improving the electron transfer between enzymes and electrodes. However, nothing is said about the comparison of purified and non-purified enzyme and their effects on the biosensor efficiency. To illustrate the effect of the enzyme purity, we studied the widely used commercial Glucose Oxidase (GOx) from *Aspergillus niger* that we are using in our miniature membrane-less biofuel cell. Our results indicate that even if additional compounds contained in the lyophilized enzyme powder do not interfere with its intrinsic catalytic properties, they could prevent a good electron transfer between the enzyme and the electrode surface. By introducing a purified glucose oxidase into a bioelectrocatalyst immobilized on an electrode surface, we show that we can increase the interaction between the enzyme and the redox polymer, forming a better homogenous, leather like gel. At 5 mM glucose concentration and under oxygen atmosphere, the current is three-fold higher when using a purified enzyme than it is when using a non-purified enzyme. Built with this novel anode, we showed that a miniature implantable membrane-less glucose–$O_2$ biofuel cell could produce, under air, twice the power density that is usually obtained when using a non-purified GOx.



## 1. Introduction

Owing to analytical and biomedical needs (Heller and Feldman, 2008), many researchers are working on engineering and developing improved enzyme-based electrochemical biosensors and miniature biofuel cells (Amir et al., 2009; Barton et al., 2004; Cooney et al., 2008a,b; Gao et al., 2007; Habrioux et al., 2008; Minteer et al., 2007; Ramanavicius et al., 2008; Sakai et al., 2009; Willner et al., 2009). The elaboration of miniature membrane-less biofuel cell is of interest because it may power, in the near future, an implanted sensor-transmitter that would broadcast, for example, the local glucose concentration, relevant to diabetes management. At the cathode, blue copper oxidases such as laccases or bilirubin oxidase (BOD) (Gallaway and Barton, 2008; Gallaway et al., 2008; Gallaway and Calabrese Barton, 2009; Mano et al., 2002a) and glucose dehydrogenase (Tsujimura et al., 2002; Yuhashi et al., 2005), and glucose oxidase (GOx) from *Aspergillus niger* at the anode, have been preferentially used (Mano et al., 2005; Yan et al., 2007). To enhance current densities or the power output of these devices, a large part of this work is focused on increasing the specific surface of the electrodes or improving the electron transfer between enzymes and electrodes (Cooney et al., 2008a,b). Different strategies such as direct electron transfer or mediated electron transfer have been explored to electrically connect these enzymes to the electrodes surfaces (Barriere et al., 2006; Courjean et al., 2009; Ivnitski et al., 2008; Kamitaka et al., 2007). However, nothing is said about the effect of enzyme purification on the elaboration of biosensors and biofuel cells, although some enzymes such as laccase, bilirubin oxidase and hydrogenase have been purified and used in biosensors or biofuel cells (Kamitaka et al., 2007; Mano et al., 2003a; Parkin et al., 2006; Tsujimura et al., 2007; Vincent et al., 2005).

Purification is a common step prior to classical enzymatic studies to accurately understand the molecular mechanisms involved in the catalytic reactions. Unless enzymes are produced, for most of the bioelectrochemical applications, lyophilized enzymes powders are purchased and used without any further purification. The specific activity in units per mg of powder is usually used as a quantitative reference of the enzymatic content. Nonetheless, little information is given about the exact chemical composition of commercial enzymes, in particular the nature and quantities of salts and stabilizers such as glycerol, PEG or proteins, and additional enzymes. The presence of such compounds varies from batch to batch, and/or from a supplier to another. The enzymatic activity measured experimentally can also be different from what is announced by the supplier (see Table 1, supporting information).

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These additional compounds are assumed to be non-interfering with the specific activity, but this is not always the case.

Nothing (or few) (Gallaway and Barton, 2008; Mano et al., 2003b) is discussed about the influence of these additional compounds in bioelectrochemistry. Even if they do not interfere with the intrinsic enzymatic reaction in solution, they may alter the electrochemical coupling with the electrode. To assess this hypothesis, we chose to study the widely used commercial Glucose Oxidase (GOx) from *Aspergillus niger*, which is frequently bought and used "as received" in electrochemical devices and that we are using in our miniature membrane-less biofuel cell. We first compared the influence of the enzyme purity on its catalytic parameters in solution and immobilized on an electrode surface. Then, we compared a miniature membrane-less glucose/$O_2$ biofuel cell when an anode was made with a purified GOx and with a non-purified GOx.

## 2. Materials and methods

### 2.1. Chemicals and materials

Glucose Oxidase (GOx) from *Aspergilus niger* (E.C. 1.1.3.4, 208 U/mg) and 2,2′-azino-di-[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS) were purchased from Fluka (Sigma–Aldrich, Saint-Louis, MO). Horseradish peroxidase from *Amoracia rusticana* (type II, E.C. 1.11.1.7), $NaIO_4$, NaCl and other chemicals were from Sigma (Sigma–Aldrich, Saint-Louis, MO). Poly(ethyleneglycol) (400) diglycidyl ether (PEGDGE) from Polyscience Inc.(Warrington, PA). Bilirubin Oxidase (BOD) (E.C. 1.3.3.5) from *Myrothecium verrucaria* was obtained from Amano Enzyme Inc. (Nagoya, Japan) and purified as described earlier (Mano et al., 2003a). All solutions were made with deionised water passed through an AQ 10 Milli-Q purification system from Millipore (Molsheim, France). Ultra-pure $O_2$, air and argon were purchased from Air Liquide (Paris, France). AKTA purifier 10 UV 900, prepacked HiTrap Phenyl HP (1 ml), HiTrap Q FF (1 ml) chromatography columns, and PD 10 desalting columns containing Sephadex G-25 medium, were from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Amicon Ultra ultrafiltration columns were from Millipore (Molsheim, France). UV–visible spectroscopic measurements were performed on a Cary 100 system from Varian Inc. (Palo Alto, CA), equipped with a peltier thermostable multicell holder. The synthesis of the GOx-wiring redox polymer PVP-[Os(N,N′-alkylanated-2,2′-bi-imidazole)$_3$]$^{2+/3+}$ (Mao et al., 2002) and of the BOD-wiring redox polymer PAA-PVI-[Os(4,4′-dichloro-2,2′-bipyridine)$_2$Cl]$^{+/2+}$ and the preparation of the "wired" BOD on 5 mm diameter glassy carbon electrodes (Pine Instruments) were previously reported (Mano et al., 2002b).

### 2.2. Instrumentation and electrodes

All the electrochemical measurements were performed using a potentiostat (CH Instruments, model CHI 660C, Austin, TX, USA) and a dedicated computer. The electrodes were rotated using a Pine Instruments rotator (Grove City, PA). The temperature was controlled by an isothermal circulator (Lab Companion, FR). The electrochemical measurements were carried out in a water-jacked electrochemical cell in PBS buffer (pH 7.2, 20 mM phosphate buffer, 0.14 M NaCl). A platinum spiral wire was used as counter electrode and all potentials were referred to a Ag/AgCl (3 M NaCl) electrode (BAS, West Lafayette, IN). A 3 mm diameter glassy carbon electrode (BAS, West Lafayette, IN) was used as working electrode for the homogenous kinetic studies. Each experiment was reproduced 3 times.

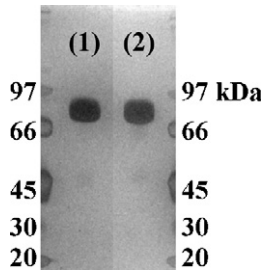


**Fig. 1.** SDS-PAGE followed by silver staining of the commercial (lane 1) and the purified GOx (lane 2).

### 2.3. Purification of commercial GOx

The GOx was purified by hydrophobic interaction chromatography, then by anion exchange chromatography using an AKTA purifier system. The purchased GOx was dissolved in the binding buffer (pH 7.5, 1 M $(NH_4)_2SO_4$, 20 mM sodium phosphate buffer), and then desalted through a PD10 column with the same binding buffer. Prior to use, the PD10 column was equilibrated with 25 ml of binding buffer for 5 times (5 ml/time), then the protein solution (2.5 ml) was loaded on the column and eluted with the same binding buffer (3.5 ml). The procedure removed part of the salts and other small molecules in the commercial enzyme powder.

The obtained enzyme solution was applied to a HiTrap Phenyl HP column (1 ml) pre-equilibrated with the binding buffer. GOx was eluted from the column at a flow rate of 1 ml/min with a linear gradient of 1–0 M $(NH_4)_2SO_4$ (in a 20 mM sodium phosphate buffer, pH 7.5). GOx elution was continuously monitored at 280 nm and 452 nm to respectively discriminate protein and its cofactor FAD (Flavin Adenine Dinucleotide). Active fractions were pooled, concentrated using an Amicon Ultra 15 (cut off 10 kDa) membrane and desalted on a PD 10 column with the binding buffer used for the second step (20 mM sodium phosphate buffer, pH 8.0).

GOx solution was then applied to a HiTrap Q FF column (1 ml) pre-equilibrated with 20 mM sodium phosphate buffer, pH 8.0. It was then eluted at 1 ml/min with a linear gradient of 0–0.4 M NaCl (in a 20 mM sodium phosphate buffer, pH 8.0). Fractions containing GOx activity were pooled, concentrated and equilibrated with the storage buffer (30 mM sodium phosphate, pH 5.1) and stored at 4 °C. The purity of GOx was evaluated on sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining. Protein concentration was measured by UV–vis spectroscopy according to absorbance at 280 nm and 452 nm. The global purification yield is 50%.

## 3. Results and discussion

### 3.1. Enzyme purification

We first measured the real total protein concentration of a commercial GOx by UV–vis spectroscopy at 280 nm ($\varepsilon = 1.67\ cm^{-1}\ mg^{-1}\ ml$) (Frederick et al., 1990). FAD concentration was also measured by absorption at 452 nm ($\varepsilon = 12.83\ cm^{-1}\ mM^{-1}$) (Swoboda and Massey, 1965) to check that two FAD molecules per GOx were bound. We found that our batches of commercial powders contained $(82 \pm 6)$ wt.% of GOx. Because only one band corresponding to the GOx monomers was observed by SDS-PAGE followed by silver staining (Fig. 1, lane 1), our results suggested that the remaining ≈20 wt.% corresponded to non-protein compounds.

The commercial GOx was then purified by fast protein liquid chromatography (FPLC) by a two-step procedure. In a typical experiment, hydrophobic interaction chromatography (HIC) was followed by anionic exchange chromatography. These two steps

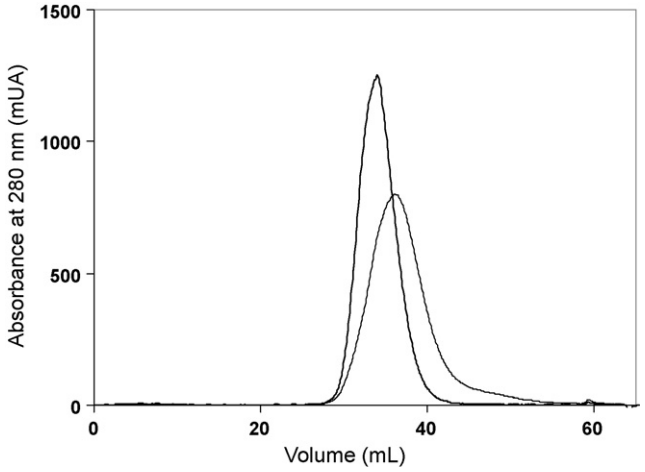


**Fig. 2.** Elution profiles obtained by anionic exchange chromatography for a pre-purified GOx on HIC (thick line) and a commercial GOx (thin line).

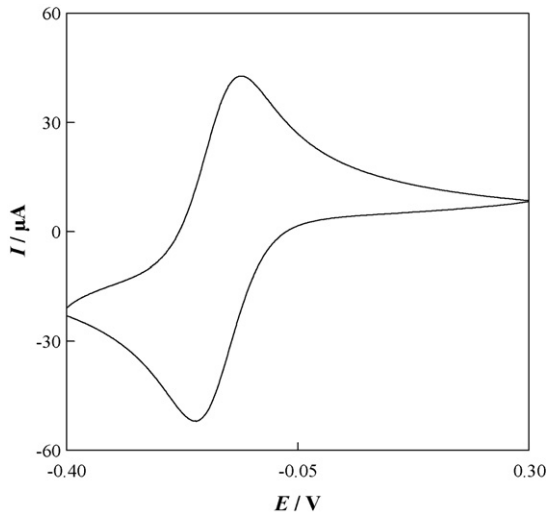


**Fig. 3.** Cyclic voltammogram of the "wired" purified GOx electrode with a total loading of 66 μg biocatalyst under argon in pH 7.4, 20 mM phosphate buffer containing 0.14 M NaCl with a scan rate of 20 mV $s^{-1}$.

were necessary to ensure a good homogeneity of the sample. Fig. 2 shows the elution profiles obtained by anionic exchange chromatography for a pre-purified GOx on HIC and a commercial GOx. When GOx was first pre-purified on HIC, a more symmetrical and narrower peak was observed compared to the commercial GOx, indicating that a more homogeneous sample was obtained (Dai et al., 2002).

The kinetic parameters measured by UV–vis spectroscopy for the commercial and the purified GOx in solution are compared in Table 1 (supporting information). Specific activity, $k_{cat}$ and $K_m$ were determined by Eadie-Hosftee plot using a large range of D-glucose concentration (from 2 μM to 1 M). After normalization by the exact protein concentration, there is no significant difference between the purified and the commercial GOx (the specific activity ≈430 U $mg^{-1}$, $k_{cat} \approx 1300\ s^{-1}$ and $K_m \approx 26$ mM). These results suggest that the purification procedure did not lead to enzyme improvement when the kinetic parameters were measured in solution using classical spectroscopic protocols. This observation may explain why enzyme purification is so rarely considered as necessary for electrochemical experiments. Nonetheless, as shown in Table 1, significant errors may be done if the real concentration in the sample is not known and if the enzyme activity is not checked prior to experiments.

We first assessed the influence of the GOx purity in solution. The homogenous reaction rate constant between glucose oxidase and ferrocenemethanol was measured by cyclic voltammetry using Bartlett's formalism (Bartlett and Pratt, 1995; Flexer et al., 2008) in the presence of 100 mM glucose concentration to find out if it could be affected by the impurities:

$$I = nFAm_{\sum}(D_m \kappa e_{\sum})^{1/2}$$

where $n$ (1) is the number of electrons; $A$ (0.0707 $cm^2$) is the electrode area; $m_\Sigma$ (2.5 mM) and $e_\Sigma$ are respectively the total concentration of ferrocenemethanol and enzyme in solution, $D_m$ ($5.73 \times 10^{-6}\ cm^2\ s^{-1}$) is the diffusion coefficient of the mediator, $F$ is the Faraday constant and $\kappa$ the reaction rate constant between glucose oxidase and ferrocenemethanol. From the slope of the plots of the limiting current versus the square root of the enzyme concentration, and after normalization by the exact protein concentration, $\kappa$ was calculated to be $7.37 \times 10^6\ M^{-1}\ s^{-1}$ for the purified GOx and $5.07 \times 10^6\ M^{-1}\ s^{-1}$ for the commercial GOx under argon. These results clearly indicate that the presence of impurities prevents a good electron transfer between the enzyme and an artificial redox mediator, indicating that the purification process may be a necessary step that can increase by up to 40% the rate constant.

### 3.2. Voltammetric characteristics

"Wired" enzyme electrodes, comprising enzymes connected electrically through redox polymers, which swell to produce electron-conducting hydrogels, are already used in experimental subcutaneously implanted electrodes and may be used in future miniature, microwatt producing, membrane-less biofuel cells (Heller, 2006). In the present study, electrodes were modified with a bioelectrocatalyst made either with purified GOx or commercial GOx and a −0.19 V vs. Ag/AgCl redox polymer, PVP-[Os(N,N′-alkylanated-2,2′-bi-imidazole)$_3$]$^{2+/3+}$. Electrostatic adducts between GOx, which is polyanionic at neutral pH, and the polycationic redox polymer was further stabilized by chemical cross-linking with PEGDGE (poly-ethylene glycol (400) diglycidyl ether). Redox hydrogels allowed the electrical "wiring" of the GOx redox centers to the electrode surface and allowed the fast permeation of water, oxygen and transport of electrons. Conduction is mainly governed by collisional electron transfer from reduced to oxidized redox centers tethered to the polymer backbone (Heller, 1992). The limiting value of the electrooxidation current is defined by the polymer/GOx ratio. It can be increased upon increasing the polymer/enzyme mass ratio, unless the electrostatic adduct between the polycationic polymer and the polyanonic GOx precipitates. The polymer/enzyme mass ratio can also be increased if the specific activity of the enzyme is higher and less enzyme mass is required (Mano et al., 2004).

Fig. 3 shows the cyclic voltammogram of a glassy carbon electrode coated with the purified GOx under argon at 20 mV $s^{-1}$ in a 20 mM phosphate buffer pH 7.4 at 37 °C. The optimum coating consisted of 67 wt.% PVP-[Os(N,N′-dialkylated-2,2′-bi-imidazole)$_3$]$^{2+/3+}$, 28 wt.% GOx and 5 wt.% PEGDGE, while it consisted of 60 wt.% PVP-[Os(N,N′-dialkylated-2,2′-bi-imidazole)$_3$]$^{2+/3+}$, 35 wt.% non-purified GOx and 5 wt.% PEDGE for the earlier reported bioelectrocatalyst (Mano et al., 2004). As shown in Fig. 3, the cyclic voltammogram exhibits the characteristic of a reversible-surface bound couple with an apparent redox potential of −190 mV vs. Ag/AgCl. The peak height varies linearly with the scan rate, showing that the redox couple is surface-confined. At 20 mV $s^{-1}$, the voltammogram exhibited a symmetrical wave with $\Delta Ep = 50$ mV separation of the oxidation

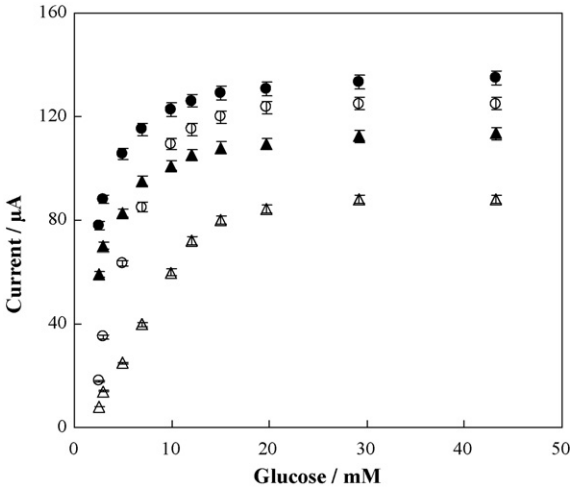


**Fig. 4.** Dependence of the steady-state current on the glucose concentration for the electrodes poised at 0 V vs. Ag/AgCl in pH 7.4, 20 mM phosphate buffer containing 0.14 M NaCl with a rotating rate of 500 rpm. Purified GOx under argon (●) and $O_2$ (○). Commercial GOx under argon (▲) and $O_2$ (△). The total biocatalyst loading is 66 μg and the relative standard deviation (R.S.D.) for three parallel measurements is about 4.3%.

and reduction peak while it was 70 mV for the non-purified GOx. This difference indicates a better charge transport in the new bioelectrocatalyst. The width of the peak at half-height, $E_{whm}$, was 112 mV whereas it was 135 mV for the earlier bioelectrocatalyst, also reflecting a better segmental mobility in the new system. By improving the enzyme purity, we were able to increase the optimal polymer/enzyme weight ratio from 1.7 for the non-purified GOx to 2.4 for the purified GOx leading to a better charge transport in the hydrogel.

### 3.3. Glucose electrooxidation

Fig. 4 shows the dependence of the current on the glucose concentration at 0 V vs. Ag/AgCl in a PBS buffer at 500 rpm when the electrode was modified with 35 wt.% of commercial GOx (i.e., 28 wt.% of pure GOx and 7 wt.% of impurities) and 35 wt.% of purified GOx, under argon and under $O_2$. Under argon, the current increased with the glucose concentration up to 50 mM, where a plateau of 135 μA was reached for the purified GOx and 125 μA for the commercial GOx. At 45 mM glucose concentration, the current was 19% lower for the commercial GOx than it was with the purified GOx; at 5 mM glucose concentration the current was 40% lower for the commercial GOx than it was for the purified GOx. The glucose concentration dependences of the current under argon were plotted as Eadie–Hofstee plots and $K'_m$, the apparent Michaelis–Menten constant (Castner and Wingard, 1984; Mano et al., 2005), was found to be $(1.9 \pm 0.2)$ mM for the purified enzyme and $(2.5 \pm 0.4)$ mM for the non-purified. This slight difference could be attributed to the lower enzyme concentration and/or a less efficient electron transfer from the enzyme to the redox polymer, when the commercial GOx was used instead of purified GOx.

In our systems, as the $O_2$ partial pressure is increased, the anodic glucose electrooxidation current usually decreases because $O_2$ competes with the redox polymer for the GOx-$FADH_2$ electrons (Eqs. (1) and (2)):

$$\beta\text{-d-glucose} + 2\,[\text{redox polymer}]^{3+} \rightarrow \delta\text{-gluconolactone} + 2\,[\text{redox polymer}]^{2+} + 2H^+ \quad (1)$$

$$\beta\text{-d-glucose} + O_2 \rightarrow \delta\text{-gluconolactone} + H_2O_2 \quad (2)$$

As seen in Fig. 4, switching the gas from argon to oxygen results in drastic changes. At 45 mM glucose concentration, the current is 10% lower for the purified GOx than it was under argon and 20% lower for the commercial GOx than it was under argon; at 5 mM glucose concentration the current is half of its value for the purified GOx than it was under argon and the current is four times lower for the commercial GOx than it was under argon. $K'_m$ was $4.9 \pm 0.3$ mM for the purified GOx and $11.9 \pm 3.3$ mM for the commercial GOx. As previously reported, the loss of current under oxygen depends on the glucose concentration but these results demonstrated that it depends also on the enzyme purity. Impurities contained in the commercial enzyme caused the screening of the charges of the electrostatically bound polycationic polymer and polyanionic enzyme, causing their dissociation and making the "wire" less efficient. As a result, as the $O_2$ partial pressure is increased, the apparent Michaelis–Menten constant increased from 2.5 mM under argon to 11.9 mM under $O_2$ because $O_2$ competes with the oxidized sites of the film for electrons. A greater glucose concentration is then required to reach half of the maximal catalytic current when the concentration of $O_2$ is higher. When the enzyme was purified, the interaction between the polycationic redox polymer and the enzyme was increased, forming a more homogenous, leather like gel. Most of the electrons were captured by the redox polymer rather than by dissolved $O_2$. This explains why at 5 mM glucose concentration, the current was three-fold higher under $O_2$ when the bioelectrocatalyst was made with a purified enzyme. We also performed control experiments with a bioelectrocatalyst made of 28 wt.% of purified GOx (not shown). At any glucose concentrations, under $O_2$ and $N_2$, the electrooxidation currents were higher than those obtained with an electrode modified with 35 wt.% of commercial GOx. This result also indicates that the better currents are a result of the better enzyme "wiring".

To asses our hypothesis and because the SDS-PAGE suggested that the remaining ≈20 wt.% corresponded to non-protein compounds, we investigate the effect of the commons stabilizers used for GOx from *Aspergillus niger*, i.e., chloride, ammonium sulphate and phosphate. Electrodes containing 28 wt.% of purified enzyme, 7 wt.% of stabilizers, 60 wt.% of the redox polymer and 5 wt.% PEGDGE were prepared. In each case, the electrooxidation current was lower than expected both under Argon and $O_2$. The observed partial loss of glucose electrooxidation current in presence of these stabilizers can be attributed to the weakening of the electrostatic bond between the polyanionic enzyme and the polycationic redox polymer, causing their dissociation and making the "wiring" less effective.

### 3.4. Biofuel cell experiments

To form a miniature membrane-less glucose/$O_2$ biofuel cell, we combine the novel purified GOx anode with a previously described $O_2$ electroreducing cathode (Mano et al., 2002b; Mano et al., 2003a). The biofuel cell was made of two 7 μm diameter, 2 cm long carbon fibers (Mano et al., 2003b). They were made hydrophilic, then the anode was coated with 28 wt.% of the purified glucose oxidase, 67 wt.% of PVP-[Os(N,N′-dialkylated-2,2′-bi-imidazole)$_3$]$^{2+/3+}$ and 5 wt.% PEGDGE. The cathode was coated with 15.5 wt.% of Bilirubin Oxidase, 77 wt.% PAA-PVI-[Os(4,4′-dichloro-2,2′-bipyridine)$_2$Cl]$^{+/2+}$ (redox potential + 0.34 V vs. Ag/AgCl) and 7.5 wt.% PEGDGE. Fig. 5 shows the dependence of the power density on the operating voltage of the miniature membrane-less biofuel cell in physiological solution under air when one anode is made with a non-purified GOx and the other with a purified GOx. In the quiescent physiological solution, the power density of the biofuel cell made with the purified GOx is 315 μW cm$^{-2}$, twice the power density obtained with the non-purified GOx. These results are consistent with the value of Fig. 4

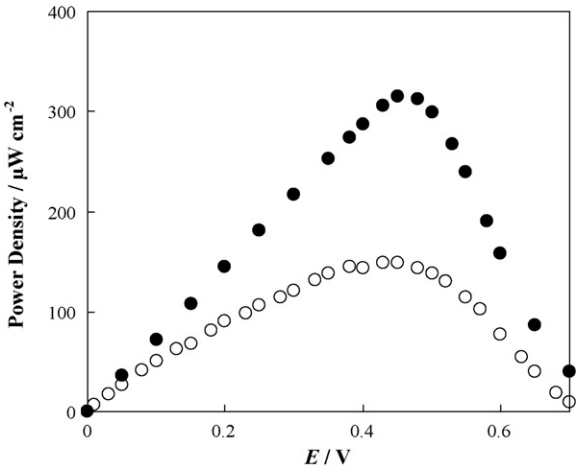


**Fig. 5.** Dependence of the power density on the operating voltage in quiescent air-saturated physiological solution (20 mM phosphate buffer, pH 7.24, 0.14 M NaCl) with 15 mM glucose at 37 °C. Purified GOx (●) and commercial GOx (○). The biocatalyst loading for each electrode is 266 μg.

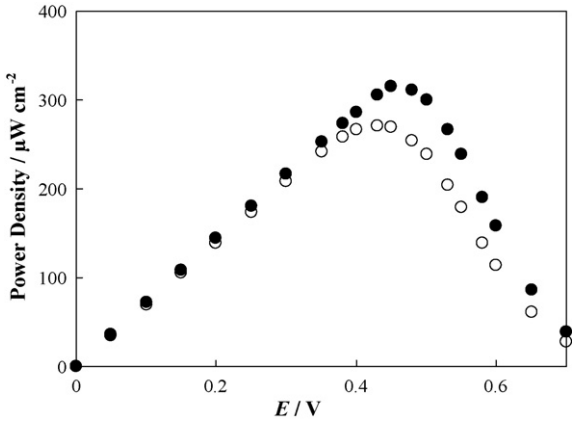


**Fig. 6.** Dependence of the power density on the cell voltage in quiescent air (●) and oxygen (○)-saturated physiological solution (20 mM phosphate buffer, pH 7.24, 0.14 M NaCl) with 15 mM glucose at 37 °C. The biocatalyst loading for each electrode is 266 μg.

because the biofuel cell is limited by its anode at low glucose concentration (Mano et al., 2003b). Fig. 6 shows the dependence of power density of biofuel cell made with the purified GOx under air and 1 atm $O_2$. Earlier (Mano et al., 2003b), we showed that under 1 atm $O_2$, the power density of a biofuel cell made with a non-purified GOx was 30% lower than it was under air while it is only 14% lower with the newly purified GOx. The biofuel cell stability was not affected by the purification process. When the cell operated continuously for a 1 week in a PBS buffer at +0.52 V, it only lost ≈6% of its power per day.

## 4. Conclusion

In summary, by coupling UV–vis spectroscopy, FPLC and SDS-PAGE, we found that our batches of commercial GOx powder only contained about 80 wt.% of GOx and 20 wt.% of non-protein species. We showed that the enzyme purification was a necessary step in our system to improve the electron transfer at the electrode surface. By introducing a purified enzyme into our bioelectrocatalyst, we increase the interaction between our enzyme and redox polymer forming a more homogenous, leather like gel. By doing so, most of the electrons are captured by the redox polymer rather than by dissolved $O_2$. At 5 mM glucose concentration and under $O_2$, the current is three-fold higher when using a purified enzyme than it is when using a non-purified enzyme. Because most glucose–$O_2$ biofuel cell are limited by their anode at glucose concentration <15 mM (Mano, 2008; Mano et al., 2003b), built with this novel anode, we showed that a miniature implantable membrane-less glucose–$O_2$ biofuel cell could produce, under air, twice the power density that is usually obtained with a non-purified GOx. In addition to possible applications in biofuel cell and glucose sensors, we believe that this strategy may be extended to others enzymatic systems and may offer a new strategy to circumvent poor electrical contact between enzymes and electrodes surfaces.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.07.015.

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