Novel Three Dimensional Macrocellular Carbonaceous Biofuel Cell

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Abstract

Here we report the first membrane-free biofuel cell obtained by using three-dimensional carbonaceous foam electrodes. We first developed a new synthetic pathway to produce a new carbonaceous foam electrode material bearing porosity both in the meso and macroporous scale. We proved that by increasing the porosity of our three-dimensional foams we could increase the current density of our modified electrodes. Then, by choosing the right combination of enzyme and mediator, and the right loading of active components, we achieved high current densities for an anodic system. Finally, we combined the improved cathode and anode to build a new membrane-free hybrid enzymatic biofuel cell consisting of a mediated anode and a mediator-free cathode.

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Introduction

Enzyme based biofuel cells convert chemical to electrical energy employing enzymes as biocatalysts, with promising advantages in terms of cost, simple construction, renewable catalysts and fuels, while operating in mild conditions. Enzymatic biofuel cells have been proposed for applications in niche areas of power generation such as implantable medical devices, or portable electronic devices under specific conditions, such as remotely located, or devices needing a non-toxic fuel. Today enzymatic biofuel cells are still limited by both lifetime (due to enzyme denaturation and desorption), and lower power densities that restrict their application. Up to now most biofuel cells involve the immobilization of enzymes on inert electrode materials such as glassy carbon. Necessary condition for a material to be a good candidate for biofuel cell construction rely on the interplay between good electrical conductivity and low cost, though these conditions alone are not enough. Carbonaceous materials, in different forms, have been proposed as good candidates because they fulfil the two previous requirements, in addition to being chemically stable and biocompatible. The design of electrode materials is critical to overcome mass transport limitations, enzyme-electrode or mediator-electrode electron transfer limitations, and optimisation of enzyme loading on the electrode surface. Circumventing these issues, will eventually lead to an improvement in current (and hence power) generation. Our group is among the firsts to have pioneered the idea of using three-dimensional electrodes for the construction of biofuel cells, as it is already common practice in classical fuel cells. The main objective behind this simple idea is to maximise the reactive surface area of the electrodes, to allow for an enhancement of enzyme loading, and consequently current production. The idea of three-dimensional electrodes automatically leads to the necessity of an interconnected hierarchical porous structure within the electrodes. Large multidirectional pores in the micrometer scale are needed for efficient fuel delivery to comply with the high demand of substrate of high...
enzymatic activity. On the other end, smaller pores in the tens of nanometres are needed to allow for larger surface area that will both maximise enzyme loading and minimize as far as possible steric hindrance. Moreover, if the right nanoporosity is chosen, enzyme molecules will then be more efficiently trapped in the porous structure, avoiding loss of catalysts, while keeping their activity.\textsuperscript{6, 7, 11} The chemical functionalization of electrode materials, and the electrode roughness in the sub-nanometer scale, will both help to stabilize the enzyme and serve as anchoring sites. Eventually they will favour the direct electron transfer (DET) from the enzyme to the electrode surface, or via a redox relay (mediated electron transfer, or MET).

With these ideas in mind, we have recently designed highly efficient carbonaceous foam electrodes with hierarchical porosity.\textsuperscript{6, 7, 12} The synthesis of the electrode material is very simple and achievable at low cost, and most important, allows a fine tuning of the pore sizes. Pore size tunability is desired in order to improve mass transport of both substrate and products and trigger the adsorption and stabilization of different enzymes. We showed that glucose electrooxidation current was 13-fold higher on these carbonaceous foams than on classical flat glassy carbon when the same enzyme and mediator loading was used on both electrodes.\textsuperscript{6} Later on, we proved that bilirubin oxidase (BOD) adsorbed onto the same electrodes was able to reduce O\textsubscript{2} to water through a direct electrons transfer from the electrode to the enzyme, without the need of additional redox mediator.\textsuperscript{7} Also, among the pioneer work on the field is that of Minteer and Cooney, who have proposed the use of chitosan scaffolds doped with carbon nanotubes as three-dimensional electrodes.\textsuperscript{8, 13} Recently, Attanasov \textit{et al.} used reticulated vitreous carbon to support similar chitosan carbon nanotubes scaffolds.\textsuperscript{14, 15} Although high current densities on these materials are yet to be shown, this approach is most interesting because it is one of the few that allows for tunable pore sizes. Cosnier \textit{et al.} fabricated three-dimensional carbon nanotube electrodes by mechanical compression of CNT and enzymes.\textsuperscript{16} The resulting fully mediator-free biofuel cell presents This is a post-print version of the following article: Flexer, Victoria, Brun, Nicolas, Destribats, Mathieu, Backov, Rénal and Mano, Nicolas (2013) A novel three-dimensional macrocellular carbonaceous biofuel cell. \textit{Physical Chemistry Chemical Physics}, 15 17: 6437-6445.
the highest power density reported to date for a glucose/O\textsubscript{2} biofuel cell. Despite this very good result, it should be noticed that in those materials there is no control over the final porosity. Kano et al. also obtained unprecedentedly high power values with a passive type biofuel cell, using piled-up commercial carbon fibre sheets as three-dimensional electrodes\textsuperscript{17} however without any control over the porosity of the material. Calabrese-Barton et al. have also proposed interesting materials based on carbon nanotubes\textsuperscript{18, 19} A few other examples of three-dimensional materials with interesting properties have also been proposed\textsuperscript{20-24}

Here we built in from the results of our previous work\textsuperscript{6, 7} First, we developed a new synthetic pathway to produce a new carbonaceous foam material with increased porosity both in the meso and macroporous scale. We proved that by increasing the porosity of our three-dimensional foams we could increase the current density in our electrodes. Then, by choosing the right combination of enzyme and mediator, addressed with the right loading of active components, we achieved high current densities for an anodic system. Finally, we combined the improved cathode and anode to build a new membrane-free hybrid enzymatic biofuel cell consisting of a mediated anode and a mediator-free cathode.

Results and discussion

We worked with two different carbonaceous foam materials, 60-Carb(PHIPE) and 40-Carb(HIPE). This last one is the same material that we have already presented in our previous work\textsuperscript{6, 7} The detailed synthetic pathways are described in the experimental section. Final carbonaceous materials can be first described as interconnected macrocellular foams (Figure 1 a, b). Their macro-structural characteristics have been quantified through mercury porosimetry and are shown in Table 1. At this stage it is of importance to mention that for
both materials the mechanical properties are good enough as to support pressurized mercury infiltration.

Considering the synthetic routes, THF is used as solvent for the resorcinol-formaldehyde carbon precursor dilution. The THF departure through the applied thermal treatment is promoting these materials micro-mesoporosity within the walls. The micro-mesoporosity was addressed through Nitrogen physi-sorption measurements (Figure 2). We can see strong adsorption-desorption curves at low relative pressure ($P/P^0$) indicating that these carbonaceous macrocellular electrodes are bearing strong microporosity within the walls. We can notice hysteresis at high relative pressure (from 0.6 to 0.9), a feature that indicates also the presence of high mesoporosity within these foam walls. This feature is particularly evident for the 60-Carb(PHIPE) material. Indeed, in such case, the high mesoporosity is certainly induced by the silica nanoparticles used to stabilize the native emulsion oil/water interface of the Si(PHIPE) hard templates. Also, BET and BJH surface areas can be extracted from these nitrogen physi-sorption experiments and the micro-mesoscopic characteristics are summarized within Table 1. We have to underline that the microscopic surface area can be deduced from BET surface area minus the BJH surface area. Normally, values calculated in this way should be close to t-plot extraction values that depict exclusively the microporosity. This is the case here where we found the t-plot surface area of the 60-Carb(PHIPE) being equal to 425 m$^2$g$^{-1}$, while the t-plot value for the 40-Carb(HIPE) is 417 m$^2$g$^{-1}$ (which compares well with 504 and 630 m$^2$g$^{-1}$, respectively from BET -BJH). We can notice that the 60-Carb(PHIPE) foam is bearing both lower bulk and skeleton densities than the 40-Carb(HIPE). This feature is explained by the fact that the 60-Carb(PHIPE) is developing an extremely high mesoscopic surface area of around 2700 m$^2$g$^{-1}$ to be compared with the 700 m$^2$g$^{-1}$ developed for the 40-Carb(HIPE) macrocellular foams. In summary, the most distinctive feature of the new 60-Carb(PHIPE) carbonaceous foams as compared to the classic...
40-Carb(HIPE) is a considerably increased mesoporosity. They also show 12% increase in the macrososcopic pore volume, while the microscopic characteristic are very similar. We can also notice that the surface area of the material 60-Carb(PHIPE) appears to be very high. To explain such high value we performed fractal analyses of the surface, considering the material surface roughness. Material roughness was evaluated from the determination of the Fractal surface Dimension (Ds), which can be deduced from the nitrogen isotherm adsorption curves. Ds was calculated according to the procedure described previously.\textsuperscript{25} The experimental adsorption isotherm is modified according to:

\[
\theta = K \left[ \log\left(\frac{P_0}{P}\right) \right]^{-\nu}, \quad (1)
\]

where \( \nu = 3 - Ds \), \( \theta \) is the relative adsorption calculated by normalizing the curve with the highest adsorption value, K is a constant and Ds is the surface fractal dimension that we are looking for. An easy way to obtain Ds is to convert Eq. (1) according to:

\[
\log(\theta) = \log(K) - \nu \log(\log\left[\frac{P_0}{P}\right]), \quad (2)
\]

Ds is deduced from the slope of the line, and it must vary between 2 (flat surface) and 3. Any value higher than 2 describes an increasing surface roughness. The adsorption range to be used for this analysis has to be taken within the partial pressure range \(0.05 < \frac{P}{P^o} < 0.3\) and limited below the Kelvin condensation step that corresponds to the voids filling.\textsuperscript{26} The curves are proposed within the Figures 3a, b where we can estimate that Ds for the 40-Carb(HIPE) is 2.21 while Ds for the 60-Carb(PHIPE) is 2.55. These results indicate that the surface roughness of the 60-Carb(PHIPE) is very high, where Nitrogen multilayer adsorption above partial pressure of 0.3 is certainly not to be excluded providing the high surface area provided trough the BET equation. To confirm this quantitative information high resolution scanning electron microscopy was performed over the electrodes surfaces to evaluate the electrode roughness (Figure 4). Considering the HR-SEM images of the Figure 4, we can observed
without any ambiguity that the surface of the 60-Carb(PHIPE) cathode is much rougher than the 40-Carb(HIPE).

After having characterized these new material void spaces at the micro-, meso and macroscopic length scales, we studied their performance as prospective electrode materials for enzymatic biofuel cells. We first analysed the results of the cathodic and anodic systems separately, in a classical 3 electrodes configuration. Subsequently, we connected the anodic and cathodic system together and we measured its joint performance in a classical membrane-free biofuel cell configuration. We started our optimization process by looking for the optimal buffer concentration, as suggested by Sakai et al.\textsuperscript{17} Because of its big geometrical size, our porous materials are very unlikely to be used in implantable devices, were micrometer sized materials such as carbon fibres or carbon nanotubes fibres would probably be preferred.\textsuperscript{27-29} Therefore, there is no point in restraining the experimental conditions to the classical 20 mM phosphate buffer experiments, normally used to mimic physiological conditions. Figure 5 (a) shows the catalytic current density for $O_2$ reduction as a function of the phosphate buffer concentration (no added salts) for a 60-Carb(PHIPE) modified BOD electrode. Indeed, the lowest current density is obtained with the less concentrated buffer, suggesting that there is not enough buffer capacity in the confined environment for the high enzyme activity. The highest current was obtained at 100 mM buffer concentration, and therefore the remaining experiments reported in this article have been performed in this buffer.

We then compared the performance of the new carbonaceous foams with improved porosity (60-Carb(PHIPE)) with the materials used in our previous work (40-Carb(HIPE)).\textsuperscript{7} Both electrodes were of the same thickness (1 mm), since we showed earlier\textsuperscript{7} that for 3-dimensional electrodes, the current is proportional to the electrode thickness. Each electrode was modified following exactly the same protocol and was measured under the same conditions. Figure 5 (b) shows the catalytic current density measured at 0 V for the 60-
Carb(PHIPE) electrode modified with BOD (thick line, 1mm thick) and for the 40-Carb(HIPE) modified electrode (thin line, 1mm thick)

The results obtained with the modified 40-Carb(HIPE) electrode are in agreement with our previous results. However, the current density is increased by 30% with the new 60-Carb(PHIPE) electrode. We hypothesise that this enhanced current density is directly related to the increased meso and macroporosity. With regards to the mesoporosity, we suggested in our previous work that enzyme molecules will most probably be trapped in the mesopores within the carbonaceous foam. We name mesopores all the pores with size distribution in the 1.7 - 50 nm range, and BOD diameter is estimated to be ~6nm. We have just shown in table 1 that, 60-Carb(PHIPE) present almost 4 times higher mesopore surface area than the old material, 40-Carb(HIPE). Therefore we expect that this higher mesoporosity has been translated into a higher enzyme loading. We have also shown in table 1 that the 60-Carb(PHIPE) also shows an increase in macroporosity. We believe that this will produced an improved mass transport both towards and out of the carbonaceous foam. Dissolved O₂ will better diffuse inside the porous material and towards all the enzyme moieties buried inside the porous structure, while the accelerated diffusion of OH⁻ ions outside the porous material will decrease the formation of pH gradients. Figure 5 (c) shows the cyclic voltammogram for O₂ reduction on the 60-Carb(PHIPE) BOD modified electrode. The onset potential for O₂ reduction is about +0.45V which corresponds to the potential of the T1 copper. The differences in the apparent current densities observed in figures 5 (a) to (c) are explained by the different thicknesses of the electrodes used in these experiments (0.5, 1.0, and 1.7 mm respectively). Moreover, figure 5 (c) has not been corrected by the background current, and therefore, a big capacitive current is superposed to the catalytic signal (for the analysis further down, the stationary current for this electrode poised at 0 V and 1000 RPM was 1.3 mA cm⁻²).
Our first communication on 3-dimensional porous foam electrodes was focused on showing that these materials could be used to immobilise enzymes and could produce a catalytic current density that was 13 times higher than the current obtained onto a flat glassy carbon electrode, for the same enzyme loading.\textsuperscript{6} However that system was far from being optimised, mainly because the potential, $E^0$, of the redox mediator was too high to build a biofuel cell. Indeed, in order to build an efficient biofuel cell, anode and cathode potentials should be as far apart as possible to maximise the voltage output of the cell.\textsuperscript{1,2} Even for biosensors applications, low operating voltages are desired to avoid interferences in complex samples.\textsuperscript{31} In this work, we have decided to try two different redox mediators with two different redox potentials: polymer I (PVP-[Os(N,N’-dialkylated-2,2’-bis-imidazole)$_3$]$^{2+/3+}$ with $E^0 = -0.19$V vs. Ag/AgCl) and polymer II (PVP-Os(1,1’-dimethyl-2,2’biimidazole)$_2$-2-[6methylpyrid-2yl]imidazole)$^{2+/3+}$, with $E^0 = -0.03$V vs. Ag/AgCl. Figure 6 (dotted line) shows the electrooxidation of glucose (50 mM) for the 40-Carb(HIPE) electrode modified with 62.7 wt\% polymer, 31.3 wt\% GOx, and 6 wt\% PEGDGE using polymer I (Anode 1). The cyclic voltammogram does not show a perfect sigmoidal shape, and therefore the current increase with potential is rather slow. This may be due to the effect of O$_2$ on the polymer.\textsuperscript{32} This phenomenon is still under investigation. The maximum value of current density is $\sim 6$ mA cm$^{-2}$, similar than previously obtained.\textsuperscript{6} However, this high current density was achieved at a lower potential than in our previous report (100 mV more negative), and we also observe a high current density at lower potentials, 1.8 mA cm$^{-2}$ when the electrode is poised at 0 V, and 3.3 mA cm$^{-2}$ when poised at 0.2 V. At these lower electrode potentials the catalytic current was negligible in our previous report. Moreover, these high values of electrocatalytic signal were obtained with a lower polymer enzyme/loading, and by only applying a mild forced convection (electrode rotating at 1000 RPM vs. 10000 RPM in our previous report)\textsuperscript{6}, showing that the change in parameters indeed improves the efficiency of our system.

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To further improve our anodes, electrodes were prepared with polymer II (anode 2). This polymer has already shown to be a more efficient redox mediator with respect to polymer I, when immobilized on a flat glassy carbon electrode. The increase in the electrooxidation current is obvious as seen on the thick line for an electrode rotating at 1000 RPM. The line of intermediate thickness shows the current response in the absence of rotation, showing that the system is still very efficient even in the absence of forced convection. Both polymers I and II possess an Os redox centre that is linked to the polymer backbone through a long carbon chain (a so called tether), that gives the redox centre more freedom to move and react with enzymes, while the polymeric backbone keeps the hydrogel structure. This long carbon chain or tether, was absent from the polymer used in our previous work. Finally, we avoided the use of NaIO4 in the catalysts solution (as opposed to our previous protocol), since we found out that its effect was detrimental when the hydrogel is immobilised inside the porous structure. All these changes, have led us to obtain current densities that are more than 2.5 higher than in our previous report. The high current values of the bioanode made of polymer II represent, to the best of our knowledge, the highest current densities measured for an enzyme modified electrode at low electrode potentials, i.e. at potentials where the anode of a biofuel cell would work, and in the presence of O2, a point of outmost importance if we think that O2 will most probably be present in a membrane-free enzymatic biofuel cell. At 0 V, the current has already reached a value of 5.7 mA cm⁻², both in the absence and in the presence of forced convection. This value is 20% higher than that reported by Cosnier et al under similar conditions. At 0.1V, the current reached already 10.1 mA cm⁻² (with or without rotation), which represents more than twice the value reported by Kano et al. at the same buffer concentration (0.1 M), and almost the same value they reported in 1 M buffer solution (the optimal conditions for those authors). At 0.2 V we measured 14.6 and 13.9 mA cm⁻² (with and without rotation respectively). The maximum value of 19.3 mA cm⁻² was obtained.
at 0.38 V while rotating the electrode; whereas we recorded 15.6 mA cm$^{-2}$ at 0.3 V under quiescent conditions. Calabrese Barton et al. reported 22 mA cm$^{-2}$ for a GOx electrode\textsuperscript{18}, at 0.55 V, in the absence of O$_2$ and at 4000 RPM, which as the author pointed out, is not suitable for biofuel cell operation. At 0.2 V their electrodes display a current density of only 1.5 mA cm$^{-2}$, while current was null at 0 V.\textsuperscript{18}

After optimization of the anodes and cathode, we combined them to build different configurations of biofuel cells. In all of these, anode and cathode were always in the same compartment, \textit{i.e.} making them membrane-free biofuel cells. Moreover, the anode was always a mediated enzyme electrode (MET), while the cathode was always a direct electron transfer enzyme electrode (DET), making it a hybrid MET-DET biofuel cell. Figure 7 shows the power density as a function of the cell voltage for the three different biofuel cells constructed. The thickest line shows the power density for the biofuel cell made with polymer I + GOx at the anode (BFC1). The intermediate line corresponds to the biofuel cell made with polymer II + GOx at the anode (BFC2). For these two biofuel cells, a mild forced convection was applied, by rotating both the anode and the cathode at a rotation rate of 1000 RPM. The thinnest line corresponds to the biofuel cell built with the anode made with polymer II + GOx under stationary conditions (BFC3). In all three cases the cathode was a mediator-less BOD electrode. For the three curves, the variation of the power density as a function of the cell voltage presents the classical bell-shaped curve characteristic to biofuel cells. The maximum power densities were 202 µW cm$^{-2}$ at 0.40 V (BFC 1); 188 µW cm$^{-2}$ at 0.35 V (BFC2); and 125 µW cm$^{-2}$ at 0.38 V (BFC3). Results shown are for individual experiments, and each biofuel cell experiment was repeated in duplicate. Duplicate results were within 15% of the data shown (the power output was always higher for the cell made using anode 1). As seen in Figure 7, under quiescent conditions, the power density reached by BFC3 (thin line) is 66.5% of the power density when mild forced convection is applied (BFC2). This result tends to
suggest that the porosity of the new 60-Carb(PHIPE) has been almost fully optimised and that the substrate delivery to the inside of the foams is very high, even under stationary conditions. By comparing the power density profiles of the biofuel cells with anodes made with different polymers, BFC 1 and BFC 2, we can note that the use of polymer I at the anode allows for a slight improved power output, ~ 10 %. However at low voltage output, the power density of both devices is the same. This result does not follow the trend of the anodes for the oxidation of glucose when tested separately in a three-electrode cell configuration (see figure 6). For that experimental setup, the performances of anodes 1 and 2 were distinctly different. The oxidation current of anode 2 was much higher than that of anode 1. However, this is not translated to the biofuel cells performances made with those anodes, even though both BFC 1 and BFC 2 were made with similar BOD cathodes. Coming back to the CVs from figure 6, we should notice that at very low potentials, in the interval -0.26 V to -0.12 V, it is the current of anode 1 that outperforms that of anode 2 (as opposed to what happens at higher potentials). This behaviour at low potential is explained by the different $E^0$ of polymer I and polymer II, -0.17 V and +0.03 V (vs. Ag/AgCl) respectively. Surprisingly, the open circuit potentials of both biofuel cells are quite close. This phenomenon is still under investigation. In our system, the current output of the biofuel cell (and hence the power output) is mainly governed by the cathode, as it is normally the case for most biofuel cells. This can be seen by comparing the cyclic voltamograms of the cathode (figure 5 c) and anodes (figure 6) measured separately in a three-electrode configuration cell. It might seem that the performance of the cathode is similar to the performance of the anode made with polymer I, however this is only an apparent impression arising from the very high capacitive current of the cathode.

The power densities of our biofuel cells are, to the best of our knowledge, among the highest of their type. They are at least one order of magnitude higher than reports on electrodes based on three-dimensional chitosan scaffolds, including a recent report with.This is a post-print version of the following article: Flexer, Victoria, Brun, Nicolas, Destraths, Mathieu, Backov, Rénal and Mano, Nicolas (2013) A novel three-dimensional macrocellular carbonaceous biofuel cell. Physical Chemistry Chemical Physics, 15 17: 6437-6445.
also a hybrid MET-DET configuration.\textsuperscript{15} The power density and the voltage are also, respectively, 10% and 150 mV higher than those obtained by Dong \textit{et al.}\textsuperscript{37} for a biofuel cell built with three-dimensional electrodes. However, none of our biofuel cell configurations, reached the highest power densities of 1.3 (DET)\textsuperscript{16} and 1.45mWcm\textsuperscript{-2} (MET) reported to date.\textsuperscript{17} It is worth noting here that in the cited work by Cosnier \textit{et al.}\textsuperscript{16}, electrodes were solely made of carbon nanotubes and enzymes. Not surprisingly, an earlier work that also reached unusually high power densities of 0.74mWcm\textsuperscript{-2}, also used exclusively carbon nanotubes, in the form of carbon nanotube fibers.\textsuperscript{35} Carbon nanotubes have diameters in the range of the nanometer and display excellent electrical conductivity, therefore it is not surprising that the highest power densities in the biofuel cell field have been achieved with pure carbon nanotubes as electrode material. In their work Sakai \textit{et al.},\textsuperscript{17} reached a power density of 1.45mWcm\textsuperscript{-2} because they used an air breathing cathode getting O\textsubscript{2} directly from the air surrounding the BFC and not from dissolved O\textsubscript{2} in solution. Other than these few examples, our biofuel cells show power densities very close to the highest power densities reported in the literature\textsuperscript{38-40}. Most importantly, power densities reported here are much higher than other devices made with three-dimensional electrodes. We prove here that three-dimensional electrodes for enzymatic biofuel cells are not only a promise of high current and power delivery, but are becoming a reality.

\textbf{Conclusions}

Here we present the first membrane-free enzymatic biofuel cell constructed using three-dimensional carbonaceous foam electrodes. Using a new Integrative Chemistry-based\textsuperscript{41,42} synthetic route, we produce new carbonaceous foam electrode materials. The hierarchical porosity has been carefully design to specifically improve the porosity both in the...
meso and macrometre scale. Improvement of the mesoporosity has allowed for a higher enzyme loading, while the increased macroporosity has allowed for an improvement in substrate delivery to the inside of the three-dimensional structure. In addition, the good mechanical properties enable the external shape and size of the electrodes to be designed on demand, or shaped by chiselling from bigger pieces, a very interesting characteristic to incorporate these electrodes into real devices.

Then, by choosing the right combination of enzyme and mediator, and the right loading of active components, we achieved high current densities for an anodic system. Finally, we combined the improved cathode and anode to build a new membrane-free hybrid enzymatic biofuel cell consisting of a mediated anode and a mediator-less cathode.
<table>
<thead>
<tr>
<th>Materials</th>
<th>N₂ Physi-sorption</th>
<th>Mercury porosimetry</th>
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<tr>
<td></td>
<td>BET (m² g⁻¹)</td>
<td>BJH* (m² g⁻¹)</td>
</tr>
<tr>
<td>60-Carb(PHIPE)</td>
<td>3280</td>
<td>2650</td>
</tr>
<tr>
<td>40-Carb(HIPE)</td>
<td>1184</td>
<td>680</td>
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</table>

**Table 1.** Summary of the electrodes porosity characteristic, obtained by N₂ physisorption (micro and mesoporosity) and mercury porosimetry (macroporosity). *BJH surface area calculated from the adsorption curves, above 1.7 -50 nm range of pore sizes.
Figure 1: SEM visualization. a) 60-Carb(PHIE) carbonaceous cathode, b) 40-Carb(HIPE) carbonaceous anode.
Figure 2: Nitrogen physisorption curves (full squares adsorption, empty circles desorption) for
the 60-Carb(PHIPE) carbonaceous cathode a) and b) for the 40-Carb(HIPE) carbonaceous
anode. The embedded figures represent the pore sizes distribution calculated from the BJH
desorption curves.
Figure 3: Surface fractal analysis of the nitrogen adoption curves. a) 60-Carb(PHIPE) carbonaceous cathode and b) 40-Carb(HIPE) carbonaceous anode.
Figure 4: HR-SEM visualisation of the electrodes surface roughness a-c) 60-Carb(PHIPE) cathode and e-f) 40-Carb(HIPE) carbonaceous anode.
Figure 5:  

a) Catalytic current density for O₂ reduction as a function of the phosphate buffer concentration (pH 7.2, no added salts) for a 60-Carb(PHP) modified electrode with BOD. Experiments at 0V vs. Ag/AgCl and at 37°C. 

b) Catalytic current density measured at 0 V vs. Ag/AgCl for the 60-Carb(PHP) modified electrode (thick line, 1mm thick) and for the 40-Carb(HIPE) modified electrode (thin line, 1mm thick). 100mM phosphate buffer, pH 7.2, 37°C, 1000 RPM.  

c) Cyclic voltammogram for the O₂ reduction on the 60-Carb(PHP) modified electrode. 1mV s⁻¹,100mM phosphate buffer, pH 7.2, 37°C, 1000 RPM

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Figure 6: Electrooxidation of glucose (50 mM) for GOx/polymer modified electrodes (62.7 wt% polymer, 31.3 wt% GOx, and 6 wt% PEGDGE) using either PVP-[Os(N,N’-dialkylated-2,2’-bis-imidazole)₃]²⁺/³⁺ (Anode 1) or (PVP-Os(1,1’-dimethyl-2,2’biimidazole)₂-2-[6methylpyrid-2yl]imidazole)²⁺/³⁺ (Anode 2). 1mVs⁻¹, 100mM phosphate buffer pH 7.2, 37°C. Anode 1: (dotted line : 0 RPM, loading : 1300 µg cm⁻² ). Anode 2: (thin line: 0 RPM; thick line: 2000RPM; loading: 1734 µg cm⁻²)
Figure 7: Power density for the three biofuel cells vs. cell voltage. Thicker line, BFC1, Anode: GOx/PVP-[Os(N,N’-dialkylated-2,2’-bis-imidazole)]_{2^{+/3^{+}}} / Cathode: BOD only, 1000 RPM. Intermediate line, BFC2: Anode: GOx/PVP-Os(1,1’-dimethyl-2,2’biimidazole)_{2^{2-}}[6methylpyrid-2yl]imidazole)^{2^{+3^{+}}}/Cathode: BOD only, 1000 RPM. Thinnest line, BFC3: same as the intermediate line but in quiescent conditions. 100mM phosphate buffer pH 7.2, 37°C, 50mM glucose.
Experimental:

Materials.

Glucose Oxidase from *Aspergillus niger* (GOx) and Billirubin Oxidase (BOD) from *Trachiderma tsunodae* were purchased from SIGMA and AMANO respectively, and were purified as previously reported.\textsuperscript{7,38} Synthesis of polymer I (PVP-[Os(N,N’-dialkyalted -2,2’-bis-imidazole)\textsubscript{3}]\textsuperscript{2+/+3} \( E^0 = -0.19 \text{V vs. Ag/AgCl} \)) has been previously reported.\textsuperscript{34} Polymer II (PVP-Os(1,1’-dimethyl-2,2’biimidazole)\textsubscript{2}-2-[6methylpyrid-2yl]imidazole)\textsuperscript{2+/+3}, \( E^0 = -0.03 \text{V vs. Ag/AgCl} \)) was a generous gift from Abbott Diabetes Care\textregistered. All other chemicals were of analytical grade or higher. Electrochemical measurements were performed using a bipotentiostat (CH Instruments, Austin, TX, CHI760C). A plastic beaker was used as an electrochemical cell and was immersed in a thermostatic bath. All experiments reported here were carried out at 37\textdegree C. Solutions were bubbled with O\textsubscript{2} gas for 30 minutes before every experiment, and O\textsubscript{2} atmosphere was kept during all the electrochemical measurements (by bubbling above the solution). Experiments performed in a 3 electrode configuration cell were carried out using a Pt gauze as a counter electrode and a Ag/AgCl reference electrode (all potentials in this paper are referred to this electrode). Some of the experiments (specified in the text) were performed using rotating electrodes (2 rotators in parallel, PINE). All experiments were performed in 100 mM Na phosphate buffer, pH = 7.2 in absence of salts except experiments of Figure 4 (a) were the concentration of phosphate was varied.

Current and power density data have been normalized by the projected surface area of the foam (*i.e.* geometrical area of the base of the cylinder). 5mm diameter glassy carbon electrodes (PINE) were used as supports for porous materials. Disks were pasted via a small drop of conductive carbon paint and let dried for at least 2 weeks before use. Before enzyme adsorption, electrodes were made hydrophilic by exposure to 1 Torr O\textsubscript{2} plasma for 15 minutes. This is a post-print version of the following article: Flexer, Victoria, Brun, Nicolas, Destrribats, Mathieu, Backov, Rénal and Mano, Nicolas (2013) A novel three-dimensional macrocellular carbonaceous biofuel cell. *Physical Chemistry Chemical Physics, 15* 17: 6437-6445.
Characterization.

SEM observations were performed with a Hitachi TM-1000 apparatus at 15 kV. The specimens were gold-palladium-coated in a vacuum evaporator prior to examination. High resolution SEM spectroscopy was performed with a JSM-6700 F field emission scanning electron microscope operating at 5.00 kV with a cold cathode. Intrusion/extrusion mercury measurements were performed using a Micromeritics Autopore IV 9500 analyzer. The specific surface area was determined by N\textsubscript{2} adsorption measurements performed on a Micromeritics ASAP 2010 apparatus. The isotherms were subjected to the Brunauer, Emmett and Teller (BET) treatment, and to the Barrett, Joyner and Halenda (BJH) method on the adsorption branch to estimate the mesopore surface area.

Carbonaceous electrode morpho-syntheses.

40-Carb(HIPE))

The hard template silica Si(HIPE) synthetic path is extrapolated from the method described by Carn et al.\textsuperscript{43} Typically, TEOS (5 g) was added to an aqueous solution of TTAB (16 g, 35 wt %) previously acidified (7 g of HCl). Hydrolysis was left going on until a monophasic medium was obtained. The oily phase constituted of dodecane (35 g) was then emulsified drop by drop into the hydrophilic continuous phase using a mortar, and the emulsion was allowed to condense for 1 week at room temperature. The as-synthesized monoliths were washed three times with a THF/acetone mixture (1:1 v/v) to extract the oily phase. Drying of the materials for a week at room temperature was followed by a thermal treatment at 650 °C (heating rate of 2 °C.min\textsuperscript{-1}) for 6 h, with a 2 h-plateau at 200 °C to remove the organic supramolecular-type template. The carbonaceous anodic synthetic path is extrapolated from Brun et al.\textsuperscript{12} Typically, a monolith of Si(HIPE) (0.5 g) was added into a 40 wt % Ablaphene® RS101 solution in THF. For a good impregnation, the suspension was...
placed under vacuum until the effervescence disappeared. After 24 hours aging at room temperature, the solution was filtered. The monolith was then quickly washed with THF and dried in an air oven at 80 °C for 24 h to favour solvent evaporation and to initiate the thermally-induced reticulation of the monomers. A second thermal treatment was performed at 155 °C for 5 h under air (heating rate of 2 °C.min⁻¹), with a first plateau at 80 °C for 12 h, and a second one at 110 °C for 3 h. The cooling process was uncontrolled and directed by the oven inertia. Silica was then removed from the composites by immersing them three times in a 10 wt % hydrofluoric acid solution, followed by extensive washing with deionised water. The monoliths were dried in an air oven at 80 °C overnight. Finally, pyrolysis was carried out at 900 °C for 1 h under N₂ flow (heating rate of 4 °C.min⁻¹). The resulting carbon monoliths was called 40 carb and labelled 40-Carb(HIPE).

60-Carb(PHIPE):

Here we used a macroporous silica template synthesized via Pickering-based emulsion, namely Si(PHIPE) macrocellular foams, where cell monodispersity is driven by limited-coalescence phenomenon. Typically, aqueous phases were prepared by adding to the particles dispersions, at various concentrations, the same volume (as the dispersion) of HCl (37%) and the same volume of TEOS under magnetic stirring. As a direct consequence, the solution obtained is therefore composed of equal volumes of the particles dispersion, HCl solution and TEOS solution, each of them corresponding to a third of the total hydrophilic volume. Then, hexadecane is emulsified at a 64% weight fraction using 2.6 mg of modified silica particles per gram of oily phase. In such acidic conditions, mineralization of the aqueous phase occurred very quickly but emulsions were left at rest for a 10 days period in order to fully complete the sol–gel process. In a second step, the monoliths were washed by immersion in a THF/acetone (7:3 v/v) mixture three times over a 24 h period in order to remove the aqueous and oily phases, and then they were dried slowly by air in a desiccator for.
one week. To remove any remaining organic traces and to mechanically strengthen by sintering the silica macrocellular foams, the materials were thermally treated as follows: the first step consisted of a heating ramp at 2 °C min⁻¹ to 200 °C. Then, this temperature was held for 2 h before a second ramp at 2 °C min⁻¹ was imposed to reach 650 °C where the materials are let for 6 hours. The kinetic of temperature of cooling is uncontrolled, being driven by the oven inertia.

A monolith of the Si(PHIPE) (0.6 g) was added into a 60 wt % Ablaphene® RS101 solution in THF. For a good impregnation, the suspension was placed under vacuum until the effervescence disappeared. After 24 hours aging at room temperature, the solution was filtered. The monolith was then quickly washed with THF and dried in an air oven at 80 °C for 24 h to favour solvent evaporation and to initiate the thermally-induced reticulation of the monomers. A second thermal treatment was performed at 155 °C for 5 h under air (heating rate of 2 °C.min⁻¹), with a first plateau at 80 °C for 12 h, and a second one at 110 °C for 3 h. The cooling process was uncontrolled and directed by the oven inertia. Carbonaceous monoliths were synthesized by beginning with a pyrolysis step, carried out at 900 °C for 1 h under N₂ flow (heating rate of 4 °C.min⁻¹). Then, the silica was removed under HF treatment by immersing the monolith three times in a 10 wt % hydrofluoric acid solution, followed by extensive washing with deionised water. Finally, the monoliths were dried in an air oven at 80 °C overnight. Final carbonaceous foams are labelled 60-Carb(PHIPE).

**Electrode preparation.**

Cathodes. Cathodes were prepared using either 60-Carb(PHIPE) foams or 40-Carb(HIPE). Electrodes were immersed in a 1.7 mg ml⁻¹ BOD solution for 1.5 hours at 4°C, abundantly rinsed in buffer, and used right after modification.⁷
Anodes. 40-Carb(HIPE) foams were used as electrode material. Electrodes were prepared as previously reported. The catalysts solution consisted of a mixture of 62.7 wt % polymer, 31.3 wt % GOx, and 6 wt% PEGDGE. Two different types of electrodes were prepared. Anode 1 was prepared using polymer I, and Anode 2 was prepared using polymer II. Slightly different total catalysts loadings were used: 1300 µg cm\(^{-2}\) for Anode 1; and 1734 µg cm\(^{-2}\) for Anode 2. The reported polymer-enzyme-crosslinker mass loading is quoted with respect to the geometrical surface area of the electrode.

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BROAD CONTEXT:

Enzyme based biofuel cells are devices dedicated toward electro-chemical energy conversion using enzymes as biocatalysts. They possess promising advantages in terms of cost, simple construction, renewable catalysts, wide flexibility of renewable fuels, operation at room temperature and ambient pressure. They have been proposed for applications in niche areas of power generation such as implantable medical devices, or portable electronic devices under specific conditions, such as remotely located, or devices needing a non-toxic fuel. Today enzymatic biofuel cells are still limited by both lifetime, and lower power densities that restrict their application. In order to overcome these limitations we propose to use three-dimensional porous electrodes bearing a hierarchical porous structure that will maximise the enzyme loading while maintaining efficient mass transport towards and out of the monolithic electrodes. By using a new synthetic protocol, that increases the electrode meso and macroporosity, we could increase the current density of our modified electrodes. Then, by choosing the right combination of enzyme and mediator, and the right loading of active

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components, we achieved unprecedentedly high current densities for an anodic system at low electrode potential. Finally, we built a new membrane-free hybrid enzymatic biofuel cell displaying promising power density for future applications.

References:

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