A 280 μW cm^{-2} biofuel cell operating at low glucose concentration†

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We report the highest power biofuel cell operating at the lowest concentration to date: 5 mM glucose concentration.

The elaboration of miniature membrane-less biofuel cells is of interest because these could power, in the near future, implanted sensor-transmitters that would broadcast, for example, the local glucose concentration, relevant to diabetes management.[1–5] Several of these are enzymatic-based.[1–5] At the cathode, blue copper oxidases such as laccases or bilirubin oxidase,[6–8] and glucose oxidase (GOx) from *Aspergillus niger* (*A. niger*), at the anode, have been preferentially used.[9,10] Different strategies such as direct electron transfer or mediated electron transfer have been explored to electrically connect these enzymes to the electrode surfaces.[11–14]‡ A common point of most of the existing biofuel cells is the low power density at low glucose concentration. For example, we described earlier a biofuel cell made by "wiring" GOx from *A. niger* at the anode and by "wiring" laccase at the cathode.[15] The cell reached 350 μW cm^{-2} (at +0.88 V) in the presence of 15 mM glucose concentration but only $\frac{1}{4}$ of that (90 μW cm^{-2}) at 5 mM.

When the cathode and anode fibers are of equal length, under saturated O_2, the power density of the biofuel cell increases with the glucose concentration until the kinetic limit of the anodic bioelectrocatalyst is reached, *i.e.* ~20 mM. Because the physiological concentration of glucose in blood is between 5 and 8 mM, the optimum power density of the biofuel cell must already be reached at 5 mM glucose concentration. Our objective is to reduce the GOx's K_m value to below this concentration and reach the V_{max} (the maximum turnover rate of the enzyme) already at 5 mM glucose. Ideally, the anodic enzyme should be stable in physiological conditions (20 mM phosphate, 0.14 M NaCl, 37 °C) with a $K_m < 5$ mM and a V_{max} over 100 U mg^{-1}.

To build a more efficient biofuel cell and to increase the power density of the biofuel cell at low glucose concentration we replaced glucose oxidase from *A. niger* by glucose oxidase from *Penicillium pinophilum* (*P. pinophilum*).

The *P. pinophilum* enzyme shares many common properties with the one from *A. niger*. The relative molecular weight of the enzyme was found to be 154 700 and consisted of two subunits of 75 000 with dimensions of 5 nm × 8 nm. It contains tightly bound flavin adenine dinucleotide (FAD) with an estimated stoichiometry of 1.76 mol/mol of enzyme. The enzyme is specific for D-glucose, for which a K_m value of 6.2 mM was found[16] and the pH optimum was determined to be in the pH 4–6 range.

To avoid phase separation, the bioelectrocatalysts are made by forming electrostatic adducts of the enzymes, which are polyanions, and the "wires", which are polycations.[17] Because both *P. pinophilum* and *A. niger* have the same isoelectric point (pI = 4.2), we used the same redox hydrogel that "wired" earlier glucose oxidase from *A. niger*.[18] Fig. 1 shows the cyclic voltammogram of the glassy carbon electrode coated with "wired" *P. pinophilum* under argon at 50 mV s^{-1}, in a 20 mM citrate buffer pH 5 at 37 °C. The coating consisted of 55 wt% PVP-[Os(*N*,*N*′-dialkylated-2,2′-bi-imidazole)$_3$]$^{2+/3+}$ (where PVP is poly(*N*-vinyl-2-pyridine)), 40 wt% *P. pinophilum* and 5 wt% poly(ethylene glycol) (400) diglycidyl ether (PEGDGE), for a total loading of 800 μg cm^{-2} and exhibit the characteristic of a reversible-surface bound couple with an apparent redox potential of −175 mV/AgAgCl. At 50 mV s^{-1}, the voltammogram exhibited a symmetrical wave with ΔE_p = 60 mV separation of the oxidation and reduction peaks. The width of the peak at half-height, E_{whm}, was 145 mV.

Fig. 2 shows the dependence of the glucose electrooxidation current density on the (polymer/*P. pinophilum*) weight percentage ratio in a 20 mM citrate buffer, pH 5, in the presence of 32 mM glucose, at 37 °C and at a fixed loading of 0.8 mg cm^{-2}. In the 0–1.25 (polymer/enzyme) weight ratio, the current density increased with the weight percentage of *P. pinophilum*, reaching 800 μA cm^{-2} at 40 wt%. At higher ratios the current density declined. The decline is attributed to the precipitation

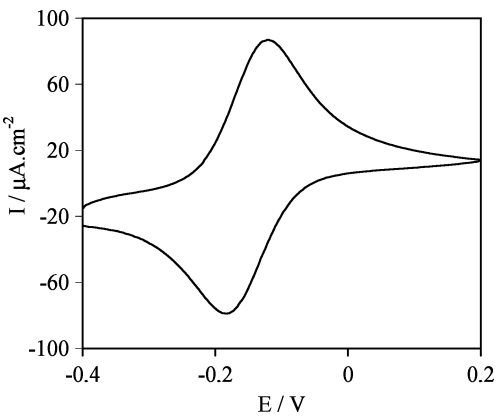

Fig. 1 Cyclic voltammogram of the "wired" *P. pinophilum* enzyme electrode under argon. 0.14 M NaCl, pH 5, 20 mM citrate buffer, 50 mV s^{-1}.

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‡ Details about the chemicals, instrumentation, enzymes and preparation of the electrodes can be found in the following papers.[15,16,19]

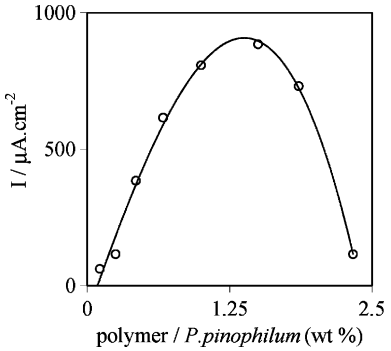

Fig. 2 Dependence of the electrooxidation current density on the (polymer/enzyme) weight percentage ratio. Electrode poised at −100 mV/AgAgCl. 32 mM glucose, 500 rpm, 20 mM citrate buffer, pH 5, 0.14 M NaCl.

of the electrostatic adduct formed between the polyanionic enzyme and the polycationic "wire".

Unlike the earlier used bioelectrocatalyst made of *A. niger* in which the wt% of PEGDGE was 1 wt%,[19] this bioelectrocatalyst has a higher PEGDGE weight fraction. In "wired" enzyme films, crosslinking reduces D_{app} (the apparent electron diffusion coefficient) because the more rigid the gel, the lesser the segmental mobility of the redox functions, on the collision rate on which D_{app} depends.[20] Because we showed earlier, that the introduction of long tethers could increase up to 100-fold D_{app} of the hydrogel formed upon crosslinking with PEGDGE, we were able to raise the PEGDGE crosslinker weight fraction of the bioelectrocatalyst to 5 wt%. We were then able to apply leather-like, thicker, better adhering and more stable hydrogels.

The pH-dependence of the steady state current density of glucose electrooxidation for the *P. pinophilum* (○) and *A. niger* (●) electrodes is shown in Fig. 3. The current was measured under argon with the electrodes poised at −100 mV *versus* Ag/AgCl in 0.14 M NaCl while the electrode rotated at 500 rpm, in the presence of 32 mM glucose. Phosphate, borate, citrate or Tris were added at 20 mM concentration to maintain the desired pH. As seen in Fig. 3, the current density increased with pH until it reached a plateau at pH 5 for *P. pinophilum* and at pH 6 for *A. niger*. It then decreased slightly above pH 7 for the *P. pinophilum* and pH 8 for *A. niger*. In the pH 4.8–6.5 range the current density was nearly independent of pH, varying by less than ±10% for *P. pinophilum*. As discussed earlier, the pH dependence of the current (under argon, at 32 mM glucose) differs from the pH dependence of V_{max}, the maximum turnover rate of the dissolved GOx.[21] When the enzyme is dissolved, the *P. pinophilum* enzyme exhibited an optimum between pH 4 and 6, reaching 50% activity both at pH 3 and at pH 7.5.[16] In contrast, the glucose electrooxidation current of the "wired" *P. pinophilum* electrode is at its maximum through the pH 4.8–6.5 range. Even though the *P. pinophilum* enzyme is highly active at low pH, the bonding in the adduct is reduced by lowering the pH near the isoelectric point of the enzyme. That results in the phase separation of the macromolecules, and a decrease of the electrooxidation current. Because *P. pinophilum* is more stable and slightly more active for pH < 5 than *A. niger*, the current density is higher for *P. pinophilum*. At high pH, the electrostatic bonding between the polyanionic GOx and the polycationic redox polymer improves, and the maximal flux of electrons from the enzyme to its "wire" is likely to increase, when more of the enzyme is ionized at a higher pH, where the polymer is, as yet, not deprotonated. Because the *P. pinophilum* is unstable at pH 7, the current decrease is associated with the denaturation of the enzyme.

The temperature dependence of both anodes when poised at −100 mV/AgAgCl in a 20 mM citrate buffer, 32 mM glucose concentration under argon is seen in Fig. 4. When the rate of increase is about 10 °C h^{-1}, the electrooxidation current increases up to 40 °C for *P. pinophilum*, then declines rapidly as the GOx is denatured.[16] The wired *P. pinophilum* is less stable than the electrode made with *A. niger* for which an optimal temperature of 45 °C was reported.[21] The apparent activation energy for the glucose electrooxidation is the same for both electrodes, *i.e.* 28.3 kJ mol^{-1}, half of other "wires"[18,22] but still well above the 14 kJ mol^{-1} for the native glucose oxidase in solution.[23] The activation energy for thermal denaturation of *P. pinophilum*, determined from the decline of the glucose electrooxidation current at >40 °C is

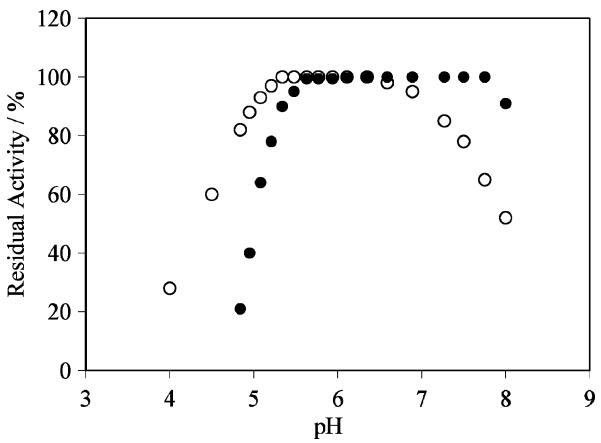

Fig. 3 pH dependence of the steady state current density under argon for the *P. pinophilum* (○) and *A. niger* (●) electrodes poised at −100 mV/AgAgCl. 32 mM glucose, 500 rpm, 0.14 M NaCl.

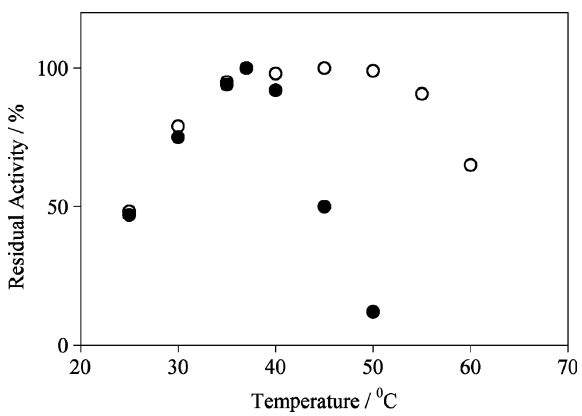

Fig. 4 Temperature dependence of the steady state current density under argon for the *P. pinophilum* (●) and *A. niger* (○) electrodes poised at −100 mV/AgAgCl. 32 mM glucose, 500 rpm, 20 mM citrate buffer pH 5, 0.14 M NaCl.

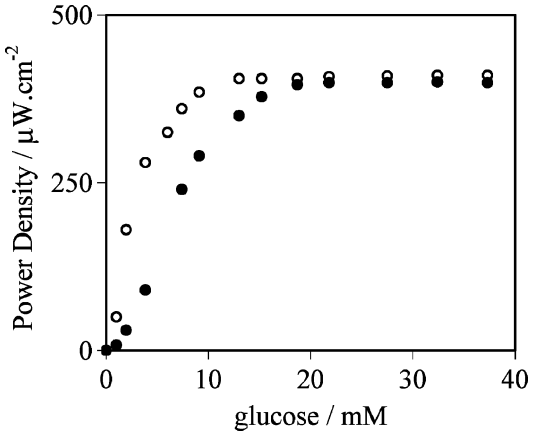

Fig. 5 Dependence of the power density on glucose concentration under air at +0.88 V. 20 mM citrate buffer, pH 5. ●: *A. niger*; ○: *P. pinophilum*.

25 kJ mol^{-1}, a value smaller than that of the *A. niger* anode (96 kJ mol^{-1}), showing that the new anode is less stable than the earlier glucose electrooxidizing anodes at pH 5.

Even though the enzyme is unstable at temperature >40 °C in the pH 7–8 range, the enzyme is extremely stable at pH 5 and exhibited half lives longer than 305 days in the 4–37 °C temperature range.[16]

To form a miniature membrane-less glucose/O_2 biofuel cell, we combine the novel *P. pinophilum* anode with a previously described O_2 electroreducing cathode.[15] The biofuel cell was made of two 7 μm diameter, 2 cm long carbon fibers.[19] They were made hydrophilic, then one was coated with 40 wt% *P. pinophilum*, 55 wt% PVP-[Os(*N*,*N*′-dialkylated-2,2′-bi-imidazole)$_3$]$^{2+/3+}$ and 5 wt% PEGDGE. The other was coated with 39.6 wt% laccase, 53.2 wt% PVP-[Os(dme-bpy)$_2$(amino-dme-bpy)]$^{2+/3+}$ (redox potential +0.55 V/AgAgCl) and 7.2 wt% PEGDGE.[24]

Fig. 5 shows the dependence of the power density on the glucose concentration at 37 °C in a 20 mM pH 5 citrate buffer under air when the anode is made with *A. niger* (●) or with *P. pinophilum* (○). The current density increased with the glucose concentration up to 12 mM for *P. pinophilum* and 20 mM for *A. niger*, where a plateau of 398 μW cm^{-2} was reached. At 5 mM glucose concentration the power density was 280 μW cm^{-2} for the biofuel cell made with *P. pinophilum* and only 90 μW cm^{-2} for the biofuel cell made with *A. niger*. The dependence of the power density on the glucose concentration is consistent with the value of K_m' (the apparent Michaelis–Menten constant) determined for both enzymes, *i.e.* 6.2 mM glucose for *P. pinophilum* and 20 mM glucose for *A. niger*.[16] The apparent Michaelis–Menten constant is a characteristic of the film on the electrode, not of the enzyme, and usually differs substantially from the enzyme's constant, measured in a homogenous solution.

Because the *P. pinophilum* enzyme is highly stable at pH 5 and at 37 °C, the cell operated continuously at +0.88 V for a month, losing only 3% power per day for the first two weeks (see ESI†).

In summary, we have reported the highest power density biofuel cell to date, 280 μW cm^{-2} (+0.88 V) while operating at 37 °C at pH 5 in the presence of only 5 mM glucose. The power density of the novel biofuel cell is three times that of the best biofuel cell reported so far.[15] It is also four times more stable.

The components enabling this new biofuel cell are first based on the substitution of glucose oxidase from *Aspergillus niger* by glucose oxidase from *Penicillium pinophilum*, a high V_{max} glucose oxidation catalyzing enzyme of K_m = 6.2 which is highly stable at pH 5. Secondly it is based on the selectivity of the bioelectrocatalysts of the two electrodes for their respective substrates, enabling the construction of a single compartment cell containing both glucose and O_2.

The availability of numerous enzymes and the possibility of tailoring their activities as well as our ability to connect enzymes to electrode surfaces through redox hydrogels, suggest that miniature membrane-less biofuel cells with enhanced performances will be designed in the near future and may be part of systems for implanting inside the body to power and control biosensors.

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