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Graphite anode surface modification with controlled reduction of specific aryl diazonium salts for improved microbial fuel cells power output

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ABSTRACT

Graphite electrodes were modified with reduction of aryl diazonium salts and implemented as anodes in microbial fuel cells. First, reduction of 4-aminophenyl diazonium is considered using increased coulombic charge density from 16.5 to 200 mC/cm^2. This procedure introduced aryl amine functionalities at the surface which are neutral at neutral pH. These electrodes were implemented as anodes in "H" type microbial fuel cells inoculated with waste water, acetate as the substrate and using ferricyanide reduction at the cathode and a 1000 Ω external resistance. When the microbial anode had developed, the performances of the microbial fuel cells were measured under acetate saturation conditions and compared with those of control microbial fuel cells having an unmodified graphite anode. We found that the maximum power density of microbial fuel cell first increased as a function of the extent of modification, reaching an optimum after which it decreased for higher degree of surface modification, becoming even less performing than the control microbial fuel cell. Then, the effect of the introduction of charged groups at the surface was investigated at a low degree of surface modification. It was found that negatively charged groups at the surface (carboxylate) decreased microbial fuel cell power output while the introduction of positively charged groups doubled the power output. Scanning electron microscopy revealed that the microbial anode modified with positively charged groups was covered by a dense and homogeneous biofilm. Fluorescence *in situ* hybridization analyses showed that this biofilm consisted to a large extent of bacteria from the known electroactive *Geobacter* genus. In summary, the extent of modification of the anode was found to be critical for the microbial fuel cell performance. The nature of the chemical group introduced at the electrode surface was also found to significantly affect the performance of the microbial fuel cells. The method used for modification is easy to control and can be optimized and implemented for many carbon materials currently used in microbial fuel cells and other bioelectrochemical systems.
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1. Introduction

The discovery of electroactive microorganisms such as bacteria or yeasts was published a hundred years ago by Potter (1911). This seminal work is now experiencing renewed interests in view of developing applications in the energy and environmental fields such as microbial fuel cells, bioelectrolysis cells, biosensors or logic gates (Rabaey et al., 2010; Li et al., 2011). In these bioelectrochemical systems, microbial metabolism and respiration are electrically linked to electrodes where oxidation/reduction of biological substrates is catalyzed and coupled to the generation/consumption of an electrical current (Schaetzle et al., 2008). In most devices, microbial catalysis only occurs at the anode although examples involving microbial cathodes are also documented (Rabaey and Keller, 2008). At a microbial anode, and in the absence of natural electron acceptors in the anolyte, some species of bacteria have indeed the ability of transferring the electrons derived from the catabolism of organic substrates to the solid electrode (Schaetzle et al., 2008). Bacterial electron transfer to an anode may involve at least one of the following mechanisms: (i) direct electron transfer from the outer membrane cytochrome intermediates of the bacteria respiratory chain (Busalmen et al., 2008), (ii) *via* protruding conducting *pili* of proteic nature (Gorby et al., 2006), (iii) mediated electron transfer by oxidation of endogeneous (Marsili et al., 2008) adventitious or artificial redox mediators and (iv) by the anodic oxidation of secondary metabolic substrates like dihydrogen or formate (Rosenbaum et al., 2006). In microbial fuel cells the oxidation of organic substrates at the anode is coupled to electrical energy production and to the reduction of a suitable electron acceptor at the cathode (Logan, 2008; Logan et al., 2006). One of the applications of microbial fuel cells hence aims at a profitable wastewater treatment process (Logan and Regan, 2006; Rozendal

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et al., 2008). The current power outputs of practical microbial fuel cells are nevertheless modest ranging around only a hundred Watts per cubic meter which is about one order of magnitude too low to be economically viable. Thus, it is necessary to study and improve the materials, microbiological, electrochemical and design aspects of bioelectrochemical systems in order to produce transdisciplinary fundamental knowledge and progress towards credible applications. Regarding the interface between biofilms and electrodes, recent research has focused on treating or modifying carbon-based substrates in order to increase microbial fuel cell performance.

For example, heat treatment of graphite fibres or carbon cloth and felt anodes in the presence of air (Feng et al., 2010a; Wang et al., 2009), ammonia (Cheng and Logan, 2007), or nitric acid (Zhu et al., 2011), have resulted in improved microbial fuel cell performance whereas heat treatment in the presence of hydrogen or methane (Cheng and Logan, 2007) showed lower performances. Immobilization on the anode surface of redox mediators of the quinone type with redox polymers (Adachi et al., 2008) encapsulated in a conducting polymer matrix (Feng et al., 2010b) or through adsorption (Lowy et al., 2006) also led to improvement of microbial fuel cell power output. Saito et al. (2011) recently introduced 4-(*N*,*N*-dimethylamino) aryl groups at electrodes through diazonium reduction and concluded on the basis of X-ray photoelectron spectroscopy studies that a low extent of modification was necessary to optimally improve microbial fuel cell performance. Previous attempts towards the modification of anode microbial fuel cells have been also summarized by Saito et al. (2011).

Building on our experience in the design of enzymatic and microbial fuel cells (Schaetzle et al., 2008, 2009; Barrière, 2010; Haslett et al., 2011) and in carbon surface modification (Pellissier et al., 2008a,b; Barrière and Downard, 2008; Boland et al., 2008) we sought to combine these fields in order to design a practical way to modify electrodes and consequently improve the performance of bacterial fuel cells.

Electrochemical reduction of aryl diazonium salts is a versatile technique for the covalent modification of graphitic carbon substrates. Hence, the grafting on carbon electrodes of a large range of groups with different chemical and physical properties can be readily achieved (Barrière and Downard, 2008; Pinson and Podvorica, 2005). In addition, the modification of carbon surfaces with an electrochemical approach (through recurrent potential sweeps or potentiostatic techniques) allows the fine tuning of the amount of modifier (i.e. thickness) grafted at the electrode surface by simply controlling the coulombic charge consumed in the modification process (Brooksby and Downard, 2004). Indeed, it is well documented that aryl diazonium reduction at carbon electrodes yield a covalently bound modifier layer of the polyphenylene type, with thicknesses up to ca. 10 nm (Pinson and Podvorica, 2005; Brooksby and Downard, 2004; Ceccato et al., 2011). We have used the *in situ* generation of aryl diazonium salts and their subsequent electrochemical reduction as reported by Baranton and Bélanger (2005) and Lyskawa and Bélanger (2006) as described in Section 2.1. The chemical structures of the *in situ* generated aryl diazonium salts used in this work are shown in Chart 1.

In this article we thus report the controlled modification of graphite anodes with the electrochemical reduction of aryl diazonium salts, and the subsequent effect of the modified electrodes on the power output of microbial fuel cells. We first discuss the modification of graphite using phenylene diamine as the starting aryl amine (corresponding 4-aminophenyl diazonium salt A in Chart 1) and show the importance of the extent of modification on the performance of the microbial fuel cells. Then the effect of charged groups (carboxylate and phosphonium) at the surface are examined at low modification extent.

2. Materials and methods

2.1. Chemicals and electrode modification procedure

Potassium ferricyanide (Accros), sodium acetate (Prolabo), *p*-phenylenediamine (Sigma), 4-aminophenylacetic acid, 98% (Accros Organics), sodium nitrite 97%, ACS reagent (Aldrich), glutaraldehyde (Aldrich), p-formaldehyde (Janssen) and hypophosphorous acid (H_3PO_2, 50 wt% in water, Aldrich) were used as received. (4-Aminobenzyl)triphenylphosphonium bromide, was obtained by the reduction of the commercial (4-nitrobenzyl)triphenylphosphonium bromide (Bellamy and Ou, 1984). Diazonium salts were generated *in situ* in acid media (0.1 M HCl, 100 mL) containing 10 mM of the starting aryl amine and followed by addition of 20 mM sodium nitrite (Baranton and Bélanger, 2005; Lyskawa and Bélanger, 2006). This solution was then directly used as the electrolyte for the modification procedure of the graphite working electrode by electrochemical reduction of the diazonium salts using a potentiostat (Autolab POSTAT302N). A three electrodes cell configuration was used with a KCl saturated calomel as the reference electrode (SCE) and a second graphite electrode as the counter electrode. Electrochemical reduction of the diazonium salts was carried out either by holding the working electrode potential at −0.2 V for different set periods, or by recurrent cyclic voltammetry sweeps between +0.2 and −0.4 V. After modification the coulombic charge consumed was retrieved. The three electrodes cell configuration was also used to record cyclic voltammograms of the pristine, modified and colonized graphite electrodes.

2.2. Microbial Fuel Cells

An “H” type reactor configuration was used as described in Schaetzle et al. (2009). Cell chambers were separated using a cation exchange membrane (Nafion™, 0.18 mm thick, Alfa Aesar). Both anode and cathode were carbon graphite plates (6 cm × 2 cm × 0.5 cm or 3 cm × 2 cm × 0.5 cm, Einsenhuth Gmbh & Co.). Potassium ferricyanide was used as an electron acceptor at the cathode side (0.1 M, 20 mM phosphate buffer). This high ferricyanide loading ensured a stable cathode potential that was monitored throughout the experiment: 0.17 V *vs.* SCE. Reactors were inoculated with domestic waste water (Beaurades Wastewater Treatment Plant, Rennes, France), and fed with sodium acetate (20 mM) in 20 mM phosphate buffer solution (pH 7) with 10 mL/L of a macronutrient solution (28 g/L NH_4Cl, 10 g/L $MgSO_4{\cdot}7H_2O$ and 0.57 g/L $CaCl_2{\cdot}2H_2O$); 1 mL/L of a trace element solution (2 g/L $FeCl_2{\cdot}4H_2O$, 1 g/L $CoCl_2{\cdot}6H_2O$, 0.5 g/L $MnCl_2{\cdot}4H_2O$, 0.05 g/L $ZnCl_2$, 0.05 g/L H_3BO_3, 0.04 g/L $CuCl_2{\cdot}2H_2O$, 0.07 g/L $(NH_4)_6Mo_7O_2{\cdot}5H_2O$, 1 g/L $NiCl_2{\cdot}6H_2O$, 0.16/L $Na_2SeO_3{\cdot}5H_2O$ and 2 mL/L of HCl 37%); and vitamins (1 mL/L). Cell voltage (*V*) and electrode potentials were measured using a digital multimeter (Velleman DVM9912 multimeter). Reactors were operated in a batch mode at room temperature (20 ± 5 °C) and under a 1 kΩ external resistor. When the biofilm had develop, power density curves were recorded under steady-state conditions (acetate saturation) using a potentiostat (Autolab POSTAT302N) and a two-electrodes cell configuration (using the anode and cathode of the microbial fuel cell) by applying ten voltages (300 s for each voltage steps) from open circuit potential to near-short circuit potential while monitoring the steady state current.

2.3. Surface angle measurements

Contact angles of 90 ± 3° were measured (Krüss, Easydrop DSA) for as-received carbon plates. The carbon plates were roughened

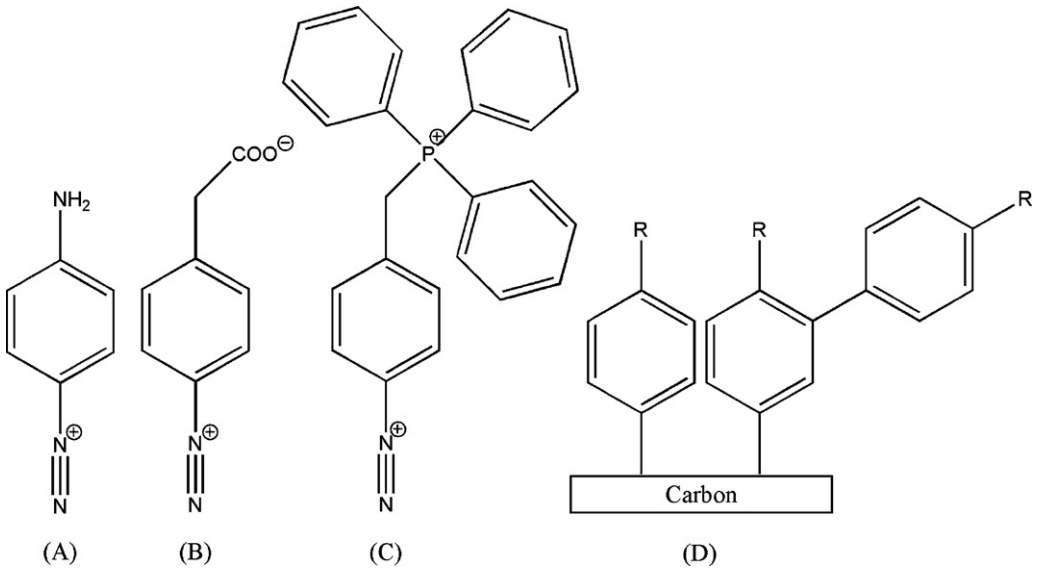

Chart 1. Chemical structure of *in situ*-generated aryl diazonium salts (A), 4-phenylamino diazonium (B), 4-benzyltriphenylphosphonium diazonium, (C) 4-phenylacetic acid diazonium (D). Scheme describing the nature of the modified carbon surface, R = NH_2, CH_2COO^- or $CH_2PPh_3^+$.

with using SiC sand paper 22 μm (Struers). After this treatment, measured contact angles were always higher than 100°. Surfaces modified with 4-phenylamino diazonium had a contact angle of 97 ± 4°, with 4-benzylcarboxylic acid diazonium: 69.6 ± 6° and with 4-benzyltriphenylphosphonium diazonium: 74.1 ± 5°. The charge consumed for modification of the electrodes was 60 ± 10 mC/cm^2.

2.4. SEM analyses

Scanning electron microscopy (SEM) experiments were carried out using the following protocol. Samples were collected and fixed overnight with a 2.5% glutaraldehyde in a buffer solution (0.1 M phosphate buffer, pH 7) at room temperature. Then, samples were washed using a phosphate buffer solution (pH 7) and immersed successively in different aqueous solutions with increased ethanol content (60, 70, 80, 90 and 100% ethanol), and was then critical-point dried. Samples were finally coated with Au/Pd before SEM observation.

2.5. Fluorescent in situ hybridization (FISH) analyses

Anode samples (2 cm × 0.5 cm × 0.5 cm) were taken from functional microbial fuel cells and washed with buffered phosphate saline (PBS) solution. The samples were covered with an adequate volume of p-formaldehyde solution (concentration 4%) and incubated for 2 h. Finally, after fixation, samples were transferred to PBS/ethanol 50/50 v/v and kept in the freezer. rRNA-targeted oligonucleotide probes were synthesized and labelled by Sigma–Aldrich (Steinheim, Germany). EUB338-I (Amann et al., 1990) EUB338-II and III (Daims et al., 1999) were used as equimolar mixture. Geo1A is specific for *G. sulfurreducens*, *G. hydrogenophilus*, *G. grbiciae*, and *G. metallireducens* (Demaneche et al., 2008). Fluorescently labelled cells were detected by a Zeiss LSM510 confocal laser scanning microscope (CLSM). An argon ion laser supplied a wavelength of 488 nm to excite fluorescein, and a helium neon laser provided the wavelengths of 543 nm for Cy3. Each hybridization probe EUB338-mix labelled with fluorescein (green) was combined with Geo1A labelled with Cy3 (red) at 35% v/v formamide concentration. Binding of both probes resulted in a yellow-orange staining of target cells. Excitation at 633 nm served as negative control, as no specific fluorescent dye was present that could be induced at this wavelength. Therefore fluorescent signals observed with this excitation indicated unspecific auto-fluorescence of the sample and not specifically labelled bacteria.

3. Results and discussion

3.1. Optimization of the anode surface modification with neutral aryl-amine groups

The resulting grafted polyphenylene film at the surface of the electrodes modified by the reduction of 4-aminophenyl diazonium (A on Chart 1) contains aryl-amine functionalities which are neutral at pH 7. Little change in the wettability of the modified electrode surface was found with respect to the untreated graphite plates (contact angles ∼100°, see Section 2.3).

Different electrodes, modified with increased coulombic charge density (respectively not modified, 16.5, 23, 28, 48, 60, 150 and 200 mC/cm^2) were simultaneously implemented as anodes in microbial fuel cell set-ups while the cell potential was left to develop. Each of the microbial fuel cell equipped with a modified anode was inoculated and left to develop concomitantly with controls equipped with unmodified anodes. The maximum power output from eight replicates control microbial fuel cells was 50 ± 6 mW/m^2. The monitoring of the microbial fuel cells start-up was carried out by regularly checking the cell voltage at closed circuit (through a 1 kΩ load). The cell voltage was very low (below 10 mV) for all fuel cells at the beginning of the experiment and then gradually increased to reach ca. 300–400 mV, consistent with the development of an electroactive biofilm at the anode surface. As the cathode had a stable potential (see Section 2.2), development of a cell potential was due to the activity at the anode. No effect of the extent of the modification of the anodes on the start-up time of the microbial fuel cells was noticed. After ca. 20 days, when the biological fuel cells had started, power curves were recorded (under acetate saturation conditions), as shown in Fig. 1. Power curves were also recorded at later stages of the experiment and no change in the performance of the microbial fuel cells was noted for a minimum of one month continuous operation. The results show that the maximum power output of the microbial fuel cell can be doubled not only by surface modification of the anode but also most importantly by the fine control of the extent of its modification. Indeed, examination of Fig. 1 shows that compared with

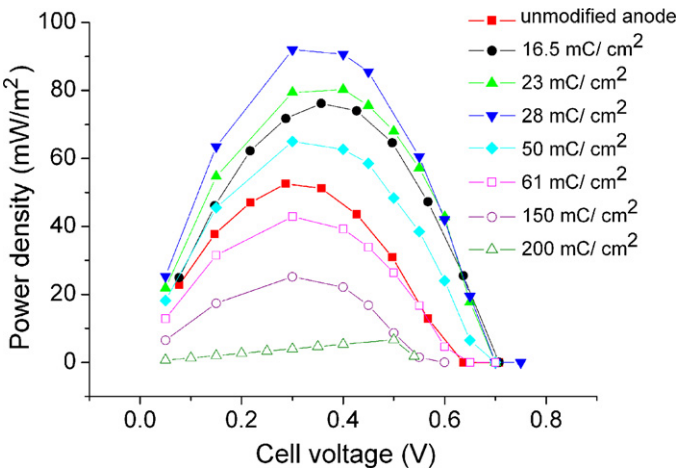

Fig. 1. Power density curves recorded for microbial fuel cells with increasingly modified anodes: unmodified or modified with the reduction of *in situ* generated 4-aminophenyl diazonium salt at different coulombic charge densities.

the performance of the microbial fuel cell with the pristine graphite anode (red squares) increasing extent of modification first results in a significant increase of the maximum power output (black circles and green triangles, respectively for 16.5 and 23 mC/cm^2 consumed in the electrochemical modification process). The optimum performance was found for a fuel cell with an anode modified by the consumption of 28 mC/cm^2 (inverted blue triangles). Above this amount, the performance of the device started to decrease (blue diamonds, 50 mC/cm^2). (For interpretation of the references to color in text, the reader is referred to the web version of the article.) It became even less efficient than the microbial fuel cell with the pristine graphite anode when the anode modification consumed 60 mC/cm^2 (open pink squares) or *a fortiori* 150 mC/cm^2 (open purple circles). The fuel cell with the most extensively modified electrode (200 mC/cm^2) did not lead to the development of an electrocatalytic biofilm at its anode and is logically the least performing device (open green triangles).

The decreased performance of microbial fuel cells equipped with anodes electrochemically modified with consumption of over 28 mC/cm^2, is most likely a consequence of the reduced rate of interfacial electron transfer between the electroactive bacteria and the electrode. Indeed it is well known that thick modifier layers at electrode surfaces may significantly affect the heterogeneous electron transfer rate to electroactive molecules (Pellissier et al., 2008b; Pinson and Podvorica, 2005; Brooksby and Downard, 2004; Ceccato et al., 2011; Baranton and Bélanger, 2005). This is now illustrated with the following experiment using the reversible reduction of ferricyanide to ferrocyanide as a redox probe ($[Fe(CN)_6]^{3-/4-}$, Fig. 2).

At an electrode modified with a small amount of coulombic charge consumed (16.5 mC/cm^2), the peak to peak potential separation for the chemically reversible reduction of ferricyanide remains almost unchanged on the cyclic voltammogram compared to that recorded at an unmodified electrode (Fig. 2A), indicating that the electron transfer rate was not altered for this redox probe. However, when the amount of modifier at the electrode surface increases, the peak to peak potential separation for the reduction of ferricyanide gradually increases until it is no longer detectable, as shown in Fig. 2B, for the highest amount of modification (200 mC/cm^2). In this extreme case the electrode is blocked with respect to the reduction of ferricyanide. This indicates a large degree of surface modification and explains why no electrocative biofilm could develop on an anode modified to that extent. One must keep in mind that the decreased electron transfer rate with increased surface modification is not only dependent on the thickness of the modifier layer but also on the respective physico-chemical properties of both the modifier and the redox probe itself. Hence, little change was noted in the peak to peak separation for the reversible oxidation of hydroxymethyl ferrocene measured at electrodes modified with different amounts of the aryl-amine modifier (not shown). This is important as it shows that the effect of the modified electrode (as a function of the nature of the modifier) is also a means of investigating the properties of a redox probe. Applied to microbial anodes, this helps in defining the physico-chemical properties of the micro-organisms outer-membrane redox proteins.

Electrocatalysis experiments were also carried out at functional microbial anodes under acetate depletion or acetate saturation conditions. Fig. 2C shows the electrochemical response of the best performing anode (modified by the consumption of 28 mC/cm^2) in these conditions. Under acetate conditions a main large reversible system is detected at a formal potential of −465 mV *vs.* SCE and ascribed to outer membrane cytochromes. Under acetate saturation, electrocatalytic acetate oxidation is evidenced with maximum catalytic current density around 400 mA/m^2. The magnitude of this maximum catalytic current recorded at a flat carbon electrode consistent with other reports (Katuri et al., 2010; Fricke et al., 2008). Most importantly, the trend of maximum catalytic current density as a function of the extent of electrode modification follows the trend shown in Fig. 1 for microbial fuel cell power density. For example the electrode modified with a large amount of modifier (150 mC/cm^2), which yielded a poorly efficient microbial fuel cell, displayed only about 40 mA/m^2 at maximum catalytic current density (not shown). This trend suggests that the interfacial electron transfer rate between the modified electrode and the electroactive biofilm is not significantly decreased at low extent of modification and that the ferri-ferrocyanide couple could be a relevant redox probe in this context.

The first trend of increased power output with increased extent of anode surface modification can be explained by the positive interactions between the introduced surface aryl-amine groups at the electrode surface and the outer membrane environment of electroactive bacteria. This includes outer membrane cytochromes with electrochemically addressable heme centres, as well as extracellular anchoring polysaccharides (Magnuson, 2011; Rollefson et al., 2011). Other authors have shown that modifying a carbon electrode surface through treatment with amino groups such as ethylene diamine, ammonia or 4-(*N*,*N*-dimethylamino)phenyl groups also increases the power output of the microbial fuel cells (Cheng and Logan, 2007; Zhu et al., 2011; Saito et al., 2011). Saito et al. (2011) noted that this beneficial effect was dependent on the extent of modification. These authors also concluded that a low amount of modification was desirable and that the surface nitrogen content should be controlled. We show here that the beneficial effect for microbial fuel cells can be easily optimized through the fine control of the charge consumed during the electrochemical modification of the electrode. The detrimental effect on the fuel cell power output at higher degrees of electrode modification is suggested to arise from the severe decrease of the heterogeneous electron transfer rate between the bacteria and the extensively modified electrode. The aryl-amine groups at optimum surface density must favour bioadhesion and development of a biofilm and also provide an efficient wiring link between the electrode and the outer membrane c-type cytochromes of electroactive bacteria. Since most bacteria possess a globally hydrophilic periphery with negatively charged outer membrane exopolysaccharides (Terada et al., 2006; Rollefson et al., 2011; Hori and Matsumoto, 2010), and given the versatility of the aryldiazonium salt reduction method for carbon surface modification, we then resorted to test the effect of electrodes bearing thin layers of differently charged groups. These groups were introduced by the

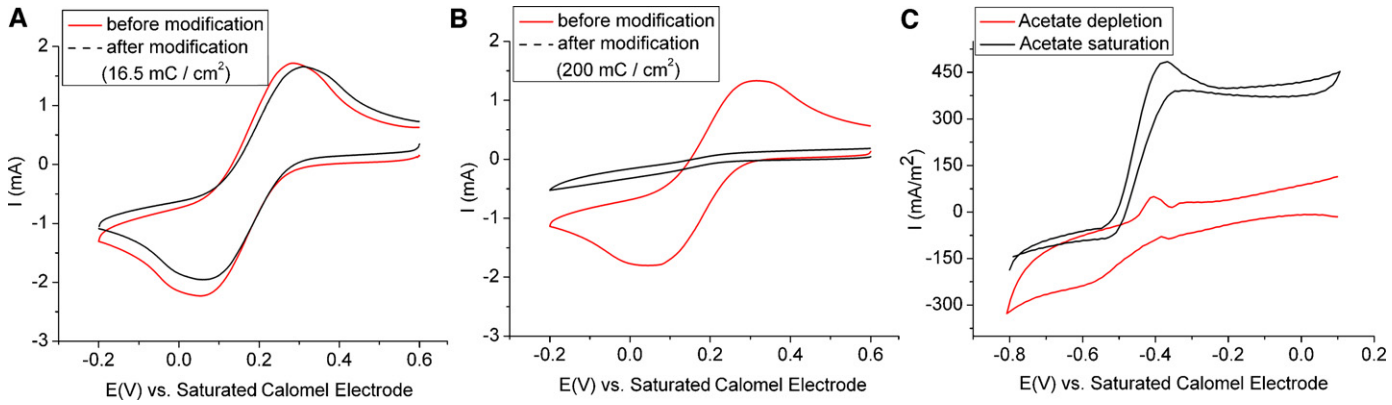

Fig. 2. (A) and (B) Reversible reduction (scan rate 0.1 V s^{-1}) of potassium ferricyanide at differently modified anodes: electrode modified by the consumption of 16.5 mC/cm^2 (A) and 200 mC/cm^2 (B) by reduction of *in situ* generated 4-aminophenyl diazonium. Both are compared to an unmodified electrode. (C) Cyclic voltammetry (5 mV s^{-1}) at the microbial anode modified by the consumption of 28 mC/cm^2 under acetate depletion and acetate saturation conditions.

cathodic reduction of 4-phenylacetic acid diazonium salt and 4-benzyltriphenylphosphonium diazonium salt, introducing surface groups that are respectively negatively charged at neutral pH (carboxylate, Chart 1B) and positively charged (phosphonium, Chart 1C).

3.2. Graphite surface modification with charged groups

3.2.1. Effect on power density at low modifier surface coverage

Graphite electrodes were modified with three recurrent sweeps between +0.2 and −0.4 V (*vs.* SCE) in the presence of either 4-phenylacetic acid diazonium or 4-benzyltriphenylphosphonium diazonium. We consider first a microbial fuel cell equipped with an anode modified by the reduction of 4-phenylacetic acid diazonium (Chart 1B) with the consumption of 11 mC/cm^2. This low extent of surface modification ensures that the interfacial electron transfer rate is not significantly affected (demonstrated in the case of the ferricyanide redox probe) and at the same time that the surface of the anode is modified with a sufficiently large amount of negative charge (phenylacetate groups at neutral pH). Results in terms of power density values are shown in Fig. 3 and compared with a control microbial fuel cell with an unmodified anode. As shown in Fig. 3 (left) the presence of negatively charged groups at the surface clearly decreases the performance of the device. The electrostatic repulsion between the modified electrode surface and the bacteria impedes the development of an efficiently wired electroactive biofilm. The presence of positively charged surface groups should logically have the opposite effect. This was confirmed with the study of a microbial fuel cell equipped with an anode modified by the reduction of 4-benzyltriphenylphosphonium diazonium, again with relatively low charge consumption during the cathodic modification process (24 mC/cm^2). Indeed, examination of Fig. 3 (right) shows that the maximum power density is doubled compared with the control microbial fuel cell equipped with a pristine graphite anode. Hence, controlled surface modification of a carbon-based electrode may significantly increase microbial fuel cell performance provided that (i) the physico-chemical properties of the modifier is adapted to that of the electroactive biofilm and (ii) that the extent of modification is optimized to avoid a possible decrease of performance due to the blocking of the electrode by a too thick layer of modifier.

3.2.2. Scanning electron microscopy (SEM) analyses

Examination of different functional bioanodes by the scanning electron microscope was carried out and showed that biofilms (up to ca. 10 μm thick) had developed on all electrodes. Bacterial colonization was however relatively less dense and less homogeneous on the surface modified by the reduc-

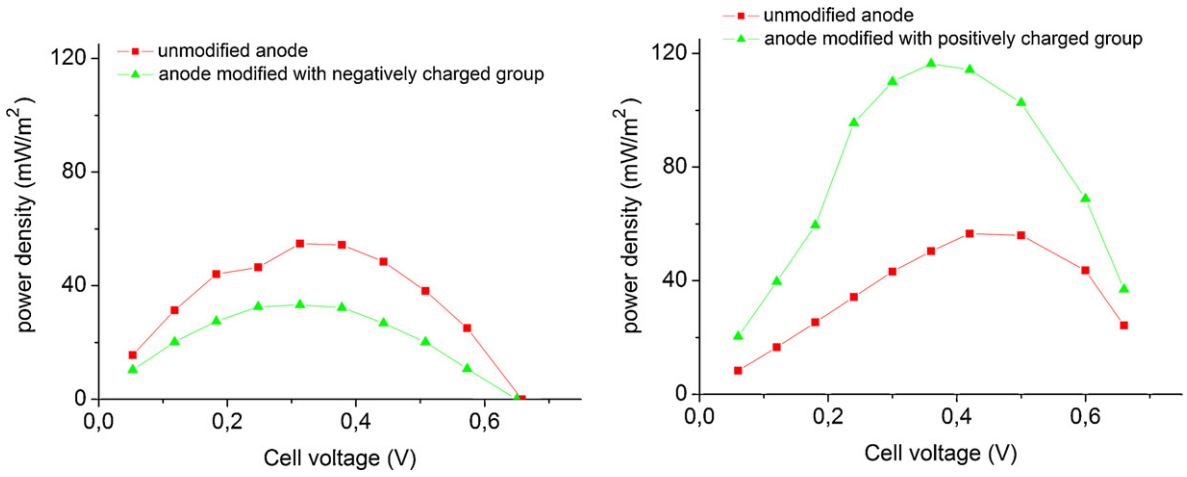

Fig. 3. Microbial fuel cell power density curves for unmodified and differently modified anodes. (A) Anode modified by the reduction of 4-phenylacetic acid diazonium and consumption of 11 mC/cm^2. (B) Anode modified by the reduction of 4-benzyltriphenylphosphonium diazonium and consumption of 24 mC/cm^2.

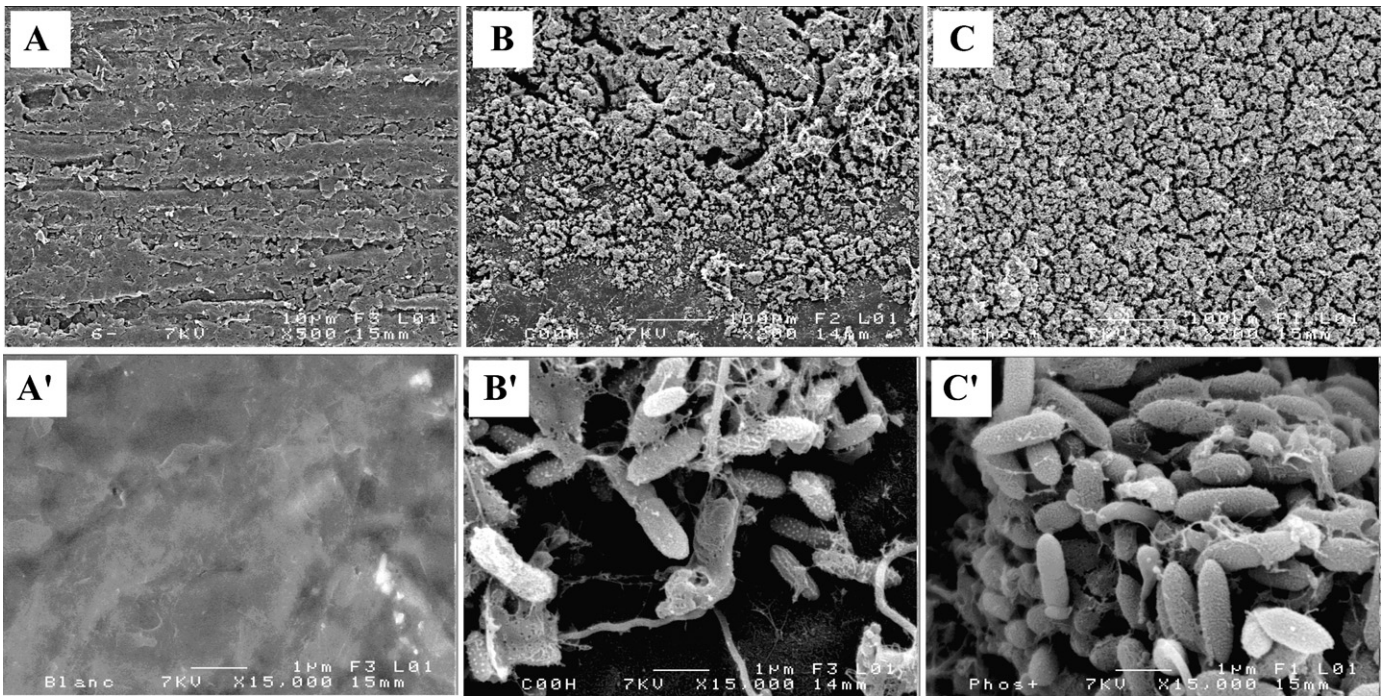

Fig. 4. Scanning electron micrographs at increased magnifications of pristine graphite (A, A′) and of colonized graphite anodes modified either by the reduction of 4-benzylcarboxylic acid diazonium (B, B′) and 4-benzyltriphenylphosphonium diazonium (C, C′).

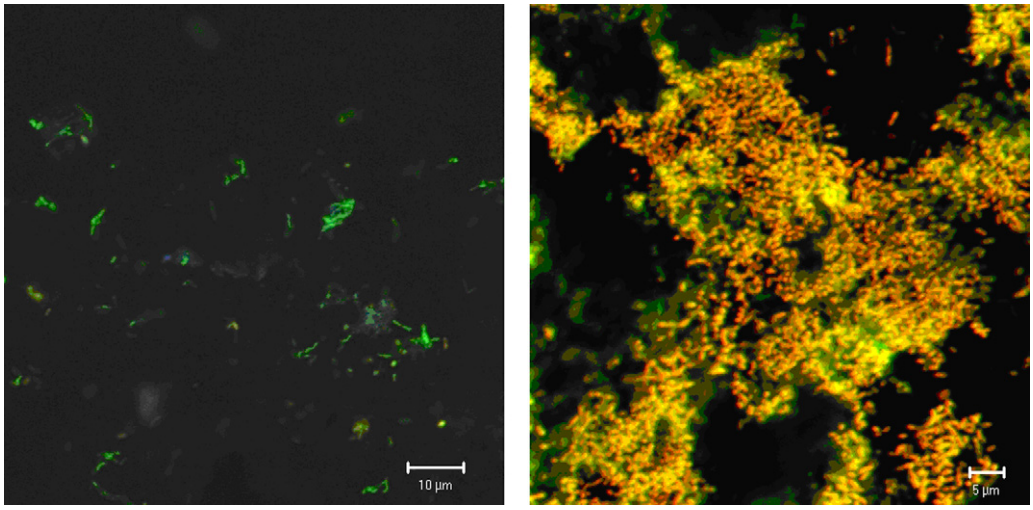

Fig. 5. FISH micrographs of anodes modified by the reduction of 4-benzylcarboxylic acid diazonium (left) and 4-benzyltriphenylphosphonium diazonium (right). The applied probes were Eub338mix-Fluos (all bacteria, green) and Geo1A-Cy3 (*Geobacter* subgroup, red). Binding of both probes results in an orange-yellow staining, positively identifying bacterial cells belonging to the *Geobacter* subgroup (*G. sulfurreducens*, *G. hydrogenophilus*, *G. grbiciae*, and *G. metallireducens*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

tion of 4-benzylcarboxylic acid diazonium (negative surface charge introduced) compared with the surface modified by the reduction of 4-benzyltriphenylphosphonium diazonium (positive surface charge introduced). This was noted at lower magnification where the colonization on the carboxylate electrode was found to be patchy rather than continuous as found for the phosphonium modified surface, Fig. 4, and is consistent with the previous discussion (Section 3.2.1).

3.2.3. *Fluorescence in situ hybridization (FISH) analyses*

Representative electrodes were subjected to fluorescent *in situ* hybridization (FISH) analysis using a combination of probes specifically detecting (i) all bacteria (probe EubMix 338) and (ii) only an electrochemically active subgroup of the *Geobacter* genus (probe Geo1A). A notable biofilm was visible on phosphonium modified anodes. In the phosphonium modified anode most cells were positively identified as belonging to the electrochemically active *Geobacter* group (probe Geo1A) with bright fluorescent signals indicating metabolically and reproductively active cells. Less bacterial cells were found on the least performing anode (modified with benzylcarboxylate groups) and none belonged to the *Geobacter* subgroup detected with probe Geo1A (see Fig. 5). Finally, the best performing aryl-amine modified anode was also analyzed and a notable biofilm was also visible. On aryl-amine modified anodes the biofilm was more heterogeneous, consisting of several cells labelled by probe Geo1A but also a considerable number of other bacte-

ria. Furthermore, the biofilm on the aryl-amine modified anodes appeared less dense and thick compared to the phosphonium modified anode.

Hence, the positive effect of optimized modification is consistent with both the development of a thick and efficient biofilm and the selection of a large amount of known electroactive bacteria at the biofilm-electrode interface. These species were also found to be evenly distributed within the biofilm thickness (ca. 5–10 μm).

4. Conclusions and perspectives

A modification procedure of carbon electrodes for microbial fuel cells has been carried out by electrochemical reduction of differently substituted aryl diazonium salts. For optimum improvement of anodic microbial electrocatalysis in microbial fuel cells it is essential to (i) introduce a surface modifier with physicochemical properties promoting the development of an efficient electroactive biofilm (demonstrated here with neutral aryl amine groups or positively charged benzylphosphonium groups) and (ii) that the amount of modifier is finely tuned, likely because the interfacial electron transfer rate between the modified electrode and the biofilm is not compromised by a low extent of modification (as suggested by the correlation between increased extent of modification, decreased heterogenous electron transfer rate to ferricyanide and decreased bioelectrocalysis). This technique is easy to implement and very versatile in that it allows the robust (covalent) grafting of a large array of chemical groups at the surface of carbon materials. We have shown that depending on the physicochemical properties of the modifier, the performance of microbial fuel cells can be significantly improved provided the amount of modifier introduced onto the surface is controlled and optimized. This is easily carried out by controlling the charge consumed during the electrode modification. We attribute the improvement of the microbial fuel cell performance both to electrostatic attraction between the negatively charged bacteria outer environment and the positively charged anode surface, and to the local physicochemical compatibility between the electroactive bacteria outer membrane cytochromes and the nature of the modifier. Hence, the critical ultimate electron transfer step at the biofilm/electrode interface involving outer membrane or free standing cytochromes is favored by a combination of a good electrostatic and hydrophobic/hydrophilic compatibility between the heme groups and the modified but conducting electrode surface. The fact that equally functional and efficient electroactive biofilms (respectively on aryl-amine and benzylphosphonium modified anode surface) were found to consist of different proportions of bacteria from the *Geobacter* genus shows that the nature of the modifier also has an effect on the microbial selection and diversity in the biofilm.

Since carbon materials used in microbial fuel cells come in many types (e.g. vitreous carbon or graphite) and forms (plates, felts, rods, granules, powder, etc.) the optimum modification conditions is to be defined for each type of electrode. The electrochemical reduction of aryl diazonium salts may not be suitable for all types of material, in particular those in the form of granules or powder for example. Alternative modification techniques involving aryl diazonium salts can nevertheless be implemented through their chemical reduction, or with the so-called spontaneous modification, or even with a mechanical approach based on ball milling functionalization (Barrière and Downard, 2008; Pinson and Podvorica, 2005; Pandurangappa et al., 2009).

The findings reported in this work have been exemplified with microbial fuel cells whose design has not been optimized (high internal resistance, Schaetzle et al., 2008). The technique detailed herein may now be implemented for the refinement of microbial fuel cells already optimized in terms of design, specific area of electrodes, etc. (Chen et al., 2011). Moreover, it can also be readily applied to other bioelectrochemical systems including microbial electrolysis cells (Rabaey et al., 2010).

Finally, we note that the reported electroactivity of biofilms from pure cultures (e.g. from the *Geobacter* or *Shewanella* genera) exhibits on a rather large potential window a number of redox couples ascribed to multiheme cytochromes (Katuri et al., 2010; Fricke et al., 2008; Liu et al., 2011; Millo et al., 2011). We propose that the surface modification reported in this study may help to discriminate between the different heme centres from electroactive outer membrane cytochromes as a function of their protein environment and the nature of the electrode surface modification. These studies are underway in our laboratory.

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References

Adachi, M., Shimomura, T., Komatsu, M., Yakuwa, H., Miya, A., 2008. Chem. Commun., 2055–2057.
Amann, R.I., Krumholz, L., Stahl, D.A., 1990. J. Bacteriol. 172, 762–770.
Baranton, S., Bélanger, D., 2005. J. Phys. Chem. B 109, 24401–24410.
Barrière, F., 2010. In: Crabtree, R.H. (Ed.), Energy Production and Storage – Inorganic Chemical Strategies for a Warming World, Encyclopedia of Inorganic Chemistry. , second ed. John Wiley & Sons, Inc., pp. 73–87.
Barrière, F., Downard, A.J., 2008. J. Solid State Electrochem. 12, 1231–1244.
Bellamy, F.D., Ou, K., 1984. Tetrahedron Lett. 25, 839–842.
Boland, S., Barrière, F., Leech, D., 2008. Langmuir 24, 6351–63588.
Brooksby, P.A., Downard, A.J., 2004. Langmuir 20, 5038–5045.
Busalmen, J.P., Esteve-Núñez, A., Berná, A., Feliu, J.M., 2008. Angew. Chem. Int. Ed. 47, 4874–4877.
Ceccato, M., Bousquet, Hinge, A.M., Pedersen, S.U., Daasbjerg, K., 2011. Chem. Mater. 23, 1551–1557.
Chen, S., Hou, H., Harnisch, F., Patil, S.A., Carmona-Martinez, A.A., Argawal, S., Zhang, Y., Sinha-Ray, S., Yarin, A.L., Greiner, A., Schröder, U., 2011. Energy Environ. Sci. 4, 1417–1421.
Cheng, S., Logan, B.E., 2007. Electrochem. Commun. 9, 492–496.
Daims, H., Brähl, A., Amann, R., Schleifer, K.H., Wagner, M., 1999. Syst. Appl. Microbiol. 22, 434–444.
Demaneche, S., Sanguin, H., Pote, J., Navarro, E., Bernillon, D., Mavingui, P., Wildi, W., Vogel, T.M., Simonet, P., 2008. Proc. Natl. Acad. Sci. U.S.A. 105, 3957–3962.
Feng, C.H., Ma, L., Li, F., Mai, H., Lang, X., Fan, S., 2010a. Biosens. Bioelectron. 25, 1516–1520.
Feng, Y., Yang, Q., Wang, X., Logan, B.E., 2010b. J. Power Sources 195, 1841–1844.
Fricke, K., Harnisch, F., Schröder, U., 2008. Energy Environ. Sci. 1, 144–147.
Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S., Culley, D.E., Reed, S.B., Romine, M.F., Saffarini, D.A., Hill, E.A., Shi, L., Elias, D.A., Kennedy, D.W., Pinchuk, G., Watanabe, K., Ishii, S., Logan, B., Nealson, K.H., Fredrickson, J.K., 2006. Proc. Natl. Acad. Sci. U.S.A. 103, 11358–11363.
Haslett, N.D., Rawson, F.J., Barrière, F., Kunze, G., Pasco, N., Gooneratne, R., Baronian, K.H.R., 2011. Biosens. Bioelectron. 26, 3742–3747.
Hori, K., Matsumoto, S., 2010. Biochem. Eng. J. 48, 424–434.
Katuri, K.P., Kavanagh, P., Rengaraj, S., Leech, D., 2010. Chem. Commun. 46, 4758–4760.
Li, Z., Rosenbaum, M.A., Venkataraman, A., Tam, T.K., Katz, E., Angement, L.T., 2011. Chem. Commun. 47, 3060–3062.
Liu, H., Matsuda, S., Kawai, T., Hashimoto, K., Nakanishi, S., 2011. Chem. Commun. 47, 3870–3872.
Logan, B.E., 2008. Microbial Fuel Cells. Wiley.
Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Environ. Sci. Technol. 40, 5181–5182.
Logan, B.E., Regan, J.M., 2006. Environ. Sci. Technol. 40, 5172–5180.
Lowy, D.A., Tender, L.M., Zeikus, J.G., Park, D.H., Lovley, D.R., 2006. Biosens. Bioelectron. 21, 2058–2063.
Lyskawa, J., Bélanger, D., 2006. Chem. Mater. 18, 4755–4763.
Magnuson, T.S., 2011. J. Bacteriol. 193, 1021–1022.
Marsili, E., Baron, D.B., Shikhare, I.D., Coursolle, D., Gralnick, J.A., Bond, D.R., 2008. Proc. Natl. Acad. Sci. U.S.A. 105, 3968–3973.

Millo, D., Harnisch, F., Patil, S.A., Ly, H.K., Schröder, U., Hildebrandt, P., 2011. Angew. Chem. Int. Ed. 50, 2625–2627.
Pandurangappa, M., Ramakrishnappa, T., Compton, R.G., 2009. Carbon 47, 2186–2193.
Pellissier, M., Barrière, F., Downard, A.J., Leech, D., 2008a. Electrochem. Commun. 10, 835–838.
Pellissier, M., Zigha, D., Barrière, F., Hapiot, P., 2008b. Langmuir 24, 9089–9095.
Pinson, J., Podvorica, F., 2005. Chem. Soc. Rev. 34, 429–439.
Potter, M.C., 1911. Proc. R. Soc. Lond. Ser. B 84, 260–276.
Rabaey, K., Angement, L., Schröder, U., Keller, J., 2010. Bioelectrochemical Systems. IWA Publishing.
Rabaey, K., Keller, J., 2008. Water Sci. Technol. 57, 655–659.
Rollefson, J.B., Stephen, C.S., Tien, M., Bond, D.R., 2011. J. Bacteriol. 193, 1023–1033.
Rosenbaum, M., Zhao, F., Schröder, U., Scholz, F., 2006. Angew. Chem. Int. Ed. 45, 6658–6661.
Rozendal, R.A., Hamelers, H.V.M., Rabaey, K., Keller, J., Buisman, C.J.N., 2008. Trends Biotechnol. 26, 450–459.
Saito, T., Mehanna, M., Wang, X., Cusick, R.D., Feng, Y., Hickner, M.A., Logan, B.E., 2011. Bioresour. Technol. 102, 395–398.
Schaetzle, O., Barrière, F., Baronian, K., 2008. Energy Environ. Sci. 1, 607–620.
Schaetzle, O., Barrière, F., Schröder, U., 2009. Energy Environ. Sci. 2, 96–99.
Terada, A., Yuasa, A., Kushimoto, T., Tsuneda, S., Katakai, A., Tamada, M., 2006. Microbiology-Sgm 152, 3575–3583.
Wang, X., Cheng, S., Feng, Y., Merril, M.D., Saito, T., Logan, B.E., 2009. Environ. Sci. Technol. 43, 6870–6873.
Zhu, N., Chen, X., Zhang, T., Wu, P., Li, P., Wu, J., 2011. Bioresour. Technol. 102, 422–426.