



HAL
open science

Electrochemical checking of aerobic isolates from electrochemically active biofilms formed in compost

Sandrine Parot, Olivier Necessian, Marie-Line Délia, Wafa Achouak, Alain Bergel

► **To cite this version:**

Sandrine Parot, Olivier Necessian, Marie-Line Délia, Wafa Achouak, Alain Bergel. Electrochemical checking of aerobic isolates from electrochemically active biofilms formed in compost. *Journal of Applied Microbiology*, 2009, 1 (4), pp.1350-1359. 10.1111/j.1365-2672.2008.04103.x . hal-03476690

HAL Id: hal-03476690

<https://hal.science/hal-03476690>

Submitted on 13 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Open Archive TOULOUSE Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author-deposited version published in : <http://oatao.univ-toulouse.fr/>
Eprints ID : 2914

To link to this article : DOI:10.1111/j.1365-2672.2008.04103.x
URL : <http://dx.doi.org/10.1111/j.1365-2672.2008.04103.x>

To cite this version :

Parot, Sandrine and Nercessian , Olivier and Délia, Marie-Line and Achouak, Wafa and Bergel, Alain (2009) [Electrochemical checking of aerobic isolates from electrochemically active biofilms formed in compost](#). Journal of Applied Microbiology, vol. 106 (n° 4). pp. 1350-1359. ISSN 1364-5072

Electrochemical checking of aerobic isolates from electrochemically active biofilms formed in compost

S. Parot¹, O. Nercessian², M.-L. Delia¹, W. Achouak² and A. Bergel¹

¹ Laboratoire de Genie Chimique, CNRS-University of Toulouse, Toulouse, France

² DSV-DEVIM, Laboratoire d'Ecologie Microbienne de la Rhizosphere et d'environnements extremes (LEMIRE), CNRS – CEA – Aix/Marseille University, CEA-Cadarache, Saint-Paul-lez-Durance, France

Keywords

biocorrosion, *Enterobacter* spp., microbial fuel cell, oxygen reduction, *Pseudomonas* spp.

Correspondence

Alain Bergel, Laboratoire de Genie Chimique,
5 rue Paulin Talabot BP1301, 31106
Toulouse, France.
E-mail: alain.bergel@ensiacet.fr

Abstract

Aims: To design a cyclic voltammetry (CV) procedure to check the electrochemical activity of bacterial isolates that may explain the electrochemical properties of biofilms formed in compost.

Methods and Results: Bacteria catalysing acetate oxidation in garden compost were able to form electrochemically active biofilms by transferring electrons to an electrode under chronoamperometry. They were recovered from the electrode surface and identification of the isolates using 16S rRNA sequencing showed that most of them were *Gammaproteobacteria*, mainly related to *Enterobacter* and *Pseudomonas* spp. A CV procedure was designed to check the electrochemical activity of both groups of isolates. Preliminary CVs suggested that the bacteria were not responsible for the catalysis of acetate oxidation. In contrast, both groups of isolates were found to catalyse the electrochemical reduction of oxygen under experimental conditions that favoured adsorption of the microbial cells on the electrode surface.

Conclusions: Members of the genera *Enterobacter* and *Pseudomonas* were found to be able to catalyse the electrochemical reduction of oxygen.

Significance and Impact of the Study: This study has shown the unexpected efficiency of *Enterobacter* and *Pseudomonas* spp. in catalysing the reduction of oxygen, suggesting a possible involvement of these species in biocorrosion, or possible application of these strains in designing bio-cathode for microbial fuel cells.

Introduction

Various electrochemical techniques (Herbert-Guillou *et al.* 1999), including cyclic voltammetry (CV) (Illsley *et al.* 1997; Vieira *et al.* 2003), have proved efficient in characterizing microbial fouling (biofouling) on electrode surfaces. In these cases, the adherent micro-organisms are not involved in the electrochemical pathway but they affect the electrochemical signal only by masking a part of the electrode surface area. In contrast, CV procedures have also been explored to characterize the electron transfer reactions observed between some microbial cells and electrodes. This kind of direct electrochemical interaction has been used to detect, or even to identify, microbial

species and to progress in the fundamental understanding of microbial electrochemistry. In the majority of cases, CV has been implemented with microbial cell suspensions. The micro-organisms were generally grown in culture media, washed and then resuspended in phosphate buffer. Micro-organism films have also been reconstituted by collecting microbial cells by filtration through a membrane and pasting them onto the electrode surface (Matsunaga and Nakajima 1985; Han *et al.* 2000). Working electrodes were commonly pyrolytic graphite and glassy carbon with different pretreatments or modified surfaces. Microbial cells have thus been found to induce either anodic peaks between 0.65 and 0.80 V vs saturated calomel reference electrode (SCE) (Matsunaga and

Namba 1984; Matsunaga and Nakajima 1985; Han *et al.* 2000) or coupled oxidation/reduction peaks with a redox potential at approximately -0.15 V vs Ag/AgCl (Kim *et al.* 1999a,b, 2002, 2005; Park *et al.* 2001; Pham *et al.* 2003). CV has allowed the number of cells in suspensions of *Saccharomyces cerevisiae* (Matsunaga and Namba 1984), *Escherichia coli* or *Lactobacillus acidophilus* (Matsunaga and Nakajima 1985; Han *et al.* 2000) to be determined. In various samples including food, drink and water, a linear relation has been established between the current of the anodic peak and the concentration of micro-organisms in the medium (Han *et al.* 2000). CV has been also used to classify microbial species into Gram-positive and Gram-negative bacteria through the difference of the anodic peak potentials, of the order of 4 mV between the two kinds of bacteria (Matsunaga and Nakajima 1985).

The electrochemical peaks have generally been attributed to a direct extracellular electron transfer involving a compound bound to the external cell wall for *Saccharomyces cerevisiae*, *Escherichia coli* and *Lactobacillus* sp. Two hypotheses have been proposed involving either coenzyme CoA (Matsunaga and Namba 1984; Matsunaga and Nakajima 1985) or cytochrome oxidase (Han *et al.* 2000). Other bacteria, including *Shewanella putrefaciens* (Kim *et al.* 1999a,b, 2002), *Clostridium butyricum* (Park *et al.* 2001), *Aeromonas hydrophila* (Pham *et al.* 2003) and *Enterococcus gallinarum* (Kim *et al.* 2005) are believed to involve c-type cytochromes. The mechanism of electron transfer to the electrode has often been shown to be direct but some bacteria have been reported to produce their own external mediator, for example, *Pseudomonas aeruginosa* is known to produce phenazines (Rabaey *et al.* 2005).

Research in this field has been stimulated anew by the recent numerous studies devoted to microbial fuel cells (MFCs). These fuel cells use the microbial biofilms that form on the electrode surfaces, most often on anodes, as catalysts of the electrochemical reactions. In this framework, CV performed with a biofilm-covered anode developed from domestic effluent has revealed two redox couples that were due to intrinsic compounds contained in the biofilm (Liu *et al.* 2005). Nevertheless, studies that perform CV on whole biofilms that were developed on the electrode surface remain very few.

A previous study has demonstrated that electrochemically active biofilms can be developed in garden compost in a quite reproducible way (Dulon *et al.* 2007; Parot *et al.* 2008a) on dimensionally stable anodes (DSA) maintained at a constant potential for several days. The purpose of this study was to extract isolates from the electrochemically active biofilms formed in compost and to design a CV procedure to check the individual activity of these isolates. The microbial characterization of the

composts is a complex scientific area, which is still in its infancy (Fracchia *et al.* 2006), and is not addressed here. The purpose was to achieve a first step in identifying and characterizing isolates from compost that may reveal interesting electrochemical properties.

Materials and method

Biofilm growth

Biofilm was grown on the surface of Dimensionally Stable Anode (DSA, 20 cm², Electro Chemical Services, St Genis, Pouilly, France) electrodes placed vertically in garden compost (Eco-terre) mixed with a solution containing 10 mmol l⁻¹ NaCl. A solution of 10 mmol l⁻¹ acetate was added to the compost either initially or during the experiment. DSA electrodes are made of titanium covered with iridium and tantalum oxides that give them their electrocatalytic properties (Trasatti 2000). Before use, they were cleaned by 5-h galvanostatic electrolysis at 0.5 A in a 0.1 mol l⁻¹ sulfuric acid solution. Biofilm growth was achieved under chronoamperometry at 0.50 V with respect to an SCE (Radiometer Analytical, TR100, Villeurbanne, France). A graphite rod (Alfa Aesar, stock#10134, Bischheim, France) was used as the auxiliary electrode. The DSA electrodes were connected to a potentiostat (VMP2, Bio-Logic SA, Claix, France) interfaced with the EC-Lab software.

Isolation and identification of bacterial strains

Each DSA electrode colonized with electrochemically active biofilm was immersed in a sterile minimum medium (medium M9) (Sambrook *et al.* 1989). The electrode surface was scraped vigorously with a sterile glass spreader and gave a suspension consisting of oxide particles released from the DSA, and microbial cells.

Serial dilutions of the cell suspension were spread on a solid artificial medium consisting of the mineral base of M9 to which tryptic soy broth (TSB, 150 mg l⁻¹), lactate (5 mmol l⁻¹) or acetate (5 mmol l⁻¹) had been added. Gellan gum (16 g l⁻¹, Sigma) was used as the solidifying agent. Microbial suspensions were also plated on agar Petri dishes containing 10-fold diluted TSB (3 g l⁻¹). Bacterial isolation was also performed in anaerobic liquid media designed to enrich anaerobic bacteria to be able to transfer electrons to extracellular electron acceptors. All cultures were grown at 30°C in the dark. The number of colony forming units (CFU) per DSA electrode was determined after 5 days of incubation. Colonies showing different morphotypes were collected with sterile tips and subsequently transferred on to new plates for purification. Isolates were stored at -80°C in minimal medium M9 containing 5% DMSO.

The isolates were identified by sequencing the 16S rRNA gene fragments obtained using primers fD1 and S17 (Heulin *et al.* 2003).

Cell preparation for cyclic voltammetry

The isolates were cultivated in liquid TSB medium at 30°C. Cultures of 30 in 100 ml flasks were stirred continuously with a Teflon-coated magnetic stir bar (250 rev min⁻¹). After 24 h of growth, the cells were harvested by centrifugation of 25 mL of culture (3140 g, 10 min, 4°C, Sigma 3K20, rotor 12158) in 30 mL sterile Nalgene centrifuge tubes. The cells were centrifuged three times, the supernatant being taken off each time and the pellet resuspended in 25 mL sodium phosphate (NaH₂PO₄) 0.1 mol l⁻¹ buffered at pH 7.5. After the last centrifugation, the pellet was resuspended in only 5 ml phosphate buffer in order to concentrate the bacterial cells. This final solution formed the working bacterial cell suspension.

Cyclic voltammetry

Cyclic voltammetry was performed at 100 mV s⁻¹ at ambient temperature with a multipotentiostat (VMP2 Bio-Logic SA). A three-electrode system was used in a 100 ml glass cell (Metrohm). The working electrode was a glassy carbon rod (Glassy Carbon V25, 3 × 150 mm, Carbone Lorraine, Gennevilliers, France) enclosed in an insulating epoxy resin (Epofix, Struers, Denmark). A new electrode was used for each CV experiment. Before each experiment, the surface was polished with successively finer grade (P120, P400, P800, P1200, P2400, P4000) abrasive discs (LAM-PLAN, Gaillard, France) until a mirror-polished surface was obtained. A platinum wire (0.5 mm diameter) and a KCl-SCE were used as auxiliary and reference electrodes, respectively. Before use, the platinum wire was heated over a gas flame until the metal became red, and the reference electrode was cleaned with ethanol and rinsed with distilled water.

The potential was scanned from open circuit voltage to oxidation potentials between -1.00 and 0.70 V *vs* SCE. The first CV without bacteria was always performed at least 10 min after the cell had been filled with the solution in order to allow the potential of the working electrode to stabilize. The effectiveness of the catalysis of oxygen reduction was compared by measuring the poten-

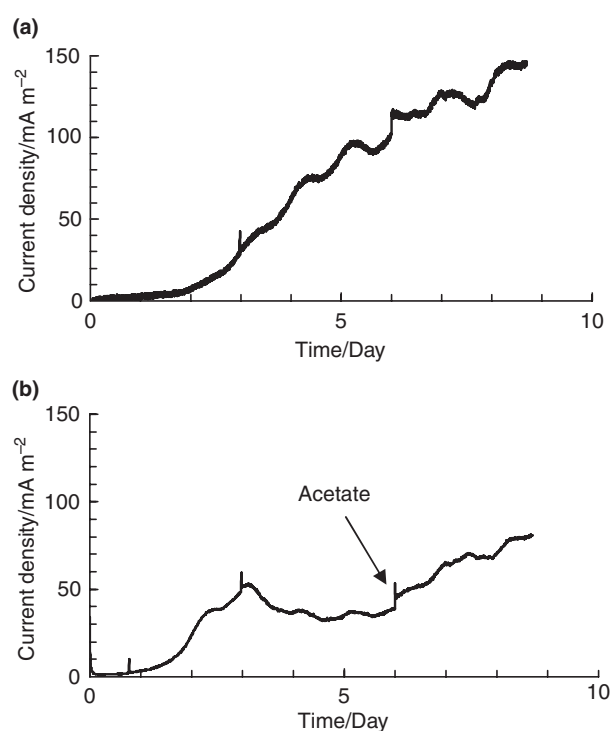


Figure 1 Chronoamperometries at 0.50 V *vs* saturated calomel reference electrode (SCE) in (a) compost mixed with acetate 10 mmol l⁻¹, (b) in compost only.

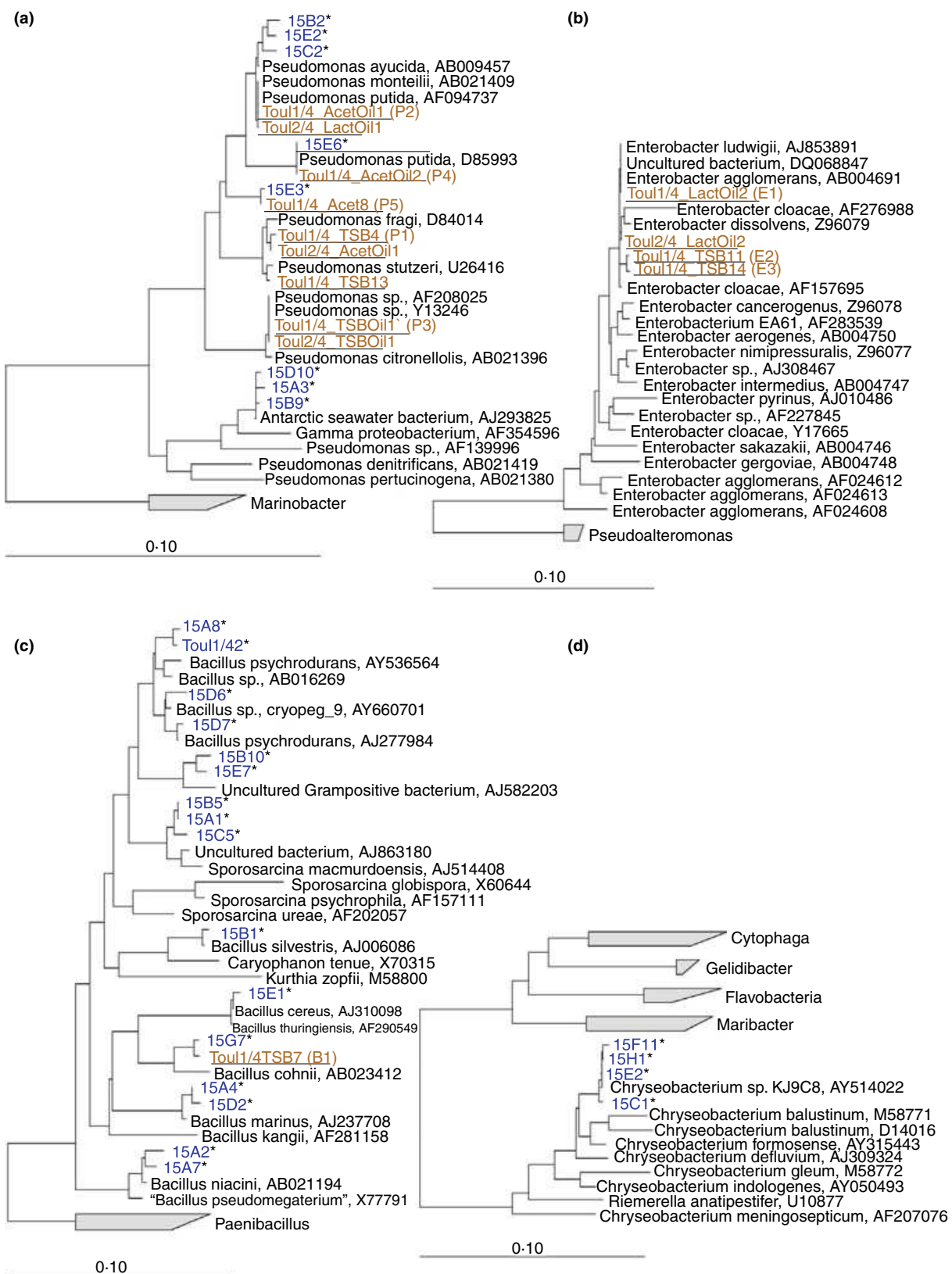
tial shift at the same value of current, arbitrary chosen at -6 μ A in all the experiments.

Results

Formation of electrochemically active biofilms in compost

Two biofilms were formed at the same time in two parallel experiments on the surface of DSA embedded in garden compost and polarized at 0.50 V *vs* SCE. The compost was mixed with a solution containing 10 mmol l⁻¹ NaCl to improve ionic conduction, and 10 mmol l⁻¹ sodium acetate was added to the compost bulk as a nutrient. In the first reactor, acetate was added from the beginning of the experiment. The current was initially close to zero because no species contained in the compost could oxidize on the clean electrode surface. The current increased after 2 days, following the development

Figure 2 Phylogenetic relationships of bacterial 16S rRNA sequences as determined by neighbour joining analysis, in the genus (a) *Pseudomonas*, (b) *Enterobacter*, (c) *Bacillus* and *Sporosarcina*, (d) *Chryseobacterium*. Sequences from isolates are underlined; sequences from the clone library are marked with a star. The scale bars represent the expected number of changes per nucleotide position.



on the electrode surface of a biofilm that gave the electrode its electro-catalytic properties. (Fig. 1a). It has been assumed that the biofilm formed on the electrode surface was able to oxidize the acetate and to transfer to the electrode the electrons produced by this metabolic oxidation (Parot *et al.* 2008b). In the second reactor, the current density increased also after 2 days but to a smaller value, because of the lack of substrate in compost. Adding acetate at day 6, when the current density had stabilized around 39 mA m^{-2} (Fig. 1b), clearly induced a new increase in current density. After 8 days, the current densities were 145 mA m^{-2} in reactor 1 and 81 mA m^{-2} in reactor 2. As previously demonstrated, the addition of acetate into the compost since the beginning of the experiment led to biofilms that showed higher electro-catalytic properties. These results were fully consistent with the results already reported on the formation of electrochemically active biofilms from compost (Parot *et al.* 2008b). The electrodes were removed from the compost before the current stabilized at a plateau value or decreased, as observed previously, to increase the chances of identifying electrochemically active bacteria in their active growth phase.

Bacterial isolation

The biofilms were scratched from the electrodes and serial dilutions of cells were performed on solid medium containing TSB, acetate or lactate. These serial dilutions were used for the enumeration of the total number of heterotrophic bacteria associated with each sample. The total number of heterotrophic bacteria ranged from 6.0×10^3 to $3.2 \times 10^4 \text{ CFU cm}^{-2}$. The highest cell density was obtained for both biofilms when the medium contained TSB.

A total of 88 isolates were obtained from the two samples. Seventy-six were obtained in aerobic conditions on solid medium and 12 were obtained in liquid medium under anoxic conditions. The sequence analyses showed that most of the isolates were *Gammaproteobacteria*. They were closely related to species belonging to the genera *Pseudomonas* (61% of the isolates) and *Enterobacter* (36% of the isolates). Based on partial 16S rDNA sequence analysis, six different *Pseudomonas* phylotypes and three different *Enterobacter* phylotypes were identified in the collection of isolates. The remaining isolates were Firmicutes and were closely related to species belonging to the genus *Bacillus* (Fig. 2). The isolates obtained under anaerobic conditions were also related to *Enterobacter* spp. and *Pseudomonas* spp., some of them being closely related to the isolates obtained under aerobic conditions. These results indicated the presence of facultative anaerobes in the sample.

As they were the predominant isolates, and because they showed close similarities to the isolates obtained under anaerobic conditions, the candidates chosen for electrochemical activity checking were one isolate of *Enterobacter* spp., called *Enterobacter* sp. E1, and a strain mixture that contained all isolates of *Pseudomonas* spp.

Electrochemical activity study of bacterial strains

Cyclic voltammetry was performed at 100 mV s^{-1} on 3-mm diameter glassy carbon electrodes, first in 5 ml of 0.1 mol l^{-1} sodium phosphate solution, pH 7.5, that contained 10 mmol l^{-1} sodium acetate, then immediately after the addition of 1 ml bacterial cell suspension of *Enterobacter* sp. E1, and finally after the electrode had been kept in the solution for 60 min in the electrochemical cell in the presence of bacteria (Fig. 3). The voltammograms revealed no anodic peak, but a cathodic peak was obtained because of the reduction of oxygen. The shift of the peak towards more positive potential values that was observed after the electrode had remained in contact with the bacterial cell suspension indicated a clear catalysis of oxygen reduction. To compare the effectiveness of the catalysis between the different experiments, it was chosen to measure the potential shift always at the same value of current, arbitrary chosen at $-6 \mu\text{A}$. This value of current was chosen because it gave the higher difference in potential, nevertheless, choosing another value of current led to the same conclusion, with identical ranking of the isolates. Here the potential shift was of $+0.23 \text{ V}$. The electrochemical experiments were highly reproducible (the potential shift being in the range $\pm 0.025 \text{ V}$) when care was taken to prepare the surface of the electrode with exactly the same procedure.

The contact between the glassy carbon electrode and the bacterial solution was studied at different times and with different methods. First, the glassy carbon electrode was kept in the cell suspension of *Enterobacter* sp. E1 for 0, 30, 60 and 90 min. The CVs performed after these different exposure times showed that the amplitude of the cathodic peak increased with the contact time (Fig. 4A). Secondly, the electrode was dipped in the bacterial cell suspension for 90 min while the suspension was either kept quiescent, or bubbled with air, or stirred with a magnetic stir bar (Fig. 4B). The peak currents reached $-9.5 \mu\text{A}$ with no stirring, $-17.9 \mu\text{A}$ with air bubbling and $-20.7 \mu\text{A}$ with magnetic stirring. For these three conditions, compared with the control voltammogram in the buffer solution, the shifts of potential at $-6 \mu\text{A}$ were 0.08, 0.29 and 0.32 V, respectively. The contact with magnetic stirring revealed the highest amplitude peak and the highest shift in potential.

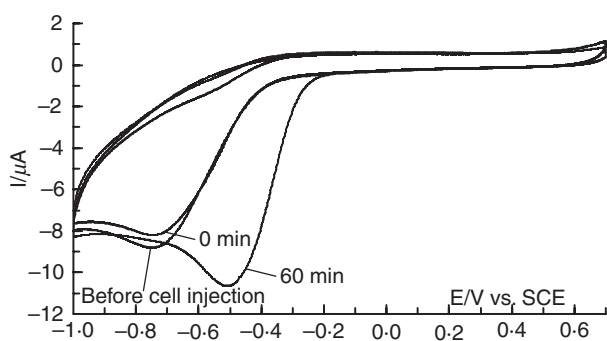


Figure 3 Cyclic voltammograms in buffer solution with 10 mmol l^{-1} acetate (before cell injection) and after addition of 1 ml cell suspension of *Enterobacter* sp. E1.

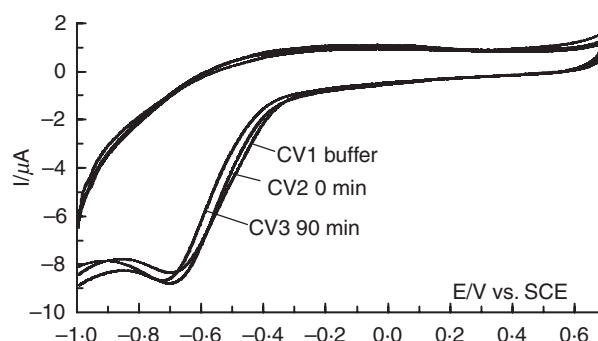


Figure 5 Standard cyclic voltammetry (CV) procedure with buffer solution. CV1 in buffer solution, CV2 after addition of 1 ml buffer solution. CV3 after 90 min with magnetic stirring.

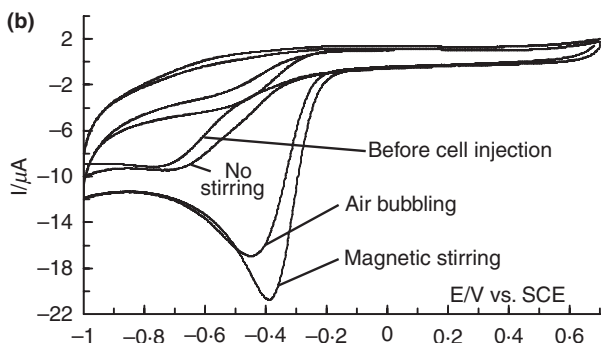
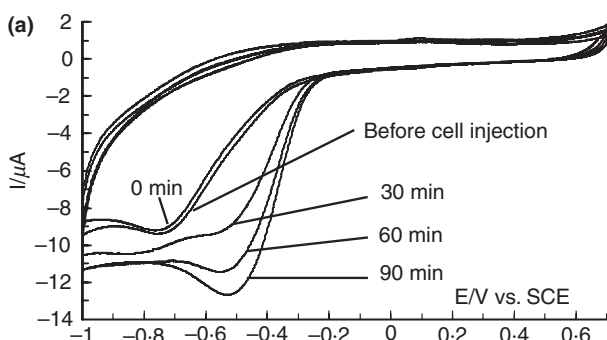


Figure 4 Influence of the conditions of contact between glassy carbon electrode and bacterial cell suspension. Cyclic voltammograms in buffer solution (before cell injection) and after addition of 1 ml of bacterial suspension of *Enterobacter* sp. E1. (a) $0, 30, 60$ and 90 min without stirring, (b) 90 min without stirring, or with air bubbling, or with magnetic stirring.

After consideration of these results, a 'standard CV procedure' was defined:

- i CV1 in phosphate buffer solution,
- ii CV2 immediately after addition of 1 ml bacterial suspension to the buffer solution,
- iii CV3 after keeping the electrode in the solution for 90 min with continuous stirring.

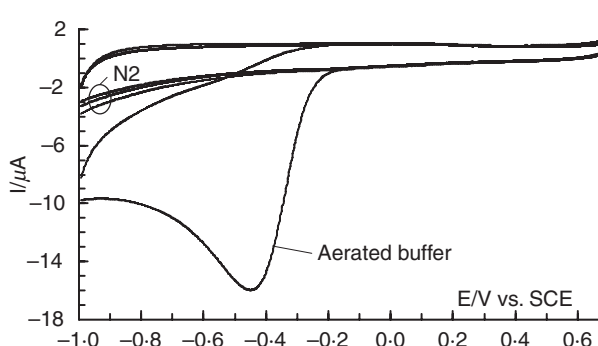


Figure 6 Standard cyclic voltammetry procedure with bacterial cell suspension of *Enterobacter* sp. E1 in anaerobic conditions, and after replacement of the solution with fresh aerated solution without bacteria.

Control experiments, run according to the standard procedure with the addition of 1 ml phosphate buffer instead of the injection of 1 ml bacterial cell suspension, revealed no modification of the oxygen reduction peak (Fig. 5), showing that there were no artefacts because of the solutions or the procedure.

Following the standard protocol, CVs were performed under anaerobic conditions with a bacterial suspension of *Enterobacter* sp. E1. In this case, nitrogen was bubbled for 15 min before performing CV1 and during the time for which the electrode was left in contact with the bacteria. The gas flow was maintained above the solution during CV recording (Fig. 6). No cathodic peak was found in the voltammograms, confirming that the peaks observed in the previous CVs were due to oxygen reduction. Replacing the deoxygenated solution that contained the bacteria with fresh aerated buffer without bacteria restored the reduction peak showing the catalysis of oxygen reduction (Fig. 6). In an additional experiment, the standard procedure was repeated, the electrode was

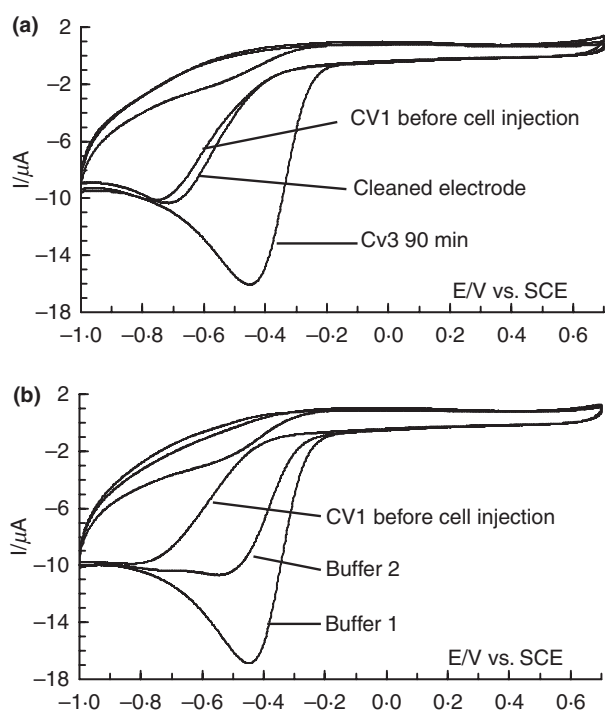


Figure 7 Standard cyclic voltammetry (CV) procedure with bacterial cell suspension of *Enterobacter* sp. E1. (a) and supplementary CV after electrode cleaning. (b) Following the standard procedure two successive CV were recorded in different fresh buffer solutions.

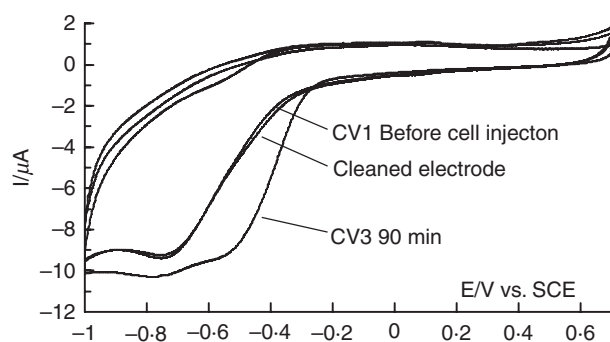


Figure 8 Standard cyclic voltammetry (CV) procedure with bacterial cell suspension of *Pseudomonas* spp. mixture and supplementary CV performed in the same bacterial solution after electrode cleaning.

cleaned with smooth paper after CV3, and a supplementary CV was performed with the cleaned electrode in the same solution containing the bacteria. The catalytic effect completely disappeared (Fig. 7A). On the contrary, when, at the end of the standard procedure, the solution was replaced with two successive fresh buffer solutions that did not contain bacteria, the catalytic effect was still observed (Fig. 7B). The potential shift was 0.29 V with

the first solution and 0.22 V with the second one (for the current value of $-6 \mu\text{A}$).

Finally, the standard CV procedure was applied on the strain mixture of *Pseudomonas* spp. (Fig. 8). The oxygen reduction was observed to be catalysed with a potential shift of 0.17 V in two different experiments. Cleaning the electrode made the shift disappear (Fig. 8). In comparison with *Enterobacter* sp. E1, which shifted the potential by 0.30 V on average, the catalysis was less effective with *Pseudomonas* spp.

Discussion

Studies on MFCs have shown that the dominant bacterial classes attached on anodes differ depending on the fuel used to feed the system (Chang *et al.* 2006; Choo *et al.* 2006). When acetate was used as the substrate, the microbial community was dominated by *Deltaproteobacteria*, particularly in sediment MFCs (Bond *et al.* 2002; Lee *et al.* 2003; Holmes *et al.* 2004; Choo *et al.* 2006). The predominance of *Gammaproteobacteria* phylotypes has been reported on MFC anodes fed with glucose and glutamate (Choo *et al.* 2006; Kim *et al.* 2006), and with cysteine (Logan *et al.* 2005). Choo *et al.*, have observed a specific enrichment from the same inoculum according to the substrate; a majority of *Deltaproteobacteria* was observed with acetate whereas *Gammaproteobacteria* dominated with glucose and glutamate. In contrast, in this study, the analysis of the bacterial community that developed in the electrochemically active biofilm from compost mixed with acetate showed that *Gammaproteobacteria* were predominant. These results suggest that the substrate is not the single key factor and the inoculum certainly also has crucial involvement in the microbial diversity, as do the operating conditions (Logan and Regan 2006). Compost rather favours *Gammaproteobacteria* development whereas marine sediments induce *Deltaproteobacteria* enrichment. Recently, electrochemical pretreatment of the anode of a marine sediment MFC has also been found to have an impact on the microbial diversity of the anode biofilm (Liu *et al.* 2007).

The CVs performed here with cell suspensions of *Enterobacter* sp. E1 and *Pseudomonas* spp. revealed no oxidation peak, even when acetate was present in the solution. It may be concluded that these species were not responsible for the electrochemical properties of the biofilm, even if they were closely related to the anaerobic species. The isolation process, which was carried out under aerobic conditions, was certainly not able to select the species that could be effective in oxidation; it may be concluded that either noncultivable bacteria or only strictly anaerobic species are likely to be responsible for this catalysis.

As the cells were separated from the culture broth by three successive cycles of centrifugation and resuspension in phosphate buffer, only the microbial cells were present in the final phosphate solution and the involvement of metabolites or any other compound from the biofilm matrix can be excluded. The catalysis required several 10-min contact times between the electrode and the bacterial solution, and it was improved by continuous stirring of the solution during exposure i.e. by increasing the probability that bacteria would encounter the electrode surface (Fig. 4B). Cleaning the electrode surface removed the catalytic effect (Fig. 7A and 8), while the effect of replacing the solution with fresh buffer without bacteria was only slight. It can be concluded that the catalysis of oxygen reduction was fully controlled by the adsorption of bacteria on the electrode surface. Hence, the slight decrease of the catalytic effect with two successive fresh buffer solutions can be attributed to partial desorption of bacteria from the electrode surface (Fig. 7B). The adsorption of bacteria on the electrode surface and their activity was not affected by nitrogen bubbling (Fig. 6). Many biological compounds adsorbed or immobilized on the surface of carbon or graphite electrodes are known to catalyse oxygen reduction through direct electron transfer with the electrode. Many iron-based proteins have been investigated; haeme proteins, such as haemoglobin, myoglobin (Li and Hu 2003; Shen and Hu 2004), cytochrome P450 (Pardo-Jacques *et al.* 2006; Shumyantseva *et al.* 2007), and enzymes such as cytochrome c oxidase (Katz *et al.* 1999; Shin *et al.* 2003; Su *et al.* 2004), peroxidase (Bogdanovskaya *et al.* 2000; Huang and Hu 2001; Li and Hu 2003; Shen and Hu 2004), catalase (Lai and Bergel 2000; Salimi *et al.* 2005), are among the most widely studied. A number of studies have focused on the oxygen reduction through the copper-containing enzymes, laccase and bilirubin oxidase, as reviewed by Shleev *et al.* (Shleev *et al.* 2005a,b; Tsujimura *et al.* 2005; Stolarczyk *et al.* 2007; Weigel *et al.* 2007). These studies resulted in a wide diversity of reduction potential values, depending on the electrode material or on the immobilization method. Bilirubin oxidase immobilized as multiple layers in a cationic polymer matrix deposited on a carbon electrode produced a cathodic peak potential at +0.25 V *vs* Ag/AgCl (Tsujimura *et al.* 2005). Laccase in solution with a boron-doped diamond electrode caused the oxygen reduction to start at +0.605 V *vs* Ag/AgCl (Stolarczyk *et al.* 2007). Among this diversity, the closest peaks, considering both the peak shape and the potential range, to those found here with *Enterobacter* and *Pseudomonas* spp. have been reported with haeme proteins such as catalase adsorbed on a glassy carbon electrode, which gave an oxygen reduction peak at -0.33 V *vs* SCE (Lai and Bergel 2000), or horseradish peroxidase incorporated in a polyester film, with a peak

at -0.36 V *vs* SCE (Huang and Hu 2001). It may thus be suggested that some haeme enzyme bound to the membrane of *Enterobacter* and *Pseudomonas* sp. could be responsible for the catalysis of the reduction of oxygen observed here.

Enterobacteriaceae are facultative anaerobic bacteria; they can grow with oxygen as an electron acceptor but also with a variety of electron acceptors such as selenate, nitrate or Fe(III) in the absence of oxygen (Jan-Roblero *et al.* 2004). This explains why *Enterobacter* sp. E1 was isolated under aerobic conditions from compost, which is an anaerobic environment. Recently, members of *Enterobacteriaceae* have been identified in biofilms responsible for anaerobic corrosion of steels in seawater (Bermont-Bouis *et al.* 2007) and pipelines (Jan-Roblero *et al.* 2004; Neria-Gonzalez *et al.* 2006). In addition, on metallic surfaces, the catalysis of oxygen reduction by a biofilm is known to provoke the increase of the free potential of the metal (phenomenon called potential ennoblement) that increases the risk of biocorrosion (Scotto *et al.* 1985; L'Hostis *et al.* 2003). According to the high efficiency in catalysing the reduction of oxygen that was demonstrated here, *Enterobacter* spp. may prove to be unexpectedly efficient participants in the ennoblement of metals when oxygen traces are present in biofilms. *Enterobacteriaceae*, which have been recently shown to be effective in anaerobic biocorrosion, should now also be suspected of being able to contribute to aerobic biocorrosion.

From another point of view, it is well known that designing efficient microbial bio-cathodes remains a key challenge to develop MFCs (He and Angenent 2006). These strains may be now considered as good candidates to form microbial oxygen-reducing bio-cathodes.

Conclusion

Garden compost has already been demonstrated to form electrochemically active biofilms efficient in catalysing acetate oxidation. These biofilms were sampled and the recovered cells were grown in aerobic and anoxic conditions. Identification of the isolates by 16S rRNA sequencing showed that most of the isolates were *Gamma*proteobacteria, mainly related to *Enterobacter* and *Pseudomonas* spp. The CV procedure that was applied on suspensions of these isolates resulted in the discovery of their unexpected ability to catalyse the electrochemical reduction of oxygen. CV did not reveal catalysis of acetate oxidation, showing that acetate oxidation was likely not achieved by cultivable aerobic or facultative anaerobic species. Work is now in progress to check the strictly anaerobic cultivable species following the same CV procedure.

Acknowledgements

This work was supported by the 6th FP of the European Community under the EA-Biofilms project (NEST 508866).

References

- Bermont-Bouis, D., Janvier, M., Grimont, P.A.D., Dupont, I. and Vallaeys, T. (2007) Both sulfate-reducing bacteria and *Enterobacteriaceae* take part in marine biocorrosion of carbon steel. *J Appl Microbiol* **102**, 161–168.
- Bogdanovskaya, V.A., Tarasevich, M.R., Fridman, V.A., Zagudaevan, N.M. and Kuznetsova, L.N. (2000) Electroreduction of oxygen on carbon black with adsorbed peroxidase or a peroxidase-nafion composite. *Russ J Electrochem* **36**, 874–878.
- Bond, D.R., Holmes, D.E., Tender, L.M. and Lovley, D.R. (2002) Electrode-reducing microorganisms that harvest energy from marine sediments. *Science* **295**, 483–485.
- Chang, I.S., Moon, H., Bretschger, O., Jang, J.K., Park, H.I., Neelson, K.H. and Kim, B.H. (2006) Electrochemically Active Bacteria (EAB) and mediator-less microbial fuel cells. *J Microbiol Biotechnol* **16**, 163–177.
- Choo, Y.F., Lee, J., Chang, I.S. and Kim, B.H. (2006) Bacterial communities in microbial fuel cells enriched with high concentrations of glucose and glutamate. *J Microbiol Biotechnol* **16**, 1481–1484.
- Dulon, S., Parot, S., Delia, M.L. and Bergel, A. (2007) Electroactive biofilms: new means for electrochemistry. *J Appl Electrochem* **37**, 173–179.
- Fracchia, L., Dohrmann, A.B., Martinotti, M.G. and Tebbe, C.C. (2006) Bacterial diversity in a finished compost and vermicompost: differences revealed by cultivation-independent analyses of PCR-amplified 16S rRNA genes. *Appl Microbiol Biotechnol* **71**, 942–952.
- Han, S., Li, X., Guo, G., Sun, Y. and Yuan, Z. (2000) Voltammetric measurement of microorganism populations. *Anal Chim Acta* **405**, 115–121.
- He, Z. and Angenent, L.T. (2006) Application of bacterial biocathodes in microbial fuel cells. *Electroanalysis* **18**, 2009–2015.
- Herbert-Guillou, D., Tribollet, B., Festy, D. and Kiene, L. (1999) In situ detection and characterization of biofilm in waters by electrochemical methods. *Electrochim Acta* **45**, 1067–1075.
- Heulin, T., Barakat, M., Christen, R., Lesourd, M., Sutra, L., de Luca, G. and Achouak, W. (2003) *Ramlibacter tataouinensis* gen. nov., sp. nov. and *Ramlibacter henchirensis* sp. nov., cyst-producing bacteria isolated from sub-desert soil in Tunisia. *Int J Syst Evol Microbiol* **53**, 589–594.
- Holmes, D.E., Bond, D.R., O'Neil, R.A., Reimers, C.E., Tender, L.R. and Lovley, D.R. (2004) Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. *Microb Ecol* **48**, 178–190.
- Huang, R. and Hu, N. (2001) Direct electrochemistry and electrocatalysis with horseradish peroxidase in Eastman AQ films. *Bioelectrochemistry* **54**, 75–81.
- Illsley, R.A., Roscoe, S.G., Jackson, E.D. and Hughes, T.J. (1997) An electrochemical investigation of the fouling of a model surface by coliform bacterium. *Biofouling* **11**, 191–199.
- Jan-Roblero, J., Romero, J.M., Amaya, M. and Le Borgne, S. (2004) Phylogenetic characterization of a corrosive consortium isolated from a sour gas pipeline. *Appl Microbiol Biotechnol* **64**, 862–867.
- Katz, E., Willner, I. and Kotlyar, A.B. (1999) A non-compartmentalized glucose/O₂ biofuel cell by bioengineered electrode surfaces. *J Electroanal Chem* **479**, 64–68.
- Kim, B.H., Kim, H.J., Hyun, M.S. and Park, D.H. (1999a) Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. *J Microbiol Biotechnol* **9**, 127–131.
- Kim, B.H., Ikeda, T., Park, H.S., Kim, H.J., Hyun, M.S., Kano, K., Takagi, K. and Tatsumi, H. (1999b) Electrochemical activity of an Fe-(III) reducing bacterium, *Shewanella putrefaciens* IR-1, in the presence of alternative electron acceptors. *Biotechnol Tech* **13**, 475–478.
- Kim, H.J., Park, H.S., Hyun, M.S., Chang, I.S., Kim, M. and Kim, B.H. (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. *Enzyme Microb Technol* **30**, 145–152.
- Kim, G.T., Hyun, M.S., Chang, I.S., Kim, H.J., Park, H.S., Kim, B.H., Kim, S.D., Wimpenny, J.W.T. et al. (2005) Dissimilatory Fe(III) reduction by an electrochemically active lactic acid bacterium phylogenetically related to *Enterococcus gallinarum* isolated from submerged soil. *J Appl Microbiol* **99**, 978–987.
- Kim, G.T., Webster, G., Wimpenny, J.W.T., Kim, B.H. and Weightman, A.J. (2006) Bacterial community structure, compartmentalization and activity in a microbial fuel cell. *J Appl Microbiol* **101**, 698–710.
- L'Hostis, V., Dagbert, C. and Feron, D. (2003) Electrochemical behavior of metallic materials used in seawater—interactions between glucose oxidase and passive layers. *Electrochim Acta* **48**, 1451–1458.
- Lai, M.E. and Bergel, A. (2000) Electrochemical reduction of oxygen on glassy carbon: catalysis by catalase. *J Electroanal Chem* **494**, 30–40.
- Lee, J., Phung, N.T., Chang, I.S., Kim, B.H. and Sung, H.C. (2003) Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses. *FEMS Microbiol Lett* **223**, 185–191.
- Li, Z. and Hu, N. (2003) Direct electrochemistry of heme proteins in their layer-by-layer films with clay nanoparticles. *J Electroanal Chem* **558**, 155–165.
- Liu, H., Cheng, S. and Logan, B.E. (2005) Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ Sci Technol* **39**, 658–662.
- Liu, J.L., Lowy, D.A., Baumann, R.G. and Tender, L.M. (2007) Influence of anode pretreatment on its microbial colonization. *J Appl Microbiol* **102**, 177–183.

- Logan, B.E. and Regan, J.M. (2006) Electricity-producing bacterial communities in microbial fuel cells. *Trends Microbiol* **14**, 512–518.
- Logan, B.E., Murano, C., Scott, K., Gray, N.D. and Head, I.M. (2005) Electricity generation from cysteine in a microbial fuel cell. *Water Res* **39**, 942–952.
- Matsunaga, T. and Nakajima, T. (1985) Electrochemical classification of Gram-negative and Gram-positive bacteria. *Appl Environ Microbiol* **50**, 238–242.
- Matsunaga, T. and Namba, Y. (1984) Detection of microbial cells by cyclic voltammetry. *Anal Chem* **56**, 798–801.
- Neria-Gonzalez, I., Wang, E.T., Ramirez, F., Romero, J.M. and Hernandez-Rodriguez, C. (2006) Characterization of bacterial community associated to biofilms of corroded oil pipelines from the southeast of Mexico. *Anaerobe* **12**, 122–133.
- Pardo-Jacques, A., Basseguy, R. and Bergel, A. (2006) Electroactive cytochromeP450BM3 cast polyion films on graphite electrodes. *Electrochim Acta* **52**, 979–987.
- Park, H.S., Kim, B.H., Kim, H.S., Kim, H.J., Kim, G.T., Kim, M., Chang, I.S., Park, Y.K. *et al.* (2001) A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum*, isolated from a microbial fuel cell. *Anaerobe* **7**, 297–306.
- Parot, S., Delia, M.-L. and Bergel, A. (2008a) a) Acetate to enhance electrochemical activity of biofilms from garden compost. *Electrochim Acta* **53**, 2737–2742.
- Parot, S., Delia, M.-L. and Bergel, A. (2008b) b) Forming electrochemically active biofilms from garden compost under chronoamperometry. *Bioresour Technol* **99**, 4809–4816.
- Pham, C.A., Jung, S.J., Phung, N.T., Lee, J., Chang, I.S., Kim, B.H., Yi, H. and Chun, J. (2003) A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. *FEMS Microbiol Lett* **223**, 129–134.
- Rabaey, K., Boon, N., Hofte, M. and Verstraete, W. (2005) Microbial phenazine production enhances electron transfer in biofuel cells. *Environ Sci Technol* **35**, 3401–3408.
- Salimi, A., Noorbakhsh, A. and Ghadermarz, M. (2005) Direct electrochemistry and electrocatalytic activity of catalase incorporated onto multiwall carbon nanotubes-modified glassy carbon electrode. *Anal Biochem* **344**, 16–24.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. NY: Cold Spring Harbor Laboratory, Cold Spring Harbor.
- Scotto, V., DiCinto, R. and Marcenaro, G. (1985) The influence of marine aerobic microbial biofilm on stainless steel corrosion behaviour. *Corrosion Sci* **25**, 185–194.
- Shen, L. and Hu, N. (2004) Heme protein films with polyamidoamine dendrimer: direct electrochemistry and electrocatalysis. *Biochim Biophys Acta* **1608**, 23–33.
- Shin, H., Lee, D.H., Kang, C. and Karlin, K.D. (2003) Electrocatalytic four-electron reductions of O₂ to H₂O with cytochrome c oxidase model compounds. *Electrochim Acta* **48**, 4077–4082.
- Shleev, S., Tkac, J., Christenson, A., Ruzgas, T., Yaropolov, Al., Whittaker, J.W. and Gorton, L. (2005a) Direct electron transfer between copper-containing proteins and electrodes. *Biosens Bioelectron* **20**, 2517–2554.
- Shleev, S., Jarosz-Wilkolazka, A., Khalunina, A., Morozova, O., Yaropolov, A., Ruzgas, T. and Gorton, L. (2005b) Direct electron transfer reactions of laccases from different origins on carbon electrodes. *Bioelectrochemistry* **67**, 115–124.
- Shumyantseva, V.V., Bulko, T.V., Rudakov, Yu.O., Kuznetsova, G.P., Samenkova, N.F., Lisitsa, A.V., Karuzina, I.I. and Archakov, A.I. (2007) Electrochemical properties of cytochromes P450 using nanostructured electrodes: direct electron transfer and electro catalysis. *J Inorg Biochem* **101**, 859–865.
- Stolarczyk, K., Nazaruk, E., Rogalski, J. and Bilewicz, R. (2007) Mediatorless catalytic oxygen reduction at boron-doped diamond electrodes. *Electrochem Commun* **9**, 115–118.
- Su, L., Hawkrige, F.M. and Rhoten, M.C. (2004) Electroreduction of oxygen by cytochrome c oxidase immobilized electrode-supported lipid bilayer membranes. *Chem Biodivers* **1**, 1281–1288.
- Trasatti, S. (2000) Electrocatalysis: understanding the success of DSA[®]. *Electrochim Acta* **45**, 2377–2385.
- Tsujimura, S., Kano, K. and Ikeda, T. (2005) Bilirubin oxidase in multiple layers catalyzes four-electron reduction of dioxygen to water without redox mediators. *J Electroanal Chem* **576**, 113–120.
- Vieira, M.J., Pinho, I.A., Giao, S. and Montenegro, M.I. (2003) The use of cyclic voltammetry to detect biofilms formed by *Pseudomonas fluorescens* on platinum electrodes. *Biofouling* **19**, 215–222.
- Weigel, M.Ch., Tritscher, E. and Lisdat, F. (2007) Direct electrochemical conversion of bilirubin oxidase at carbon nanotube-modified glassy carbon electrodes. *Electrochem Commun* **9**, 689–693.