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Electrochemical reduction of oxygen catalyzed by *Pseudomonas aeruginosa*

Amandine Cournet [a,b], Mathieu Bergé [a], Christine Roques [a], Alain Bergel [b], Marie-Line Délia [b,*]

[a] *Université de Toulouse; UPS; LU49, Adhésion bactérienne et formation de biofilms; 35 chemin des Maraîchers, 31062 Toulouse Cedex 09, France*
[b] *Laboratoire de Génie Chimique CNRS UMR5503; 4 allée Emile Monso, BP 84234, 31432 Toulouse Cedex 04, France*

ABSTRACT

Pseudomonas aeruginosa has already been shown to catalyze oxidation processes in the anode compartment of a microbial fuel cell. The present study focuses on the reverse capacity of the bacterium, i.e. reduction catalysis. Here we show that *P. aeruginosa* is able to catalyze the electrochemical reduction of oxygen. The use of cyclic voltammetry showed that, for a given range of potential values, the current generated in the presence of bacteria could reach up to four times the current obtained without bacteria. The adhesion of bacteria to the working electrode was necessary for the catalysis to be observed but was not sufficient. The electron transfer between the working electrode and the bacteria did not involve mediator metabolites like phenazines. The transfer was by direct contact. The catalysis required a certain contact duration between electrodes and live bacteria but after this delay, the metabolic activity of cells was no longer necessary. Membrane-bound proteins, like catalase, may be involved. Various strains of *P. aeruginosa*, including clinical isolates, were tested and all of them, even catalase-defective mutants, presented the same catalytic property. *P. aeruginosa* offers a new model for the analysis of reduction catalysis and the protocol designed here may provide a basis for developing an interesting tool in the field of bacterial adhesion.

1. Introduction

Electroactive bacteria are able to transfer electrons to an electrode and thus produce current [1]. They have recently generated considerable interest concerning bioelectricity production in Microbial Fuel Cells (MFCs), in which they offer the possibility of converting chemical energy from a wide range of organic wastes into electrical energy [2]. Various bacteria have already been shown to be electroactive [3] and a lot of work is being done to try to decipher the cellular mechanisms by which such an electron transfer is possible. Many studies are devoted to oxidation processes that occur in the anode compartment of MFCs in an attempt to understand how bacteria transfer electrons to electrodes. Three different processes of transfer have been described. Some bacteria produce electrochemically active mediator metabolites that serve as electron shuttles between cells and electrodes [4]. Other bacteria have been shown to transfer electrons directly to electrodes without the implication of metabolites but through membrane-bound redox compounds such as C-cytochromes [5]. Finally, in some thick biofilms, involvement of conductive nanowires produced by the cells has been demonstrated with *Shewanella* and *Geobacter* species [6,7]. These various studies have been mostly performed for anodic processes.

Microbially catalyzed reductions also exist [8–10] but have not been so largely studied. The catalysis of oxygen reduction by microbial biofilms was first identified in the field of microbial corrosion [11]. Biofilms formed in seawater have been shown to be efficient in catalyzing the reduction of oxygen on stainless steels and other materials but, for now, the mechanisms remain a subject of debate. Several hypotheses have been proposed, including direct catalysis by adsorbed enzymes like catalase [12,13] or indirect catalysis via the production of hydrogen peroxide [14] or the production of manganese oxides/hydroxides by manganese oxidizing bacteria [15]. In recent years, attempts have been made to exploit the electrocatalytic properties of aerobic seawater biofilms to design biofilm-cathodes in MFCs [10,16,17]. Marine electroactive biofilms are composed of natural microbial consortia, in which microbial diversity is very large. It is suspected that mechanisms similar to those identified in corrosion studies may be involved but these mechanisms alone cannot explain the high current density values that are observed with natural seawater biofilms operating at controlled potential.

Several studies have been performed on the electroactivity of *Pseudomonas* species but most of them have focused on the ability of these microorganisms to give electrons to electrodes using their own electron shuttles. In 1996, pyocyanin, a blue pigment produced by several strains of *P. aeruginosa*, was studied by adsorptive stripping voltammetry [18]. This work revealed that pyocyanin had a redox activity of around −0.20 V *vs* Ag/AgCl. Some work on *Pseudomonas chlororaphis* assessed the necessity

of phenazine-1-carboxamide (PCN) production for this strain to keep its ability to reduce Fe(III) [19]. More recently, *P. aeruginosa* was isolated from the anodic compartment of an MFC [20]. This bacterium was shown to produce soluble components called phenazines that served as electron shuttles between the bacteria and the electrode [4]. *P. aeruginosa* has already been used to improve electricity generation in the anodes of MFCs [21] but, to our knowledge, this bacterium has never been shown to catalyze a cathodic reaction. However, previous data have been published showing the increment of cathodic currents by an unidentified specie of *Pseudomonas*, correlated to its catalase activity [22].

P. aeruginosa is an aerobic bacterium, so its usual electron acceptor is oxygen. For this reason, we focused on the ability of *P. aeruginosa* to catalyze the electrochemical reduction of oxygen. This catalysis cannot be used in cathodic compartments of MFCs since the potential of the oxygen reduction catalyzed by bacteria is still too low for such an application. However, a specific experimental protocol was designed to study the reaction using cyclic voltammetry (CV). This method has already been shown to be efficient for rapid detection of the electroactivity of bacterial cells [20,23]. The objectives of the present study were (i) to see whether *P. aeruginosa* was able to catalyze the electrochemical reduction of oxygen (ii) to progress in understanding the mechanisms of the electron transfer, and (iii) to discuss the potential applications of such a property.

2. Experimental

2.1. Bacterial strains, culture conditions and chemicals

Experiments were performed on the reference strain PA01 of *P. aeruginosa*. Clinical isolates from patients with cystic fibrosis obtained from the Hospital Bacteriology and Hygiene Laboratory in Toulouse (France) were also tested (Table 1). Four *P. aeruginosa* PA14 catalase mutants were provided by Sogang University, Korea: PA14 KatA−, PA14 KatB−, PA14 KatE− and PA14 KatABE− [24]. Catalase activity was assessed with the ID color Catalase test (Biomérieux, France). All the strains were stored at −80 °C in Eugon medium (10% glycerol). They were plated on Trypcase Soy Agar (Biomérieux, France) under aerobic conditions. Before each experiment, the strains were grown overnight in 20 mL Trypcase Soy Broth (Biomerieux, France) at 37 °C under gentle stirring (200 rpm). The bacterial suspensions were then centrifuged (10 min, room temperature, $3600 \times g$), washed twice in phosphate buffer (K_2HPO_4/KH_2PO_4, 0.1 M, pH 7), and resuspended in the same buffer. All experiments were performed in the same buffer at room temperature.

Table 1
Phenotypic characterization of various electroactive strains of *P. aeruginosa*.

Name	Origin	Phenotype
PA01	Pasteur institute (France)	Green pigmentation
7844M	Clinical isolates	Mucoid
7844S	Clinical isolates	Non-mucoid revertant
7645M	Clinical isolates	Mucoid
A01	Clinical isolates	No pigmentation
A02	Clinical isolates	Brown pigmentation
A03	Clinical isolates	No pigmentation
A04	Clinical isolates	No pigmentation
A05	Clinical isolates	Blue pigmentation
A06	Clinical isolates	Brown pigmentation
PA14	Sogang University (Korea)	Catalase +
PA14 Kat A−	Sogang University (Korea)	Catalase −/+
PA14 Kat B−	Sogang University (Korea)	Catalase +
PA14 Kat E−	Sogang University (Korea)	Catalase +
PA14 Kat ABE−	Sogang University (Korea)	Catalase −

2.2. Cyclic voltammetry method

Cyclic voltammetry consists in performing a potential scan on a solution while recording the current obtained. Currents due to oxidation processes are conventionally noted as positive and those due to reduction as negative. Experiments were monitored by a Princeton Applied Research potentiostat and the Power Suite software. Working electrodes were 0.07 cm² glassy carbon rods inserted in an inert resin cylinder of approximately 1 cm diameter. Prior to use, the rods were polished with abrasive silicon carbide paper and cleaned in distilled water. The counter-electrode was a platinum wire and a saturated calomel electrode (Radiometer Analytical) was used as a reference (SCE).

2.3. Protocol conditions

Experiments were conducted in a 40 mL cell. A first voltammogram was run with 20 mL buffer to check the quality of the working electrode surface. A second was performed immediately after the addition of bacterial cells (resuspended culture in buffer) at the final concentration of 10^8 bacteria/mL. Then the suspension was stirred for 15 min, 30 min or 1 h to maintain a homogeneous oxygen concentration and the CV scan was performed again. Measurements under anaerobic conditions were carried out after removing oxygen from the solution by 20 min of nitrogen bubbling. There was no stirring or gas bubbling during current recording.

Similar CV experiments were done on the filtrate of *P. aeruginosa* PA01. After 1 h of stirring, 20 mL of bacterial suspension was filtered through a 0.45 μm sterile filter then through a 0.20 μm one. The resulting filtrate was tested by CV as described above.

CVs were also performed on dead cells of PA01. After an overnight culture, bacteria were washed as described above and resuspended in pure methanol (Aldrich, France) for 30 min. The dead cells were then washed again in phosphate buffer and tested in CV in the same way as live cells. In another experiment, cells were killed after the 1-h contact time between electrodes and bacteria directly on the surface of the working electrode: the glassy carbon rod was dipped into pure methanol (Aldrich, France) for 30 min. CV was also performed on this electrode with adhered dead cells.

Each experiment was independently performed at least three times.

2.4. Scanning electron microscopy (SEM)

Scanning electron microscopy was used on small pieces of working electrodes to analyze the coverage of the surface by microorganisms. Prior to observations, the samples were fixed in 2.5% glutaraldehyde at 4 °C for 1 h and dehydrated in a graded series of aqueous ethanol solutions (30, 50, 80 and 99%). Finally, samples were sputtered with gold and examined in a LEO 435 VP microscope (Carl Zeiss SMT).

3. Results

3.1. Preliminary measurements

Before the measurements, voltammograms of *P. aeruginosa* PA01 in buffer were analyzed with different scan rates: 1000, 750, 500, 200, 100, 50, 20 and 10 mV s⁻¹. The shape of the curves was the same whatever the scan rate, the only differences being the background signals, the time the measurement lasted, and the intensity of the response. The scan rate of 100 mV s⁻¹ was chosen as the best compromise between quality, intensity and rapidity of the response obtained. Voltammograms starting at 0.1 V/SCE, continuing with three cycles from 0.7 V/SCE to −1 V/SCE and ending at the initial
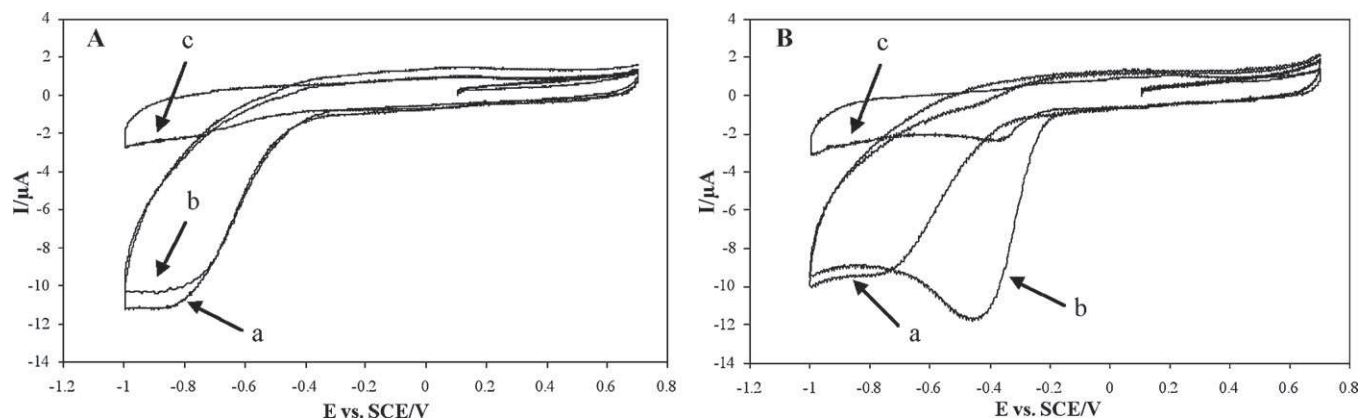
Fig. 1. Cyclic voltammograms of (A) phosphate buffer alone and (B) *P. aeruginosa* PA01 in phosphate buffer (line a), after 1 h of gentle stirring (line b) and after 20 min of nitrogen bubbling (line c).

potential were reproducible and allowed easy observation of oxygen reduction. In the figures, only the first cycle is shown, to make the curves clearer.

3.2. Control voltammograms on phosphate buffer

The voltammograms performed on phosphate buffer under aerobic conditions presented a reduction wave (Fig. 1A, line a). This wave was confirmed to correspond to the electrochemical reduction of the dissolved oxygen ($O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$ (1) and $H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$ (2)) since it disappeared after oxygen was removed by nitrogen bubbling (Fig. 1A, line c). It has previously been shown that the balance between the two reactions depends on the nature of the carbon electrode and of its pretreatment. The balance between reactions (1) and (2) controls the general shape of the current–potential curve obtained by voltammetry. Numerical modelling has also indicated that, at high scan rate (as used here in voltammetry experiments), hydrogen peroxide is almost completely reduced by reaction (2) before it is able to move far from the electrode surface by diffusion [12]. So, in a first approach, an overall equation can be used to explain the phenomenon ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$). The voltammogram in phosphate buffer under anaerobic conditions showed neither a current peak nor a current wave (Fig. 1A, line c). The flat curve corresponded to the capacitive (non-Faradic) current induced by the charging and discharging of the electrochemical double layer, but no electron exchange (oxidation–reduction phenomenon) took place between the electrode and dissolved compounds. Under aerobic conditions, oxygen reduction started at -0.37 ± 0.01 V/SCE, and a steady state current was established from -0.81 ± 0.02 V/SCE due to mass transfer limitation of oxygen to the electrode surface (diffusion-limited current). The average values and standard deviations were the result of 7 independent experiments. Moreover, oxygen bubbling led to an augmentation of the curve amplitude (data not shown). After 1 h of stirring, the voltammogram remained the same (Fig. 1A, line b). Stirring ensured that the solution was regularly re-oxygenated and led to reproducible voltammograms.

3.3. Electrochemical reduction of oxygen by P. aeruginosa PA01

The voltammogram recorded immediately after the addition of the suspension of *P. aeruginosa* PA01 (Fig. 1B, line a) was identical to the first voltammogram performed in phosphate buffer alone (Fig. 1A, line a). In contrast, after 1 h of stirring, a displacement of the oxygen reduction wave was observed (Fig. 1B, line b). The presence of bacteria clearly modified the shape of the current–potential curve. In these new conditions, the wave started

at -0.18 ± 0.01 V/SCE, reached its maximum at -0.44 ± 0.03 V/SCE (average values and standard deviations from 7 independent experiments) and finally returned to the same diffusion-limited current. For a given value of the potential, the current generated in presence of *P. aeruginosa* PA01 after 1 h of stirring was higher than immediately after bacteria addition. It was observed to rise to as much as four times the current obtained without bacteria: -11.4 ± 0.4 µA (-162 ± 5 µA/cm^2) compared to -2.4 ± 0.5 µA (-34 ± 7 µA/cm^2) for the same potential value (7 independent experiments). After 1 h of stirring in presence of bacteria and 20 min of nitrogen bubbling, very weak catalysis was observed (Fig. 1B, line c), probably due to traces of oxygen remaining in the solution.

Different durations of stirring were tested. As shown in Fig. 2E (only the part of the voltammogram that is of interest is shown in order to make the phenomenon more clearly visible) the amplitude of the oxygen reduction wave increased with the contact duration. Weak catalysis was observed after 15 min (Fig. 2E, line b) but was maximal after 1 h of contact (Fig. 2E, line d). Beyond 1 h (3 h, 6 h, and 24 h), the wave amplitude did not increase further and it appeared that a steady state had been reached. Scanning microscopy was used to assess the working electrode surface coverage by bacteria. Observations were made after 15 s, 15 min, 30 min and 1 h of contact between electrodes and bacteria in phosphate buffer under gentle stirring (Fig. 2A–D). There were not more adhered bacteria after 1 h of contact between electrodes and the bacterial suspension than after 15 s (Fig. 2A and D). These pictures show that the coverage did not significantly increase with the contact duration (Fig. 2A–D). The catalytic effect observed on the voltammogram was consequently not directly correlated to the bacterial surface coverage.

3.4. Characterization of the electrochemical activity

Three other tests were performed on *P. aeruginosa* PA01 to understand the phenomenon further. The bacterial suspension was stirred for 1 h without any contact with the electrodes. After this delay, when the electrodes were plunged into the suspension, the voltammogram recorded was identical to the control curves recorded in buffer alone; no catalytic effect could be observed (data not shown). Thus, a certain time of contact between microorganisms and electrodes was absolutely necessary. In a second set of experiments, the bacteria were filtered out of the suspension, and the electrodes were plunged for 1 h in the filtrate under stirring. Voltammograms revealed only a very weak catalytic effect compared with that of the bacterial suspension (Fig. 3). This demonstrated that a mediator alone was not responsible for the catalysis and that the presence of bacteria was necessary. The final test eval-
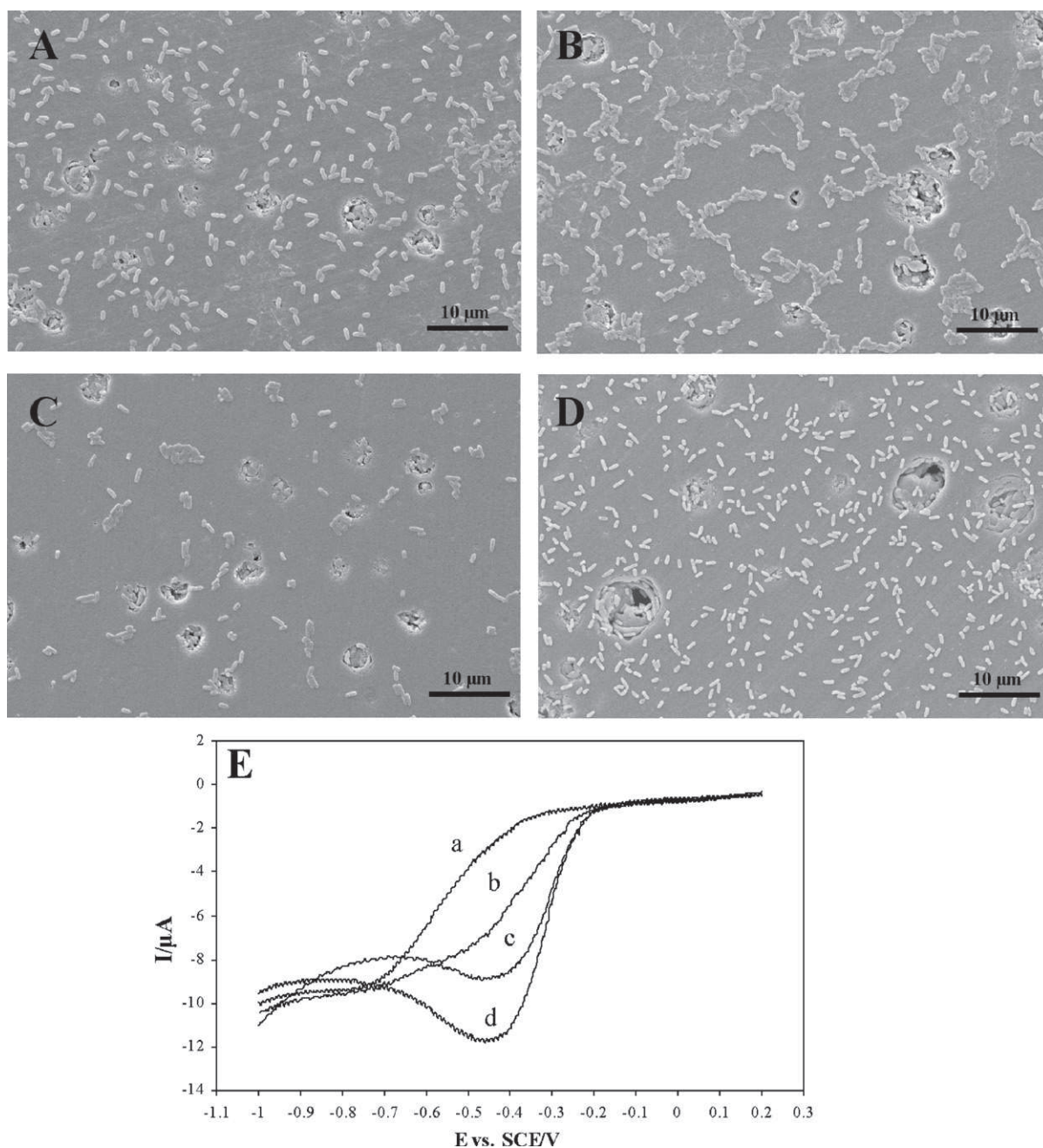
Fig. 2. Scanning electron micrographs of the working electrode surface after 15 s (A), 15 min (B), 30 min (C) and 1 h (D) of contact with bacteria, magnification 5000, and corresponding cyclic voltammograms of *P. aeruginosa* PA01 in phosphate buffer (E) after 15 s (line a), 15 min (line b), 30 min (line c), and 1 h (line d) of contact. Beyond 1 h (3 h, 6 h and 24 h), the wave amplitude does not increase further.

uated the activity of the matter adhering to the working electrode. After 1 h of contact with bacteria under stirring, the electrodes were removed from the suspension, rinsed in distilled water and tested again in 20 mL fresh phosphate buffer (Fig. 4). The curve in the fresh buffer presented the same catalysis of oxygen reduction. Bacteria, proteins or other molecules they produced, and which became attached to the working electrode surface, were consequently fully responsible for the catalytic effect.

Tests were performed to assess the necessity for metabolic activity of the cell during the transfer of electrons. Voltammograms performed on dead cells even after 1 h of contact between electrodes and dead bacteria revealed no catalysis of the oxygen reduction (Fig. 5A, line c). The catalysis was, however, observed
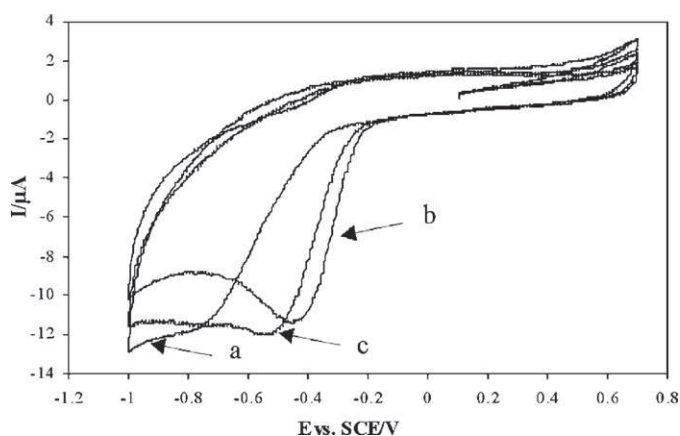
when bacteria were killed on the electrode surface after 1 h of contact (Fig. 5B, line c).

3.5. Electrochemical reduction of oxygen by other strains of P. aeruginosa

All the strains of *P. aeruginosa* tested in this study by the cyclic voltammetry method (Table 1) presented the same behavior as PA01. The difference between the maximal intensity of the oxygen reduction peak in presence of PA01 and other strains of *P. aeruginosa* was lower than 5% (3 independent experiments on each strain). The clinical strains were chosen because of their different phenotypes so as to assess a possible implication of alginate and
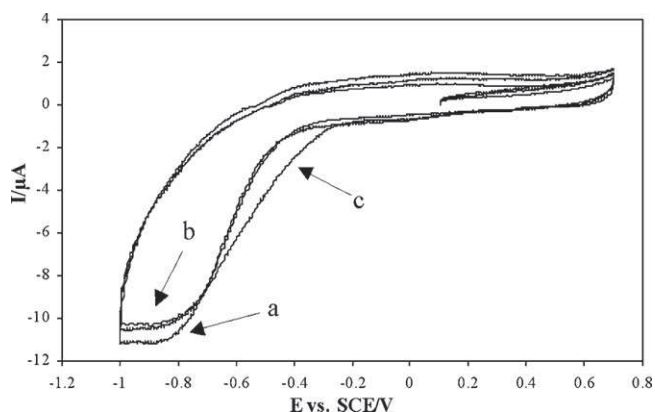
Fig. 3. Cyclic voltammograms in phosphate buffer (line a), with *P. aeruginosa* PA01 filtrate (line b) and *P. aeruginosa* PA01 filtrate after 1 h of stirring (line c). After 1 h of stirring, the bacterial suspension was filtered through a 0.45 μm sterile filter then through a 0.20 μm one. CV scans were performed on this filtrate (lines b and c).

Fig. 4. Cyclic voltammograms of *P. aeruginosa* PA01 in phosphate buffer (line a), after 1 h of stirring (line b) and after the electrodes were removed and plunged into a fresh phosphate buffer (line c).

differences in pigmentation: green (pyoverdin), blue (pyocyanin), brown (pyomelanin and pyorubin) and three with no pigmentation at all. All of them presented the same electrochemical activity. Finally, no significant electrochemical differences were observed between the PA14 catalase-defective mutants and the wild type strain. The catalase activity of the mutants was tested and revealed that PA14 KatB− and PA14 KatE− presented a catalase activity similar to the wild type one, PA14 KatA− presented a weak catalase activity, and the triple mutant PA14 KatABE− no catalase activity at all (Table 1).

4. Discussion

In the presence of bacteria, the reduction of oxygen started for lower potentials and higher currents were obtained (Fig. 1). For the first time, *P. aeruginosa* has been shown to catalyze the electrochemical reduction of oxygen. Previous studies on *P. aeruginosa* have been devoted to anodic processes only, where the bacterial cell transfers electrons to the electrodes. In the present work, the reverse phenomenon has been demonstrated (Fig. 1B).

A certain time of contact between the bacterial suspension and electrodes is necessary for the full catalytic effect to be observed (Fig. 1B). A weak effect was detected in the first few minutes and was complete only after 1 h (Fig. 2E). Microscopic images showed that a few seconds were enough to get a bacterial coverage of the electrode surface similar to that observed after 1 h (Fig. 2A and D). Nevertheless, it was necessary to wait for 1 h to obtain the full catalytic effect even though there were not more bacteria on the electrode surface after this delay. On the other hand, experiments performed in fresh buffer after adhesion indicated that bacteria, proteins or other produced molecules adhering to the electrode surface were responsible for the phenomenon (Fig. 4). Adhesion is necessary but not sufficient; a prolonged contact time is also required.

The attachment of bacteria to a solid surface implies two steps: reversible and irreversible attachment [25]. In the first step, the bacteria are transported close enough to the surface, involving van der Waals forces, electrostatic forces and hydrophobic interactions. The second stage of adhesion is the locking phase and employs molecularly mediated binding between the surface and specific adhesins, like exopolysaccharides (biofilm matrix), pili, fimbriae, etc. On the SEM images, no difference was observed between bacteria adsorbed on the electrode surface after 15 s or after 1 h of contact. The 1-h contact time did not lead to a visual modification of the adsorption pattern, which would have indicated biofilm development. Moreover, the tests on mucoid and non-mucoid strains indicated that alginate, which is the main component of the biofilm

pigments in the catalytic effect. The strain 7844M is mucoid; it produces a lot of alginate. The 7844S is a non-mucoid revertant obtained after plating 7844M several times. Both presented the same restriction card and differed only by the production of alginate. These two strains showed similar positive activity, suggesting that alginate production was not involved in the electrochemical effect. Four strains of *P. aeruginosa* were tested because of their
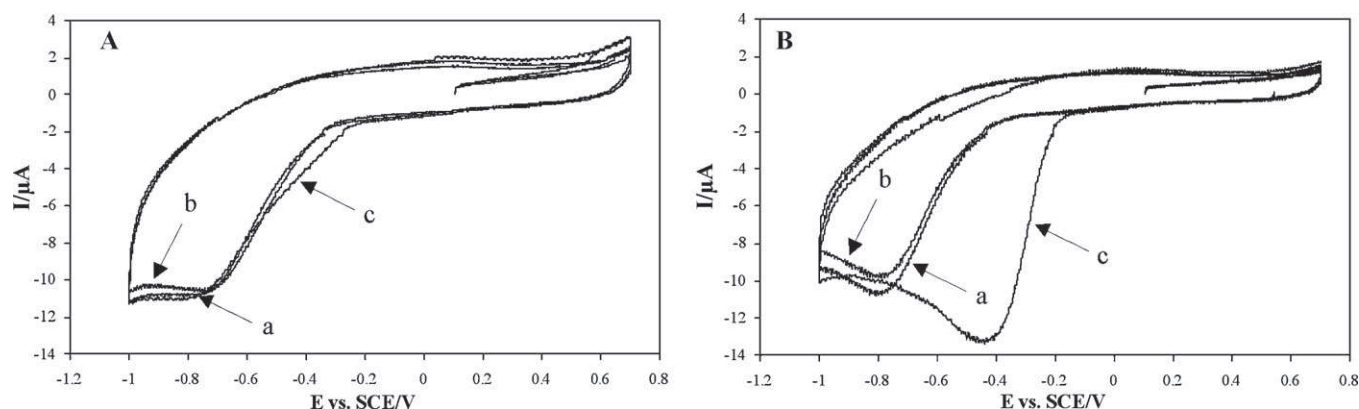

Fig. 5. Effects of different methanol treatments on the catalysis of oxygen reduction by *P. aeruginosa* PA01. (A) CV on phosphate buffer alone (line a), after addition of PA01 cells previously treated with methanol (line b), and after 1 h of stirring (line c). (B) CV on phosphate buffer alone (line a), after the addition of PA01 cells (line b), and after the working electrode was removed, treated with methanol, and then plunged into a fresh phosphate buffer (line c).

matrix formed by *P. aeruginosa* [26], had no effect on the catalysis (Table 1). Preliminary work on a *P. aeruginosa pilA* mutant (deficient in biogenesis of type IV pili) did not show any difference with respect to the wild type strain in cyclic voltammetry (data not shown). Like alginate, these pili are crucial for irreversible attachment and maturation of biofilm [25,27]. In addition, beyond 1 h of contact (3 h, 6 h or 24 h), no increase in the catalysis was observed. All these data suggest that the pure process of adhesion and biofilm formation is not entirely responsible for the transfer of electrons. Something more than adhesion is needed.

A real contact between bacterial cells and the electrode surface was required since, after 1 h of stirring of the bacterial suspension without any contact with the electrode, no catalytic effect was observed on the voltammogram. So the catalysis does not depend on compounds that bacteria produce and release into the medium, like phenazines. The negative result on the filtrate test confirmed this hypothesis (Fig. 3). Previous studies that have demonstrated the involvement of phenazines in anodic processes related to *P. aeruginosa* showed a strong activity of the filtrate [4]. Then, whatever the pigmentation of the strain, due to the production of phenazines [28], detection became possible, even for strains with no pigmentation at all (Table 1). Phenazines are definitively not involved in the transfer of electrons described here. Hence, unlike in anodic catalysis, *P. aeruginosa* does not use released electron shuttles.

Another explanation could be that bacteria do produce electroactive molecules but that they are bound into the membrane or are diffusible only for a short distance from the bacteria. This may explain why contact with the electrodes is required during the delay time if the catalytic effect is to be observed. The weak effect obtained with the filtrate would be explained by a small release of this compound from the cell membrane to the solution. For instance, type IV pili produced at the surface of *Geobacter sulfurreducens* were responsible for the transfer of electrons [7]. As presented above, *P. aeruginosa* mutants deficient in type IV pili biogenesis were able to catalyze the electrochemical reduction of oxygen. These pili were consequently not involved in the electronic transfer observed here. Experiments performed on dead cells showed that bacteria had to be alive during the 1 h contact time. After this delay, probably necessary for the production or relocation of proteins or other bacterial compounds on the surface of cells, the bacteria could be killed without their death influencing the catalysis. This observation supports the hypothesis of the involvement of membrane-bound proteins having no direct link with the metabolic activity of the cells. Several biological compounds adsorbed alone on an electrode surface are known to catalyze the electrochemical reduction of oxygen without any involvement of bacterial cells. The most studied are iron-based proteins: haeme proteins [29], cytochrome [30], cytochrome c oxidase [31], catalase [12,32]. Here, we focused on catalase, which had already been demonstrated to induce the catalysis of electrochemical reduction of oxygen on glassy carbon electrodes [13] and which activity had been shown to be correlated to the increment of cathodic currents for *Pseudomonas* sp. [22]. Catalase is an enzyme located on the outer membrane of the majority of Gram-negative and on several Gram-positive bacteria [33]. It catalyzes hydrogen peroxide dismutation. Catalase adsorbed on glassy carbon electrodes has been tested with cyclic voltammetry under conditions similar to those in the work presented here. The voltammograms obtained had the same general shape as those plotted here with *P. aeruginosa*. The oxygen reduction wave was displaced and started around −0.20 V/SCE in presence of catalase. We consequently suspected that this protein was responsible for the phenomenon observed here. In *P. aeruginosa*, there are three differentially evolved monofunctional catalase genes, *katA*, *katB*, and *katE* [34]. PA14 KatABE− strain does not present any catalase activity [24], but this strain is able to catalyze

the electrochemical reduction of oxygen like the wild type strain, PA14 (Table 1). Therefore catalase is not entirely responsible for the electrochemical catalysis of oxygen reduction.

The concept of microbial electro-catalysis of oxygen reduction on metallic surfaces was first identified in the field of microbial corrosion with biofilms formed in fresh seawater. The work presented here, which was carried out with pure cultures of different *P. aeruginosa* strains, opens a new route for investigation. Actually, the different assumptions that have been evoked to explain the electro-catalysis of oxygen reduction by natural biofilms: production of hydrogen peroxide, involvement of manganese oxides/hydroxides, direct catalysis by adsorbed catalase, can no longer explain the electro-catalytic effect observed here with pure strains. Data obtained in the present work suggest that electro-catalysis is sustained by the production of a compound that remains linked to the cells or in the cell vicinity, with only weak release and with no direct link with the cell's metabolism after the 1 h contact time. Catalase cannot be responsible but other small iron-binding proteins, such as ferredoxin, cytochrome, rubredoxin, etc., could play this role. The common denominator among all these proteins is their active site called porphyrin, and such haeme proteins are known to catalyze oxygen reduction on metallic cathodes [35]. Involvement of porphyrin molecules in the catalytic effect observed here would explain the similarity of the effect for all the *P. aeruginosa* strains tested. Moreover, recent work devoted to the identification of the microbial composition of the seawater biofilms that catalyze oxygen reduction has evidenced the large microbial diversity of these biofilms (Vandecandelaere, personal communication, to be published). The involvement of such ubiquitous molecules as porphyrins would also explain the fact that a large number of various species are able to catalyze the electrochemical reduction of oxygen. The present study will be completed by focusing on possible production of haeme compounds during the contact period.

In a more technical approach, although the catalysis of oxygen reduction demonstrated here is not interesting for MFC applications (potential too low), the protocol allows the detection of the early stages of *P. aeruginosa* biofilm formation, i.e. the first step of adhesion. All the tested strains of *P. aeruginosa*, including clinical isolates, were able to catalyze the electrochemical reduction of oxygen. This property seems to be universal among *P. aeruginosa* strains. The catalytic effect allowed a detection of the bacterial adhesion to the electrode after a few minutes only (1 h for the full catalytic effect). The signal obtained was easily identifiable and highly reproducible. Therefore this property may provide an opportunity for early detection of bacterial adhesion. Indeed, the majority of the electrochemical methods already proposed to detect biofilm colonization has focused on biofilm growth and can only detect a deposit on the electrode surface, without indication of its biotic or abiotic character [36,37]. In contrast, the technique presented here detects the presence of bacteria on the surface. The method does not only measure surface fouling; it requires a real interaction between the bacteria and the electrode. Further work is now in progress to assess whether the cyclic voltammetry protocol highlights a property present in most aerobic bacteria or a specificity of *P. aeruginosa*. Preliminary results have shown that other opportunistic bacteria, like *Escherichia coli*, *Enterobacter cloacae* and *Shigella* sp., are detectable using the CV method in the same way as *P. aeruginosa*.

5. Conclusions

It has been demonstrated here that *P. aeruginosa* is able to catalyze the electrochemical reduction of oxygen. This phenomenon implies a prolonged contact between bacteria and the working electrode. The transfer of electrons is not due to electron shut-

tles released into the medium by bacteria and phenazines are not involved. Type IV pili are not involved in the process either. The metabolic activity of the cell is necessary during the 1-h contact time but, after this delay, the catalysis is possible even if the bacteria are killed. Finally, the activity of membrane-bound redox compounds having no direct link with the cell's metabolism seems the most probable explanation because of the similarity of results reported in the literature with adsorbed proteins. Tests performed on catalase mutants showed that this enzyme was not necessary for the catalysis described. *P. aeruginosa* now offers a new, easy-to-handle model to advance in deciphering the mechanism of microbial electro-catalysis of oxygen reduction. Moreover, the procedure designed here may become a suitable technique for detecting the first steps of bacterial adhesion.

Acknowledgments

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