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Forming microbial anodes under delayed polarisation modifies the electron transfer network and decreases the polarisation time required

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ABSTRACT

Microbial anodes were formed from compost leachate on carbon cloth electrodes. The biofilms formed at the surface of electrodes kept at open circuit contained microorganisms that switched their metabolism towards electrode respiration in response to a few minutes of polarisation. When polarisation at −0.2 V/SCE (+0.04 V/SHE) was applied to a pre-established biofilm formed at open circuit (delayed polarisation), the bacteria developed an extracellular electron transport network that showed multiple redox systems, reaching 9.4 A/m^2 after only 3–9 days of polarisation. In contrast, when polarisation was applied from the beginning, bacteria developed a well-tuned extracellular electron transfer network concomitantly with their growth, but 36 days of polarisation were required to get current of the same order (6–8 A/m^2). The difference in performance was attributed to the thinner, more heterogeneous structure of the biofilms obtained by delayed polarisation compared to the thick uniform structure obtained by full polarisation.

1. Introduction

Electro-active (EA) biofilms have been widely exploited to design microbial fuel cells (MFCs) (Lefebvre et al., 2011; Logan, 2010) microbial electrolysis cells (Geelhoed et al., 2010; Logan et al., 2008) and other related technologies. Numerous studies have identified EA bacteria (Logan, 2009) and deciphered the different electron transfer pathways that can be used inside microbial biofilms (Rabaey et al., 2007; Reguera et al., 2005; Schaetzle et al., 2008). In contrast, the mechanisms of formation, structuring and ageing of the EA biofilms have rarely been investigated. Wang et al. (2009) have observed that the start-up time of an MFC was significantly decreased when a constant potential was applied to the anode, in comparison with the same MFC implemented without applied potential. They postulated that the applied potential increased the positive charge on the anode surface and thus favoured the primary adhesion of negatively charged bacteria. The adhesion of EA species on anodes might consequently depend on the surface charge of the electrode as it has sometimes been suggested (Busalmen et al., 2008; Cheng and Logan, 2007). In contrast, Aelterman et al. (2008) did not observe significant difference in start-up time with applied potentials in the range from −0.4 to 0 V vs. Ag/AgCl. Actually Wang et al. (2009) have inoculated their MFCs with domestic wastewater, while Aelterman et al. (2008) have taken their inoculum from an operating MFC, which means that the microbial community was already adapted to generating electricity. Comparing the two studies suggests that the time necessary for wild microbial communities to adapt to electrochemical conditions depends on the applied potential, while the time necessary for already-adapted bacteria to form EA biofilms does not. When using wild communities as inoculum, the adaptation phase necessary for the cells to develop their EA capacity may consequently be an essential parameter in controlling the formation of EA biofilms, rather than electrostatic interactions between bacteria and electrode surface. The occurrence of an initial phase during which the cells must optimize attachment or the electron transfer chain to the surface has also been observed with pure cultures of *Geobacter sulfurreducens* (Marsili et al., 2010). Nevertheless, little is known so far about the way a clean electrode surface catches microbial species from a natural environment and how they shift from the conventional respiration mechanism to an anode respiring mechanism.

The purpose of this work was to give some insights into EA biofilm construction with the practical target of improving the performance of microbial anodes. Garden compost was used as the source of the inoculum. Soils are a very rich source of microorganisms (Liu et al., 2006; Torsvik et al., 1996) and garden compost has proved its excellent capacity to form EA biofilms. Microbial anodes can be formed by simply embedding polarised electrodes in a soil (Parot et al., 2008), but it is then difficult to use the resulting anodes out of their initial medium. A new procedure has been proposed recently, which consists of producing a leachate by percolating the garden compost with an ionic solution and then using the

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leachate obtained as the inoculum. This procedure has given promising results for the treatment of dairy wastes (Cercado-Quezada et al., 2011, 2010a). The same procedure has led to current density so high as 66 A/m^2 for acetate oxidation when the biofilms were formed around ultra-microelectrodes (Pocaznoi et al., 2012). Obviously this result has been reached with particular laboratory electrodes, but it demonstrates the promising potential of the inoculum source.

The present study used compost leachate as inoculum and acetate as substrate. Comparing biofilms formed at open circuit and biofilms formed under constant polarisation showed very different structures and allowed the phases of biofilm formation and of development of the extracellular electron transfer network to be distinguished. These observations were exploited to define an optimal procedure for the formation of microbial anodes.

2. Methods

2.1. Soil biofilm

One litre of Garden compost (Cultura, Lombricompost) was mixed with a 60 mM KCl solution and left for 24 h under stirring at room temperature. The mix was then centrifuged and the resulting leachate, supplemented with 10 mM acetate, was used for the formation of EA biofilms.

2.2. Electrochemical instrumentation and set-up

Electrochemical experiments were carried out in closed vessels that contained 150 mL leachate. Carbon cloth (supplied by PaxiTech SAS, France) of 2 cm^2 projected surface area was used for the working electrodes and a platinum grid for the auxiliary electrode. All potentials were controlled via a conventional 3-electrode set-up vs. a saturated calomel reference electrode (SCE, Radiometer, +0.241 V vs. SHE) by means of a VMP potentiostat (Bio-logic SA). Polarisations were performed at −0.2 V vs. SCE because this potential was around the most negative value that could provide the maximum current density (Cercado-Quezada et al., 2010b). Cyclic voltammetries were performed at 1 mV s^{-1}. Electrochemical experiments were carried out at room temperature, around 22 °C.

2.3. Microscopy and image analysis

For scanning electron microscopy, the microbial structures were stabilised on the electrode surfaces by fixation in phosphate buffer (400 mM, pH 7.0) with 4% glutaraldehyde. The electrodes were then rinsed in phosphate buffer with saccharose (0.4 M), treated with 2% osmium tetroxide in phosphate buffer and saccharose for 1 h, dehydrated in an ascending series of acetone solutions (50%, 70%, 100%), then in acetone and hexamethyldisilazane (50:50) and, finally, in 100% hexamethyldisilazane. Anodes were examined with an LEO 435 VP scanning electron microscope.

3. Results and discussion

3.1. Biofilm formed at open circuit

Five 2 cm^2 carbon cloth electrodes were left at room temperature in separate bioelectrochemical reactors containing 150 mL of the same compost leachate supplemented with 10 mM acetate, for 0 (control), 8, 12, 15 and 19 days. No polarisation was applied and the open circuit potential (OCP) was recorded as a function of time (Fig. 1A). The OCP exhibited similar evolution for each electrode, with initial values around +0.05 V vs. SCE and a fast decrease during a 4-day period, followed by a stabilisation at close to −0.5 V vs. SCE at day 5.

The OCP values are the result of the delicate balance between electron exchanges that are spontaneously established between the electrodes and the species from the bulk environment. For instance, cathodic currents (electrons lost by the material) may be due to the natural slow reduction of dissolved oxygen on the electrode surface, and anodic currents (electrons gained by the material) may result from the slow oxidation of the electrode surface itself. With graphite and carbon electrodes, the presence of functional groups like phenol, carbonyl, carboxyl and quinine on the surface (Cabaniss et al., 1985; Nagaoka and Yoshino, 1986) makes the anodic process complex. Moreover, in a solution such as compost leachate, which contains a large diversity of dissolved organic compounds, anodic currents can also be due to the slow spontaneous oxidation of some of them. For instance, OCP recording is commonly exploited to measure the redox potential of solutions. In this case, a platinum electrode is used because no significant oxides form on its surface.

From a general point of view, OCP decrease is due to vanishing of the reduction current and/or to an increasing of the anodic current. For instance, in redox potential measurements with a platinum electrode, it is generally postulated that OCP decrease is mainly due to vanishing of the cathodic current because of oxygen depletion. Here OCP decrease can be due to both the oxygen depletion in the closed electrochemical reactor and the increase of the anodic current resulting from the catalysis of acetate oxidation. Actually, it should be borne in mind that OCP values are controlled by the very low exchange currents due to spontaneous reactions only.

In this framework, the adhesion of only a few EA bacteria is sufficient to catalyse low acetate oxidation and to cause OCP decreases observed here. To check this assumption, two experiments were performed with, in each reactor, one electrode dipping in the leachate and another hanging in the gas free space. In both experiments, the OCP of the immersed electrodes followed the same evolution as previously recorded, with an initial OCP around +0.1 V vs. SCE which decreased to −0.65 V vs. SCE after 20 days of immersion. At day 20, the hung clean electrodes were plunged into the solutions. The OCPs of the clean electrodes were in the range of −0.1 to −0.2 V vs. SCE in both experiments, i.e. around 0.2 mV less than the initial OCP value. The difference between the initial OCP value (+0.1 V vs. SCE) and OCP measured at day 20 with the clean electrode can only be attributed to changes in the solution composition, and most probably to the consumption of oxygen by the aerobic microorganisms during the first few days of the experiment. The final stable OCP values being around −0.65 V vs. SCE, it can be concluded that oxygen depletion was responsible for around one third of the whole OCP decrease, while the catalysis of the oxidation reaction supported around two thirds. These experiments confirmed that a major part of OCP decrease was due to the primary settlement of EA microbial cells that started to catalyse acetate oxidation.

Going back to the five-electrode experiment, each electrode was polarised at −0.2 V vs. SCE for 3 h at the end of its open circuit phase (Fig. 1B). The control electrode that was polarised immediately upon immersion in the compost leachate did not produce any current during the 3 h of polarisation. In contrast, every other electrode gave an oxidation current density of about 50 mA/m^2 after only 30 min of polarisation. This time was too short for the current increase to be attributed to the growth of EA microbial species. It indicated an activation phase of the EA cells that were already present on the anode surface and switched their metabolism towards an electrode-respiring mechanism in response to the polarisation.

In conclusion, the microbial communities that adhered to the surface of non-polarised electrodes contained some microbial