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Short communication

Electrochemical growth of *Acidithiobacillus ferrooxidans* on a graphite electrode for obtaining a biocathode for direct electrocatalytic reduction of oxygen

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ABSTRACT

An aspect in microbial fuel cell research that is currently of great interest is the development of bacterial cathodes. Bacterial cathodes that catalyze oxygen reduction to water at low pH have the advantage of overcoming the kinetic limitations due to the requirement of 4 protons per molecule reduced. In this work we have studied the performance of a biocathode using as electrocatalyst an acidophile microorganism: *Acidithiobacillus ferrooxidans*. Growth of the microorganism directly on the electrode took place using an applied voltage of 0 V *vs.* SCE as the only energy source and without adding redox mediators to the solution. Current densities of up to 5 A m^{-2} were measured for O_2 reduction in the *At. ferrooxidans* cathode at pH 2.0 and the electrocatalytic wave was shifted 300 mV to higher potential compared to the control graphite electrodes without the bacterium.

1. Introduction

In a microbial fuel cell (MFC) the oxidation of the fuel at the anode is catalyzed by microorganisms. Up to date MFCs are not economically feasible because they have very low efficiency. They produce power very slowly and with low density (Pham et al., 2009). There are several bottlenecks that need to be eliminated in MFC development, one of these being the performance of the cathode. Various catholytes such as ferricyanide or acidic permanganate have been used in MFCs. However, these electron acceptors are impractical and unsustainable because they need to be regenerated. Alternatively, the use of O_2 as final electron acceptor in the cathode greatly improves the sustainability of MFCs (Oh et al., 2004; Zhao et al., 2006). The reduction of O_2 at a carbon cathode is kinetically hampered, thus a catalyst, normally platinum, is required to accelerate the reaction. The problem is that platinum is a very scarce and expensive noble metal, which contributes in making the MFCs economically unfeasible.

An interesting alternative in MFCs is the use of microorganisms as biocatalysts not only in the anode but also in the cathode. The study of biocathodes for MFC applications is very recent (Bergel et al., 2005; Rabaey et al., 2008). Special interest has the study of microbial cathodes that may operate at low pH because the electrocatalytic reduction of O_2 to H_2O is kinetically limited by the availability of protons (Zhao et al., 2006; Erable et al., 2009). Therefore, acidophilic microorganisms that may be reduced directly at an electrode and use O_2 as final electron acceptor are of great interest.

Acidithiobacillus ferrooxidans is an autotrophic, aerobic and strictly chemolithotrophic bacterium. This microorganism uses CO_2 as a carbon source and obtains its energy from the oxidation of ferrous iron, elemental sulphur or reduced sulphur compounds at low pH, using O_2 as final electron acceptor (Malki et al., 2006). It has been shown that *At. ferrooxidans* cells are able to grow using electricity as sole energy source by using soluble iron as an electron-transfer mediator (Nakasono et al., 1997; Thrash and Coates, 2008). In the present work we have studied the performance of an *At. ferrooxidans* biocathode without using redox mediators. The goal has been to grow the microorganism directly on the electrode using applied voltage as the only energy source and to study the electrocatalytic properties of the bacterial cathode for O_2 reduction at low pH.

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2. Materials and methods

2.1. Microbial growth and culture manipulations

At. ferrooxidans cells were grown at pH 2.0 in Mackintosh liquid medium composed by three solutions: Sol A, which contained basalt salts; Sol B, which contained $FeSO_4$ as energy source; and Sol C, which contained $MnCl_2$, $ZnCl_2$, $CoCl_2$, $CuCl_2$, H_3BO_3 and $NaMoO_4$ as trace elements. These solutions were prepared as reported (González-Toril et al., 2006). The culture preparation before the bacterium inoculation into the electrochemical cell involved the following steps: (a) 150 ml of exponential phase growth culture was filtered through a filter paper in sterile conditions to take out the Fe^{2+} traces from the medium; (b) the filtered culture was centrifuged at 15,000 × *g* during 6 min to sediment the bacteria; (c) all the pellet was suspended in 1 ml of Sol A from Mackintosh medium.

2.2. Electrochemical measurements

A sterilized glass electrochemical cell from Radiometer thermostatized at 30 °C was used. The working electrodes were graphite felt (9 μm fibre diameter; 3500 $cm^2\ g^{-1}$ surface area, RVG 4000, Le Carbon Lorraine) prisms of 11.8 cm^2 projected area. Before use, the prisms were cleaned in 1 M H_2SO_4 and then sterilized at 120 °C and 1 atm for 30 min. A standard calomel electrode (SCE) was used as the reference electrode and a platinum electrode as the counter electrode; both of them were supplied by Radiometer. Redox potential values mentioned in the text are *vs.* SCE if not specified. *At. ferrooxidans* was inoculated into the electrochemical cell that contained 90 ml of Sol A and the trace elements of Sol C at a final concentration of 60 $\mu g\ l^{-1}$. A constant voltage of 0 V was applied on the working electrode with an Autolab PGSTAT 30 potentiostat (Eco-Chemie) while the solution was stirred with a magnetic bar. Cyclic voltammetry experiments were performed at different scan rates with stationary solution. Polarization curves were performed applying the linear voltammetry pGES Autolab procedure at 1 $mV\ s^{-1}$ scan rate with magnetic stirring of the solution. In some experiments O_2 or N_2 (99.999% purity, Air Liquide) was bubbled into the electrochemical solution for at least 10 min.

2.3. Scanning electron microscopy

Scanning electron microscopy (SEM) of modified electrodes was done according to a protocol described previously (Alphenaar et al., 1994) with a JEOL-5600LV scanning electron microscope.

2.4. X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) spectra were recorded with a SPECS Phoibos 150 electron spectrometer and a nine segments delay line detector, using Al Kα radiation (1486.61 eV) in an ultrahigh vacuum chamber with a base pressure of 10^{-9} mbar. Prior to the XPS measurements, the samples were dried out at room temperature by pumping down at a vacuum pressure of 10^{-3} mbar. All samples were analyzed by XPS no later than 24 h after removal from solution. The overall surface composition was determined from survey scans. Additionally, high-resolution XPS spectra were recorded for the C 1s, Fe 2p, N 1s and O 1s core-level peaks at normal emission.

3. Results and discussion

3.1. Electrochemical growth of At. ferrooxidans cells

At. ferrooxidans was grown in an electrochemical cell at a constant applied potential of 0 V, pH 2.0 and in absence of redox mediators. Negative currents were registered that increased slowly during 20 days after bacterial inoculation, and then the current oscillated around a mean value of -2.3 ± 0.3 mA (Supplementary Fig. S1). The slow increase of the current is typical of electrodes that are being colonized by electrochemically active bacteria (Malki et al., 2008; Erable et al., 2009). In the control experiments without bacterial cells inoculated into the solution the measured currents were negligible during the same time period (Supplementary Fig. S1).

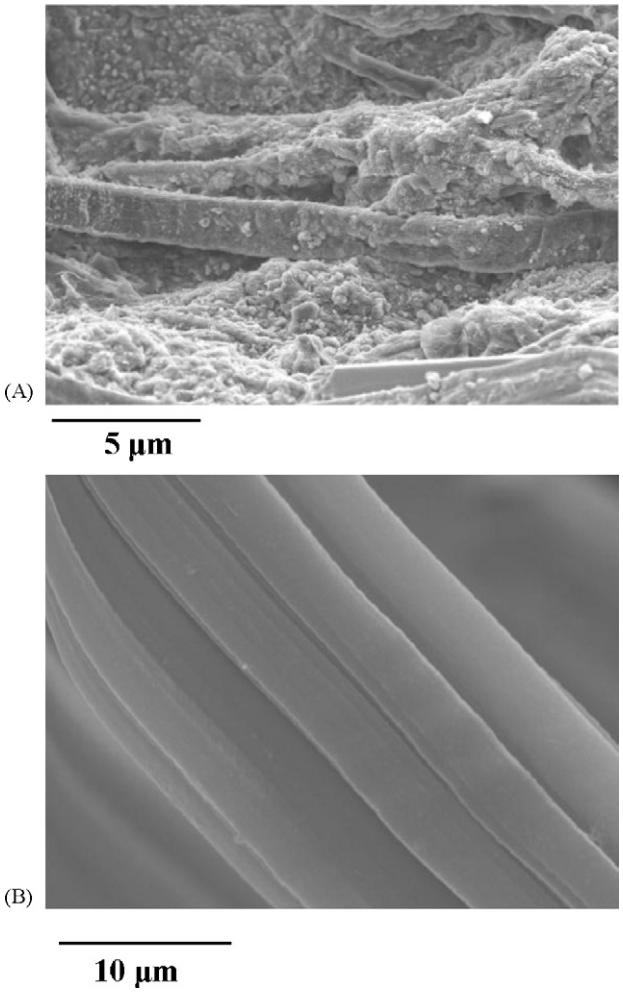

Fig. 1. Bacterial electrode characterization by SEM. (A) Image of a fiber of a carbon felt electrode 28 days after inoculation of the electrochemical cell with *At. ferroxidans* and a applied redox potential of 0 V. (B) Control image of a carbon felt electrode fiber before inoculation with *At. ferroxidans*.

The SEM image of Fig. 1A shows that bacterial cells had colonized the graphite felt electrode after 28 days of 0 V applied potential. A biofilm formation can be observed with cells embedded in extracellular polymeric substances (EPS).

Although it has been demonstrated that *At. ferrooxidans* can grow oxidizing Fe^{2+} regenerated at an electrode as the only energy source of the system (Nakasono et al., 1997), to our knowledge it has not been reported before that this bacterium could accept electrons directly from the electrode without the need of adding iron to solution.

3.2. Electrocatalytic reduction of oxygen at the bacterial cathode

The electrocatalytic properties of the bacterial cathode were studied by cyclic voltammetry at low scan rate. In this way, the background capacitive current and the kinetic limitations due to