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The current provided by oxygen-reducing microbial cathodes is related to the composition of their bacterial community

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ABSTRACT

Oxygen reducing biocathodes were formed from sludge under constant polarization at −0.2 and +0.4 V/SCE. Under chronoamperometry at pH 10.3 ± 0.3, current densities of 0.21 ± 0.03 and 0.12 ± 0.01 A m^{-2} were displayed at −0.2 V/SCE by the biocathodes formed at −0.2 and 0.4 V/SCE, respectively. Voltammetry revealed similar general characteristics for all biocathodes and higher diffusion-limited current densities (0.84 ± 0.26 A m^{-2}) than chronoamperometry. Up to 3.7 A m^{-2} was reached under air bubbling. A theoretical model was proposed to show the consistency of the chronoamperometric and voltammetric data.
The biocathodes formed at −0.2 V/ECS that gave the highest electrochemical performance showed a homogeneous selection of Deinococcus–Thermus and Gemmatimonadetes, while the biocathodes formed at 0.4 V/SCE were enriched in different bacteria. The biocathode that led to the worst electrochemical characteristics, while formed at −0.2 V/SCE, showed the largest bacterial diversity. The biocathode performance was consequently related to the enrichment in specific microbial phyla. Moreover, the strong presence of bacteria parented to Deinococci may also have some interest in biotechnology.

1. Introduction

Microbial fuel cells (MFCs) may represent a promising technology to extract electrical energy directly from the chemical energy contained in low cost and widely available organic matters. Amazing advances have been done on fundamental understanding and practical development of microbial anodes, but the low efficiency of oxygen reducing (OR) cathodes still limits MFC performance. Microbial cathodes have been identified as an interesting alternative to the abiotic air-cathodes that are implemented in most MFCs [1]. Such biocathodes have been formed from various natural environments including seawater [2,3], soils [4], sludge and wastewater [5–7]. Aerated sludge and nitrifying biomass have been asserted to be particularly appropriate inocula due to their richness in autotrophic bacteria [8–11].

Despite the various possibilities to form OR microbial cathodes, this research field is still in its infancy and a lot remains to be done to understand the mechanisms and increase the performance. In particular, the factors that may influence the selection of the microorganisms that colonize the electrode surface, or even if a microbial selection occurs or not, remain to be established.

Different works are available in the literature that studied the microbial population of OR biocathodes. They analyzed the microbial diversity by mean of different techniques: DGGE [6], clone library [12–14], phylochip [15] and in more recent studies 16S-DNA pyrosequencing [16–18]. However, these studies were realized on biocathodes formed during MFC operation, so that no potential control was applied. As the potential of the cathode conditions the energy recoverable by the bacteria, it may play a crucial role in bacterial selection. To establish the possible impact of the potential on the selection of microorganisms, experiments should be performed using analytical 3-electrode set-ups and potentiostatically controlled conditions [19].

Two studies have been published that used such an electroanalytical system with the aim to link the applied potential to the bacterial selection on OR biocathodes. Vandecandelaere et al. have analyzed biocathodes formed under polarization at −0.2 V/SCE in seawater, using clone libraries [3]. They observed that the bacterial population of the biocathode was similar to the population of the surrounding seawater and concluded to the absence of any bacterial selection on the biocathode. Nevertheless, it should be mentioned that only the cultivable part of the population was addressed in this study. Xia et al. have analyzed the population of OR biocathodes formed under three different potentials, 0.2, 0.06, and −0.1 V/SCE, from a mix of aerobic sludge and a previously enriched biocathode consortia [11]. They observed higher proportions of Bacteroidetes and *Thiorhodospira* sp. as the potential decreased, showing a clear impact of the applied potential on the bacterial selection. Eventually, these studies presented only one population analysis for each of experimental condition, while microbial electrochemical systems are known for their considerable versatility [20]. Further studies are

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consequently required to clearly establish the impact, or not, of the polarization potential on the selection of bacteria on OR biocathodes.

The objective of the present work was to investigate whether a bacterial selection occurred when an OR biocathode is formed from a complex inoculum and how it could impact the performance of the biocathode. Biocathodes were formed in a synthetic medium inoculated by long-term aerated sludge, using two distant polarization potential, −0.2 and +0.4 V/SCE. The first potential has already been identified as producing high performance OR biocathodes [7,10,11] and so consequent current densities were expected. The second one was chosen due to its closeness to the standard potential for oxygen reduction at pH 10. At this potential, no current production was expected from the electrode. These two values of potential represent two very different situations, which could affect the biocathode formation. Replicates were realized for each experimental condition: four electrodes were formed at −0.2 V/SCE, two electrodes were formed at +0.4 V/SCE. The biocathodes were electrochemically characterized by chronoamperometry and cyclic voltammetry and the communities present in each biofilm were identified by 16S-DNA pyrosequencing. The replicates used here showed that the applied potential had a clear impact on the bacterial communities of oxygen-reducing biocathodes, although it did not allow fully controlling them. The electrochemical performance was linked to the enrichment in specific microbial phyla that can happen at −0.2 V/SCE, while the biocathode that exhibited the lowest performance, while formed at the same potential, showed deficient microbial selection.

2. Materials and methods

2.1. Electrochemical setup and tests

Each electrochemical cell was a three-electrode set-up in a two-compartment H-cell equipped with an anion exchange membrane (Fumasep® FAA-PK, Germany) of 7.1 cm^2 surface area (Scheme 1). The working electrode was a 2 cm^2 of carbon cloth (Paxitech®, France) connected by a platinum wire, the counter-electrode was a 10 cm^2 platinum grid and the reference electrode was a saturated calomel electrode (SCE, potential +0.241 V/SHE). Four holes were drilled in the cap that covered each electrochemical compartment; they were used to introduce the electrodes and the tubes for air or nitrogen bubbling. When the electrochemical cell was air-opened one of these apertures on the anodic and cathodic compartments was not sealed during the experiments. Aerated sludge was collected at a sewage treatment plant (Evry, France) just before the nitrifying step. Its initial pH was 7.0. A nitrification medium was consequently chosen, which contained a high concentration of ammonium ions. The anolyte was composed as follow: 1.908 g L^{-1} NH_4Cl, 0.7 g L^{-1} K_2HPO_4, 0.7 g L^{-1} Na_2HPO_4, 28.6 g L^{-1} $KHCO_3$ and 0.5 mL L^{-1} of a mineral solution [21]. The final pH was adjusted at 7.8 with HCl 37%. The catholyte was same medium inoculated with 10% (v/v) aerated sludges (500 mL final volume). Each compartment was air-opened without stirring.

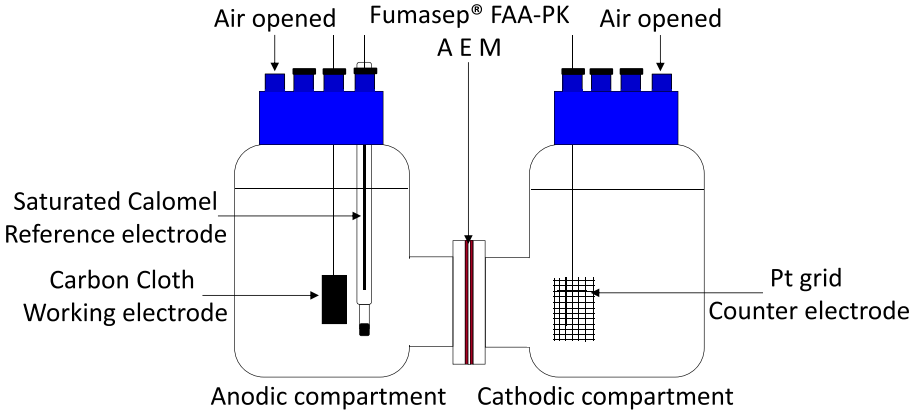
The pH was daily measured in each compartment but not controlled. Two reactors without electrodes were used as control experiments. When indicated, nitrogen or air bubbling was punctually performed into the catholyte. All experiments were conducted in a stove thermostated at 40 °C. This temperature was chosen to favor fast high bacterial growth, even if it lowered the concentration of dissolved oxygen in the medium (0.41 mM against 0.52 mM at 25 °C).

Microbial cathodes were formed under constant polarization (chronoamperometry) using a multichannel potentiostat (Biologic, France, EC-Lab software). Four electrodes (numbered 1 to 4) were polarized at −0.2 V/SCE, two electrodes (numbered 5 and 6) at +0.4 V/SCE. At some times the chronoamperometry was interrupted and cyclic voltammetry was recorded at 1 mV s^{-1}, starting at the polarization potential and scanning to 0.3 V (upper limit for the electrodes polarized at −0.2 V/SCE) or 0.5 V (for the electrodes polarized at +0.4 V/SCE) and back down to −0.6 V/SCE. Three voltammograms were successively recorded each time, only the second was reported here for the sake of simplicity. The current densities discussed in the text were average values calculated for each group of electrodes formed at the same potential.

2.2. Bacterial community analysis

The bacterial populations of the cathodic biofilms were analyzed using 16S rDNA-pyrotags sequencing. DNA was extracted with reagent kit MOBIO PowerSoil® DNA Isolation. The DNA extraction was quantified by fluorometry (QuBit™ fluorometer with Quant-it™ assay kit, Invitrogen) and spectrophotometry (WPA Biowave™ II) which also permitted to check DNA quality thanks to the ratio of UV absorbance at 260/280 nm and 260/230 nm. DNA samples were finally sent to Research and Testing Laboratory (RTL — Texas, USA) where 454 pyrosequencing (Roche) with 28F (*5′- GAG TTT GAT YMT GGC TC -3′*) and 519R (*5′- GWA TTA CCG CGG CKG CTG -3′*) primers was performed.

Resulting data were analyzed with the open source software package QIIME "Quantitative Insights Into Microbial Ecology" [22]: 16S DNA sequence quality was controlled using a sliding window 50 nt long requiring an average quality above 25. The sequences were thus trimmed to the end of the last window with required average quality and discarded if their final length was less than 150 nt. In addition, remaining reads where the longest homopolymer was greater than 6 nt or containing an ambiguous base were also discarded. Sequences were then aligned with PyNAST [23] using the Silva 108 database core-aligned set formatted for QIIME as a template [24]. Putative chimeric sequences were identified with ChimeraSlayer and removed from dataset [25]. Remaining sequences were clustered in operational taxonomic



Scheme 1. Experimental set-up.