Bioelectrochemical Dechlorination of 1,2-DCA with an AQDS-Functionalized Cathode Serving as Electron Donor[1]

P. Leitão; H. Nouws; A. S. Danko; F. Aulenta

Abstract

In the present study we describe a simple method to immobilize the redox mediator anthraquinone-2,6-disulfonate (AQDS) at the surface of graphite electrodes, by means of a commercial anion exchange membrane. Cyclic voltammetry experiments confirmed the efficacy of the immobilization protocol and the long-term (over 70 days) electrochemical stability of the AQDS-functionalized electrode. Potentiostatic (–300 mV *vs*. SHE) batch experiments proved the capability of the electrode in accelerating the bioelectrochemical reductive dechlorination of the groundwater contaminant 1,2-dichloroethane (1,2-DCA) to harmless ethene by a mixed microbial culture, by serving as electron donor in the process. Considering the reported broad range of anodic and cathodic reactions catalyzed by AQDS, the herein described functionalized electrode has a remarkable potential for application in the environmental and industrial sector.

1 Introduction

The chlorinated compound 1,2-dichloroethane (1,2-DCA), nowadays industrially used to produce vinyl chloride (VC), a precursor in the synthesis of polyvinylchloride (PVC), is one of the most frequent and harmful soil and groundwater contaminants **1**. This compound is highly toxic and a suspected human carcinogen, therefore its presence in the environment poses important health risks **1**. Anaerobic bioremediation is increasingly being recognized as an effective and sustainable technology for the cleanup of sites contaminated by halogenated compounds, including 1,2-DCA **2**, **3**. A typical bioremediation approach involves the biostimulation of autochthonous 1,2-DCA degrading microorganisms through the subsurface addition of H_2-releasing fermentable substrates capable to promote the anaerobic reductive dechlorination of contaminants, eventually to harmless, non-chlorinated end products, such as ethene **3**. A major disadvantage of this approach is the need for continuously supplying the fermentable

[1] paper presented at the 3rd Meeting of the International Society for Microbial Electrochemistry and Technology (EU-ISMET 2016); held 26–28 September 2016 in Rome, Italy; organized by www.is-met.org

electron donors into the subsurface, which may eventually result in the accumulation of fermentation products (including potentially explosive methane gas), in groundwater acidification, as well as in increased operational costs and longer remediation times [4]. Recently, a new approach has been pursuit to tackle some of these disadvantages, which is based on the use of a potentiostatically controlled graphite electrode to selectively and efficiently deliver the needed electrons to the reductively dechlorinating microbial communities [5, 6]. So far, the efficacy of this bioelectrochemical approach has been verified, at least at the laboratory scale, on several different chlorinated aliphatic hydrocarbons (CAHs), namely perchloroethene (PCE) [7], trichloroethene (TCE) [8-10], and even 1,2-DCA [11, 12]. In this respect, a previous study from our group has shown that 1,2-DCA can be reductively dechlorinated to harmless ethene, *via* dihaloelimination, using a polarized graphite cathode (from –300 mV to –900 mV *vs*. standard hydrogen electrode, SHE) serving as the sole electron donor [12]. Notably, high values (up to 70%) of coulombic efficiency (i.e., the yield of current utilization for the reductive dechlorination process) were obtained when the cathode was maintained at –300 mV *vs*. SHE, whereas substantially lower and decreasing values were observed at lower cathode potentials. By contrast, the rate of dechlorination was extremely low at –300 mV *vs*. SHE and increased steadily at lower cathode potentials. In principle, the possibility to operate the bioelectrochemical dechlorination process with the cathode set at –300 mV *vs*. SHE is extremely appealing from a practical and economical point of view, since at this potential electrolytic hydrogen generation is prevented and, accordingly, side reactions such as hydrogen-dependent methanogenesis or homoacetogenesis are minimized [13]. In a follow-up study, we examined the possibility to boost 1,2-DCA dechlorination rates by using a soluble redox mediator (i.e., the humic acid analogue anthraquinone-2,6-disulfonate, AQDS) ($E^{\circ\prime}$ = –184 mV *vs*. SHE) [11]. Addition of AQDS (0.5 mmol L^{-1}) to the cathode compartment of a bioelectrochemical system resulted in 2.5- to 7-fold higher 1,2-DCA dechlorination rates relative to those obtained in the mediatorless system. Clearly, while the use of soluble redox mediators is feasible in lab-scale reactors operating in batch regime, it is extremely problematic in continuous-flow systems operating at the field-scale, due to the environmentally unacceptable need for a continued supply of the redox mediator, as well as for the associated economic implications and regulatory restrictions [14]. To overcome these limitations, in this study we developed a simple, yet reproducible method to immobilize AQDS at the surface of a graphite electrode using a commercial anion exchange membrane. The effectiveness of the immobilization protocol was thoroughly assessed by means of a suite of electrochemical experiments. Finally, the capability of the AQDS-functionalized electrode to

stimulate the bioelectrochemical reductive dechlorination of 1,2-DCA was verified by means of replicated batch experiments.

2 Experimental

2.1 Preparation of AQDS-Functionalized Electrodes

The redox mediator AQDS was immobilized at the surface of 6-mm diameter graphite rods (Sigma-Aldrich, Italy), according to the following protocol:

i. A weighted (0.025 g) piece of fumasep® FAD (FuMA-Tech GmbH, Germany) anion exchange membrane was placed in glass vial containing 5 mL of ethanol and 5 mL of an aqueous solution containing AQDS (0.5 mmol L^{-1}).

ii. The vial was closed and heated at 60 °C for ten minutes to favor dissolution of the membrane (harboring ammonium ion exchange groups) and its separation from the polyester backbone.

iii. The graphite rod, pre-treated as described previously [15], was completely immersed in this solution, and maintained therein for 1 hour at 60 °C.

iv. The so-prepared AQDS-functionalized electrode was air dried overnight in vertical position and then thoroughly rinsed with DI water prior to being used.

An AQDS free-functionalized electrode was also prepared following the same procedure described above, with the only exception that during the first step the anion exchange membrane was immersed in a glass vial containing ethanol only.

2.2 Bioelectrochemical Cell Setup

The bioelectrochemical cell used in this study consisted of two gastight borosilicate glass bottles (with a total volume of about 270 mL per bottle) separated by a 3-cm^2 cross-sectional area Nafion™ 117 proton exchange membrane (PEM). The PEM was boiled successively in H_2O_2 (3% vol vol^{-1}), deionized (DI) water, then in 0.5M H_2SO_4 and DI water each for 2 h, and stored in DI water. The working electrodes (cathodes) had a nominal surface area, calculated taking into account only the part of the electrode that was immersed in the liquid phase, of 9.7

cm^2. The counter electrode (anode) was a graphite rod, also having a nominal surface area of 9.7 cm^2. The distance between the anode and the cathode was around 10 cm. A KCl-saturated Ag/AgCl reference electrode [+200 mV *vs*. the standard hydrogen electrode (SHE)], maintained within a Luggin capillary, was also placed in the cathode compartment of the cell. The catholyte and anolyte consisted of anaerobic medium, prepared as described below.

2.3 Cyclic Voltammetry

The working electrodes were characterized primarily by cyclic voltammetry (CV). CV tests were carried out in the 2-compartment bioelectrochemical cell previously described, under both stirring and no stirring conditions and at different scan rates (from 1 mV s^{-1} to 50 mV s^{-1}). Electrochemical measurements were performed using IVIUMnSTAT, multichannel electrochemical analyzer (Ivium Technologies, The Netherlands) and data were processed with Ivium Soft.

2.4 Bioelectrochemical 1,2-DCA Dechlorination Experiments

The capability of the AQDS-functionalized electrode to stimulate the reductive dechlorination of 1,2-DCA by a mixed dechlorinating culture was assessed by means of batch experiments. The experiments were carried out in the 2-chamber bioelectrochemical cell described previously **12**. Initially, the working electrode's compartment of the bioelectrochemical cell was filled with 110 mL of anaerobic mineral medium and a 40 mL inoculum consisting of a 1,2-DCA dechlorinating enrichment culture **11**. The medium contained the following components: NH_4Cl (0.5 g L^{-1}), $MgCl_2 \cdot 6H_2O$ (0.1 g L^{-1}), K_2HPO_4 (0.4 g L^{-1}), $CaCl_2 \cdot 2H_2O$ (0.05 g L^{-1}), trace metal solution (10 mL L^{-1}) **16**, vitamin solution (10 mL L^{-1}) **17**, and $NaHCO_3$ (15 mL L^{-1}, 10% w vol^{-1}). All solutions were purged for at least 0.5 h with a N_2/CO_2 (70:30% vol vol^{-1}) gas mixture before use. The pH value of the medium was approximately 7.5. The counter electrode's compartment was filled with 150 mL of anaerobic mineral medium with the same composition.

A parallel bioelectrochemical cell, serving as a control, was set up under identical conditions, but for the presence of the AQDS free-functionalized electrode in place of the AQDS-functionalized electrode.

Prior to the start of each batch experiment, the working and counter electrode compartments of the bioelectrochemical cells (i.e., the cell housing the AQDS-functionalized electrode and the one housing the AQDS free-functionalized electrode) were purged (30 min) with a N_2/CO_2 (70:30 vol vol^{-1}) gas mixture to remove volatile compounds and to maintain anaerobic conditions.

Thereafter, the working electrode's compartment was spiked with 1,2-DCA at a nominal concentration (i.e., neglecting partitioning into the gas phase) of approximately 0.12 mmol L^{-1}. Afterwards, the working electrode was either polarized at -300 mV *vs*. SHE (by means of the IVIUMnSTAT multichannel potentiostat) or kept at open circuit potential (OCP). Over the course of the tests (which had a duration of approximately 7 days), the cell was monitored for volatile components (i.e., the spiked chlorinated contaminant and its reductive dechlorination products) and for the consumed electric current and cumulative charge. Cumulative reducing equivalents (μeq) that were used for 1,2-DCA dechlorination were calculated from the measured amounts of reductive dechlorination products formed (i.e., ethene), considering that 2 μeq are required for the formation of 1 μmol of ethene.

Throughout the tests, the bioelectrochemical cells were maintained at 25 °C, with the liquid phase of each compartment vigorously stirred (400 rpm) with a magnetic stirrer. Notably, the bioelectrochemical cells were inoculated only once, prior to the start of the first batch test. At the end of each test, a fixed volume of liquid phase (approx. 20 mL) was removed from each compartment of the cells and replaced with fresh anaerobic medium in order to maintain an average hydraulic and biomass retention time of approximately 50 days.

2.5 Analytical Methods

Volatile components (1,2-DCA, VC, ethene, methane) were quantified by injecting 50 μL of headspace (taken with a gas-tight syringe) into a Shimadzu GC-2014 gas chromatograph (2.4 m × 2.1 mm metal packed column 60/80 Carbopack B/ 1% SP-1000; N_2 carrier gas 40 mL min^{-1}; oven temperature 60 °C with a ramp of 40 °C min^{-1} until 180 °C hold for 1.25 min; FID temperature 200 °C).

Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants **18**. Volatile fatty acids (VFA) (i.e., acetate, propionate) were analyzed by injecting 1 μL of filtered (0.22 μm porosity) liquid sample, previously acidified with formic acid (10 % vol vol^{-1}), into a Shimadzu GC-2014 gas chromatograph (3 m × 2.1 mm stainless steel column packed with 60/80 mesh Carbopak C/ 0.3% Carbowax 20 M/ 0.1% H_3PO_4; N_2 carrier gas 40 mL min^{-1}; oven temperature 120 °C; injector temperature and FID temperature 200 °C).

2.6 Scanning Electron Microscopy (SEM)

SEM was used to analyze the surface of the AQDS-functionalized electrode, at the end of the batch dechlorination experiments. To this aim, the electrode was initially immersed in a petri

dish containing 3% (w vol^{-1}) glutaraldehyde in cacodylate buffer (pH 7.2) for 3 hours at room temperature. Thereafter, it was dehydrated in an ethanol series (50, 60, 70, 80, 90, and 2 × 100% vol vol^{-1}, 10 min per step). Treated samples were coated with gold powder and were viewed with a SEM/EDS system (FEI Quanta 400FEG ESEM/EDAX Genesis X4M, FEI Company, USA) in high-vacuum mode at 10 or 15 kV to observe size, morphology and distribution.

2.7 Chemicals

All the chemicals were analytical grade or higher and used as received.

3 Results and Discussion

3.1 Electrochemical Characterization of AQDS-Functionalized Electrodes

The AQDS-functionalized electrodes, prepared as described in the materials and methods section, were preliminarily characterized by means of cyclic voltammetry (CV). The CV of freshly prepared electrodes, recorded at a scan rate of 10 mV s^{-1}, displayed a well-defined couple of redox peaks characterized by a formal redox potential (E^f) of –172 mV *vs*. SHE, which could be uniquely ascribed to AQDS reduction and oxidation processes (Figure 1). This value was close to that (–196 mV *vs*. SHE) retrieved from a CV recorded with AQDS in solution at 1 mmol L^{-1}, using a neat graphite electrode (Figure 1).

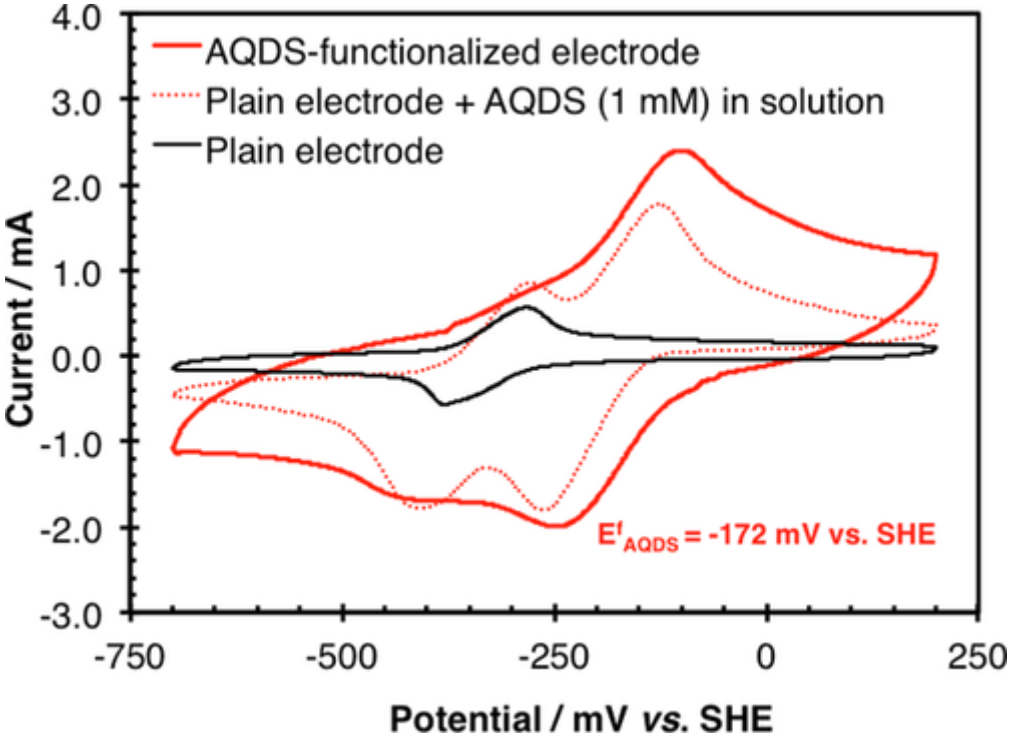

Figure 1

Cyclic voltammograms recorded at a scan rate of 10 mV s^{-1} with no stirring of the liquid phase. Black line: plain graphite electrode in reduced mineral medium; Red dotted line: plain graphite electrode in reduced mineral medium supplemented with AQDS at a final concentration of 1 mM; Red line: AQDS-functionalized electrode in reduced mineral medium.

The AQDS surface concentration (Γ_{AQDS}) of functionalized electrodes was calculated according to the equation 1:

$$\Gamma_{\mathrm{AQDS}} = Q_C / \mathrm{nFA} \quad (1)$$

where Q_C is the electric charge (in Coulombs) from the area under the AQDS reduction peak (corrected for the baseline); n is the number of electrons exchanged per reactant molecule (n =2); F is Faraday's constant; and A is the nominal area of the electrode (in cm^2). The estimated Γ_{AQDS} was in the range of 1×10^{-8} mol cm^{-2}, a value which compares favorably with that reported in the literature for other AQDS-modified electrodes, prepared, however, via chemical bonding of AQDS to conductive polymers (e.g., polyethylenimine, polyaniline, polypyrrole) or materials (e.g., glassy carbon) 19-21.

The CV of functionalized electrodes also revealed the presence of another redox couple (E^f = –340 mV *vs*. SHE), which was, however, related to a redox active component (e.g., Fe^{2+}/Fe^{3+}) of the mineral medium.

For practical applications, the formal potential of the AQDS-functionalized electrode is of interest because it is sufficiently high to avoid interference from hydrogen evolution ($E^{\circ\prime}$ = –440 mV at pH 7.5), a main competitive reaction in aqueous media.

In order to gain a deeper understanding of the electrochemical behavior of the AQDS-functionalized electrode, CVs at different scan rates were recorded, under stirring and no stirring conditions (Figure 2a). The peak separation substantially decreased from 240 mV to 69 mV, when the scan rate was decreased from 50 mV s^{-1} to 1 mV s^{-1}, hence suggesting that AQDS oxidation and reduction processes were severely mass-transport limited and, in turn, that the electrochemical behavior of the modified electrode did not recall that of an ideal surface-bound redox system 22. Interestingly, plotting values of the anodic and cathodic peak current as a function of the square root of the scan rate (Figure 2b) yielded a substantially better linear correlation (R^2 = 0.99) compared to when they were plotted *vs.* the scan rate (R^2 < 0.97), thereby proving an additional line of evidence of the occurrence of mass-transport limitations. In spite of that, CVs of AQDS-functionalized electrodes recorded with no stirring of the liquid phase or in the presence of vigorous magnetic stirring at 600 rpm were very similar (Figures 2a and 2b), hence indicating that the redox processes taking place at the electrode were not significantly influenced by transport phenomena occurring in the liquid phase but rather by the slow diffusion of reactants (most likely the protons contributing to the maintenance of charge

balance) through the thick polymeric film consisting of the fumasep® anion exchange membrane surrounding the electrode surface.

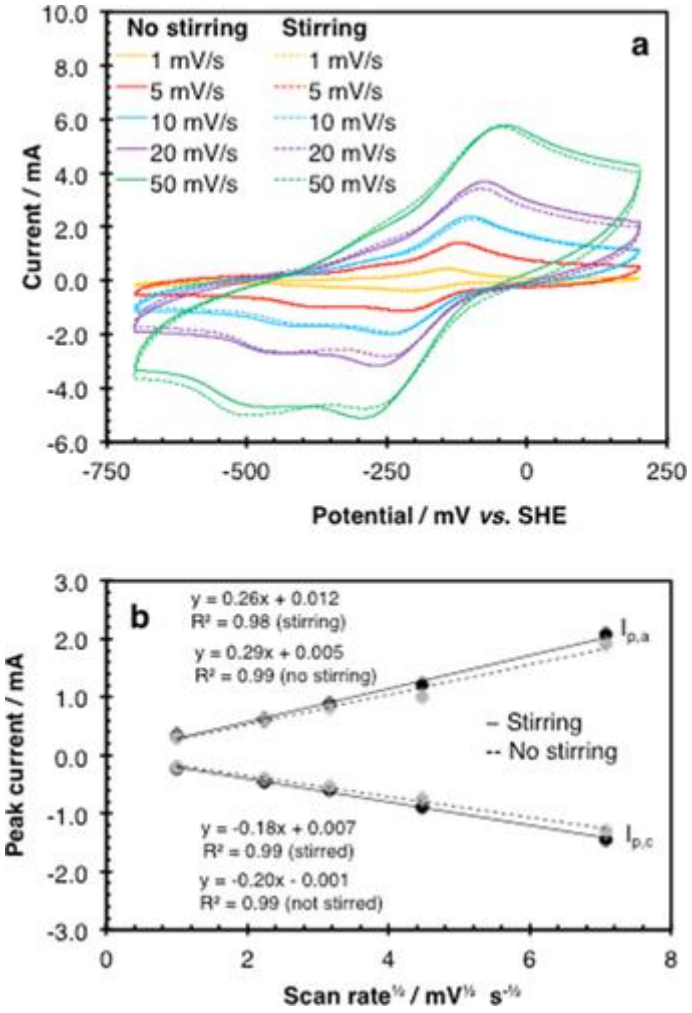

Figure 2

(a) Effect of the scan rate (1–50 mV s^{-1}) on the cyclic voltammetric response of AQDS-functionalized electrodes, under magnetic stirring (600 rpm) and no-stirring of the liquid phase. (b) Linear dependency of cathodic ($I_{p,c}$) and anodic ($I_{p,a}$) peak current, under stirring and no stirring conditions, on the square root of scan rate.

3.2 Long-Term Application of the AQDS-Functionalized Electrode to Stimulate the RD of 1,2-DCA

The capability of the AQDS-functionalized electrode (cathode) to stimulate the reductive dechlorination of 1,2-DCA by a mixed microbial culture was investigated by means of batch experiments. The batch experiments were carried out in an H-type bioelectrochemical cell, which was inoculated at the cathode with an anaerobic culture capable to dechlorinate 1,2-DCA using a polarized (–300 mV *vs*. SHE) graphite rod as electron donor and soluble AQDS (0.5

mmol L^{-1}) as a redox mediator. In total 8 batch experiments were conducted, each having a duration of 7 days. The tests were alternatively carried out with the AQDS-functionalized electrode kept polarized at –300 mV *vs*. SHE (4 tests) or kept at open circuit potential (OCP) (4 tests). Figure 3a show the time course of dechlorination products during typical batch tests carried with the AQDS-functionalized electrode set at –300 mV *vs*. SHE and at OCP, as well as with the AQDS free-functionalized electrode set at –300 mV *vs*. SHE. Under all the tested conditions, 1,2-DCA was dechlorinated (via dichloroelimination) to primarily ethene, which accounted for over 98% of the formed dechlorination products, and to a minor extent vinyl chloride (<2%), this latter being formed via 1,2-DCA dehydrochlorination. Negligible methane formation was detected throughout the tests. Notably, the observed dechlorination patterns mirrored that observed with the same culture in the presence of soluble AQDS at 0.5 mmol L^{-1} 11. Despite the slow dechlorinating activity observed during tests at OCP, which was most probably sustained by the H_2 deriving from the slow anaerobic degradation of the acetate and propionate initially present in the inoculum at concentrations of 400 mg L^{-1} and 75 mg L^{-1} respectively, polarization of the AQDS-functionalized electrode at –300 mV *vs*. SHE resulted in a remarkable enhancement of the dechlorination process. Indeed, the average rate of 1,2-DCA dechlorination at –300 mV *vs*. SHE (13.1 ± 1.8 μeq L^{-1} d^{-1}) was 2 times greater than that observed at OCP conditions (6.3 ± 0.3 μeq L^{-1} d^{-1}). Notably, the rate of 1,2-DCA dechlorination in the presence of the AQDS free-functionalized electrode (7.4 ± 3.2 μeq L^{-1} d^{-1}) was also substantially lower than that observed with the AQDS-functionalized electrode, and very similar to that observed in OCP tests. Collectively, these results provide a clear indication on the key role of AQDS and polarization in accelerating the bioelectrochemical reductive dechlorination process. On the other hand, it should be noted that the 1,2-DCA dechlorination rate obtained with the AQDS-functionalized electrode was substantially lower than that obtained, in the presence of the same microbial culture, when AQDS was supplied in solution at a concentration of 0.5 mM (i.e., 43 ± 7 μeq L^{-1} d^{-1}) 11, hence clearly indicating a lower accessibility of AQDS to microorganisms, when entrapped within the polymeric structure of the Fumasep membrane.

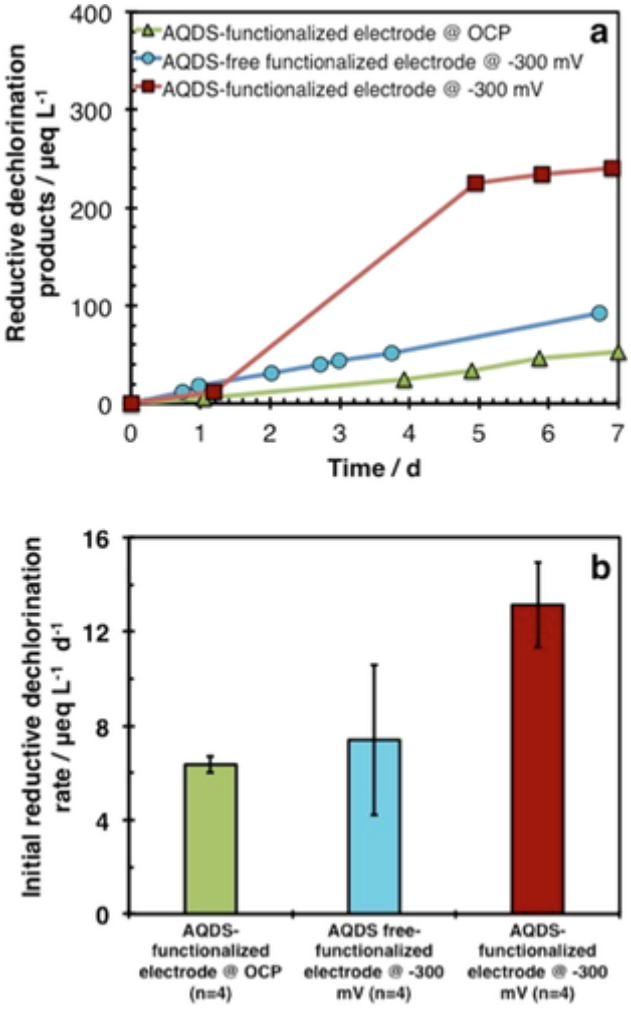

Figure 3

(a) Time course of 1,2-DCA dechlorination products during typical batch experiments with the AQDS-functionalized electrode polarized at –300 mV *vs*. SHE or kept at open circuit potential (OCP), and with the AQDS free-functionalized electrode polarized at –300 mV *vs*. SHE. (b) Average values of the initial 1,2-DCA dechlorination rates with the AQDS-functionalized electrode at –300 mV *vs*. SHE and at OCP, and with the AQDS free-functionalized electrode at –300 mV *vs*. SHE. Error bars represent the standard error of replicated (n = 4) batch experiments.

The long-term redox activity of the AQDS-functionalized electrodes, in the presence of the microbial culture, was primarily verified by means of CV. Interestingly, after approximately 70 days of operation the AQDS cathodic peak current (Ip,c) was reduced by only approximately 25% compared to time zero, although peak separation increased by nearly 3.5 times.

3.3 SEM Analysis

At the end of the above-described batch experiments, the bioelectrochemical cell was dismounted and the AQDS-functionalized electrode was analyzed by scanning electron

microscopy (SEM). Remarkably, the electrode surface was almost completely covered by a biofilm, presumably involved in the bioelectrochemical 1,2-DCA reductive dechlorination process. Predominant cellular morphologies were rods (resembling the morphology of *Desulfitobacterium* spp., a known 1,2-DCA dechlorinating microorganism) and, although less abundant, cocci.

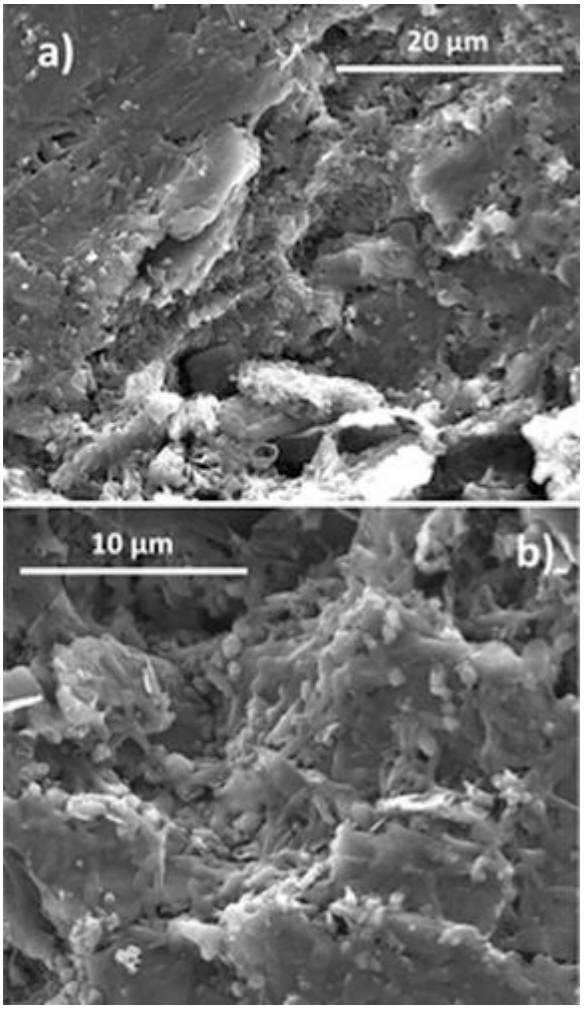

Figure 4

SEM micrographs of the AQDS-functionalized electrode at the end of the 1,2-DCA batch dechlorination tests (a, b).

4 Conclusions

Fumasep® anionic membrane allowed immobilization of the redox mediator AQDS at the surface of graphite electrodes. Most likely, AQDS was retained within the membrane via the establishment of electrostatic interactions of sulfonic groups of AQDS with ammonium groups of the membrane. The herein described immobilization method was simple, rapid, and effective. Potentiostatic batch experiments with a mixed dechlorinating culture spiked with 1,2-DCA

revealed a 2-fold enhancement of the average reductive dechlorination rate compared to OCP experiments. Over time (> 70 days and upon complete replacement of the catholyte with fresh medium), AQDS remained stably attached to the electrode, with the AQDS-functionalized electrode retaining most of its electrocatalytic activity while promoting the formation of a dense microbial biofilm.

Considering the reported broad range of anodic and cathodic reactions catalyzed by AQDS, the herein described functionalized electrode has a substantial potential for application in the environmental and industrial sector. Clearly, depending on the envisaged application, a technical-economical analysis will have to verify the actual viability of the process and, in case, drive the search for alternative and possibly cheaper, ammonium ion-bearing anion exchange membranes.

Acknowledgements

The authors would like to thank FCT (Portuguese Foundation for Science and Technology) for the financial support of Patrícia Leitão through the PhD grant SFRH/BD/87312/2012 and to CEMUP – Centro de Materiais da Universidade do Porto for expert assistance and helpful discussions about SEM measurements.

References

[1] B. Huang, C. Lei, C. Wei, G. Zeng,Environ. Int.2014,71,118–138.

[2] F. Aulenta, M. Majone, V. Tandoi,J. Chem. Technol. Bio-technol.2006,81, 1463–1474.

[3] M. Majone, R. Verdini, F. Aulenta, S. Rossetti, V. Tandoi,N. Kalogerakis, S. Agathos, S. Puig, G. Zanaroli, F. Fava,N. Biotechnol.2015,32, 133–146Crossref CAS PubMed Web of Science®Google ScholarTexto Integral @ b-on

[4] F. Aulenta, A. Pera, S. Rossetti, M. Petrangeli Papini,M. Majone,Water Res.2007,41, 27–38.

[5] F. Aulenta, S. Rossetti, B. Matturro, V. Tandoi, R. Verdini,M.Majone,inOrganohalide-Respiring Bact.(Eds.L. Adrian, F. E. Loffler), Springer Berlin Heidelberg,2016, pp. 499–516.

[6] A. Lai, F. Aulenta, M. Mingazzini, M. T. Palumbo, M. P.Papini, R. Verdini, M. Majone,Chemosphere2017,169,351–360.

[7] S. T. Lohner, D. Becker, K. M. Mangold, A. Tiehm,Envir-on. Sci. Technol.2011,45, 6491–6497.

[8] F. Aulenta, A. Catervi, M. Majone, S. Panero, P. Reale,S. Rossetti,Environ. Sci. Technol.2007,41, 2554–2559.

[9] F. Aulenta, P. Reale, A. Canosa, S. Rossetti, S. Panero,M. Majone,Biosens. Bioelectron.2010,25, 1796–1802.

[10] F. Aulenta, A. Canosa, P. Reale, S. Rossetti, S. Panero,M. Majone,Biotechnol. Bioeng.2009,103, 85–91.

[11] P. Leita˜o, S. Rossetti, A. S. Danko, H. Nouws, F. Aulenta,Bioresour. Technol.2016,214, 426–431.

[12] P. Leita˜o, S. Rossetti, H. P. A. Nouws, A. S. Danko,M. Majone, F. Aulenta,Bioresour. Technol.2015,195,78–82.

[13] F. Aulenta, L. Tocca, R. Verdini, P. Reale, M. Majone,Environ. Sci. Technol.2011,45, 8444–8451.

[14] F. Aulenta, V. Di Maio, T. Ferri, M. Majone,Bioresour.Technol.2010,101, 9728–9733.

[15] K. B. Gregory, D. R. Bond, D. R. Lovley,Environ. Micro-biol.2004,6,596–604.

[16] J. G. Zeikus,Bacteriol. Rev.1977,41, 514–541.

[17] W. E. Balch, G. E. Fox, L. J. Magrum, C. R. Woese, R. S.Wolfe,Microbiol. Rev.1979,43, 260–296.

[18] J. M. Gossett,Environ. Sci. Technol.1987,21, 202–208.

[19] M. Adachi, T. Shimomura, M. Komatsu, H. Yakuwa,A. Miya,Chem. Commun.2008, 2055–2057.

[20] F. Aulenta, T. Ferri, D. Nicastro, M. Majone, M. P. Papini,N. Biotechnol.2011,29, 126–131.

[21] G. Zhang, W. Yang, F. Yang,J. Electroanal. Chem.2007,602, 163–171.

[22] F. Harnisch, S. Freguia,Chem. – An Asian J.2012,7, 466–475