Bioelectrochemical Systems for In Situ Treatment of Groundwater Contaminated by Hexavalent Chromium

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ABSTRACT: Groundwater resources are highly vulnerable to heavy metals contamination, in particular hexavalent chromium. In-situ bioremediation is based on microbiological communities and their metabolic capabilities that allow contaminant transformation, hopefully reducing their toxicity and mobility in groundwater. Bioremediation technologies usually aim to ensure bacterial metabolism and biodegradation of contaminants by dosing suitable electron donors/acceptors in the subsurface. Critical issues are related to prediction of dosage, frequency of injection and distribution in the aquifer. In bioelectrochemical processes (BioElectrochemical Systems - BESs), electrochemically active microorganisms develop as biofilms on electrodes, catalyzing reactions unfavourable from a purely electrochemical point of view. Compared to traditional bioremediation, the main advantage of such systems is, therefore, the improvement/control in the electron transfer by applying a current or a voltage between two bioelectrodes, without the addition of external chemical agents. In the present study, the chance of using Cr(VI) as a terminal electron acceptor for an anaerobic biocathode in a Microbial Electrolysis Cell (MEC) has been investigated, at the laboratory scale. Tests in batch operating MECs with poised abiotic and biocathodes and open circuit controls, at 1 mg L^{-1} initial Cr(VI) concentration have been performed. A decrease in Cr(VI) concentration was observed at the end of the tests, both in the polarized reactors and in the control systems. The biocathode, operating at -300 mV, showed a removal efficiency over 90% in 6 d. The results from microbial characterization showed that the bacterial community in the MEC-300 was affected by the cathodic polarization, and it was different from the biomass in the open circuit control.

INTRODUCTION

Groundwater resources are highly vulnerable to heavy metals contamination, hexavalent chromium Cr(VI) in particular. Hexavalent chromium Cr(VI) and trivalent chromium Cr(III), among the wide range of valence states of chromium (from -4 to +6), are the dominant ions in the environment. Cr(VI) is highly soluble, mobile and toxic, mutagenic and carcinogenic to all living organisms, while Cr(III) is less toxic and minimally soluble in the neutral pH range. Accordingly in-situ Cr(VI) remediation approaches aim to promote Cr(VI) reduction to Cr(III).

In-situ bioremediation is based on microbiological communities and their metabolic capabilities that allow contaminant transformation, hopefully reducing their toxicity and mobility in groundwater. Bioremediation processes usually provide for substances (electron donors/acceptors) to ensure bacterial metabolism and biodegradation of contaminants. Critical issues are related to prediction of dosage, frequency of injection and distribution in the aquifer. In bioelectrochemical processes (BioElectrochemical Systems - BESs), electrochemically active microorganisms develop as biofilms on electrodes, catalyzing reactions unfavourable from a purely electrochemical point of

view. Compared to traditional bioremediation, the main advantage of such systems is, therefore, the improvement/control in the electron transfer by applying a current or a voltage gradient between two bioelectrodes, without the addition of external chemical agents. The transformation of pollutants is related to the electrical signal, so the process can be monitored in real time. In BES for the removal of metals, cathode is used as an electron donor by electroactive microorganisms that catalyzed electrons transfer to oxidized metallic ions (Nancharaiah et al., 2015; Huang et al., 2011; Lovley et al., 2011). Tandukar et al. (2009) observed for the first time biological chromium reduction at the cathode of a Microbial Fuel Cell (MFC). Huang et al. (2011b) tested a Microbial Electrolysis Cell (MEC) with a biocathode poised at -300 mV (vs. Standard Hydrogen Electrode - SHE), which was able to efficiently reduce Cr(VI). Unlike previous works, the aim of this study was to evaluate the possibility to remove Cr(VI) in a biocathodic chamber of a dual-chamber (2C-MEC) with cathode as the sole electron donor, selecting and exploiting only autotrophic microorganisms. Since selection of electroactive biofilms under anaerobic conditions is generally more difficult at the cathode than at the anode, in this study we used the acclimatization method proposed by Wu et al. (2015), involving the transfer of a mature bioanode of an MFC to the cathode of a MEC.

The electrical inversion of a bioelectrode without significant reductions in the electron transfer efficiencies has already been reported in previous studies (Rozendal et al., 2008; Pisciotta et al., 2012, Zaybac et al., 2013). It was also shown that some microorganisms are able to exploit an electrode as either electron acceptor or electron donor (Yang et al., 2015; Xafenias et al., 2015).

In this study, to shorten the time required for the development of electroactive biofilm on the electrode and to increase the Cr(VI) reduction efficiency (Xafenias et al., 2013; Wang et al., 2008), cathode was first acclimated in anode chamber of a 2C-MFC (Wu et al., 2015). However, for this research area further extensive studies into efficient biocathodes for the enhancement of Cr(VI) reduction and electricity generation in MFC are required.

MATERIALS AND METHODS

Microbial Fuel Cell setup, Inoculation and Operation (for biocathodes production)

Dual-chamber H-shaped reactors (2C-MFC), each one made of a couple of 1.2 L Pyrex-glass bottles, separated by a Cation Exchange Membrane (CEM, 4.52 cm^2, Membranes International Inc., USA), were used in this study. Graphite cylinders (ATAL Grafiti, Italy, 6 cm length, 1 cm diameter, geometric area 18.85 cm^2) served as electrodes. To increase the surface for exoelectrogenic biofilm enrichment, three graphite cylinders were placed into the anode chamber and only a single one into the cathodic chamber (anode to cathode ratio =3). The distance between anodes and cathode was about 10 cm. Stainless steel or titanium wires (1 mm diameter) fixed in the centre of the graphite cylinders, were used as current collectors.

Before use, the CEM was soaked in 5% NaCl solution for at least 24 h, as described in Daghio et al. (2016). The anodic and cathodic chambers were filled with sterile M9 minimal medium (7 g L^{-1} $NaH_2PO_4 \cdot 12H_2O$, 3 g L^{-1} KH_2PO_4, 1 g L^{-1} NH_4Cl and 0.5 g L^{-1} NaCl). The anodic chamber was inoculated with 0.24 L anaerobic sludge from an anaerobic digester of a wastewater treatment plant in Cremona (Italy) (Figure 2).

At the beginning of the experiment, the anodic chamber was flushed for 15 min with sterile-filtered N_2 to establish anaerobic conditions. The anode chamber was sealed by

a screw cap with PTFE-coated silicone septum; the cathode chamber was kept open to air to let oxygen diffuse into the solution.
The MFC operated in a batch-fed mode at a constant temperature (18 ± 0.5 °C) and pH (7.4 ± 0.1). Sodium acetate, in concentration up to 0.1 g L^{-1}, acted as the carbon source for anodic bacterial growth and was periodically added into the medium, with a syringe, every time the current density dropped below 0.5 mA m^{-2}. Throughout the whole test, the voltage drop across an external resistor (500 Ω) was continuously recorded using a data logger (Picolog 1012, Pico Technology Ltd., UK).
The test lasted about 16 days, until stable maximum output voltage increased up to 220 mV (about 230 mA m^{-2}), indicating bacteria have colonized the electrodes. At the sixteenth day, the three bioelectroactive anodes were disconnected and 40% of the anolyte volume collected to be used, respectively, as biocathodes and inoculum, in the Cr(VI)-reducing MECs and control reactor. At the same time, three new electrodes replaced those removed and the initial volume in MFC was re-established with fresh medium.

Analyses and calculations
Current (I) was calculated as I = U/R, where U is the measured voltage across the external resistor [V] and R is the external resistance [Ω] (Logan et al., 2006). Current density [mA m^{-2}] was calculated as the ratio of the recorded current, I [A], and the electrode's surface area. MFC performance was evaluated by means of Coulombic efficiency (CE), i.e. the efficiency of the conversion of a substrate into electrical current (Logan et al., 2006), calculated as the fraction of the electrons theoretically available through oxidation of substrates (the sole acetate in this case) in the anodic chamber that are actually transferred to the anode:

$$CE = \frac{MI\Delta t}{FV_{an}\, b\Delta S}$$

where: M (59 g mol^{-1}) is the molecular weight of acetate (CH_3COO^-), I [A] is the recorded current within time Δt [sec], F the Faraday's constant (96,485.3 Coulombs mol^{-1} of electrons), ΔS is the substrate removed [g L^{-1}] over time, b is the number of electrons exchanged per mole of substrate (in case of acetate b = 8) and V_{an} [L] is the working volume of the anode chamber.

Cr(VI)-reducing MEC set up and Operation
The bioelectrochemical Cr(VI)-reducing experiments were carried out using H-shaped reactors equal to those used in 2C-MFC. Also, the Cr(VI)-reducing MEC set up was the same, except CEM was replaced by Proton Exchange Membrane (PEM, 4.52 cm^2, Nafion117, FuelCellsEtc, USA). To increase the membrane's proton conductivity, prior to use, the membrane (about 50 cm^2) underwent a purification procedure by successive boiling, for about 30 minutes, in 5% solution of H_2O_2, deionized water, H_2SO_4 solution 1M, air cooling at room temperature overnight and final storage in deionized water (Casalegno et al., 2014).
Three different MECs were prepared and bioelectrochemical experiments were conducted at temperature 18 ± 0.5 °C by means of home-made (Politecnico di Milano I3N–DICA, 2016 Cariplo-BEvERAGE) dual channel Arduino-based potentiostats.
All the MECs had new graphite cylinders as anodes, whereas the anodes previously acclimatized in the 2C-MFC, were used as the biocathodes; furthermore, 240 mL of the inoculum enriched in 2C-MFC was added to the catholyte (up to 20% final volume). As catholyte a solution of M9 minimal medium, $KHCO_3$ (2 g L^{-1}) as the sole carbon source and hexavalent chromium (1000 µg L^{-1}) was used. The biocathodes were poised at

+700 mV (MEC700) and -300 mV (MEC-300) vs. SHE. The current profiles were recorded using chronoamperometry. Two abiotic controls (A-300 and Anp) and an open circuit system (OC) were also set up in reactors all equal to those used for MECs. The first abiotic control (A-300) set up was the same as Cr-reducing MEC-300, to assess the effects of electrochemical reduction of hexavalent chromium. In the second one (Anp), unpoised abiotic electrodes were used to investigate possible Cr(VI) loss due to adsorption on glass, graphite and PEM. Finally, the OC with MFC acclimated electrode served to compare biological Cr(VI) removal in bioelectrochemical and non bioelectrochemical systems. The MEC700, Anp and OC were operated for 12 days, instead, MEC-300 and A-300 for 6 days.

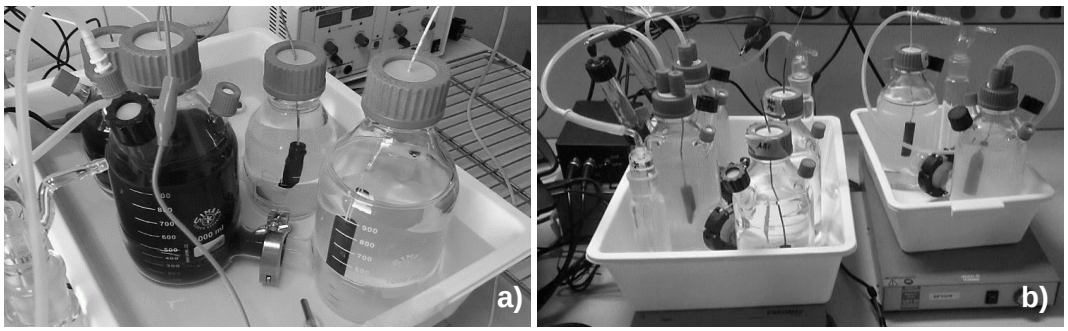

FIGURE 1: a) MFCs and b) MECs and one related control, at the end of the set-up phase

Analyses, Calculations and data processing

Periodical sampling of the catholyte was performed, using a syringe, for optical density at 600 nm (OD600) and chromium analyses. Aliquots were immediately 0.45 µm filtered and analysed for residual soluble Cr(VI) and total soluble chromium using colorimetric standard methods (Method APHA 3500-Cr D, ISO 11083:1994). The amount of soluble Cr(III) was estimated as the difference between total chromium and hexavalent chromium concentrations.

To characterize the structure of microbial communities, samples of the anaerobic sludge, the anodic solution of the MFC and the cathodic solutions of MEC-300 and OC were filtered on 0.45 µm sterile paper. DNA from filters was extracted by means of FastDNA® SPIN Kit for Soil (MP) and, subsequently, amplified by PCR using bacterial primers for the 16S rRNA gene with adapters for the Illumina platform (Illumina Miseq).

A quality filter was applied to the sequencing results and the remaining sequences have been grouped into Operational Taxonomic Units (OTUs); each OTU was made up of 97% identical sequences. For each cluster of sequences, a single representative sequence was identified, which, through RDP classifier, was categorized according to the taxonomic unit (Order). The relative abundances of each OTUs were calculated on the total number of OTUs of each sample.

RESULTS

Current density trends in the MFCs

A relationship between substrate availability and current production was observed, as rapid increase in the circulating current was recorded after each acetate addition. Following the first two additions of acetate, current density showed small peaks, lasting 10-15 minutes. In the two following cycles maximum current density increased to 227 and 232 mA m^{-2}, with longer-lasting peaks. This trend suggests an electroactive biofilm has developed on the electrodes. Coulombic Efficiency also increased in time, from

0.5% in the second cycle to above 15% in the third and fourth cycles. After 16 days, the decrease in the coulombic efficiency (to about 5%) is due to the anodes withdrawal and the renewal of 40% of the medium for the set up of Cr(VI)-reducing MECs and OC system.

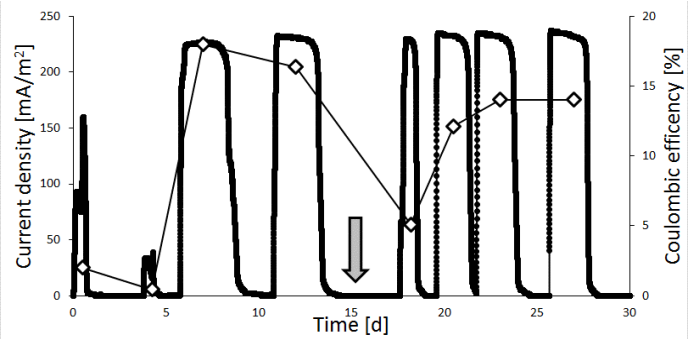

FIGURE 2: Current density and Coulombic efficiency during the anode acclimation phase in 2C-MFC. The grey arrow indicates a 40% replacement of anolyte

Cr(VI) Reduction in MECs

Cr(VI) concentrations in the biocathode of MEC700 and in OC decreased gradually with time (Figure 3). Faster Cr(VI) removal in the OC than in the MEC700 biocathode was observed, with 38% residual chromium in OC and 55% in MEC700. In the final sampling, after twelve days operation, however, residual dissolved chromium is about the same (5%) in both the systems. In the abiotic control (Anp), no significant changes were observed throughout the test.

In the systems a decrease in the optical density from the beginning (0.66 in both OC and MEC700) to the end of the test was observed. In the MEC700 final OD (0.57) was however markedly lower than in OC (0.64).

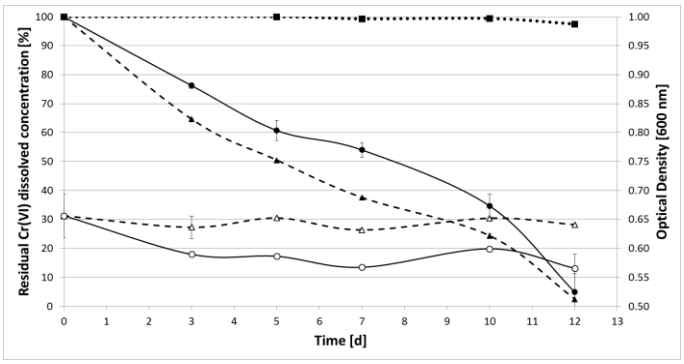

FIGURE 3: Cr(VI) concentration (black symbols) and optical density trends (white symbols); OC control (dashed line and triangles), MEC700 (solid line and circles), and Anp (dotted line and squares).

The most rapid decrease in Cr(VI) concentration was observed in the MEC-300 reactor, with -300 mV vs SHE poised biocathode (Figure 4). In fact, just in six days, Cr(VI) in MEC-300 had reduced to a residual 7% of the initial concentration. In the same timeframe, in the OC control residual Cr(VI) was about 45%. In the abiotic control, A-300, Cr(VI) rapidly decreased to about 35% of the initial concentration, but no further reduction was observed, probably as purely electrochemical Cr(VI) reduction in fact is thermodynamically poorly favoured at neutral pH. Furthermore, Cr(III) precipitation on the electrode, as reported in Li et al. (2008), may passivate it,

preventing any further reduction of the dissolved Cr(VI). MEC-300 showed higher Cr(VI) removal efficiency (93%) and a faster rate than the other systems, likely thanks to the selection of a bacterial community containing electro-active and/or Cr(VI) reducing/resistant bacteria.

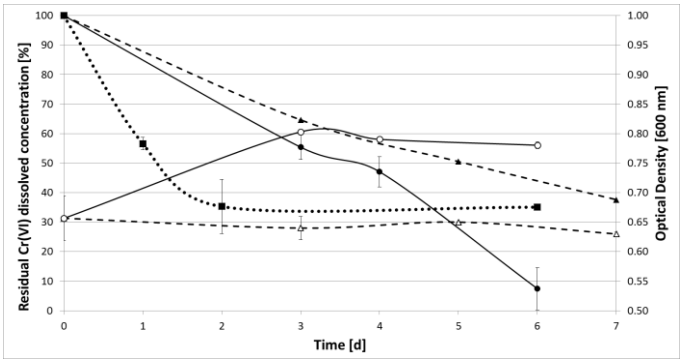

FIGURE 4: Cr(VI) concentration (black symbols) and optical density trend (white symbols); OC control (dashed line and triangles), MEC-300 (solid line and circle), and A-300 (dotted line and squares).

Microbial Communities Structure

The results of the microbiological analyses showed an evolution from anaerobic digester community compared to the final time (MEC-300 and OC) (Figure 5). The microbial communities, from the beginning to the end of the tests, show an increase in the relative abundance of bacteria belonging to the orders: *Burkholderiales*, *Bacteroidales* and a reduction of the bacteria belonging to the orders *Spirochaetales, Methanosarcinales* and *Methanomicrobiales.* Representatives of the *Burkholderiales* and *Bacteroidales* orders have been previously described as bacteria capable of transferring electrons to the anode (Barbosa et al., 2018; Kumar, Malyan, Basu, & Bishnoi, 2017). The availability of substrate and a solid electron acceptor/donor has promoted electroactive bacteria, to the detriment of heterotrophic microorganisms and methanogens. This confirms that the increase in the Coulombic efficiency over time has been promoted by the selection of an electroactive community. In MEC-300, the presence of the cathode as the main electron donor favoured the order *Xanthomonadales*, already reported as components of the electroactive communities enriched from anaerobic digester sludge (Im et al., 2018)(Paiva et al., 2015).

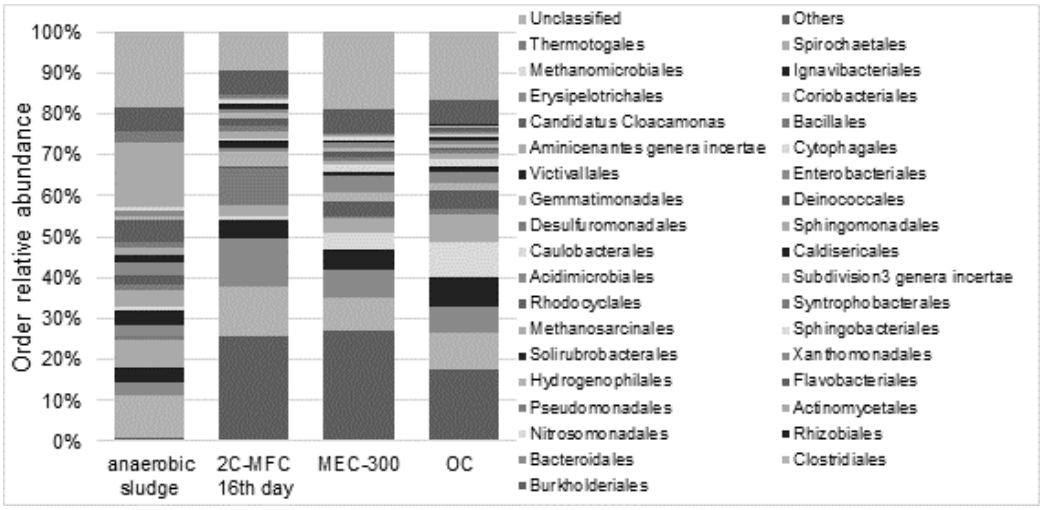

FIGURE 5: Comparison of the bacterial communities from the inoculum (anaerobic sludge) to the end of the test (MEC-300, OC) at the Order level.

CONCLUSIONS

The acclimation at the anode of the MFC allowed to shorten time for the electroactive biofilm to colonize the electrode and to increase the efficiency of the Cr(VI)-reducing MEC with -300 mV vs SHE poised biocathode. The bioelectroactive film at the cathode was essential for high performance chromium removal: MEC-300 showed the fastest chromium removal compared to MEC700 and purely electrochemical and biological control. Although BESs require further laboratory testing and scale up, the use of bioelectrochemical systems for removing hexavalent chromium is a new, sustainable and promising approach for remediation of water polluted with Cr(VI), as well as other contaminants. The results presented in this study reinforce earlier works that suggest the potential of biocathode to stimulate chromium reduction in contaminated waters (Xafenias et al., 20013; Huang et al., 2011).

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