

Sequential Reductive/Oxidative Bioelectrochemical Process for groundwater perchloroethylene removal

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Chlorinated aliphatic hydrocarbons (CAHs) are common groundwater contaminants, microbial communities naturally present in groundwater can reduce CAHs as perchloroethylene (PCE) and trichloroethylene (TCE) to ethylene through reductive dechlorination (RD) reaction while low chlorinated CAHs like cis-dichloroethylene (cis DCE) and vinyl chloride (VC) can be oxidized by aerobic pathways. A combination of reductive and oxidative dechlorination results an effective strategy for the complete mineralization of CAHs. Bioelectrochemical systems (BES) are innovative processes which can be adopted to stimulate both reductive and oxidative dechlorination biomass through polarized electrodes. The present study describes the performances of a an oxidative bioelectrochemical reactor composed by a membrane-less microbial electrolysis cell (MEC) equipped with an internal graphite counterelectrode. In the oxidative reactor the oxygen provided by a mixed metal oxides (MMO) anode stimulated the oxidative dechlorination of the cisDCE contained in synthetic groundwater. Throughout the experimental period, both reductive and oxidative dechlorination pathways were identified due to presence of an internal counter electrode that acted as electron donor. Reductive and oxidative bioelectrochemical reactions, including anions reduction were determined and their relative contribution to the overall flowing current has been quantified in terms of oxidative and reductive coulombic efficiencies.

1. Introduction

Chlorinated aliphatic hydrocarbons (CAHs) are nowadays common groundwater contaminant due to their improper use in the past. In recent years, to make remediation technologies more sustainable and cost-effective, the utilization of groundwater's indigenous microorganisms has been widely reported (Dolinová et al., 2017). Microbial communities are able to reduce CAHs as perchloroethylene (PCE) and trichloroethylene (TCE) to ethylene through reductive dechlorination (RD) reaction (Matturro et al., 2017). Because only the *Dehalococcoides* *McCarty* results able to complete reduce PCE and TCE to ethylene (Matturro et al., 2013), VC accumulation is usually observed even in presence of nutrients injection (Devlin et al., 2004). Indeed, low chlorinated compounds like cis-DCE and VC are more susceptible to the aerobic degradation. Moreover, cis-DCE and VC aerobic biodegradation can be metabolic, in which the chlorinated compound is used as direct carbon and energy source and co-metabolic in which the degradation of the chlorinated compound occurs in the presence of a growth substrate (Aulenta et al., 2013). The oxidative dechlorination of low chlorinated compounds proceed through the oxygenase (or monooxygenase) enzyme which produces chlorinated epoxides, unstable intermediates which easily degrade into CO₂ (Frascari et al., 2015). The utilization of the oxidative dechlorination in combination with the RD reaction represent an effective bioremediation strategy for the complete mineralization of chlorinated compounds. Several studies have been reported in which the stimulation of the RD reaction by nutrient injection is combined with oxygen bio-sparging to create an oxidative environment. An innovative strategy for the biological activity control is offered using bioelectrochemical systems (BES), in which the direct or indirect interaction of microorganism and polarized electrode can be exploit for environmental applications (Zeppilli et al., 2016; Zeppilli et al., 2015). The use of microbial electrolysis cells (MECs) for the bioremediation of CAHs contaminated groundwater did not require the insertion of chemicals and permits the stimulation of both reductive and oxidative bioelectrochemical reactions (Wang et al., 2020), indeed a biocathode can provide the reducing power for the RD reaction while an anode can be used for the oxygen supply to the aerobic dechlorinating biomass (Lai et al., 2017). Regarding the oxidative dechlorination, two main bioelectrochemical approaches have been proposed including the in-situ

oxygen electrolytic generation or the adoption of the polarized anode as direct terminal electron acceptors to promote the anaerobic oxidation of contaminants (Gregory et al., 2004). The first approach resulted the most efficient when a mixed metal oxides (MMO) electrode is used as anodic material for the oxygen evolution as already shown in the literature, i.e. the oxygen-scavenging properties of a graphite electrode negatively affect the oxygen availability with a consequent limitation of the dechlorination rate (Zeppilli et al., 2020). In the present study, an innovative membrane-less configuration of a bioelectrochemical reactor, equipped with a MMO anode and an internal graphite cathode, has been continuously operated with a synthetic groundwater contaminated by cis DCE. In the experimental study, both reductive and oxidative bioelectrochemical reactions have been characterized and described including competitive anions reduction.

2. Material and methods

2.1 Oxidative Bioelectrochemical Reactor

The oxidative reactor (Figure 1) consisted in a 3.14 L borosilicate glass cylinder which contains an internal graphite counter electrode (CE) of 0.38 L already described in previous work (Zeppilli et al., 2019). The external chamber of the oxidative reactor was filled with silica beds containing three slices of 10X5 mm of a mixed metal oxides (MMO) electrode (Magneto Special Anodes, The Netherlands) connected through a titanium wire which constituted the working electrode (WE) of the cell. A reference Ag/AgCl electrode (RE) was inserted in the external chamber to control the bioelectrochemical cell through a three-electrode configuration. The anode potential was controlled at +1.4 V vs SHE through a VSP 300 BioLogic potentiostat. The synthetic groundwater was fed by a peristaltic pump with an average flow rate of 1.97 L/d corresponding to an HRT of 1.8 days. The synthetic groundwater consisted in tap water in which 460 mg/L Na₂SO₄, and 20 mg/L NaNO₃ were added and contaminated with cisDCE 15 µM. During the open circuit period the application of the potential to the anode was interrupted.

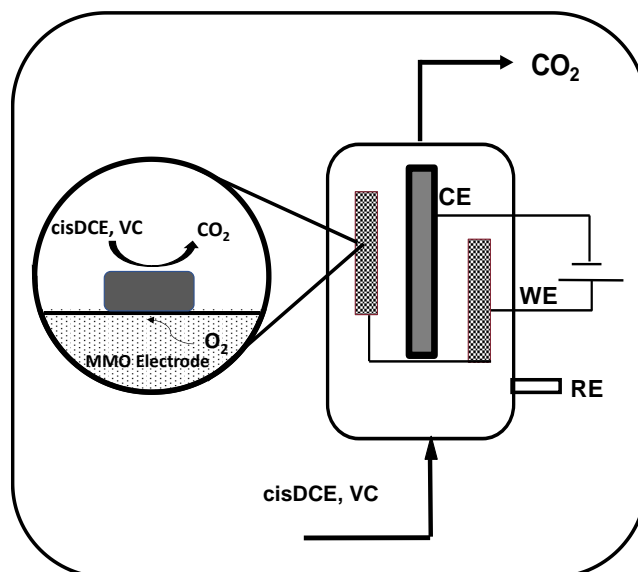


Figure 1 Schematic view of the bioelectrochemical oxidative reactor

2.2 Analytical methods

VC and cisDCE were determined by a DANI MASTER GasChromatograph (Zeppilli et al., 2019), sulphate and nitrate were determined using an ion chromatograph (Dionex ICS-1000 IC, Sunnyvale, California) equipped with a conductivity detector (Zeppilli et al., 2020).

2.3 Calculation

The cis DCE removal and the VC production rate has been evaluated by the following equations:

$$\begin{aligned} \text{cisDCE removal rate } (\mu\text{mol/Ld}) &= Q_{\text{liquid}}/V_{\text{oxidative}} * [\text{cisDCE}]_{\text{in}} - [\text{cisDCE}]_{\text{out}} \\ \text{VC production rate } (\mu\text{mol/Ld}) &= Q_{\text{liquid}}/V_{\text{oxidative}} * [\text{VC}]_{\text{out}} \end{aligned}$$

The cisDCE removal efficiency was calculated by the following equation:

$$\text{cisDCE removal efficiency (\%)} = ([\text{cisDCE}]_{\text{in}} - [\text{cisDCE}]_{\text{out}}) / [\text{cisDCE}]_{\text{in}} * 100$$

Sulphate and nitrate removal rate were evaluated by

$$\begin{aligned} \text{SO}_4^{2-} \text{ removal rate (mmol/Ld)} &= Q_{\text{liquid}}/V_{\text{oxidative}} * [\text{SO}_4^{2-}]_{\text{in}} - [\text{SO}_4^{2-}]_{\text{out}} \\ \text{NO}_3^- \text{ removal rate (mmol/Ld)} &= Q_{\text{liquid}}/V_{\text{oxidative}} * [\text{NO}_3^-]_{\text{in}} - [\text{NO}_3^-]_{\text{out}} \end{aligned}$$

The coulombic efficiencies of the reductive reactions, i.e., the percentage of current involved in the VC production from cisDCE (RD), the sulphate (RS) and the nitrate reduction (RN), were evaluated according to the following equations

$$\begin{aligned} \text{RD (\mu eq/d)} &= \text{VC production rate (\mu mol/Ld)} * 2 * V_{\text{oxidative}} \rightarrow \text{RD (mA)} = \text{RD (\mu eq/d)} * 96485/86400/1000 \rightarrow \text{CE}_{\text{RD}}(\%) = \text{RD (mA)} / \text{Current (mA)} * 100 \\ \text{RS (meq/d)} &= \text{SO}_4^{2-} \text{ removal rate (mmol/Ld)} * 8 * V_{\text{oxidative}} \rightarrow \text{RS (mA)} = \text{RS (meq/d)} * 96485/86400 \rightarrow \text{CE}_{\text{RS}}(\%) = \text{RS (mA)} / \text{Current (mA)} * 100 \\ \text{RN (meq/d)} &= \text{NO}_3^- \text{ removal rate (mmol/Ld)} * 5 * V_{\text{oxidative}} \rightarrow \text{RN (mA)} = \text{RN (meq/d)} * 96485/86400 \rightarrow \text{CE}_{\text{RN}}(\%) = \text{RN (mA)} / \text{Current (mA)} * 100 \end{aligned}$$

The coulombic efficiencies for the oxidative reactions, i.e. the cisDCE oxidation to CO₂, has been evaluated according to previous experiments in which the current flowing in the circuit is converted into molecular O₂ while the stoichiometric ratio for the complete oxidation of cisDCE is used to determine the O₂ necessary for the oxidation

$$\begin{aligned} \text{cisDCE removal rate (mmolO}_2\text{/d)} &= \text{cisDCE removal rate (\mu mol/Ld)} * V_{\text{oxidative}} * 2/1000 \\ \text{Current (mmolO}_2\text{/d)} &= \text{Current (mA)} / 4/96485 * 86400 \\ \text{CE}_{\text{OD}} &= \text{cisDCE removal rate (mmolO}_2\text{/d)} / \text{Current (mmolO}_2\text{/d)} * 100 \end{aligned}$$

3. Results and discussion

3.1 Reactor Operation under continuous flow conditions

As reported by previous work, the oxidative reactor microbial community analysis showed the presence of both anaerobic and aerobic dechlorinating microorganisms (Zeppilli et al 2020). The latter evidence was related to the previous reductive/oxidative configuration adopted by the bioelectrochemical process in which the oxidative reactor continuously received the reductive reactor effluent as feeding solution, including the anaerobic reductive biomass (Zeppilli et al 2019). After the reactor separation, the oxidative reactor was operated by feeding a synthetic groundwater solution contaminated with cisDCE, a medium chlorinated compound with an average concentration of $13.6 \pm 0.5 \mu\text{mol/L}$. During the entire oxidative reactor operation, the current profile and the cell voltage remained similar during both Reductive + Oxidative and Oxidative periods as reported in Figure 2. The average current that flowed in the cell resulted on average $8 \pm 2 \text{ mA}$ while an average cell voltage of $2.03 \pm 0.05 \text{ V}$ was necessary to operate the bioelectrochemical reactor.

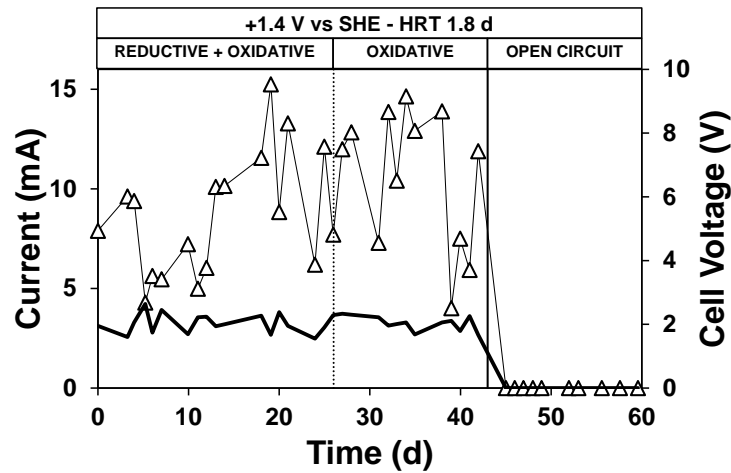


Figure 2: Current and Cell Voltage time course during the oxidative reactor operation

The daily energy consumption of the bioelectrochemical oxidative reactor, which in turn does not include the energy for pumping, resulted on average 0.2 kWh/m³ of treated solution. As reported in figure 3, both reductive and oxidative mechanisms have been identified by the production of 2.2 ± 0.9 $\mu\text{mol/Ld}$ of VC and the removal of 7.7 ± 1.9 $\mu\text{mol/Ld}$ of cis DCE. During this period, named Reductive + Oxidative period, an average cis DCE removal efficiency of 89 ± 7 % was obtained. Starting from day 27 the VC concentration drop below the detection limit, with a corresponding increase in cisDCE effluent concentration, which resulted on average 4.3 ± 0.5 $\mu\text{mol/L}$ giving a cisDCE removal efficiency of 68 ± 5 %. During this second period, also named oxidative period, the effluent VC decrease and the cis DCE removal efficiency decrease was probably caused by the inhibition of the anaerobic dechlorinating biomass present in the graphite counterelectrode, which was inhibited by the aerobic environment created by the anodic water oxidation. It is noteworthy to highlight that during the oxidative period, the cis DCE removal rate resulted 5.5 $\mu\text{mol/Ld}$, which corresponded to the difference between the cisDCE removal rate and the VC production rate of the previous condition, as reported in table 1.

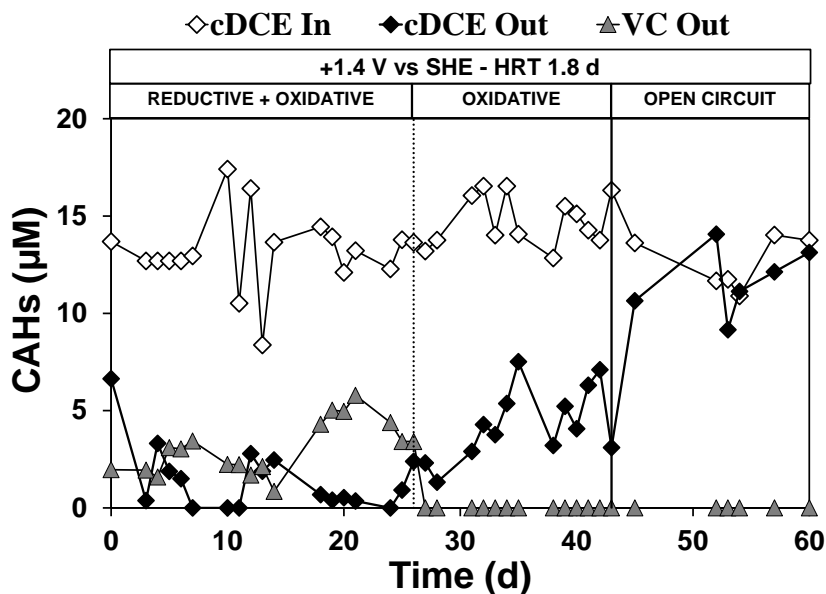


Figure 3: cis DCE and VC time course in the oxidative reactor during the operation of the oxidative reactor with the synthetic groundwater

In order to identify any other possible cisDCE removal pathway, an open circuit period, in which no potential was provided to the cell, was conducted confirming the effect of the applied potential on the oxidative dechlorination pathway.

Table 1: Oxidative reactor performances with the synthetic groundwater contaminated by cisDCE

Period	Reductive + Oxidative	Oxidative	Open Circuit
cisDCE removal rate ($\mu\text{mol/Ld}$)	7.7 ± 1.9	5.2 ± 1.4	0.6 ± 0.4
VC production rate ($\mu\text{mol/Ld}$)	2.2 ± 0.9	-	-
cisDCE removal efficiency (%)	89 ± 7	68 ± 5	7 ± 4

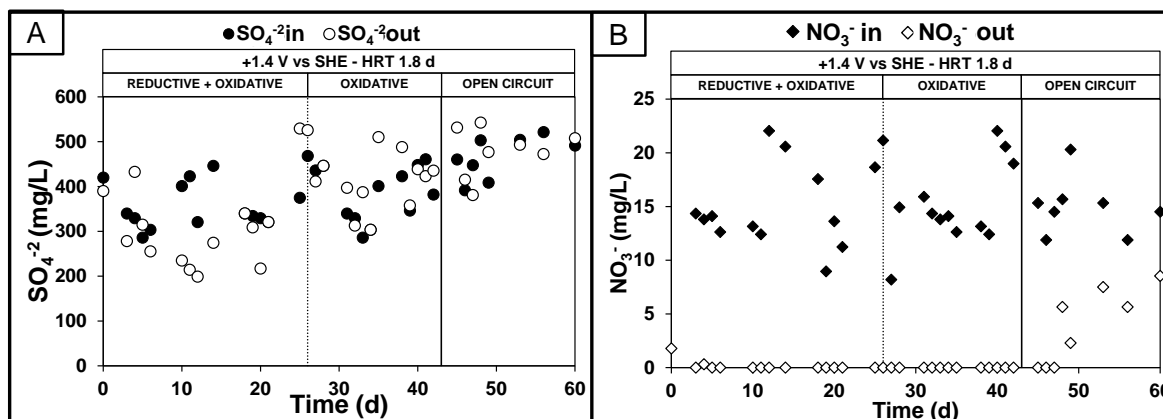


Figure 4: Sulphate (A) and Nitrate (B) time course during the oxidative reactor operation

Figure 4 shows the sulphate (Figure 4 - A) and nitrate (Figure 4 - B) time course during the operation of the oxidative reactor, while sulphate was slightly removed during the Reductive + Oxidative period with an average removal rate of 0.26 ± 0.07 mmol/Ld, nitrate was completely removed with an average removal rate of 0.15 ± 0.03 mmol/Ld during all over the operating periods in which the +1.4 V vs SHE potential was applied. Both nitrate and sulphate removal were probably driven by a reductive pathway performed in the graphite internal counter electrode. It is important to underline the correspondence of the sulphate reduction and VC production during the reductive+ oxidative period, i.e., some sulphate reducing microorganisms can perform both reductive dechlorination and sulphate reducing reactions.

3.2 Coulombic efficiencies of the reductive and oxidative reactions

The flowing current in the bioelectrochemical oxidative reactor, as in conventional electrochemical devices, represent the reaction rate of both reductive and oxidative processes. Because of the presence of both reductive (i.e. cisDCE reduction to VC, SO_4^{2-} and NO_3^- reduction), and oxidative (cisDCE oxidation to CO_2) bioelectrochemical reactions, the coulombic efficiency of reductive and oxidative processes have been evaluated throughout the oxidative reactor operation. As reported in table 2, during the Reductive + Oxidative period the current promoted the occurrence of the reductive dechlorination and the oxidative dechlorination with an efficiency of 0.06 ± 0.01 and 1.4 ± 0.2 %, respectively.

Table 2: Reductive and oxidative bioelectrochemical dechlorination reaction their relative coulombic efficiencies during the oxidative reactor operation

Period	Reductive + Oxidative	Oxidative	OPEN CIRCUIT
RD rate ($\mu\text{eq/Ld}$)	4.4 ± 1.8	-	-
OD rate ($\mu\text{mol/Ld}$)	7.7 ± 1.9	5.2 ± 1.4	-
CE RD (%)	0.06 ± 0.01	-	-
CE OD (%)	1.4 ± 0.2	0.9 ± 0.1	-

Regarding the SO_4^{2-} and NO_3^- reduction, as reported in table 3, their coulombic efficiencies corresponded to a small percentage below the 1 % during the Reductive + Oxidative period, while, during the oxidative period nitrate reduction consumed the 0.16 ± 0.06 % of the current.

Table 3: Anions reduction in the oxidative reactor counterelectrode and their coulombic efficiency during the oxidative reactor operation

Period	Reductive + Oxidative	Oxidative	OPEN CIRCUIT
SO_4^{2-} removal rate (mmol/Ld)	0.26 ± 0.07	-	-
NO_3^- removal rate (mmol/Ld)	0.15 ± 0.03	0.16 ± 0.06	-
CE RS (%)	29 ± 2	-	-
CE RN (%)	10 ± 3	10 ± 2	-

4. Conclusions

The oxidative bioelectrochemical process highlighted the possibility to stimulate the oxidative dechlorination of medium chlorinated compounds by the oxygen in-situ production. The experimental study showed that by the use of the membrane-less configuration with an internal graphite counter electrode, both reductive and oxidative bioelectrochemical reaction can be simultaneously stimulated. The effect of the potential application, and the consequent current generation, on the cisDCE dechlorination has been further confirmed by the adoption of an open circuit period in which no cisDCE removal has been observed accordingly to the potential application interruption.

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