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Chromate fate and effect in bioelectrochemical systems for remediation of chlorinated solvents

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Highlights

- Simultaneous reduction of trichloroethylene and Cr(VI) demonstrated in a bioelectrochemical reactor (BES)
- In the BES, Cr(VI) removal was due to both applied reducing potential and microbial activity
- Cr(VI) exerted partial inhibition on reductive dechlorination in unacclimated dechlorinating consortium
- Previous acclimation period increased both dechlorination performance and removal rate
- Co-contamination microbial consortia adaptation confirmed by a long-term, continuous-flow experiment

Abstract

A continuous-flow bioelectrochemical reactor was developed in a previous study to address the bioremediation of groundwater contaminated by trichloroethene (TCE). The present report investigated the applicability of the same system in the presence of Cr(VI) and its possible inhibitory effect on dehalorespiring bacterial populations. Preliminary batch tests were performed at the optimal cathodic reducing potential for the reductive dechlorination (RD) of TCE (-0.65 V vs. the standard hydrogen electrode) with two different dechlorinating microorganism consortia. The results demonstrated that Cr(VI) removal efficacy was increased by microorganisms that had been previously acclimatised to Cr(VI). Specifically, Cr(VI) was completely reduced only in the presence

of acclimated microorganisms. The presence of chromate negatively affected RD performance, by either (i) limiting the TCE transformation to cis-dichloroethene at lower concentrations, or (ii) completely inhibiting RD at higher concentrations. In contrast, after the acclimation period, RD was extended down to vinyl chloride, which is the main TCE daughter product. Finally, the continuous flow reactor was fed by synthetic groundwater contaminated with TCE (50 µM) and Cr(VI) (45 µM), and the experimental results showed that Cr(VI) was completely reduced under RD conditions. Moreover, TCE removal was complete, with vinyl chloride and ethene as the main intermediates, thus indicating that chromate inhibition was decreased by Cr(VI) removal.

List of abbreviations

CAHs: Chlorinated aliphatic hydrocarbons; Cr(VI): hexavalent chromium; Cr(III): trivalent chromium; RD: reductive dichlorination; TCE: trichloroethylene; cisDCE: cis dichloroethylene; VC: vinyl chloride; ETH: ethylene; ETA: ethane; BES: bioelectrochemical systems; PEM: proton exchange membrane; SHE: standard hydrogen electrode.

Keywords: Chlorinated solvents; bioremediation; Cr(VI) reduction, bioelectrochemical system

Introduction

Anthropogenic activities have caused severe contamination of groundwater, resulting in major environmental problems. Chlorinated aliphatic hydrocarbons (CAHs) such as trichloroethylene (TCE) are among some of the most widespread contaminants that are difficult to remediate. Over the last 30 years, the approaches applied to the remediation of groundwater contaminated with CAHs have shifted from traditional pump-and-treat systems to contain and treat the plumes [1] towards *in situ* source treatment technologies that are more economical and environmentally effective. Indeed, the aim of *in situ* bioremediation is to encourage indigenous degradative bacterial communities through the addition of electron donors (reduced compounds) or acceptors (oxidised compounds) in order to promote the degradation of contaminants by respiration or fermentation pathways.

Typically, highly chlorinated CAHs are degraded via anaerobic respiration by a hydrogenolytic reaction, namely, reductive dechlorination (RD) [2–5]. Organohalide-respiring bacteria use highly chlorinated CAHs as terminal electron acceptors in their energy metabolism, in which chlorine atoms are sequentially replaced by hydrogen atoms. As an example, the microbial RD of TCE proceeds via the formation of cis-dichloroethene (cis-DCE), vinyl chloride (VC), and finally, non-toxic ethene (ETH) and ethane (ETA). However, the RD of TCE occurs until less-chlorinated CAHs (cis-DCE or VC) result in stalled or incomplete degradation, which can be due to many factors, including an insufficient negative redox condition, a less energetic/favourable degradation pathway of less-chlorinated CAHs, or the absence of *Dehalococcoides* spp. The latter is the only known bacteria that can achieve complete dechlorination of chlorinated ethenes [6, 7]. Therefore, a co-metabolic or metabolic aerobic oxidative dechlorination pathway can also be necessary to complete the degradation of DCE and VC [8, 9].

Under natural conditions, the metabolic electron acceptors and donors, and/or nutrient-limited bioavailability, cause low rates of biodegradative reactions. Such limitations can be overcome by the continuous addition of chemical amendments below ground, e.g. oxygen (or oxygen-releasing chemicals), hydrogen (or hydrogen-releasing chemicals), fermentable organic substrates and/or nutrients.

In the last 15 years, bioelectrochemical systems (BESs) have made a remarkable emergence as novel technologies that are able to increase the effectiveness and sustainability of remediation [9]. A BES is an electrochemical system that enhances microbial activity through control of the electrochemical conditions, either directly by the interaction between the electrode and microorganisms, or indirectly by the action of electron mediator compounds [10]. The use of BESs in environmental remediation

can employ electrodes as non-exhaustible electron acceptors or donors to eliminate the external chemical amendments and associated costs. Accordingly, in previous work an RD bioelectrochemical stimulation was thoroughly investigated, whereby a flow-through sequential reactor was used to demonstrate that the dechlorination rate of TCE can be directly controlled by fine-tuning of the cathode potential [7, 11]. However, in a real environmental matrix, the implementation of bioremediation may be complicated by competition and/or inhibitory effects caused by the possible presence of a variety of contaminants [12]. In fact, a large number of sites are also contaminated by heavy metals (e.g. Cr(VI)) due to their extensive previous use for various industry-related technological processes, thus resulting in a significant environmental liability [13–16].

In water, Cr is present as two stable oxidation states, trivalent Cr(III) and hexavalent Cr(VI). Due to its low solubility, the former is less bioavailable for animals and humans; hence, the majority of *in situ* remediation methods are based on the formation of insoluble Cr(III) (e.g., as Cr_2O_3 or $Cr(OH)_3$) by shifting the oxidation state from hazardous Cr(VI) to the less toxic Cr(III) [17–20]. BESs have been satisfactorily applied to the reduction of a range of metal ions including Cr(VI), Au(III), Ag(I), Cu(II), V(V), Fe(III), and Hg(II), [13,21,22]. Many factors may affect the metal reduction in BESs, such as pH, applied potential and electrical resistance or medium conductivity.

The Cr(VI) cathodic reduction in a BES depends on different mechanisms [21]: (i) direct electrodic reduction, because the reaction from Cr(VI) to Cr(III) is spontaneous at 1.36 V vs. the standard hydrogen electrode (SHE) (i.e. standard redox potential (E0)), or (ii) microbial reduction, which is independent of the use of an external potential. In BES technology, the introduction of metal ions could cause several complications. For example, the deposition of reduced metals on a cathode surface could cause a passivation or increase in resistance due to the precipitation of metal hydroxides on the solid electrode, decreasing the effective electrodic area and creating over-voltage [23].

Subsequently, these metal-electrode surface interactions may affect the bioremediation technology of a BES. Furthermore, the dehalorespiring bacterial populations may potentially be affected by inorganic compounds or inter-/intra-species competition for growth substrates (including the contaminants themselves) [24, 25]. It is clear that the outcome of bioremediation in a real environmental matrix is difficult to predict in the presence of multiple contaminants. The previous study determined that it is possible to remediate TCE via a BES with good performance; hence, here the feasibility of this approach in the presence of Cr (VI) as a co-contaminant has been investigated.

Materials and methods

Bioelectrochemical reactor setup and operating conditions

Batch bioelectrochemical cell

A batch of bioelectrochemical tests were conducted in borosilicate glass H-cell reactors, as described elsewhere [7, 12]. The half-cells were separated by a 12 cm^2 treated Nafion 117 proton exchange membrane (PEM). The cathode and anode chambers were filled with 140 mL of anaerobic minimal mineral medium (NH_4Cl, 0.5; $MgCl_2.6H_2O$, 0.1; $CaCl_2.2H_2O$, 0.05; K_2HPO_4, 0.4 g L^{-1}) supplemented with a metal and vitamin solution [26, 27]. Anaerobic conditions were guaranteed by purging the gas and liquid phases with O_2-free gas, namely N_2:CO_2 (70:30 v/v%). A $NaHCO_3$ solution (10% w/v) was added to maintain the pH in the range 7–7.5. In each chamber, a graphite rod was used as a solid-state electrode, connected to a potentiostat by titanium wires (Sigma Aldrich, Italy). A KCl saturated Ag/AgCl reference electrode (+199 mV vs. the SHE) (Amel S.r.l., Milan, Italy) was also placed in the cathode chamber.

The chronoamperometry tests were conducted using a multichannel Ivium potentiostat (Ivium Technologies, Eindhoven, The Netherlands) at -0.65 V (vs. the SHE). The cells were maintained at

room temperature (22±3 °C). At regular intervals during the test, the pH, Cr(VI), CAHs, and headspace concentrations of O_2, H_2 and CO_2 were measured, as described below. The purpose of these tests was to evaluate i) the ability of the dechlorinating microorganisms to remove Cr(VI) as a function of the Cr(VI) concentration, and ii) microbial acclimation in the presence of CAHs only or CAHs and Cr(VI) (**Table 1**). The bacteria used in the batch experiments were cultivated for a long period of time (10 years) in a cathodic chamber of bioelectrochemical reactors at -0.65 V vs. the SHE using TCE as a sole carbon source in the absence (inoculum 'a': unacclimated microorganisms) or presence of Cr(VI) (inoculum 'b': acclimated microorganisms). Control tests were performed in the absence of potential (9, 10), microorganisms (11), and both (12).

Bench-scale continuous flow reactor

As previously reported [7], the continuous flow bioelectrochemical reactor was composed of a 0.8 L cathodic cylinder chamber and a 0.9 L anodic cylinder chamber, with Nafion® PEM separating the compartments. Conductive graphite granules with a graphite rod connector acted as the cathode and completely filled the cathodic chamber. The anode was a helicoidally rutile type electrode (Magneto Special Anode) enclosed in a bed of silica beads. All the electrical connections to the potentiostat (Amel Model 549, Milan, Italy) were ensured by titanium wiring. Finally, the reference electrode (Ag/AgCl +0.199 vs. the SHE, Amel, Milan, Italy) was inserted into the cathodic chamber. The tests were conducted at -0.65 V vs. the SHE (cathodic potential) and an ambient temperature (22 ± 3 °C).

To prove the stability of the bioelectrochemical reactor, the TCE-contaminated O_2-free mineral medium (with metals and vitamins traces) was added at a flow rate of 0.58 L d^{-1} with an alternate addition of Cr(VI). To avoid the partitioning of the TCE into the gas phase, the medium was maintained in a collapsible Tedlar® bag.

At the start of the experiment the reactor was inoculated with specialised dechlorinating microorganisms. Halorespiring microorganisms and an oxidative dechlorinating aerobic bacterial community were introduced into the cathodic and anodic chambers, respectively [7, 28, 29]. The continuous flow bioelectrochemical reactor was monitored daily by taking the gas- and liquid-phases from the sampling cells positioned at the inlet and outlet of the reactor and between the chambers.

Analytical methods

The headspace of the reactors was analysed by a DANI master gas chromatographer (GC) equipped with a thermal conductivity detector (TCD) (DANI Instruments, Contone, Switzerland) to determine the H_2, O_2, and CO_2 concentrations [7]. The concentration of CAHs was measured using a Dani GC 1000 (Contone, Switzerland) equipped with a flame ionisation detector (FID), while ethylene, ethane, methane, and VC concentrations were measured by a Varian 3400 (Palo Alto, CA) GC–FID [7, 10]. The aqueous-phase concentrations were calculated by converting the headspace concentrations using tabulated Henry's law constants assuming liquid–gas equilibrium conditions [30, 31]. The molar compound concentration was reported as the total amount with respect to the volume of the liquid phase, which represented the ''nominal'' concentration.

The addition of 1,5 diphenylcarbazide for spectrophotometric measurements was used to determine the Cr(VI) concentration according to the standard method [32] The total Cr concentration was determined by inductively coupled plasma mass spectrometry (ICP–MS; 820-MS, Bruker, Bremen, Germany) equipped with a collision-reaction interface and glass nebuliser (0.4 mL min^{-1}; MicroMistTM, Analytik Jena AG, Jena, Germany). A five-point matrix-matched external calibration was performed in the concentration range of 0.5–10 μg L^{-1}. Yttrium (Y) was used as internal standard for all measurements to control the nebuliser efficiency, as reported previously [33, 34]. Details of the instrumental conditions and the method performance are reported in [35].

Organic chlorine release

The cumulative release of organic chlorine (i.e. Cl bound to the ethylene skeleton) was introduced to assess the overall dechlorinating activity in each batch test. It was then estimated by the sum of the amount produced or by the amount of oxidised RD intermediates [34]. A high background Cl concentration (> 1 mM) in the mineral medium did not allow the direct measurement of the released ions.

Calculations

The average rates (Eqs. 1 and 2) and coulombic efficiency (Eq. 3) of RD and Cr(VI) reduction were calculated in the continuous-flow reactor, similar to that reported previously [7].

$$r_{RD}\left[\frac{\mu\ eq}{Ld}\right] = \frac{(2x[cisDCE]+4x[VC]+6x\ [ETH]+\ 8x[ETA])\ xQ}{V_C} \quad (1)$$

$$r_{Cr(VI)}\left[\frac{\mu\ eq}{Ld}\right] = \frac{3x[Cr(VI)]\ xQ}{V_C} \quad (2)$$

where [cisDCE], [VC], [ETH] and [ETA] are the average liquid-phase compound concentration (μM); 2, 4, 6, and 8 are the number of moles of electrons required for the formation of RD intermediates from 1 mole of TCE, while 3 is the equivalent moles required for the reduction of Cr(VI) to Cr(III); Q is the flow rate (L d^{-1}); VC is the empty volume of the cathode chamber (L) (i.e. the volume without the electrode).

$$\varepsilon_{RD}\ (\%) = \frac{\frac{r_i x V_C}{24x3600} xF}{I} x100 \quad (3)$$

where r_i is the average rate of formation of the ith RD compound or reduction of Cr(VI) (μM d^{-1}), F is Faraday's constant (96485 C mol eq^{-1}), and I is the electric current (μA) [10].

Chemicals

The analytical standard and feed solutions were prepared using analytical grade chemicals. Ethylene, methane, and CAHs were purchased from Sigma–Aldrich (Milan, Italy). The calibration curve for

the ICP–MS was performed by diluting a standard stock solution of Cr (1000 ± 3 mg L^{-1}; Exaxol Italia Chemical Manufactories S.r.l., Genoa, Italy) in HNO_3 1% v/v (assay > 67%; residue < 1 mg L^{-1}; Promochem, LGC Standards GmbH, Wesel, Germany). The concentration of Y in the samples introduced to the ICP–MS was 5 µg L^{-1} and was prepared fresh before each run from the standard stock solution (1000 ± 2 mg L^{-1}; Panreac Química, Barcelona, Spain).

Results and Discussion

TCE-dechlorination in batch tests

The bioelectrochemical reduction of TCE in the presence of the TCE-acclimated inoculum (inoculum 'a') was tested preliminarily in the absence of Cr(VI) by determining the TCE and RD daughter products over the duration of the experiment (**Figure 1**, batch 1). Figure 1 shows a typical profile of sequential RD, whereby TCE was mainly dechlorinated to VC and ETH after passing through an intermediate accumulation of DCE. The formation of VC and ETH indicate the presence and activity of *Dehalococcoides* spp. The results of the reduction of TCE in the absence of Cr(VI) can be taken as being representative of the optimal performance of the dechlorinating inoculum.

To investigate the effect of Cr(VI) on the dechlorinating metabolism of bacteria with respect to TCE, batch experiments were conducted in the presence of increasing Cr(VI) concentrations (Figure 1, batches 2–4). From the profiles of TCE and its daughter products, it is evident that Cr(VI) inhibited RD and that the inhibition effect was dependent on the Cr(VI) concentration. In particular, cis-DCE was the only TCE daughter product observed at a Cr(VI) concentration of ~25 µM, while it was absent at higher Cr(VI) concentrations. Moreover, the RD beyond cis-DCE was absent even at the lowest Cr(VI) concentration, suggesting a stronger inhibitory effect of Cr(VI) on *Dehalococcoides* spp activity. The results from the control experiments (Figure 1, batches 9 and 11 compared to batch

2) showed the absence of cis-DCE and any other daughter products, thus indicating that both applied a negative potential and that dechlorinating microorganisms were necessary to support any RD.

The set of RD batch tests at increasing Cr(VI) concentrations was repeated using Cr(VI) acclimated inoculum 'b', i.e. the dechlorinating consortium taken from the continuous-flow reactor after a period of acclimation to Cr(VI) (batch tests 5–8 and 10, **Figure 2**). Figure 2 shows that some inhibitory effects still occurred as the Cr(VI) concentration increased, although a comparison with Figure 1 shows that the inhibition was lower with inoculum 'b' than with the unacclimated inoculum 'a'. Indeed, with the acclimated inoculum 'b', cis-DCE was observed at all the tested Cr(VI) concentrations, and VC was also present to a significant extent, although only at the lowest Cr(VI) concentration.

To summarise RD performance as a function of the operating conditions, **Figure 3** presents the distribution of the RD products on day 14 after the start of the test. This confirms both the increasing inhibitory effect with increasing Cr(VI) concentration and the lower inhibition with the acclimated inoculum. The removal of TCE decreased as a function of the Cr(VI) concentration, and was > 80% in the absence of Cr(VI) in inoculum 'a'. As the concentration of Cr(VI) increased, only 44%, 37%, and 27% of TCE was removed in batches 2, 3, and 4, respectively. As reported, inoculum 'b' exhibited the best performance, with TCE almost completely removed in all cases.

Cr(VI) reduction in batch tests

The reduction of Cr(VI) was also determined in the batch tests (see Table 1 for the operating conditions). **Figure 4** shows that some reduction of Cr(VI) occurred over time, either in the presence of the unacclimated dechlorinating consortium (inoculum 'a', part a) or the Cr(VI) acclimated consortium (inoculum 'b', part b). However, it is evident that the reduction of Cr(VI) occurred at a

higher rate in the presence of the Cr(VI) acclimated dechlorinating consortium, which suggests that Cr(VI) removal was at least partially microbially catalysed.

From the relevant literature, the microbial reduction mechanisms for Cr(VI) are known to vary significantly among various organisms, both intracellularly and extracellularly. It has been reported that Cr(VI) might act as a terminal electron acceptor under anaerobic conditions and in the presence of an exogenous electron donor [35-37], thus explaining the improvement in the reduction rate with the possible selection of Cr-respiring bacteria in the Cr(VI) acclimated dechlorinating consortium. In fact, the Cr(III) formed by the reduction of Cr(IV) can be retained in soluble form or precipitated intracellularly or extracellularly as $Cr(OH)_3$ or a carbonate-based insoluble compound (depending on the microbial strains and carbon source types) [38]. Accordingly, also in the present case, the total Cr concentration decreased along with the Cr(VI), thereby indicating that the Cr(III) that was formed was subsequently either precipitated or adsorbed onto the electrode surface (or a combination thereof). These findings also suggest that the lower effect of RD inhibition by Cr(VI), which was observed with the acclimated inoculum, could be due to a faster removal of the inhibitory agent from the medium.

Cr(VI) reduction kinetics in batch tests

Based on the evidence of Cr(VI) removal from the medium, which also affected the RD performance, the reduction kinetics of Cr(VI) were investigated in short-term (8 h) batch tests as a function of the operating conditions (**Table 2**).

In the presence of an applied potential, the removal kinetics of Cr(VI) were well represented by a first-order equation as:

$$[\mathrm{Cr(VI)}] = [\mathrm{Cr(VI)_0}]\mathrm{e}^{-\mathrm{kt}} \quad (4)$$

which can be made linear by taking the natural logarithm of both sides as:

$$\ln \frac{[Cr(VI)]0}{[Cr(VI)]} = kt \quad (5)$$

Hence, the rate constant (k) values and their corresponding linear regression correlation coefficients (R^2) were calculated by the linear regression between ln([Cr(VI)0]/[Cr(VI)]) and the working time (**Figure 5**), as reported in Table 2.

As mentioned, the Cr(VI) reduction rate was found to follow first-order kinetics with high correlation coefficients ($R^2 > 0.9$) in the presence of an applied cathode potential. Among the three conditions where the negative potential was applied, the slowest was in batch test B where no microorganisms were present, which confirmed that the Cr removal rate increased in the presence of microbial activity. It is also noteworthy that the highest removal rate was observed in batch test D where TCE was absent, exceeding that in batch test A where both Cr(VI) and TCE were present. This suggests that RD and Cr(VI) removal mechanisms were in competition for available electron donors, i.e. the electrons directly released at the cathode (direct electron transfer) and/or molecular hydrogen, which could have formed as part of the reduction reaction (indirect electron transfer).

The direct electron transfer from electrodes to microorganisms could be limited by the low surface of the electrode (graphite rod) and related attached biofilm. Moreover, the indirect electron transfer required the formation of H_2 as an electron shuttle, whereby the associated formation rate could have been the limiting factor [10–12; 39]. As mentioned, during each batch test, the total Cr concentration also decreased steadily, as determined by the correspondence of the Cr(VI) determined by the diphenylcarbazide method and the total soluble Cr measured by ICP–MS analysis (data not shown). In summary, under these test conditions, Cr removal appeared to be due to two main mechanisms: (i) the electrochemical reduction of Cr(VI) and the subsequent precipitation of Cr(III) as an oxide or hydroxide (due to the reducing conditions generated by the potential applied at the cathode surface), and (ii) microbial activity, which may have been due either to the microbial reduction of Cr(VI), bio-

precipitation (intracellular or extracellular), biosorption onto the biomass, or a combination of these factors.

It can be assumed that acclimation on the electrode in the biocathode positively affected the removal rate. It has been reported that it is possible to improve Cr (VI) reduction ability by more than an order of magnitude [40]. However, in the BES approach, the kinetic constant increased 10-fold with respect to the traditional approach with the addition of organic carbon, thus indicating the electrical stimulation of the microbial community [41, 42].

In the absence of an applied potential (batches C and E), Cr(VI) was removed at a lower rate and followed near zero-order kinetics (Table 2; Figure 5b) that were similar to conventional biological Cr(VI) reduction [43] . Indeed, in abiotic conditions, the reduction of Cr (VI) to Cr (III) is spontaneous only at very low pH, while in this study, the solution pH was approximately 7. Moreover, the slight decrease in Cr(VI) in the control reactors under open-circuit conditions might be attributable in part to adsorption by graphite. In this case, the short experimental period (8 h) could also obscure a further effect of biofilm creation on the electrode surface.

Continuous Flow bench-scale reactor tests

Long-term experiments were performed using the bench-scale continuous-flow bioelectrochemical reactor, which was maintained under the same operating conditions (cathode potential of -0.65 V vs. the SHE; influent TCE of 31–54 µM; TCE organic load rate of 23–40 µM d^{-1}). The experiments involved either feeding Cr(VI) with the influent (runs II and IV, at a Cr(VI) concentration of ~40 µM), or not (runs I and III). **Figure 6** shows the time profile of TCE and its daughter products in the effluent from the cathodic compartment.

Average steady state performances (**Table 3**) were calculated for a period of at least 20 days (15 residence times) in each run. In all runs, TCE was almost completely removed, and VC and ethylene were detected in the cathode effluent as the main RD products. As reported in Figure 6, the main TCE cathodic dechlorination product was VC, which represented 84%, 78%, 86%, and 87% of the total compounds in the cathodic effluent (**Figure 7**) for runs I, II, III, and IV, respectively. The second most abundant RD product (ETH) was similarly produced and accounted for 14%, 13%, 12%, and 11% of the total compounds in runs I, II, III, and IV, respectively. In contrast, TCE and cis-DCE were only present in the effluent of run II as a consequence of the introduction of Cr(VI). Indeed, the removal of TCE decreased from 100% to 95% with a negative effect on the overall RD rate, which fell by 31%. Moreover, the presence of cis-DCE corresponded to a lower VC concentration in the effluent (comparison of runs I and II in Figure 6). These findings suggest a Cr(VI) inhibitory effect on dechlorinating microorganisms, especially *Dehalococcoides* spp, which is in agreement with the batch tests. However, this was less evident in the continuous flow reactor because of its plug-flow behaviour [11] and high Cr(VI) reduction kinetics, which exposed the microbial consortium to decreasing Cr(VI) concentrations along the hydraulic flow of the reactor.

In run III, which returned to the previous conditions in the absence of co-contamination, the same performance of run I was reached again, thus demonstrating that the inhibition was reversible. Finally, in run IV, the Cr(VI) co-contamination was reintroduced and the process maintained a high performance with neither TCE nor cis-DCE present in the effluent. This confirmed the progressive acclimation of the dechlorinating consortium to the presence of Cr(VI), which agrees with the batch tests (Table 3).

In all conditions, the Cr(VI) concentration in the cathodic effluent was below the limit of detection . As no Cr(III) was observed, it was confirmed that Cr(VI) was first reduced, and then Cr(III) was

precipitated and/or adsorbed onto the graphite surface and/or biofilm. The cathode current decreased from 3.85 ± 0.00 to 2.94 ± 0.12 mA from run I to run IV, while the cell voltage maintained the same value (-2.16 ± 0.06 and -2.14 ± 0.07 V), suggesting that the electrical resistance increased due to the precipitation/adsorption of Cr(III) on the electrode. With regard to the performance of the anodic compartment, almost all the residual chlorinated daughter products were eliminated due to oxidation. Indeed, previous research [7] has demonstrated the ability of anodic microorganisms to oxidise chlorinated solvents if molecular oxygen is formed at the anodic surface.

As a summary, **Figure 8** presents the overall molar concentration of organic Cl (i.e. any Cl linked to the ethylene skeleton) along the reactor flow. In run I, only 2.5% of the initial organic Cl in the reactor feed was present in the anodic effluent, due both to the transformation of TCE into VC (and ethylene/ETA) in the cathodic compartment and subsequently the 91% oxidation of VC in the anodic compartment. As Cr(VI) was introduced in run II, the presence of residual TCE and cis-DCE in the cathodic compartment also negatively influenced the anodic oxidation. Indeed, it is well known that TCE and cis-DCE are less prone to oxidation than VC, whereby this condition has been found to lead to ~7% of the initial organic Cl remaining in the anodic effluent [8, 44, 45]. Surprisingly, the performance of the anodic compartment in run III remained lower than that in run I, whereas VC was almost completely removed in run IV. Consequently, less than 1% of the initial organic Cl in the feed was present in the reactor effluent. The co-metabolic degradation of cis-DCE and VC (using ETH as a growth substrate) has been previously demonstrated using this reactor [7].

Conclusion

The applicability of bioremediation by a BES was investigated with reference to the simultaneous reduction of two contaminants of different natures: TCE and Cr (VI), with particular attention on the possible mutual influence of the reduction reactions. The dechlorinating microbial community was found to be extremely adaptable to the operating conditions. The acclimation of a dechlorinating

consortium by a relatively short exposure to a moderate concentration of Cr(VI) (~50μM for 20 days) resulted in an increased performance for both RD and Cr(VI) reduction. In fact, the unacclimated microorganisms (inoculum 'a') showed a strong inhibition of RD at all tested Cr(VI) concentrations, which was strongly reduced with the acclimated inoculum (inoculum 'b'). The positive effect of inoculum acclimation on RD in the presence of Cr(VI) was likely due to the parallel increase of the Cr removal rate by the acclimated inoculum, which also indicated that the removal of Cr(VI) was due to the combination of the applied reducing potential and microbial activity. The long-term continuous-flow tests confirmed the hypothesis that microbial consortia can effectively adapt to co-contamination scenarios. Elsewhere [11] it was shown that the cathode chamber had a plug flow with dispersion behaviour; thus, TCE and Cr(VI) concentrations varied along the flow direction and resulted in different ecological niches where the microbial biofilm was exposed at different inhibitor concentrations.

In general, metal ions can affect the performance of BESs as the associated conductivity, redox, deposition and biotoxicity may act as an electrode passivator. In turn, BESs can also affect the redox state of metal ions in the system. A possible scaling-up of the process must consider the precipitation of metals on the electrode surface. The exact fate of metal ions in BESs still remains unclear and requires further investigation.

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Figure Captions

Figure 1: TCE and its daughter products' concentrations, as function of time in batch experiment with unacclimated inoculum (batches 1-4, 9 and 11, see Table 1 for operating conditions).

Figure 2: TCE and its daughter products' concentrations, as function of time in batch experiment with Cr(VI) acclimated inoculum (batches 5-8 and 10, see Table 1 for operating conditions).

Figure 3: Distribution of TCE and daughter products at the end of batch experiments (day 14) (see Table1 for operating conditions).

Figure 4: Concentration of $Cr(VI)/Cr(VI)_0$ in batch experiment, as function of time with (a) no acclimated and (b) acclimated inoculum tests.

Figure 5: Concentration ratio of Cr(VI) and linear regression of batch kinetics in (a) presence or (b) absence of cathodic potential.

Figure 6: Profiles of TCE and its daughter products in the continuous-flow experiments of the different experimental conditions. The vertical lines indicate the change of TCE and Cr(VI) concentration in the feeding solution.

Figure 7: TCE and daughter products' distributions in the cathodic effluent under the different operating conditions explored in the continuous flow experiment.

Figure 8: Total concentration of organic chlorine in different stages of continuous-flow reactor, i.e. reactor influent (influent); cathodic effluent; anodic effluent.

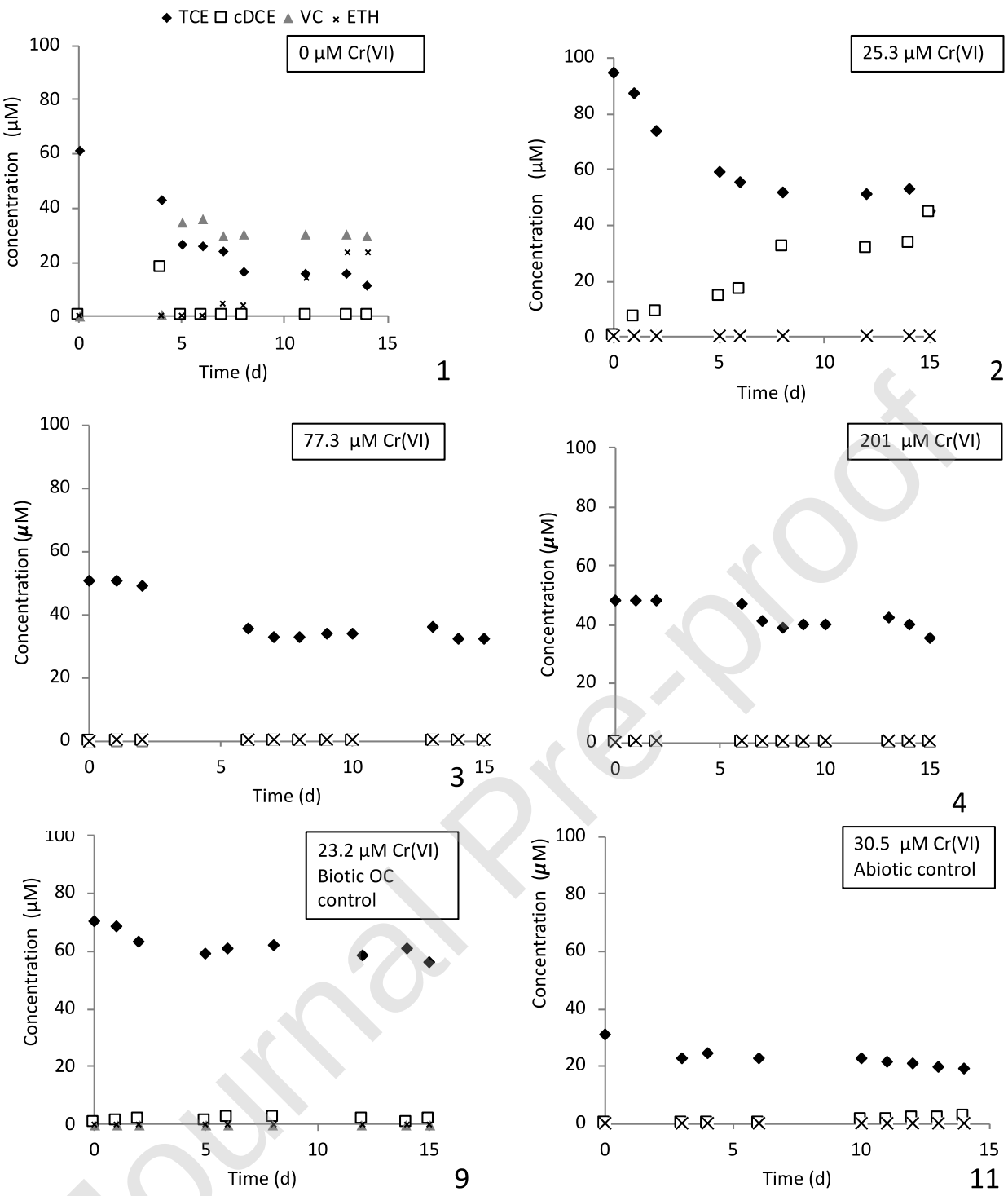


Figure 1

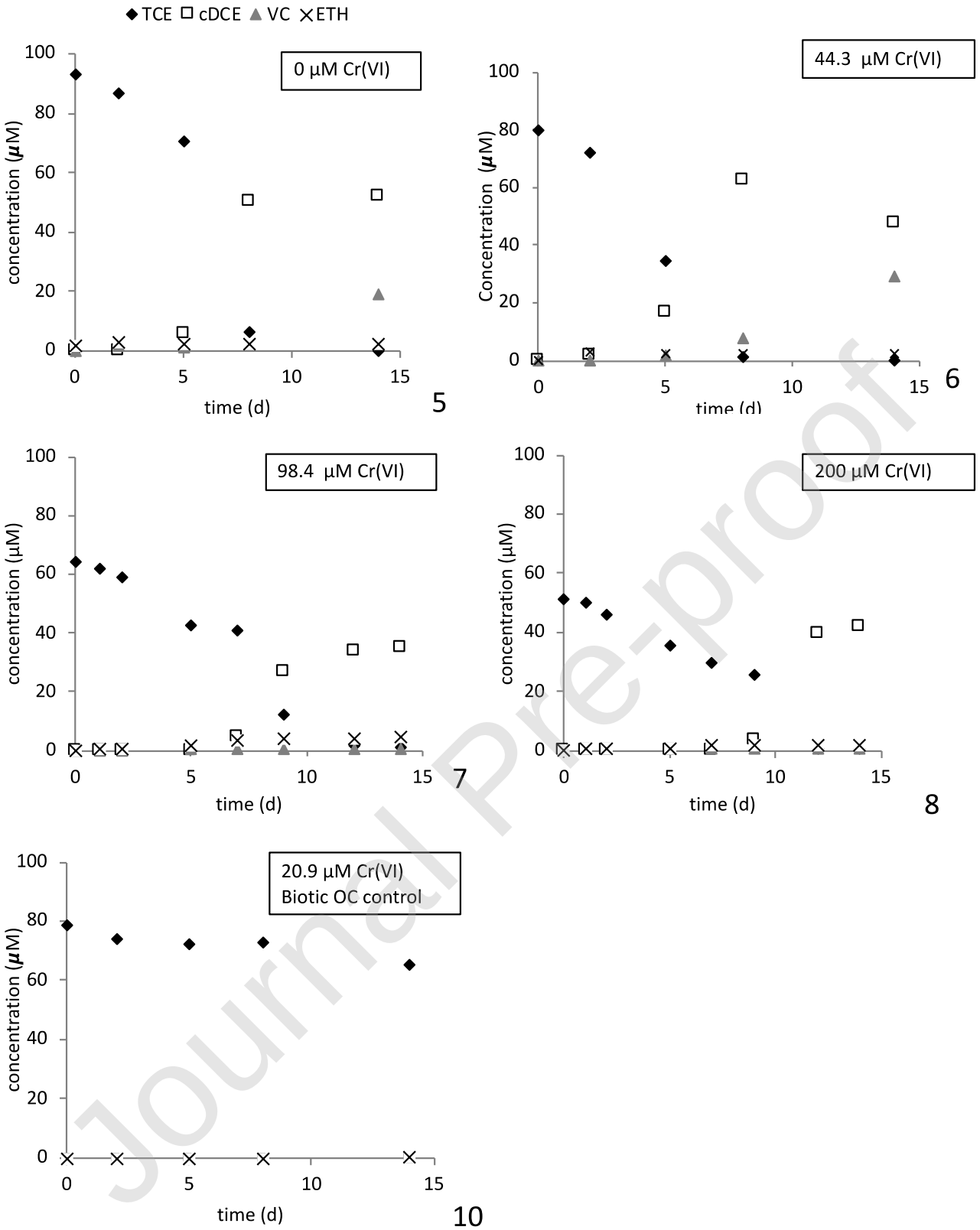

TCE
cDCE
VC
ETH
0 µM Cr(VI)
44.3 µM Cr(VI)
98.4 µM Cr(VI)
200 µM Cr(VI)
20.9 µM Cr(VI)
Biotic OC control
concentration (µM)
Concentration (µM)
time (d)
5
6
7
8
10

Figure 2

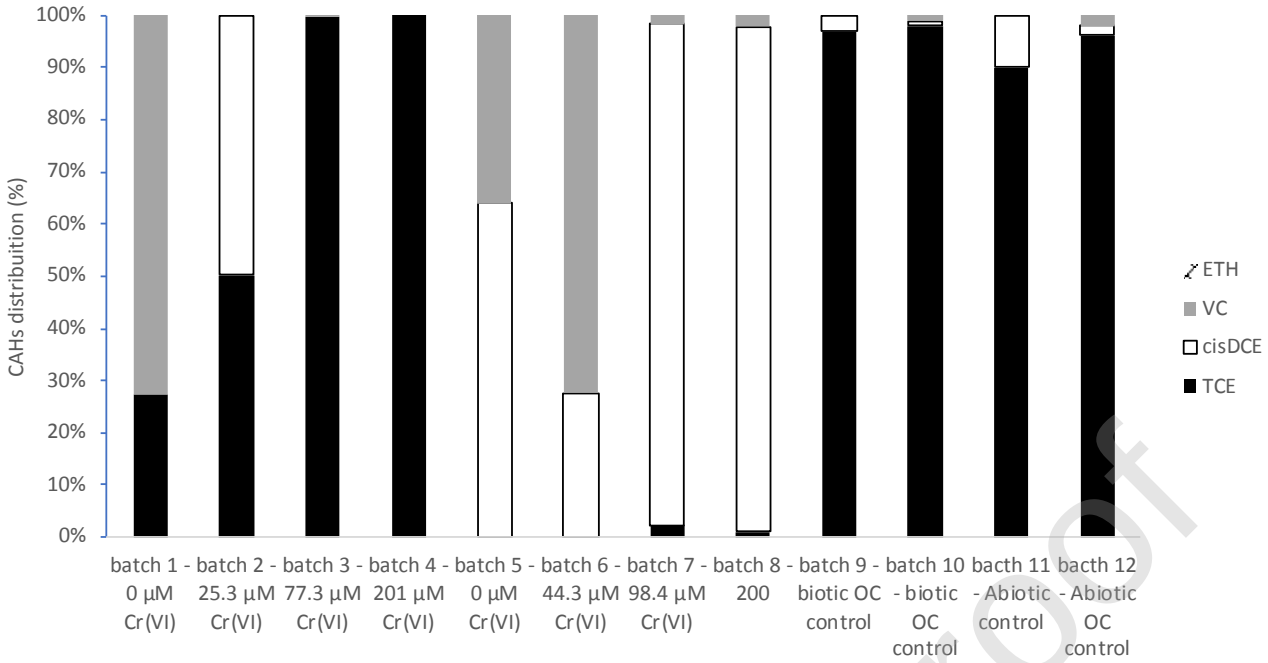


Figure 3

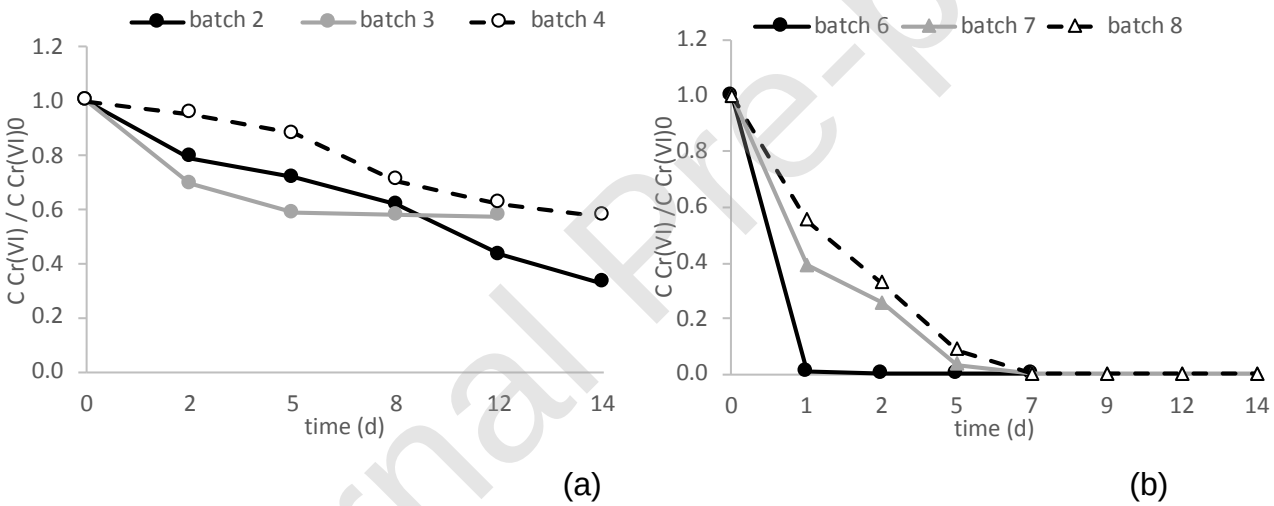
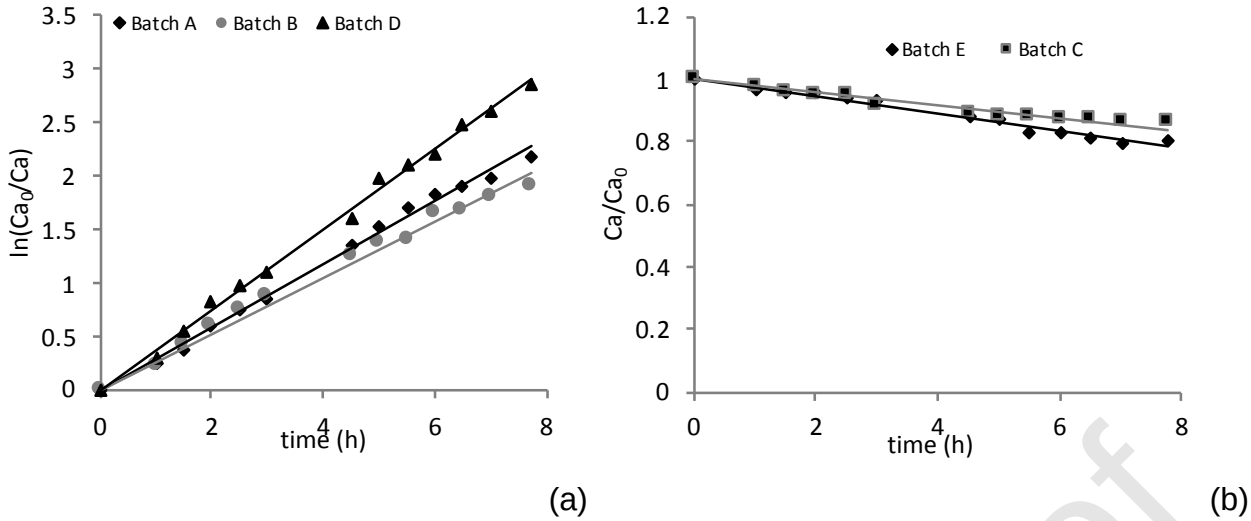


Figure 4



(a) (b)

Figure 5

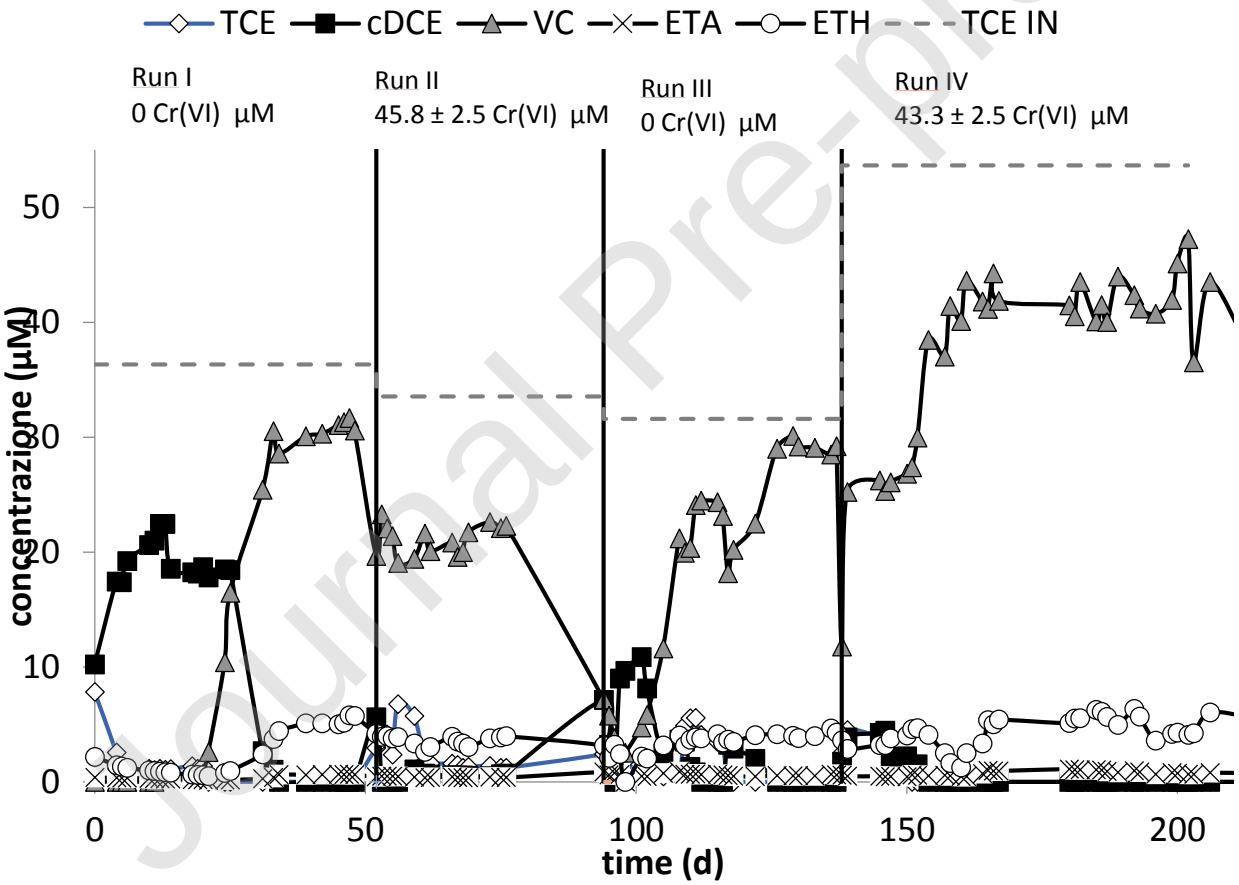


Figure 6

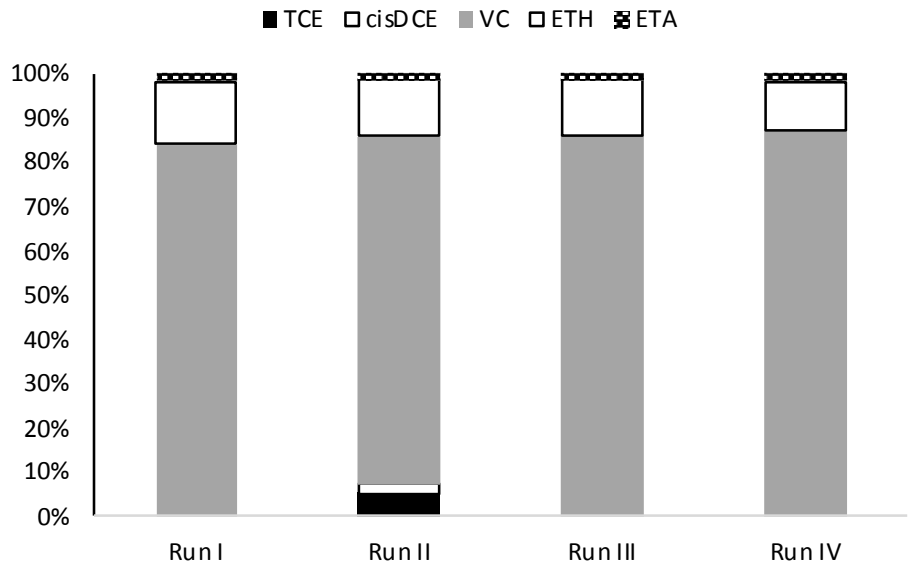


Figure 7

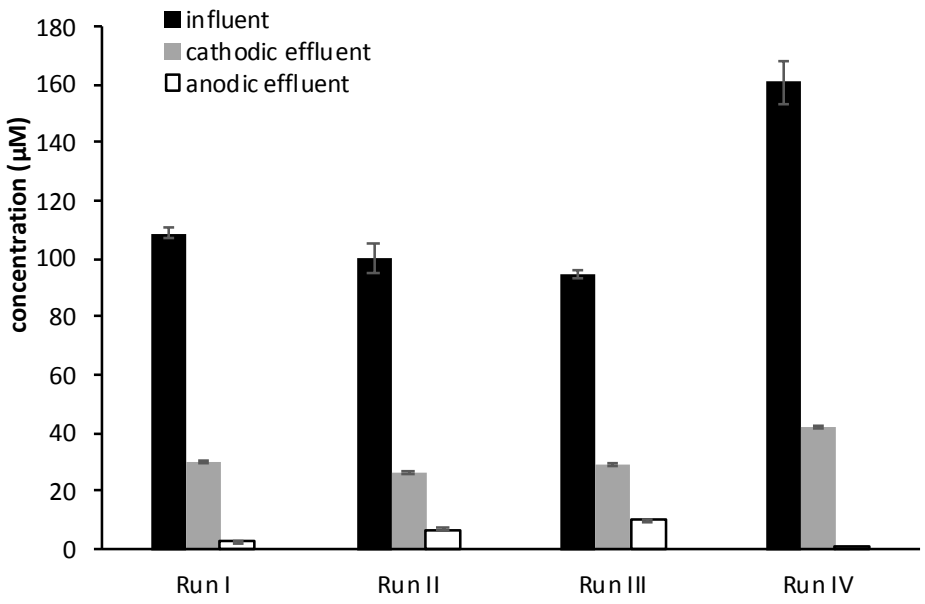


Figure 8

	TCE (µM)	**Cr (VI)** (µM)	**Potential**	**Inoculum**
batch 1	61.0	-	+	a
batch 2	94.5	25.3	+	a
batch 3	50.5	77.3	+	a
batch 4	48.0	201	+	a
batch 5	93.0	-	+	b
batch 6	79.4	44.3	+	b
batch 7	64.1	98.4	+	b
batch 8	51.0	200	+	b
Batch 9	70.3	23.2	-	a
batch 10	78.8	30.4	-	b
batch 11	21.9	30.5	+	-
batch 12	41.0	20.9	-	-

Table 1: Batch Experimental conditions. a: bioelectrochemical active dechlorinating; b: bioelectrochemical active dechlorinating acclimated on Cr(VI) microorganisms.

	TCE (µM)	**Cr (VI)** (µM)	**Potential**	**Microrganisms**	**Kinetics order**	**k** (h^{-1})	**R^2**	**Cr(VI) removed** %
batch A	81.7	19.4	+	+	I	0.293	0.99	89
batch B	74.9	19.0	+	-	I	0.262	0.99	85
batch C	68.0	18.6	-	+	0	0.020	0.91	14
batch D	0	18.1	+	+	I	0.375	0.99	94
batch E	72.3	19.2	-	-	0	0.027	0.98	20

Table 2: Experimental conditions used in kinetics tests.

	TCE inlet	Cr (VI) inlet	TCE removed (%)	Cr(VI) removed (%)	RD rate (μeq $L^{-1}d^{-1}$)	Cr(VI) reduction rate (μeq $L^{-1}d^{-1}$)	Coulombic Efficency RD (%)	Coulombic Efficency Cr(VI) (%)
Run I (52 d)	36.3 ±0.7	-	100	-	111 ±2	-	2.67 ±0.12	-
Run II (42 d)	33.5 ± 1.7	45.8 ± 2.5	96	100	77.0 ±1.5	95.7 ±5.0	1.86 ±0.05	2.00 ±0.32
Run III (44 d)	31.6 ± 0.5	-	100	-	102 ±0.6	-	3.27 ±0.19	-
Run IV (64 d)	53.7 ± 2.5	43.3 ±2.5	100	100	146 ±1	91.1 ±5.2	4.66 ±0.2	2.72 ±0.19

Table 3: Experimental conditions in continuous flow bench-scale reactor tests.