Sustainable remediation: electrochemically assisted microbial dechlorination of tetrachloroethene-contaminated groundwater

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Summary

Microbial electric systems (MESs) hold significant promise for the sustainable remediation of chlorinated solvents such as tetrachloroethene (perchloroethylene, PCE). Although the bio-electrochemical potential of some specific bacterial species such as Dehalococcoides and Geobacteraceae have been exploited, this ability in other undefined microorganisms has not been extensively assessed. Hence, the focus of this study was to investigate indigenous and potentially bio-electrochemically active microorganisms in PCE-contaminated groundwater. Lab-scale MESs were fed with acetate and carbon electrode/PCE as electron donors and acceptors, respectively, under biostimulation (BS) and BS-bioaugmentation (BS-BA) regimes. Molecular analysis of the indigenous groundwater community identified mainly Spirochaetes, Firmicutes, Bacteroidetes, and γ and δ-Proteobacteria. Environmental scanning electron photomicrographs of the anode surfaces showed extensive indigenous microbial colonization under both regimes. This colonization and BS resulted in 100% dechlorination in both treatments with complete dechlorination occurring 4 weeks earlier in BS-BA samples and up to 11.5 μA of current being generated. The indigenous non-Dehalococcoides community was found to contribute significantly to electron transfer with ~61% of the current generated due to their activities. This study therefore shows the potential of the indigenous non-Dehalococcoides bacterial community in bio-electrochemically reducing PCE that could prove to be a cost-effective and sustainable bioremediation practice.

Introduction

Chlorinated ethenes such as tetrachloroethene (perchloroethylene, PCE), trichloroethene (TCE) and dichloroethene (DCE) are among the most frequently detected groundwater pollutants (Moran et al., 2007), posing a serious threat to the environment and human well-being because of their carcinogenic properties (ATSDR, 2007). The current in situ and onsite bio reactor-engineered approaches for the bioremediation of chlorinated contaminants typically involve the addition of molecular hydrogen (H2) or H2-releasing organic substrates to stimulate the metabolism of reductive dechlorinating microorganisms. This stimulation facilitates the reduction of PCE to environmentally benign ethene. The problems often associated with this approach include the extensive competition for carbon and H2 between dechlorinators and non-dechlorinating sulphate reducers, methanogens and homoacetogens, and accumulation of large amounts of fermentation products in the subsurface. These problems can result in deterioration of groundwater quality, possible aquifer clogging because of excessive biomass growth and even explosion hazards through excessive methane production (Aulenta et al., 2009a).

A ground-breaking alternative to this approach is the use of insoluble electrodes to directly and selectively deliver electrons instead of chemicals via microbial electric system (MES) to dechlorinating communities growing as biofilms at the electrode surfaces (Lohner and Tiehm, 2009; Lovley, 2011). A wide diversity of microorganisms is able to convert the chemical energy stored in the chemical bonds of organic compounds to electrical energy through the catalytic reactions under anaerobic conditions (Lovley, 2012). The most important step in MES is the transfer of electrons from bacteria to the electrode (Rabaey et al., 2004). During this process, some microorganisms require soluble redox mediators such as methylene blue, viologens, thionines, ferrocyanides and quinoid compounds that serve as an electron shuttle between the cells and the electrodes to stimulate the bio-electrochemical conversion process. As an example, Aulenta and
colleagues (2007) reported the cessation of TCE dechlorination in the absence of the low-potential redox mediator, methyl viologen. However, TCE degradation resumed when methyl viologen was added. The proposed MES process carries several advantages resulting from the use of electrodes to stimulate biological reduction in the subsurface. Among them are continuous monitoring and direct delivery of electrons to dechlorinating microorganisms in terms of current and potential. In addition, no chemicals are required to be injected, which eliminates the need for transport, storage, dosing and post-treatment (Aulenta et al., 2009b).

Reductive dechlorination or dehalogenation (i.e. the substitution of chlorine by a hydrogen atom) is the main pathway used by dechlorinating microorganisms for the stepwise reduction of PCE to TCE, cis-DCE (cDCE) and vinyl chloride (VC) before forming the environmentally safe end-product ethene (Futagami et al., 2008). A clear understanding of how microbial ecology within MES brings about reductive dechlorination is important for its wider application (Rabaey et al., 2004). Pronounced enrichment of microorganisms from Geobacteraceae, Desulfuromonas, Desulfitobacteria and Dehalococcoides (Dhc) groups in mixed consortia have been extensively observed to electrochemically interact with electrodes. This interaction that involves directly donating or accepting electrons from electrode surfaces is exploited in MES to assist in the reductive dechlorination of chlorinated compounds with energy production (Bond et al., 2002; Bond and Lovley, 2003; Aulenta et al., 2007; 2008; 2009a,b; Strycharz et al., 2008). However, a wide diversity of other, as-yet-undefined microorganisms may function in a similar manner. The ability of other dechlorinating populations compared with the mentioned ‘classical’ bacterial groups associated with bi-electrochemical reductive dechlorination has to date been poorly investigated (Lovley, 2012). Hence, we developed a system where MES were fed with PCE-contaminated groundwater consisting of a biostimulated natural microbial population [biostimulation (BS) treatment] and a stimulated population augmented with a dechlorinating consortia Dhc strains BAV1, GT and FL2 [BS-bioaugmentation (BS-BA) treatment]. These treatments were compared with control MES with no inoculum or nutrient stimulation. We postulated that it is important to understand the multispecies interactions among the dechlorinating community in order to successfully assess the potential for stimulating the process of decontamination of groundwater. If stimulation of indigenous microbial community can lead to bio-electrochemical PCE transformation, then it could serve as a cost-effective in situ remediation practice as it would restrict the need for BA of contaminated subsurfaces. Furthermore, given the recent move in some countries to discourage the use of bio-augmenting agents (Ball, 2013), this approach may reduce the risk of damaging or causing mutation in the natural biome.

The purpose of this study was therefore to identify and evaluate the ability of an indigenous non-Dhc dechlorinating community present in PCE-contaminated groundwater that could evolve in MES to accomplish reductive dechlorination along with bioenergy production. In addition, an assessment of the contribution of this indigenous non-Dhc dechlorinating population in comparison with classical dechlorinating microorganisms such as Dhc was performed. These investigations were carried out using electrochemical analysis and culture-independent polymerase chain reaction (PCR)–denaturing gradient gel electrophoresis (DGGE)-based molecular techniques.

Results and discussion

**MES-assisted reductive dechlorination of PCE**

In this study, we employed a bio-electrochemical system (Fig. 1) to study the microbial reductive dechlorination of PCE under BS and BA regimes. Figure 2 illustrates the cumulative formation of PCE-dechlorinating intermediate products and simultaneous current flow during both BS and BS-BA treatments, when MES were fed with acetate as an electron donor and PCE/electrodes as acceptors. During BS treatment, PCE was completely reduced to ethene over a period of 16 weeks (Fig. 2A). PCE was consumed by week 4, with the subsequent production of TCE. As the TCE concentration was reduced to 15 μmol l⁻¹, cDCE was detected by week 6. Daughter products, cDCE and VC co-existed until ethene was formed. In week 16, only after VC was respired did ethene concentration reach its peak. Current production was negligible for first 3 weeks, but as dechlorination progressed, current production increased from week 4 and stabilized between 6.27 and 6.98 μA over the period of 16 weeks of complete dechlorination (Fig. 2A). In contrast, PCE dechlorination did not progress beyond TCE, and current generation was also negligible in the control 1 MES without acetate stimulation (Table 1). These findings showed that BS was beneficial to dechlorination and that the indigenous microbial community (BS) were most likely involved in complete reductive dechlorination given that no Dhc were detected in the groundwater samples used for this study. Reductive dechlorination was also accompanied by simultaneous bioenergy production (Fig. 2A).

In BS-BA-treated MES, dechlorination was faster, and the current production was ∼1.6-fold higher than MES run on BS-only treatment (Fig. 2B). PCE dechlorination started immediately, as indicated by the rapid accumulation of TCE by week 2. During BS-BA treatment, PCE was transformed into ethene over 12 weeks (Fig. 2B). Current generation started from week 2 and was stabilized.
between 11.08 and 11.45 μA by the end of dechlorination in week 12 (Fig. 2B). The Dhc and non-Dhc communities altogether led to complete PCE dechlorination, where ~61% of energy production (from Eqn 1 in experimental procedures) was observed to be contributed by non-Dhc activities. This indicated the significant role played by non-Dhc community in association with the Dhc consortia to optimize dechlorination and current output. In contrast, over the experimental period, PCE dechlorination stopped at TCE, and negligible current was observed in control 2 MES, when the Dhc microbial culture and acetate were omitted from the poised electrode system (Table 1). Wei and colleagues (2012) have also reported K₃Fe(CN)₆ as an excellent cathodic electron acceptor for two-chambered MES to obtain high power output. While there are some reports on the benefits of BS or BA, or the combined treatment on PCE dechlorination (Aulenta et al., 2007; 2008; 2009a,b), most of these studies have been on samples with Dhc, with scant attention being paid to the role of indigenous non-Dhc species. The potential roles of these non-Dhc species have therefore been comparatively poorly investigated to date. In addition, most studies have been focused on either BS or...
BA, or BS-BA alone in different systems. Hence, one highlight of this research is that both BS and BS-BA treatments were set up with the same sample under similar experimental conditions allowing for objective comparison of these strategies (unlike in most studies).

Community analysis

Microbial community fingerprints were obtained from DGGE analysis during the MES-assisted reductive dechlorination of PCE from BS-only, BS-BA and control treatments (Fig. 3). No bands were obtained from the control MES (Fig. 3) presumably because of either PCR detection constraints relating to the small volume used for DNA extraction or the absence of nutrient stimulation. Similarly, no amplicons were obtained from archaea-specific PCR indicating the absence or below-detection level of a methanogenic archaea community in the groundwater. No Dhc species were detected in PCR-based assays carried out on groundwater samples. To identify the likely electrochemically active bacterial species carrying out reductive dechlorination in BS and BS-BA MES, the dominant bands (operational taxonomic units) based on strong band intensity were excised from the DGGE gel and sequenced. Sequencing results indicated significant phylogenetic diversity in the species identified. Microbial community belonging to facultative anaerobic bacteria including the taxa *Spirochaetes*, *Firmicutes*, *γ-Proteobacteria*, *δ-Proteobacteria* and *Bacteroidetes* were detected in both BS-only- and BS-BA-treated MES, while *Chloroflexi* was detected only in BS-BA MES (Table 2).

Community detected within BS-only-treated MES. In addition to the non-detection of Dhc in the original groundwater samples, no Dhc amplicons were obtained from optimized PCR assays during the dechlorination process, within BS-only treated MES. This indicated either the absence of Dhc species or their presence below PCR detection limits and possible ecological insignificance in groundwater sample. However, the *Spirochaetes* showed 96–98% similarity to uncultured bacterial clones DCE33, TANB18, DPF05 and *Spirochaeta* sp. (Table 2). DGGE bands that showed 96% similarity with an unidentified bacterial clone DCE25 was grouped under *Firmicutes*, while bands putatively assigned to the phyla *γ-Proteobacteria* showed 98% and 96% sequence similarity to *Enterobacter* species and the bacterial clone ALO1_GLFRUDD03F0MQ1, respectively. Other bands

| Chloroethene | BS only | BS-BA | Control 1 (without acetate) | Control 2 (without acetate and Dhc inoculum) | Control 3 (without catholyte) | Control 4 (without electrodes) |
|--------------|---------|-------|----------------------------|---------------------------------|-------------------------------|--------------------------------|
| PCE          | +       | +     | +                          | +                               | +                             | +                              |
| TCE          | +       | +     | +                          | +                               | +                             | +                              |
| cDCE         | +       | +     | −                          | −                               | −                             | −                              |
| VC           | +       | +     | −                          | −                               | −                             | −                              |
| Ethene       | +       | +     | −                          | −                               | −                             | −                              |

* Presence; − Absence; Control MES (1) medium, electrodes, PCE-contaminated groundwater, catholyte but no acetate; (2) medium, electrodes, PCE-contaminated groundwater, catholyte but no acetate and Dhc inoculum; (3) medium, electrodes, PCE-contaminated groundwater, acetate but no catholyte; (4) medium, PCE-contaminated groundwater, acetate, catholyte, but no electrodes.
Table 2. Overview of the bacterial species identified based on the occurrence of a dominant DGGE pattern obtained from MES-assisted PCE dechlorination.

| Excised DGGE Bands | Accession No. | Closest matches overall (NCBI database) | Maximum % similarity | Phylum | Detected within (treatment) |
|--------------------|--------------|----------------------------------------|----------------------|--------|----------------------------|
| 1                  | AF349763.2   | Uncultured bacterium DCE33 16S         | 97                   | Spirochaetes | BS and BS-BA               |
| 2                  | AY667253.1   | Uncultured bacterium clone TANB18 16S | 96                   | Spirochaetes | BS and BS-BA               |
| 3                  | GQ377125.1   | Bacterium enrichment culture clone     | 96                   | Spirochaetes | BS and BS-BA               |
| 4                  | AF357916.2   | Spirochaeta sp. 16S ribosomal RNA gene, partial sequence | 98                   | Spirochaetes | BS and BS-BA               |
| 5                  | AJ249227.1   | Bacterium DCE25 16S rRNA gene          | 96                   | Firmicutes   | BS and BS-BA               |
| 6                  | JF920024.1   | Enterobacter sp. 16S ribosomal RNA gene, partial sequence | 98                   | γ-Proteobacteria | BS and BS-BA        |
| 7                  | JF689075.1   | Bacterium enrichment culture clone     | 96                   | γ-Proteobacteria | BS and BS-BA               |
| 8                  | DO903931     | Desulfovibrio Sp. GmS2 (SRB enrichment clone) 16S ribosomal RNA gene, partial sequence | 97                   | δ-Proteobacteria | BS and BS-BA               |
| 9                  | HM488066.1   | Uncultured bacterium clone ZM4-54 16S | 97                   | Bacteroidetes | BS and BS-BA               |
| 10                 | AY165308.1   | Dehalococcoides sp BAV1 16S rRNA gene, partial sequence | 100                  | Chloroflexi | BS-BA only                  |
| 11                 | AY914178.1   | Dehalococcoides sp GT, 16S rRNA, partial sequence | 100                  | Chloroflexi | BS-BA only                  |
| 12                 | AF357918.2   | Dehalococcoides sp FL2, 16S rRNA gene, partial sequence | 100                  | Chloroflexi | BS-BA only                  |

showed 97% similarity to the well-known dechlorinator Desulfovibrio species under the taxonomic group of δ-Proteobacteria and uncultured bacterial clones ZM4-54 within the phylum Bacteroidetes.

The detection of Spirochaetes group in this study is not unusual as they have also been reported in other PCE-reducing cultures inoculated with a sample from a chloroethene-contaminated site (Gu et al., 2004; Macbeth et al., 2004; Dong et al., 2011). Spirochaetes either utilize H₂ or ferment carbohydrates and other complex substrates to acetate and other substances that are utilized during organohalide respiration. Under the γ-Proteobacteria group, ALO1_GLFRUDD03F0MQ1 is a known 1,2-dichloroethene dechlorinator (Low et al., 2011), while Enterobacter species are the only facultative anaerobes reported so far to reductively dechlorinate PCE to cDCE (Holliger et al., 1999). Studies by Löffler and colleagues (2003), and Sun and colleagues (2000) have shown the contribution of a marine dechlorinating Desulfovibrio species in the reductive dechlorination that forms syntrophic associations with other dechlorinating bacteria to produce hydrogen by the transformation of organic compounds added to the medium. The hydrogen produced can then be transferred to dehalogenating bacteria and thus support microbially mediated reductive dechlorination (Drzyzga et al., 2001; Eydal et al., 2009).

Bacterial clones ZM4-54 under the Bacteroidetes may, through fermentation, supply small organic molecules or H₂ necessary for the growth of dechlorinating bacteria (Tancsics et al., 2010); however, the metabolic function of this organism is still unclear. The bacterium DCE25 in TCE and cDCE cultures acts as an acetate-fermenting organisms providing energy and a carbon source for the dechlorinating microbes (Flynn et al., 2000).

Community detected within BS-BA-treated MES. In addition to the earlier H₂-utilizing bacterial communities, only in MES run with BS-BA treatment augmented with Dhc strains BAV1, GT and FL2 were the Chloroflexi phyla detected (Table 2). The Dhc strains GT, FL2 and BAV1 have previously been well documented to cometabolically transform PCE to ethene (Futagami et al., 2008).

The faster dechlorination and almost twofold increase in current flow in BS-BA-treated MES when compared with the BS-only MES could be due to synergistic activity between the dechlorinating Dhc and non-Dhc species. Members of the indigenous non-Dhc community were believed to have contributed significantly to electron transfer as calculations using Eqn 1 showed that they could have been responsible for ~61% of the total current generated within BS-BA-treated MES. In spite of the absence of Dhc strains, BS-MES cultures containing an indigenous non-Dhc community were equally capable of completely reducing PCE. Overall, these comparative.
treatments highlighted the potential of mixed non-Dhc bacterial communities evolved in MES, which most likely contributed to the electron transfer mechanism supporting complete reductive dechlorination of PCE with and without Dhc. A study by Aulenta et al. (2009b) also reported the key role played by β-, δ- and γ-Proteobacteria, and Firmicutes besides Dhc in a mixed culture carrying out the dechlorination of TCE to non-chlorinated end-products within MES. Although the dechlorinating capabilities of these detected indigenous non-Dhc population have been well-studied (Gu et al., 2004; Macbeth et al., 2004; Dong et al., 2011), knowledge about their electrochemical properties is currently limited.

**Anode biofilms**

The analysis of the anode electrode surfaces via environmental scanning electron microscope (ESEM) conducted at the end of the experiment revealed that the surfaces of carbon fibre electrodes in both BS-only- and BS-BA-treated MES had been colonized (Fig. 4A and B). In contrast, no bacterial cells were observed at the anode surface of the controls 1–3 MES (Fig. 4C) that could be due to the lack of nutrient stimulation and/or biologically active microorganisms. This could have led to incomplete PCE degradation observed in these control samples unlike in BS-only and BS-BA samples. Mixed bacterial culture in BS-BA-treated MES formed complex cellular aggregates compared with sparsely distributed cells in BS-only MES (Fig. 4A and B). Microbial cells did not support complete dechlorination and energy production when the supply of electrons to the electrode was discontinued in control 4 MES (Table 1). Overall, this result demonstrated that the bacterial community present in BS-only and BS-BA sets of MES could bio-electrochemically interact with electrodes as electron acceptors by forming a stable, attached population that can produce electrical current via reductive dechlorination coupled to electron transfer to the electrodes.

**Reductive dechlorination and bacterial electrochemical activity**

In this study, DGGE band sequencing yielded sequences similar to those of several previously described H₂-oxidizing Dhc and non-Dhc bacteria. These findings suggest that facultative anaerobic bacterial species are capable of growing in MES using the electrode as an acceptor, further indicating their electrochemical potential or at least redox controlling properties. MES enhances the growth of bacteria that can use the electrode as an electrode acceptor as bacteria have been observed to gain more energy when using an electrode as an electron acceptor than when they use protons (Rabaey et al., 2013).
2004). In recent years, it has been demonstrated that a direct electron transfer between bacteria and electrodes is possible within MES (Strycharz et al., 2008). This study seems to indicate that a similar approach might have pursued by non-Dhc bacteria especially with regards to ethene formation that strive to access electrons throughout PCE dechlorination that was utilized for energy production.

As previous reports on this topic have been limited to a few microbes (Lovley, 2012), this study focused on other organisms to elucidate their electrochemical mechanism during PCE remediation. We report here that non-Dhc microorganisms within MES supplied with acetate as an electron donor and PCE/electrode as acceptors seemed to play a role in the complete PCE dechlorination with current production. However, to implement this strategy competitively with BS-BA, a clear understanding about electron transfer mechanisms between non-Dhc species and electrodes with nutrient stimulation is necessary to optimize the dechlorination rates and current output. In order to scale-up this strategy for successful in situ application, extensive testing based on subsurface characteristics and site-specific design needs to be studied. Williams and colleagues (2010) for the first time demonstrated the in situ applicability of graphite electrodes in the subsurface serving as electron acceptors for microbial stimulation during uranium bioremediation at Rifle site in Colorado. A similar approach may be employed for the remediation of chlorinated solvents. Preliminary investigations of environmental factors and complex microbial interactions at contaminated sites will decide the potential of MES for PCE bioremediation. If a native dechlorinating community was found to be capable of self-mediated electrochemical conversion of PCE, it would further eliminate the need for bio-augmenting the subsurface that will eventually reduce remediation cost. Future research necessitates investigating the possibility of electrode-dependent, microbially catalysed PCE degradation where non-Dhc bacteria can utilize the electrode as the sole electron donor. Altogether, this strategy could prove advantageous, especially where electron donor delivery to subsurface has always been a challenge. Nevertheless, it will be of fundamental importance to focus on mechanisms involved in the extracellular electron transfer process between microorganism and the electrodes to develop strategies to maximize dechlorination rates.

To conclude, this study highlighted the electrochemical potential of indigenous non-Dhc dechlorinators compared with Dhc species during the complete dechlorination of PCE to ethene via MES. Although the direct involvement of mixed Dhc culture in the electron transfer process was expected in BS-BA run MES, the potential of non-Dhc dechlorinators exhibiting similar mechanisms within BS-MES was observed. Microbial communities in the MES evolved specifically as an optimized biocatalyser generating a stable power output, opening a perspective for the development of a new sustainable bioremediation strategy. Clearly, research is needed to further elucidate the electrochemical mechanism of these as-yet-undefined non-Dhc dechlorinators in order to advance this field in a rational manner.

Experimental procedures

Materials

All chlorinated ethenes, ethene and other chemicals including potassium hexacyanoferrate (III), sulphuric acid and hydrogen peroxide required for the experimental setup and analytical measurements were purchased from Sigma-Aldrich (Sydney, NSW, Australia) with a minimum purity of 99.5%. All gases were ordered from Coregas (Melbourne, Vic., Australia).

Groundwater sample collection

For this study, we selected a chloroethene contaminated site located in Victoria, Australia. A sample of contaminated groundwater (4 l) with a PCE concentration of 130 μg l⁻¹ was collected from the monitoring well as per the protocol suggested by Ritalahti et al. (2010). A flow-through cell (YSI, Melbourne, Vic., Australia) recorded pH, oxidation-reduction potential, specific conductance, temperature, dissolved oxygen and turbidity of groundwater. When geochemical parameters were stabilized, the flow-through cell was disconnected and replicate samples collected consecutively without flow interruption. Sample containers consisted of sterile and N₂-purged high-density polyethylene Nalgene 4 l bottles with polypropylene screw caps (Thermo Fisher Scientific Australia, Sydney, NSW, Australia). During sampling, bottles were filled to capacity and stored on ice that was then express-shipped to the analytical laboratory. Upon arrival, samples were placed in the dark at 4°C.

Media preparation

An anoxic PCE-dechlorinating mineral media was enriched and maintained as per the guidelines presented by Löffler and colleagues (2005) and the American Type Culture Collection (ATCC; http://www.atcc.org). Media was prepared in Wheaton serum bottles of 125 ml nominal volume containing 75 ml of growth medium and 20 ml of PCE-contaminated groundwater as an inoculum amended with 5 mM acetate as an electron donor and 5 μl of PCE as an electron acceptor. The bottles were sealed with Teflon-coated butyl rubber septa and aluminium crimp caps (Altech, Melbourne, Vic., Australia). Hydrogen (5% in 95% nitrogen) was added in the headspace (5–10% of the headspace volume of a bottle) at a low partial pressure of 9 kPa. Cultures were prepared under strict anaerobic conditions maintained in an anaerobic glove box (La-Petite, Thermo Fisher Scientific Australia) using N₂ : CO₂ at the ratio of 80%:20%. Resazurin redox indicator was added to the groundwater to denote reduced conditions. Immediately upon setup, media turned clear from pink tint
(given by the resazurin redox indicator added to the groundwater) indicating establishment of reduced conditions.

**MES construction and operation**

For this study, we employed typical two-chamber NCBE-type MES (National Centre for Biotechnology Education, Reading, UK). The MES chamber (7.5 × 9.0 × 5.5 cm) consisted of two electrode compartments (60 × 70 × 10 mm; 10 ml each) separated by a reinforced Nafion® proton exchange membrane (PEM) 0.007” thickness (Sigma-Aldrich) (Fig. 1A; Bennet, 1990). Compartments were kept watertight by placing rubber gaskets between chambers and by bolting two Perspex sheets together. The PEM was pretreated by boiling in H₂O₂ (30%), then in 0.5 M H₂SO₄ and finally in de-ionized (DI) water, each for 1 h, and then stored in DI water prior to being used. The carbon fibre electrodes (3.2 × 4 cm) were soaked in DI water prior to use (Aulenta et al., 2007). Sampling ports were sealed with rubber stoppers, while carbon electrodes were attached to copper wires fed through rubber stoppers in the sampling port.

Two out of the four MES were run on a BS-only approach where MES were fed with groundwater comprising an indigenous microbial population. The remaining two MES were dedicated to the BS-BA treatment where the same groundwater was amended with a dechlorinating mixed consortia of *Dhc* species FL2 (Dehalococcoides sp. ATCC® BAA-2098™), BAV1 (Dehalococcoides sp. ATCC BAA-2100™) and GT (Dehalococcoides sp. ATCC BAA-2099™) outsourced from ATCC (http://www.atcc.org). MES were established by supplying acetate as electron donor and PCE/electrodes as acceptors. Ten millilitres of anoxic media inoculated with *Dhc* strain FL2 (Begin et al., 1995) was injected only into the working electrode chambers of all four MES using a gas-tight syringe. In parallel, the counter electrode was filled with an equal electrode chambers of all four MES using a gas-tight syringe. The working electrode was poised at +450 mV (versus standard hydrogen electrode). Electrochemical measurements were taken using a Fluke 289 digital true RMS multimeter (RS Components, Melbourne, Vic., Australia). Cells were monitored over 16 weeks (112 days) with current and voltage being recorded throughout the dechlorination process. The energy production (%) from BS and BS-BA treatments was calculated as follows:

\[
a = (a_1 + c) \times 100; \quad b = 100 - a
\]

where \(a_1\) = current from BS, \(a\) = % current from BS, \(b\) = % current from BA, and \(c\) = total current from BS-BA. The experiment was followed by multiple control MES with: (i) medium, electrodes, PCE-contaminated groundwater, catholyte but no acetate; (ii) medium, electrodes, PCE-contaminated groundwater, catholyte but no acetate and *Dhc* inoculum; (iii) medium, electrodes, PCE-contaminated groundwater, acetate but no catholyte; (iv) medium, PCE-contaminated groundwater, acetate, catholyte, but no electrodes.

**Microbial community analysis**

Genomic DNA extraction from groundwater samples treated with MES was carried out as described by Löffler and colleagues (2005) and 16S rRNA gene fragments were amplified with bacterial universal primer set 341 F-GC/518R (Muyzer et al., 1993) to detect the presence of indigenous and likely dechlorinating bacterial communities. The Archaea-specific primers A109f and A934b were also used for detection of methanogens under domain Archaea as described by Hej et al. (2008). *Dhc*-specific primers 1F-GC and 259R with *Dhc* strain GT, FL2 and BAV1 (positive controls) were used for the detection of *Dhc* in groundwater samples using touchdown PCR that was optimized as described by Kim and colleagues (2010). Amplified 200 bp PCR fragments were further analysed by DGGE as described by Patil and colleagues (2010). Dominant DGGE bands were excised using sterile razor blades and incubated in two volumes of DNA elution buffer (0.5 mmol l⁻¹ ammonium acetate, 10 mmol l⁻¹ magnesium acetate, 1 mmol l⁻¹ ethylendiamine tetraacetic acid pH 8 and 0.1% sodium dodecyl sulfate) overnight at 37°C. DNA was then precipitated with two volumes of absolute ethanol, air-dried, resuspended in 20 μl nuclease-free water and stored at −20°C until re-amplification (McKew et al., 2007). Re-amplification was performed using 341F/518R primers. Re-amplified PCR products were purified using the Wizard® SV gel and PCR clean up system (Promega, Madison, WI, USA) as per the manufacturer’s instructions. The eluted DNA was checked for concentration and purity using a Nanodrop Lite spectrophotometer (Thermo Scientific Australia). Samples were then sent to the Australian Genome Research Facility for sequencing using an automated sequencer, ABI 3730. Nucleotide sequences were analysed using SEQUENCHER software (Sequencer Version 4.1.4, GeneCodes Corp., Ann Arbor, MI, USA), and homology searches were completed with the BLAST server of the National Centre for Biotechnology Information (NCBI) using a BLAST algorithm (http://www.ncbi.nlm.gov/BLAST) for the comparison of a nucleotide query sequence against a nucleotide sequence database (blastn).

**Analytical procedures**

Every 2 weeks, 50 μl chlorinated ethenes were removed from the gas headspace of both working and counter electrode compartments using a gas tight, sample-lock Hamilton syringe (Alltech) and analysed by an HP 6890 gas chromatographic (GC) system equipped with a 5973 mass spectrometry and flame ionizing detector and a Porabond-Q column (0.32 mm by 25 m) (Agilent Tech, Melbourne, Vic., Australia). The GC settings were: injector temperature 200°C; detector temperature 300°C; oven temperature 3 min at 40°C, followed by an increase of 10°C min⁻¹ to 70°C, followed by an...
increase of 15°C min⁻¹ to 250°C for 7 min; and carrier gas (He) with a flow rate of 2 ml min⁻¹. External standards at six different concentrations from 0 to 30 μM were used for calibration. Electron donors were replenished every time analyses indicated they were exhausted.

Microscopy

At the end of the experiment, anodes from all MES were removed, cut into small pieces using a sterile razor blade and washed with phosphate buffer (pH 7.0) to remove loosely attached cells. Subsequently, samples were observed using a Quanta 200 ESEM (FEI Company, Melbourne, Vic., Australia). The ESEM was operated at 10–20 kV, and images were captured digitally.

Nucleotide sequence accession numbers

All bacterial sequences have been deposited in the NCBI database under accession numbers JX495100–JX495111.

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Conflict of interest

None declared.

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