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Reductive dechlorination of 2-chlorophenol by *Anaeromyxobacter dehalogenans* with an electrode serving as the electron donor

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Summary

Electrodes poised at potentials low enough to serve as an electron donor for microbial respiration, but high enough to avoid the production of hydrogen, have been proposed as an alternative to the use of soluble electron donors for stimulating the bioremediation of chlorinated contaminants and/or metals. However, this form of respiration using pure cultures of microorganisms has only been reported in *Geobacter* species. To further evaluate this bioremediation strategy studies were conducted with *Anaeromyxobacter dehalogenans*, which has previously been reported to reductively dechlorinate 2-chlorophenol to phenol with acetate as the electron donor. *Anaeromyxobacter dehalogenans* could oxidize acetate with electron transfer to a graphite electrode poised at a positive potential, demonstrating its ability to directly exchange electrons with electrodes. *Anaeromyxobacter dehalogenans* attached to electrodes poised at –300 mV versus standard hydrogen electrode reductively dechlorinated 2-chlorophenol to phenol. There was no dechlorination in the absence of *A. dehalogenans* and electrode-driven dechlorination stopped when the supply of electrons to the electrode was disrupted. The findings that microorganisms other than *Geobacter* species can accept electrons from electrodes for anaerobic respiration and that chlorinated aromatic compounds can be dechlorinated in this manner suggest that there may be substantial potential for treating a diversity of contaminants with microbe–electrode interactions.

Introduction

It has been suggested that direct electron transfer from electrodes to attached microorganisms may be preferable to soluble electron donors for stimulating the bioremediation of chlorinated organic compounds and toxic metals in subsurface environments (Gregory *et al*., 2004, Gregory and Lovley, 2005; Lovley, 2006; Aulenta *et al*., 2009). As recently reviewed (Thrash and Coates, 2008), the concepts of employing electrodes to generate hydrogen as an electron donor for microbial metabolism or to electrochemically reduce contaminants via abiotic reactions are well established. Furthermore, the potential for stimulating reductive dechlorination via the reduction of electron shuttling molecules that can then serve as an electron donor for microbial reductive dechlorination has been documented (Aulenta *et al*., 2007; Thrash and Coates, 2008). However, direct electron transfer from electrodes to microorganisms offers several potential advantages including: (i) effective delivery of electrons specifically to the microorganisms carrying out the bioremediation reactions; (ii) eliminating non-specific, environmentally detrimental reactions that can result from abiotic reduction of contaminants with electrodes (Skadberg *et al*., 1999); (iii) the ability to specifically and permanently locate the source of the electron donor within or near contaminant source zones; and (iv) the ability to remove contaminant metals reduced and precipitated at the electrode surface from the subsurface by withdrawing the electrode and extracting the precipitated metals (Gregory and Lovley, 2005). Furthermore, the propensity for graphite to adsorb organic contaminants (Cho *et al*., 2007; Diaz *et al*., 2007) may help concentrate contaminants on graphite electrodes, localizing the contaminants with the source of electrons and bioremediating organisms.

The list of priority pollutants identified under the Clean Water Act which pose a risk to human health contains a

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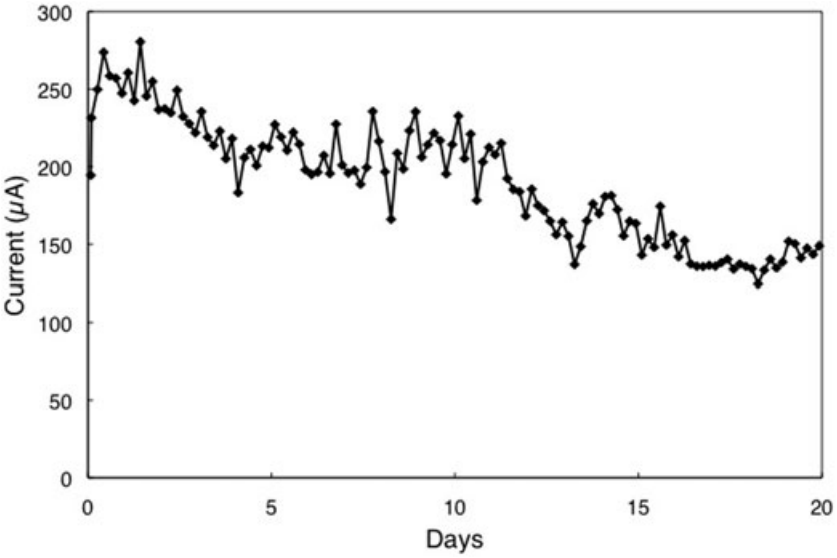

Fig. 1. Current produced by *A. dehalogenans* strain 2CP-1 with acetate as the electron donor and poised graphite electrode (+500 mV versus SHE) as the electron acceptor. Cultures of *A. dehalogenans* were grown at room temperature (*c.* 22–25°C) under N_2 : CO_2 (80:20) in 200 ml of stirred (180 r.p.m.) medium in the 265 ml working electrode chamber of a sterile, anaerobic, dual-chambered electrode system as previously described (Bond and Lovley, 2003; Gregory *et al*., 2004; Holmes *et al*., 2006). Acetate (10 mM) was provided as the electron donor and initially both fumarate (40 mM) and the electrode (+500 mV versus SHE) were available as electron acceptors. Time 0 in the figure represents the time at which the medium was exchanged with fumarate-free medium and the electrode served as the sole electron acceptor. The results shown are a representative data set from duplicate incubations.

broad range of chlorinated organic contaminants, such as mono- and poly- chlorinated ethenes, ethanes, biphenyls and phenols including 2-chlorophenol (US EPA *et al*., 2009). Bioremediation has been implemented as a strategy to remediate a number of these contaminants (AFCEE, 2004; Loeffler and Edwards, 2006), but limitations to microbial stimulation, such as the availability of a specific electron donor, hinder the success of this approach (Lee *et al*., 2007; Robinson *et al*., 2009).

Evaluation of organisms in pure culture using bioelectric systems for which a fully sequenced genome is available provides a valuable tool for researchers in the field of environmental microbiology who wish to understand the physiological basis of electron transfer. To date, the potential for stimulating bioremediation of chlorinated contaminants and metals with direct electrode–microbe electron transfer in pure culture has only been studied with a few contaminants and only with *Geobacter* species. *Geobacter sulfurreducens* reduced U(VI) to U(IV) (Gregory and Lovley, 2005) and *Geobacter lovleyi* reduced tetrachloroethene to *cis*-dichloroethene (Strycharz *et al*., 2008) with an electrode serving as the sole electron donor. In order to determine whether organisms other than *Geobacter* might be able to reductively dechlorinate using the electrode as an electron donor, we conducted studies with *A. dehalogenans*, an organism with the capacity to reductively dechlorinate the model chlorinated aromatic compound, 2-chlorophenol (Sanford *et al*., 2002).

Results and discussion

The potential for *A. dehalogenans* to make electrochemical connections with a graphite electrode was first evaluated by determining its potential for current production. *Anaeromyxobacter dehalogenans* strain 2CP-1 (ATCC #BAA-258) was grown with acetate as the electron donor and a poised graphite electrode [+500 mV versus standard hydrogen electrode (SHE)] as the electron acceptor (Fig. 1). Maximum current produced in duplicate incubations was 280 μA (Fig. 1) and 350 μA (data not shown). This is somewhat less than previously reported power production by *Geobacter* and *Geopsychrobacter* species in the same culturing system (Bond *et al*., 2002; Bond and Lovley, 2003; Holmes *et al*., 2004; 2006; Strycharz *et al*., 2008). Biofilm development on the surface of the electrode was patchy, with what appeared to be a single layer of cells covering the electrode surface in some areas (Fig. 2). This amount of biomass on the electrode surface is consistent with what has been observed for *G. sulfurreducens* at similar current levels (Bond and Lovley, 2003; Reguera *et al*., 2006). When the acetate medium was removed and replaced with fresh medium current production resumed immediately, suggesting that current production could be attributed to cells attached to the anode and that soluble electron shuttles did not significantly contribute to electron transfer to the anode (data not shown).

In order to determine whether *A. dehalogenans* could use an electrode as an electron donor for reductive dechlorination, *A. dehalogenans* was inoculated into the working electrode chamber of a dual-chambered cell (Gregory *et al*., 2004; Strycharz *et al*., 2008) in which both acetate (10 mM) and a graphite electrode (–300 mV versus SHE) were available as electron donors, and *c.* 80 μM 2-chlorophenol was added as the sole electron acceptor. As previously described (Strycharz *et al*., 2008) acetate was then progressively removed with several medium exchanges until the electrode served as the sole electron donor.

With the electrode as the sole donor there was a loss of 2-chlorophenol with a concomitant accumulation of

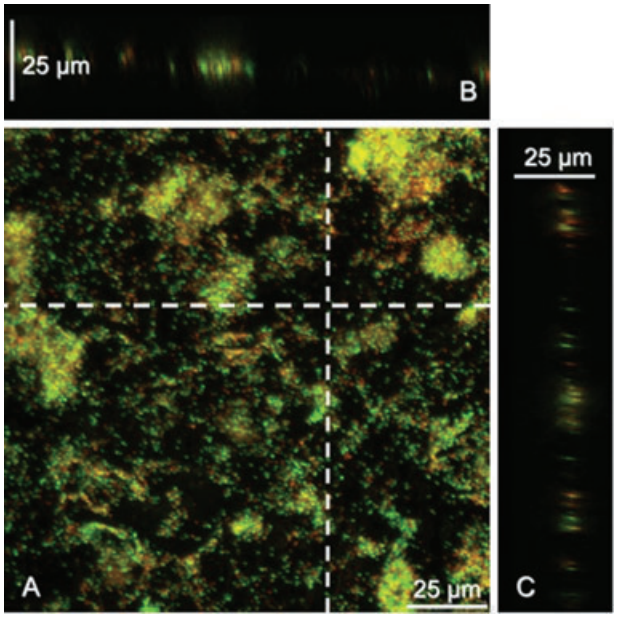

Fig. 2. Three dimensional top down (A) and perpendicular (B and C) confocal laser scanning microscopy images of a biofilm of *A. dehalogenans* growing with the electrode as an electron acceptor from the incubation summarized in Fig. 1 and imaged on day 20. The biofilm was stained with the BacLight viability kit (Molecular Probes) and imaged as previously described (Reguera *et al*., 2006) using a Leica TCS SP5 microscope with a HCX PL APO 20× (NA 0.7) objective (Leica Microsystems GmbH, Wetzlar, Germany). Images were analysed using Leica LAS AF software (Leica Microsystems GmbH, Wetzlar, Germany). Dashed white lines represent location of the perpendicular slices.

phenol over time (Fig. 3A and B). The rate of phenol production varied among replicate cultures (Fig. 3A and B). Such differences in initial rates of metabolism are common in electrode cultures and presumably represent differences in the lag periods of metabolism and the metabolic status of the electrode biofilms.

The stoichiometry of 2-chlorophenol dechlorinated and current consumed could not be determined. For example, the most rapid rates of dechlorination were *c*. 40 µM over 24 h (Fig. 3A, inset). The 2 electron reduction of the 8 µmoles (40 µM in 200 ml) of 2-chlorophenol that were dechlorinated in 24 h requires 9.63×10^{18} electrons (8×10^{-6} moles of 2-chlorophenol × 2 moles of electrons/mole of 2-chlorophenol × 6.02×10^{23} electrons/mole of electrons); 9.63×10^{18} electrons is equivalent to 1.54 coulombs (9.63×10^{18} electrons × 1 coulomb/6.24×10^{18} electrons); and 1.54 coulombs produced over a 24 h period provides an average current flux of 18 µA (1.54 coulombs/24 h × (1 amp × sec)/coulomb × 1 h/3600 s). Currents less than 40 µA could not be accurately measured with the available electronics (Gregory *et al*., 2004; Strycharz *et al*., 2008). Thus, even under the most rapid rates of dechlorination, the current consumed was well below the detection limit.

However, dechlorination was clearly dependent upon the supply of electrons from the cathode and the presence of *A. dehalogenans* because when the cathode and potentiostat were disconnected, the production of phenol stopped immediately (Fig. 3B) and there was no phenol production with a connected cathode in the absence of *A. dehalogenans*. A slow abiotic loss of both 2-chlorophenol and phenol were observed in uninoculated cells (Fig. 3C). This loss can likely be attributed to the adsorption of these compounds to the graphite. As noted in similar previous studies (Gregory *et al*., 2004, Gregory and Lovley, 2005; Strycharz *et al*., 2008), hydrogen did not accumulate in uninoculated cathode chambers, suggesting that *A. dehalogenans* directly accepted electrons from the electrode.

The low cell density of the biofilms that microorganisms typically produce when growing with an electrode serving as the electron donor generally requires scanning electron microscopy in order to image the cells appropriately (Gregory *et al*., 2004; Strycharz *et al*., 2008). Scanning electron microscopy of the dechlorinating cathodes revealed cells of *A. dehalogenans* scattered on the surface of the graphite (Fig. 4A and B).

These results suggest that the capacity for current-driven, biologically catalysed dechlorination could be possible with a variety of microorganisms and diversity of chlorinated compounds. The reductive dechlorination of 2-chlorophenol by *A. dehalogenans* represents the first description of a pure culture other than a *Geobacter* species capable of accepting electrons from an electrode for anaerobic respiration and is the first example of dechlorination of a chlorinated aromatic compound by a microorganism using an electrode as the electron donor.

Some studies have attempted to treat chlorinated phenols with abiotic electrochemical reduction (Berrios *et al*., 2008; Wang and Wang, 2008), or by producing hydrogen to stimulate the activity of hydrogen-oxidizing dechlorinating microorganisms (Skadberg *et al*., 1999). However, as previously discussed in detail (Strycharz *et al*., 2008), the low potentials required for such non-discriminate abiotic reduction adversely impacts on the environment by: (i) increasing pH; (ii) stimulating the growth of a wide diversity of hydrogen-consuming microorganisms that do not contribute to dechlorination; and (iii) consuming much more electricity than is required for direct dechlorination. This can be avoided with the specific delivery of electrons to dechlorinating microorganisms and the low energy requirement of this approach offers the possibility of installing systems that can continuously provide electron donor to dechlorinating microorganisms colonizing the electrode surface with renewable power sources such as solar panels.

It is becoming increasingly apparent that a wide diversity of microorganisms may be capable of directly interacting with electrodes (Logan and Regan, 2006; Lovley,

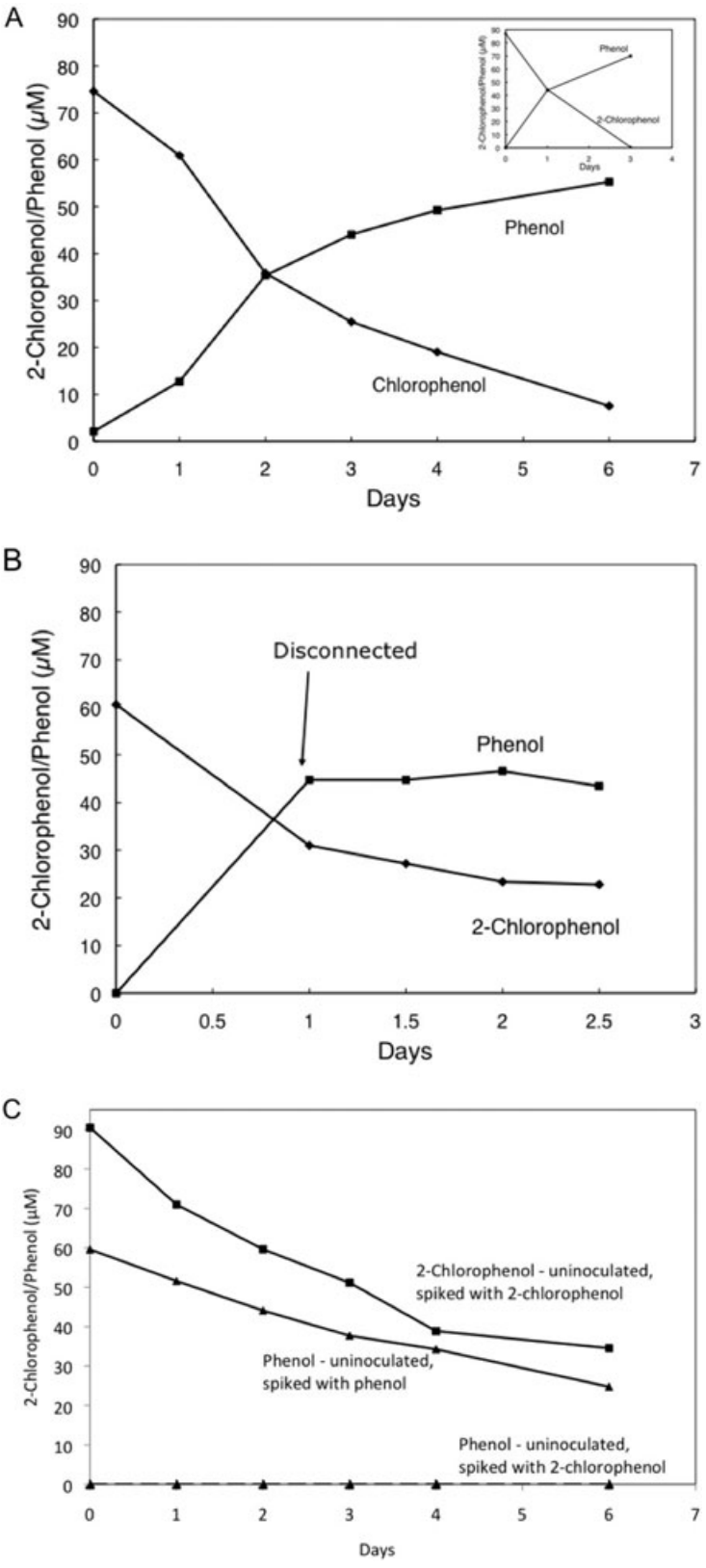

Fig. 3. Reduction of 2-chlorophenol to phenol by *A. dehalogenans* strain 2CP-1 with a poised graphite electrode (−300 mV versus SHE) as the sole electron donor.
A. Time-course for two duplicate incubations (main plot and inset) showing the variability of rates in duplicate incubations. Plots begin when medium was exchanged in the working electrode chamber with fresh medium containing *c.* 80 μM 2-chlorophenol.
B. Phenol production stopped immediately when the supply of electrons to the electrode was eliminated.
C. Abiotic loss and/or reduction of 2-chlorophenol, and loss of phenol in an identical, sterile system were monitored separately over time. Phenol and 2-chlorophenol were analysed with high-performance liquid chromatography as previously described (Sanford *et al*., 2002).

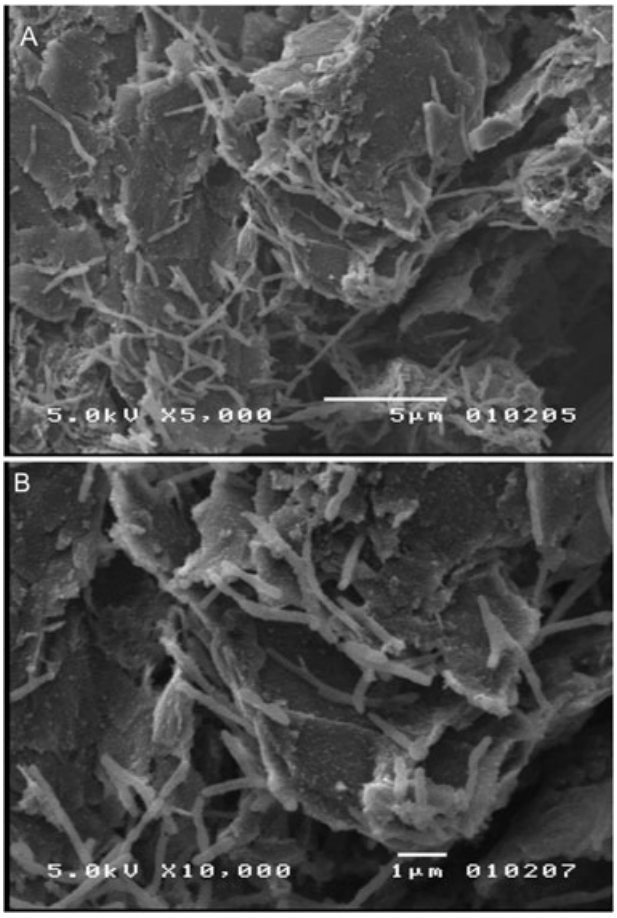

Fig. 4. Scanning electron micrograph of *A. dehalogenans* using the electrode as an electron donor and 2CPh as the electron acceptor (A). Once dechlorination in the incubation shown in the inset of Fig. 3A was complete (Day 3), the working electrode was removed from the electrode chamber, fixed, dehydrated, mounted and examined as previously described (Gregory *et al*., 2004). Panel B is the same image as A at 2× higher magnification.

2008). Thus, it seems likely that the possibilities for stimulating the bioremediation of contaminants with electrodes serving as the electron donor, as described here, or with the electrodes as the electron acceptor (Bond *et al*., 2002) may be extensive. Further work is underway to evaluate the performance of an electrode as an electron donor *in situ*.

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