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Enhanced biodegradation of pentachlorophenol in unsaturated soil using reversed field electrokinetics

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ABSTRACT

This study investigated the use of electrokinetics in unsaturated soil to promote biodegradation of pentachlorophenol through increased contact between bacteria and contaminant. Soil microcosms, contaminated with approximately 100 mg kg^{-1} pentachlorophenol (containing [^{14}C]-PCP as a tracer), and inoculated with a specific pentachlorophenol-degrading bacterium (*Sphingobium* sp. UG30–1×10^8 cfu g^{-1}) were subjected to constant and regularly reversed electric currents (10 mA). The former caused large pH and moisture content changes due to water electrolysis and electroosmotic effects, with subsequent negative impacts on biodegradation parameters including enzyme activity and contaminant mineralisation (as measured by $^{14}CO_2$ evolution rate). The reversed field caused little change in pH and moisture content and led to more rapid contaminant mineralisation, lower soil contaminant concentration in the majority of the microcosms and increased soil enzyme activity (with the exception of soil immediately adjacent to the anode). The presence of an electric field, if suitably applied, may therefore enhance contaminant biodegradation in unsaturated soil.

1. Introduction

Application of an electric field to soil can instigate a number of chemical processes, collectively known as electrokinetic phenomena. These include electromigration, where charged species present in solution move towards the electrode of opposite charge, and electroosmosis, where a flow of water is generated, usually towards the cathode, in soils with charged surfaces (e.g. clays). The transport of both organic and inorganic contaminants in soils through application of electrokinetic phenomena has been studied extensively (Pamukcu and Wittle, 1992; Acar and Alshawabkeh, 1993; Probstein and Hicks, 1993; Khodadoust et al., 2006) although its application in the field to date has been limited (Lageman et al., 1989). Potential difficulties in remediating soils in this way include achieving relatively long distance transport through heterogeneous material, changes in contaminant state due to soil chemistry effects and remediation of insoluble or strongly sorbed contamination. Electrokinetics has also been used to introduce materials (e.g. limiting nutrients) into soils that can facilitate biodegradation of organic contaminants (Budhu et al., 1997; Acar et al., 1997; Thevanayagam and Rishindran, 1998), move contaminants towards a microbial treatment zone (Jackman et al., 2001) or can be combined with microbial oxidation or reduction to facilitate treatment of inorganics (Maini et al., 2000). Microorganisms as well as chemicals can also be moved by these processes, particularly by electroosmosis (Deflaun and Condee, 1997; Wick et al., 2004).

Electrokinetics has the potential to enhance bioremediation of organic contaminants through control and movement of both contaminant and bacteria, facilitating greater interaction and hence contaminant bioavailability (Wick et al., 2007). Bioavailability is a critical factor in bioremediation, as the sorption, sequestration and heterogeneous distribution of chemicals can lead to their persistence within soils (Reid et al., 2000). Electrokinetics may have the ability to overcome these factors through contaminant desorption and redistribution on both a micro- and a macro-scale. However, soil properties such as pH and moisture content have a significant effect on biodegradation and contaminant behaviour, and can be rapidly altered by an applied field (Acar and Alshawabkeh, 1993). They may also affect the health of the soil microbial community and its response to contamination. An electric current in liquid culture can have a detrimental effect on cell viability at high enough levels, for example Jackman et al. (1999) found a current density of 200 A m^{-2} inactivated acidophilic bacteria. However, lower current densities have little effect (e.g. Wick et al., 2004 [current density of 1.57 A m^{-2}]) or may even stimulate cell

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activity (She et al., 2006). This latter occurrence was attributed to the formation of hydrogen and oxygen *via* water electrolysis. Jackman et al. (1999) found that the presence of solid particles offers protection from the current, even at high levels, whilst Lear et al. (2004, 2007) reported that a low electric current (3.14 A m^{-2}) in soil detrimentally impacted communities only near the anode. This was largely attributed to changes in pH or contaminant distribution. No discernible effect of the electric current itself was observed.

Luo et al. (2005, 2007) and Fan et al. (2007) applied a direct current to phenol-contaminated soil, and by frequently changing the direction of the current produced an enhanced degradation rate. This minimized changes in soil physical properties, particularly pH, whilst generating contaminant movement. Similarly, Niqui-Arroyo et al. (2006), Niqui-Arroyo and Ortega-Calvo (2007) found that pH control helped electrokinetics enhance polyaromatic hydrocarbon degradation, with electroosmosis improving contaminant availability and subsequent degradation.

The aim of the work described here was to investigate the effects of electrokinetics on degradation of a persistent contaminant, pentachlorophenol (PCP). It was hypothesised that small movements of contaminant or bacteria through application of a low direct current would increase contact between the two, for example by moving contaminants sequestered in micropores to a nearby area containing degrading bacteria. Using laboratory microcosms, the study examined the effects of electric current regime and subsequent pH and moisture content conditions on a clayey soil artificially contaminated with PCP, and inoculated with PCP-degrading bacteria (*Sphingobium* sp. UG30; Leung et al., 1997a,b; Lear et al., 2007). PCP is an organic biocide and a U.S. EPA priority pollutant. It has several human health effects, including probable carcinogenicity (Proudfoot, 2003). It is a persistent environmental contaminant, toxic to many microorganisms (Chaudri et al., 2000) and biodegradation in natural situations is often restricted. Several microbial species are able to degrade the chemical (e.g. Leung et al., 1997a; Yang and Lee, 2008) and here a single degrading strain was employed in combination with a recalcitrant contaminant. Contaminant radiolabelling was used to enable detection of $^{14}CO_2$ evolved through biodegradation (Niqui-Arroyo et al., 2006; Lear et al., 2007). Sequential chemical extractions provided indications of the relative availability of PCP remaining in the soil, and dehydrogenase activity was monitored as a proxy for degradative activity. It was found to be necessary to control physical changes to the soil (pH and moisture content) in order to prevent negative impacts on the microbial community.

2. Materials and methods

2.1. Microcosm preparation

A silty clay soil was obtained from Wytham, Oxfordshire, UK (Table 1). Soil was prepared by air-drying before sieving past 2.0 mm. This was then rehydrated to a moisture content of 19% (w:w), thoroughly mixed and allowed to equilibrate overnight. Soil microcosms (dimensions 130 × 59 × 54 mm; 500 g moist soil) were prepared in plastic cartridges (Fig. 1) with compaction at 50 kPa as described by Lear et al. (2007).

2.2. Microcosm contamination and inoculation

The soil was contaminated with an aqueous pentachlorophenol (PCP, sodium salt, Sigma–Aldrich Ltd., UK) solution (700 μM). The method of contamination involved repeated cycling of this solution through the prepared soil, as described by Lear et al. (2007), to give a final concentration of approximately 100 mg kg^{-1}. The solution also contained [^{14}C]-PCP (Sigma–Aldrich Ltd., UK; radiochemical purity 37–555 MBq $mmol^{-1}$) as a marker, which gave final radiolabel levels of between 3.4 and 19.8 kBq per microcosm. The microcosm was left for 1 day in a fume hood to permit loss of excess moisture then sealed in a plastic bag for 5 days to equilibrate. At the initial soil pH it would be expected that most PCP was in the form of negatively charged pentachlorophenate (pKa = 4.35). PCP-degrading bacteria (rifampicin-resistant *Sphingobium* sp. UG30) were grown in a minimal salts medium (detailed in Lear et al., 2007) containing glutamic acid (a precursor of glutathione, which PCP-degrading organisms have to synthesise; Leung et al., 1997a,b) and inoculated into soil microcosms following the 6-day equilibration period. Inoculum (100 μl) was injected at each of 96 regularly spaced points within the soil (at depths of 5 mm [36 points over 120 × 50 mm], 30 mm [24 points over 105 × 35 mm] and 55 mm [as for 5 mm]), and gave an initial inoculation of the order of 1×10^8 cfu (colony-forming units) *Sphingobium* sp. UG30 g^{-1} dry soil.

Table 1
Soil properties (for prepared soil, as used in all experiments).

Soil organic matter content (%)	8
Soil pH	8
Cation exchange capacity (cmol kg^{-1} dry soil)	22
Specific gravity	2.63
Particle size distribution	
Clay (% <2 μm)	10
Silt (2 μm < % <60 μm)	45
Sand (60 μm < % <2 mm)	45
Atterberg limits	
Plastic limit	27
Liquid limit	58
K_d (PCP sorption) (mL g^{-1})	
No added acid	13
Added acid (pH 2.5)	22

2.3. Experimental protocol

Three experiments are presented, with their initial conditions and further information summarised in Table 2.

Two electrode configurations were used. In experiments I and II, acrylic electrode chambers (Fig. 1) were slotted into either end of the soil cartridge. Each contained a compressed graphite electrode (50 × 50 × 8 mm) with de-aired, de-ionised water as the electrolyte solution. The chambers and the soil were separated by a semi-permeable membrane (Daramic Inc., USA) that allowed water to pass by electrokinetic forces, but presented sufficient resistance to prevent hydraulic flow due to the water head in the chamber. In experiment III, electrodes were placed directly in contact with the soil, such that no electrolyte fluid was required. Each soil microcosm was enclosed in an air-tight plastic container (5.7 L) to enable monitoring of $^{14}CO_2$ evolution. Air-tight ports were provided in these containers to enable cycling and pH control of electrolyte fluids where necessary. Electrolyte pH was controlled in experiment I at the cathode only (to maintain electroosmotic flow) by adding concentrated sulphuric acid, in experiment II by mixing anode and cathode electrolyte fluids (as in Lee and Yang, 2000), and in experiment III by reversing the current daily. In each case, the electric current magnitude was 10 mA (3.14 A m^{-2}); this was unlikely to have had a negative impact on bacterial viability in the soil. The voltage necessary to maintain this constant current varied during the experiments as conditions changed, but was generally in the range of 1 V cm^{-1}. All experiments were performed in triplicate, with triplicate controls (no current applied) in identical cartridges although without electrode chambers. In experiment III, anode and cathode positions were changed daily. Subsequent references to the anode or cathode for this experiment refer to the position at the start of testing.