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Experimental design for assessment of electrokinetically enhanced delivery of lactate and bacteria in 1,2-cis-dichloroethylene contaminated limestone

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HIGHLIGHTS

- Electrokinetically induced delivery of amendments in bryozoan limestone is proposed.
- For the application, an experimental set-up was successfully designed and evaluated.
- Fermentation of donor indicated successful pH control and anaerobic conditions.
- Enhanced delivery of lactate and bacteria by electromigration and electrophoresis.
- Essential contact between bacteria, donor and contaminant achieved within matrix.

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ABSTRACT

Bacterial dechlorination of chlorinated solvents often causes accumulation of the intermediate cis-DCE. Back diffusion of e.g. cis-DCE, due to the dual porosity of limestone, often limits the remediation efficiency. A remediation scheme capable of establishing contact between contaminant, degrading bacteria and electron donor within the low permeable limestone matrix is required. The technology EK-BIO, which combines enhanced reductive dechlorination and electrokinetics (EK), was assessed. This novel technology has not previously been tested in limestone. An experimental set-up was designed to meet the requirements of anaerobic bacteria and to manage the volatile contaminants and extreme pH development prompted by electrolysis. The experimental set-up was tested and recommendations for design improvements presented. In this study, supplementary methods were developed for e.g. sampling of intact bryozoan limestone cores and for saturation and contamination of the cores with cis-DCE. EK induced transport processes for delivery of the donor lactate and mixed bacteria culture KB-1® were studied. EK was shown to enhance delivery of lactate and bacteria resulting in fermentation of lactate in the limestone. Lactate was delivered by electromigration causing an increase in electric conductivity. No indications of establishment of electro-osmotic flow in limestone were observed. Presence of specific cis-DCE degraders, *Dehalococcoides*, in the limestone could not be verified. However, the results indicated that fermentative bacteria were distributed by electrophoresis. This study suggests that EK application can establish the essential contact and overcome back diffusion. Thereby, EK-BIO may be superior to advection-based technologies for bioremediation of chlorinated solvent contaminated limestone matrices.

Abbreviations: EK, electrokinetics; Dhc, Dehalococcoides; DC, direct current; EM, electromigration; EOF, electroosmotic flow; EP, electrophoresis.

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1. Introduction

Leakage of chlorinated solvents into limestone aquifers from contamination in overlying deposits and long-lasting back diffusion from the limestone matrix pose a threat to groundwater resources (Broholm et al., 2013; Hinsby et al., 2007; Lipson et al., 2005). In Denmark, approximately one third of the groundwater, which is used for drinking water, is extracted from limestone aquifers (Nygaard, 1993).

Assessment and treatment of groundwater contaminated with chlorinated solvents is a challenge due to the related physiochemical properties and transport mechanisms (Damgaard et al., 2013; Pankow et al., 1996). Dechlorination of PCE and TCE often accumulates the intermediate cis-DCE due to absence of the specific degraders *Dehalococcoides* (*Dhc*) (Lee et al., 2013). In low permeable media, such as the limestone matrix, one relevant remediation technique is enhanced reductive dechlorination (ERD), where a fermentable carbon source, e.g. lactate, is injected together with a bacterial culture containing *Dhc* carrying the *vcrA* gene to create optimal conditions for biological degradation of chlorinated solvents to the non-harmful compounds ethene and ethane (Scheutz et al., 2008, 2010). Transport into the finest pores by advection-based techniques such as injection in boreholes and cross recirculation is slow due to diffusion limited transport in the matrix. This delays establishment of the essential contact between the contaminant and the bioremediation amendments; electron donor and degrading bacteria (Lipson et al., 2005; Scheutz et al., 2010; Venkatraman et al., 1998).

Electrokinetics (EK) has shown potential for counteracting the transport limitations in low permeable media, such as clay, by application of a low-level direct current (DC) (Mao et al., 2012; Reddy, 2013; Reynolds et al., 2008; Wu et al., 2012). The unique transport processes offered by EK include electromigration (EM)—transport of charged ions, electro-osmotic flow (EOF)—transport of pore fluid, and electrophoresis (EP)—transport of charged colloids (Reddy, 2013; Acar et al., 1997; Alshawabkeh, 2009) (Fig. 1). In addition, electrolysis occurs at the electrodes, generating an aerobic and acidic environment at the anode, whereas reduced and alkaline conditions evolve at the cathode (Acar and Alshawabkeh, 1993; Mosavat et al., 2012). Such conditions can be severe for the strict anaerobic bacteria *Dhc* (DeFlaun and Condee, 1997; Dennis et al., 2008; Parsons Coporation, 2014).

Generally, EM is the dominating transport mechanism in an electric field (Reddy, 2013; Acar and Alshawabkeh, 1993; Arnerdal and Neretnieks, 2002). Rates of EM from 10 to 300 times that of mass transport by EOF have been observed in clay (Acar and Alshawabkeh, 1993; Arnerdal and Neretnieks, 2002). In limestone, it is uncertain whether EOF can be established (Alam et al., 2010). EP is often a minor contributor to the overall transport (Reddy, 2013).

A new technology, EK-BIO, combines enhanced reductive dechlorination and EK to attain more effective bioremediation in low permeable media (Alshawabkeh, 2009; Gill et al., 2014). The chlorinated solvents can be transformed electrochemically at the cathode to chloride ions and ethane (Mao et al., 2012) or form positively charged ion pairs with protons present in the dissolved phase (Chen et al., 2002). Thus, chlorinated solvents can be subject to EM (Mao et al., 2012; Chen et al., 2002).

Most bacterial strains carry a weak negatively charged cell surface at near neutral pH causing EP towards the anode (DeFlaun and Condee, 1997; Acuña et al., 2012; Tahmasbian and Nasrazadan, 2001). However, the bacteria can also be subject to advection in the EOF in the opposite direction of EP (Mao et al., 2012; Lee and Lee, 2001). Similarly, EM of the electron donor lactate can be retarded by EOF, but the negative charge of lactate is capable of establishing a net movement towards the anode (Mao et al., 2012; Wu et al., 2012, 2007). At bench scale, EK has demonstrated net transport rates of lactate in clay of 1.8–3.7cm d^{-1} (Mao et al., 2012; Wu et al., 2012, 2007). In sand, rates for lactate of 5.0 cm d^{-1} have been observed (Wu et al., 2007). Bench scale studies of EK-BIO in clay have demonstrated promising results and a full scale remediation is currently operating in Denmark (Mao et al., 2012; Wu et al., 2012; NIRAS, 2012).

Few field scale studies have been published on ERD of chlorinated solvents in limestone (Landry and Stone, 2005; Riis et al., 2010, 2014). The main objective of this study is to design an experimental set-up for assessment of the potential for EK to deliver lactate and the culture KB-1® into limestone contaminated with cis-DCE. This is the first study of EK-BIO in limestone. For the design and assessment, several supplementary techniques have been developed in this study for handling of the limestone cores, e.g. sampling of intact bryozoan limestone cores, saturation and contamination of cores under vacuum conditions, and suspension of limestone for analytical procedures.

2. Materials and methods

2.1. Design of experimental set-up

The experimental set-up was carefully developed and adapted to assess the application of EK-BIO in bryozoan limestone while meeting the special needs of the bacteria. The different compartments included in the experimental set-up and the major considerations behind the decisions on the final design are presented in the following. A comprehensive description is available in Hansen and Nedergaard (2014).

The limestone reactor was an acrylic cylinder with an inner diameter of 10.3 cm and a length of 20.3 cm (Fig. 2). The cylindrical shape was chosen to minimize boundary effects and facilitate the collection of intact limestone. The cores were wrapped in self vulcanizing tape to prevent boundary effects. Attached to each end of the cylinder were the acrylic electrode reservoirs with an inner diameter of 10.3 cm and a length of 5 cm resulting in working volumes of approximately 0.4 L. Membranes, consisting of fabric and filter paper, were placed at the limestone core-electrode reservoir interface. Platinum coated titanium electrodes with a working area of 0.5 cm^2 were placed in the electrode reservoirs and connected to a power supply regulated to a constant current of 39 mA (0.5 mA cm^{-2}). Another two electrodes were installed horizontally in the limestone matrix approximately 4 cm from the membranes, serving as voltage measurement ports. Fermentation locks were installed on top of the electrode reservoirs to allow for release of gasses produced at the electrodes due to electrolysis.

A mixing reservoir serving for neutralization of pH was connected to the reactor set-up through four connections and was magnetically stirred continuously in 24 h operation. The system solution had a working volume of 10 L and contained the donor lactate (10g L^{-1}) and KH_2PO_4 (0.05 g L^{-1}), K_2HPO_4 (0.10 g L^{-1}) and $(NH_4)_2SO_4$ (0.50 g L^{-1}) to ensure availability of N and P for the bacteria. For the recirculation of the system solution, peristaltic pumps with a flow rate of 25 ml min^{-1} were applied to pump from the mixing reservoir into the two electrode reservoirs and back from the electrode reservoirs into the mixing reservoir. Keeping the conditions in the system anaerobic was important to ensure proper conditions for the strict anaerobic bacteria *Dhc*. Therefore, this key issue was carefully considered when designing the different components of the experimental set-up. All tubes and couplings were designed to fit tightly to prevent oxygen intrusion and all air filled compartments were flushed with nitrogen prior to assembling the experimental set-up.