Influence of electron donors and acceptors on the bioremediation of soil contaminated with trichloroethene and nickel: laboratory- and pilot-scale study

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Abstract Laboratory- and pilot-scale studies were conducted in order to adjust and optimize the in-situ conditions for bioremediation of a soil contaminated with trichloroethene (TCE) and nickel. Results from laboratory studies showed that the indigenous microorganisms of the soil were limited by the type of electron donor. A better TCE dechlorination was obtained when the electron donor was composed of a mixture of methanol and lactate, as compared to that with methanol alone. Addition of up to 10 mM of sulphate as external electron acceptor (in combination with TCE) and with a mixture of methanol and lactate as electron donor had no significant effect on the TCE reducing activity of indigenous microorganisms of the soil, while higher concentrations (15 and 20 mM) yielded a lower dechlorination. Long term operation of a large pilot-scale soil reactor demonstrated the feasibility of a single-process in situ soil remediation. Results showed that, on one hand, TCE was progressively and stepwise reduced to cis-dichloroethene (DCE), vinyl chloride (VC) and finally to ethene, using only the indigenous microorganisms of the soil. On the other hand, stimulating the activity of sulphate-reducing bacteria of the soil with the addition of sulphate as electron acceptor was efficient in precipitating nickel as nickel sulphide.

Keywords Anaerobic reductive dechlorination; chlorinated ethenes; co-substrate; electron donors; lactate; methanol; reducing equivalents

Introduction Chlorinated ethenes, such as trichloroethene (TCE), have been used extensively as industrial solvents and they have become common and persistent soil pollutants throughout Europe. Since soils contaminated with chloroethenes often cannot be approached directly due to the presence of buildings or commercial activities, in situ bioremediation is an attractive and cost effective technology suitable for the removal of such pollutants. The dechlorination process in which TCE acts as a terminal electron acceptor for selected anaerobic bacteria is called halorespiration. Complete dechlorination of TCE all the way to ethene and/or ethane can be done either by a variety of microorganisms acting in concert or even by a single bacterium, Dehalococcoides ethenogenes (Fathepure et al., 1987; Mohn and Tiedje, 1992; Holliger et al., 1993; El Fantroussi et al., 1998; Horber et al., 1999; Flynn et al., 2000; Maymo-Gatell et al., 2001). Different organic compounds acting as electron donors such as complex organic materials, lactate, methanol, ethanol, propionate, butyrate and hydrogen have been used to sustain dechlorination activity (Fennell and Gossett, 1997; Gerritse et al., 1999). However, the degree of dechlorination differs by several orders of magnitude depending on the type of electron donors used and varies from one site to another. These observations point out the influence of microbial environments on the dechlorination process. We have studied both the influence of methanol and lactate as electron donors and that of sulphate as an alternative electron acceptor on the indigenous microorganisms of a soil industrially polluted with TCE and nickel. After that, a demonstration at a sufficiently large pilot-scale reactor was conducted.
to define a single-process bioremediation strategy to treat such a soil contaminated with TCE and nickel.

**Materials and methods**

**Microcosm batch tests**

In order to evaluate the rate of the microbial dechlorination process, a series of microcosm batch tests were conducted. For this, 20 grams of soil from the contaminated site (province of Utrecht, The Netherlands) were placed in 250 ml glass bottles with 100 ml of nutrient solution. The latter contained in mg/L: KH₂PO₄, 1.02; K₂HPO₄, 1.31; NH₄HCO₃, 13; MgSO₄.7H₂O, 1.54. A trace metal solution devoid of chloride (containing in mg/L: FeSO₄.7H₂O, 195; MgSO₄.7H₂O, 307; MnSO₄.H₂O, 39; CoSO₄.7H₂O, 15; (NH₄)₆Mo₇O₂₄.4H₂O, 12; ZnSO₄.7H₂O, 12; CuSO₄, 4; H₃BO₃, 3; Na₂SeO₃.5H₂O, 1) was added at 10 mL/L of the nutrient solution. The bottles were then flushed with nitrogen gas for 20 minutes and sealed with a Teflon rubber stopper. TCE was subsequently added to a final concentration of about 10 mg/L. The bottles were incubated at 20°C and 150 rpm on a rotary shaker.

**Reactor design**

The pilot-scale reactor used in this study consist in a 680 L stainless steel parallelepiped. The inside of the reactor was coated with a Teflon film to avoid the adsorption of pollutants. The reactor was composed of a soil chamber and two liquid zones (wells) situated at its inlet and outlet. These wells, which allowed liquid homogenization, were separated from the soil chamber by a grid permeable only to the liquid feed solution. A horizontal flow system was chosen to simulate the plume flow. The nutrient solution was fed to the reactor by gravity and an ultrasonic probe controlled the liquid level inside the reactor.

**Analytical methods**

Measurements of TCE, DCEs, VC and ethene either in the gas phase or in the liquid phase were performed by gas chromatography (GC) (Shimadzu GC-14A) equipped with a flame ionization detector (FID) and CPSil 5 CB capillary column. Nitrogen was used as a gas carrier at a rate of 8 mL/min. The column temperature program was 40°C for 5 min ramped to 120°C at 10°C/min and held for 5 min. The injector and detector temperatures were 240 and 250°C, respectively. For aqueous phase determinations, 5 mL of liquid sample were added to 60 mL serum bottles, capped with a Teflon rubber septum and aluminium crimp cap. The serum bottles were then heated in a 80°C water bath for one hour to release dissolved gas. Following equilibration between the aqueous phase and headspace, a headspace sample of 100 µL was injected onto the GC. Compounds were identified by comparison of their retention times with those of external standards. Sulphate, sulfide and chemical oxygen demand (COD) were determined according to *Standard Methods* (APHA, 1998).

**Results and discussion**

**Laboratory studies**

Influence of methanol and lactate. A series of batch microcosms (250 mL bottles) were incubated using soil from the contaminated site. Results showed that indigenous microorganisms of the site are active in TCE dechlorination. When methanol (100 mg COD/L) was used as the only electron donor, the anaerobic dechlorination process of TCE was very slow since 40 days of incubation were necessary for complete dechlorination of TCE (Figure 1A). However, with 100 mg COD/L of a methanol and lactate mixture (1:1, g COD:g COD), TCE was completely dechlorinated within 15 days and the degree of dechlorination was
higher (Figure 1B). Based on these data, a mixture of methanol and lactate was thus selected as an electron donor in order to sustain TCE dechlorination.

The influence of different methanol and lactate concentrations on TCE dechlorination showed that dechlorination was rather stimulated when the concentration of the co-substrate mixture was in the range of 100–1,000 mg COD/L, as compared to that with higher COD concentrations of 2,000–5,000 mg/L (Figure 2). The results are consistent with the hypothesis that electron donor precursors fed at a low concentration, thus generating presumably a slow and steady release of hydrogen, allow halorespirators to compete better against the other hydrogen-consuming bacteria (Yang and McCarty, 1998).

**Influence of sulphate.** A series of batch microcosms were set up in order to assess the influence of sulphate as an alternative electron acceptor on TCE dechlorination. The concentration of electron donor precursors (mixture of methanol and lactate) was fixed at 1,000 mg COD/L. Results obtained after 50 days of incubation showed that up to 10 mM sulphate had

![Figure 1 Influence of electron donors on TCE dechlorination in batch microcosms. A) methanol. B) methanol and lactate](image1)

![Figure 2 Influence of co-substrate mixture concentrations on TCE dechlorination in batch microcosms](image2)
no significant effect on the TCE reducing activity of indigenous microorganisms of the soil (Figure 3). However, when higher concentrations (15 and 20 mM) were used a decrease in the dechlorination process was observed, as indicated by the lower portion of vinyl chloride (VC) and ethene produced.

Liquid analysis at the end of the experiments (day 50) showed that sulphate was completely used in microcosms amended with up to 5 mM, whereas a residual sulphate was found in those amended with either 10, 15 or 20 mM (Table 1). A positive correlation was observed between the amount of sulphate added and that of sulfide produced. Furthermore, COD removal was adversely affected in microcosms amended with high amount of sulphate, reaching 76% when the added sulphate concentration was 20 mM, as compared to COD removal of 88–90% in microcosms amended with up to 10 mM (Table 1). Apparently, this high sulfide accumulation partly inhibited dechlorinating as well as methanogenic bacteria, thus yielding a poor TCE dechlorination and a lower COD removal. This study indicated that sulphate-reducing and dechlorinating activities may occur simultaneously when the amount of sulphate is lower than 10 mM.

**Pilot-scale demonstration**

The pilot-scale reactor was filled with 1,100 kg of soil from the contaminated site. The soil apparent specific weight and porosity were 1.9 g/mL and 36.2% (v/v), respectively. The organic matter content of the soil was 0.25% (w/w). The net liquid volume of the pilot reactor was 216 L. Electron donors used consisted of a mixture of methanol and lactate (1:1, g COD/g COD). Inlet TCE and nickel were fixed, respectively, at 10 and 1 mg/L, matching the plume data at the site. The pilot reactor was operated at a hydraulic retention time of 7 days and at liquid flow velocity of 1 m/d. During the first 6 months of operation, when the inlet COD concentration was 1,000 mg/L, TCE was only dechlorinated to cis-dichloroethene (DCE) (Figure 4). After the concentration of inlet COD decreased from 1,000 down to 100 mg COD/L, cis-DCE was further dechlorinated to VC and ethene, as a result of a low

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**Figure 3** Influence of sulphate concentrations on TCE dechlorination in batch microcosms

**Table 1** Sulphate, aqueous sulfide and COD removal in batch microcosms

<table>
<thead>
<tr>
<th>Initial sulphate concentration in microcosms (mM)</th>
<th>0.1</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual sulphate (mM)</td>
<td>0</td>
<td>0</td>
<td>1.1</td>
<td>2.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Aqueous sulfide (mM)</td>
<td>0.045</td>
<td>2.1</td>
<td>3.9</td>
<td>4.7</td>
<td>6.2</td>
</tr>
<tr>
<td>COD removal (%)</td>
<td>90</td>
<td>89</td>
<td>87</td>
<td>80</td>
<td>76</td>
</tr>
</tbody>
</table>
hydrogen production (Yang and McCarty, 1998). Mass balance after 450 days of operation showed that cis-DCE remained the main accumulated metabolite, representing 71% of added TCE, while VC and ethene represented, respectively, 7 and 2%, yielding a total recovery of 80%. The operation of the pilot reactor is still going on and a longer incubation period appears to favor adaptation and growth of cis-DCE halorespirators.

Addition of 0.1 mM of sulphate to the feed solution promoted the growth of sulphate-reducing bacteria. This led to the production of sulfide which was efficient in sequestering nickel since more than 92% of nickel added to the pilot reactor was precipitated within the soil (Figure 5).

**Dechlorination specific activities**

In order to evaluate the rate of dechlorination activity during the course of pilot reactor operation, microcosm batch tests were conducted by incubating a soil sample from the pilot reactor at regular time intervals. Such tests are illustrated by the data from a microcosm on a soil sample taken from the reactor on day 420. As shown in Figure 6, this sample performed a rapid and complete dechlorination of TCE to less chlorinated metabolites such as cis-DCE, VC and ethene. Dechlorination of TCE can be divided into two steps: a high-rate and stoichiometric transformation of TCE to cis-DCE followed by a rate-limiting dechlorination step of cis-DCE to both VC and ethene. The rate of these two steps seem to be at least 44 nmoles/g_soil.d and 7 nmoles/g_soil.d, respectively. An abiotic dechlorination test was also conducted by incubating an autoclaved soil sample taken from the pilot reactor. This test confirmed that abiotic phenomena did not contribute to TCE dechlorination (data not shown).

**Conclusions**

Laboratory studies showed that electron donors composed of a mixture of methanol and lactate was more efficient than methanol alone in sustaining TCE dechlorination. TCE dechlorination was found to be favored when the co-substrate COD concentration was low (i.e. 100 mg/L). This result is consistent with the hypothesis that electron donor precursors...
fed at a low concentration, thus generating a slow and steady release of hydrogen, allow halorespirators to compete better against the other hydrogen-consuming bacteria. Up to 10 mM sulphate had no significant effect on the TCE reducing activity of indigenous microorganisms of the soil, while higher concentrations (15 and 20 mM) yielded a lower dechlorination, as a result of sulfide accumulation. The pilot study demonstrated the feasibility of a single-process in situ remediation of a soil contaminated with TCE and nickel. Dechlorination of TCE can be divided in a high-rate dechlorination step to cis-DCE followed by a rate-limiting dechlorination step of cis-DCE to VC and ethene. Addition of sulphate in the feed solution promoted the growth of sulphate-reducing bacteria. This led to the production of sulfide and subsequent precipitation of nickel as nickel sulfide within the soil. This project pointed out the importance of defining an appropriate strategy to develop indigenous mixed populations in order to clean up soils industrially polluted by several contaminants. Implementation of this in situ soil remediation technology on the field scale is currently under way.

References


