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Aerobic/anaerobic/aerobic sequenced biodegradation of a mixture of chlorinated ethenes, ethanes and methanes in batch bioreactors

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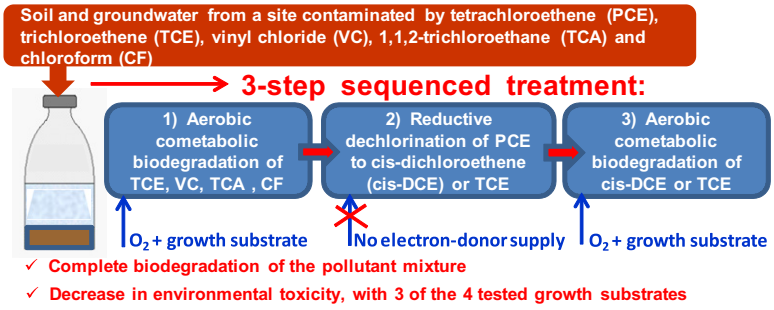
HIGHLIGHTS

- Aerobic/anaerobic/aerobic treatment biodegrades complex chlorinated solvent mixture.
- First aerobic step: low- and medium-chlorinated solvents degraded by cometabolism.
- Anaerobic step: tetrachloroethene degraded to cis-dichloroethene or trichloroethene.
- Second aerobic step: cis-dichloroethene or trichloroethene degraded by cometabolism.
- The anaerobic step is likely fed with dead cells grown during the aerobic step.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel aerobic/anaerobic/aerobic treatment was implemented in batch reactors containing aquifer materials from a site contaminated by tetrachloroethylene (PCE), trichloroethylene (TCE), vinyl chloride (VC), 1,1,2-trichloroethane (1,1,2-TCA) and chloroform (CF). Consortia grown aerobically on methane, propane, *n*-pentane and *n*-hexane completely biodegraded the chlorinated solvent mixture, via aerobic cometabolism of VC, CF, TCE and 1,1,2-TCA, followed by PCE reductive dechlorination (RD) to 1,2-cis-dichlorothylene (cis-DCE) or TCE, and cis-DCE/TCE cometabolism in a further aerobic phase. *n*-Hexane was the best substrate. No electron donor was supplied for RD, which likely utilized cellular material produced during the aerobic phase. Chloride release was stoichiometric with chlorinated solvent biodegradation. According to the *Lepidium sativum* ecotoxicity test, a decreased toxicity was observed with propane, *n*-pentane and *n*-hexane, but not methane. A kinetic study of PCE RD allowed to estimate the PCE maximum specific rate (0.57 ± 0.07 mg $mg^{-1}_{protein}$ day^{-1}) and half-saturation constant (6.7 ± 1.5 mg L^{-1}).

1. Introduction

Chlorinated aliphatic hydrocarbons (CAHs) can be biodegraded through aerobic cometabolic oxidation, direct metabolic oxidation and anaerobic reductive dechlorination (RD). Aerobic cometabolism allows the rapid transformation of the vast majority of CAHs by bacteria grown on methane, propane (Frascari et al., 2006a), butane (Ciavarelli et al., 2012), ammonia (Kocamemi and Çeçen, 2009), benzene, toluene, and xylene (Wu et al., 2008), vinyl chloride (VC) (Mattes et al., 2010), and phenol (Hopkins and McCarty, 1995). Aerobic cometabolism presents inherent limits, such as competition between CAHs and primary substrate, the toxic effect of some metabolites and the risk that an excessive microbial growth clogs the aquifer porosity (Tiehm and Schmidt, 2011). Although the pulsed injection of growth substrate can partly

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Notation

c_{CAH} aqueous phase concentration of a given CAH (mg L^{-1})
$k_{1,CAH}$ first-order constant of net biodegradation of a given CAH (L $mg^{-1}_{protein}$ day^{-1})
$k_{CAH,abiotic}$ first-order constant of abiotic depletion of a given CAH (day^{-1})
$k_{max,PCE}$ PCE maximum specific biodegradation constant (mg $mg^{-1}_{protein}$ day^{-1})
$K_{s,PCE}$ PCE half-saturation constant (mg L^{-1})
PCE/S PCE mass dechlorinated during an anaerobic phase/mass of growth substrate consumed during the preceding aerobic phase (μg_{PCE} $mg^{-1}_{substrate}$)
r_{CAH} initial net biodegradation rate of a given CAH (mg L^{-1} day^{-1})
$\overline{r_{CAH}/c_{CAH}}$ ratio of the initial net aerobic biodegradation rate of a given CAH to the corresponding initial CAH concentration: average value obtained in the last 2 CAH pulses of the G1 tests (day^{-1})
$\overline{r_{PCE}/c_{PCE}}$ ratio of the initial net PCE dechlorination rate to the corresponding initial PCE concentration: average value obtained in the last 2 PCE pulses of each anaerobic phase of the G2 and G3-a tests (day^{-1})
X cell concentration ($mg_{protein}$ L^{-1})
X_{ta} concentration of total aerobic biomass in the G3-b microcosms at the end of the first aerobic phase ($mg_{protein}$ L^{-1})
$Y_{X/S}$ biomass growth yield on the generic substrate S ($g_{protein}$ g_s^{-1})

overcome such drawbacks (Frascari et al., 2012; Goltz et al., 2001), aerobic cometabolism is rarely utilized in full scale applications of CAH bioremediation. Direct aerobic metabolism is attractive, as it does not require an external growth substrate and it does not produce toxic intermediates (Mattes et al., 2010). However, only VC, cis-1,2-dichloroethylene (cis-DCE), dichloromethane and 1,2-dichloroethane were shown to be biodegradable through direct oxidation (Mattes et al., 2010; Miyake-Nakayama et al., 2006). RD represents the bioremediation approach that found the largest application for CAH-contaminated sites (Tiehm and Schmidt, 2011). The complete tetrachloroethylene (PCE) dechlorination to ethene can only be performed by strains belonging to the genus *Dehalococcoides* (Aulenta et al., 2006; Tiehm and Schmidt, 2011). Although *Dehalococcoides* are common in PCE-contaminated sites, they are very sensitive to oxygen and less robust than other halorespirers (Tiehm and Schmidt, 2011). Thus, several studies report an incomplete PCE dechlorination, with accumulation of cis-DCE and VC (Bradley, 2003; Sharma and McCarty, 1996).

In the frequent cases of sites contaminated by PCE and/or trichloroethylene (TCE), an interesting alternative is represented by sequential anaerobic/aerobic treatments, typically characterized by a first anaerobic zone where PCE and/or TCE are dechlorinated to cis-DCE, followed by an aerobic zone where cis-DCE is oxidized. cis-DCE oxidation generally occurs via aerobic cometabolism, as cis-DCE-growing bacteria are rare (Mattes et al., 2010). This alternative, appealing for the elimination of the risk of accumulating a carcinogenic and volatile compound as VC, was successfully applied in several studies (Devlin et al., 2004; Guiot et al., 2008; Tiehm and Schmidt, 2011).

In this work, an alternative aerobic/anaerobic/aerobic sequential treatment was implemented in batch bioreactors containing aquifer materials sampled from an Italian site contaminated by a mixture of PCE, TCE, VC, 1,1,2-trichloroethane (1,1,2-TCA) and chloroform (CF). The idea of the 3-step process derived from preliminary experiments which showed that cells or metabolites produced as a result of growth substrate uptake during the initial aerobic step can be utilized as electron donors by the RD process. The addition of the initial aerobic step can thus avoid the need to supply a specific electron donor for the anaerobic phase. Thus the entire process can be fed with a single substrate, with a consequent simplification in terms of reagents, equipment and analytical procedures.

The general goal of this work was to perform an evaluation of the applicability of the above-described 3-step process to the studied site. More specifically, this research was aimed at (i) identifying the most suitable primary substrate for the aerobic phases; (ii) evaluating the possible selection of PCE-degrading aerobic strains; (iii) testing the effectiveness of bioaugmentation; (iv) testing the feasibility of feeding the RD process with metabolites or cells accumulated during the aerobic phase. Methane, propane, *n*-pentane and *n*-hexane were tested as aerobic growth substrates. While *n*-pentane was never tested before as a substrate for CAH aerobic cometabolism, the use of *n*-hexane as a cometabolic substrate was investigated only in one previous study (Frascari et al., 2006b), to the best of the authors' knowledge.

2. Methods

2.1. Experimental scheme and microcosm operation

The experimental work was divided into four groups of 119-mL batch bioreactors for a total of 32 experimental conditions, each studied in duplicate. An overview of the experimental scheme, with the goals of each microcosm group, is provided in Table 1, and the main characteristics of the four microcosm groups are described in this section. A detailed description of each experimental condition is reported in Tables S1and S2 in the Supplementary data. All the tests were sealed with Teflon-lined rubber septa and maintained in agitation in an orbital shaker (180 rpm) at 25 °C.

The first three microcosm groups consisted of 119-mL slurry bioreactors (dry soil 19.7 g, groundwater 50.3 mL). Soil and groundwater were sampled in an Italian site contaminated by a 5-CAH mixture (average groundwater concentrations, in mg L^{-1}: VC 0.7, CF 0.16, TCE 0.36, 1,1,2-TCA 0.18, PCE 0.52). Other site characteristics are reported in Table S2. The contamination affects mainly the shallow aquifer, a 5-m sandy layer characterized by a low groundwater velocity (20–40 m y^{-1}).

The first group of slurry microcosms (G1) was aimed at evaluating the possibility to biodegrade the 5-CAH mixture via aerobic cometabolism, with (sub-group G1-b) or without (G1-a) initial bioaugmentation with 2 CAH-degrading exogenous consortia (see Table S1 for details). Methane, propane, *n*-pentane and *n*-hexane were tested as growth substrates. As a result of the gas-phase partitioning of the CAHs initially present in soil and groundwater, the initial CAH aqueous concentrations were (mg L^{-1}; average ±95% confidence interval): VC 0.350 ± 0.038; CF 0.120 ± 0.012; TCE 0.190 ± 0.030; 1,1,2-TCA 0.100 ± 0.015; PCE 0.200 ± 0.022. The G1 tests were exposed to consecutive pulses of oxygen (9 mg) and growth substrate. After the complete biodegradation of the initial amounts of VC, CF, TCE and 1,1,2-TCA, 3–4 further pulses of the CAH mixture were supplied, in order to evaluate possible