Journal of Hazardous Materials 340 (2017) 417–426

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

In-situ biodegradation potential of 1,2-DCA and VC at sites with different hydrogeological settings

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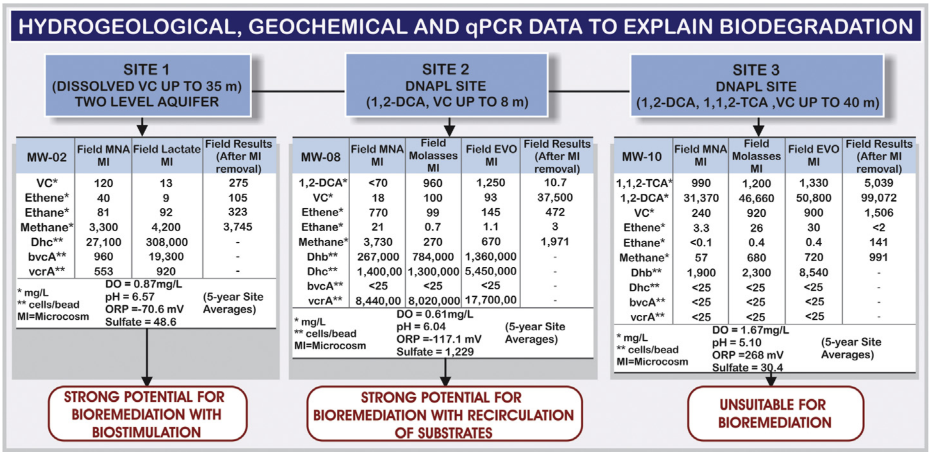
HIGHLIGHTS

- The biodegradation potential of 1,2-DCA and VC is proposed at three field sites.
- Site-Specific Limiting Factors are presented to Biodegradation of 1,2-DCA and VC.
- Hydrogeological, geochemical and qPCR data are presented to explain biodegradation.
- *Dehalococcoides* proved to be a key dechlorinator to VC and chlorinated ethanes.
- Biodegradation is a viable strategy to chlorinated ethanes and VC.

GRAPHICAL ABSTRACT



MW-02	Field MNA MI	Field Lactate MI	Field Results (After MI removal)
VC*	120	13	275
Ethene*	40	9	105
Ethane*	81	92	323
Methane*	3,300	4,200	3,745
Dhc**	27,100	308,000	-
bvcA**	960	19,300	-
vcrA**	553	920	-

* mg/L
** cells/bead
MI=Microcosm
DO = 0.87mg/L
pH = 6.57
ORP =-70.6 mV
Sulfate = 48.6
(5-year Site Averages)

MW-08	Field MNA MI	Field Molasses MI	Field EVO MI	Field Results (After MI removal)
1,2-DCA*	<70	960	1,250	10.7
VC*	18	100	93	37,500
Ethene*	770	99	145	472
Ethane*	21	0.7	1.1	3
Methane*	3,730	270	670	1,971
Dhb**	267,000	784,000	1,360,000	-
Dhc**	1,400,00	1,300,000	5,450,000	-
bvcA**	<25	<25	<25	-
vcrA**	8,440,00	8,020,000	17,700,00	-

* mg/L
** cells/bead
MI=Microcosm
DO = 0.61mg/L
pH = 6.04
ORP =-117.1 mV
Sulfate = 1,229
(5-year Site Averages)

MW-10	Field MNA MI	Field Molasses MI	Field EVO MI	Field Results (After MI removal)
1,1,2-TCA*	990	1,200	1,330	5,039
1,2-DCA*	31,370	46,660	50,800	99,072
VC*	240	920	900	1,506
Ethene*	3.3	26	30	<2
Ethane*	<0.1	0.4	0.4	141
Methane*	57	680	720	991
Dhb**	1,900	2,300	8,540	-
Dhc**	<25	<25	<25	-
bvcA**	<25	<25	<25	-
vcrA**	<25	<25	<25	-

* mg/L
** cells/bead
MI=Microcosm
DO = 1.67mg/L
pH = 5.10
ORP =268 mV
Sulfate = 30.4
(5-year Site Averages)

ARTICLE INFO

Article history:
Received 5 May 2017
Received in revised form 12 July 2017
Accepted 13 July 2017
Available online 15 July 2017

Keywords:
Bioremediation
1,2 Dichloroethane
Vinyl chloride
qPCR
Field data
Dehalococcoids

ABSTRACT

This paper investigates the feasibility of applying in-situ Bioremediation (ISB) to three sites contaminated with vinyl chloride and/or chlorinated alkanes such as 1,2-DCA and 1,1,2-TCA, presenting distinct hydrogeological settings and history of contaminant loading. Biotransformation of these compounds is well established in laboratory studies and pure cultures. Due to confidential aspects, however, few field data are available to support real case studies to the predictability of their fate and lifetime in soil and groundwater. Bio-Trap® In Situ Microcosm (ISM) studies were performed in selected monitoring wells, and consisted of a control unit which simulated Monitored Natural Attenuation conditions and other units which were amended with either lactate, emulsified vegetable oil (EVO) or molasses as electron donors. For wells with moderate *Dhc* counts, the ISM study demonstrated that electron donor addition could stimulate further growth of *Dhc* and enhance reductive dechlorination. Conversely, for wells with high population counts, substrate addition did not alter results significantly. Site-specific determining factors that most influenced the biodegradation results were microbial activity, soil texture and presence of organic matter, site pH, redox conditions and presence of free phase.

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http://dx.doi.org/10.1016/j.jhazmat.2017.07.035
0304-3894/

1. Introduction

The widespread use of chlorinated alkanes such as 1,1,2-trichloroethane (1,1,2-TCA) and 1,2 dichloroethane (1,2-DCA or EDC) in industrial processes, their relative recalcitrance in the subsurface and adverse health effects has led to an increased interest in cost-effective remediation approaches for these contaminants. 1,1,2-TCA is used as a chemical intermediate and a solvent and is primarily used in the production of 1,1-dichloroethene. 1,2-DCA is used as a precursor in the manufacture of vinyl chloride (VC), which in turn is used to make polyvinyl chloride (PVC) plastic and vinyl products. VC is characterized as a human carcinogen (Group A) by USEPA whereas 1,2-DCA and 1,1,2-TCA as probable (Group B2) and possible (Group C) human carcinogen, respectively by USEPA.

The biotransformation of vinyl chloride and chlorinated alkanes such as 1,2-DCA and 1,1,2-TCA is well documented. As a pure phase, these compounds are dense non-aqueous phase liquids (DNAPLs) with high solubility in relation to current environmental standards. The alkanes 1,2-DCA and 1,2-TCA can be degradable under aerobic, anaerobic and cometabolic conditions [1]. The aerobic transformations are usually biotic and occur through oxidation. The oxidation products are not easily detectable in the field as they are readily degraded. The anaerobic biotic processes generally are reductions that involve either hydrogenolysis – reductive dechlorination, the substitution of a chlorine atom from the molecule by a hydrogen atom, or dihalo-elimination, where two adjacent chlorine atoms are removed, leaving a double bond between the respective carbon atoms to form ethene, which may then undergo hydrogenolysis to ethane [2]. Dihaloelimination of 1,2-DCA directly to ethene is the dominant mechanism for DCA degradation and can be mediated by a variety of micro-organisms such as the *Dehalococcoides*, *Dehalobacters*, *Dehalogenimonas*, *Desulfitobacterium* and others (Fig. 1). Although abiotic transformations are slow, these are significant within time scales commonly associated with groundwater movement and remediation. 1,2-DCA, for instance, undergoes abiotic reductive dechlorination to VC and has been observed in the field in a faster rate than previously expected [3]. In addition, during an isotope fractioning study using *Dehalococcoides* strains, Schmidt et al. [4] suggest that VC is a minor side product resulting from branching of the 1,2-DCA dihaloelimination to ethene, in which both end-products are yielded simultaneously into the aquifer. Maymó-Gatell et al. [5] also observed small amounts of VC produced from 1,2-DCA, indicating a dehydrochlorination reaction.

Many types of microorganisms have been shown to use 1,2-DCA and 1,1,2-TCA as a terminal electron acceptor: *Dehalobacter* [6], *Desulfitobacterium* [6–9], *Dehalogenimonas* [10–12], and *Dehalococcoides* reported in the case of 1,2-DCA only [4,5,13,14]. Maymó-Gatell et al. [5,13] originally described the *Dehalococcoides mccartyi* strain 195 (formerly *Dehalococcoides* ethenogens strain 195) and demonstrated that this bacterial strain was able to dihalo eliminate 1,2-DCA as a sole electron acceptor. In the experiment, ethene was the primary product of 1,2-DCA utilization and VC production from 1,2-DCA was approximately 1% of the ethene production (a dehydrochlorination reaction), with no monochloroethane detected in cultures utilizing 1,2-DCA. *Dehalococcoides*, *Dehalobacters* and *Dehalogenimonas* isolates are highly specialized bacteria that strictly depend on organohalide respiration for growth, in most cases coupled to hydrogen as the sole electron donor. They present slow growth rates, and are often maintained and studied in microbial consortia for H_2 supply. The *Geobacter* and *Desulfitobacterium* genera, on the other hand, are relatively easy to culture and more metabolically versatile in relation to electron donors and acceptors [15].

Electron acceptors are frequently the limiting resource as well as a major determinant of species composition of anaerobic communities such as the ones described above. Complete dechlorination of alkanes into nontoxic end products such as ethane and ethene is believed to be faster and more likely to occur under methanogenic conditions [2]. Although methanogens compete with *Dehalococcoides* and can inhibit reductive dehalogenation, there are some exceptions. Methanogenesis is required for degradation in most cases as methanogens simultaneously metabolize natural substrates and the chlorinated organics. Moreover, the dehalogenating organisms are dependent on methanogens.

The quantification of vinyl chloride reductase genes (*bvcA* and *vcrA*) by molecular tools confirms the potential for biodegradation of VC. The *bvcA* gene encodes the vinyl chloride reductase enzyme responsible for reductive dechlorination of VC to ethene by *Dhc spp*. strain BAV1 [16]. The *vcrA* gene encodes the vinyl chloride reductase enzyme responsible for reductive dechlorination of VC by *Dhc spp*. strain VS.

Most studies related to the biotransformation of VC and chlorinated alkanes such as 1,2-DCA and 1,1,2-TCA focus on individual groups of bacteria in pure cultures or laboratory studies. Due to confidential aspects, few field data are available to be used in real case studies to the predictability of fate and lifetime of contaminants in soil and groundwater [3,17–20]. In this regard, this paper investigates the feasibility of applying in-situ Bioremediation (ISB) to three contaminated sites, located in Brazil, with distinct hydrogeochemical settings and history of contaminant loading. These sites share in common the fact of being mostly impacted by chlorinated ethanes (1,2-DCA, 1,1,2-TCA) and/or vinyl chloride. Multiple lines of evidence were used to document the potential for natural attenuation and biostimulation of these compounds. An appropriate field monitoring program was designed, in addition to source control measures, to accelerate the overall remediation process. For the biostimulation pilot system design, environmental molecular diagnostics were used. This method proved to be a useful tool for assessing the efficiency of engineered bioremediation, in identifying specific degraders of importance such as *Dehalobacter* spp. and *Dehalococcoides*, a key reductive dechlorinator and the only bacteria capable of utilizing VC as growth-supporting electron acceptor. These molecular tools, although essential to characterize microbial and understand microbially mediated processes, were associated to hydrological and geochemical data to provide a more defensible framework to explain contaminant biodegradation and its variations in the subsurface. Due to higher subsurface temperatures observed at these sites, in comparison with those in North America and Europe with temperate climates, tropical soils are more oxidized and, in general, not mineral and nutrients rich [21]. Thus, complete reductive dechlorination to non-toxic compounds may not be achieved easily. Electron donor availability is a common limitation but does not explain all. The identification of the most relevant processes or driving forces of dechlorination is an essential part of a bioremediation program.

2. Materials and methods

2.1. Study sites and hydrogeology settings

Table 1 summarizes the main aspects of the three sites where Bio-Trap® In Situ Microcosm (ISM) studies were performed in selected monitoring wells to evaluate biodegradation of source products under existing site conditions. All sites share in common the presence of VC, either as a source compound (Site 1) or a byproduct from chlorinated alkanes (Sites 2 and 3).

2.1.1. Site 1: former PVC manufacturing plant with a VC-dissolved plume

This is a groundwater contaminated site with vinyl chloride as single major contaminant. The former industrial process in this site