Journal of Hazardous Materials 268 (2014) 92–101

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Application of polycolloid-releasing substrate to remediate trichloroethylene-contaminated groundwater: A pilot-scale study

T.T. Tsai [a], J.K. Liu [b], Y.M. Chang [c], K.F. Chen [d], C.M. Kao [a,*]

[a] Institute of Environmental Engineering, National Sun Yat-Sen University, Kaohsiung, Taiwan
[b] Department of Biological Science, National Sun Yat-Sen University, Kaohsiung, Taiwan
[c] Institute of Environmental Engineering and Management, National Taipei University of Technology, Taipei, Taiwan
[d] Department of Civil Engineering, National Chi Nan University, Nantou, Taiwan

HIGHLIGHTS

- A slow polycolloid-releasing substrate for continuous carbon supplement is developed.
- The developed substrate contains vegetable oil, molasses, and two types of surfactants.
- Addition of developed substrate can create anaerobic conditions.
- Gene analysis is useful in evaluating the effectiveness of TCE biodegradation.
- Complete TCE removal is obtained after injecting the slow polycolloid-releasing substrate.

ARTICLE INFO

Article history:
Received 22 October 2013
Received in revised form
30 December 2013
Accepted 5 January 2014
Available online 8 January 2014

Keywords:
Anaerobic bioremediation
Groundwater contamination
Real-time polymerase chain reaction (PCR)
Reductive dechlorination
Slow polycolloid-releasing substrate

ABSTRACT

The objectives of this pilot-scale study were to (1) evaluate the effectiveness of bioremediation of trichloroethylene (TCE)-contaminated groundwater with the supplement of slow polycolloid-releasing substrate (SPRS) (contained vegetable oil, cane molasses, surfactants) under reductive dechlorinating conditions, (2) apply gene analyses to confirm the existence of TCE-dechlorinating genes, and (3) apply the real-time polymerase chain reaction (PCR) to evaluate the variations in TCE-dechlorinating bacteria (*Dehalococcoides* spp.). Approximately 350 L of SPRS solution was supplied into an injection well (IW) and groundwater samples were collected and analyzed from IW and monitor wells periodically. Results show that the SPRS caused a rapid increase of the total organic carbon concentration (up to 5794 mg/L), and reductive dechlorination of TCE was significantly enhanced. TCE dechlorination byproducts were observed and up to 99% of TCE removal (initial TCE concentration = 1872 μg/L) was observed after 50 days of operation. The population of *Dehalococcoides* spp. increased from 4.6×10^1 to 3.41×10^7 cells/L after 20 days of operation. DNA sequencing results show that there were 31 bacterial species verified, which might be related to TCE biodegradation. Results demonstrate that the microbial analysis and real-time PCR are useful tools to evaluate the effectiveness of TCE reductive dechlorination.

1. Introduction

Groundwater is a very valuable water resource. In Taiwan, more than 20% of all water usage come from groundwater. However, at many existing and former industrial areas and disposal sites, subsurface environment is contaminated by different types of contaminants [e.g., chlorinated ethenes (CEs)] due to the illegal dumping or accidental spills. CEs such as trichloroethylene (TCE) had been widely used as solvents for more than 50 years. As a result of heavy use of TCE, many aquifers in the industrialized countries are contaminated by TCE [1,2]. Because of its complex distribution in the subsurface, remediation of TCE-contaminated sites is a challenge. Enhanced bioremediation has been applied as a remedial strategy to cleanup TCE-contaminated aquifers [3–7].

In situ reductive dechlorination of TCE is operated under anaerobic conditions, which results in the production of daughter products including dichloroethene (DCE) isomers [(1,1-DCE), 1,2-cis-DCE (cis-DCE), 1,2-trans-DCE (trans-DCE)], vinyl chloride (VC), and ethane. The bioremediation rates of these chlorinated ethenes (CEs) can be enhanced by the supplement of soluble primary substrates [8–12], or immobile substrates, such as bark mulch, compost, and peanut shell [13,14]. Soluble substrates must be added to the aquifer frequently, which increases capital as well as operation and maintenance costs. In recent years, soluble slow-releasing liquid substrates using edible oils have been developed to release primary organic substrates, which can enhance the anaerobic reductive

* Corresponding author. Tel.: +886 7 525 2000; fax: +886 7 525 4449.
E-mail address: jkao@mail.nsysu.edu.tw (C.M. Kao).

0304-3894/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2014.01.004

dechlorination of chlorinated solvents [15–18]. These products containing significant amounts of organic carbons in the molecular structures can provide substrates for the enhancement of the reductive dechlorination of chlorinated solvents [19–22]. Soybean oil and other food-grade edible oils can provide an effective, long-lasting substrate for enhancing anaerobic biodegradation processes [14,23–25]. However, injection of edible oils into the subsurface might clog soil pores due to the large globule diameters of the oils. Thus, edible oils must be emulsified to reduce the globule diameters. Researchers developed EOS® (Edible Oil Substrate – Emulsified Soybean Oil) process to remediate chlorinated solvent contaminated groundwater [6,20,26,27]. EOS® has become widely accepted as a primary commercial treatment method by the environmental consulting/contracting industry.

Except for the injection of edible oils, more easily biodegradable carbon sources are required at the early stage of the reductive dechlorination so the biodegradation process would not be delayed. In our previous study, slow polycolloid-releasing substrate (SPRS) has been developed to continuously provide biodegradable substrates for the enhancement of TCE reductive dechlorination [28]. The produced SPRS contained vegetable oil (used as slow-released substrate), cane molasses [used as early-stage (fast-degradable) substrate] and surfactants [Simple Green™ (SG) and soya lecithin (SL)]. The SPRS with a uniformly small droplet size (0.93 μm for D_{10}) is able to continuously supply primary substrates and the addition of SRPC into the subsurface can create anaerobic conditions and reach a thorough TCE removal through biodegradation and sorption mechanisms.

Molecular biology techniques [e.g., polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), nucleotide sequence analysis; real-time PCR (quantitative PCR, qPCR)] have been applied in bioremediation studies to confirm the feasibility and efficiency of the bioremediation [29,30]. These techniques can be used to identify the trends in the biodegradation process and can provide a direct evaluation of microbial diversity and dominant microorganisms, which can contribute to the contaminant biodegradation [31]. Real-time PCR is the technique of collecting data throughout the PCR process as it occurs, thus combining amplification and detection into a single step [32,33]. This is achieved using a variety of different fluorescent chemistries that correlate PCR product concentration to fluorescence intensity [34]. Reactions are characterized by the point in time (or PCR cycle) where the target amplification is first detected. This value is usually referred to as cycle threshold (Ct), the time at which fluorescence intensity is greater than background fluorescence. Consequently, the greater the quantity of target DNA in the starting material. The faster a significant increase in fluorescent signal will appear, yielding a lower Ct [35]. Real-time PCR has been widely applied in medical researches, however, its application to environmental researches has not caught the attention [36]. In real-time PCR, amplicons are detected by measurement of a fluorescence signal without post-PCR sample processing such as gel electrophoresis [37].

Researchers claim that the main bacterial group, which could degrade TCE under reductive dechlorination is *Dehalococcoides* [38–40]. Among the *Dehalococcoides* groups, *Dehalococcoides ethenogenes* could use hydrogen as the electron donor for dechlorination process [39,41]. *Dehalobacter restrictus* is able to obtain energy through the dechlorination reaction [39]. *Dehalococcoides* strain 195 could reduce tetrachloroethylene (PCE) to VC and *Dehalococcoides* sp. strain FL2 could convert TCE to VC then VC could be further reduced to ETH via the reductive dechlorination [42]. Different *Dehalococcoides* species has different dechlorinating gene, which is responsible for the reductive dechlorination of TCE. Both *D. ethenogenes* strain 195 and *D. ethenogenes* strain FL2 contain *tceA*, which could reduce TCE to VC. The *Dehalococcoides* strain BAV1 contains *bvcA* and *Dehalococcoides* strain VS and *D. ethenogenes* strain GT contain *vcrA*, which could reduce TCE to ETH [42,43]. Thus, gene analysis can be applied to evaluate the feasibility of applying reductive dechlorination for TCE site remediation by analyzing the appearance of appropriate TCE-degrading genes.

The main objectives of this pilot-scale study include the following: (1) evaluation of the effectiveness of bioremediation of TCE contaminated groundwater with the supplement of SPRS for the enhancement of reductive dechlorination of TCE under anaerobic conditions, (2) development of a specific genes analytical method to confirm the existence of TCE-dechlorinating gene at the studied TCE-contaminated site, (3) application of the real-time PCR technique to evaluate the variations in the populations of TCE-dechlorinating bacteria (*Dehalococcoides* spp.) during the bioremediation process, and (4) evaluation of the dominant native microorganisms during the bioremediation process. In this study, a series of molecular biology techniques including DNA extraction, PCR amplification, DGGE, real-time PCR, and nucleotide sequence analysis were applied to monitor the variations in activity-dependent microbial diversity and dominant microorganisms.

2. Materials and methods

2.1. Site description

An industrial park site located in southern Taiwan was selected for the pilot-scale study. At this site, a leakage of TCE storage tank was discovered in early 2005, which resulted in groundwater contamination with TCE. Groundwater samples from monitor wells were collected and analyzed to determine the local hydrogeology and delineate the TCE plume during a previous site investigation study. Site investigation results show that the components of the site soils are consistent with a sandy loam texture. The water table is generally found at depths ranging from 13 to 15 m below ground surface. The site groundwater flows to the northeast at a velocity of 7.2 cm/day and with a hydraulic conductivity of 0.006 cm/s. A test area within a TCE-spill site was selected for this study. Fig. 1 presents the site map showing the groundwater flow direction, injection well (IW), background well (BW), and three monitor wells (MW-1 to MW-3) at the test site.

The BW and monitor wells were located at the upgradient and downgradient areas of the IW. The SPRS was injected into the injection well located at the upgradient area of the test site to evaluate the feasibility of applying SPRS for groundwater remediation. Before the injection, the SPRS needs to be diluted with groundwater

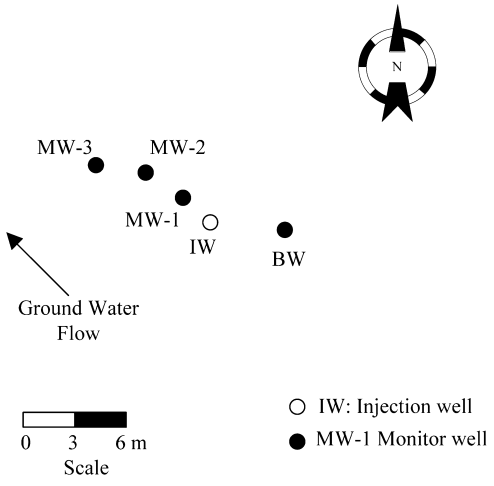

Fig. 1. Site map showing the groundwater flow direction, injection well (IW), background well (BW), and three monitor wells (MW-1 to MW-3) at the test site.