International Biodeterioration & Biodegradation xxx (2017) 1–8

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Trichloroethene removal by separately encapsulated and co-encapsulated bacterial degraders and nanoscale zero-valent iron

Sai Sharanya Shanbhogue [a], Achintya Bezbaruah [b], Senay Simsek [c], Eakalak Khan [b, *]

[a] Crestwood Midstream Partners LP, Tupman, CA 93276, USA
[b] Department of Civil and Environmental Engineering, North Dakota State University, Fargo, ND 58108, USA
[c] Department of Plant Sciences, North Dakota State University, Fargo, ND 58108, USA

ARTICLE INFO

Article history:
Received 21 May 2017
Received in revised form
9 August 2017
Accepted 9 August 2017
Available online xxx

Keywords:
Bacterial degraders
Encapsulation
Nanoscale zero-valent iron
Remediation

ABSTRACT

Degradation of trichloroethene (TCE) by separately encapsulated and co-encapsulated nanoscale zero-valent iron (nZVI) and bacterial degraders was investigated. *Pseudomonas putida* F1 and *Dehalococcoides* species BAV1 were used in the separate encapsulation and co-encapsulation, respectively. Results from batch experiments showed that the encapsulation systems were able to degrade 100% of TCE (10 mg/L to less than a detection limit of 0.2 μg/L) in 3 h. After 3 h, 10 mg/L of TCE was re-dosed and the co-encapsulation system was able to again completely remove TCE. Common TCE degradation by-products, dichloroethene and vinyl chloride, were not detected. The first order model was suitable for describing TCE degradation kinetics. The initial TCE degradation was mainly chemical while after the re-dosing biodegradation dominated due to exhaustion of nZVI caused by nitrate and possibly phosphate and chloride in the test medium. The encapsulation systems can overcome problems associated with limited activity longevity of nZVI under field conditions, residual TCE and chlorinated degradation by-products. The systems can potentially be a technique for in-situ remediation of groundwater.

1. Introduction

Nanoscale zero-valent iron (nZVI) particles have been used for environmental remediation particularly for degradation of chlorinated compounds in groundwater including trichloroethene (TCE) (Grieger et al., 2010; Liu et al., 2005; Theron et al., 2008). However, nZVI particles tend to agglomerate and settle in the aquifer. Several studies used polymers to modify nZVI to increase its stability in water and consequently TCE degradation efficiency such as surface coating by amphiphilic triblock copolymers (Saleh et al., 2008) and amphiphilic polysiloxane graft copolymers (Krajangpan et al., 2012), and nZVI embedment in alginate (Bezbaruah et al., 2009). Bezbaruah et al. (2011) employed an encapsulation technique to enhance the performance of nZVI. nZVI encapsulated in alginate was able to remove TCE up to 91%. However, there are other disadvantages associated with TCE degradation by nZVI which are limited longevity of activity of nZVI under field conditions due to its reactivity with nitrate, sulfate, phosphate, chloride, and other chemical species in groundwater, and generation of chlorinated by-products.

Bioremediation is preferred over other processes as it is less costly and can result in complete mineralization of organic contaminants or benign products. A number of successful bioremediation techniques including reductive dechlorination of TCE, co-metabolism of TCE and direct oxidation of TCE have been studied using different bacterial strains such as *Pseudomonas*, *Dehalococcoides*, and *Bacillus* species (Pant and Pant, 2010). For example, *Pseudomonas putida* F1 can degrade TCE aerobically (Wackett and Gibson, 1988). *Dehalococcoides* species BAV1 is a strain capable of degrading TCE to ethene under anaerobic condition (Lu et al., 2008). Bioremediation therefore can be used along with nZVI to treat TCE left over due to possible exhaustion of nZVI, and chlorinated degradation by-products.

A combined nZVI-microorganism system for TCE remediation will result in more removal efficiency and benign end products because of microbial actions. The system will allow TCE degradation to continue once nZVI gets exhausted. When nZVI is completely used up, there is a potential risk of the presence of TCE break down by-products such as dichloroethene (DCE) and vinyl chloride (VC). *Dehalococcoides* sp. is known to thrive on these by-

* Corresponding author. Civil and Environmental Engineering Department (# 2470), PO Box 6050, Fargo, ND 58108-6050, USA.
E-mail address: eakalak.khan@ndsu.edu (E. Khan).

http://dx.doi.org/10.1016/j.ibiod.2017.08.005
0964-8305/

products and break them down to harmless end products such as ethene (Cupples et al., 2003; He et al., 2003; Krajmalnik-Brown et al., 2004; Maymó-Gatell et al., 1997).

To use the combined nZVI-microorganism system, it is essential to understand the interactions between the two components. There are two schools of thought associated with microorganism-nZVI interactions. Some researchers reported that nanoparticles are harmful to microorganisms (Auffan et al., 2008; Barnes et al., 2010; Lee et al., 2008; Tilston et al., 2013; Velimirovic et al., 2015; Zabetakis et al., 2015) whereas some others reported that microorganisms are not negatively affected by the presence of nZVI (Grieger et al., 2010; Kirschling et al., 2010). Zabetakis et al. (2015) reported that nZVI is toxic to a TCE-degrading anaerobic microbial community. Kirschling et al. (2010) concluded the possibility to utilize microorganisms and nZVI together to synergistically engineer a novel remediation system as they found that reducing conditions and hydrogen created by nZVI stimulate both sulfate reducer and methanogen populations.

The activity of sulfate reducing bacteria in aquifer sediment can be enhanced or inhibited depending on the dose of nZVI (Kumar et al., 2014). Similarly, Jiang et al. (2015) reported that low and high doses of nZVI (50 and 1000 mg/L) can promote and inhibit denitrification by *Paracoccus* sp., respectively. A study on effect of nZVI on TCE dechlorination by a mixed anaerobic culture containing *Dehalococcoides* sp. was conducted (Xiu et al., 2010). Hydrogen produced from nZVI through cathodic corrosion stimulated methanogens and dechlorinating bacteria. Ethene production was negatively affected during a lag period but after that increased to the same level observed in a system with no nZVI. An et al. (2009) studied a combined nZVI and *Alcaligenes eutrophus* system for autotrophic denitrification. After bacterial acclimation, the system was able to denitrify all nitrate without ammonium production. Recently, Chaithawiwat et al. (2016) demonstrated that negative impact of nZVI on *Pseudomonas putida* KT2440 is growth phase dependent.

The objective of this study was to examine the effectiveness of encapsulated nZVI-bacteria systems for aqueous contaminant removal with TCE as a model contaminant. Batch experiments were conducted to examine TCE removal using encapsulated nZVI and TCE degrading bacteria. Both separate encapsulation and co-encapsulation of nZVI and bacteria were attempted. The first part of the experiments involved separate encapsulation between nZVI and bacteria to avoid potential negative impact of nZVI on bacteria. The second part focused on the co-encapsulation.

2. Materials and methods

2.1. Chemicals

Iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, 99%, Alfa Aesar), sodium borohydride ($NaBH_4$, 98%, Aldrich), calcium chloride ($CaCl_2$, ACS grade, BDH), sodium alginate (production grade, Pfaltz & Bauer), methanol (production grade, BDH), maltodextrin (food grade, Aldrich), and TCE (ACS Grade, 99.5% pure) were used as received.

2.2. Bacterial cultures

Pseudomonas putida F1 (pure culture, ATCC # 700007) and *Dehalococcoides* (*Dhc*) species BAV1 (pure culture, ATCC # BAA-2100) were used as received. The cultivation procedures for these two bacterial strains are described in Subsection 2.4.

2.3. Synthesis of nZVI

nZVI particles were synthesized using the borohydride reduction of ferrous iron and passivation technique according to Bezbaruah et al. (2009), and Liu and Lowry (2006). The characteristics of synthesized nZVI included mostly spherical with an average diameter of 35 nm in diameter, > 80% in a size range of 10–50 nm, an average Brenauer-Emmett-Teller surface area of 25 m^2/g, and clustered chain agglomeration (Bezbaruah et al., 2011).

2.4. Bacterial cultivations and stock suspensions

A 1 mL aliquot of *P. putida* F1 was inoculated into 20 mL of tryptic soy broth (TSB) media (Sigma-Aldrich Tryptic Soy Broth for microbiology) and grown overnight at 30 °C and 150 rpm orbital shaking. This is a stock suspension. One hundred microliter aliquots were collected from the stock suspension periodically during a 24 h incubation and subjected to the plate count method using Standard Methods Agar (Plate Count Agar). A growth curve was constructed based on the plate count data. A stationary phase was observed from 12 to 24 h and in later cultivations the culture was grown until that time interval for use in encapsulation. The preparation of *Dhc* BAV1 for encapsulation followed the same procedure except for the followings. Mineral salt medium (MSM, composition shown in supplementary data) supplemented with methanol (27.7 mg C/L) was used for the cultivation and the shaking was at 100 rpm. The cultivation was for a total of 36 h and reached at a stationary phase at 15 h. In addition, the entire cultivation was performed under a hood, with N_2 purged media to ensure anaerobic conditions.

2.5. Encapsulations of nZVI, P. putida F1, and Dhc BAV1, and co-encapsulation of nZVI and Dhc BAV1

nZVI was encapsulated in calcium alginate using a procedure described in Bezbaruah et al. (2011) by mixing 30 mg of nZVI with a combination of 0.25 g $CaCl_2$ and 4.0 g maltodextrin in 6 mL of deionized water and dropping the mixture in a sodium alginate solution. For the encapsulations of *P. putida* F1 (PpF1) and *Dhc* BAV1, the same procedure was followed except that the bacterial stock suspension (1 mL) containing a specific number of bacterial cells (10^7 and 10^8 cells assuming 1 CFU = 1 cell for PpF1 and *Dhc* BAV1, respectively) was encapsulated instead of 30 mg of nZVI. The number of bacterial cells were ensured based on the growth curve data obtained from the procedure described in Subsection 2.4 (The PpF1 and *Dhc* BAV1 cell concentrations during the stationary phase were 10^7 and 10^8 CFU/mL, respectively).

The same encapsulation procedure described above was also used to co-encapsulate nZVI and *Dhc* BAV1 (30 mg of nZVI and 1 mL of the stock bacteria suspension combined together in the encapsulation process). Anaerobic conditions were maintained through N_2 purging during the encapsulation of *Dhc* BAV1 and co-encapsulation of nZVI and *Dhc* BAV1. Capsules produced from each batch of the encapsulation and co-encapsulation were entirely used in one batch of TCE degradation experiment (see the following subsection). All the capsule products were spherical with a diameter of 3.96 ± 0.01 mm and a skin thickness of 0.27 ± 0.00 mm, and were stored in a deoxygenated 2% $CaCl_2$ solution until used in degradation experiments.

2.6. TCE degradation experiments

TCE degradation experiments consisted of two parts that had two different focuses and approaches. The experiments in the first part were conducted under aerobic conditions and focused on

Please cite this article in press as: Shanbhogue, S.S., et al., Trichloroethene removal by separately encapsulated and co-encapsulated bacterial degraders and nanoscale zero-valent iron, International Biodeterioration & Biodegradation (2017), http://dx.doi.org/10.1016/j.ibiod.2017.08.005