Nitrate removal by entrapped zero-valent iron nanoparticles in calcium alginate
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ABSTRACT

Zero-valent iron nanoparticles (nZVI) were successfully entrapped in calcium alginate beads. The potential use of this technique in environmental remediation using nitrate as a model contaminant was investigated. Kinetics of nitrate degradation using bare nZVI (~35 nm dia) and entrapped nZVI were compared. Calcium alginate beads show promise as the entrapment medium for nZVI for possible use in permeable reactive barriers for groundwater remediation. Based on scanning electron microscopy images it can be inferred that the alginate gel cluster acts as a bridge that binds the nZVI particles together. Kinetic experiments with 100, 60, and 20 mg NO$_3$-N L$^{-1}$ indicate that 50–73% nitrate-N removal was achieved with entrapped nZVI as compared to 55–73% with bare nZVI over a 2 h period. The controls ran simultaneously show little or no NO$_3$-N removal. Statistical analysis indicates that there was no significant difference between the reaction rates of bare and entrapped nZVI. The authors have shown for the first time that nZVI can be effectively entrapped in Ca-alginate beads and no significant decrease in the reactivity of nZVI toward the model contaminant (nitrate here) was observed after the entrapment.

Key words | alginate beads, entrapment, iron nanoparticle, Nitrate, permeable reactive barrier

INTRODUCTION

In recent years, zero-valent iron nanoparticles (nZVI) have been used for the removal of various groundwater contaminants including chlorinated compounds (Liu & Lowry 2006; Cheng et al. 2007), pesticides (Feitz et al. 2005; Joo & Zhao 2008; Thompson et al. 2008), heavy metals (Blowes et al. 1997; Alowitz & Scherer 2002), and explosive materials (Gregory et al. 2004; Oh et al. 2005). Advantages of nZVI over other zero-valent iron (ZVI) such as microparticles (mZVI) and iron filings include higher reactive surface area (25–54 m$^2$g$^{-1}$ for nZVI) (Chen et al. 2004; Li et al. 2006), faster and more complete reactions, and injectability into aquifer (Cantrell et al. 1995; Wang & Zhang 1997). Due to their small particle size (<100 nm) (Wang & Zhang 1997) and high reactivity, nZVI has also been proposed to be used in the remediation of contaminated soils (Martin et al. 2008), sediments (Zhang & Frankenberger 2006), and biosolids (Li et al. 2007).

Zero-valent iron nanoparticles are highly reactive and nonselective. They react rapidly with surrounding media in the subsurface and significantly lose their reactivity (Zhang 2003). Further, because of smaller particle size and relatively higher dispersibility (as compared to other ZVI materials), nZVI becomes mobile in the aquifer (Cantrell et al. 1995; Wang & Zhang 1997; Martin et al. 2008). As such it is difficult to use nZVI in a permeable reactive barrier (PRB) while iron filings (Phillips et al. 2000) and mZVI (Johnson et al. 1996) have been used in PRBs with varying degree of success. Keeping in view the advantages of nZVI over other
ZVI materials, it is expected that the use of nZVI in a PRB will significantly increase the performance of the barrier (Elliot & Zhang 2001). However, the higher mobility of nZVI and oxidation by non-target compounds in groundwater remain as major challenges (Krajangpan et al. 2008). To overcome these problems, nZVI can be entrapped in a porous polymeric hydrogel and the entrapped particles can then be used effectively.

Entrapment within calcium (Ca) alginate beads is one of the most common methods for immobilizing living cells in food and beverage industries (Kobaslija & McQuade 2006; Olivas & Barbosa-Canovas 2008). Ca-alginate has also been used to prepare alginate hydrogels and microbeads for drug delivery (Morch et al. 2006). In addition, calcium alginate entrapped bacterial cells have been used in environmental remediation (Fundueanu et al. 1999; Mishra & Bajpai 2004; Zala et al. 2004; Pongjanyakul & Puttipipatkhachorn 2007; Hill & Khan 2008). Immobilization of cells in calcium alginate is a simple and cost effective technique (Zala et al. 2004). The porosity in calcium alginate allows solutes (contaminants and substrate) to diffuse through the beads and come in contact with the entrapped cells (Huang & Zhihui 2002). Moreover, alginate is nontoxic, biodegradable, and nonimmunogenic. Because of the divalent calcium ions alginate produces thermally irreversible gels that are insoluble in water (Vold et al. 2006). Alginate and calcium crosslinking has been described in details elsewhere (Velings & Mestdagh 1995).

The objective of this paper is to demonstrate that iron nanoparticles can be effectively entrapped in a biopolymer matrix without significant reduction in their reactivity. The study described in this paper targeted at retaining nZVI within alginate beads to reduce nZVI mobility in the flowing fluids (groundwater) and, thus, increase nZVI contact time with target contaminant to ensure better contaminant removal. Nitrate was used as a test contaminant to examine the effectiveness of the entrapped nanoparticles in degrading environmental contaminants. Nitrate was selected because of simplicity of analysis and availability of literature on nitrate degradation using ZVI (Siantar & Schreier 1996; Cheng et al. 1997; Westerhoff & James 2005; Choe et al. 2004; Su & Puls 2004; Huang & Zhang 2006). In addition, groundwater contamination by nitrate remains an ongoing problem of concern (Schnobrich et al. 2007; Kapoor & Viraraghavan 1997; Alowitz & Scherer 2002). The major sources of nitrate pollution in groundwater are the use of nitrogen fertilizers and nitrogen pesticides, animal wastes, and drainage of domestic wastewater (Su & Puls 2004). Chemical reduction of nitrate by ZVI is a highly energetic reaction. It has long been known as a potential technology to remove nitrate from water (Siantar & Schreier 1996; Huang & Wang 1998; Huang & Zhang 2006). ZVI has been used for nitrate removal from groundwater under different experimental conditions (Yang & Lee 2005). Bare nZVI has been used in remediating nitrate under different environmental conditions (Chen et al. 2004; Li et al. 2006; Liu & Lowry 2006; Sohn et al. 2006).

**MATERIALS AND METHODS**

**Chemicals**

Iron(II) sulfate heptahydrate (FeSO₄·7H₂O, 99%, Alfa Aesar), sodium borohydride (NaBH₄, 98%, Aldrich), calcium chloride (CaCl₂, ACS grade, BDH), sodium alginate (production grade, Pfaltz & Bauer), methanol (production grade, BDH), and ethanol (ACS grade, Mallinckrodt Chemicals) were purchased from VWR (West Chester, PA, USA) and were used as received unless otherwise specified.

**Synthesis of nZVI**

Iron nanoparticles were synthesized by borohydride reduction of ferrous ion in FeSO₄·7H₂O in an aqueous phase (Liu et al. 2005). The use of this method is well documented (Liu & Lowry 2006). The method is inexpensive and requires no specialized equipment. Additionally, the nanoparticles synthesized by this method have shown both high reactivity and durability (Liu & Lowry 2006). The nZVI synthesis method of Liu et al. (2005) was used with two modifications: (1) the ZVI synthesized in the reaction between FeSO₄·7H₂O and NaBH₄ is dried under alternating N₂ and vacuum overnight, and (2) the dried ZVI is air stabilized (passivated) overnight. Similar passivation techniques were employed by Sohn et al. (2006) for increased stability of nZVI. The resulting nZVI was stored in a
glovebox (Innovation Technology, Inc.) under nitrogen environment for future use.

Entrapment of nZVI

The method developed by Aksu et al. (1998) for cell entrapment in alginate was modified for nZVI entrapment in the present study. One gram of sodium alginate was dissolved in 50 mL deionized deoxygenated water (DDW) at room temperature (22 ± 2°C). The deionized water was deoxygenated by purging it with nitrogen gas (ultra high purity grade) for about 15 minutes to remove dissolved oxygen (DO). DO removal was necessary to ensure that nZVI is not oxidized during entrapment. The alginate-water mixture was stirred until complete dissolution was achieved (~20–30 minutes). Then, the mixture was left at room temperature for 30 minutes to allow the air/gas bubbles generated from the mixing to escape. This step is necessary to ensure that the alginate beads do not float on the aqueous solution. The alginate solution (2% w/v) was gently mixed with 1 g of nZVI. The mixture was promptly dropped into a 3.5% (v/v) deoxygenated aqueous solution of CaCl₂ at room temperature (22 ± 2°C) using a peristaltic pump (Masterflex, Cole Parmer Instrument, with 0.5 mm internal diameter tubing at 2.5 mL min⁻¹ flow rate). As soon as the drops came in direct contact with the CaCl₂ solution, calcium alginate gel beads were formed. The gel beads were retained in the deoxygenated solution of CaCl₂ for 9 h for hardening and then washed with DDW. A minimum of 6 h hardening ensures that the beads allow optimal diffusion of substrates into and out of them (Aksu et al. 1998; Garbayo et al. 2002).

Nitrate degradation kinetics experiments

Nitrate degradation experiments were conducted through a series of batch trials. The experiments were performed in anaerobic batch reactors made of 500 mL commercial grade polyethylene terephthalate bottles with sleeve type silicone septum seal. Four hundred fifty milliliters of DDW were spiked with a stock nitrate solution to obtain initial concentrations of 20, 60, and 100 mg NO₃⁻-N L⁻¹. Alginate entrapped nZVI (1.0 g) were added to each reactor. The reactors were rotated end-over-end at 28 rpm in a custom made shaker. Aliquots were withdrawn periodically (at 0, 10, 15, 30, 45, 60, 90, and 120 minutes) and analyzed for nitrate and pH. Reactors with nitrate in DDW but no nZVI were run as controls. Similar experiments were conducted with bare nZVI. All experiments were conducted in triplicates.

ZVI characterization and analytical methods

The particle sizes of nZVI were analyzed through transmission electron microscopy (TEM, JEOL JEM-100CX II). The scanning electron microscopy with x-ray microanalysis (SEM/EDX, JEOL JSM-6300) was used to observe surface morphology and to analyze percent mass element composition of nZVI. The specific surface area of the synthesized nZVI was measured with a micromeritics analyzer (ASAP 2000, GA, USA) using Brunauer–Emmett–Teller (BET) gas adsorption with N₂. Nitrate and pH were determined according to APHA et al. (1998). The concentration of NO₃⁻-N was measured with a nitrate electrode and a Ag/AgCl reference electrode (both SympHony, VWR). The pH was measured using a pH electrode (Orion 250A⁺ ORION 91-07).

For TEM and SEM/EDX analyses, the nZVI was stored in ethanol. A 200 mesh copper grid coated with carbon was carefully dipped into the mixture to adsorb nZVI from ethanol which was later dried under ambient temperature. The adsorbed nZVI was used for TEM analyses. The sample preparation for TEM and SEM/EDX was done under atmospheric and under high vacuum conditions, respectively. The entrapped nZVI particles were collected and stored in a 0.1 M CaCl₂ solution at 4°C before SEM analysis and prepared in accordance with Hill & Khan (2008). The samples were attached to aluminum mounts by silver paint and coated with gold/palladium using a Balzers SCD 030 sputter coater. Images were obtained by SEM under high vacuum condition.

RESULTS AND DISCUSSION

Characteristics of bare nZVI

Analysis of TEM image (Figure 1) indicates that nZVI particle size ranges from 10 to 100 nm with an average size of 35 nm (Figure 2). Figure 1 shows a cluster of nZVI as the...
particles agglomerate because of a strong magnetic interaction between them. This agglomeration limits the availability of surface area on the particle and hence available reactive surface area for contaminant degradation (Schlicker et al. 2003). A higher magnification TEM shows ∼2.5 nm of passivating oxide shell around the nZVI core (inset of Figure 1). The shell was formed during the passivation process of nZVI. Similar nZVI core/shell geometry has been reported earlier (Nurmi et al. 2005; Sohn et al. 2006). Sohn et al. (2006) found the passivating oxide shell to be ∼5 nm and reported a 50% reduction in nZVI reactivity as compared to fresh particles. However, the passivation is a trade off between high reactivity and longer shelf life of the particles. It is important to have a longer particle shelf life for real world applications. The reactivity of passivated nZVI was still reported to be higher than commercial grade mZVI (Sohn et al. 2006).

SEM/EDX analysis of the nZVI indicated Fe⁰ as the most abundant element (∼76%) with a smaller amount of oxygen (∼24%) forming the oxide shell. Researchers have reported the oxide shell as magnetite (Fe₃O₄) (Liu & Lowry 2006; Sohn et al. 2006; Martin et al. 2008).

**Characteristics of entrapped nZVI**

nZVI entrapped alginate beads were analyzed with SEM (Figures 3a–d) and TEM (Figures 3e–f). The images are used to qualitatively understand the morphology inside the alginate beads. The dispersibility and location of nZVI inside the alginate bead were also investigated from these images. The images show that pore size of alginate beads is not identical because crosslinking between Ca and alginate is not uniform throughout the bead. In some parts, Ca-alginate formed densely and entrapped more nanoparticles. In higher density areas there is a possibility of nZVI agglomeration and hence reduction in their reactive surface area. The higher magnification TEM images (Figures 3e–f) confirm high agglomeration of nZVI in parts of the beads. Further, SEM analysis revealed that the bead surface has undulations, folds, and pores, which were also reported by Benerjee et al. (2007). Benerjee et al. (2007) reported the pore size in similar alginate beads to be 3.17–5.07 nm. Reported small pore size ensures retention of nZVI used in this study.

**Kinetics of nitrate degradation**

Kinetic studies were conducted to find out the effectiveness of bare nZVI and entrapped nZVI on nitrate removal at three initial concentrations without any pH adjustment (Figures 4–6). With bare nZVI, the NO₃⁻-N concentration decreased from 100 mg L⁻¹, 60 mg L⁻¹, and 20 mg L⁻¹ to 27 mg L⁻¹, 23 mg L⁻¹, 9 mg L⁻¹, respectively, over a 2 h period. The batch studies were continued for 24 h. However, this paper reports the results for the initial 2 h period only as the degradation curve leveled off beyond that time and no significant NO₃⁻-N reduction was observed. The slightly
lower or similar NO$_3^-$-N reductions were observed with entrapped nZVI than with bare nZVI for the same initial concentrations and the same reaction time. The initial NO$_3^-$-N of 100, 60, and 20 mg L$^{-1}$ reduced to 27 mg L$^{-1}$, 26 mg L$^{-1}$, 10 mg L$^{-1}$, respectively, with entrapped nZVI. Nitrate degradation by bare nZVI and entrapped nZVI followed first order reaction for all concentrations. The reaction rate constants (k) and the coefficient of determination ($R^2$) of the fit are summarized in Table 1.

For entrapped nZVI, a marked drop in nitrate concentration during the first fifteen minutes was observed in the control (calcium alginate beads only). This initial drop in nitrate in the entrapped systems was a physical phenomenon. The initial drop in nitrate can be attributed to sorption into the calcium alginate bead due to the NO$_3^-$-N gradient that existed between the aqueous (bulk) solution and the beads. Similar substrate sorption by Ca-alginate beads were reported by Hill & Khan (2008) in experiments involving cell entrapment.

The NO$_3^-$-N reduction by entrapped nZVI was observed to be slightly lower compared to bare nZVI in some cases (Figures 4–6). However, two-way analysis of variance test on the reaction rate data indicates that there is no significant difference between the reaction rates of bare
nZVI and entrapped nZVI ($\alpha = 0.05$, $p$-value = 0.142). The authors expected some reduction in degradation rate due to the presence of alginate coating on the nZVI leading to diffusion limitations. It seems that there was no degradation reduction due to diffusion limitation. Nitrate diffusion in alginate beads is non-Fickian in nature and depends on the concentration of alginate and extent of crosslinking of calcium (Garbayo et al. 2002). The alginate beads used in this experiment were gelled for more than 6 h. Therefore, they should have reached optimal nitrate diffusion characteristics (Aksu et al. 1998) and should not restrict substrate (nitrate here) diffusion through them (Garbayo et al. 2002).

The results clearly indicate that the reactivity of entrapped nZVI was comparable to bare nZVI. Reduced mobility of iron nanoparticles can be achieved through entrapment in Ca-alginate. With further improvement in dispersibility of the nZVI within the beads, the alginate entrapment technique may possibly offer a way to effectively use nZVI in permeable reactive barriers. The entrapped nZVI will have the advantage of being stationary in the aquifer under dynamic groundwater conditions as compared to the bare particles.

**CONCLUSIONS**

The results from this study indicate that nZVI entrapment in a biopolymer may increase the overall efficacy of permeable reactive barriers for groundwater remediation. The authors have shown for the first time that nZVI can be effectively entrapped in Ca-alginate beads and comparable reactivity of nZVI toward a model contaminant (nitrate here) was observed after the entrapment. The
reduction in nitrate concentration using bare nZVI and entrapped nZVI were 55–73% and 50–73%, respectively, over a 2 h period. There was no significant difference between bare nZVI and entrapped nZVI reactivity. Calcium alginate entrapped nZVI would be a good alternative for in-situ remediation of nitrate and other contaminants in groundwater. Ca-alginate can be used as the entrapment media for nZVI to make the nanoparticles relatively stationary as compared to bare nZVI. Bare nZVI either migrate with groundwater flow or settle into soil pore and hence become unavailable for contaminant degradation. The entrapped nZVI can possibly be used in permeable reactive barriers. Further work needs to be conducted to investigate surface chemistry of the entrapped iron particle. Clogging due to iron corrosion byproducts was not examined in this study and is also recommended for future research.

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