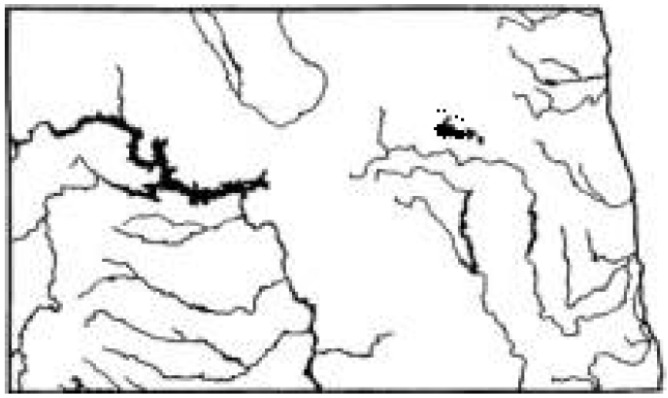

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ALGINATE ENCAPSULATED NANOPARTICLE-MICROORGANISM SYSTEM FOR TRICHLOROETHYLENE REMEDIATION

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TABLE OF CONTENTS

LIST OF TABLES iv

LIST OF FIGURES iv

ABSTRACT 1

ACKNOWLEDGMENTS 2

BACKGROUND 3

Nanoscale Zero-valent Iron (NZVI) 3

Remediation with Zero-valent Iron 3

TCE Remediation using entrapped NZVI 4

TCE Bioremediation 5

Combined metal-microorganism system 5

DESCRIPTION OF A CRITICAL STATE 5

SCOPE AND OBJECTIVES 6

MATERIALS AND METHODS 6

Chemicals and reagents 6

Synthesis of NZVI 6

Preparation of alginate capsules and characterization 6

Encapsulation of iron nanoparticles 7

Diffusion studies 7

TCE degradation studies 8

Shelf-life studies 8

Bacterial Growth Study 8

Encapsulation of *Dehalococcoides sp.* 8

Combined metal-microorganism system 9

Batch TCE degradation Experiments 9

Analytical methods 10

Quality Control 10

RESULTS AND DISCUSSIONS 10

NZVI characteristics 10

Ca-alginate capsule characteristics 11

Diffusion studies 11

TCE degradation 12

TCE degradation kinetics 14

Shelf-life of NZVI 15

Bacterial Growth Characteristics 16

Batch TCE degradation studies 16

TCE Degradation Kinetics 18

CONCLUSIONS 19

REFERENCES 20

LIST OF TABLES

Table Page

Table .1: Reaction rate constants calculated based on the results obtained during this study.....15

Table 2: Reaction rate constants calculated based on the results obtained during this study.....19

LIST OF FIGURES

Figure Page

Fig.1: Schematic of Ca-alginate capsule preparation process. 7
Fig.2: Encapsulation under anaerobic conditions.....9
Fig.3: NZVI and Ca-alginate capsule characteristics……. 11

Fig.4: TCE diffusion characteristics for the Ca-alginate capsules……..... 12

Fig.5: Reduction of TCE by bare and encapsulated NZVI over time……... 14

Fig.6: *D.BAV*1 growth curve at room temperature…… 16

Fig 7: TCE removal using: (a) Encapsulated NZVI, (b) Encapsulated NZVI (re-dosed), (c) Encapsulated *D.BAV1*, and (d) Combined metal-microorganism system……. 17

ABSTRACT

Nanoscale zero-valent iron (NZVI) particles (10-90 nm) were encapsulated in biodegradable calcium alginate capsules for application in environmental remediation. Trichloroethylene (TCE) removal by encapsulated NZVI was 89-91% in 2 h, and the reaction followed first order kinetics for encapsulated NZVI systems with an observed reaction rate constant (k_{obs}) of 1.92-3.23x10^{-2} min^{-1} and a surface normalized reaction rate constant (k_{sa}) of 1.02-1.72x10^{-3} L m^{-2} min^{-1}. TCE degradation reaction rates for encapsulated and bare NZVI were similar indicating no adverse affects of encapsulation on degradation kinetics. The shelf-life of encapsulated NZVI was found to be four months with little decrease in TCE removal efficiency. *Dehalococcoides BAV1* (*D.BAV*1) a bacterial strain capable of degrading TCE was co-encapsulated with NZVI and used in this study. The combined metal-microorganism system is engineered with the expectation to increase TCE degradation efficiency, reducing the contaminant to its very benign forms. Batch re-dosing experiments were performed to test the efficacy of the combined *iron-DBAV*1 system over a 36-h time period. TCE re-dosing was done at 3 h and the experiment was continued up to 36 h. During the first three hours of the experiment 100% TCE removal was achieved apparently by the NZVI, and again 100% removal was achieved post re-dosing where *D.BAV*1accomplished the treatment. The conclusions on the role of NZVI and the microorganisms were drawn by comparing the data from the re-dosing experiments and the data from batch experiments conducted with only NZVI, or *D.*BAV1 encapsulated in Ca-alginate. However, the observations on the distinct roles played by NZVI and microorganisms should be viewed with the caveat that additional research is needed to establish them more conclusively.

The results from this study are expected to contribute to the body of knowledge for successful use of engineered nanoparticles for environmental remediation. The proof of concept experiments conducted for the first time with a combined microorganism-NZVI system have established that viable remediation systems can be designed where the beneficial aspects of both physico-chemical and biochemical processes can be harnessed.

ACKNOWLEDGMENTS

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BACKGROUND

Chlorinated solvents such as trichloroethylene (TCE) in particular represent one of the most problematic class of volatile organic compounds found in groundwater (Russell et al. 1992). A number of studies have demonstrated that the widespread presence of TCE in groundwater is a serious public concern due to the hazardous nature of this contaminant (Ellis and Rivett 2007; Pant and Pant 2010; Tsai et al. 2011).The MCL for TCE in drinking water is 5 μg/L or parts per billion (ppb) (USEPA 1997).

TCE is a dense non-aqueous-phase liquid (DNAPL), and has the ability to penetrate deep into the aquifer much below the water table. Once in the aquifer TCE gets dissolved in water to form plumes and may remain in the aquifer over several decades depending on the concentration. The size of these plumes may even be a few kilometers depending on the sorption capacity of aquifer materials and reactivity of TCE (Jackson 1998; Rivett et al. 2001). Improper storage and use of TCE has resulted in a number of spills which ultimately found their way to the local regional aquifers. Spills can result in extremely high concentrations (approaching TCE solubility limits) which would call for remedial action. TCE is among the most pervasive chlorinated solvents in groundwater at hazardous waste sites in the United States (National Research Council; Lu et al 2005; Knox and Canter 1996) and its wide spread use, mobility and persistence causes the greatest risk of groundwater contamination.

Nanoscale Zero-valent Iron (NZVI)

Iron nanoparticles used for environmental remediation have average particle sizes in the range of 12.5- 80 nm ((Bezbaruah et al. 2009; Bezbaruah et al. 2011). NZVI particles have significantly much higher reactive surface areas when compared to other larger iron particles (micro particles and iron fillings)(Zhang 2003). The average BET surface areas of NZVI reported in literature range between 25-54 m^2g^{-1} (Bezbaruah et al. 2011; Zhang 2003) compared to 1-2 m^2g^{-1} for micro iron particles (Sigma-Aldrich, 2007).

Remediation with Zero-valent Iron

Nanoscale Zero-valent Iron (NZVI) particles have been used to remediate a wide range of environmental contaminants including chlorinated compounds (Bezbaruah et al. 2011; (Kim et al. 2010a; Wang and Zhang, 1997), heavy metals (Kanel et al., 2005; Klimkova et al. 2011; pesticides (Thompson et al. 2010); (Joo and Zhao 2008) and explosives (Gregory et al. 2004).

The zero-valent iron (ZVI) remediation process is a two-electron redox reaction. The standard electrode pote

ntial of the zero-valent iron system is -0.44V (Milazzo et al., 1978). A wide range of environmentally problematic contaminants (especially chlorinated hydrocarbons like Trichloroethylene) get oxidized in a thermodynamically feasible oxidation-reduction reaction. In this reaction the $Fe^{(0)}$ gets oxidized (loses electrons) whereas the target contaminant gains the electrons from the iron thereby getting reduced.

(Johnson et al., 1996, Kim et al., 2010). Matheson and Tratnyek (1994) have described the iron-contaminant reaction (oxidation-reduction) as follows:

$$Fe^{(0)} \rightarrow Fe^{+2} + 2e^{-} \quad (2\text{-}1)$$

$$R\text{-}X + 2e^{-} + H^{+} \rightarrow R\text{-}H + X^{-} \quad (2\text{-}2)$$

Matheson and Tratnyek (1994) also established that NZVI may possibly have two other competing pathways while reacting with water/ dissolved oxygen as indicated below:

$$Fe^{(0)} + 2H_2O \rightarrow Fe^{+2} + H_2 + 2OH^{-} \quad (2\text{-}3)$$

$$2\,Fe^{(0)} + O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4\,OH^{-} \quad (2\text{-}4)$$

From equations (2-3) and (2-4) it is evident that the reactive iron gets consumed/used up by non-target contaminants. Therefore it is essential to eliminate any dissolved oxygen in water in order to maximize contaminant removal efficiency using NZVI.

NZVI particles are inherently highly mobile with the tendency to settle down and potentially agglomerate. This property may cause them to settle down or agglomerate into the aquifer pores which is highly undesirable. The agglomeration of NZVI takes place due to the interparticulate magnetic and van der Waals forces between the particles(Bezbaruah et al. 2009). In a possible effort to overcome the mobility and settlement problems associated with NZVI, the NZVI particles can be "encapsulated" in a polymer, for the effective delivery of reactive materials to the target contaminant. "Encapsulation can be defined as a process of confining active compounds within a matrix or membrane in particulate form to achieve one or more desirable effects" (Chan et al., 2009)..Encapsulation technique, for the remediation of TCE was investigated for the first time in 2011 by Bezbaruah et al., using nano scale zero-valent iron encapsulated in Ca-alginate capsules. The TCE removal efficiency of the encapsulated iron system was found to be ~ 90 % over a 2 hr reaction interval, establishing the efficiency of the Ca-alginate encapsulated iron system and usability of the encapsulation technique for environmental remediation applications.

Alginates, which are derived from seaweeds, have been extensively used for encapsulation. This is because the encapsulation process that involves the material is simple, mild and non-toxic (Chan et al., 2009). Ca-alginate is non-toxic, biodegradable, and sparsely soluble in water making it an ideal polymer for use in environmental applications (Bezbaruah et al. 2009; Chan et al. 2010; Lai et al. 2008). The porous nature of Ca-alginate allows solutes to diffuse and come in contact with the encapsulated reactive materials (Bezbaruah et al. 2009; Huang and Zhihui 2002).

TCE Remediation using entrapped NZVI

Entrapment of NZVI has been effectively used for the remediation of TCE. He et al. 2010 used bi-metallic NZVI/Pd system entrapped in carboxymethyl cellulose (CMC) for TCE removal. The CMC entrapped NZVI/Pd system removed TCE with an 80% efficiency. The bi-metallic NZVI/Pd system has also been used by Kim et al. 2010 who achieved a 99.8% TCE removal efficiency using alginate for NZVI/Pd entrapment. This system has a high removal efficiency, however, Pd is considered to be toxic to the environment. Alginate entrapped NZVI/powdered

activated carbon (PAC) system removed only 62% TCE (Kim et al. 2010a). Wang et al 2010 used a Poly (methyl methacrylate) (PMMA) entrapped NZVI system to remediate TCE from an aqueous solution with a TCE removal efficiency of ~ 62 %.

TCE Bioremediation

Bioremediation of TCE using microbial strains (capable of metabolizing TCE) is a cost effective remedial strategy (Capiro et al. 2011). A number of research studies have shown that dechlorinating bacterial strains are capable of reductive dechlorination within close proximity of the DNAPL zones. In line with these findings, the biodegradation potential of TCE and its daughter products have been thoroughly studied and investigated. Research has revealed that chlorinated ethenes can be biologically degraded by three types of metabolic processes. Maymo-Gatell et al. (1999) have pointed out that TCE undergoes complete dechlorination under anaerobic conditions yielding ethane or ethene as the final products depending on the microbial strain used to bring about the degradation. Different anaerobic strains have been explored for reductive dechlorination of halogenated hydrocarbons with the most popular strain being Dehalococcoides. The popularity of the *Dehalococcoides sp.* for TCE degradation is attributed to the fact that this strain can degrade TCE, reducing the contaminant to benign by-products (i.e. Ethane) (Maymo-Gatell et al. 1999; Krajmalnik-Brown et al. 2004; Cupples et al. 2003; Pant and Pant 2010).

Combined metal-microorganism system

Research is in progress to synergistically utilize the combined effects of NZVI and microbes in order to establish a remediation system for effective dechlorination of TCE (Kirschling et al., 2010). In order to engineer a remediation system which uses the combined desired effects of NZVI and microorganisms, it is essential to understand the interactions between the metal and microorganism system. There is limited literature to address NZVI-microorganism interaction. The available literature indicates that there is a strong possibility to use NZVI and microorganisms together in a single system for increased dechlorination of halogenated organics. Shabnam (2011) established that the NZVI-microorganism interactions are dependent on the: (i) NZVI concentration; (ii) bacterial phase (i.e. whether the microorganism is in lag, growth or death phase while NZVI is being dosed); and (iii) experimental conditions (i.e. stirring speed). In general different bacterial strains responded differently to NZVI dosage. There was found to be a negative correlation between NZVI dose and bacterial growth. NZVI-microorganism interaction is bacterial growth phase dependant. Microorganisms in active growth phase are not affected by NZVI. On the other hand, microorganisms in their lag phase of growth (i.e. non-dividing) cells are adversely affected by NZVI.

DESCRIPTION OF A CRITICAL STATE

The United States Environmental protection Agency (USEPA) reports that 61% of the Superfund Sites are contaminated with TCE. The groundwater in Valley City,

North Dakota has been adversely affected by TCE contamination due to industries, landfills and factories. Sheyenne River at Valley City has TCE levels beyond the acceptable limits (5µg/L) as prescribed by USEPA. The City of Lisbon, ND is also affected by high TCE contamination. West Fargo, ND has a huge contaminated site. This contamination occurred due to the leak from a dry cleaning facility and has affected the city's water supply chain for which the city has started an expensive clean-up process. The results from a survey was conducted in Valley City on 6826 people who consumed water contaminated with TCE revealed some sort of health impairment in 4 out of 6 people. The development of new TCE spill remediation technologies can potentially abate these issues.

SCOPE AND OBJECTIVES

The broad objective of this research is to test the efficacy of a metal-microorganism system for TCE remediation.

The specific objectives of this research are as follows:

- Encapsulation of NZVI in Ca-alginate capsules.
- Determine whether alginate encapsulated NZVI (in alginate polymer) is effective for TCE remediation.
- Encapsulation of TCE degrading bacteria in Ca-alginate capsules.
- Determine whether encapsulated TCE degrading bacteria (*Dehalococcoides BAV1*) are effective for TCE remediation.
- Test the efficacy of the combined metal-microorganism system for TCE remediation.
- Quantify the reaction kinetics of TCE degradation:
  - Using NZVI
  - Using bacterial strains
  - Combined metal-microorganism system.

MATERIALS AND METHODS

Chemicals and reagents

Calcium chloride ($CaCl_2$, ACS grade, BDH), sodium alginate (production grade, Pfaltz & Bauer), methanol (production grade, BDH), maltodextrin (food grade, Aldrich), *Dehalococcoides* sp. strain BAV1 (pure culture, ATCC) and trichloroethylene (TCE, ACS Grade, 99.5% pure) were used as received.

Synthesis of NZVI

NZVI particles were synthesized using the borohydride reduction of ferrous iron and passivation technique reported by others (Eq. 1, Liu and Lowry 2006; Bezbaruah et al. 2009).

$$2Fe^{2+} + BH_4^- + 3H_2O \rightarrow 2Fe^0\downarrow + H_2BO_3^- + 4H^+ + 2H_2 \quad (1)$$

Preparation of alginate capsules and characterization

A sodium (Na) alginate solution was prepared by dissolving 10g of Na-alginate in 1 L of de-ionized (DI) water. Alginate capsules were made using a variable flow

mini-pump (VWR, 0.1 mm ID tubing, 1.5 mL min^{-1} flow rate). $CaCl_2$ (0.25 g) and maltodextrin (4.0 g) were dissolved in DI water (6 mL). Fifty milliliters of the Na-alginate solution (10 g L^{-1}) was transferred to a 250 mL glass beaker and continuously stirred at 600 rpm using a magnetic stirrer. The $CaCl_2$/maltodextrin mixture was then pumped drop-wise into the Na-alginate solution from a height of 6 cm from the solution surface (Fig. 1). The capsules formed were continuously stirred in the Na-alginate solution for ~10 min and rinsed several times using DI water. They were then transferred into a 2% $CaCl_2$ solution for 30 min with constant stirring. The resulting capsules were allowed to harden in a 2% $CaCl_2$ solution for 6 h before being used in batch studies. To store the capsules for longer time, 2% $CaCl_2$ was used. All procedures were carried out at room temperature (22±2°C).

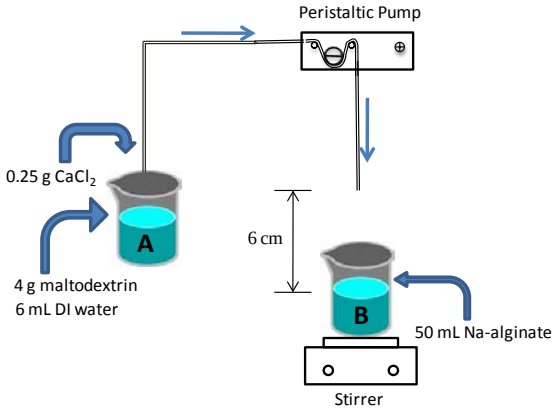


Fig.1: Schematic of Ca-alginate capsule preparation process. For NZVI encapsulation, 30 mg NZVI particles were added to the solution in beaker A and deoxygenated solutions were used

Encapsulation of iron nanoparticles

Encapsulation of NZVI in Ca-alginate was done following the capsule preparation method described earlier. The $CaCl_2$ (0.25 g) and maltodextrin (4.0 g) were mixed with 30 mg of NZVI in 6 mL deoxygenated DI water, and the mixture was stirred to ensure homogeneity. The alginate-maltodextrin-NZVI mixture was then purged with N_2 gas (ultra high purity grade) for ~20 min to remove any air bubbles present before being dropped into the Na-alginate solution.

NZVI loss during the encapsulation was estimated by flushing the pipes and the pump with copious amount of methanol. The methanol flushed NZVI was collected and dried in the oven for a short period and the NZVI was weighed. This exercise was repeated for 5 independent batches of encapsulated NZVI and the results were averaged.

Diffusion studies

Diffusion studies were conducted in reactors (40 mL amber glass vials) with 25 mL. TCE solution (30 and 40 mg L^{-1}) and 300 alginate capsules without NZVI. The reactor caps were fitted with a Teflon septum seal to avoid possible sorption by the plastic caps. The diffusion of TCE from the bulk solution into the capsules was monitored over time. The reactors were shaken in a custom-made end-over-end

rotary shaker (28 rpm) to reduce mass transfer resistance. Aliquots (40 µL) of bulk solution were collected over time and analyzed for TCE.

TCE degradation studies

Batch TCE degradation experiments were conducted with bare and encapsulated NZVI at room temperature in 40 mL amber glass vials (reactors) fitted with Teflon septum. Deoxygenated TCE solution (25 mL) of specific concentration (1, 10, 30, and 40 mg L^{-1}) was used in each reactor along with a definite amount of encapsulated NZVI (30 mg NZVI). The reactor headspace was purged with N_2 gas. All the reactors were rotated end-over-end at 28 rpm in the custom-made rotary shaker. Experiments with (a) only TCE (blank), (b) alginate capsules (with no NZVI) and TCE (control), (c) bare NZVI (not encapsulated) and TCE, and (d) encapsulated NZVI and TCE were conducted. Samples were withdrawn over time and then analyzed for TCE.

Shelf-life studies

Shelf-life study was conducted for the encapsulated NZVI for a 6-month period. NZVI particles were synthesized in a single batch (~3 g) and encapsulated in Ca-alginate (30 mg NZVI in each batch of capsules). Each batch of encapsulated NZVI was stored in a 45-mL vial containing 2% CaCl2 (made with deoxygenated DI water). The vials were purged with N2 gas and closed air tight to prevent possible NZVI oxidation. The vials were wrapped in aluminum foils to prevent any possible photo reactions and stored in a cabinet at room temperature. At least two sacrificial vials were taken out every month and TCE (initial concentration 30 mg L-1) degradation batch studies were conducted using the encapsulated NZVI as described earlier (see TCE degradation studies).

Bacterial Growth Study

Bacterial growth study was conducted to understand the growth behavior of *D.*BAV1 and determine the optimal time for the collection of bacteria for the NZVI-bacteria batch experiments. The pure bacterial culture obtained from ATCC was grown in mineral salt media (MSM). Aliquot (1 mL) of *D.*BAV1 from ATCC supplied pure culture was inoculated into 50 mL of MSM and grown at 30^{o}C overnight under constant shaking at 100 rpm in an orbital shaker (stock solution). Aliquots (100 µL) were collected using sterilized/disposable pipette tips from the stock solution over a 0-36 h time interval for bacterial count. Following the process of serial dilution, the spread plate method was used to count the number of bacterial colonies and thus, the, viable cells. The bacterial growth was monitored over a 36 h time period. The growth studies were performed under a hood, with N_2 purged media to ensure anaerobic conditions. During aliquot withdrawal there may have been some exposure to air (oxygen), but utmost effort was taken to ensure anaerobic conditions. All experimental glassware and work spaces were sterilized prior to use.

Encapsulation of *Dehalococcoides sp*.

The encapsulation of *D.*BAV1 was done following the methods described by Bezbaruah et al (2011). Bacterial sample (1mL) along with 0.25 g $CaCl_2$ and 4g

maltodextrin mixed with 6 mL of distilled de-ionized water ("the solution"). Alginate solution (50 mL of 1% alginate) was stirred in 100 mL Erlenmeyer flask and stirred using a sterilized magnetic pellet. The flask was sealed on the top using two layers of parafilm and was taped to reduce oxygen transfer. The solution was added drop wise using a 10 mL sterilized syringe, the tip of which was inserted through the parafilms on the beaker .Capsules were formed almost instantly. The capsules were rinsed using de-oxygenated, de-ionized water and allowed to harden in 2% deoxygenated $CaCl_2$ solution made up in media 30 min.

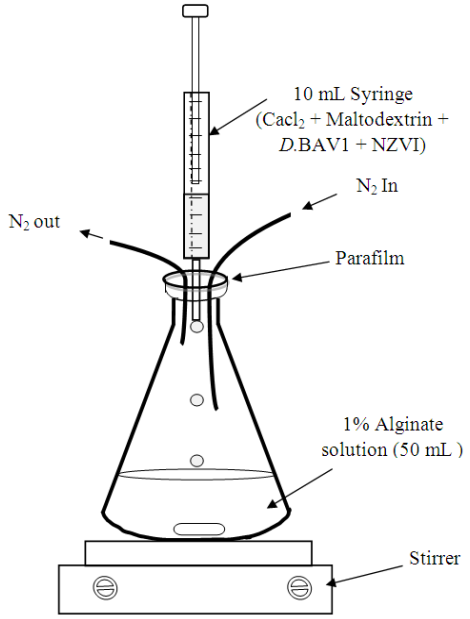


Fig.2: Encapsulation under anaerobic conditions

Combined metal-microorganism system

NZVI (0.75g/L) was co-encapsulated with 1mL of *D.*BAV1 taken along with 4 g of Maltodextrin and 0.25 g $CaCl_2$ made up in 6mL of distilled, de-ionized, and de-oxygenated water. The system was continuously purged with N_2 in an effort to maintain an anaerobic environment (Figure 2). All glassware was sterilized prior to use.

Batch TCE degradation Experiments

Using Encapsulated NZVI

Batch TCE degradation experiments were conducted with encapsulated NZVI at room temperature ($22\pm2^{o}C$) in 40 mL amber glass vials (reactors) fitted with Teflon septum. TCE solution (25 mL, 10mg/L) made with MSM media was used in each of the reactors. A definite amount of encapsulated NZVI (0.75g NZVI/L) was added

into each reactor. Reactors with only TCE in MSM media (blank) were run. Samples were withdrawn at specific time intervals. Re-dosing experiments were performed to find the efficacy of encapsulated NZVI beyond the initial 3 h time period. Following 3 h of reaction the supernatant in the reactor was drained. New TCE solution (25 mL, 10mg/L) was added retaining the encapsulated NZVI. Aliquots were withdrawn periodically up to 36 hr time period.

*Using Encapsulated D.*BAV1

Batch experiments were conducted using encapsulated (1mL) of *D.*BAV1 in 25 mL of 10mg/L TCE in 40mL amber vials with Teflon septum seal. The system was rotated using a custom made rotary shaker at a speed of 28 rpm. Aliquots were withdrawn periodically over a 36 h time interval.

Using the combined metal-microorganism system

Batch experiments were conducted using 0.75 g/L of NZVI co-encapsulated with 1mL of *D.*BAVI in 25 mL of 10 mg/L TCE (in MSM media).The experiments were conducted in 40 mL amber vials over a 36-h time period. After 3 h of the experiment, the TCE-media solution was flushed from the reactor and new TCE-media solution (25 mL of 10 mg/L concentration) was added. Aliquots were collected periodically and analyzed for TCE.

Analytical methods

A gas chromatography (GC, Agilent 6890A PLUS with a capillary column, HP-5MS, 30 m long, and 0.25 mm inner diameter) and mass selective detector (Agilent 5973 Network) coupled with a purge and trap auto sampler system (Tekmar Dohrmann trap concentrator with Tekmar 2016 autosampler) was used for TCE analysis (APHA et al. 2005; USEPA 1992). A five point TCE calibration was performed with 5 $\mu g\ L^{-1}$ to 50 $\mu g\ L^{-1}$ standards ($R^2 = 0.9794$). The method detection limit for TCE was ~0.2 $\mu g\ L^{-1}$. The internal standard was fluorobenzene and a response factor method was used for the calibration and estimation of TCE in the samples.

Quality Control

All experiments were performed in triplicates and average values are reported. While performing experiments involving bacterial samples all work spaces were sterilized with alcohol prior use.

RESULTS AND DISCUSSIONS

NZVI characteristics

NZVI synthesized (Fig. 3a-c) in the laboratory had a size < 100 nm (average size 35 nm) and an average BET surface area of 25 $m^2\ g^{-1}$. The synthesized particles were black in color (Fig. 3a) and majority of them were $\leq$ 50 nm (Fig. 3b). Transmission electron microscope (TEM) image (Fig. 3c) showed the particles as clustered chains.

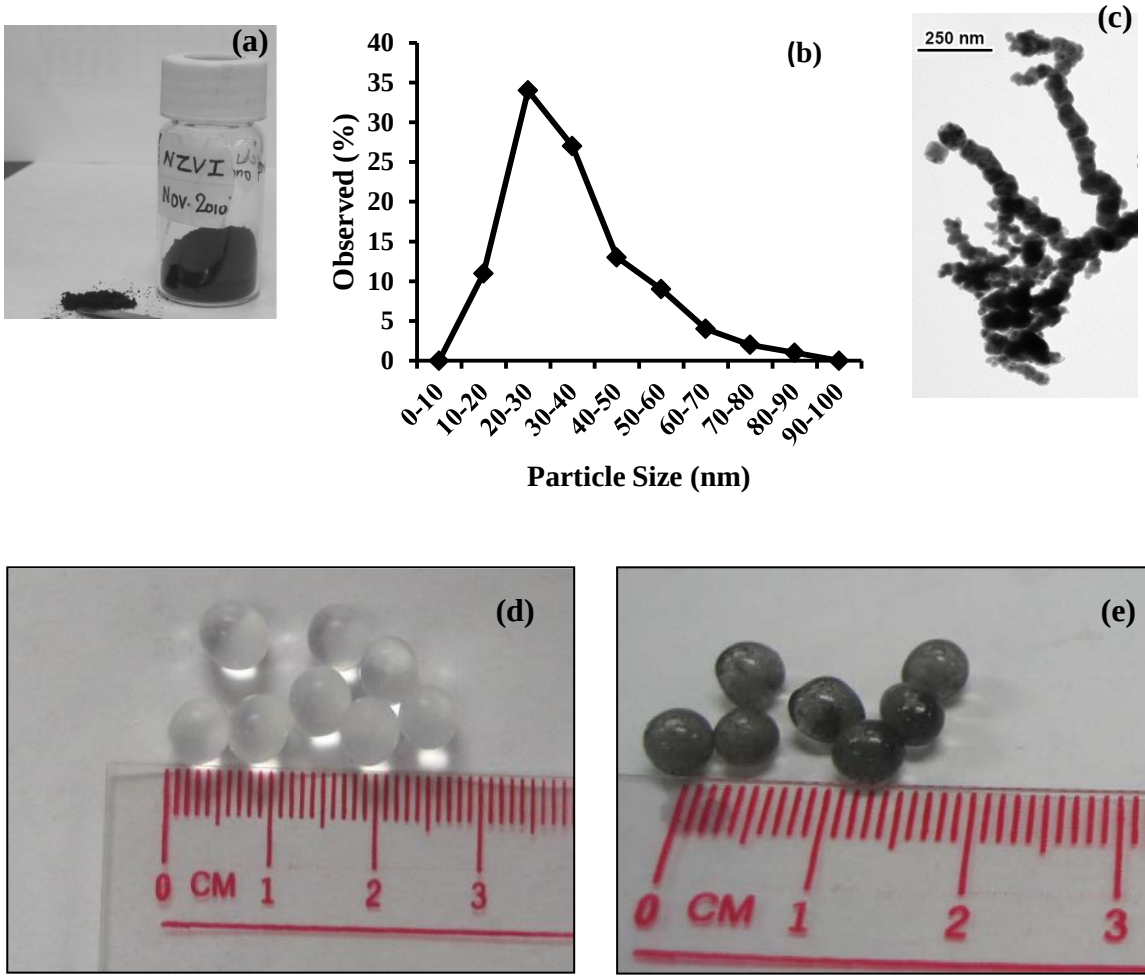


Fig.3: NZVI and Ca-alginate capsule characteristics (a) NZVI synthesized in the laboratory; (b) NZVI particle size distribution. The particle size distribution (average ~35 nm) was determined by measuring individual particles in TEM images (n = 200); (c) TEM image of clustered NZVI particles; (d) Bare Ca-alginate capsules (without NZVI); and (e) NZVI encapsulated in Ca-alginate. Average capsule size = 3.96 mm

Ca-alginate capsule characteristics

Alginate capsules (Fig. 3d-e) were successfully prepared in the laboratory and had an average diameter of 3.96±0.01 mm (average of 25 capsules from 5 batches) and skin thickness of 0.2736±0.0036 mm. Capsule diameter of ~ 2.96 mm and skin thickness of ~0.11 mm have been reported by other researchers (Wang et al. 2010). During NZVI encapsulation, there was negligible loss (~0.15%) of NZVI. A very small number of particles were stuck in the tubing and the beaker.

Diffusion studies

During the diffusion studies conducted with bulk TCE concentrations of 30 and 40 mg L^{-1}, the TCE concentration in bulk solution decreased gradually and leveled off within ~60 min to attain equilibrium (Fig. 4a-b). It can be inferred from the

results from the diffusion studies that there was no major mass-transfer resistance for contaminant diffusion through Ca-alginate. The diffusion characteristics observed within this research are comparable with similar results obtained by others (Garbayo et al. 2002; Lu et al. 2005; Srimornsak and Sungthongjeen 2007; Wang et al. 2011). The controls run with only the capsule skins did show a small initial decrease in bulk TCE concentration, but no further decrease was observed. Similar decreases were reported by others (Bezbaruah et al. 2009; Hill and Khan 2008 and have been attributed to physical sorption by Ca-alginate.

Diffusion into Ca-alginate (beads) has been reported to be a function of the residence time (for hardening) of the beads in the $CaCl_2$ solution. Diffusion can be optimized with a long enough residence time in the solution (Garbayo et al. 2002). A 6-h residence time was used in this study to ensure proper hardening and, hence, contaminant diffusion into the alginate capsules.

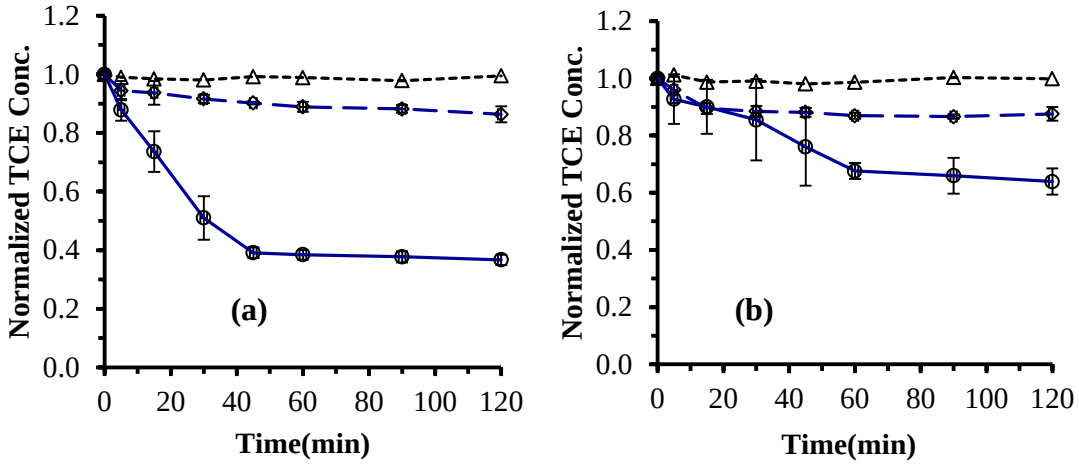
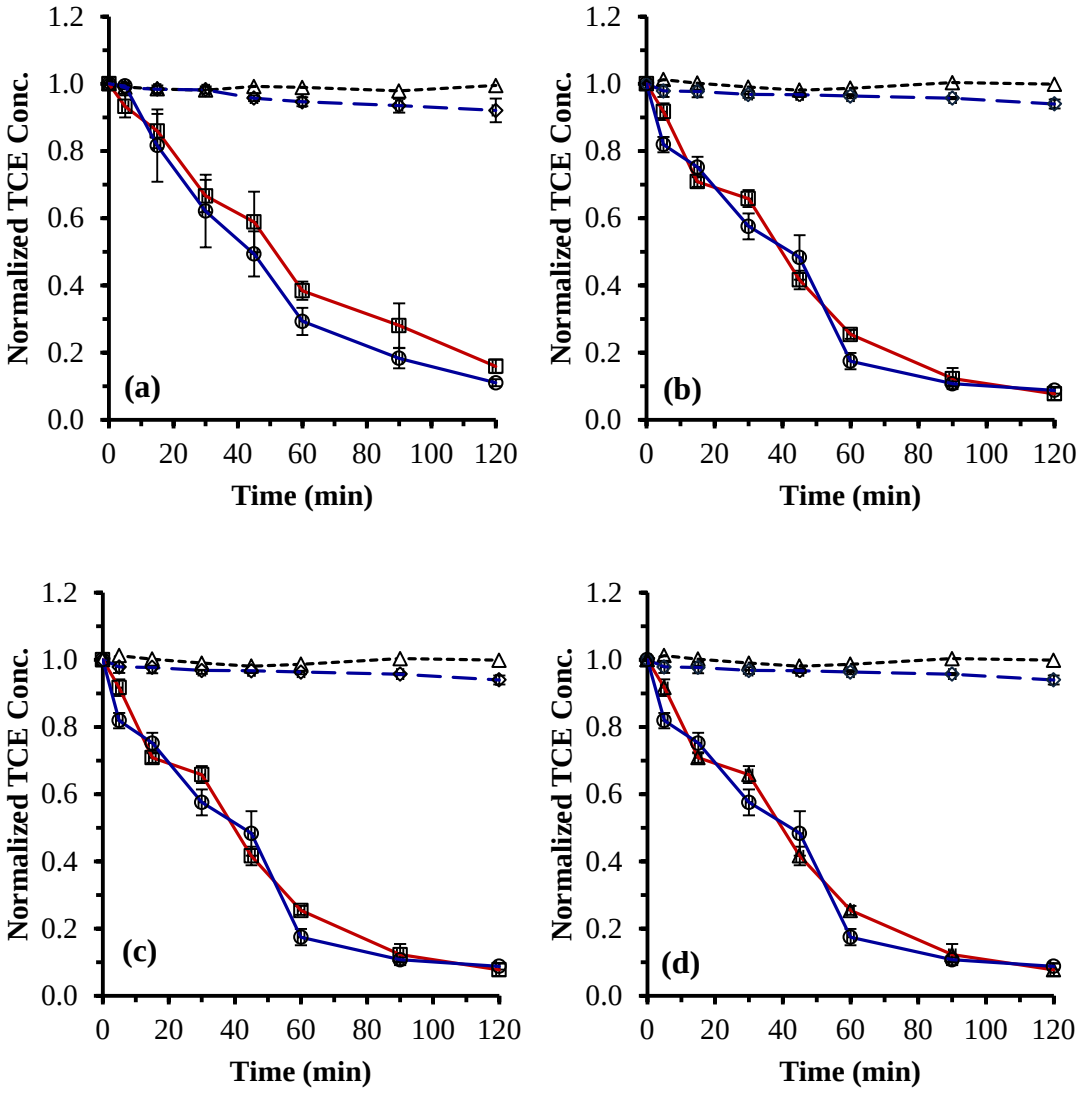


Fig. 4: TCE diffusion characteristics for the Ca-alginate capsules
(a) Initial TCE concentration = 30 mgL^{-1}; **(b)** Initial TCE concentration = 40 mgL^{-1}. Legends: Blank (only TCE solution), (TCE solution with capsule skins), and Ca-alginate capsules in TCE solution. The vertical error bars indicate ± standard deviations. The data points are joined by straight lines for ease of reading only and they do not represent any trend.

TCE degradation

The encapsulated NZVI removed 89-91% of TCE in a 2-h period during the batch experiments. Bare NZVI also showed similar decrease (88-90%) over the same time period (Fig. 5a-d). The pH was not adjusted during the experiment and the pH of the bulk solution changed from 6.4 to 8.9 during the 2-h period (Fig. 5e). These results suggest that the encapsulated iron performed similar to bare NZVI. Comparable TCE degradation efficiencies with bare and encapsulated NZVI indicate that Ca-alginate did not create a barrier for contaminant transport. The controls (capsules with no NZVI) did not show any marked TCE decrease except a minor

reduction (compared to the blank) possibly due to physical adsorption onto the Ca-alginate (Bezbaruah et al. 2009; Hill and Khan 2008).



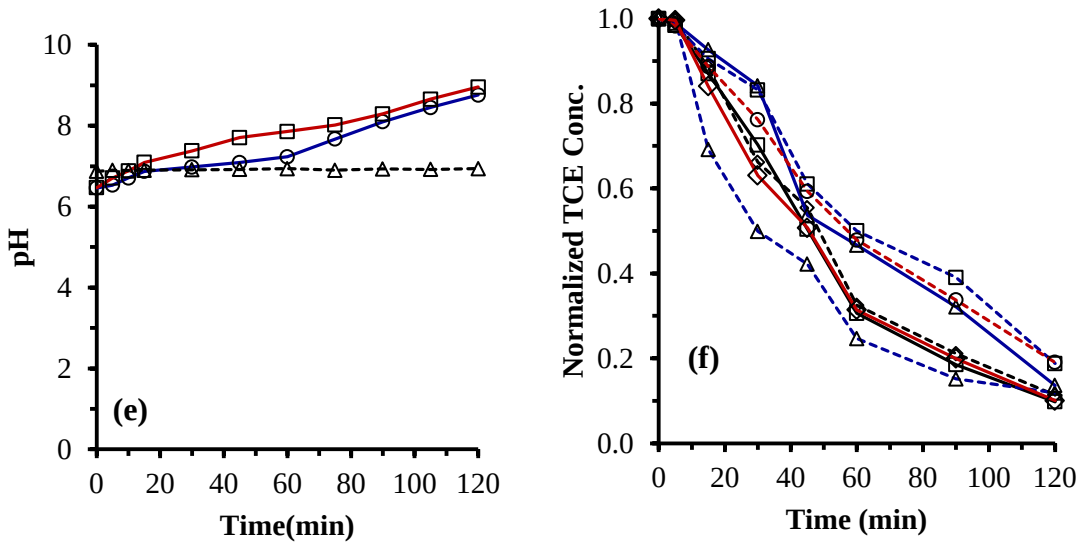


Fig. 5: (a-d) Reduction of TCE by bare and encapsulated NZVI over time **(a)** The initial TCE concentration was 40 mg L^{-1}; **(b)** Initial TCE concentration was 30 mgL^{-1}; **(c)** The initial TCE concentration was 10 mgL^{-1}; **(d)** The initial TCE concentration was 1 mgL^{-1} **(e)** Representative pH trend during TCE degradation. The pH plot for 30 mg TCE/L is shown here. Legends (for **a-e**): TCE solution, TCE + Bare NZVI, TCE + Encapsulated NZVI. The vertical error bars indicate ± standard deviations. **(f)** Reduction of TCE by encapsulated NZVI system over a time span of 6 months (shelf-life study). Legends: Month 0, Month 1, Month 2, Month 3, Month 4, Month 5, Month 6. The data points are joined by straight lines for ease of reading only and they do not represent any trend.

TCE degradation kinetics

TCE degradation by both bare and encapsulated NZVI has been found to follow first order kinetics (Table 1). The observed reaction rate constant (k_{obs}) for the bare NZVI system was found to be $1.53x10^{-2}$ to $2.92x10^{-2}$ min^{-1}. The value of k_{obs} ranged from 1.92 $x10^{-2}$ to 3.23 $x10^{-2}$ min^{-1} for the encapsulated system. Statistical analyses (two-way ANOVA) indicate that there is no significant difference between the TCE degradation reaction rate constants when bare and encapsulated NZVI particles were used (α =0.005, p-value = 0.211). The reactions are known to be surface area controlled in NZVI, and it is, therefore, prudent to normalize the reaction rate constants to the NZVI surface area used per unit volume of treated water (Matheson, L.J., Tratnyek 1994; Thompson et al. 2010). Surface normalized reaction rate, k_{sa} (Eq. 2, Johnson et al. 1996), for bare and encapsulated NZVI are presented in Table 1

$$dC/dt = - k_{sa} \rho_{np} C \qquad (3\text{-}2)$$

Where dC/dt = reaction rate (mg L^{-1} min^{-1}), k_{sa} = surface area normalized reaction rate constant (L m^{-2} min^{-1}), C = contaminant concentration (mg L^{-1}), t = time (min), and ρ_{np} = concentration of iron surface area (m^2 L^{-1}).

The results from the present research indicate no significant difference in the values between bare and encapsulated NZVI possibly because the particles are only restrained within the confined space and no surface modification was observed. Such a confinement reduces the mobility of the particles without sacrificing their reactivity and, hence, will be ideal for in-situ applications for groundwater remediation (e.g., in permeable reactive barriers). As durability of the capsules and long-term effectiveness of the NZVI are important for such applications, shelf-life studies (see below) were conducted.

Table .1: Reaction rate constants calculated based on the results obtained during this study

Batch	**Initial TCE concentration**	**Reaction rate constant**		**R^2**
	mg L^{-1}	**k_{obs}** 10^{-2} min^{-1}	**k_{sa}** 10^{-3} L m^{-2} min^{-1}	
Bare NZVI	1	2.92	1.6	0.9689
	10	2.35	1.3	0.9801
	30	1.53	0.8	0.9897
	40	2.24	1.2	0.9868
Encapsulated NZVI	1	3.23	1.7	0.9832
	10	2.45	1.3	0.9491
	30	1.92	1.0	0.9921
	40	2.21	1.2	0.9425

Shelf-life of NZVI

The NZVI particles are expected to have long shelf-life to be commercially viable. Long shelf-life would ensure that they can be stored for an extended period of time after production, and shipped out to distant remediation sites without the change in their characteristics. Results from the shelf-life study experiments revealed that the efficiency of the encapsulated NZVI for TCE removal did not decrease in the first 4 months (~89% TCE removal) and decreased marginally by 5-7% over the fifth (84%) and the sixth (82%) months (Fig. 5f). The first order reaction rate constant (k_{obs}) for TCE removal decreased from $1.95x10^{-2}$ to $1.37x10^{-2}$ min^{-1} over the six month study period. A 4-month shelf-life can be considered to be very good for transportability and storage of the encapsulated NZVI and increases the relevance of the present technique for real world applications.

Bacterial Growth Characteristics

The bacterial growth was monitored using the plate count method over a 36-h time period. The results from the bacterial growth study (Figure6) indicate that the bacteria are in the lag phase between 0-4 h, followed by stationary growth phase from 4-36 h. The growth study was not continued beyond 36 h as the objective of this effort was to find out the active growth phases. Knowing the growth phases provides a basis for the time during which the bacterial sample should be collected for TCE batch degradation experiments. The optimum time (phase) to collect the bacterial sample is 15 h and beyond as the culture is in stationary growth phase.

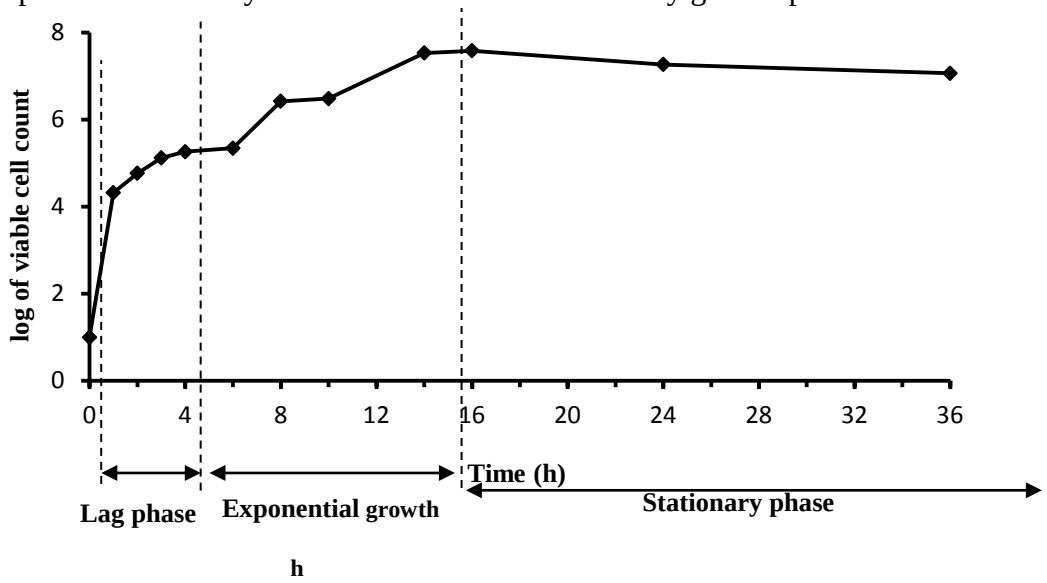


Fig. 6.: *D.*BAV1 growth curve at room temperature 22±2^{o}C and in MSM growth media.

Batch TCE degradation studies

Using Encapsulated NZVI

Batch TCE degradation studies were conducted using encapsulated NZVI in MSM in an effort to understand whether the media interferes with TCE degradation using NZVI. The results indicate that encapsulated NZVI had an efficiency of ~100% for the removal of TCE in MSM media over a 3-h time period. When re-dosed at 3 h with additional TCE there was no further removal of TCE. This indicates that NZVI gets used up in 3 h of reaction (Figure 7 a).When the data from this study is compared with that reported by Bezbaruah et al. (2011) it is apparent that there was no interference due to the use of MSM.

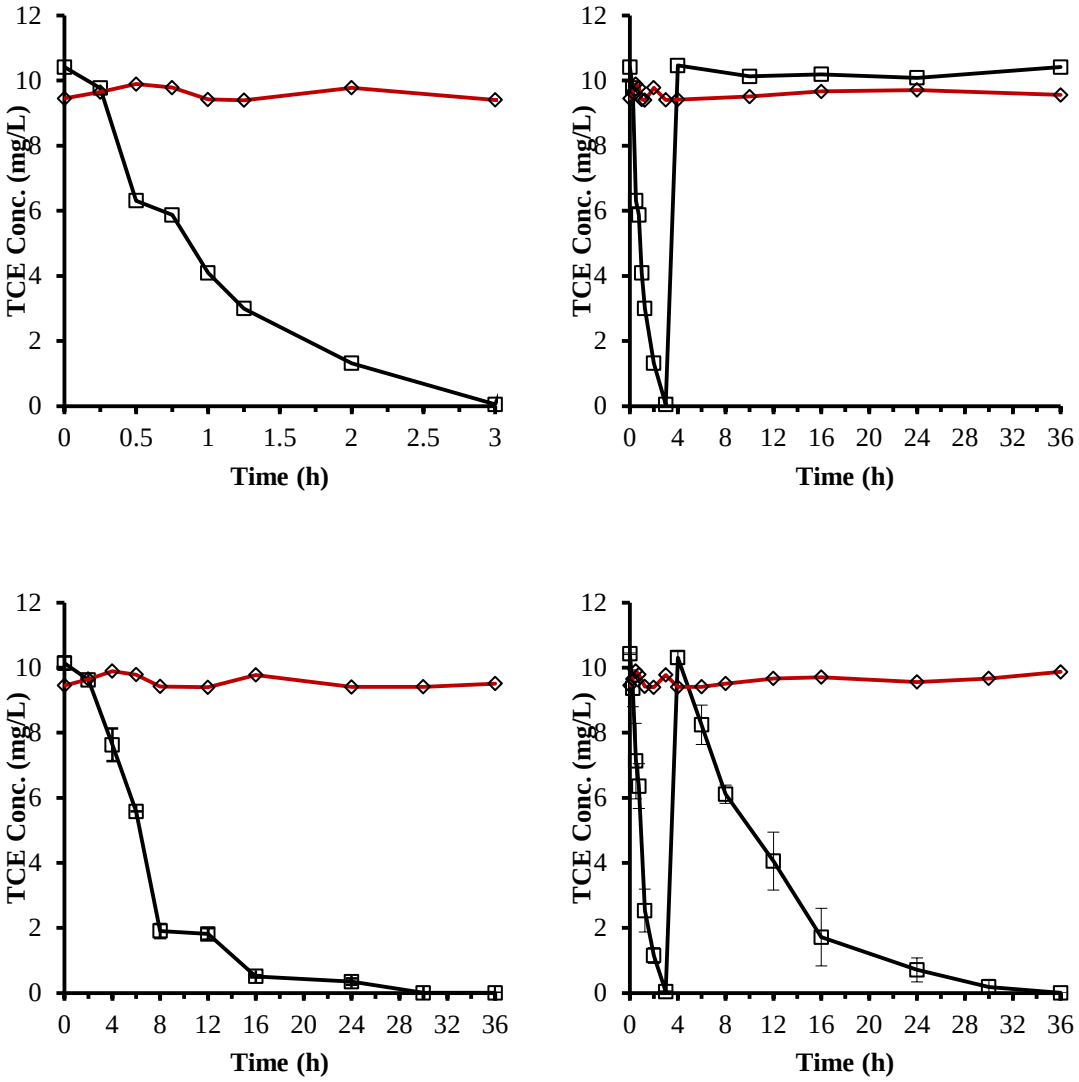


Fig 7: TCE removal using: (a) Encapsulated NZVI, (b) Encapsulated NZVI (re-dosed), (c) Encapsulated *D.BAV1*, and (d) Combined metal-microorganism system. Blank (—◇—) TCE in MSM media.

Using Encapsulated D.BAVI

Batch experiments were conducted using encapsulated *D.*BAV1 in 25 mL of 10mg/L TCE in MSM media over a 36-h time period. The results of this study revealed encapsulated *D.BAV1* had a TCE removal efficiency of ~100% over a 36-h time period. The by-products of TCE removal were not observed during the entire course of the 36-h reaction. The degradation trend for TCE removal using encapsulated *D.BAV1* indicated that the bacterial degradation was relatively slow during first two hours. This can probably be explained by the fact that *D.*BAV1 was

in the initial lag phase during the first few hours of the reaction. Beyond 16 h, the reaction slowed down and achieved a 100% TCE removal at 36 h.

Using combined metal-microorganism system

Batch TCE degradation experiments were carried out using the co-encapsulated NZVI-*D.BAV*1 system over a 36-h time period. The TCE removal was found to be 100% efficient during the first 3 h, which was dominated by NZVI. The dominance of NZVI for TCE removal can be justified by the microorganism lag period during the initial stages of the reaction. After TCE re-dosing, the TCE removal trend resembled that of bacterial degradation (Figure 7 b- c). Any removal of TCE which occurred after re-dosing is attributed to bacterial degradation because NZVI is completely consumed in the first three hours of the reaction. Control re-dosing experiments were run, with only encapsulated NZVI. The results indicate that NZVI was consumed during the first three hours of the reaction, and showed no further TCE removal after re-dosing (Figure 7a).

TCE Degradation Kinetics

TCE degradation by encapsulated NZVI and *D.*BAV1 followed first order kinetics. The observed reaction rates were found to be 2.76×10^{-2} min^{-1} and 4.90×10^{-3} min^{-1} respectively. The reaction rate for the combined system was calculated under the assumption that the first 3-h of the reaction was dominated by NZVI and from 3-36 h the reaction was bacteria dominated. Under this assumption the experiment was divided into two stages and separate reaction rates were calculated for each stage (reaction dominated by NZVI, and reaction dominated by bacteria). Both the stages of the reaction followed first order kinetics **(Table .2)**. The reaction rate for the first 3-h for the combined metal-microorganism was found to be very similar to the reaction rate of encapsulated NZVI, with a k_{obs} of 2.93×10^{-2} $min^{-1.}$ This result suggests that it is most likely that NZVI dominated the first 3 h of TCE reduction for the combined metal-microorganism system in line with the above assumption. The reaction rate constant for the latter part of the experiment (3-36 h) also followed first order kinetics with k_{obs} of 3.80×10^{-3} min^{-1}, which is very similar to the reaction rate constant of encapsulated *Pseudomonas putida* F1.

Table 2: Reaction rate constants calculated based on the results obtained during this study.

Reaction order		E-NZVI	E-B	C-NZVI	C-B
Zero	K_{obs} ($mg \cdot L^{-1} \cdot min^{-1}$)	5.83×10^{-2}	6.6×10^{-3}	6.01×10^{-2}	5.00×10^{-3}
	R^2	0.863	0.33	0.89	0.82
1st	K_{obs} (min^{-1})	**2.76×10^{-2}**	**4.90×10^{-3}**	**2.93×10^{-2}**	**3.80×10^{-3}**
	R^2	**0.912**	**0.92**	**0.93**	**0.86**
2nd	K_{obs} ($L \cdot mg^{-1} \cdot min^{-1}$)	8.35×10^{-2}	2.85×10^{-1}	1.08×10^{-1}	6.05×10^{-1}
	R^2	0.636	0.63	0.64	0.41

E-NZVI: Encapsulated NZVI, E-B: Encapsulated Bacteria, C-NZVI: Combined System (NZVI dominated), C-B: Combined system (bacteria dominated)

CONCLUSIONS

This study on co-encapsulation of nanoscale zero-valent iron (NZVI) and bacteria has demonstrated that a combined physico-biochemical process is feasible for contaminant removal from aqueous environment. NZVI particles were encapsulated in Ca-alginate without significant reduction in their reactivity. The TCE removal using encapsulated NZVI was 89-91% when compared to 88-90% removal using bare NZVI over a 2-h period. The TCE degradation followed first order kinetics for both the encapsulated NZVI systems. The shelf-life of the encapsulated NZVI was four months within which there was little decrease in its TCE degradation efficiency.The encapsulation technique has been effectively used to entrap TCE degrading microorganisms in Ca-alginate for TCE remediation. In an effort to exploit the combined advantages of NZVI and microorganisms, an effective remediation system was successfully designed. Batch experiments revealed that the combined NZVI-microorganism system could efficiently function with the first stage of the remediation performed by encapsulated NZVI while the second stage was preferentially taken over by encapsulated microorganisms. By-products of TCE degradation such as dichloroethylene, and vinyl chloride were not observed while using the combined NZVI-microorganism system. Non-detection of toxic by-products is considered positive and the system has potential for field application. The following are the conclusions which can be drawn from this study:

1. Encapsulation of NZVI in alginate polymer is a viable, technique for TCE dechlorination. It has a potential for in-situ remediation applications.
2. Encapsulation of microorganisms in Ca-alginate capsules is an efficient technique for TCE removal and converts it to benign end products. Toxic byproducts (DCE and VC) were not detected during degradation of TCE.
3. The combined metal-microorganism system worked very well for TCE removal and had the advantages of both NZVI and microorganisms. Such a system has potential for use in Permeable Reactive Barriers (PRBs) for environmental remediation.

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