ALGINATE ENCAPSULATED NANOPARTICLE-MICROORGANISM SYSTEM FOR TRICHLOROETHYLENE REMEDIATION

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ABSTRACT

Nanoscale zero-valent iron (NZVI) particles were encapsulated in calcium alginate capsules for application in environmental remediation. TCE degradation rates for encapsulated and bare NZVI were similar indicating no adverse effects of encapsulation on degradation kinetics. Microorganisms were separately encapsulated and used along with encapsulated NZVI and co-encapsulated in calcium alginate capsules. Batch experiments were performed to test the efficacy of the combined iron-Pseudomonas *sp*. (*PpF1)* system. The combined system removed 100% TCE over the first three hours of the experiment followed by 70% TCE removal post TCE re-dosing. Complete reduction of TCE was achieved by NZVI between 0-3 h and the second phase of treatment (3-36 h) was mostly achieved by microorganisms. Experiments conducted with co-encapsulated NZVI-*D.BAV1* achieved 100% TCE removal. During the first three hours of the experiment 100% TCE removal was achieved by NZVI, and 100% removal was achieved post re-dosing where *D.BAV1* accomplished the treatment.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Cells/mL- Number of viable cells/mL

Fe –Iron

Fe^0 –Zero-valent iron

Fe^{2+} -Ferrous

Fe^{3+} - Ferric

nm- nanometer

mM -millimoles

mg -milligrams

mL -milliliter

NP-Nanoparticles

NZVI-Nanoscale-Zero-valent iron nanoparticles

PCE -Tetrachloroethene

PpF1- *Pseudomonas putida F1*

PRB -Permeable reactive barrier

TCE -Trichloroethene

TEM -Transmission electron microscopy

Viable bacteria - living bacteria

NPL- National Priority List

ZVI- Zero-valent iron nanoparticles

D.BAV1 – *Dehalococcoides* BAV1

LIST OF APPENDIX TABLES

CHAPTER 1. INTRODUCTION

1.1 Background

Volatile organic compound (VOC) contamination of groundwater is an environmental concern. Chlorinated solvents such as trichloroethylene (TCE) in particular represent one of the most problematic class of VOCs 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). TCE is listed by the United States Environmental Protection Agency (USEPA 1992) as a Class A hazardous waste, believed to be a carcinogen and a mutagen. Improper storage and disposal of chlorinated degreasing solvents have resulted in a number of cases of TCE contamination of groundwater. The maximum contaminant level (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). TCE is a widely used industrial solvent and a degreasing agent. Its improper storage and use have 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 solubility limit in groundwater is 1000 mg/L (Russell et al. 1992)

The USEPA has reported a number of National Priority List (NPL) Sites which require immediate attention under Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) that are contaminated with TCE. 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 widespread use, mobility and persistence cause the greatest risk of groundwater contamination. In the event of TCE contamination immediate remedial action is needed due to the hazardous nature of this contaminant.

1.2 Need statement

There are a number of technologies presently being used for TCE remediation. They are chemical reaction mechanisms, physical treatment methods and bioremediation. Chemical treatment techniques utilize chemicals such as Fenton's reagent, ozone, and iron to degrade TCE. Chemical oxidation using permanganate/ Fenton's reaction has been used to remediate TCE (Tsai et al. 2011) but due to the high reduction potential associated with TCE a reductive pathway (where the contaminant is an electron acceptor) may be more preferable for TCE contaminant remediation as opposed to an oxidative pathway (where the contaminant is an electron donor) as reported by a number of researchers (Kim et al. 2010a; Russell et al. 1992). However, chemical reaction techniques have a number of associated problems including short life span, and harmful by-product production.

Physical treatment methods such as air stripping are expensive and have recently been replaced by chemical/physico-chemical remediation techniques. Bioremediation is another attractive option as it is inexpensive and known to reduce TCE to benign end products such as ethane and CO_2. However, bacterial degradation is slow and time consuming. Thus, the combined advantages of a chemical reductive technique (using nanoscale zero-valent iron particles) and a bioremediation technique (use of bacterial cultures) could create a unique niche for in-situ remediation of TCE with high dechlorination rates, with the potential to mitigate the hazardous by-products of TCE degradation.

1.3 Research objective

The broad objective of this study is the co-entrapment of nanoscale zero-valent iron (NZVI) and TCE degrading bacteria in alginate polymer to design a remedial system for potential groundwater applications to achieve complete degradation of TCE. The specific objectives of this research are as follows:

- Encapsulate NZVI in Ca-alginate capsules.
- Determine whether alginate encapsulated NZVI (in alginate polymer) is effective for TCE remediation.
- Encapsulate TCE degrading bacteria in Ca-alginate capsules.
- Determine whether encapsulated TCE degrading bacteria (*Pseudomonas putida F1* and *Dehalococcoides BAV1*) are effective for TCE remediation.

- Determine the interferences between NZVI and bacterial growth media.
- Test the efficacy of the combined metal-microorganism system for TCE remediation.
- Quantify the reaction kinetics of TCE degradation by NZVI, bacterial strains and combined metal-microorganism system.

1.4 Hypothesis

NZVI will reduce TCE in the first step in the degradation process and then the microorganisms will preferentially take over the process. This will result in benign end products without the generation of harmful intermediate by products.

1.5 Expectations of this study

This study is expected to effectively dechlorinate TCE to harmless by products utilizing the combined advantages of a chemical and biological system. However, toxicity of NZVI to microorganisms is not well understood. Interaction between NZVI and microorganisms is essential to ensure functionality of the combined metal-microorganism system.

CHAPTER 2. LITERATURE REVIEW

2.1 Trichloroethylene contamination

Trichloroethylene (C_2HCl_3) is a chlorinated hydrocarbon most commonly used as an industrial solvent, popularly abbreviated as TCE. TCE is a clear, volatile, non-flammable liquid, with a sweet smell (ATSDR 1997). The use of TCE as an industrial degreasing agent occurred until the mid 1970's when TCE was replaced by other chemicals due to environmental and health impacts associated with it. Disposal of TCE included burial of TCE drums in trenches often unlined and direct deposits within fire pits in designated landfills often with improper lining. Inappropriate methods of disposal resulted in substantial releases of TCE as DNAPLs to groundwater (Truex et al. 2011). A number of superfund sites (61%) listed under CERCLA are contaminated with TCE requiring immediate remedial action plans. (USEPA 1992) lists TCE as a Class A hazardous waste and a likely human carcinogen with the MCL of 5 ppb in drinking water.

2.2 Overview of TCE remediation

Remediation of TCE contaminated groundwater is receiving a lot of attention due to the potential magnitude of health impacts associated with TCE. The TCE remediation methods can be broadly classified under three distinct categories: (i) chemical methods, (ii) physical methods; and (iii) bioremediation. Chemical methods for TCE remediation includes reaction mechanisms such as oxidation (using potassium ferrate), reduction (using iron), and dehalohydrolysis. Surface treatment technologies like air stripping and soil excavation come under physical treatment

technologies to remediate TCE. Physical methods for the treatment of TCE may incur a lot of cost thus calling for newer technologies with improved cost efficiency at the same time delivering efficient TCE removal. Bioremediation (using bacterial strains capable of metabolizing TCE) is widely used due to the fact this method is economical and reduces TCE to benign end products (ethane and CO_2). Chemical methods for TCE removal especially using iron have received major attention. The reasons for the high success rate for TCE remediation using iron includes its availability (inexpensive), non-toxic nature, ease of use and high efficiency for TCE removal. Exhaustive research has been carried out exploring the feasibility of TCE removal using iron (Bezbaruah et al. 2009; Bezbaruah et al. 2011; Chen et al. 2010; Grieger et al. 2010; Kim et al. 2010a; Krajangpan et al. 2008; Long and Ramsburg 2011; Tang et al. 2011; Truex et al. 2011; Xiu et al. 2010; Zhang 2003).Bioremediation of TCE is also extensively used as the process is cheap, efficient and believed to result in benign by products. (See section 2.5)

2.3 Nanoscale zero-valent iron (NZVI)

NZVI was introduced in 1994 by Gillham and Ohannesin (1994) for the remediation of halogenated aliphatics. The most basic form of NZVI is spherical $Fe^{(0)}$, which has dimensions less than 100 nm. 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; Cullen et al. 2011; Li et al.2006). 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).

There are a number of wet chemistry techniques for the synthesis of NZVI. Liu et al. (2005) demonstrated that NZVI from the borohydroxide reduction process has the unique ability to generate H_2, thus enhancing hydrodehalogenation and hydrogenation. Li et al. (2006) listed a number of wet chemistry techniques used for the synthesis of NZVI and indicated that NZVI particles synthesized using different methods had very different physical properties. Literature review focuses on the borohydroxide method for NZVI synthesis **(See Section 3.2)**

2.4 Remediation with zero-valent iron

Nanoscale zero-valent iron particles have been used to remediate a wide range of environmental contaminants including chlorinated compounds (Bezbaruah et al. 2011a; Kim et al. 2010a; Lien and Zhang, 1999; Liu et al. 2005; Liu et al. 2005; Lowry and Johnson, 2004; Song and Carraway, 2005; Wang and Zhang, 1997), heavy metals (Alowitz and Scherer 2002; 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 potential of the zero-valent iron system is -0.44V (Milazzo et al. 1978). A wide range of environmentally problematic contaminants (especially chlorinated hydrocarbons such as 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:

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

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

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.

In the past traditional zero-valent iron has been employed in hundreds of NPL Sites to remediate chlorinated hydrocarbons. ZVI (non-nano) has been used for the purpose of site remediation since 1990s. The technique employed while using ZVI is the creation of permeable reactive barriers (PRBs) by filling out trenches with ZVI, designed in order to allow groundwater to pass through the PRB while removing the contaminants. (EPA 2008; ITRC 2005). Macroscale ZVI has also been extensively used and recognized to be an excellent electron donor with the capability of releasing

electrons irrespective of its particles size (Zhang and Elliott, 2006). Research indicates that using NZVI in the place of macroscale iron will accomplish the same remedial action with more efficiency and much less cost. In the recent years researchers are exploring new techniques for the injection of NZVI directly into the source of contamination; which is believed to be a quicker and more effective means of groundwater treatment as opposed previously existing methods of contaminant treatment such as pump-and-treat (U.S. EPA 2008).

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 addition there are also some increasing concerns about the high mobility of nanoparticles and them possibly reaching undesired receptors like aquatic organisms, microorganisms and even human beings (Phenrat 2009). 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 basically targets confining a group of particles within hollow membranes or spheres without individually restraining the particles (i.e. particles are free to move within the sphere) as opposed to the "entrapment" technique. In the "entrapment" technique groups of reactive particles are "embedded" within a

membrane or sphere and are not free to move. Bezbaruah et al. (2009) affirmed the efficacy of a Ca-alginate entrapped iron system for nitrate remediation with a nitrate removal efficiency of up to 73 % while Bee et al. (2011) established the efficiency of magnetic alginate beads for the removal of Pb (II) ions from wastewater (Bee et al. 2011). 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.

The use of a number of biopolymers such as alginate, poly(methyl methacrylate) (PMMA), chitosan, and carrageenan have been explored and tested for environmental remediation applications (Bezbaruah et al. 2009; Crini 2005; Krajangpan et al. 2008; Krajewska 2005; Ngila 2011; Vieira et al. 2011; Youssef et al. 2010).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). The process does not require complicated instrumentation and it can be performed at ambient temperature and pressure. 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). The easiest way to encapsulate

active compounds within alginate matrix is by using the dripping-gelation method. In this method, alginate liquid droplets containing active compounds are extruded from an orifice and are allowed to fall into a gelling bath (Calcium chloride solution has been proven to be a good gelling bath by Bezbaruah et al., 2011) under gravity (Chan et al., 2009). The droplets surface will solidify almost instantly when in contact with the gelling bath and the droplets will fully gelled by prolonging the gelation time. An optimum gelation time of ~6 hr was optimized by Bezbaruah et al., in 2011. This method has been widely used to encapsulate many heat sensitive active ingredients such as living cells, enzymes, drugs, plant extracts and nano particles (Bezbaruah et al., 2011; Chan et al. 2009; Deladino et al. 2008; Idris and Suzana 2006; Kosaraju et al. 2006; Kulkarni et al. 2000).

2.5 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 (Rauch et al. 2004). Other entrapped NZVI systems have less TCE removal efficiencies when compared to the entrapped bi-metallic NZVI/Pd system. 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 %.

2.6 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. Over the past decades, laboratory and field studies have revealed that subsurface microorganisms can degrade a wide range of chlorinated hydrocarbons (Bouwer et al. 1981; Harker and Kim 1990; De Bont, 1992; Wilson and Wilson 1985; Capiro et al. 2011). 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. These processes can be broadly classified as: (i) Reductive dechlorination of TCE; (ii) Co-metabolism of TCE and (iii) Direct oxidation of TCE. A summary of different microbial strains used to degrade TCE is shown in Table 2.1.

Reductive dechlorination generally occurs under anaerobic conditions. Mc-Carty (1994) elucidated the reductive dechlorination process for chlorinated solvents, where chlorinated ethenes including TCE are used as electron acceptors. 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

Table 2.1: Comparison between different bacterial strains, methods of removal, and end products for TCE bioremediation

Microorganism	Method of Removal	End Product	Reference
Pseudomonas putida F1	Direct Oxidation Co-metabolism	ETH	(Sun and Wood 1996); (Kim et al. 2010b); (Radway et al. 1998)
Clostridium bifermentans DPH-120	Co-metabolism	c-DCE	(Chang et al. 2000)
Dehalospirillum multivorans	Direct Oxidation	c-DCE	(Neuman et al.1994)
Dehalococcoides ethenogenes 195	Reductive dechlorination	ETH	(Maymo-Gatell et al. 1999), (Fennell et al. 2001)
Dehalococcoides BAV1	Reductive dechlorination	ETH	(Krajmalnik-Brown et al. 2004; Maymo-Gatell et al. 1999),(He et al. 2003)
Dehalococcoides VS	Reductive dechlorination	ETH	(Cupples et al. 2003)
Dehalococcoides FL2	Reductive dechlorination	ETH	(El Fantroussi et al. 1998)

ETH:Ethane, **c-DCE**: cis Dichloroethene. The bacterial strains used in this study are highlighted in bold

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) (Gerritse et al. 1999; MaymoGatell et al. 1997; Maymo-Gatell et al. 1999; Krajmalnik-Brown et al. 2004; Cupples et al. 2003; Pant and Pant 2010).

The problem associated with the reductive dechlorination is that the presence of other electron acceptors in the water can negatively impact TCE dechlorination. According to Byl and Williams (2000) the electron acceptors which could interfere or delay the process of dechlorination are oxygen, nitrate, manganese, sulfate, and carbon dioxide, listed in the order of bacterial preference. Noell (2009) reported the presence of some competing electron acceptors such as dissolved oxygen, nitrate and sulfate during the pilot test conducted in contaminated groundwater at the Naval Weapons Industrial Reserve Plant in Dallas, Texas. To counter the problem associated with electron acceptor competition, effective delivery of the microbial strain to the target contaminant is essential. Encapsulation/ entrapment of the reactive microbial strains within a biopolymer to ensure effective delivery of microbial strain to the contaminant plume is one of the potential delivery method researchers are targeting for the years to come. (See section 2.3)

The process of aerobic co-metabolism of TCE was brought to light after the work of Wilson and Wilson (1985). In the case of TCE co-metabolism, a co-solvent is added (for example toluene) in order to increase the efficiency of TCE biodegradation. The contact time between the microorganism and the co-solvent has

been found to play an important role in the biodegradation process. Methane oxidizing bacteria have found to be very efficient in the co-metabolism of TCE. Apart from methane oxidizing bacteria, toluene oxidizers and phenol oxidizers have received tremendous attention. These bacteria produce oxygenases in response to phenol or toluene, which initiate the oxidative degradation and mineralization of TCE. Fan and Scow (1993) reported that that the ratio of TCE and co-metabolites such as phenol and toluene control the co-metabolism process by influencing the microbial growth (Fan and Scow 1993). Research indicates that *Pseudomonas putida F1* strain is capable of degrading TCE by the production of toluene (a dioxygenase) which will help in the complete mineralization of TCE. Other researchers have also published work verifying the efficiency of *Pseudomonas putida F1* to mineralize TCE (Heald and Jenkins 1994; Kim et al. 2010b; Radway et al. 1998).

Direct oxidation is another method for TCE removal where less chlorinated reductive dechlorination products can serve as electron donors (primary substrates) for some respiratory bacteria. (Olaniran et al. 2008; Bradley and Chapelle 1996; McCarty and Semprini 1994; Fennell et al. 2001). Any of these processes could be occurring at a given site depending on the form of chlorinated ethenes and the site environmental characteristics.

2.7 Combined metal-microorganism system

Research is in progress to synergistically utilize the combined effects of NZVI and microorganisms in order to establish a two stage 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. To date there are at least two schools of thought in regards to nanoparticle-microorganism interactions. Some researchers believe that nanoparticles are detrimental to microorganisms (Auffan et al. 2008; Lee et al., 2008) while some believe that microorganisms are unaffected by the presence of NZVI (Grieger et al. 2010; Kirschling et al. 2010; Li et al. 2010). Kirschling et al. (2010) conducted experiments with microbial community obtained from real groundwater and established that there was no decrease of microbial abundance while being exposed to 1.5±0.1 g/L of NZVI. When a polyspartate coating was added to the NZVI, microbial population increased due to the ability of the biopolymer coating to enhance microbial growth by supplying N_2 and carbon. There is limited literature to address NZVI-microorganism interaction (Table 2.2). 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. It was found to be a negative correlation between NZVI dose and bacterial growth. NZVI-microorganism interaction is bacterial growth phase dependent. Microorganisms in active growth phase are not affected by NZVI. Microorganisms in their lag phase of growth (i.e. non-dividing) cells are adversely affected by NZVI.

Table 2.2: Relevant research reported on NZVI-microorganism interactions

Test organism	NZVI concentration	Solution	Interaction condition	Notes	Reference
Escherichia coli strain Qc1301 and its mutant *sod*A *sod*B Qc2472	7,70,175,350, 700 g/L	Ultrapure water (pH5-5.5)	Aerobic	Dose-dependent toxicity (>70mg NZVI/L).	Auffan et. al. (2008)
Escherichia coli (ATCC strain 8739)	1.2–110 mg/L	2 mM Carbonate buffer		No explicit signs of growth inhibition.	Lee et al. (2008)
Bacillus subtilis, -Gram positive, *Pseudomonas fluorescens*-Gram negative *Aspergillus versicolor Fungus*	0.1,1,10 g/L	DI water	Aerobic	Complete inactivation in *P. fluorescens*. when treated with 10 mg/ml of NZVI	Diao and Yao (2009)
Escherichia coli (ATCC strain 33876)	100 mg/L	5mM bicarbonate buffer solution	Aerobic Anaerobic	Aerobic exposure to 100 mg/L of NZVI (28% Fe^0 and rest oxides) led to 0.8-log inactivation after 60 min. Toxicity is decreased in anaerobic conditions.	Li et al. (2010)
Mixed culture (microsoms-from aquifer materials)	1.5±0.1 g/L	Real groundwater sample	Anoxic	No adverse effect on bacterial growth using polyspartate coated NZVI.	Kirschling et al. (2010)

CHAPTER 3. ENCAPSULATION OF IRON NANOPARTICLES IN ALGINATE BIOPOLYMER FOR TRICHLOROETHYLENE REMEDIATION

3.1 Introduction

NZVI particles have been used to remediate a wide range of groundwater contaminants including chlorinated compounds (Liu and Lowry 2006), pesticides (Bezbaruah et al. 2009; Joo and Zhao 2008), heavy metals (Alowitz and Scherer 2002), and explosives (Gregory et al. 2004). NZVI particles are ideal for the degradation of environmental contaminants because of their environment friendly nature, high reactivity, and low cost (Zhang 2003). The mode of degradation by which the NZVI breaks down such contaminants is reductive dehalogenation and sorption (Matheson and Tratnyek 1994). The small sized (< 100 nm) NZVI particles have very high reactive surface area (25-54 $m^2 g^{-1}$) (Bezbaruah et al. 2009; Liu and Lowry), and that makes them highly efficient for contaminated water remediation. However, NZVI particles are highly mobile in the aquifer or they settle down into the aquifer pores once they agglomerate due to interparticulate magnetic and van der Waals forces (Bezbaruah et al. 2009). There are also increasing concerns about high mobility of the small nanoparticles and their reaching undesired receptors including endemic microorganisms and humans (Phenrat et al. 2009).

In order to overcome the inherent mobility problem, NZVI can be encapsulated in calcium (Ca) alginate. In encapsulation, groups of particles are put inside hollow Ca-alginate capsules without individually restraining the particles. Encapsulation ensures that the particles do not come out of the capsule and become

mobile. This is in contrast to entrapment (Bezbaruah et al. 2009) where particles are embedded into a polymer matrix for the same purpose. Encapsulation also needs less alginate as compared beads (from entrapment), and may result in major material savings.

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 entrapped NZVI (Bezbaruah et al. 2009; Huang and Zhihui 2002). Results from previous studies indicate that entrapped nanoparticles perform equally well as bare nanoparticles with little change in their reactivity (Bezbaruah et al. 2009). While entrapment has been tried for environmental remediation (Bayramoğlu and Arica 2009; Bezbaruah et al. 2009; Bleve et al. 2011; Hill and Khan 2008; Lin et al. 2005; Önal et al. 2007; Pramanik et al. 2011), encapsulation has not been reported as a possible remediation technique. Encapsulation is expected to ensure better contacts between contaminants and encapsulated nanoparticles. In addition successful encapsulation of NZVI is expected to lead to the development of more effective and robust environmental remediation techniques involving co-encapsulation of nanoparticles, microorganisms, and/or enzymes.

The objective of the work described in this chapter is to examine the effectiveness of the encapsulated iron nanoparticles for aqueous contaminant remediation with TCE as the test contaminant. Comparisons between TCE remediation by bare and encapsulated NZVI were made to see if the NZVI particles

lose their reactivity due to encapsulation. Diffusion studies were performed to check whether Ca-alginate creates any additional mass transfer resistance for contaminant diffusion. Shelf-life studies were conducted for the encapsulated NZVI to see whether the particles lose their reactivity upon storage and during transport.

3.2 Materials and methods

3.2.1 Chemicals and reagents

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

3.2.2 Synthesis of NZVI

NZVI particles were synthesized using the borohydride reduction of ferrous iron and passivation technique reported by others (Eq. 3-1, Liu and Lowry 2006; Bezbaruah et al. 2009).

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

3.2.3 Preparation of alginate capsules and characterization

A sodium (Na) alginate solution was prepared by dissolving 10 g of Na-alginate in 1 L of distilled 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). Maltodextrin was added to control the viscosity and obtain spherical shape of capsules (Tanriseven

and Doan 2001). 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). Capsules were formed as soon as the $CaCl_2$/maltodextrin mixture hit the stirred alginate solution. The capsules formed were continuously stirred in the Na-alginate solution for approximately 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^oC). To know the number of capsules synthesized in each batch, the capsules formed during the synthesis of 10 different batches were manually counted and the results were averaged. The diameter and skin thickness of the capsules were measured using a vernier caliper for a number of capsules (n = 25) from different batches and the average value is reported. The values reported closely match the values reported by other researchers.

3.2.4 Encapsulation of iron nanoparticles

Encapsulation of NZVI in Ca-alginate was performed 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.

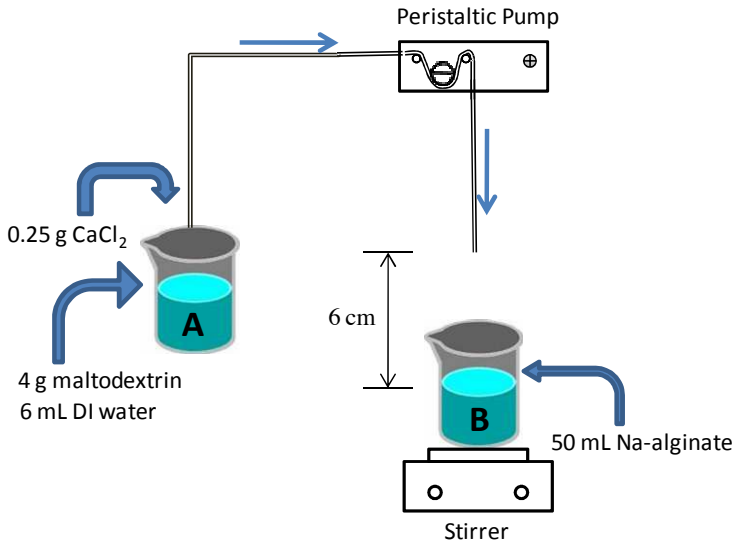


Figure 3.1: Schematic of Ca-alginate capsule preparation process. For NZVI encapsulation, 30 mg NZVI particles were added to the solution in beaker A and deoxygenated solutions were used

There were possibilities that NZVI particles might have got attached to the pipes and the pump system, and these particles would not be accounted for in the final results. This 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.

3.2.5 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 at 0, 5, 15, 30, 45, 60, 90, and 120 min and analyzed for TCE. All experiments were performed in triplicates and average values are reported.

3.2.6 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 at 0, 5, 15, 30, 45, 60, 90, and 120 min, and then analyzed for TCE. All experiments were performed in triplicates and average values are reported.

3.2.7 Shelf-life study

A 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% $CaCl_2$ (made with deoxygenated DI water). The vials were purged with N_2 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).

3.2.8 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). The samples were purged with helium gas at a flow rate of 35 mL min^{-1} for 11 minutes at ambient temperature after they were loaded into the purge and trap concentrator. Desorption of the trapped sample components was done by heating the trap column at 225°C for 2 minutes. The purge and trap concentrator was in a bake mode between the analyses of samples for 6 minutes at 270ºC. For GC, the carrier and split gases (Helium in both cases) had flow

rates of 1.5 and 28 mL min^{-1}, respectively. The analyses were performed with an initial oven temperature of 40°C for 1 min, followed by ramping up at 5°C min^{-1} to 45°C, 8°C min^{-1} to 125°C, and 25°C min^{-1} to a final temperature of 180°C where it was held for 1 min. The injector and detector temperatures were 250°C and 275°C, respectively. A five point TCE calibration was performed with 5 μg L^{-1} to 50 μg L^{-1} standards ($R^2 = 0.9794$). The method detection limit for TCE was 0.2 μg L^{-1}. The internal standard was fluorobenzene and a response factor method was used for the calibration and estimation of TCE in the samples.

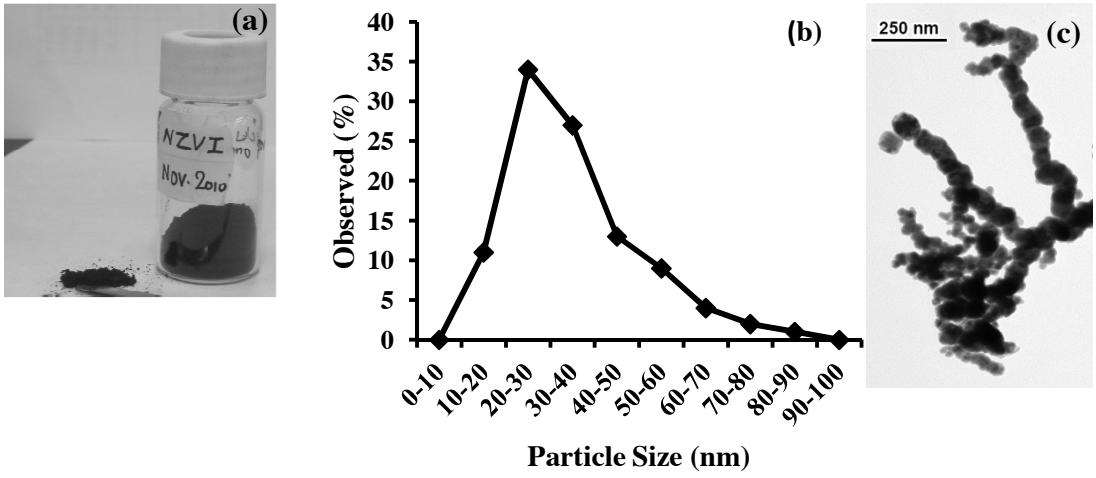
3.2.9 Statistical analysis

Standard deviations for results were calculated and have been reported in section 3.3. Two-way analysis of variance (ANOVA) was used to determine whether there is any significant difference between the reaction rates of bare and encapsulated NZVI for TCE removal. Statistical analyses were performed using Minitab software (Version 14, Minitab Inc.).

3.3 Results and discussions

3.3.1 NZVI characteristics

NZVI synthesized (**Figure. 3.2a-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 (**Figure. 3.2a**) and majority of them were ≤ 50 nm (**Figure. 3.2b**). Transmission electron microscope (TEM) image (**Figure. 3.2c**) showed the particles as clustered chains.



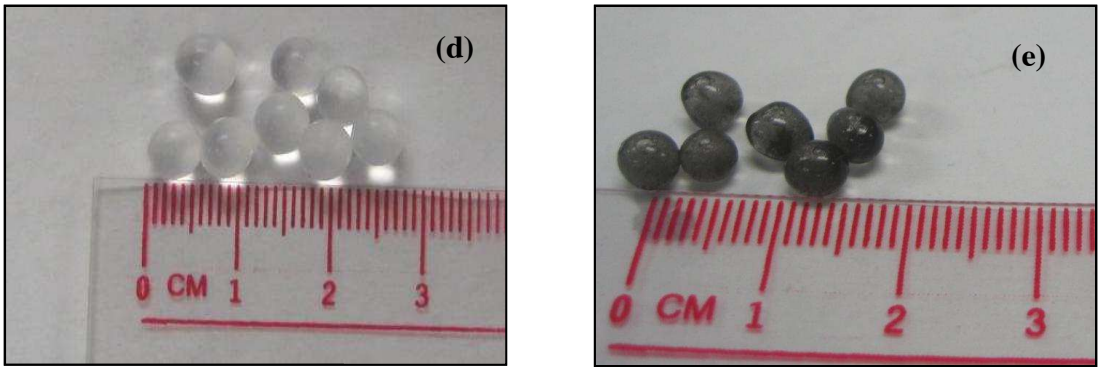


Figure 3.2: NZVI and Ca-alginate capsule characteristics. (a) NZVI synthesized in the laboratory; (b) NZVI particle size distribution. The particle size distribution (average ~35 nm) was determined by measuring individual particles in TEM images (n = 200); (c) TEM image of clustered NZVI particles; (d) Bare Ca-alginate capsules (without NZVI); and (e) NZVI encapsulated in Ca-alginate. Average capsule size = 3.96 mm

3.3.2 Ca-alginate capsule characteristics

Alginate capsules (**Figure. 3.2d-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. It is important to have skin as thin as possible to reduce contaminant mass transfer resistance during diffusion

and materials used. A capsule diameter of 2.96 mm and a skin thickness of 0.11 mm have been reported by other researchers (Wang et al. 2010). Literature indicates that the concentration of Na-alginate influences the characteristics of the capsules as Ca-alginate gel formation depends on the cross linking between alginate from Na-alginate and Ca^{2+} from $CaCl_2$ (Augst et al. 2006). In this study different concentrations of Na-alginate (4, 6, 8, 10, and 12 g L^{-1}) were tried in order to obtain the optimum capsules characteristics which include small diameter, thin membrane, ease of capsule preparation, and effective NZVI particle retention. A 10 g L^{-1} of Na-alginate was found to be the optimum concentration for capsule preparation. It is worth noting that $CaCl_2$ used in capsule formation remained inside the capsules, and possibly helped in better hardening of the capsules (Aksu et al. 2002; Garbayo et al. 1998).

The stirring speed of the Na-alginate solution and dropping height of $CaCl_2$-maltodextrin-NZVI mixture (onto the stirred Na-alginate solution) also influenced the shape and size of the capsules formed. A stirring speed of 600 rpm and 6 cm dropping height from the solution surface were selected as the optimal values for capsule formation based on a number of trials.

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.

3.3.3 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 (**Figure. 3.3a-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). While TCE is a low molecular weight (MW 131.5) non-polar compound, there are reports of effective diffusion of polar and other non-polar compounds with a wide range of MW through Ca-alginate capsule (Wang et al. 2011) and beads (Lu et al. 2005; Westrin and Axelsson 1991). Diffusion of vitamin B12 (MW 1355.37) through Ca-alginate has been reported by Wang et al. (2011). Nicotinamide adenine dinucleotide (NADH, MW 709.4) diffused from the bulk solution into Ca-alginate beads and attained equilibrium in 30 min (Lu et al. 2005).

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.

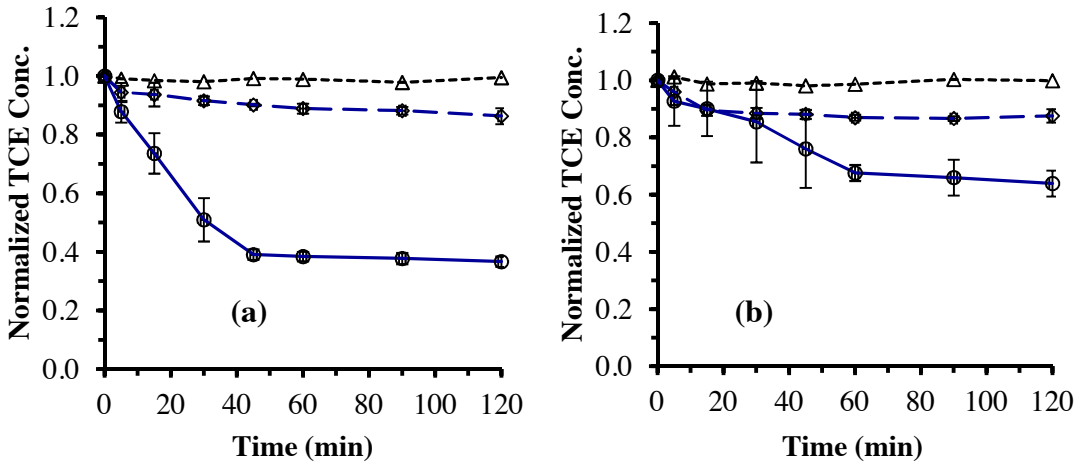


Figure 3.3: TCE diffusion characteristics for the Ca-alginate capsules. **(a)** Initial TCE concentration = 30 mg L^{-1}; **(b)** Initial TCE concentration = 40 mg L^{-1}. Legends: ---△--- Blank (only TCE solution), – –◇– – (TCE solution with capsule skins), and —○— Ca-alginate capsules in TCE solution. 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

3.3.4 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. 3.4a-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 (**Figure.3.5**). 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). Results obtained by Kim et al.

(2010) indicate 60% reduction in TCE with NZVI immobilized along with PAC in alginate beads. They also reported that entrapment of NZVI and palladium (Pd) in alginate beads achieved 99.8% TCE removal. TCE removal efficiency in this study without PAC or Pd was 90%.

3.3.5 TCE degradation kinetics

TCE degradation by both bare and encapsulated NZVI has been found to follow first order kinetics (**Table 3.1**). The observed reaction rate constant (k_{obs}) for the bare NZVI system was found to be 1.53×10^{-2} to 2.92×10^{-2} min^{-1}. The value of k_{obs} ranged from 1.92×10^{-2} to 3.23×10^{-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 ($\alpha = 0.005$, 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 and Tratnyek 1994; Thompson et al. 2010). Surface normalized reaction rate, k_{sa} (Eq. 3-2, Johnson et al. 1996), for bare and encapsulated NZVI are presented in Table 3.1 and compared with results reported by others in Table 3.2.

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

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}).

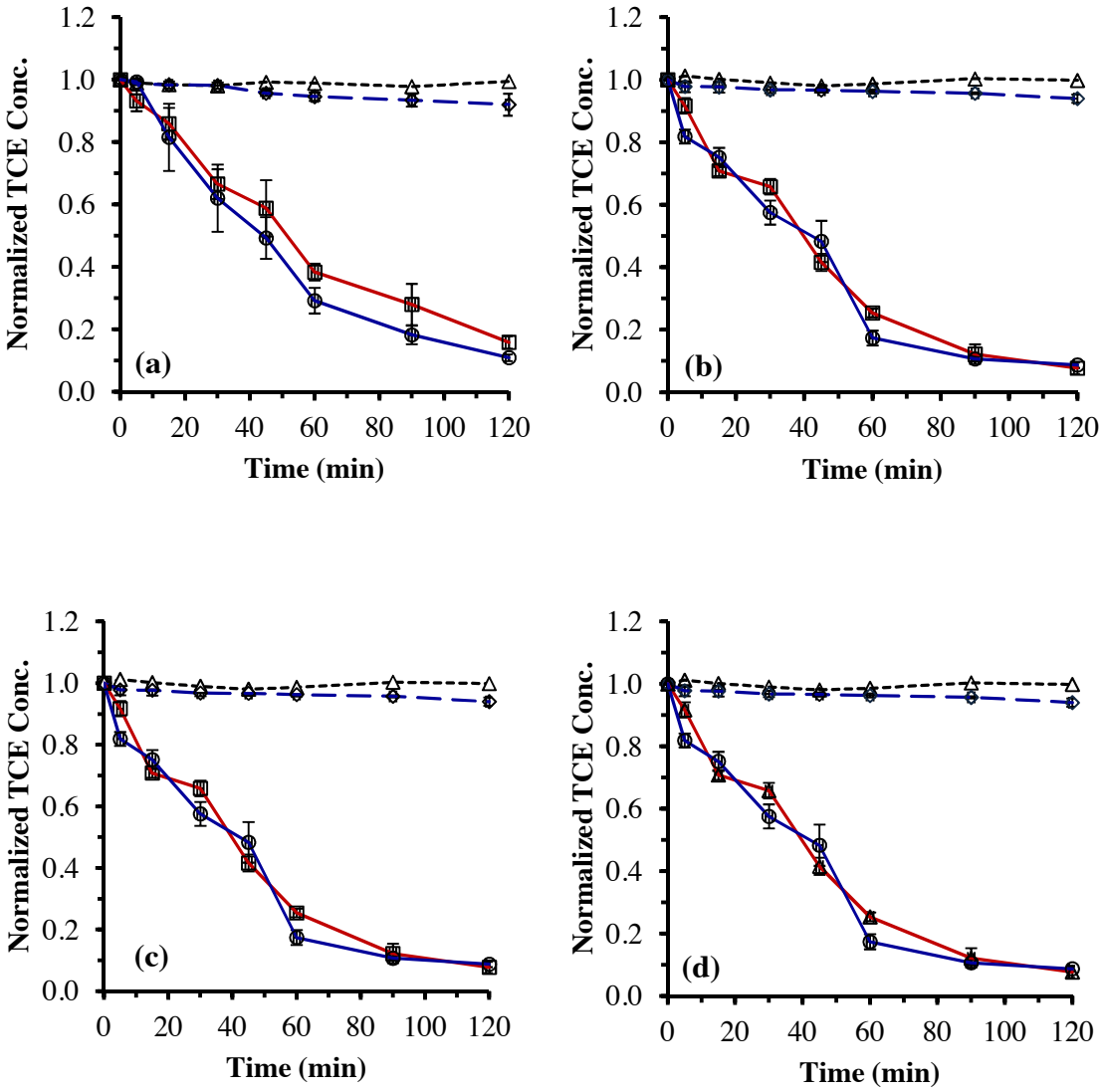


Figure 3.4: (a-d) Reduction of TCE by bare and encapsulated NZVI over time. **(a)** Initial TCE concentration was 40 mg L^{-1}; **(b)** Initial TCE concentration was 30 mg L^{-1}; **(c)** Initial TCE concentration was 10 mg L^{-1}; **(d)** Initial TCE concentration was 1 mg L^{-1}. Legends (for **a-d**): ---△---TCE solution, —□—TCE + Bare NZVI, —○— TCE + Encapsulated NZVI. 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

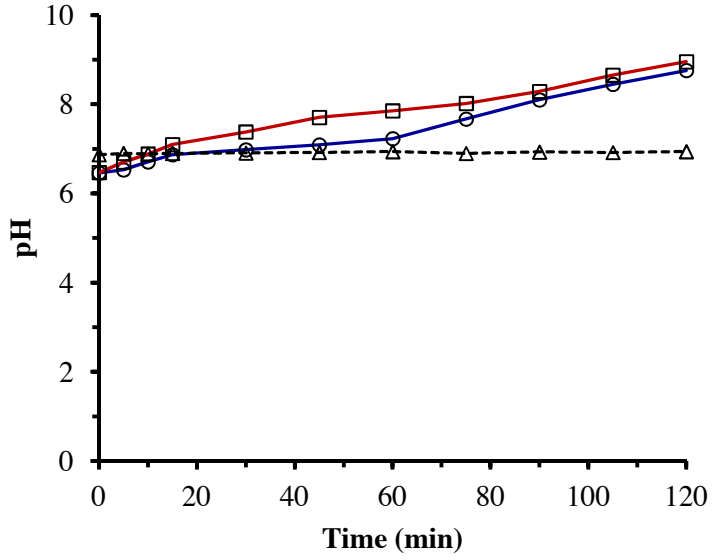


Figure 3.5: Representative pH trend during TCE degradation. The pH plot for 30 mg TCE/L is shown here. Legends ---△---TCE solution, —□—TCE + Bare NZVI, —○— TCE + Encapsulated NZVI. The vertical error bars indicate ± standard deviations. The data points are joined by straight lines for ease of reading only and they do not represent any trend

Typically NZVI coated with polymers or electrolytes show reduced reaction rates possibly because of reduction in exposed reactive surface area or reduced diffusion through the coatings (Phenrat et al. 2009a; Wang et al. 2010). 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 3.1: Reaction rate constants calculated based on the results obtained during this study

Batch	Initial TCE concentration	Reaction rate constant		R^2
		k_{obs}	k_{sa}	
	mg L^{-1}	10^{-2} min^{-1}	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

3.3.6 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 (**Figure. 3.6**). 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.

Table 3.2: Comparison of results from the present research with other reported studies on TCE removal with NZVI

Source	Type of NZVI	TCE removal (%)	Reaction Rate Constant k_{obs} (min^{-1})	Reaction Rate Constant k_{sa} ($L\ m^{-2}\ min^{-1}$)
He et al. 2010	NZVI/Pd in CMC	80	$2.40x10^{-1}$	-
Kim et al. 2010	NZVI/Pd in alginate beads	99.8	$1.01x10^{-1}$	$1.06x10^{-2}$
	NZVI/PAC in alginate beads	~62	-	-
Wang et al. 2010	Bare NZVI	69.1	$1.96x10^{-4}$	-
	NZVI in PMMA	62.3	$5.67x10^{-5}$	-
Liu et al. 2005	NZVI	100	-	$2.33x10^{-4}$
Schrick et al. 2002	Bare NZVI	-	$1.5x10^{-3}$	$3.3x10^{-5}$
Wang and Zhang 1997	Bare NZVI	~100	-	$5.0x10^{-5}$
Present work	Bare NZVI	88-90	$1.53x10^{-2}$-$2.92x10^{-2}$	$8.16x10^{-4}$-$1.56x10^{-3}$
	NZVI in alginate capsules	89-91	$1.92x10^{-2}$-$3.23x10^{-2}$	$1.02x10^{-3}$-$1.72x10^{-3}$

CMC: Carboxymethyl cellulose; **Pd:** Palladium; **PAC:** Powdered activated carbon; **PMMA:** Poly (methyl methacrylate)

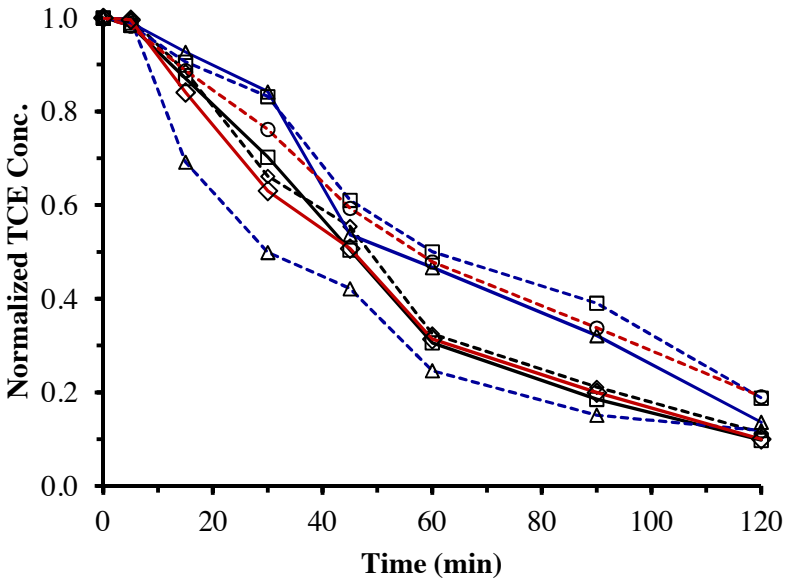


Figure 3.6: Reduction of TCE by encapsulated NZVI system over a time span of 6 months (shelf-life study). Legends: Month 0, Month 1, Month 2, Month 3, Month 4, Month 5, Month 6. The data points are joined by straight lines for ease of reading only and they do not represent any trend

3.4 Summary

This study has demonstrated that NZVI particles can be 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 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 use of Ca-alginate encapsulated NZVI can overcome the mobility and settlement problems associated with bare NZVI and can be a potential technique for in-situ remediation of groundwater. This study provides a potential technique viable for the encapsulation of microorganisms in alginate capsules for possible remediation applications.

CHAPTER 4. ENCAPSULATION OF MICROORGANISMS AND NANOSCALE ZERO-VALENT IRON PARTICLES IN ALGINATE POLYMER FOR TRICHLOROETHYLENE REMEDIATION

4.1 Introduction

TCE is a halogenated VOC which is a human carcinogen (USEPA 1997). A large number of NPL sites are contaminated with TCE (Zachritz et al. 1996) and many states in the United States have problems related to TCE contamination. (www.epa.gov/superfund/sites/npl). TCE is a widely used industrial solvent and degreasing agent. Inappropriate disposal of TCE contaminant has resulted in widespread soil and water contamination raising serious public concerns (Doucette et al. 2007). There are several methods for the remediation of TCE contaminant including physical processes (e.g. soil excavation, pump and treat method), chemical reduction and bioremediation. However, environmental and economical concerns are associated with these methods. Research indicates that treatment systems such as coagulation, sedimentation, precipitative softening, filtration and chlorination are ineffective in reducing TCE contaminant to non-hazardous levels. (Russell et. al. 1992; Robeck and Love 1983). Newer cost effective and efficient treatment technologies are needed to remediate TCE.

In recent years, NZVI particles have received a lot of attention for environmental remediation (Bezbaruah et al. 2009; Bezbaruah et al. 2011; Joo and Zhao 2008; Liu et al. 2005). NZVI particles are ideal for environmental remediation because of their environmental friendly nature, low cost, high reactivity and large surface area (Bezbaruah et al. 2011). However, due to their high mobility NZVI

particles may settle down in the aquifer once they agglomerate due to their interparticulate magnetic and van der Waals forces (Bezbaruah et al. 2009). The "encapsulation" technique has been successfully employed, in order to overcome the inherent mobility problems associated with NZVI. NZVI particles encapsulated in Ca-alginate capsules have achieved TCE removal efficiency up to ~ 91% (Bezbaruah et al. 2011).

In-situ bioremediation of TCE is gaining importance over the last two decades. The bioremediation technology is preferred over other processes as it results in complete mineralization of TCE to benign chemical forms such as CO_2, H_2O and inorganic chlorine (Russell et al. 1992). 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 microbial strains such as *Pseudomonas species*, *Dehalococcoides species*, and *Bacillus species* (Pant and Pant 2010).

Bioremediation techniques are much slower when compared to chemical reduction processes and hence a new system which combines the advantages of both chemical and biological processes is called for. A combined metal-microorganism system will incorporate fast reaction speed and higher efficiency when chemical processes are used at the same time resulting in benign end products because of microbial actions.

To use the combined metal-microorganism system, it is essential to understand the interactions between the two. There are two schools of thought

associated with microorganism-metal interactions. Some researchers believe that nanoparticles are harmful to microorganisms (Auffan et al., 2008; Lee et al., 2008) whereas some others believe that microorganisms are unaffected by the presence of NZVI (Grieger et al. 2010; Kirschling et al. 2010; and Li et al. 2010). Kirschling et al. (2010) affirmed the possibility to utilize microorganisms and NZVI together to synergistically engineer a novel remediation system.

The objective of the research presented in this chapter is to examine the effectiveness of the combined encapsulated metal-microorganism system for aqueous contaminant removal with TCE as the test contaminant. Batch experiments were conducted to compare TCE removal using encapsulated microorganisms and TCE removal using encapsulated NZVI separately. These experiments served as controls to provide an overall understanding of the efficacy of the combined system. TCE re-dosing experiments were conducted to elucidate the individual role of NZVI and microorganisms throughout the 36-h experimental duration.

4.2 Materials and methods

4.2.1 Chemicals and reagents

Calcium chloride ($CaCl_2$, ACS grade, BDH), sodium alginate (production grade, Pfaltz & Bauer), methanol (production grade, BDH), maltodextrin (food grade, Aldrich), *Pseudomonas putida F1* (pure culture, ATCC) and trichloroethylene (TCE, ACS Grade, 99.5% pure) were used as received.

4.2.2 Synthesis of NZVI

NZVI particles were synthesized using the borohydride reduction of ferrous iron and passivation technique reported by others (Eq. 4-1, (Liu et al. 2005, Liu and Lowry 2006; Bezbaruah et al. 2009a).

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

4.2.3 Preparation of alginate capsules and characterization

Ca-alginate capsules were prepared and characterized as described by Bezbaruah et al. 2011.The capsules were spherical in shape with a diameter of 3.96±0.1mm. The prepared capsules were stored in 2% $CaCl_2$ solution as needed for later use.

4.2.4 Encapsulation of iron nanoparticles

NZVI was synthesized in the laboratory and had the average particle size of 35 nm. NZVI was encapsulated as described in Section 3.2.3. NZVI (0.75 g/L) was encapsulated in Ca-alginate capsules and preserved in a 2% $CaCl_2$ solution.

4.2.5 Bacterial growth studies

A 1 mL aliquot of *Pseudomonas putida F1* was inoculated into 20 mL of Tryptic soy broth (TSB) media and grown at 30^{o}C overnight under constant shaking at 150 rpm. This is a "stock solution." One hundred micro liter aliquots were collected (using sterilized/disposable pipette tips) from the stock solution over 1, 2, 3, 4 , 5, 6, 7 , 8 , 9 , 10 , 12 , and 24 h time intervals. Following the process of serial dilution, the spread plate method was used to visually count the number of bacterial colonies and thus count the viable bacterial cells. The bacterial growth was monitored

over a 24 h time period. Bacterial growth studies were performed under two conditions: (i) without NZVI (ii) with NZVI.

4.2.6 Encapsulation of *Pseudomonas putida* F1

Encapsulation of *Pseudomonas putida* F1 (PpF1) was performed the standard method as described earlier (Bezbaruah et al. 2011). Bacterial stock solution (1 mL) containing a specific number of bacterial cells was added to a mixture of 0.25 g $CaCl_2$ and 4.0 g maltodextrin taken in 6 mL of DI water, and the mixture was stirred to ensure homogeneity. The standard encapsulation process was adopted as described in **Section 3.2.3**. The $CaCl_2$-maltodextrin-microorganisms 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 to make capsules.(See Preparation of alginate capsules and characterization in **Section 3.2.3**).

4.2.7 Preparation of combined metal-microorganism system

Batch degradation experiments were performed using the combined metal-microorganism system over a 36-h time period. Encapsulated NZVI (750 mg/L) was taken along with 1 mL of encapsulated bacterial cells (containing a specific number of cells, estimated using plate count method) in a 40 mL amber glass vial (reactor) containing 10 mg/L TCE made up in TSB media.

4.2.8 TCE degradation studies

4.2.8.1 Using NZVI

Batch TCE degradation experiments were conducted with encapsulated NZVI at room temperature (25±2^{o}C) in 40 mL amber glass vials (reactors) fitted with

Teflon septum. A TCE solution (25 mL) of 10 mg/L concentration made up in TSB media was used in each of the reactors along with a definite amount of encapsulated NZVI (0.75g/L). The reactors were rotated end-over-end at 28 rpm in a custom-made rotary shaker. Experiments with (a) only TCE made up in nutrient media (blank), and (b) encapsulated NZVI and TCE in nutrient media were conducted. Samples were withdrawn at 0, 5, 15, 30, 45, 60, 90, 120 and 180 min, and then analyzed for TCE. All experiments were performed in duplicates and average values are reported.

4.2.8.2 Using Pseudomonas putida F1

Batch TCE degradation experiments were conducted with encapsulated *Pseudomonas putida* F1 in 40 mL amber glass vials fitted with Teflon septum. TCE solution (10 mg/L) was made with TSB media. The TCE solution (25 mL) was used in each of the reactors along with 1 mL of encapsulated bacterial cells was taken in each of the reactors. Experiments with (a) only TCE in TSB media (blank), and (b) encapsulated *Pseudomonas putida* F1 and TCE in TSB media were conducted. Samples were withdrawn periodically over a 36-h time period and analyzed for TCE.

4.2.8.3 Combined metal-microorganism system

Batch experiments were conducted using the combined metal-microorganism system and monitored over a 36-h time period. Encapsulated NZVI (750 mg/L) was taken along with encapsulated *Pseudomonas putida* F1 (1 mL of bacterial solution with a specific number of cells) in a 40 mL amber vial containing 10 mg/L of TCE in TSB media. The reactors were rotated end- over-end in a custom made rotary shaker, and aliquots were withdrawn periodically over a 36-h time period and analyzed for

TCE. The by-products of TCE break down (DCE and VC) were also analyzed using the combined-metal microorganism system.

4.2.9 Re-dosing experiments

Batch experiments were conducted with the combined metal-microorganism system as described in the section above. Encapsulated NZVI (0.75 g/L) was taken along with encapsulated *Pseudomonas putida* F1 (1 mL of bacterial solution containing a specific number of cells) in a 40 mL amber vial containing 10 mg/L TCE in TSB media. Following the completion of 3 h of the experiment bulk TCE solution was drained and new TCE solution of 10 mg/L concentration was added. The experiment was continued and monitored over a 36-h time period. (**Figure 4.1**) Re-dosing experiments were performed for (a) only the encapsulated NZVI system and TCE in the TSB media solution and (b) combined metal-microorganism system and TCE in the TSB media. Aliquots were collected at specific intervals of time as described in the above section and analyzed for TCE.

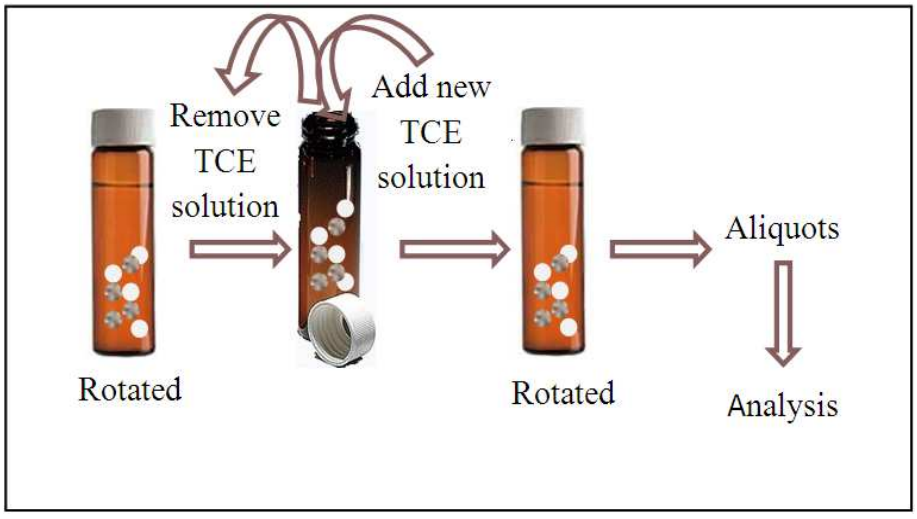


Figure 4.1: Schematic of re-dosing experiments

4.2.10 Analytical methods

The TCE samples were analyzed using the technique described in **Section 3.2.8.**

4.2.11 Statistical analysis

All experiments were conducted in duplicates and the average values are reported. EXCEL programming was used to compute the standard deviations.

4.2.12 Quality control

All glassware was sterilized prior to use. Work spaces were cleaned using 70% ethanol to ensure that there was no cross contamination.

4.3 Results and discussions

4.3.1 NZVI characteristics

NZVI synthesized in the laboratory was black in color with an average particle size of 35 nm. NZVI characterization results are described in **Section 3.3.1**.

4.3.2 Ca-alginate capsule characteristics

Ca-alginate capsules were successfully prepared in the laboratory and had an average diameter of 3.96±0.01 mm and skin thickness of 0.2736±0.0036 mm. The results are described in detail in **Section 3.3.2**.

4.3.3 Bacterial growth studies

4.3.3.1 Without NZVI

The bacterial growth was monitored using the plate count method over a 24-h time period. Results indicate that the bacteria are in stationary growth phase between

12-24 hours (**Figure 4.2a**). Based on this result, bacteria were withdrawn within this time frame to be used for TCE batch degradation experiments in order to ensure the presence of a growing culture for the TCE degradation experiments.

4.3.3.2 With NZVI

The bacterial growth was monitored using the plate count method for a 36-h time period in the presence of NZVI. During the first 5 h of the experiment the bacterial growth was slower when compared to the latter half of the experiment. A growth phase was observed between 5-12 h, while beyond 12h up to 24 h the bacteria remained in stationary growth phase. The bacterial death phase prevailed after 24 h (**Figure 4.2b**). There was no detrimental effect caused on the bacteria by NZVI.

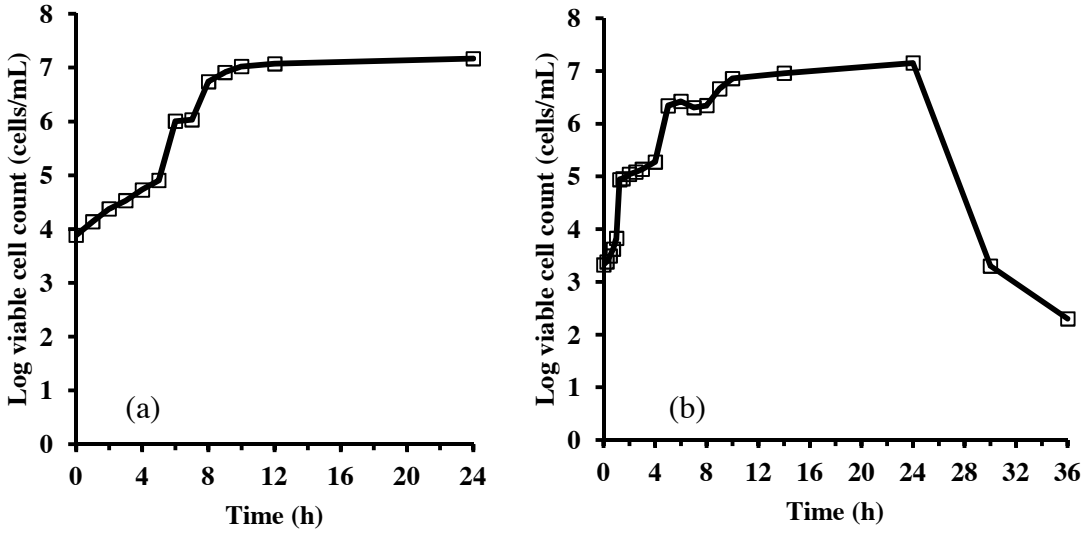


Figure 4.2: *Pseudomonas putida* F1growth studies. (a) only TSB nutrient media, (b) TSB nutrient media + encapsulated NZVI

4.3.4 TCE Degradation studies

4.3.4.1 Using NZVI

In this study, NZVI is used to remove TCE in (TSB media) in order to understand whether TSB media interferes with the TCE removal using encapsulated NZVI. Results show that bare and encapsulated NZVI removed up to 100% TCE over a 3-h time period. **(Figure 4.3a).** TCE degradation batch study results revealed that that TSB media has no effect on TCE degradation using NZVI. Earlier publications have reported up to 91% removal of TCE using encapsulated NZVI from aqueous solutions (not TSB media) over a 2-h time period (described in **Section 3.3.4**). The ANOVA results revealed that there is no significant difference between the TCE removal efficiencies using bare and encapsulated NZVI (See **Section 3.3.5**). There was a minor decrease in TCE concentration in controls (capsules in TSB media) which can be attributed to physical adsorption by the Ca-alginate capsules. Similar findings were reported by (Bezbaruah et al. 2009; Bezbaruah et al. 2011; Hill and Khan 2008).

4.3.4.2 Using Pseudomonas putida F1

Pseudomonas putida F1 was successfully encapsulated in Ca-alginate capsules. TCE degradation efficiency using *Pseudomonas putida* F1 (not encapsulated) was 70% **(Figure 4.4)** over a 36-h time period. Similar results were obtained while using encapsulated *Pseudomonas putida* F1. However there was no significant TCE removal after 24 h. This result can be explained by bacteria approaching death phase **(Figure 4.3c)** after 24 h.

4.3.4.3 Combined: metal-microorganism system

Batch-series experiments showed that the combined metal-microorganism system removed TCE to 100% over a 3-h time period. The re-dosing with TCE at 3 h, the TCE removal trend was similar to the case of TCE removal using encapsulated *Pseudomonas putida F1* alone **(Figure 4.3d)**. The TCE removal efficiency was 70%. The re-dosing experiments were performed to provide a clear understanding of the individual role played by encapsulated NZVI and encapsulated *Pseudomonas putida* F1 cells and estimate the efficacy of the combined metal-microorganism system. **Figure 4.3c** indicates that the removal of TCE during the first 3 h is predominantly due to NZVI. NZVI lost its reactivity after the 3-h time period and got used up for TCE reduction. This result can be inferred from the flat curve after TCE re-dosing, for the experiment conducted using encapsulated NZVI alone. The large spike in **Figure 4.3d** indicates the re-dosing of TCE following which the reaction resembles that of encapsulated microbial degradation with a TCE removal efficiency of 70% **(Figure 4.3c)**. The by-products of TCE degradation (dichloroethylene and vinyl chloride) were not detected during the experiment.

4.3.5 TCE degradation kinetics

TCE degradation by encapsulated NZVI followed first order kinetics while encapsulated *Pseudomonas putida* F1 followed second order kinetics. The observed reaction rates were found to be 2.33×10^{-2} min^{-1} and 8.0×10^{-5} $L{\cdot}mg^{-1}{\cdot}min^{-1}$, 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

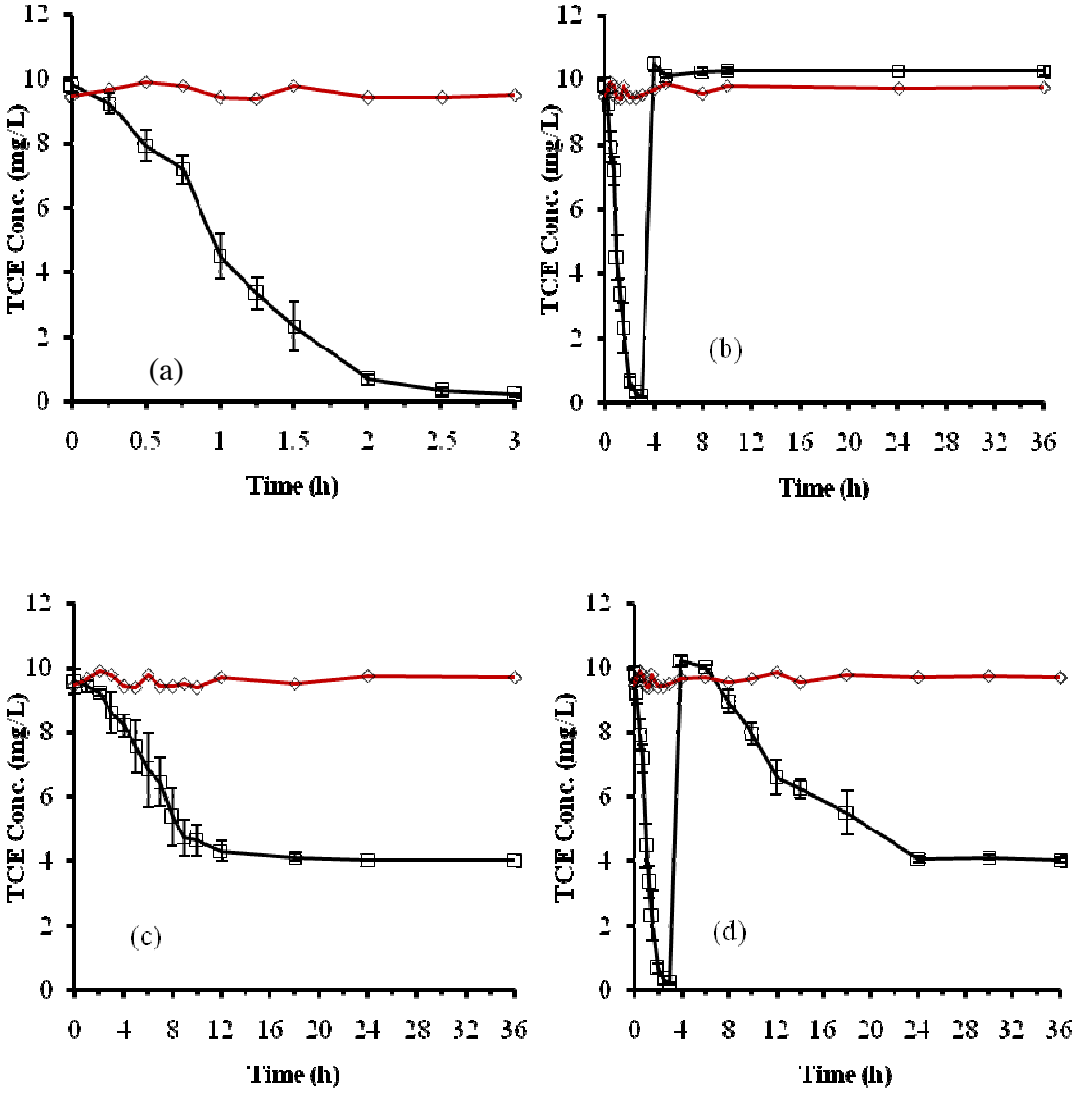


Figure 4. 3: TCE removal using (a) Encapsulated NZVI, (b) Encapsulated NZVI (re-dosed), (c) Encapsulated *Pseudomonas putida* F1, and (d) Combined metal-microorganism system. Blank (—◇—) TCE in TSB media

(reaction dominated by NZVI, and reaction dominated by bacteria). 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 2.47×10^{-2} min^{-1} **(Table 4.1)**.

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) followed second order kinetics with a k_{obs} of (9.0×10^{-5} $L \cdot mg^{-1} \cdot min^{-1}$), which is very similar to the reaction rate constant of encapsulated *Pseudomonas putida* F1.

Table 4.1: Reaction rate constants calculated based on the results obtained during this study

Reaction order		E-NZVI	E-B	C-NZVI	C-B
Zero order	K_{obs} ($mg \cdot L^{1} \cdot min^{-1}$)	5.92×10^{-2}	2.0×10^{-2}	6.05×10^{-2}	3.5×10^{-2}
	R^2	0.8764	0.57	0.90	0.84
First order	K_{obs} (min^{-1})	2.33×10^{-2}	4.0×10^{-2}	2.47×10^{-2}	5.0×10^{-2}
	R^2	0.980	0.66	0.96	0.90
Second order	K_{obs} ($L \cdot mg^{-1} \cdot min^{-1}$)	2.31×10^{-2}	8.0×10^{-5}	2.80×10^{-2}	9.0×10^{-5}
	R^2	0.778	0.74	0.78	0.93

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

4.4 Summary

NZVI and F1 were successfully encapsulated in separate Ca-alginate capsules. The growth and TCE removal efficiency of the bacteria were not affected in the presence of NZVI. The combined metal-microorganism system removed 100% TCE during the first 3-h of the reaction. After TCE re-dosing, the removal pattern of the combined system resembled that of encapsulated *Pseudomonas putida* F1 with a removal efficiency of 70%. TCE degradation kinetic studies affirmed that the first 3-h of the reaction was NZVI dominated followed by bacterial dominance from 3-36 h. The by-products of TCE (Dichloroethene and vinyl chloride) were not observed during the entire span of the experiment. This study utilizes for the very first time the

combined effects of a metal-microorganism system for enhanced dechlorination of TCE. The co-encapsulation of NZVI and bacteria will not only help to mitigate the mobility and settlement problems associated with bare NZVI but also provide a viable in-situ remediation technique for groundwater contaminated with TCE.

CHAPTER 5. CO-ENCAPSULATION OF NZVI AND *Dehalococcoides* IN ALGINATE POLYMER FOR TRICHLOROETHYLENE REMEDIATION

5.1 Introduction

NZVI particles have been used to treat a wide range of groundwater contaminants including chlorinated compounds (Sakulchaicharoen et al. 2010; Liu and Lowry 2006); pesticides (Bezbaruah et al. 2009b; Bezbaruah et al. 2011; and Joo and Zhao 2008); heavy metals (Alowitz and Scherer 2002), and explosives (Gregory et al. 2004). NZVI particles are ideal for the degradation of environmental contaminants because of their environment friendly nature, high reactivity, and low cost (Zhang 2003). The mode of degradation by which the NZVI degrades such contaminants is reductive dehalogenation.(Matheson and Tratnyek 1994); (Kim et al. 2010a). The small sized (< 100 nm) NZVI particles have very high reactive surface area (25-54 $m^2 g^{-1}$) (Bezbaruah et al. 2009; Liu and Lowry 2005) which makes them highly efficient for contaminated water remediation. However, the challenges associated with NZVI particles are their highly mobility and tendency to settle down in the aquifer pores. To overcome the mobility and settlement problems associated with NZVI, the encapsulation technique has been employed and the efficacy of the encapsulated NZVI system has already been established and accepted (Bezbaruah et al. 2011). Nanoscale zero-valent iron particles (NZVI) have been effectively encapsulated and used for the remediation of TCE with 90% removal efficiency over a 2 h time period. (Bezbaruah et al. 2011).

Bioremediation of sites contaminated with chlorinated hydrocarbons is one of the most cost effective methods for site cleanup. Reductive dechlorination is one of

the most efficient bioremediation techniques because of harmless end products, ethenes or ethanes depending on the bacterial strain employed. (MaymoGatell et al. 1997; Maymo-Gatell et al. 1999). During the process of reductive dehalogenation, each chlorine atom is replaced by a hydrogen atom, during each of the reaction step **(Figure 5.1)** .The reported intermediates that are produced in this process are cis-1,2-dichloroethene ,(cis-DCE), trans-1,2-dichloroethene (trans-DCE), 1,1-dichloroethene (1,1-DCE) and vinyl chloride before final reduction of VC to ethane. (Lu et al. 2008; Maymo-Gatell et al. 1999).

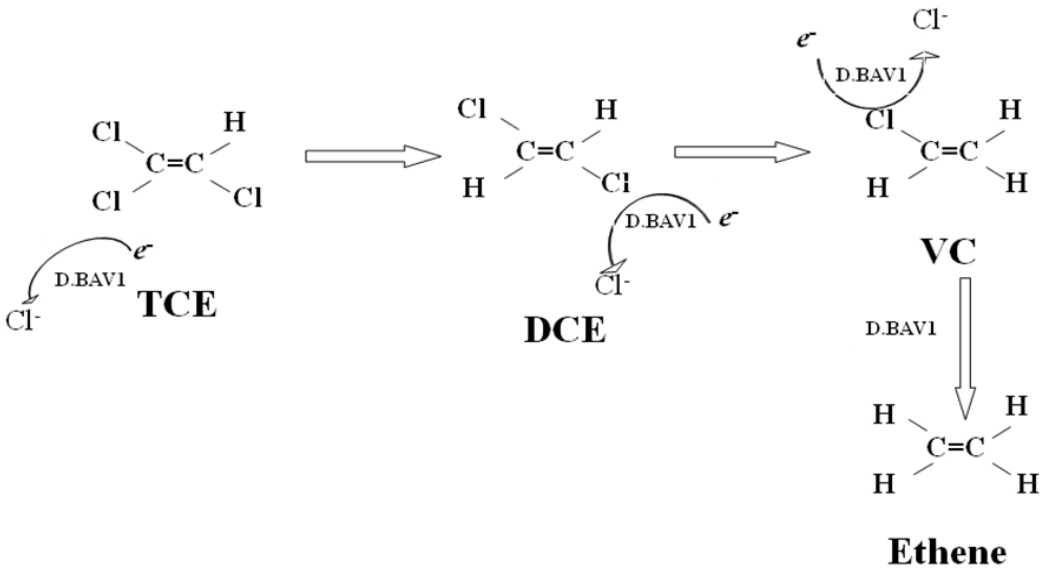


Figure 5.1: Mechanism of TCE degradation using *D*.BAV1

The effectiveness of reductive dechlorination depends on the species/strain used and bacterial strain differs significantly in their ability and potential to dechlorinate TCE. Research reveals that not all TCE dechlorinating strains are capable of degrading TCE to completely benign end products (References).

MaymoGatell et al. (1997) reported that *Dehalococcoides ethenogenes* strain 195 is capable of reducing TCE to ethane. Similar results were obtained by other researchers(Fennell et al. 2001; Hendrickson et al. 2002). *Dehalococcoides* species BAV1 is another strain capable of degrading TCE to ethane. (Duhamel et al. 2004; Krajmalnik-Brown et al. 2004; Lu et al. 2008).

Bioremediation techniques are however very slow in comparison to chemical processes. A novel technology which uses the coupled advantages of chemical reduction as well as biological techniques is necessary. A combined metal-microorganism will integrate the reaction speed of NZVI while reducing the TCE contaminant to harmless end products attributed to microbial degradation. Co-encapsulation of microorganisms and NZVI in the same capsule will equally expose the contaminant to both NZVI and the microorganism, which will allow the TCE degrading microbe to preferentially take over the reaction 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 DCE and VC. *Dehalococcoides* sp. is known to thrive on these by-products and break them down to harmless end products such as ethane. In the case of co-encapsulation in separate capsules, there is a possibility that TCE may be over exposed to either one of the systems (encapsulated NZVI/encapsulated microorganism) with little to no contact with the other.

The objective of this section is to combine the advantages of the NZVI system along with the technique of anaerobic bioremediation in an effort to engineer a remediation system for the complete degradation of TCE to ethane.

5.2 Materials and methods

5.2.1 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.

5.2.2 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). Other researchers (Kasi et al. 2011) have used MSM (also referred to as synthetic groundwater to grow bacteria, Table 5.1). An 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.

Table 5.1: Composition of mineral salt media (MSM) (after Kasi et al. 2011)

Compound	Concentration used in g/L
$CaCl_2$	0.07
FeCl2	0.625
K_2HPO_4	0.348
KH_2PO_4	0.272
KNO_3	0.505
$MgCl_2$	0.02
$NaHCO_3$	2.6
NH_4Cl	0.535
Vitamins	
B_{12}	0.0001
Bentothemic acid	0.0005
Biotin	0.0002
Folic acid	0.0002
Nicotinic acid	0.0005
p-aminobenzoate	0.0005
Pyrodoxine HCl	0.001
Riboflavin	0.0005
Thiamine	0.0005
Trace Metals	
$COCl_2$	0.003
$MnCl_2$	0.002
$NiCl_2$	0.00015
$ZnCl_2$	0.0002

5.2.3 Encapsulation of NZVI

Encapsulation of NZVI was performed using the standard encapsulation technique as described in the earlier sections. The peristaltic pump was replaced using a 10 mL syringe (**See Figure 5.2**) in an effort to ease the instrumental setup to maintain an anaerobic, sterile environment for bacterial growth. The capsules were more or less spherical in shape with a diameter of 3.96±0.1mm.

5.2.4 Encapsulation of *Dehalococcoides* sp

The encapsulation of *D*.BAV1 was done following the method described in **Section 3.2.4**. A bacterial sample (1 mL) along with 0.25 g $CaCl_2$ and 4 g maltodextrin were mixed with 6 mL of distilled de-ionized water ("the solution"). An alginate solution (50 mL of 1% alginate) was stirred in 100 mL Erlenmeyer flask 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.

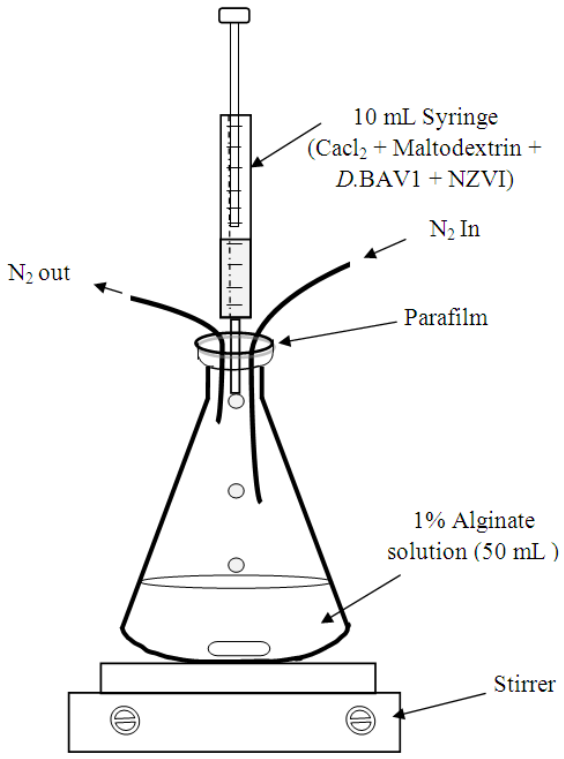


Figure 5.2: Encapsulation under anaerobic conditions

5.2.5 Combined metal-microorganism system

NZVI (750 mg/L) was co-encapsulated with 1 mL of *D*.BAV1 ($4*10^8$ cells/mL) taken along with 4 g of Maltodextrin and 0.25 g $CaCl_2$ made up in 6 mL of distilled, de-ionized, and de-oxygenated water. The same technique of encapsulation as described in **Section 5.2.3** was employed. The system was continuously purged with N_2 in an effort to maintain an anaerobic environment **(Figure 5.2)**. All glassware was sterilized prior to use.

5.2.6 Batch TCE degradation experiments

5.2.6.1 Encapsulated NZVI

Batch TCE degradation experiments were conducted with encapsulated NZVI at room temperature (22 ± 2^oC) in 40 mL amber glass vials (reactors) fitted with Teflon septums. TCE solution (25 mL, 10 mg/L) made with MSM media was used in each of the reactors. A definite amount of encapsulated NZVI (750 mg NZVI/L) was added into each reactor. The reactors were rotated end-over-end at 28 rpm in a custom-made rotary shaker. Reactors with only TCE in MSM media (blank) were run. Samples were withdrawn at 0, 5, 15, 30, 45, 60, 90, 120 and 180 min. Re-dosing experiments were performed to find the efficacy of encapsulated NZVI beyond the initial 3 h time period. Following 180 min (3 h) of reaction the supernatant in the reactor was drained. A new TCE solution (25 mL, 10mg/L) was added while retaining the encapsulated NZVI. Aliquots were withdrawn periodically up to 36 hr time period. All experiments were performed in duplicates and average values are reported.

5.2.6.2 Encapsulated D.BAV1

Batch experiments were conducted using encapsulated (1mL) of *D*.BAV1 in 25 mL of 10 mg/L TCE in 40 mL 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.

5.2.6.3 Combined metal-microorganism system

Batch experiments were conducted using 750 mg/L of NZVI co-encapsulated with 1 mL 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. The by-products of TCE break down (DCE and VC) were also analyzed using the combined-metal microorganism system. The pH-ORP trend for the reaction was monitored using a pH-ORP probe setup. The pH-ORP probes were immersed into a beaker containing the co-encapsulated *NZVI-D.BAV1* system. The system was maintained under anaerobic conditions and stirred constantly using a magnetic stirrer.

5.2.7 Analytical methods

All samples were analyzed for TCE as described in **Section 3.2.8**.

5.2.8 Quality control

All experiments were conducted in duplicates. All glassware and work spaces were sterilized using 70% ethanol prior to any experiments.

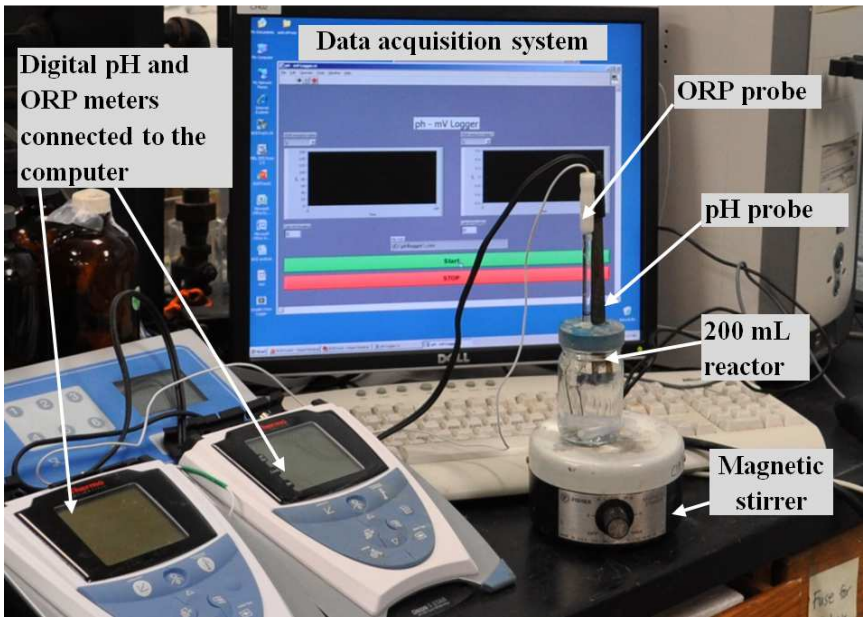


Figure 5.3: pH-ORP data acquisition system setup

5.3 Results and discussions

5.3.1. NZVI characteristics

The synthesized NZVI was black in color and had an average particle size of 35 nm as described in **Section 3.3.1**.

5.3.2. Encapsulation of NZVI and bacteria

The NZVI particles, microorganisms, and NZVI and bacteria together were successfully encapsulated and Ca-alginate capsules were mostly spherical in shape. The characteristics of the capsules were the same as described in **Section 4.3.2.**

5.3.3 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 **(Figure 5.1)** indicate that the

bacteria were possibly in the lag phase between 0-4 h (slower growth when compared to the latter portion of the experiment), 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 phase. Knowing the growth phase 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 growth phase.

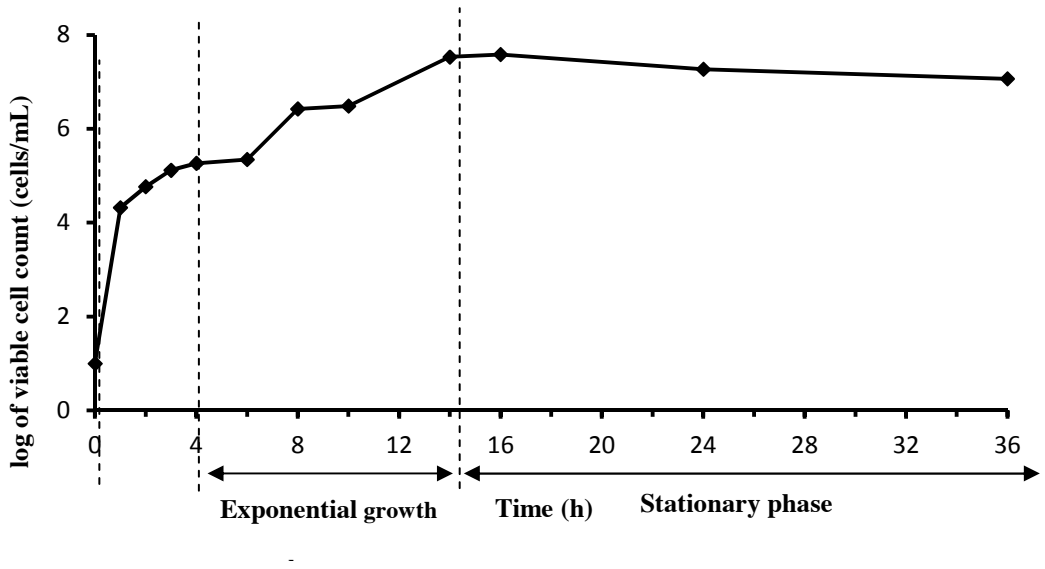


Figure 5.4: *D*.BAV1 growth curve at room temperature 22±2°C and in MSM growth media

5.3.4 Batch TCE degradation studies

5.3.4.1 Encapsulated NZVI

Batch TCE degradation studies were conducted using encapsulated NZVI in MSM 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-dosing TCE at 3 h, there was no further removal of TCE. This indicates that NZVI was consumed in 3 h of reaction **(Figure 5.4a)**. 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.

5.3.4.2 Encapsulated D.BAVI

Batch experiments were conducted using encapsulated *D*.BAV1 in 25 mL of 10 mg/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* **(Figure 5.5c)** indicated that the bacterial degradation was relatively slow during the 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. Literature indicates that as the number of chlorine atoms decreases the rate of the reductive dechlorination will decrease (i.e. there exists a linear correlation between number of chlorine atoms and reductive dechlorination rate) (Bouwer 1994; Mohn and Tiedje 1992; Pant and Pant 2010; Vogel and McCarty 1985).

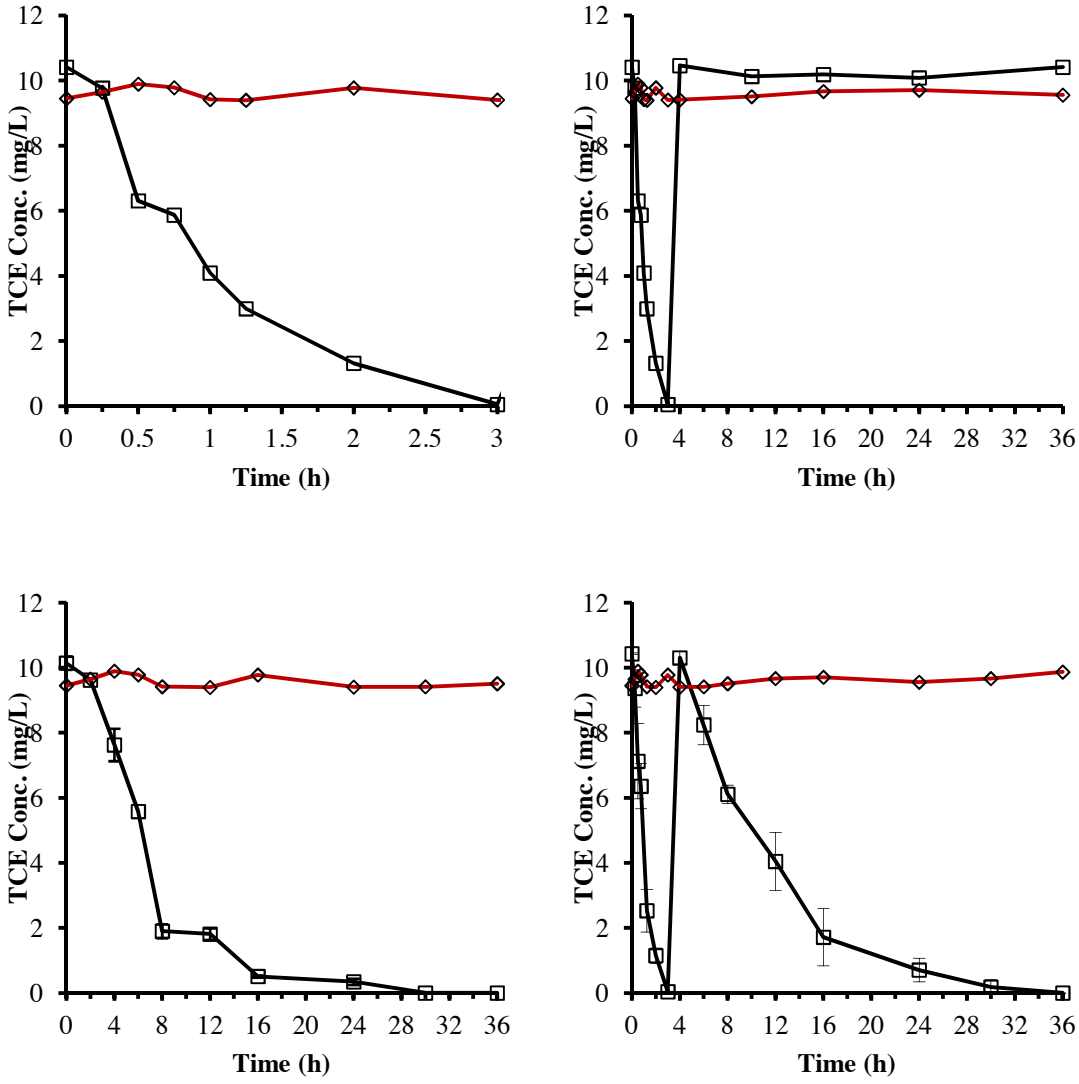


Figure 5.5: TCE removal using: (a) Encapsulated NZVI, (b) Encapsulated NZVI (re-dosed), (c) Encapsulated *D.BAV1*, and (d) Combined metal-microorganism system. Blank (—◇—) TCE in MSM media

5.3.4.3 Combined metal-microorganism system

Batch TCE degradation experiments were carried out using the co-encapsulated NZVI-*D.BAV1* system over a 36-h time period. The TCE removal was 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 stage of the reaction. After TCE re-dosing, the TCE removal trend resembled that of bacterial degradation **(Figure 5.5b- 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 5.5a**). The by-products of TCE (DCE and VC) were not observed throughout the entire experiment.

The pH and ORP were monitored throughout the 36 h reaction period for the co-encapsulated *NZVI-D.BAV1* system. The ORP values (Figure 5.3) were below zero for the first 3 hours of the re-dosing experiment. Low ORP values indicate reducing conditions. The mechanism for TCE removal using the co-encapsulated NZVI-*D*.BAV1 is apparently reductive dehalogenation. After 3 h, the reactor was opened and the bulk solution was drained and replaced with new TCE (10 mg/L) solution. The sudden rise in ORP (**Figure 5.6**) is because of the exposure of the ORP probe and the new bulk solution to air. Once the system is closed, the ORP was seen to approach negative values indicating a reestablishment of reducing conditions within the system. There was a slight increase in the pH during the first three hours of the reaction followed by a steady pH through the completion of the experiment. The slight increase in pH during the first three hours is due to the reaction between NZVI and TCE. A similar result was obtained by Bezbaruah et al. (2011).

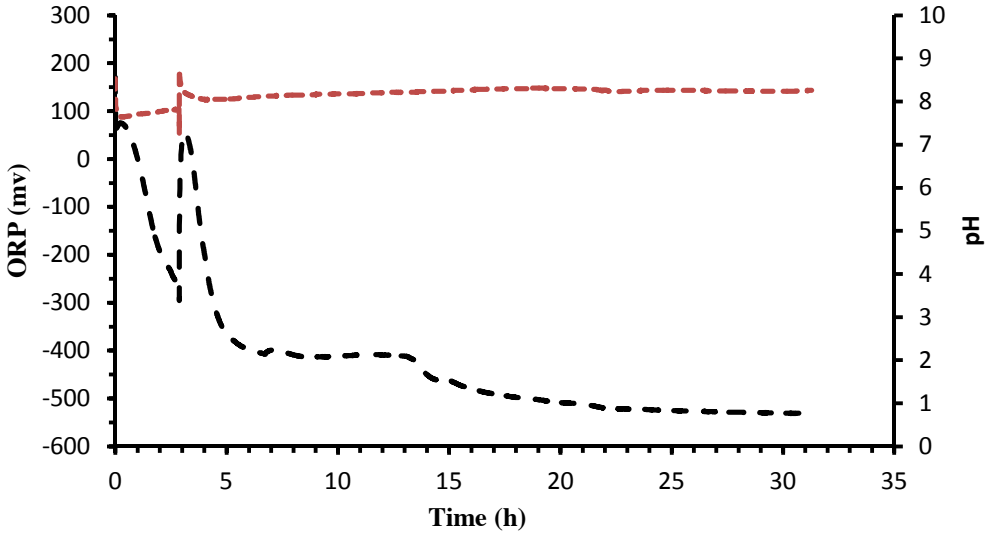


Figure 5.6: pH and ORP data of combined metal-microorganism. pH (·····), ORP (– – –)

5.3.5 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 5.2)**. 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 k_{obs} of $2.93 \times 10^{-2} min^{-1}$. 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 *Dehalococcoides* BAV*1*.

Table 5.2: 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
First	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
Second	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)

5.4 Summary

A combined metal-microorganism system was successfully engineered and tested for TCE removal. The TCE removal efficiency during the first 3 h of the experiment was 100%, with the TCE removal being dominated by NZVI. After re-dosing TCE, all the TCE removal was attributed to bacterial degradation, which yielded100% TCE removal after 36 h. The by-products of TCE degradation such as DCE and VC were not observed during the experiment. The pH-ORP trend for the combined indicated reducing condition throughout the experimental duration. This

study utilizes a novel encapsulation technique designed to provide valuable insights for potential in-situ TCE remediation applications.

CHAPTER 6. CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

This study on co-encapsulation of nanoscale zero-valent iron (NZVI) and bacteria has demonstrated that a combined physicochemical-biological 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% compared to 88-90% removal using bare NZVI over a 2-h period. The TCE degradation followed first order kinetics for both the encapsulated and bare 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 confine TCE degrading microorganisms in Ca-alginate for TCE remediation. Two distinct bacterial strains (*Pseudomonas putida* F1 and *Dehalococcoides* BAV1) capable of TCE dechlorination were tested in this study. The TCE removal efficiency in the case of encapsulated *PpF1* was found ~ 70% while encapsulated *D. BAV1* had a TCE removal efficiency of 100%.

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 DCE, and VC were not observed while using the combined

NZVI-microorganism system. The non-detection of toxic by-products is considered positive and the system has potential for field application.

The following main conclusions 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 PRBs for environmental remediation.

6.2. Future work

In order to strengthen the applicability of the combined NZVI-microorganism for in-situ applications the following topics should be exhaustively investigated.

1. Interferences of other groundwater contaminants (electrons acceptors such as nitrate, and dissolved oxygen) should be examined.
2. Shelf-life of the combined NZVI-microorganism system should be determined.
3. The distinct roles of NZVI and microorganisms in a combined system need further investigation such that the process can be optimized for contaminant (TCE) removal.

4. The NZVI , microbial, and NZVI-microorganism systems developed here should be tested for other contaminants to make them versatile.

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APPENDIX

Table A.1: TCE concentration data for experiments on diffusion characteristics for the Ca-alginate capsules (corresponding to **Figure 3.3a**)

Time (min)	Blank (mg/L)	Control ±Standard deviation (mg/L)	Capsules in TCE solution ± Standard deviation (mg/L)
0	30.30	30.42 ± 0.90	30.03 ± 0.89
5	29.99	28.74 ± 1.85	26.39 ± 1.45
15	29.83	28.52 ± 2.07	22.11 ± 2.15
30	29.72	27.87 ± 1.26	15.34 ± 2.52
45	30.05	27.44 ± 0.94	11.73 ± 0.34
60	29.96	27.05 ± 1.32	11.54 ± 0.30
90	29.64	26.83 ± 1.20	11.33 ± 0.41
120	30.14	26.28 ± 1.61	11.01 ± 0.28

Table A.2: TCE concentration data for experiments on diffusion characteristics for the Ca-alginate capsules (corresponding to **Figure 3.3b**)

Time (min)	Blank (mg/L)	Control ±Standard deviation (mg/L)	Capsules in TCE solution ± Standard deviation (mg/L)
0	38.91	39.94 ± 0.54	30.03 ± 0.77
5	39.38	38.33 ± 2.35	26.39 ± 4.12
15	38.41	35.76 ± 1.26	22.11 ± 4.40
30	38.52	35.34 ± 1.24	15.34 ± 6.20
45	38.16	35.19 ± 1.12	11.73 ± 5.68
60	38.38	34.72 ± 0.88	11.54 ± 1.38
90	39.04	34.60 ± 0.79	11.33 ± 2.52
120	38.85	34.98 ± 1.42	11.01 ± 1.74

Blank: Only TCE solution; **Control**: TCE solution with capsule skins

Table A.3: TCE concentration data for experiments on reduction of TCE by bare and encapsulated NZVI over time (corresponding to **Figure 3.4a)**

Time (min)	Blank (mg/L)	Control ± Standard deviation (mg/L)	Bare NZVI ± Standard deviation (mg/L)	Encapsulated NZVI ± Standard deviation (mg/L)
0	38.91	40.01 ± 0.08	39.27 ± 0.40	37.43 ± 0.46
5	39.38	39.14 ± 0.79	36.03 ± 1.34	30.64 ± 0.55
15	38.98	39.07 ± 0.77	27.83 ± 0.90	28.13 ± 0.91
30	38.52	38.72 ± 0.47	25.87 ± 1.25	21.55 ± 1.71
45	38.16	38.68 ± 0.33	16.32 ± 0.92	18.10 ± 2.63
60	38.38	38.52 ± 0.33	9.98 ± 0.64	6.52 ± 0.98
90	39.04	38.29 ± 0.20	4.78 ± 1.18	4.00 ± 0.31
120	38.85	37.60 ± 0.60	3.01 ± 0.70	3.28 ± 0.37

Table A.4: TCE concentration data for experiments on reduction of TCE by bare and encapsulated NZVI over time (corresponding to **Figure 3.4b)**

Time (min)	Blank (mg/L)	Control ±Standard deviation (mg/L)	Bare NZVI ± Standard deviation (mg/L)	Encapsulated NZVI ± Standard deviation (mg/L)
0	30.30	30.25 ± 0.21	30.76 ± 0.19	30.16 ± 0.18
5	29.99	29.90 ± 0.04	28.68 ± 1.05	29.95 ± 0.07
15	29.83	29.75 ± 0.13	26.44 ± 1.59	24.61 ± 3.33
30	29.72	29.69 ± 0.10	20.48 ± 1.49	18.74 ± 3.33
45	30.05	28.95 ± 0.06	18.10 ± 2.79	14.88 ± 2.04
60	29.96	28.61 ± 0.21	11.81 ± 0.83	8.82 ± 1.25
90	29.64	28.27 ± 0.42	8.62 ± 2.05	5.51 ± 0.91
120	30.14	27.83 ± 0.87	4.89 ± 0.63	3.31 ± 0.30

Table A.5: TCE concentration data for experiments on reduction of TCE by bare and encapsulated NZVI over time (corresponding to **Figure 3.4c)**

Time (min)	Blank (mg/L)	Control ±Standard deviation (mg/L)	Bare NZVI ± Standard deviation (mg/L)	Encapsulated NZVI ± Standard deviation (mg/L)
0	9.59	10.15±0.37	9.53±0.46	10.68±1.15
5	9.77	9.87±0.17	8.48±0.54	9.31±0.67
15	9.85	9.66±0.07	7.42±1.19	7.41±1.30
30	9.93	9.42±0.07	6.95±0.95	4.56±1.01
45	9.97	9.00±0.02	4.35±1.13	3.19±0.70
60	9.57	8.67±0.45	2.62±1.34	1.50±0.69
90	9.79	8.46±0.35	1.14±0.62	0.82±0.09
120	9.68	8.24±0.34	0.63±0.17	0.69±0.25

Table A.6: TCE concentration data for experiments on reduction of TCE by bare and encapsulated NZVI over time (corresponding to **Figure 3.4d)**

Time (min)	Blank (mg/L)	Control ±Standard deviation (mg/L)	Bare NZVI ± Standard deviation (mg/L)	Encapsulated NZVI ± Standard deviation (mg/L)
0	1.21	1.13 ± 0.11	1.14 ± 0.09	1.13 ± 0.09
5	1.19	1.06 ± 0.06	0.95 ± 0.04	0.91 ± 0.04
15	1.07	1.03 ± 0.06	0.74 ± 0.08	0.72 ± 0.06
30	1.12	0.99 ± 0.08	0.60 ± 0.15	0.50 ± 0.08
45	0.97	0.91 ± 0.03	0.37 ± 0.12	0.32 ± 0.11
60	1.06	0.86 ± 0.01	0.23 ± 0.05	0.18 ± 0.05
75	1.11	0.81 ± 0.01	0.11 ± 0.05	0.09 ± 0.02

Blank: Only TCE solution; **Control**: TCE solution with capsules

Table A.7: pH trend during TCE degradation. The TCE concentration used here is 30 mg/L (corresponding to **Figure 3.4e)**

Time (min)	pH of blank± Standard deviation (SU)	pH of TCE + bare NZVI ± Standard deviation (SU)	pH of TCE + encapsulated NZVI ± Standard deviation (SU)
0	6.87 ± 0.01	6.39 ± 0.10	4.99 ± 0.03
5	6.9 ± 0.07	6.5 ± 0.04	5.2 ± 0.05
10	6.89 ± 0.14	6.7 ± 0.01	5.45 ± 0.09
15	6.9 ± 0.08	6.89 ± 0.02	5.61 ± 0.22
30	6.91 ± 0.08	7± 0.03	5.81 ± 0.08
45	6.92 ± 0.08	7.13 ± 0.06	5.865 ± 0.05
60	6.94 ± 0.03	7.33 ± 0.01	5.92 ± 0.06
75	6.9 ± 0.01	7.71 ± 0.04	5.945 ± 0.07
90	6.93 ± 0	8.11 ±0.04	5.97 ± 0.01
105	6.92 ± 0	8.48 ± 0.01	5.98 ± 0.01
120	6.94 ± 0.05	8.73 ± 0.04	6.085 ± 0.13

Blank : Only TCE solution

Table A.8: TCE concentration data for experiments on reduction of TCE by encapsulated NZVI system over a time span of 6 months (shelf-life study) (corresponding to **Figure 3.4f)**

Time (min)	Month 1 (mg/L) ± Standard deviation	Month 2 (mg/L) ± Standard deviation	Month 3 (mg/L) ± Standard deviation	Month 4 (mg/L) ± Standard deviation	Month 5 (mg/L) ± Standard deviation	Month 6 (mg/L) ± Standard deviation
0	30.08 ± 0.04	30.36 ± 0.01	30.02 ± 0.15	30.21 ± 0.06	30.16 ± 0.06	30.31 ± 0.03
5	30.02 ± 0.07	29.88 ± 0.03	29.96 ± 1.21	29.89 ± 0	29.60 ± 0.07	29.78 ± 0.09
15	26.63 ± 0.08	26.43 ± 0.07	20.77 ± 0.32	27.99 ± 0.08	270.13 ± 0.21	26.80 ± 0.03
30	19.9 ± 0.03	21.33 ± 0.06	14.98 ± 0.65	25.43 ± 0.07	25.09 ± 0.18	23.0 ± 0.07
45	16.68 ± 0.1	15.3 ± 0.06	12.66 ± 0.12	16.21 ± 0.06	18.41 ± 0.26	10.78 ± 0.03
60	9.78 ± 0.23	9.29 ± 0.06	7.4 ± 0.03	14.1 ± 0.76	15.09 ± 0.56	14.501 ± 0.05
90	6.35 ± 0.17	5.63 ± 0.23	4.55 ± 0	9.71 ± 0.08	11.78 ± 0.32	10.23 ± 0.09
120	3.41 ± 0.12	2.97 ± 0.45	3.56 ± 0.09	4.12 ± 0.1	5.67 ± 0.16	5.78 ± 0.14

Table A.9: *Pseudomonas putida* F1growth studies data with only TSB nutrient media (corresponding to **Figure 4.2a)**

Time (h)	Log viable cell count (cells/mL)
0	3.89
1	4.14
2	4.37
3	4.53
4	4.73
5	4.91
6	6.01
7	6.03
8	6.74
9	6.91
10	7.02
12	7.07
24	7.17

Table A.10: *Pseudomonas putida* F1growth studies data with TSB nutrient media and encapsulated NZVI (corresponding to **Figure 4.2b)**

Time (h)	Log viable cell count (cells/mL)
0	3.32
0.25	3.38
0.5	3.49
0.75	3.62
1	3.83
1.25	4.94
1.5	4.96
2	5.04
2.5	5.08
3	5.14
4	5.27
5	6.34
6	6.42
7	6.31
8	6.34
9	6.66
10	6.86
14	6.96
24	7.15
30	3.30
36	2.30

Table A.11: TCE concentration data for experiments on TCE removal using: (a) Encapsulated NZVI, (b) Encapsulated NZVI (re-dosed), (c) Encapsulated *Pseudomonas putida* F1, and (d) Combined metal-microorganism system(corresponding to **Figure 4.3a-d)**

Time (h)	**Blank (mg/L)**	**Encapsulated NZVI (mg/L) ±Standard deviation**	**Encapsulated NZVI (re-dosed) (mg/L) ± Standard deviation**	**Encapsulated *Pseudomonas putida* F1 (mg/L) ± Standard deviation**	**Encapsulated NZVI + PpF1 re-dosed (mg/L) ± Standard deviation**
0	9.45	9.98 ± 0.25	9.81 ± 0.37	9.59 ± 0.43	9.81 ± 0.18
0.25	9.65	9.01 ± 0.32	9.23 ± 0.19	9.43 ± 0.32	9.23 ± 0.13
0.50	9.90	8.25 ± 0.48	7.91 ± 0.10	9.24 ± 0.25	7.91 ± 0.08
0.75	9.79	7.49 ± 0.43	7.18 ± 0.67	8.60 ± 0.48	7.18 ± 0.03
1.00	9.42	4.97 ± 0.70	4.48 ± 0.35	8.21 ± 0.70	4.48 ± 0.54
1.25	9.40	3.67 ± 0.48	3.33 ± 0.79	7.54 ± 0.76	3.33 ± 0.18
1.50	9.78	2.86 ± 0.76	2.32 ± 1.13	6.82 ± 0.16	2.32 ± 0.04
2.00	9.41	0.54 ± 0.16	0.65 ± 0.75	6.45 ± 0.13	0.65 ± 0.02
2.50	9.42	0.21 ± 0.13	0.30 ± 0.91	5.37 ± 0.04	0.30 ± 1.2
3.00	9.51	0.15 ± 0.04	0.18 ± 0.56	4.71 ± 0.25	0.18 ± 0.91
4	9.45	0.14 ± 0.23	10.49 ± 0.50	4.64 ± 0.32	10.22 ± 0.40
5	9.45	0.14 ± 0.06	10.11 ± 0.31	4.29 ± 0.48	10.02 ± 0.04
8	9.65	**--- *ND**	10.23 ± 0.11	4.09 ± 0.43	8.97 ± 0.09
10	9.90	**--- *ND**	10.25 ± 0.04	4.01 ± 0.25	7.94 ± 1.31
24	9.79	**--- *ND**	10.26 ± 0.07	3.98 ± 0.32	4.05 ± 1.04
36	9.42	**--- *ND**	10.27 ± 0.18	3.95 ± 0.48	4.02 ± 0.84

***ND:** Non-detect

Table A.12: *Dehalococcoides BAV*1growth studies data with only MSM nutrient media. (corresponding to **Figure 5.4)**

Time (h)	**Log viable cell count (cells/mL)**
1	1.05
2	4.13
3	5.08
4	5.51
6	5.83
8	5.99
12	6.21
16	6.31
18	7.63
20	7.78
24	7.41
36	7.54

Table A.13: TCE concentration data for experiments on TCE removal using: (a) Encapsulated NZVI, (b) Encapsulated NZVI (re-dosed), (c) Encapsulated *Dehalococcoides BAV*1 *(DBAV*1*)*, and (d) Co-encapsulated metal-microorganism system. (corresponding to **Figure 5.5a-d)**

Time (h)	**Blank (mg/L)**	**Encapsulated NZVI (mg/L) ±Standard deviation**	**Encapsulated NZVI (re-dosed) (mg/L) ± Standard deviation**	**Encapsulated *Dehalococcoides* (mg/L) ± Standard deviation**	**Co-Encapsulated NZVI + *DBAV*1 re-dosed (mg/L) ± Standard deviation**
0	9.45	10.08 ± 0.15	10.42 ± 0.37	10.14 ± 0.43	10.43 ± 0.18
0.25	9.65	9.11 ± 0.02	9.77 ± 0.19		9.37 ± 0.13
0.5	9.90	8.25 ± 0.18	6.31 ± 0.10		7.13 ± 0.08
0.75	9.79	6.49 ± 0.43	5.87 ± 0.67		7.18 ± 0.03
1	9.42	4.67 ± 0.70	4.09 ± 0.35		6.36 ± 0.54
1.25	9.40	3.97 ± 0.48	3.00 ± 0.79		2.53 ± 0.18
2	9.78	2.36 ± 0.76	1.32 ± 1.13	9.62 ± 0.32	1.15 ± 0.04
3	9.41	0.34 ± 0.16	0.06 ± 0.75		0.05 ± 0.02
4	9.42	0.11 ± 0.13	10.47 ± 0.91	7.63 ± 0.25	10.31 ± 1.2
10	9.51	0.15 ± 0.04	10.13± 0.56	1.90 ± 0.70	8.24 ± 0.91
16	9.45	**--- *ND**	10.19 ± 0.50	1.81 ± 0.76	1.72 ± 0.40
24	9.45	**--- *ND**	10.09 ± 0.31	0.347 ±0.12	0.18 ± 0.04
36	9.65	**--- *ND**	10.41 ± 0.11	0.01 ± 0.35	0 ± 0.00

***ND:** Non-detect