INTERACTIONS OF IRON NANOPARTICLES WITH MICROORGANISMS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Rabiya Shabnam

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Environmental and Conservation Sciences

December 2010

Fargo, North Dakota

North Dakota State University
Graduate School

Title

INTERACTIONS OF IRON NANOPARTICLES WITH MICROORGANISMS

By

Rabiya Shabnam

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

North Dakota State University Libraries Addendum

To protect the privacy of individuals associated with the document, signatures have been removed from the digital version of this document.

ABSTRACT

Shabnam, Rabiya, M.S., Environmental and Conservation Sciences Program, College of Graduate and Interdisciplinary Studies, North Dakota State University, December 2010. Interactions of Iron Nanoparticles with Microorganisms. Major Professor: Dr. Achintya N. Bezbaruah.

Nanoparticles (<100nm) are being used in different applications such as biomedical, personal care, industrial, and environmental remediation. NZVI particles have unique physico-chemical properties like high surface area and high reactivity. High reactivity of nanoparticles is a concern when it comes to various ecosystem components. The impacts of NZVI particles on ecosystem components including endemic microbial community are not well studied. This research is an effort to elucidate the interactions between NZVI and some environmentally significant gram negative bacteria. Experiments were designed to study the interactions of three model gram negative bacterial species (*Escherichia coli* 8739, *E. coli* JM 109, and *Pseudomonas putida* F1). The growth and viability of all bacteria were evaluated in the presence of NZVI particles with nutrients (in nutrient media) and without nutrients (in buffer solution). The bacterial species were exposed to various NZVI concentrations (0.09-10 mg/mL) under stirring (800 rpm) and shaking (150 rpm) conditions at different temperatures (4, 22, and 37°C). Microorganisms exposed to 0.09, 0.2, 0.5, 0.8 and 1.0 mg/mL of NZVI in buffer (stirring condition), showed variable viability. At very low NZVI concentration (0.09 mg/mL) viable cells were seen until 60 min, and at higher concentration (0.2-1.0 mg/mL) no viable cells were observed after 5 min. NZVI had no significant effect on the three strains at lower concentrations (1 and 2 mg/mL) in the nutrient media. *E. coli* JM109 and *P. putida* F1 were significantly reduced at environmentally significant NZVI concentrations. However, following an initial reduction *E. coli* 8739 recovered back to the same level as the control. The recovery of *E. coli* 8739 was not due to a reduction in

the toxicity of NZVI as redosing with NZVI at 3 and 6 h did not affect the regrowth. Hence it was postulated that the observed regrowth in the presence of NZVI was a result of growth characteristics of the microorganism. Further experiment at suboptimal growth temperatures of 22°C and 4°C resulted in reduction in bacterial viability for both 5 and 10 mg/ml of NZVI. Based on the results obtained from experiments done at suboptimal temperature and comparing them with the results from experiments conducted at optimal temperature of 37°C it can be concluded that NZVI toxicity on microorganisms are microbial species/strain specific and in some cases depends on the growth of the microorganisms. Actively growing *E. coli* 8739 are not affected by NZVI toxicity while non-dividing cells are adversely affected.

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my adviser Dr. Achintya Bezbaruah for accepting me as an M.S. student and encouraging me throughout my study. I also acknowledge the valuable advice and support from Dr. Eakalak Khan and Dr. John McEvoy during my research work. My sincere thanks to Dr. Senay Simsek for allowing me to work in her laboratory and helping me in my research. Thanks to Dr. Marinus Otte for serving on my research committee.

I acknowledge North Dakota Water Resource Research Institute (NDWRRI) for the fellowship and funding throughout this project. Also, I acknowledge the Environmental and Conservation Sciences program (ECS) for the ECS Summer Award, which helped me in the completion of this project. I extend my thanks to Scott Payne (Electron Microscopy Laboratory, NDSU) for the TEM images. Acknowledgments are also extended to my research group members, Sita Krajangpan, Jay Thompson, and Harjyoti Kalita and all Environmental Engineering Laboratory friends.

My deep sense of gratitude to my little one and my husband, my mom, family and friends for their support and encouragement throughout my graduate study, especially during the final month of completion of my thesis.

TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGMENTS v

LIST OF TABLES viii

LIST OF FIGURES ix

LIST OF ABBREVIATIONS xi

CHAPTER 1. INTRODUCTION 1

1.1 Definition of Nanoparticles 1

1.2 Application of Nanoparticles 1

1.3 Environmental Applications of Nanoparticles 4

1.4 Iron Nanoparticles for Environmental Applications 5

1.5 Nanoparticles and Microorganisms 7

1.5.1 Fate of nanoparticles 7

1.5.2 Nanoparticles toxicity 8

1.6 Nanoparticles Impact on Microorganism 9

1.7 Need Statement 13

1.8 Hypothesis 14

1.9 Objective 14

1.10 Organization of the Thesis 14

CHAPTER 2. MATERIALS AND METHODS 15

2.1 Chemicals 15

2.2 Media Preparation 15

2.2.1 Cultures 15

2.3 Preparation of Nanoscale Zero-valent Iron (NZVI) for Microbial Studies . 15

2.4 Experiments with Buffer Solutions 16

2.4.1 Bacteria growth study in buffer solution with NZVI (stirring condition) 16

2.4.2 Bacteria growth study in buffer solution with NZVI (shaking condition) 17

2.5 Experiments with Nutrient Media 20

2.5.1 Bacteria growth study in nutrient media with NZVI (stirring condition) 20

2.5.2 Bacterial growth study in nutrient media with NZVI (shaking condition) 20

2.5.3 Growth of *E.coli* 8739 in nutrient media with intermittent dosing with 10 mg/mL of NZVI 23

2.5.4 Growth of *E. coli* 8739 at sub-optimal temperatures 23

2.6 Transmission Electron Microscopy (TEM) Studies 25

CHAPTER 3. RESULTS AND DISCUSSION 26

3.1 Effect of NZVI on Bacteria in Buffer Solution 27

3.1.1 Microbial growth in carbonate buffer under stirring condition 27

3.1.2 Microbial growth in carbonate buffer under shaking condition 30

3.2 Effect of NZVI on Bacteria in Nutrient Media 34

3.2.1 Microbial growth in nutrient media under shaking condition 34

3.2.2 Microbial growth in nutrient media under stirring condition 38

3.2.3 Microbial growth in nutrient media under shaking condition with intermittent exposure to additional NZVI 38

3.2.4 Microbial growth at sub-optimal temperatures in nutrient media under shaking condition 41

3.3 Electron Microscopy Analysis of Bacteria Samples 44

CHAPTER 4. CONCLUSIONS AND FUTURE WORK 52

4.1 Conclusions 52

4.2 Future Work 53

REFERENCES 55

LIST OF TABLES

Table Page

1.1: Examples of a few nanoparticles used in environmental remediation 5

1.2: Effects of iron-based nanoparticles reported in the literature..........................12

LIST OF FIGURES

Figure Page

1.1: The core-shell model of Zero-Valent iron nanoparticles. (Modified from Li et al., 2006) 6

2.1: Schematic for the growth study of bacteria in buffer solution with NZVI (stirring condition) 18

2.2: Schematic for the growth study of bacteria in buffer solution with NZVI (stirring condition) 19

2.3: Schematic for growth effects of *E. coli* 8739 in nutrient media with NZVI (stirring condition) 21

2.4: Schematic for the bacterial growth study in nutrient media with NZVI (shaking condition) 22

2.5 Schematic for the bacterial growth redosing of *E. coli* 8739 with NZVI (shaking condition) 24

3.1: *E. coli 8739* growth with NZVI in carbonate buffer solution under stirring condition. 28

3.2: *E. coli JM 109* growth with NZVI in carbonate buffer solution under stirring condition. 28

3.3: *Pseudomonas putida* F1 growth with NZVI in carbonate buffer solution under stirring condition. 29

3.4: *E. coli 8739* growth with NZVI in carbonate buffer solution under shaking condition. 31

3.5: *Pseudomonas putida* F1 growth with NZVI in nutrient media under shaking condition. 35

3.6: *E. coli* JM 109 growth with NZVI in nutrient media under shaking condition. 36

3.7: *E. coli* 8739 growth with NZVI in nutrient media under shaking condition. 37

3.8: Growth of microorganism (*E. coli* 8739) at 22°C in nutrient media under stirring (800 rpm) condition 39

3.9: Growth of microorganism (*E. coli* 8739) in the control unit (with no initial NZVI). Introduction of 10 mg/mL of NZVI at 3 h did not affect the growth pattern of the microorganisms 41

3.10: Growth of microorganism (*E. coli* 8739) in the treatment reactor (with 10 mg/mL initial NZVI). Introduction of 10 mg/mL of NZVI at 3 h and again at 6 h did not affect the growth pattern of the microorganisms. 42

3.11: Growth of microorganism (*E. coli* 8739) at sub-optimal 22°C in nutrient media under shaking (150 rpm) condition. 42

3.12: Growth of microorganism (*E. coli* 8739) at sub-optimal 4°C in nutrient media under shaking (150 rpm) condition............................. 43

3.13: TEM image that indicate that nanoparticles (circled) had adsorbed onto the cell surface............................. 45

3.14. TEM image showing NZVI particles deposition on the cell surface. 47

3.15: NZVI particles appear to have penetrated through the cell membrane into the cell in this TEM image............................. 48

3.16: TEM images does not give clear idea about the location of NZVI. While the cell on the bottom shows that particles are inside. 49

3.17: Magnified TEM image of a part of an *E. coli* cell. 50

3.18: TEM image *E. coli* cells collected from a control (no NZVI). 51

LIST OF ABBREVIATIONS

CFU/mL	Colony forming units
Cells/mL	Number of viable cells/mL
CNTs	Carbon NanoTubes
DNAPL	Dense Non-aqueous phase liquids
E. coli	*Escherichia coli*
Fe	Iron
Fe^0	Zerovalent iron
Fe^{2+}	Ferrous
Fe^{3+}	Ferric
H_2O_2.	Hydrogen peroxide
Inactivation	Cell death
nm	nanometer
mM	millimoles
mg	milligrams
mL	milliliter
NP	Nanoparticles
NZVI	Nanoscale-Zerovalent iron nanoparticles
OH^+	Hydroxy radical
PCE	Tetrachloroethene
P. pF1	*Pseudomonas putida* F1
PRB	Permeable reactive barrier
ROS	Reactive oxygen species
Suscep tibility	Sensitive or Likely to be affected
TCE	Trichloroethene

TEM	Transmission electron microscopy
TiO_2	Titanium dioxide
Viable bacteria	Living bacteria
ZVI	Zero-valent iron nanoparticles

CHAPTER 1. INTRODUCTION

1.1 Definition of Nanoparticles

Nanoparticles are particles smaller than 100 nanometers in two or three dimensions (ASTM international, 2006). Nanoparticles are small colloidal particles which can be transported freely, and they are typically reactive species. Nanoparticles size-related properties significantly differ from other fine particles or bulk materials. They have unique physical and chemical properties such as particle aggregation, photoemission, electrical and heat conductivity and catalytic activity (Liu W.T et al., 2006). The classification of nanoparticles is based on their morphology, composition, dimension, and agglomeration behavior (Buzea et al., 2007). Some of the common nanoparticles are metallic (gold, iron, nickel, cobalt), semiconductor (quantum dots), and polymeric (poly (L-lactic acid or PLA) (Liu W.T et al., 2006). In nature, nanoparticles are formed either biotically or abiotically by weathering, precipitation and biomineralization and are ubiquitous in the environment (He et al., 2008). Since the early 1990s, synthetic nanoparticles have drawn interest of the scientific communities and industry. There are numerous uses of nanoparticles in specialized and everyday commercial products.

1.2 Applications of Nanoparticles

Nanoparticles are finding applications in personal care, energy, and biomedical products in addition to other industrial applications. Products and applications where nanoparticles have widespread uses include surface disinfectants, fabrics, filtration, hazardous chemical neutralizers, automotive components, electronics, scientific instruments, sports equipment, and flat panel displays.

Nanoparticles are widely used in personal-care products. For example, titanium dioxide (TiO_2) and zinc oxide (ZnO) are being used in beauty care products, toothpastes, sunscreens and opacifiers (Reijenders, 2006; Serpone et al., 2007). Metal oxide nanoparticles (TiO_2, Fe_2O_3, and ZnO) are being introduced in commercial products such as cosmetics and sunscreens (Nowack and Bucheli, 2007). Nanosized iron oxides are also used in some lipsticks as pigments (Royal Society, 2004). Silver nanoparticles are known to have antibacterial properties and are used in cosmetics, antimicrobial hand sanitizers, and hand washes. Silver nanoparticles are also used in fabric, and in medical textiles to reduce bacterial contamination (Bosetti et al., 2002; Ju-Nam and Lead, 2008).

Titania nanoparticles (TiO_2) are suitable for photocatalytic activities and photoelectric energy conversion (Schukin et al., 2003). Carbon nanotubes (CNTs), quantum dots, and graphene are also widely used in solar cells. Cerium oxide (CeO_2) nanoparticles are finding use as an additive in fuels and as a component in oxygen sensors (Robinson et al., 2002; Laosiripojana et al., 2005; Ju-Nam and Lead, 2008; Zhu et al., 2009). Gold/silica nanoparticles are used in optical sensing (Kumar et al., 2006).

Nanomaterials are used in medical and biological sciences. Different nanoparticles are used in areas such as drug delivery, gene therapy, fluorescent labeling, imaging (as contrasting agents), tissue engineering, separation and purification (of cells and biological molecules), and products such as DNA probes and microsurgical tools. Silicon dioxide (SiO_2) nanoparticles are being used in dental fillers, prosthetic implants, and as drug carriers (Calvo et al., 2001; Akerman et al., 2002., Chen et al., 2004; Salata et al., 2004; Balamurugan et al., 2006, Harris et al., 2006; Nel et al., 2006; Reijnders, 2006). Functionalized gold nanoparticles (<10 nm) show evidence of higher affinity for amino acids and nucleic acids, and, hence, are used as molecular delivery vehicles (Rosi

et al., 2006). Magnetic nanoparticles are being used in biomedical and biological applications such as targeted drug delivery, hyperthermia treatment, and protein separation and biosensing. Magnetic nanoparticles are also used as contrasting agents in magnetic resonance imaging (Chanana et al., 2009). For example, iron oxide nanoparticles are being used in imaging tumors and in therapy (Peng et al., 2008). Magnetic nanoparticles have a unique property to function at cellular and molecular level of biological interactions, making them popular for biomedical applications.

Metal oxide nanoparticles (TiO_2) are used in ceramics, coatings, catalysis semiconductors, microelectronics, sensing, transport, and other applications in biological and medical sciences (Daniel and Astruc, 2004; Aitken et al., 2006; Nel et al., 2006; Reijnders, 2006; Murphy et al., 2008).

Silver nanoparticles are used in dental resins, in coatings of medical equipment such as catheters, infusion systems (Bosetti et al., 2002; Markarian, 2006). In addition, silver nitrate and titanium dioxide nanoparticle-coated facemasks are used to protect against infections (Li.Y et al., 2006). Bimetal nanoparticles [CdSe/Cds, CdSe/ZnS, ZnSe/ZnS, CdTe/CdS] are used for fluorescent bioimaging (Schreder et al., 2000).

Fullerens are being used in combination with polymers as thin-films in electro-optical devices and biomedical applications (e.g., drug delivery). Functionalized CNTs are being used in biomedical applications like delivery of candidate vaccine antigens, drugs, peptidomimetics, proteins, and oligo-nucleotides as well as in field of peptide chemistry (Prato, 1999; Bianco and Prato, 2003; Bosi et al., 2003). Quantum dots are finding uses in medical imaging and medical devices. Also, Quantum dots are finding uses in photovoltaics, telecommunications, and sensors (Ju-Nam and Lead, 2008, Klaine et al., 2008).

Additionally, nanoparticles are used in identifying plant-pathogen interactions and are expected to result in new ways for crop protection (Nair et al., 2010).

There are numerous instances where nanoparticles have been used for environmental pollution control and contaminant remediation. As the present research specifically deals with environmental applications of nanoparticles a separate section has been devoted to discuss this topic (See section 1.3).

1.3 Environmental Applications of Nanoparticles

Because of their unique physico-chemical properties, such as high surface to mass ratio, reactivity, and small size, there is a growing interest in the use of nanoparticles for environmental remediation. Nanomaterials are used for the removal of the contaminants or pollutants from the environment (U.S. EPA, 2007). Nanoparticles are used for water quality improvement, groundwater remediation, air pollution prevention and control, and hazardous waste treatment (Masciangioli et al., 2003; Balbus et al., 2006; U.S.EPA, 2007). Nanoparticles have been used in both in-situ and ex-situ applications for the treatment of contaminated water, soil, sediment, and solid waste (Zhang, 2003; U.S.EPA, 2007 b; U.S.EPA, 2009).

Chloro-organics, considered to be a major group of contaminants in the US and around the world, can be effectively remediated using different nanoparticles (Table 1.1). Other contaminants that can be remediated by various nanoparticles/nanomaterials include halogenated hydrocarbons, certain pesticides (atrazine, alachlor), polychlorinated hydrocarbons, dyes, and inorganics such as nitrate, perchlorate, dichromate, and arsenate (Zhang 2003; USEPA 2007 b, Thompson, et al., 2010). Nanometals are already being commercially used for groundwater remediation (Gavaskar et al., 2005). Among the number of nanoscale materials explored so far for

environmental remediation are nanoscale products of zeolites, metal oxides (TiO_2, iron oxide), carbon nanotubes (CNTs), and a variety of noble metals (palladium, platinum, nickel, silver, and gold, mainly used as bimetallic nanoparticles (Xu and Zhang, 2000; Zhang 2003; Mauter and Elimelech 2008; Karn et al., 2009).

Table 1.1: Examples of nanoparticles used in environmental remediation

Nanoparticles	Contaminant	Reference
Nano zeolites	Toluene,nitrogen dioxide	Song et al., (2004)
Carbon nanotubes (CNTs)	p-nitrophenol benzene, toluene, dimethylbenzene, Heavy metal ions	Jin et al., (2007)
Bimetallic nanoparticles (Pd/Fe)	PCBs, chlorinated methane, ethane	Xu and Bhattacharyya (2005) Xu and Zhao (2007) Zhang and Wang (1997)
Ni/Fe and Pd/Au nanoparticles	TCE and PCBs	He and Zhao (2005)
TiO_2 photocatalyst	Heavy metal ions Pentachlorophenol (PCP)	Pena et al., (2005) Quan et al., (2005)

Iron-based nanoparticles are most commonly used in environmental remediation. Iron nanoparticles are discussed in more details in the next section (1.4).

1.4 Iron Nanoparticles for Environmental Applications

Iron is an inexpensive, and environmentally compatible. Among the iron, zero-valent (Fe^0) is very extensively used in environmental remediation. Nanoscale zero-valent iron (NZVI) is a good sorbent and a reducing agent which can breakdown the contaminants into benign form or less toxic substances (Liu et al., 2005). NZVI application is of great interest in the remediation industry because of its unique properties (Zhang, 2003; Tratnyek and Johnson, 2006; Zhang and Elliot, 2006). NZVI particles are very reactive, and have large surface area (22-54 m^2g^{-1}) which gives them an advantage compared to microscale ZVI (surface area 1-2 m^2g^{-1}) (Liu et al., 2005; Krajangpan et al., 2008; Thompson, 2008; Bezbaruah et al., 2009 b).

NZVI has a core-shell structure which makes it relatively stable yet reactive in the environment (Liu et al., 2005; Nurmi et al., 2005). The oxide of the outer layer protects the core Fe^0 (Figure 1.1) from accelerated oxidation and helps degradation reaction to proceed at a reasonable speed. Because of this unique structure, NZVI exhibits characteristic of both Fe^0 and oxides/hydroxides (Li et al., 2006; Bezbaruah et al., 2009 a). Iron oxides act as sorbent and metal core behave as reductant. The oxide/hydroxide shell may also provide sites for chemosorption via chemical complex formation.

NZVI particles are used in the remediation of groundwater contaminants like chlorinated compounds including dense non-aqueous phase liquid (DNAPL) (Zhang and Wand, 1997; Lien and Zhang, 1999; Zhang, 2003; Liu et al., 2005; Liu and Lowry, 2006; Liu et al., 2007), pesticides like DDT and alachor (Joo and Zhao et al., 2008; Thompson and Bezbaruah, 2008; Bezbaruah et al., 2009 b, Thompson et al., 2010), and metals and heavy metals like Ag, As, Cd, Cr, Hg, Ni, Pb, and Zn (Blowes et al, 1997; Ponder et al., 2000; Farrel et al., 2001; Alowitz and Scherer, 2002; Cao et al., 2005; Li.X.Q et al., 2006; Sohn et al., 2006). Iron Nanoparticles are also used for the remediation of contaminated soils (Martin et al., 2008), sediments (Zhang and Frankenberger, 2006), and biosolids (Li et al., 2007).

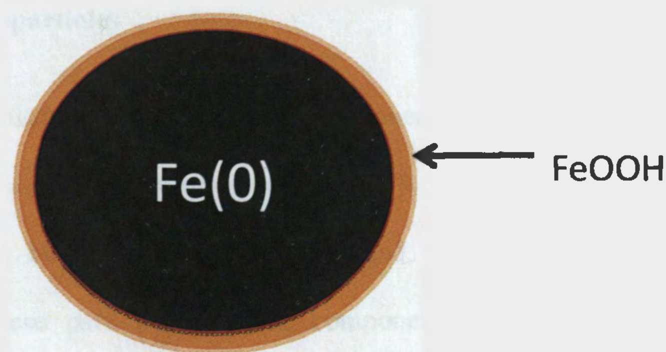


Figure 1.1: The Core-Shell model of Zero-Valent iron nanoparticles. (Modified from Li et al., 2006)

The reported mode of contaminant degradation by NZVI is reductive dehalogenation (Matheson and Tratnyek, 1994). In a typical remediation situation Fe^0 gets oxidized to Fe^{2+} and gives out electrons in the process (Vogel et al., 1987; Matheson and Tratnyek, 1994). Contaminants accept the electrons donated by Fe^0 and undergo reductive degradation.

$$Fe^0 \rightarrow Fe^{2+} + 2e^- \quad [1.1]$$

For example in case of chlorinated compounds:

$$RCl + H+ + 2e^- \rightarrow RH + Cl^- \quad [1.2]$$

Combining the reactions [1] and [2], the following representative reaction is obtained to represent the degradation process.

$$RCl + Fe^0 + H^+ \rightarrow RH + Fe^{2+} + Cl^- \quad [1.3]$$

Microscale iron and iron filings have been used in permeable reactive barriers (PRBs) for groundwater remediation with varying degrees of success (Matheson and Tratnyek, 1994; USEPA, 1999; Farrell et al., 2001). Given the advantages of NZVI, it can be expected that the use of NZVI in PRBs will significantly increase the performance of the barriers (Elliot and Zhang, 2001).

1.5 Nanoparticles and Microorganisms

1.5.1 Fate of nanoparticles

Microorganisms are the principle components of ecosystems and often they mediate redox reactions in the environment. With the increasing popularity of nanoparticles for biomedical, commercial, and environmental applications, the interactions of these particles with the components of the environment need to investigated. There are concerns about safety of human exposure of nanoparticles during handling. Among the most commonly used nanomaterials TiO_2, carbon (carbon

black and nanotubes), and transition metal nanoparticles are reported to pose health impacts. National Institute for Occupational Safety and Health (NIOSH), (2009) has recommended the use of respirators for protection against nanoparticles. (Biswas and Wu, 2005; NIOSH, 2009). There are also reports about nanoparticles affecting the aquatic environment. Certain commercial nanoscale metals like zinc oxide (ZnO), cerium dioxide (CeO_2), titanium dioxide (TiO_2) and also fullerenes (nano-C_{60}) are shown to have potential impacts on the aquatic population, food web dynamics, and the overall functioning and health of the ecosystem. (Lovern et al., 2007; Battin et al., 2009; Johnston et al., 2010). However, only limited studies have been reported on possible impact of nanoparticles on endemic microorganisms and more particularly microorganisms that are typically used for environmental contaminant removal. The routes through which microorganisms may be exposed to nanoparticles can be either direct or indirect. Nanoparticles that are used in biomedical and commercial products may find their way to the environment via wastewater and surface water flows. Also very reactive nanoparticles may come in contact with the components of the environment (e.g., microorganism) when such particles are used in environmental remediation (e.g., nanoparticles directly injected into contaminant plumes).

1.5.2 Nanoparticles toxicity

There are concerns about the toxic effects of nanoparticles on microorganisms (Neal, 2008). A variety of nanoparticles has promising antibacterial properties and, therefore, can be beneficial for human uses. Silver (Ag) nanoparticles are typically used as antimicrobial agents. The antibacterial activity of silver depends on the size of silver particles, where nanoparticles attaches to the surface of cell membrane and disrubts its proper cell functions, like respiration and permeability, and finally causing death of the

cell. (Panacek et al., 2006; Morones et al., 2005). According to Sondi and Salopek-Sondi (2004), bacterial inactivation depends on the concentration of nanoparticles. They have demonstrated that when Ag-NP concentration is increased from 10 µg/mL to 100 µg/mL there is a delay in growth of *E. coli*. In another study, Pal et al., (2007) has also shown that silver nanoparticles disrupt membrane activity and damages the membrane. Membrane impairment in microorganisms has been reported by others for ZnO (Brayner et al., 2006), Mg (Stoimenov et al., 2002), CeO_2 (Thill et al., 2006) nanoparticles. Studies with ZnO nanoparticles and *E. coli* show biocidal effects and membrane disorganization, as well as cellular internalization of nanoparticles by the bacteria (Brayner et al., 2006). Substantially decreased viability of *E. coli* and *S. aureus* has been reported in the presence of TiO_2 nanoparticles (Tsuang, 2008). But, $Fe_2O_3$and Al_2O_3 demonstrated moderate toxicity on *E. coli.*. *E. coli* could attract lower charged cations and, therefore, cytotoxicity might be exerted via the lower valent cations. Hence in case of NZVI, the decreased toxicity is seen in NZVI as the cationic charges begins to increase (Fe^0 to Fe^{2+} and finally to Fe^{3+}) (Hu et al., 2009).

1.6 Nanoparticles Impact on Microorganism

Researchers have reported that NZVI particles inactivated gram-negative bacteria *E. coli* (Auffan et al., 2008; Lee et al., 2008). Additionally, the toxicity of different iron nanoparticles (Fe_3O_4, γ-Fe_2O_3, Fe^0 nanoparticles) towards gram negative bacteria *E. coli* and its mutants *sod* A and *sod* E have been reported by Auffan et al., (2009). The toxic effect of reduced forms of iron has been reported to be higher than that of highly oxidized nanoparticles (Auffan et al., 2008).

NZVI has been used in groundwater remediation by injecting them into the aquifer at concentrations of 1g/L (i.e., 1mg/mL) to 10g/L (i.e., 10 mg/mL) This range

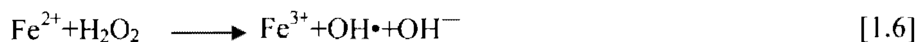
is typically ideal for the remediation depending on contaminant concentration (Henn and Waddill, , 2006; Li et al., 2006).

There is not reported studies on impacts of nanoparticles on subsurface microbial activities in the sites where NZVI was injected However, laboratory scale studies show bactericidal effects (pure cultures) of NZVI even at low concentrations in the buffer solution (Lee et al., 2008; Auffan et al., 2008). These studies indicate possible adverse effects of NZVI on microorganism but are not conclusive as the tested microorganisms are deprived of essential nutrients in a buffer solution.

Auffan et al., (2008) studied *E. coli* cells attach to NZVI and found NZVI toxicity to be dose dependent. They also compared toxicity of NZVI and iron oxide nanoparticle (magnetite and maghemite) and concluded NZVI to be more toxic. NZVI toxicity on microorganisms depend on the redox state of iron .The main source of toxicity is oxygenation of Fe species, either Fe(II) or Fe^0, inducing an oxidative stress or by Fe(II) release into the solution (Auffan et al., 2008). Nanoparticles may also enhance redox reactions by generating reactive oxygen species (ROS) (Eqs. 1.4-1.6, Auffan et al., 2008). The toxicity of NZVI is hypothesized to be due to the oxidative stress on the microorganisms caused ROS. ROS may cause the disruption of the cell membrane or electron/ion transport chain.

$$Fe^0 + O_2 + 2H^+ \longrightarrow Fe^{2+} + H_2O_2 \quad [1.4]$$

$$Fe^0 + H_2O_2 \longrightarrow Fe^{2+} + 2OH^- \quad [1.5]$$



$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH\bullet + OH^- \quad [1.6]$$

Lee et al., (2008) observed more bactericidal effects of NZVI under anerobic conditions than under aerobic conditions. They also concluded that *E. coli* inactivation is NZVI dose and size dependent. NZVI was found to be more toxic than the regular ZVI which concluded that it the nanosize of the particles that imparts toxicity. The

toxicity of NZVI in the oxygen saturated conditions is less than deoxygenated conditions, possibly because of passivating oxide layer formation around NZVI particles. Li et al., (2010) also reported that if there is complete oxidation of Fe^0 NZVI loses bactericidal effects. Aged NZVI (partial oxidized) particles were less toxic to *E. coli* than fresh NZVI particles. (Li et al., 2010). There are studies that report no signs of toxicity in *E. coli* cell proliferation by different concentrations of iron oxide nanoparticles (**Table 1.2**) at optimal conditions of growth in the Luria Bertani (LB) media (Williams et al., 2006). But in the presence of iron oxide shell, NZVI particles show decreased reactivity (Baer et al., 2008; Sarathy et al., 2008). However, Fe (II) was found to be toxic under anaerobic conditions, and in the case of NZVI, the released Fe(II) from nanoparticles may contribute to toxicity.

However, there is also a school of thought that Fe^0 does not directly contribute to the cytotoxicity of the cell where Fe (II) is a prerequisite for cell damage and (Grieger et al., 2010). In another study oxidized NZVI particles have shown no effects on *P. fluorescens*, a gram negative bacterium but hydrated iron oxide FeO(OH) nanoparticles have shown 100% inactivation. In *Bacillus subtilis* the inactivation was 95% (1 mg/mL NZVI) and 80% (0.1 mg/mL NZVI). As the concentration of the NZVI decreases the chances of survival of the bacteria increases (Diao and Yao, 2009).

Table 1.2: Effects of iron-based nanoparticles reported in the literature

Nano particle Type	Test organism	Concentrations	Suspensions used	Observation	Reference
NZVI, Fe_2O_3 (maghemite) Fe_3O_4 (magnetite)	*Escherichia coli* strain Qc1301 and its mutant *sod*A *sod*B Qc2472	7,70,175,350,700 mg/mL	Ultrapure water (pH 5-5.5)	Dose-dependent toxicity (>70mg NZVI/L) the toxicity is induced due to oxidative stress, by oxygenation of reduced Fe species (FeII and Fe0).	Auffan et. al. (2008)
NZVI	*Escherichia coli* (ATCC strain 8739)	1.2– 110 mg/L	2 mM Carbonate buffer	Strong bactericidal effect under deaerated conditions, dose dependent, physical disruption of cell membranes, Fe (II) creating oxidative stress producing ROS.	Lee et al., (2008)
Silica /iron oxide,	*Escherichia coli*	Si- iron oxide 2.2×10^{-3} g/mL Si- 3.3×10^{-2} g/mL	L.B. media	The interactions were non-specific, no overt signs of growth inhibition.	Williams et al., (2006)
NZVI, Oxidized NZVI [FeO(OH)]	*Bacillus subtilis*, -Gram positive, *Pseudomonas fluorescens*- Gram negative, *Aspergillus versicolor*- *Fungus*	0.1,1,10 mg/mL	DI water	Complete inactivation in *P. fluorescens*. when treated with 10 mg/ml of NZVI particles under aerobic condition with a vigorous shaking.	Diao and Yao (2009)
NZVI, Surface coated NZVI, (polystyrene sulfonate, polyaspartate and NOM) Aged NZVI (Partially Oxidation)	*Escherichia coli* (ATCC strain 33876)	Initial NZVI concentration 100 mg/L, Minimum inhibition concentration (MIC) 0.001-2 g/L	5mM Bicarbonate buffer solution	In NZVI, 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 anerobic conditions: it may be due to oxide layer on the Fe^0 surface.	Li et al., (2010)

1.7 Need Statement

There are at least two schools of thought regarding relationship between microorganisms and nanoscale zero-valent iron (NZVI) particles. Some researchers (Auffan et al., 2008; Lee et al., 2008) have shown that NZVI adversely affects microbial growth. However, others (Li et al., 2010; Grieger et al., 2010) argue that microorganisms can survive in the presence of NZVI as the toxicity of the nanoparticles depends on various factors including the oxidation state of NZVI. To date researchers have looked at microorganism-NZVI interactions from the perspective of iron chemistry rather than looking at it from microbial perspective. Microorganisms are in the environment for millions of years and they are very resilient and adaptive to environmental changes. While the published results on microorganism-NZVI interactions are not questioned, they need careful interpretation vis-à-vis the methodologies used by the researchers. For example, most of the growth studies were conducted either in buffer solutions (Lee et al., 2008; Li et al., 2010) or deionized (DI) water (Diao and Yao, 2009). Unfortunately, these study conditions are not representative of real life situations (e.g., contaminant plume in the aquifer or wastewater treatment plant) where microorganisms will have basic growth nutrients while getting exposed to NZVI. Further, some of the studies were conducted were under unrealistic stirred (800 rpm) conditions (Lee et al., 2008). Such extreme agitation is not expected in any treatment situation presently in use.

Based on the observations above, there is a need to look at microorganism-NZVI interactions from microbiological perspective and under more realistic environmental conditions. This study is an effort to bridge the existing knowledge gap in the area of NZVI-microorganism interactions. The needs to monitor possible toxicity

of NZVI on native microbial population and consequent ecological impacts have been emphasized by researchers (Wiesner et al., 2009).

1.8 Hypothesis

It is hypothesized in this research that microorganisms can survive and grow undisturbed in the presence of nanoscale zero-valent iron (NZVI) particles under typical environmental conditions.

1.9 Objective

The general objective of this research is to better understand the interactions between nanoscale zero-valent iron (NZVI) particles and endemic microorganisms.

The specific objectives of this study are:

1. To study growth of environmentally relevant microorganisms in the presence of NZVI in buffer (no nutrient) solution, and
2. To study growth of environmentally relevant microorganisms in the presence of NZVI in nutrient media.

1.10 Organization of the Thesis

This thesis is divided into four chapters. Chapter 1 is the introduction which includes background of this research, need statement, and research objectives. Chapter 2 discusses materials and a method used in this research, Chapter 3 presents the results from NZVI-microorganisms interactions studies in buffer and nutrient media and interprets the results. Chapter 4 includes conclusions and recommendations for future work. This thesis also includes additional information in the form of list of abbreviations.

CHAPTER 2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used for experiments were reagent grade. Ferrous sulphate ($FeSO_4.7H_2O$, 99%, Alfa Aesar, USA), sodium borohydride ($NaBH_4$, 98%, Sigma Aldrich, USA), sodium hydroxide (NaOH, Alfa Aesar, USA), ethanol (C_2H_5OH, J.T Baker,bUSA), and carbonate buffer 2 mM (pH 8.0) (VWR, USA), and phosphate buffered saline (pH 7.2) (Ambersco, USA) used as received unless otherwise specified. All materials were used as received unless otherwise stated. Chemicals were stored at room temperature (22 ±2°C) or at temperatures specified by the suppliers.

2.2 Media Preparation

Tryptic soy broth (TSB), brain heart infusion (BHI), and nutrient agar were prepared as described by the supplier (Difco, USA).

2.2.1 Cultures

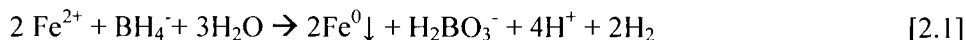
Microbial cultures of *Escherichia coli* (ATCC strain 8739*), Pseudonomas putida* F1 (ATCC strain 70007) were purchased from American Type Culture Collection (ATCC, USA). *Escherichia coli* K-12 JM109 was purchased from Promega, Inc. (Catalog number P9751, Madison, WI, USA). *E. coli* (37°C) and *P .putida* F1 (30°C) were grown in a brain heart infusion (BHI) and tryptic soy broth (TSB), respectively, for 20 h before using in the experiment.

2.3 Preparation of Nanoscale Zero-valent Iron (NZVI) for Microbial Studies

Iron nanoparticles were synthesized using the sodium borohydride reduction of ferrous ion ($FeSO_4.7H_2O$) in an aqueous phase (Li et al., 2006; Bezbaruah et al., 2009 a;

Krajangpan et al., 2008). The synthesized NZVI was dried under flowing N_2 and vacuum overnight (>5 h). The dried NZVI was air-stabilized (passivated) overnight (>5 h) and stored in the sealed vial.

The reaction scheme for the synthesis is as follows:



$$2\,Fe^{2+} + BH_4^- + 3H_2O \rightarrow 2Fe^0\downarrow + H_2BO_3^- + 4H^+ + 2H_2 \quad [2.1]$$

The nanoparticles were not characterized within this research but during previous work done by others (Thompson and Bezbaruah, 2008; Bezbaruah et al., 2009 a; Krajangpan et al., 2008). The particle size (diameter) of the synthesized nanoparticles ranged from 10 to 90 nm (average diameter = 35 nm), and they were spherical in shape (Thompson and Bezbaruah, 2009). The surface area was reported to be 25 m^2/g. For this research, the NZVI particles were autoclaved in a vial sealed with a Teflon-coated rubber septum for sterility before performing any microbial study. The autoclaving procedure included 20 min sterilization at 121°C.

2.4 Experiments with Buffer Solutions

2.4.1 Bacteria growth study in buffer solution with NZVI (stirring condition)

The goal of this experiment was to study the growth of different bacterial strains under stirring (800 rpm) conditions when exposed to different concentrations of NZVI in carbonate buffer.

A 100 µL aliquot of overnight (~18 h) bacterial cultures *(E. coli* 8739, *E. coli* JM109, and *P. putida* F1) were inoculated into 50 mL of BHI and TSB grown at 37°C (both *E. coli* strains) and 30°C (*P. putida*) for 18 h. Bacteria were harvested by centrifugation at 1000 g for 10 min, washed twice with 50 mL of 150 mM phosphate-buffered saline (PBS, pH 7.2) and resuspended in 50 mL of PBS. A 1 mL aliquot of

this bacterial-PBS stock was transferred to 50 mL of 2 mM carbonate buffer (pH 8.0) to get a final concentration of approximately $1x10^6$ CFU/mL.

Required amounts of NZVI were added to the buffer to achieve NZVI concentrations to 0.09, 0.2, 0.5, 0.8, and 1.0 mg/mL. The bacteria with NZVI in carbonate buffer were incubated at room temperature (22°C±2°C) for 60 min while stirring at 800 rpm in a magnetic stirrer. The 50mL Pyrex bottles remained uncapped (exposed to air) throughout the experiment. Samples (1 mL) were collected at definite intervals (0,5, 10,30,60 min), and bacteria was enumerated following spread plating of serial dilutions on nutrient agar plates. The agar plates were incubated at 37°C and 30°C for 24 h and colonies were counted. The counted colonies were plotted as viable cells/mL versus time. Experiments were performed in triplicate. The experimental procedure is presented in **Figure 2.1.**

2.4.2 Bacterial growth study in buffer solution with NZVI (shaking condition)

The goal of this experiment was to study the growth pattern of the bacteria when exposed to different concentrations of NZVI in carbonate buffer under shaking conditions (150 rpm).

The previous procedure (Section 2.4.1) was followed to wash the bacteria (*E. coli* 8739) and 1 mL aliquot was transferred from the PBS-bacterial stock to 50 mL of carbonate buffer (pH 8.0) and brought it to approximately $1x10^6$ CFU/mL. The required amount of NZVI was added to the buffer to bring the final concentrations to 1, 2, 5, and 10 mg/mL. The suspension of bacteria and NZVI in carbonate buffer was incubated at 22°C for 120 min and shaked at 150 rpm. Aliquots (1 mL) were collected at definite intervals (0, 5,10,30,60,120 min), following spread plating of serial dilutions on nutrient agar plate.

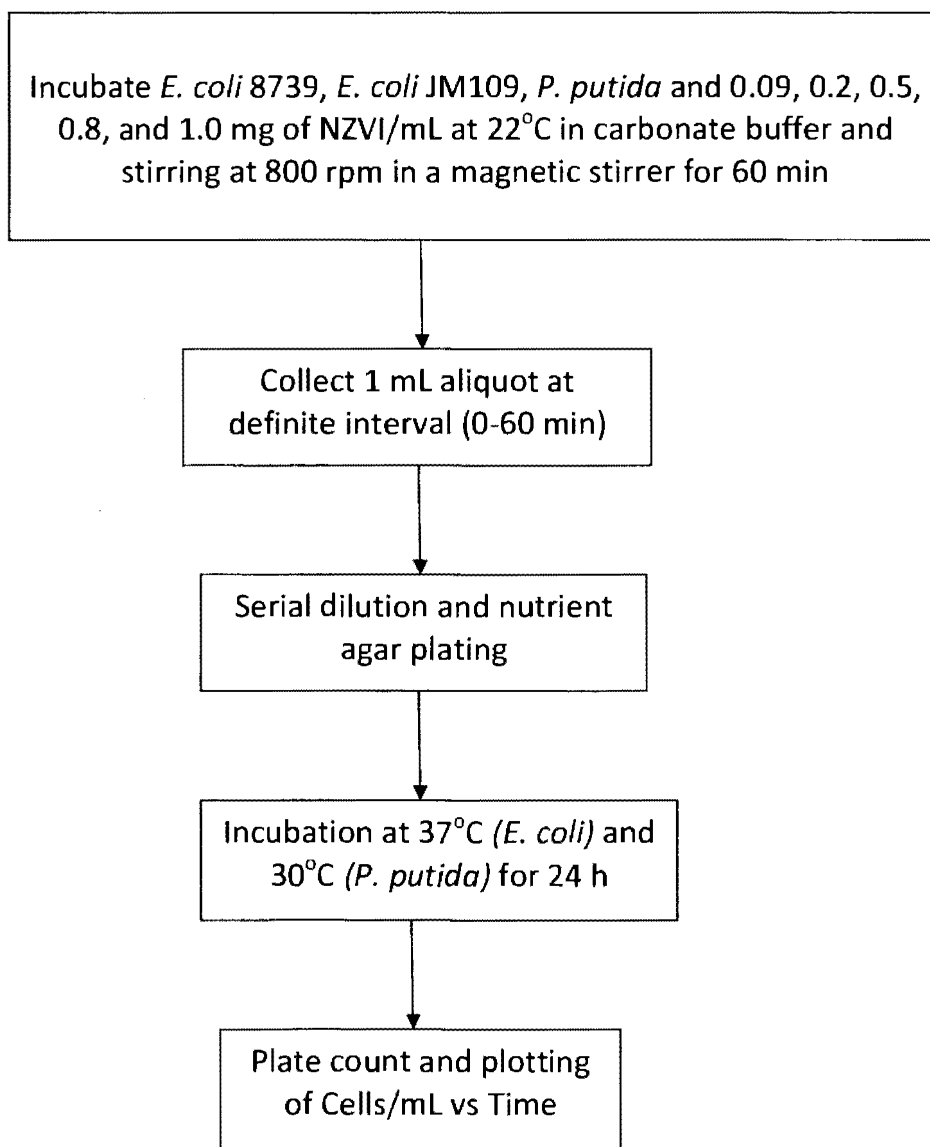


Figure 2.1: Schematic for the growth study of bacteria in buffer solution with NZVI (stirring condition).

The agar plates were incubated at 37°C (*E. coli*) and 30°C (*P. putida* F1) for 24 h and colonies were counted. The counted colonies were plotted as viable cells/mL versus time. Experiments were performed in triplicate. The experimental procedure is presented in **Figure 2.2**.

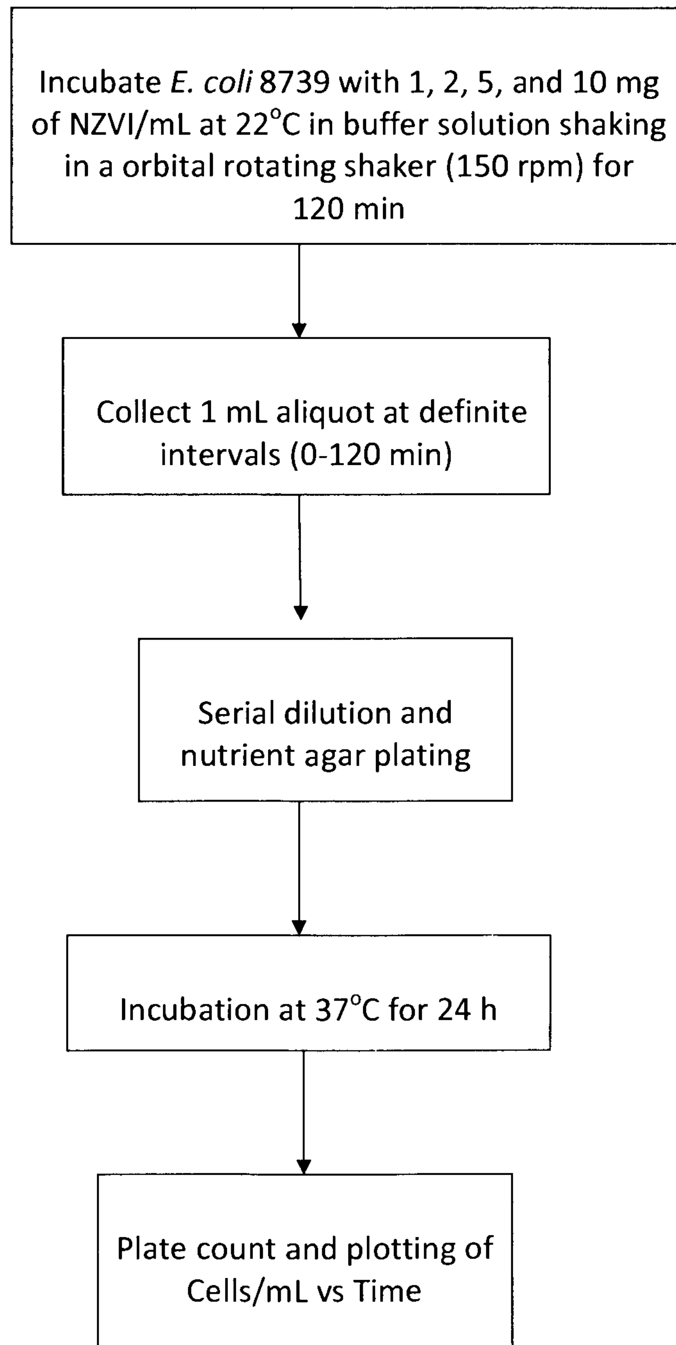


Figure 2.2: Schematic for the growth study of bacteria in buffer solution with NZVI (shaking condition).

2.5 Experiments with Nutrient Media

2.5.1 Bacteria growth study in nutrient media with NZVI (stirring condition)

The goal of this experiment was to study the growth pattern of the bacteria when exposed to different concentrations of NZVI in nutrient media under stirring conditions (800 rpm).

A 250 μL aliquot of an *E. coli* 8739 culture was inoculated into 25 mL of BHI medium and incubated at 37°C for 6 h with NZVI (1, 2, 5, 10 mg/mL). Cultures exposed to the different concentrations of NZVI were stirred using a magnetic stirrer at 800 rpm. Aliquots (1 mL) were collected at one hour intervals for 6 h followed spread plating of serial dilutions on nutrient agar plates. The agar plates were incubated at 37°C for 24 h and colonies were counted. The counted colonies were plotted as viable cells/mL versus time. All the experiments were performed in triplicate. The experimental procedure is presented in **Figure 2.3.**

2.5.2 Bacterial growth study in nutrient media with NZVI (shaking condition)

The goal of this experiment was to study the growth pattern of different bacteria when exposed to different concentrations of NZVI in nutrient media under shaking conditions.

A measured volume (250 μL) of 2-3x10^7 CFU/mL of *E. coli* 8739, *E. coli* JM109 and *Pseudomonas putida* F1 were inoculated into 25 mL of BHI medium and TSB medium, respectively. They were incubated at 37°C and 30°C respectively for 10 h with NZVI (1, 2, 5, and 10 mg/mL). The inoculation temperatures were chosen as these are optimal temperatures for *E. coli* (37°C) and *Pseudomonas putida* F1 (30°C)

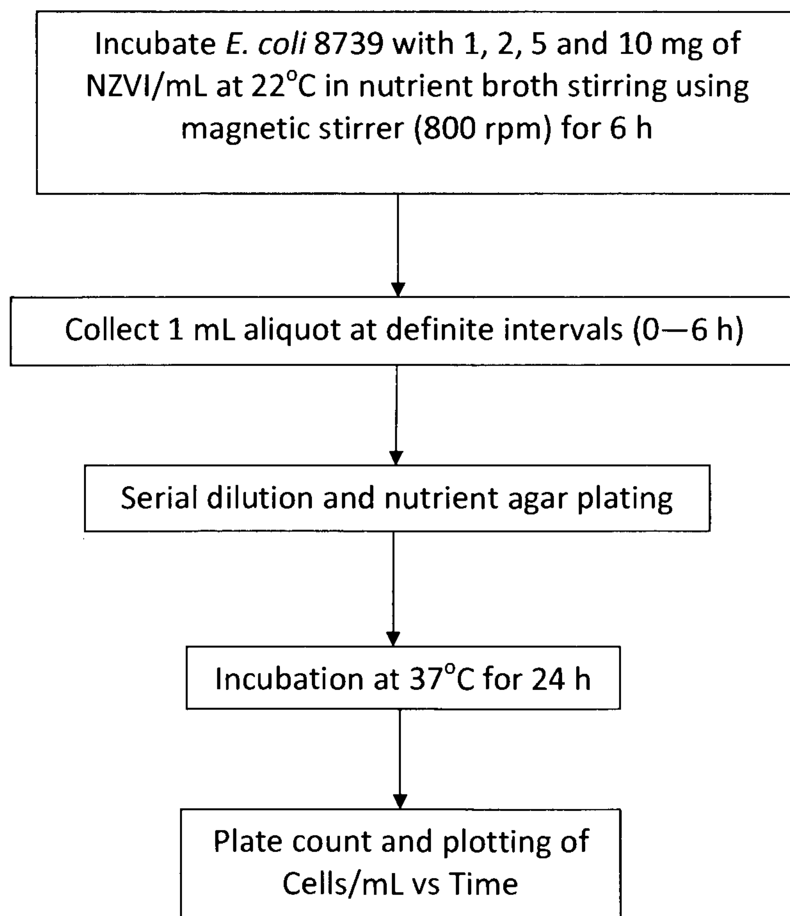


Figure 2.3: Schematic for growth effects of *E. coli* 8739 in nutrient media with NZVI (stirring condition).

growth. Controls were also incubated at 30/37°C without NZVI for all the three strains. All the cultures incubated with different NZVI concentrations were shaken at 150 rpm using an orbital rotating shaker. Aliquots (1 mL) were collected at approximately every 1 h for 10 h followed by spread plating of serial dilutions on nutrient agar plates. The agar plates were incubated at 37°C and 30°C for 24 h and colonies were counted. The counted colonies were plotted as cells/mL versus time. All the experiments were performed in triplicate. The experimental procedure is presented in **Figure 2.4.**

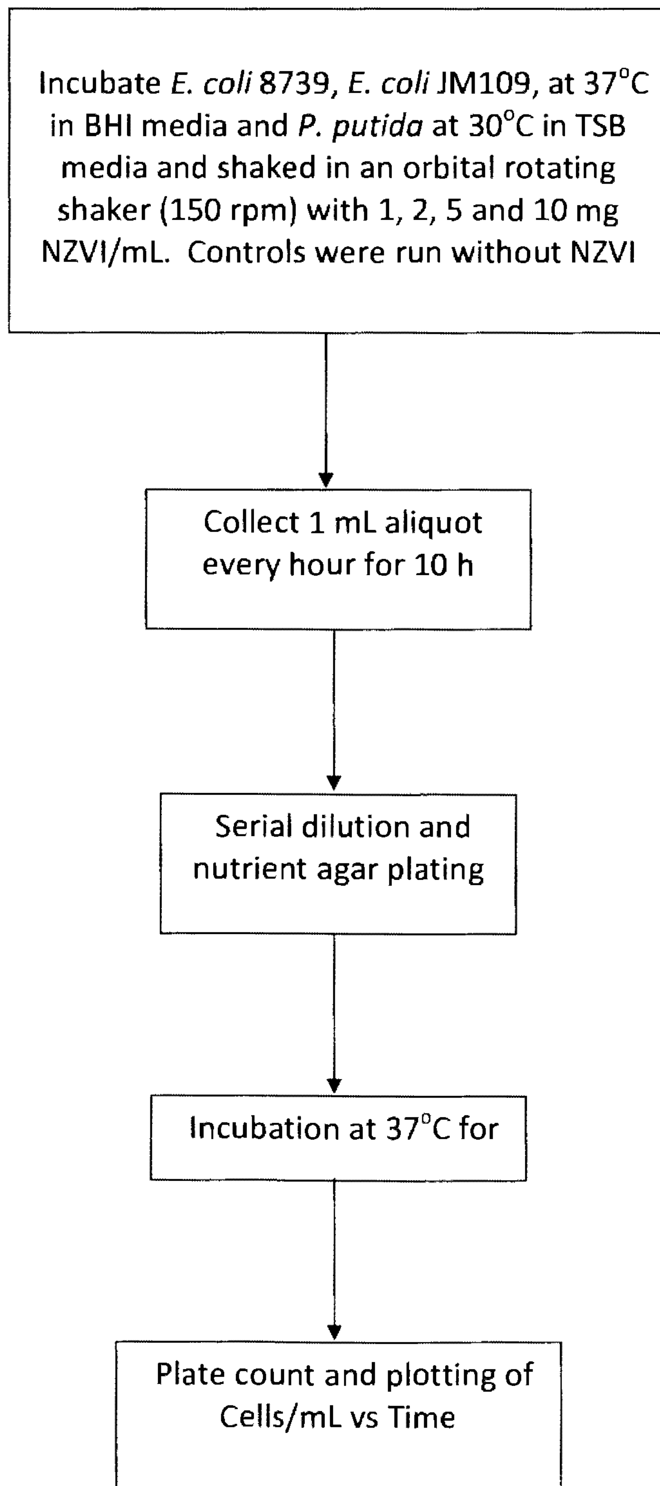


Figure 2.4: Schematic for the bacterial growth study in nutrient media with NZVI (shaking condition).

2.5.3 Growth of *E. coli* 8739 in nutrient media with intermittent dosing with 10 mg/ml of NZVI

The goal of this experiment was to further study the growth characteristic of the strain *E. coli* 8739 with repeated dosing with 10 mg/mL NZVI.

E. coli 8739 was inoculated into BHI media containing 10 mg/mL of NZVI and incubated at 37°C (shaking speed of 150 rpm). Aliquots (1 mL) were collected every 1 h for 10 h followed by spread plating of serial dilutions on nutrient agar plates. The agar plates were incubated at 37°C for 24 h and colonies were counted. The counted colonies were plotted as cells/mL versus time. At 3 h and 6 h, additional 1 mL samples of the culture were taken and the NZVI was removed using a magnet (Dynal, MPC-S, Norway). The sample (without NZVI) was transferred to a sterile nutrient broth containing 10 mg NZVI/mL and was incubated at 37°C for additional 7 h and then for another 4 h shaking at 150 rpm).

As a control, *E. coli* 8739 was grown for 3 h in nutrient broth without NZVI. 10 mg/mL NZVI was added to this control at 3 h and further incubated for 7 h at 37°C. Aliquiots (1 mL) were collected every hour for the entire length of the experiment followed by spread plating of serial dilutions on nutrient agar plates. Experiments were performed in duplicates. The experimental procedure is presented in **Figure 2.5.**

2.5.4 Growth of *E. coli* 8739 at sub-optimal temperatures

The goal of this experiment was to study the growth characteristics of *E. coli* 8739 under suboptimal temperatures (22°C and 4°C) in nutrient media. The growth studies were performed at the suboptimal temperatures at 22 ± 2°C on the bench top, and in 4±2°C in a cold room. Experimental procedure similar to the one enumerated in section 2.5.2 was followed.

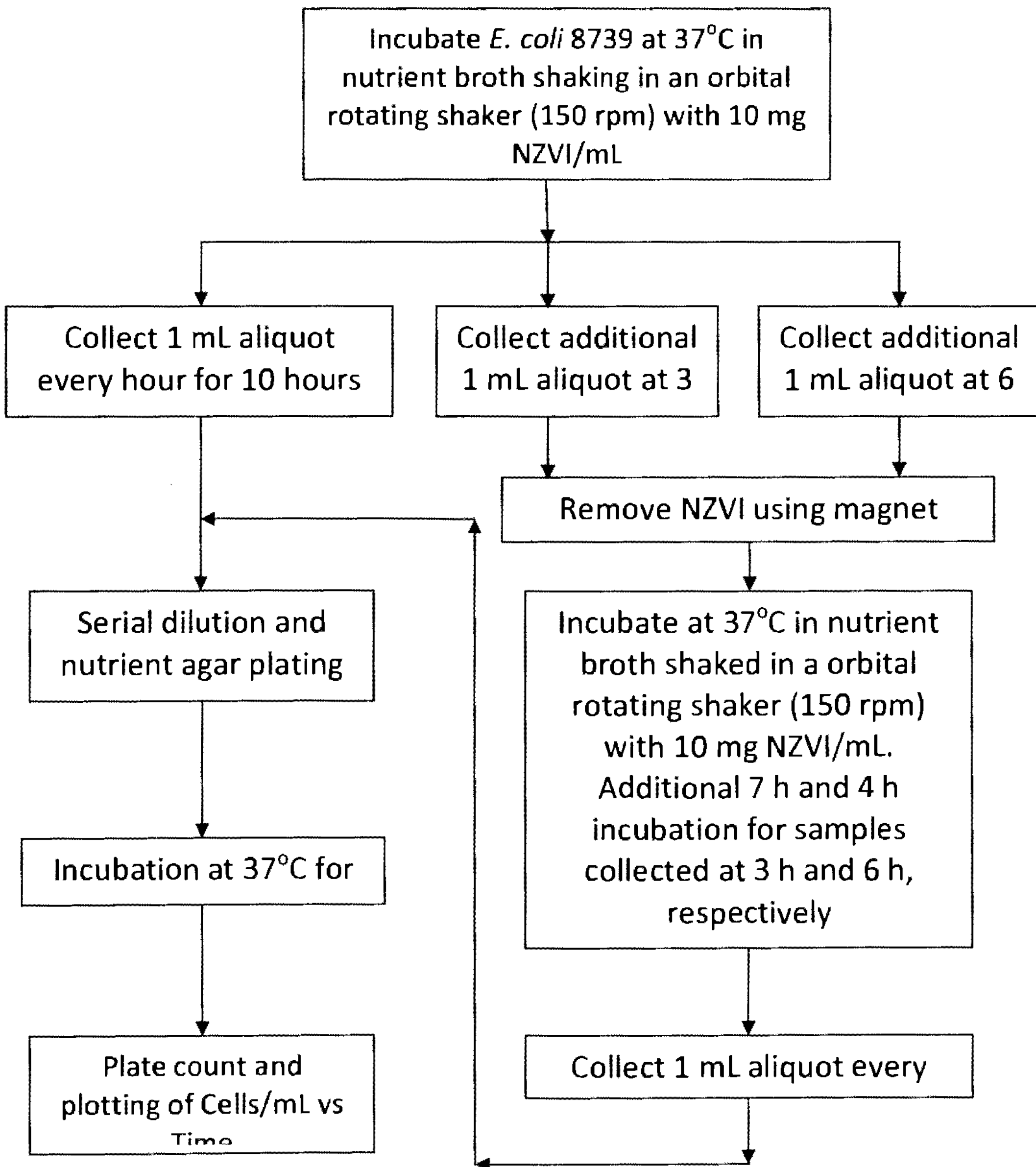


Figure 2.5: Schematic for the bacterial growth redosing of *E .coli* 8739 with NZVI (shaking condition).

2.6 Transmission Electron Microscopy (TEM) Studies

The goal of this experiment was to visualize the NZVI along with bacteria. The 5 h and 2 h sample were used to capture bacteria-NZVI interactions, while the bacteria numbers were actively diving.

The strains (*E. coli 8739*) were incubated at 37°C with 10 mg/mL of NZVI for 2h and 5 h, by shaking at 150 rpm. The same bacteria incubated at 37°C without NZVI were used as controls. Transmission electron microscopy (TEM) was done at NDSU's Electron Microscopy Laboratory. The bacteria was washed three times in ultrapure water, fixed with 2.5 % glutaraldehyde in Millonig's phosphate buffer (pH 7.4) for 24 h at 4°C then fixed with osmium tetraoxide at 25°C and dehydrated in a graded series of acetone (30, 50, 70, 90, and 100%) with en bloc saturated uranyl acetate staining during the 70% acetone step. After completion of the dehydration, the bacteria were embedded in an epon-araladite epoxy resin. Ultra-thin sections of 60 nm were cut using an RMC Powertome XL ultramicrotome equipped with a diamond knife. The sections were post-stained with lead citrate and examined using a JEM-100CX II transmission electron microscope.

CHAPTER 3. RESULTS AND DISCUSSION

Experiments within this research were conducted with two *E. coli* strains (ATCC 8739 and JM 109) and *Pseudomonas putida* F1. *E. coli* was selected because it is a well studied microorganism. The species has been used by other researchers (Lee et al., 2008; Auffan et al., 2008; Li et al., 2010) and, thus, it would be easier to compare the data obtained from this research with that obtained by others. *Pseudomonas putida* F1 was selected for this experiment as it is a heterotrophic degrader with a track record in bioremediation applications (Parales et al., 2000).

Auffan et al., (2008), Lee et al., (2008), Diao and Yao (2009), Williams et al., (2009), and Li et al., (2010) have studied the effects of different iron-based nanoparticles [e.g., NZVI, γ-Fe_2O_3, Fe_3O_4, FeO(OH)] on the gram-negative bacteria and gram-positive bacteria. Studies reported so far have tested the effects of NZVI on the bacteria in either buffer solution or deionized (DI) water. Buffer solution and DI water do not contain nutrients needed for microbial growth and it was felt necessary to study the impacts of NZVI on the bacteria in environmentally relevant media (e.g., media where basic nutrients for microbial growth are present). It was also felt that experiments with buffer solution generated valuable data for comparison. Even though buffer solutions do not remotely represent real environmental system, the decision to work on buffer was taken such the results obtained by others can be reproduced and additional inferences made if possible. It was decided to work on microorganisms in both buffer solution and nutrient media and expose them to environmentally relevant concentrations of NZVI. The experiments involved exposing gram negative bacteria to NZVI in a carbonate buffer system and nutrient broth separately. Growth of the bacteria was monitored over time and the collected data were analyzed to draw conclusions.

3.1 Effect of NZVI on Bacteria in Buffer Solution

3.1.1 Microbial growth in carbonate buffer under stirring condition

The growth studies in buffer solution (see Section 2.4.1) were carried out in the air saturated conditions to ensure adequate supply of oxygen to the microorganisms. Based on work by others (Lee et al., 2008) the microorganisms were exposed to a concentration of 0.09 mg/mL NZVI. Experiments were also conducted with higher NZVI concentrations (0.2-1.0 mg/L) to determine NZVI-microorganism interactions in environmentally relevant concentrations of NZVI. The samples in the reactor and controls (no NZVI) were stirred at 800 rpm using a magnetic stirrer. The number of active cells was monitored over time, and the results are represented in **Figures 3.1**, **3.2**, and **3.3**. The exposure of NZVI at concentrations as low as 0.09 mg/mL in carbonate buffer with stirring resulted in 2-log or more reduction of *E. coli* (from $2.1x10^{-6}$ to $6.8x10^{-4}$ cells/mL) and *Pseudomonas putida* F1 (from $1.8x\ 10^{-6}$ to $7.0x10^{-4}$ cells/mL) in 60 min. The experiments within this research were carried out under similar conditions to those reported previously by Lee et al., (2008) and similar reductions were observed (they reported 2.6 and 3.6 log inactivation of *E. coli* in 60 min). At higher NZVI concentrations (1.0 mg/mL) no viable cells were detected after 5 min. Similar reductions were observed with *E. coli* JM109 and *Pseudomonas putida* F1. The controls run without NZVI for all microorganisms have also shown marked reduction (1.1-log) in 60 min in the buffer solution indicating the lack of a conducive environment for microbial survival.

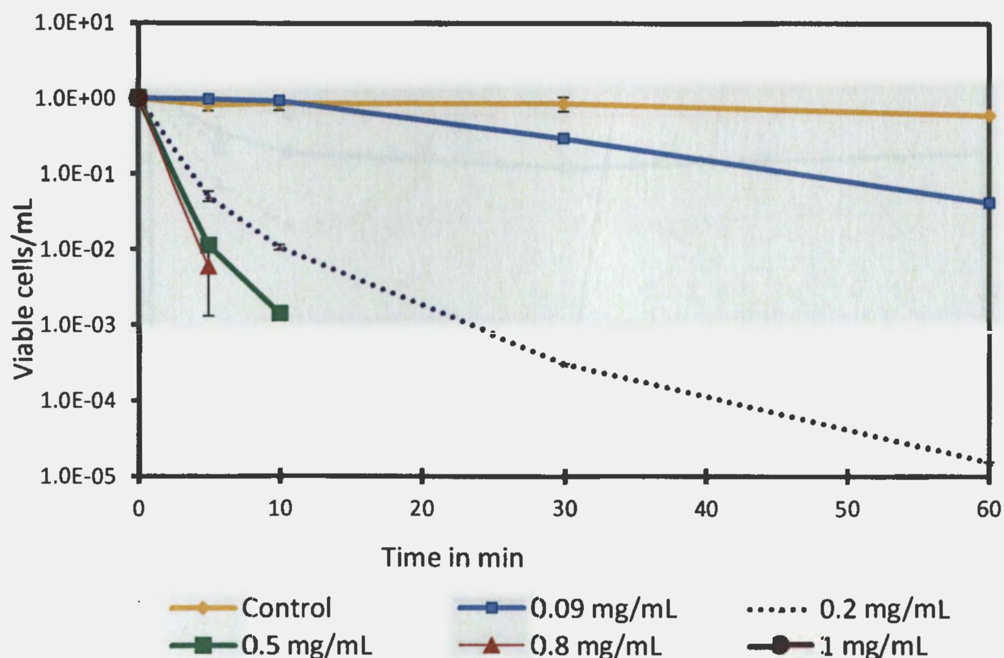


Figure 3.1: *E. coli 8739* survival with NZVI in carbonate buffer solution under stirring condition.

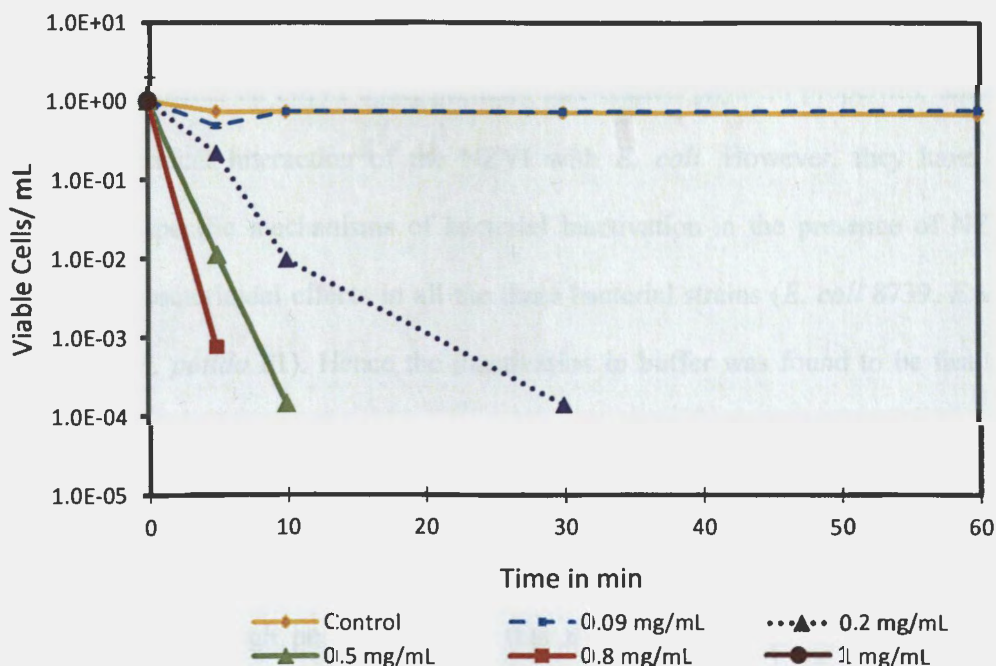


Figure 3.2: *E. coli JM 109* survival with NZVI in carbonate buffer solution under stirring condition.

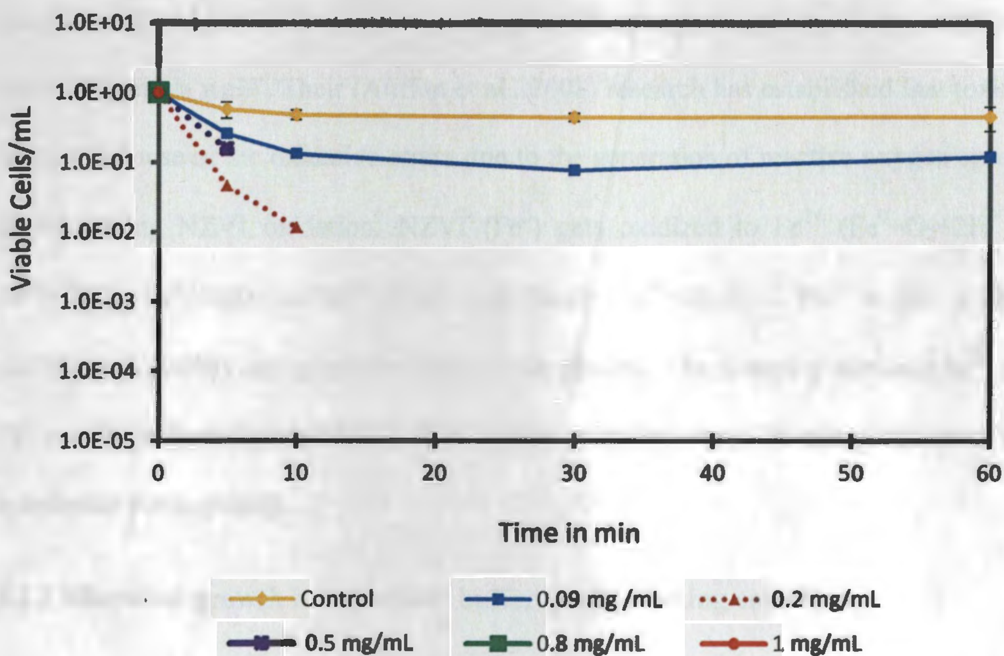


Figure 3.3: *Pseudomonas putida* F1 survival with NZVI in carbonate buffer solution under stirring condition.

Lee et al., (2008) reported biocidal effects of NZVI on *E. coli* 8739 in buffer solution and found that NZVI was more toxic than ZVI. They proposed that *E. coli* inactivation depends on NZVI dose, particles size-related physical properties, and the nature of chemical interaction of the NZVI with *E. coli.* However, they have not reported any specific mechanisms of bacterial inactivation in the presence of NZVI. NZVI shows bactericidal effects in all the three bacterial strains (*E. coli* 8739, *E. coli* JM 109 and *P. putida* F1). Hence the inactivation in buffer was found to be fast and dose dependent. Lee et al., (2008) suggested that stirring might have induced physical damage in bacterial cells. They specifically reported possible physical disruption of the cell membrane. Li et al., (2010) also suggested that NZVI causes physical disruption of cell membranes through penetration, and that might have led to enhanced biocidal effects. Auffan et al., (2008) suggested that microbial toxicity of NZVI may be observed in the form of "the disruption of the membrane integrity or the disturbance of

the chains of transport of electron and ions after the strong adsorption of iron-based NPs onto the bacteria wall". Their (Auffan et al., 2008) research has established that toxicity may be because of the oxidative stress due to the generation of reactive oxygen species (ROS) during NZVI oxidation. NZVI (Fe^0) gets oxidized to Fe^{2+} ($Fe^0+O_2+2H^+ \rightarrow Fe^{2+}+H_2O_2$, $Fe^0+H_2O_2 \rightarrow Fe^{2+}+2OH^-$, and finally $Fe^{2+}+H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$, Auffan et al., 2008) and generates ROS in the process. The interplay between Fe^{2+} and Fe^0 results in formation of ROS. ROS create oxidative stress in microorganisms and inactivates them quickly.

3.1.2 Microbial growth in carbonate buffer under shaking condition

While stirring experiments done by others were reproduced well in this research, it is felt that stirring experiments do not accurate. It represent NZVI-microorganism interactions in the environment as such high speed (800 rpm) of stirring will not be encountered in the environmental situations. It is acknowledged that the NZVI particles and microorganisms should be in suspension to ensure proper contacts between them but a milder form of mixing would serve the purpose. So, the bacteria and NZVI particles were shaked at 150 rpm in orbital shaker within this experiment.

Experiments were performed by shaking *E. coli* 8739 at room temperature (22±2°C) for 120 min. The hypothesis for this study was that the microorganisms will survive better if stirring at very high speed is avoided. The experiments were conducted with higher concentrations of NZVI (1, 2, 5 and 10 mg/mL). The initial trial was done with 1 mg/mL which was the highest concentration for the stirring experiments (see Section 3.1.1 above). It is important to note that 1-10 mg/mL (i.e, 10 g/L) are environmentally relevant concentrations and typical values for environmental remediation applications. With 10 mg/mL, no viable cells were seen after 5 min

(**Figure 3.4**). At 5 mg/mL NZVI concentration, there was a quick initial reduction (in the first 30 min) in bacterial viability and then the reduction rate showed down. For 1 and 2 mg/mL NZVI, the initial inactivation was minimal but there were sharper decreases after 1 h. Overall increased viability of *E. coli* 8739 was observed while the samples were shaken as compared to when they were stirred (see Section 3.1.1).

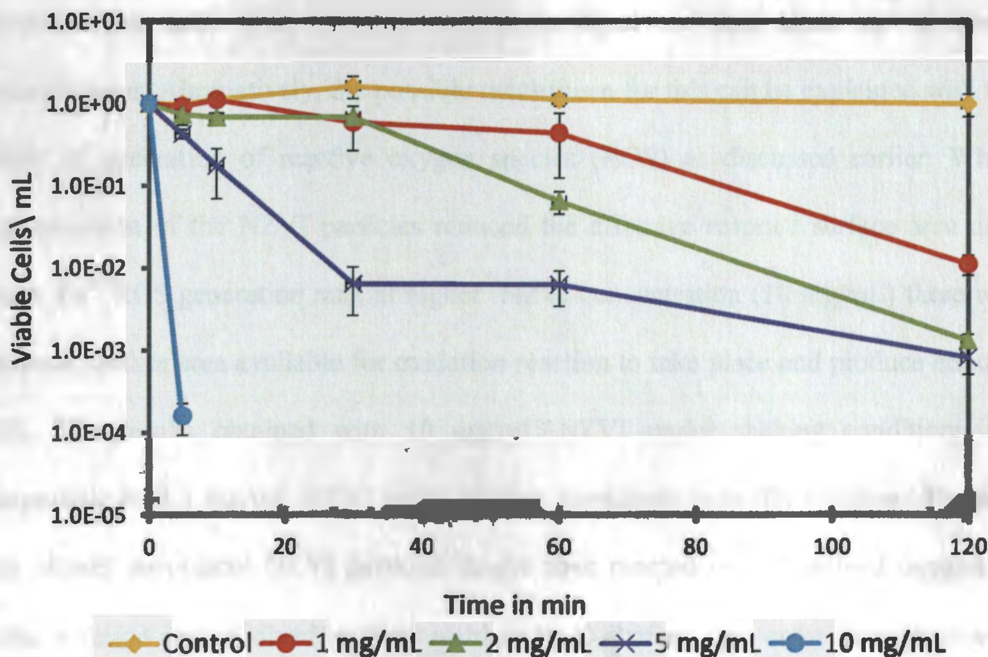


Figure 3.4: *E. coli 8739* growth with NZVI in carbonate buffer solution under shaking condition.

Various factors may explain why survival was greater for shaking than stirring. During shaking settlement of NZVI was observed. Bare NZVI often undergoes aggregation and agglomeration forming micro-sized fractal aggregates (Phenrat et al., 2007). The NZVI particles might have agglomerated and settled down to the bottom of the flask while bacteria remained in suspension. Low shaking speed might have facilitated agglomeration and eventual settling of the NZVI particles. This might have reduced NZVI contact with microorganisms and, hence, less toxicity resulting increased

survival cells was observed. Auffan et al., (2008) also observed that NZVI showed dose (concentration) dependent toxicity on *E. coli* Qc1301 as did Diao and Yao (2009). This NZVI concentration specific behavior of bacteria may be explained based on possibilities of contact. At higher concentrations more NZVI came in contact with the bacteria while at lower concentrations chances of contact (collision) were markedly reduced. However, agglomeration would have been even higher at higher NZVI concentrations and, thus, should have been equal or less toxic as in lower concentrations. Alternatively, the possible mechanism for this can be explained with the theory of generation of reactive oxygen species (ROS) as discussed earlier. While agglomeration of the NZVI particles reduced the effective reactive surface area and, hence, Fe^{2+}/ROS generation rate, at higher NZVI concentration (10 mg/mL) there was adequate surface area available for oxidation reaction to take place and produce enough ROS. The results obtained with 10 mg/mL NZVI under shaking conditions are comparable with 1 mg/mL NZVI under stirring conditions in buffer solution. Further, with slower movement NZVI particles might have reacted with dissolved oxygen in buffer solution and oxidized to more stable γ-Fe_2O_3 before they came in contact with bacteria. NZVI gets oxidize in the presence of oxygen and this may reduce its toxic effect on the bacteria (Li et al., 2009). γ-Fe_2O_3 is known to be less toxic than Fe^0 and other Fe^{2+} oxide (Auffan et al., 2008). It well established that the reactivity of NZVI decreases because of the iron oxide shell formation (Baer et al., 2008, Sarathy et al., 2008). Studies by Auffan et al., (2008) and Lee et al., (2008) have indicated that oxidized NZVI show no effects on *E. coli* 8739. Diao and Yao (2009) have reported that completely oxidized (to Fe^{3+}) NZVI particles show no effects on *P. fluorescens*, a gram negative bacterium.

An analysis of the buffer study results obtained from stirring (800 rpm) and shaking (150 rpm) experiments leads to the following observations:

1. NZVI is very toxic to microorganisms even at very low concentrations (0.5-1.0 mg/mL) in buffer solution if the samples are stirred at high speed (800 rpm). However, similar concentrations (1 and 2 mg/mL NZVI) are less toxic under shaking conditions.. NZVI is toxic only at higher concentration (10 mg/mL NZVI) when shake at a moderate 150 rpm. The toxicity of 10 mg/mL NZVI under shaking conditions is comparable to the toxicity of 0.5-1.0 mg/mL NZVI under stirring conditions.
2. The microbial toxicity of NZVI is dose dependent in buffer solution. Toxicity of NZVI particles towards microorganisms increases with increased in particle concentration.
3. The toxicity of NZVI may be bacterial species or strain specific. Some species or strains of bacteria are more resistant to the adverse affects of NZVI than others.

While it could reproduce most bacteria inactivation results reported by others, bacteria would not typically be in a nutrient deficient situation (like buffer or DI water). While the experiments done in buffer solution throws some light on possible mechanism of inactivation of bacteria by NZVI and results are non-conclusive. From the experiments in buffer solution it is, however, very apparent that NZVI is less toxic in environmentally relevant low speed shaking condition than in high speed stirring condition. High speed stirring condition is not expected in the environmental remediation. During this study, variability in toxicity was observed depending on the species or strain of microorganism used. However, more experiments were warranted to conclusively supporting that NZVI toxicity is bacterial species or strain specific. To

conduct such experiments in environmentally relevant conditions, it was decided to run rest of the experiments in nutrient solution such that microorganisms are not deprived of their basic growth nutrients. To further evaluate the growth characteristics of the bacteria in the presence of NZVI, studies may need to be conducted in the nutrient media at optimum temperature (for microbial growth) and followed by sub-optimal temperatures.

3.2 Effect of NZVI on Bacteria in Nutrient Media

3.2.1 Microbial growth in nutrient media under shaking condition

Growth studies were carried out for the three gram negative bacteria (*E. coli* 8739, *E. coli* JM109, and *P. putida* F1) by growing them in nutrient broth with different concentrations of NZVI (treatment reactors) and without NZVI (control). These experiments (as per method described in Section 2.5.2) were carried at at 37°C and 30°C which is the optimal temperature for *E. coli* and *P.putida* F1 growth. The gram negative bacteria were found to be not affected by the low NZVI concentration (1 and 2 mg/mL, Figures 3.5, 3.6, and 3.7). While the microbial growth (of all three species) in controls and in samples with 1 mg/mL NZVI followed almost the same pattern of growththe growth of *P. putida* F1 with 2 mg/mL NZVI was initially reduced but recovered back towards the end of the experiment. It was observed that there was no or little to the same level as the control reduction in growth for *E. coli* strains even with 2 mg/mL NZVI. Exposure of *P. putida* F1 and *E. coli* JM 109 to 5 and 10 mg/mL NZVI resulted in a significant reduction in bacterial numberrelative to the control (**Figures 3.5 and 3.6**). The population of *P. putida* F1 (**Figure 3.5**) was found to suffer a non-recoverable damage with 5 and 10 mg/mL NZVI while *E. coli* strains recovered over time (*E. coli* 8739, **Figure 3.7**) or maintained more or less the same populations (*E. coli*

JM109, **Figure 3.6**). Interestingly the two *E. coli* strains behaved differently under high NZVI concentrations (5 and 10 mg/mL) *E. coli* JM109 numbers decreased during the first 3h of exposure and recovered back to the initial cell concentration (**Figure 3.6**). In contrast *E. coli* 8739 not only recovered from the initial reduction in numbers during the first 2 h of treatment but recovered to the same levels as the control after 10 h (**Figure 3.7**). Sondi and Salopek-Sondi (2004) have also seen a growth delay in *E. coli* at higher concentrations of the Ag nanoparticles.

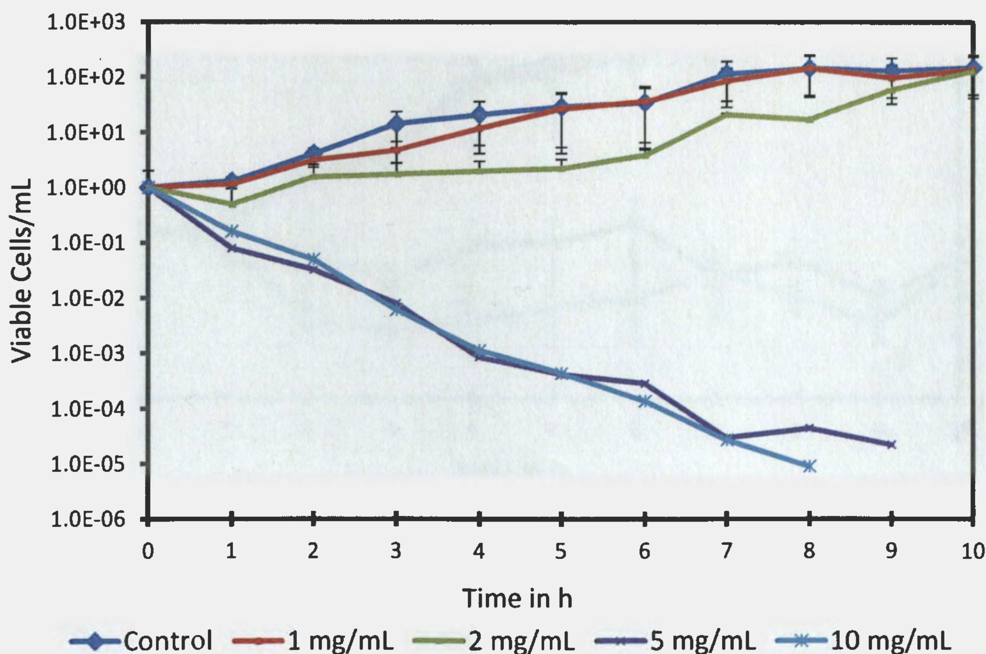


Figure 3.5: *Pseudomonas putida* F1 growth with NZVI in nutrient media under shaking condition.

Interpretation of the results from the growth studies in nutrient media indicates that growth of microorganisms (all three types) under optimal growth conditions (nutrient media, and mild shaking) is not affected when NZVI is present in low concentrations (1-2 mg/mL). The bacterium *E. coli* JM109 does not grow well and *Pseudomonas putida* F1 shows significant or total inactivation. While *E. coli* 8739 in

the presence of high concentrations (5-10 mg/mL) of NZVI did not show any signs of toxicity even with 10 mg/mLof NZVI consolidates the inference drawn from buffer studies that toxicity of NZVI may be bacterial species or strain specific, and some bacteria are more resistant to the adverse affects of NZVI than others. The characteristics specific to the strain of *E. coli*

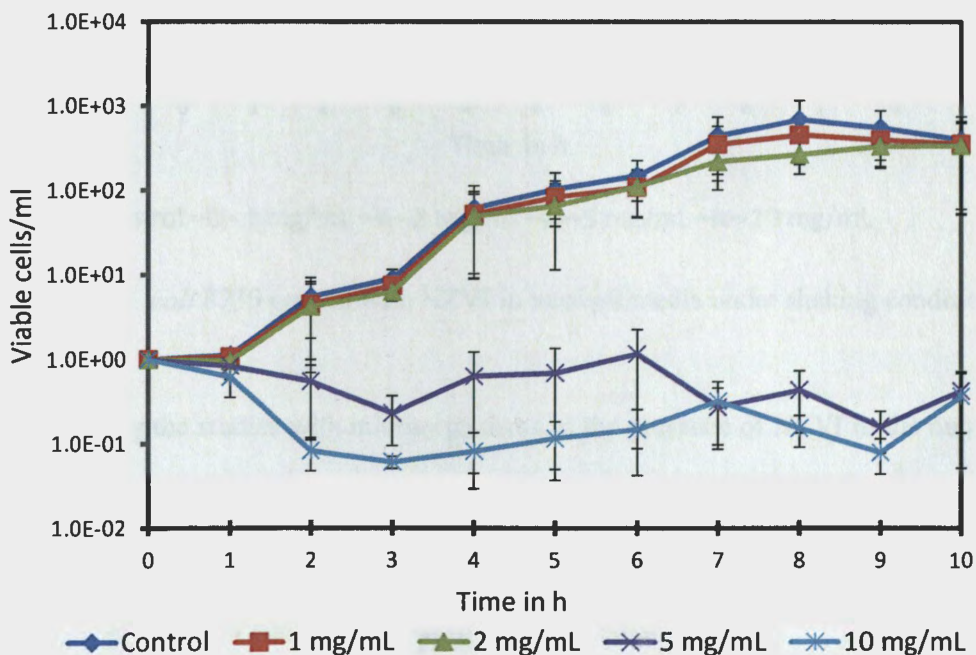


Figure 3.6: *E. coli* JM 109 growth with NZVI in nutrient media under shaking condition.

that allows the bacteria to resist NZVI toxicity are not apparent from the experiments so far conducted within this research.

Additional experiments were necessary to understand whether the recovery in growth of *E. coli* 8739 in 5 and 10 mg/mL NZVI (**Figure 3.7**) is due to a reduction in toxicity of NZV1 or some growth characteristics of the *E. coli* strain.

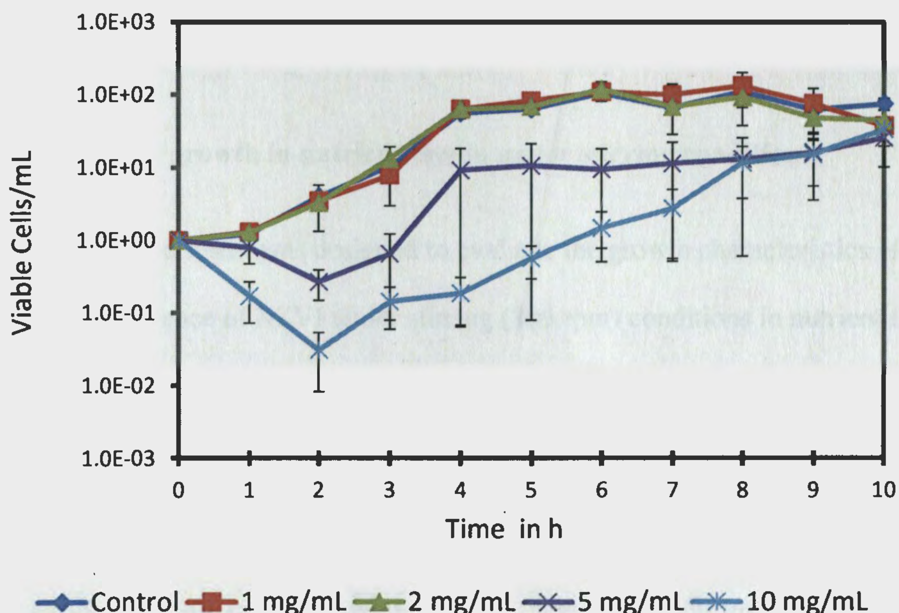


Figure 3.7: *E. coli* 8739 growth with NZVI in nutrient media under shaking condition.

During the studies with microorganisms in the presence of NZVI in the nutrient media a number of observations were made which include:

1. NZVI toxicity on microorganisms is particle concentration dependent, and higher NZVI concentrations showed increased toxicity.
2. NZVI toxicity is microbial species/strain specific. Different species/strains of microorganism behave differently in the presence of NZVI.
3. Some microorganisms have the ability to recover back from the initial toxicity affect of NZVI. This ability to recover from the initial shock may have to do with their growth. Possibly actively growing bacteria are less affected by NZVI.

The first two observations were established during NZVI-microorganism studies conducted in nutrient solution under shaking conditions (Section 3.2.1). It was necessary to see whether similar inferences can be drawn under stirring conditions

(Section 3.2.2). Further, the third observation needs additional investigation and separate experimental design would be needed to support the inference (Section 3.2.3).

3.2.2 Microbial growth in nutrient media under stirring condition

This experiment was designed to evaluate the growth characteristics of *E. coli* 8739 in the presence of NZVI under stirring (800 rpm) conditions in nutrient media at room temperature (22±2°C).

The microbial growth curve obtained for various NZVI concentrations are presented in **Figure 3.8**. At lower NZVI concentrations there was no marked difference in microbial growth in the samples (1 and 2 mg/mL NZVI) and the control (i.e., no NZVI). However, the microorganisms exposed to 5 and 10 mg/mL NZVI affected. With 5 mg/mL NZVI, *E. coli* 8739 did not show either any active growth or inactivation till ~4 h from the start of the experiment and then started actively growing until the end of the experiment (at 6 h). The microorganism suffered major inactivation with 10 mg/mL NZVI and there was recovery over time.

3.2.3 Microbial growth in nutrient media under shaking condition with intermittent exposure to additional NZVI

Active growth of *E. coli* 8739 in 10 mg/mL NZVI in nutrient solution under shaking conditions was intriguing because this is the typical dose of NZVI (10 g/L or 10 mg/mL) that is injected into the aquifers for groundwater remediation (Henn and Wadill, 2006; Li et al.,, 2006). As can be seen from **Figure 3.7**, *E. coli* 8739 recovers after initial 2 h of reduction in numbers and there is no difference in the final cell population for microorganisms exposed to different concentrations of NZVI and the control.

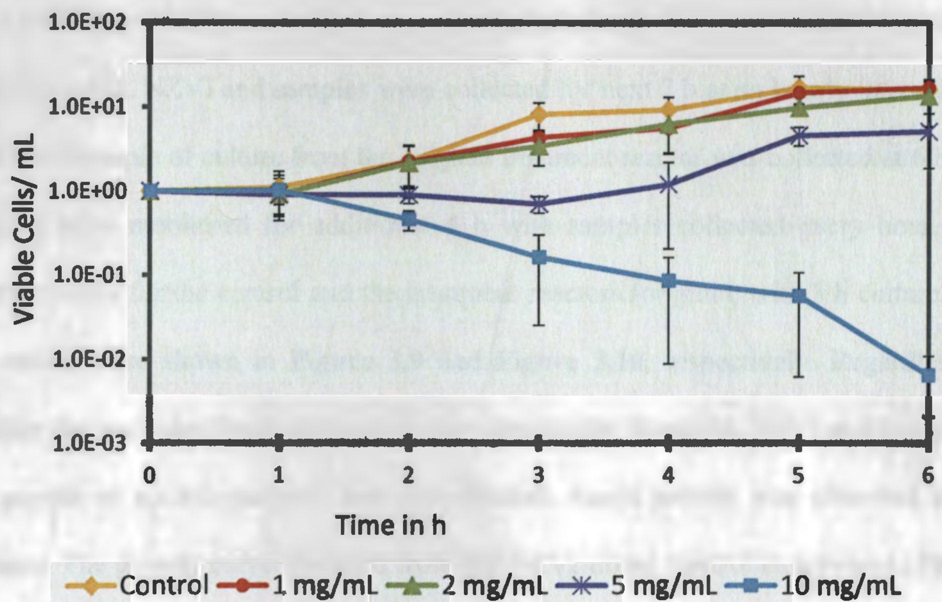


Figure 3.8: Growth of microorganism (*E. coli* 8739) at 22°C in nutrient media under stirring (800 rpm) condition.

There are three possibilities for such a setback behavior of *E. coli* 8739. (1) First, the nanoparticles were transformed from Fe^0 to a different (very stable) oxidation state within the first two hours and they were no longer toxic to the microorganisms; (2) Alternatively, the microorganisms might have developed resistanceand adapted to the new environment where nanoparticles would no longer affect them; (3) Finally, actively growing cells are resistant. An experiment was designed to determine whether growth of *E. coli* 8739 in 10 mg/mL NZVI is due to a reduction in toxicity of NZVI or the growth of the microorganism. The experiment was started as per procedure discussed in section 2.5.3 and samples were collected every hour. After collecting samples in 3 h, the control (no initial NZVI) was dosed with 10 mg/mL NZVI and sampled on an hourly basis thereafter. An additional sample of culture from the treatment reactor (with initial 10 mg/mL NZVI) was collected at 3h, the NZVI particles were removed, and the sample (with only the microorganism) was transferred to a new reactor (see Section

2.5.3 and **Figure 2.5**) for detailed experimental method). The new reactor was dosed with 10 mg/mL NZVI and samples were collected for next 7 h at an hourly interval. An additional sample of culture from the original treatment reactor was collected at 6 h and reactors were monitored for additional 4 h with samples collected every hour. The growth curves for the control and the treatment reactors (original, with 3 h culture, and 6 h culture) are shown in **Figure 3.9** and **Figure 3.10**, respectively. Regardless of whether the control or treatment reactor was dosed with 10 mg/mL NZVI at 3 h and 6 h, the growth of microorganisms was not affected. Rapid growth was observed in all reactors. The growth curve obtained from the NZVI dosed control experiment (**Figure 3.9**) was similar to one obtained from the control experiment without NZVI in nutrient media under shaking condition (**Figure 3.7**, Section 3.2.1). Further, the growth curve obtained from cultures collected at 3 h and 6 h (**Figure 3.10**) replicated the growth pattern of the control and the original treatment reactor.

Apparently fresh (reactive) NZVI could not stop or reduce the growth of *E. coli* 8739 once it the bacteria has grownin the media for3-6h. This indicates that the undisturbed growth after additional NZVI introduction is due to the characteristics of this strain of *E. coli* rather than some reduction in NZVI toxicity. Looking at the plots it can be inferred that the microorganisms were in an active growth phase when additional NZVI particles (10 mg/mL) were introduced. This observation paves the path to speculate that the possibility that when microorganisms are in an active growth phase, bacteria are resistant to the effects on NZVI. Non-replicating or minimally multiplying bacteria are more susceptible to NZVI toxicity as can seen in the initial period of this study and with the microorganisms grown in buffer solution.

3.2.4 Microbial growth at sub-optimal temperatures in nutrient media under shaking condition

The goal of this experiment was to verify the postulation made in the previous section (Section 3.2.1) that actively growing bacteria are not affected by NZVI. The experiment was designed in such a way that the active growth of *E. coli* 8739 can either be reduced or stopped. The optimal growth temperature for *E. coli* 8739 is 37°C. While experiments done at 37°C would result in maximum growth of bacteria, experiments at 22(±2) and 4(±2)°C would be expected to reduce and stop bacterial growth, respectively. Four concentrations of NZVI (1, 2, 5, and 10 mg/mL) were used during this study and the results are presented in **Figures 3.11 and 3.12.**

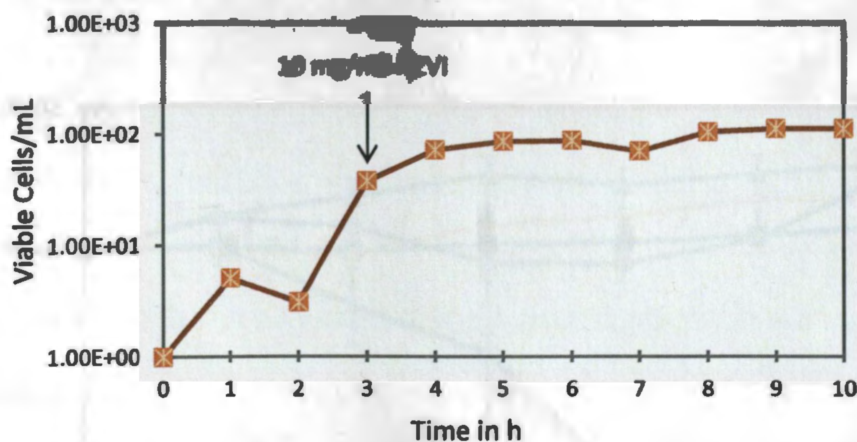


Figure 3.9: Growth of microorganism (*E. coli* 8739) in the control unit (with no initial NZVI). Introduction of 10 mg/mL of NZVI at 3 h did not affect the growth pattern of the microorganisms

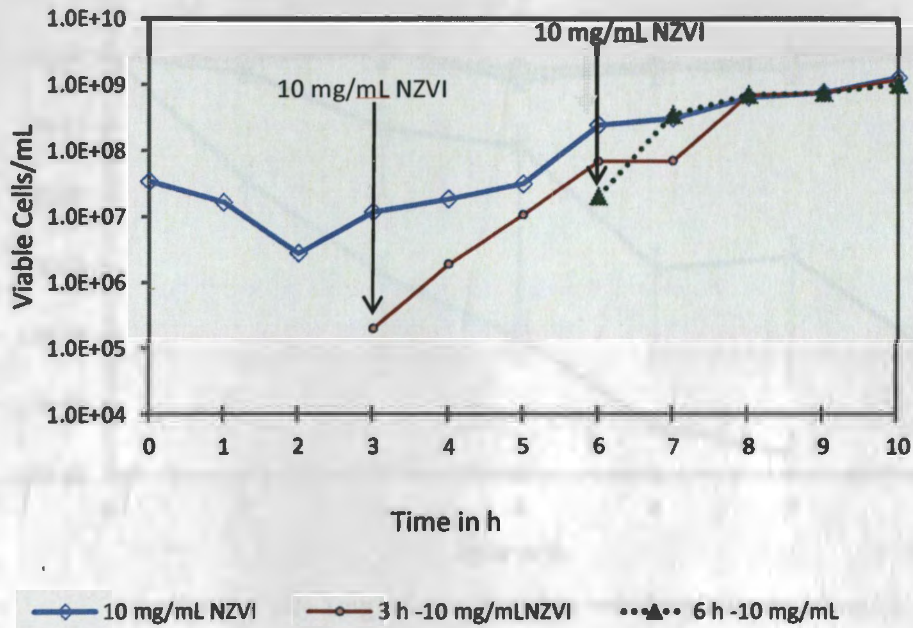


Figure 3.10: Growth of microorganism (*E. coli* 8739) in the treatment reactor (with 10 mg/mL initial NZVI). Introduction of 10 mg/mL of NZVI at 3 h and again at 6 h did not affect the growth pattern of the microorganisms.

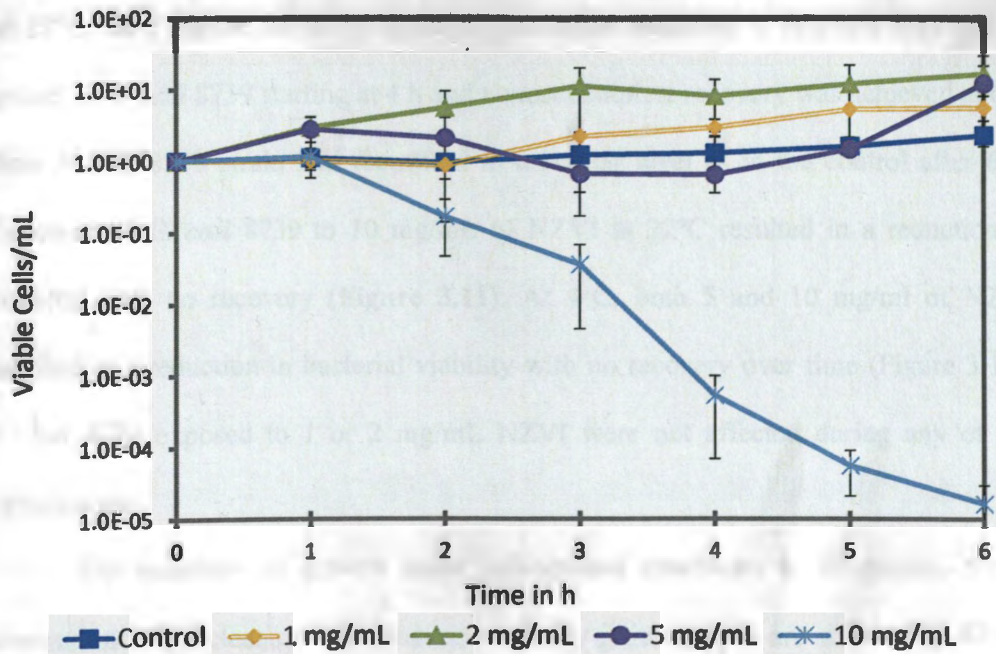


Figure 3.11: Growth of microorganism (*E. coli* 8739) at sub-optimal 22°C in nutrient media under shaking (150 rpm) condition.

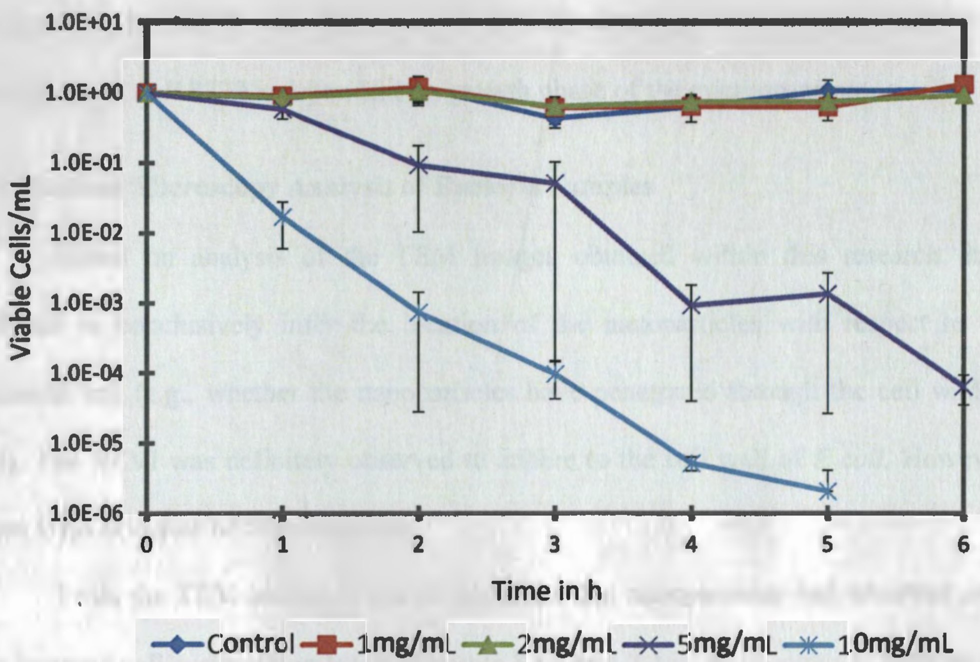


Figure 3.12: Growth of microorganism (*E. coli* 8739) at sub-optimal 4°C in nutrient media under shaking (150 rpm) condition.

At 22°C, the 5 mg/mL of NZVI resulted in an initial reduction in numbers followed by growth of *E. coli* 8739 starting at 4 h and almost complete recovery was achieved at 6 h. Here *E. coli* 8739 strain had recovered to a similar level as in the control after 6 h. Exposure of *E. coli* 8739 to 10 mg/mL of NZVI at 22°C resulted in a reduction in viability with no recovery (**Figure 3.11**). At 4°C, both 5 and 10 mg/ml of NZVI resulted in a reduction in bacterial viability with no recovery over time (Figure 3.12). *E. coli* 8739 exposed to 1 or 2 mg/mL NZVI were not affected during any of the experiments.

The reduction in growth under sub-optimal conditions at 10 mg/mL NZVI concentration is a clear indicator that non-actively growing cells are affected by NZVI. The reduction in growth at lower temperatures corresponded with an increased

susceptibility to NZVI when they are not actively dividing. This indicates that NZVI toxicity on *E. coli* 8739 is dependent on growth phase of the microorganism.

3.3 Electron Microscopy Analysis of Bacteria Samples

Based on analysis of the TEM images obtained within this research, it is difficult to conclusively infer the location of the nanoparticles with respect to the bacterial cell (e.g., whether the nanoparticles have penetrated through the cell wall or not). The NZVI was definitely observed to adhere to the cell wall of *E coli.* However, there is no evidence of cell disruption.

From the TEM images it can be observed that nanoparticles had adsorbed onto the bacterial cell surface/membrane (**Figures 3.13 and 3.14**), while some of them might have penetrated into the cells (**Figures 3.13 and 3.15**). Smaller particles might have entered into the cell and some of them got deposited between the outer cell and cytoplasm. In some of the images (e.g., **Figures 3.13** and **3.16**), the NZVI particles appear to have gone inside the cytoplasm but that could not be conclusively proved or disproved. Other researchers (Li et. al., 2010; Williams et. al., 2006) have claimed that NZVI particles have entered the cell but their conclusions were drawn based on similar images as within this research. Looking at **Figure 3.17**, one can see the bulging of the cell wall possibly because of internalization of NZVI. It appears from the image that NZVI was deposited in a space between the cell wall and cytoplasm. However, there is no explanation on the mechanism how they penetrated into the cell wall. There are not enough data to interpret the TEM properly. Further, stains used in sample preparation makes it difficult to locate NZVI in and around the cells. Samples were usually stained by heavy metal-based (osmium tetraoxide) and organic (glutaraldehyde) compounds to improve the contrast. The osmium tetraoxide stain may interfere in locating the NZVI particles within the cell or outside. There is hardly any difference between TEM images

of controls (no NZVI, **Figure 3.18**) and those where NZVI particles are apparently present. Also, there is no adequate control over the sectioning of cells and nanoparticles may find their way into the cells during that process. William et al., (2006) have reported specific interactions between the nanoparticles and membrane based TEM images. They believed that NZVI particles interact with bacterial cell surface expressed proteins. Based on TEM images, Lee et al., (2008) have stated that there was physical disruption of the membrane in the presence of NZVI and that caused the biocidal effects on the bacteria. Based on analysis of TEM images from this research such inferences could not be arrived at.

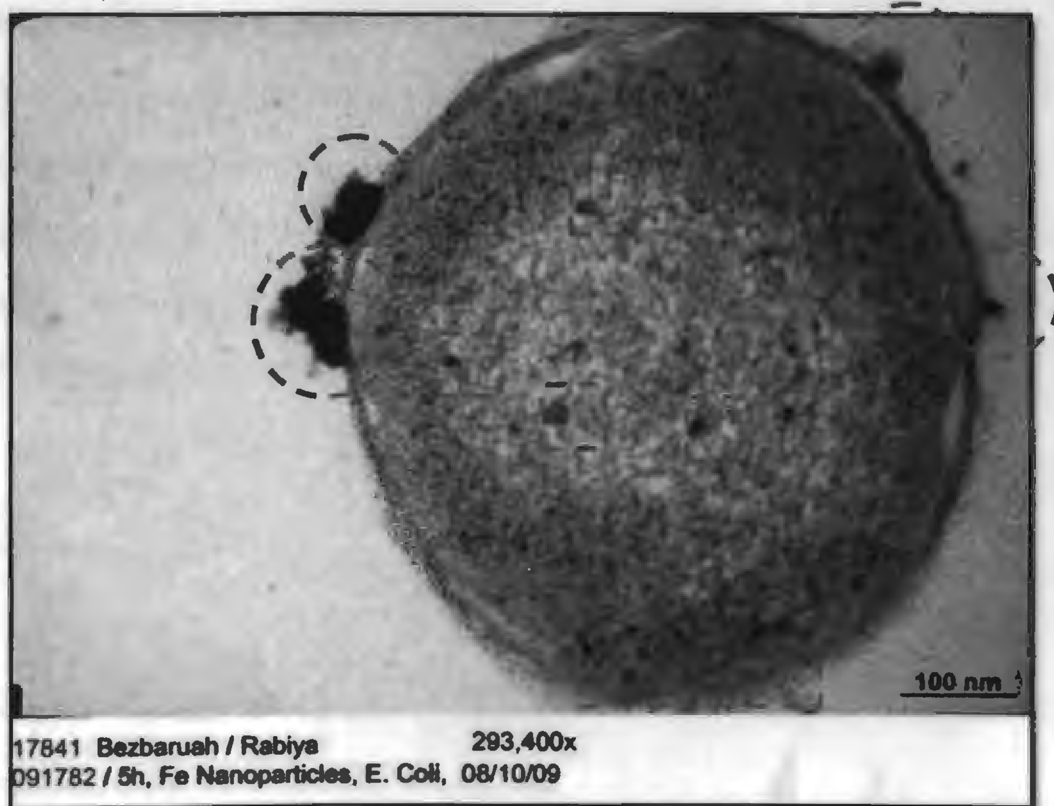


Figure 3.13: TEM image that indicate that nanoparticles (circled) had adsorbed onto the cell surface. However, there are some NZVI particles inside the cell as well (enclosed by squares). The NZVI particles inside the cell might have penetrated into it or showing up in image because of imperfect sectioning of the cell. Another image is shown in Figure 3.14.

This study has demonstrated that there is toxic effect on all the three types of bacteria grown in nutrient media at lower NZVI concentrations. At higher NZVI concentrations the effects were strain specific. The results from the effects in buffer indicate the dose dependent activity and faster inactivation as the cells remain inactive without nutrients. Further, with nutrients the cells tend to grow and show either susceptibility or resistance towards the NZVI. In the *E. coli* 8739 interactions, it was observed that actively growing bacteria are not affected by NZVI, i.e., Non-dividing cells/ bacteria are more susceptible to NZVI toxicity at higher concentrations (5, 10 mg/mL). The effect of NZVI on bacteria is prevented when bacteria were in the nutrient broth, but *E. coli* 8739 shows a significant growth in presence of NZVI(10 mg/mL). The redosing result indicates that the NZVI effects may depend on the characteristics of the strain. In the TEM images, NZVI particles were seen in the cytoplasm, though it is not clear how the NZVI have entered the cells (**Figure 3.16 and 3.17**). Finally, this study shows that NZVI has no effects on the specific strain at optimal growth conditions, but shows bactericidal effects in a buffer solution.

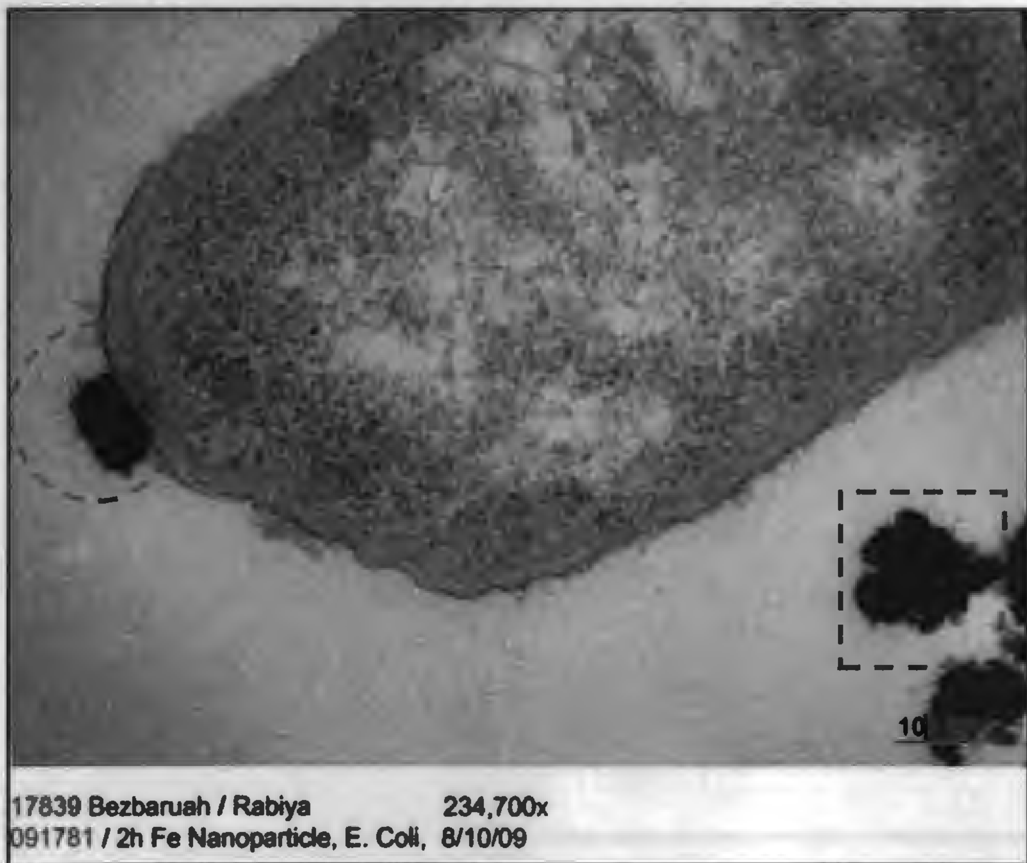


Figure 3.14: TEM image showing NZVI particles deposition on the cell surface. The circled nanoparticle seems to be ~100 nm in the longest visible direction. The image within the square is an NZVI particles or a cluster of NZVI particles attached to the bacterial cell.

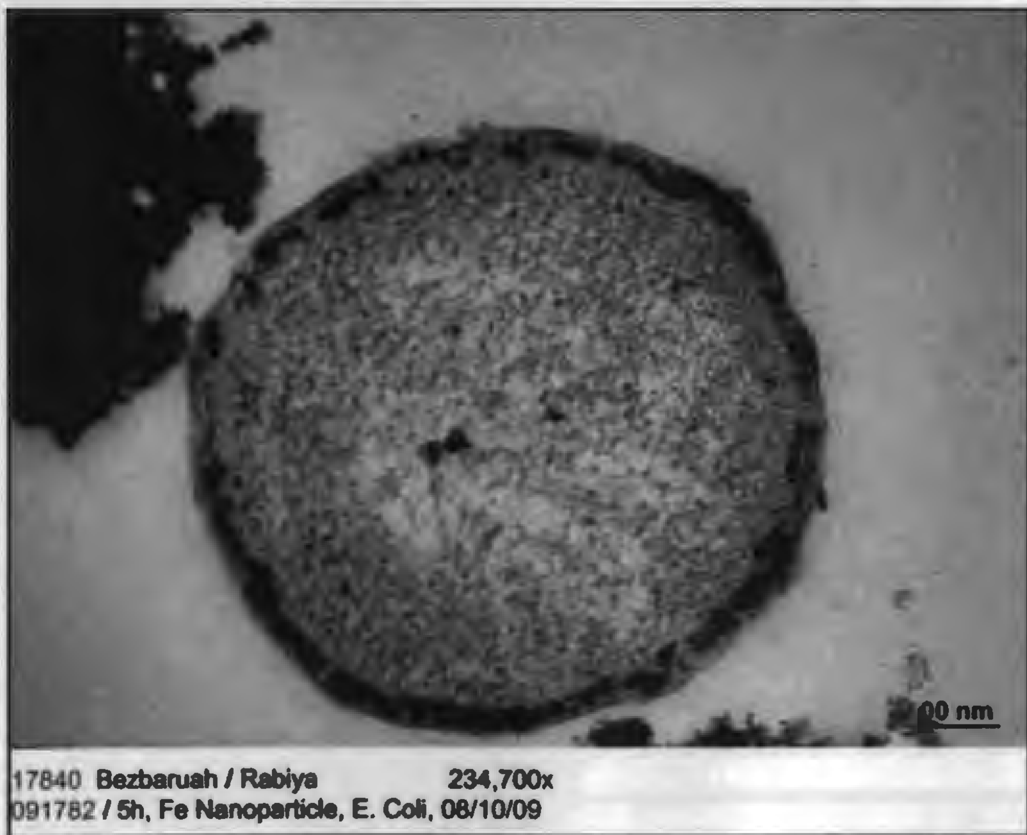


Figure 3.15: NZVI particles appear to have penetrated through the cell membrane into the cell in this TEM image. Also see Figure 3.16.

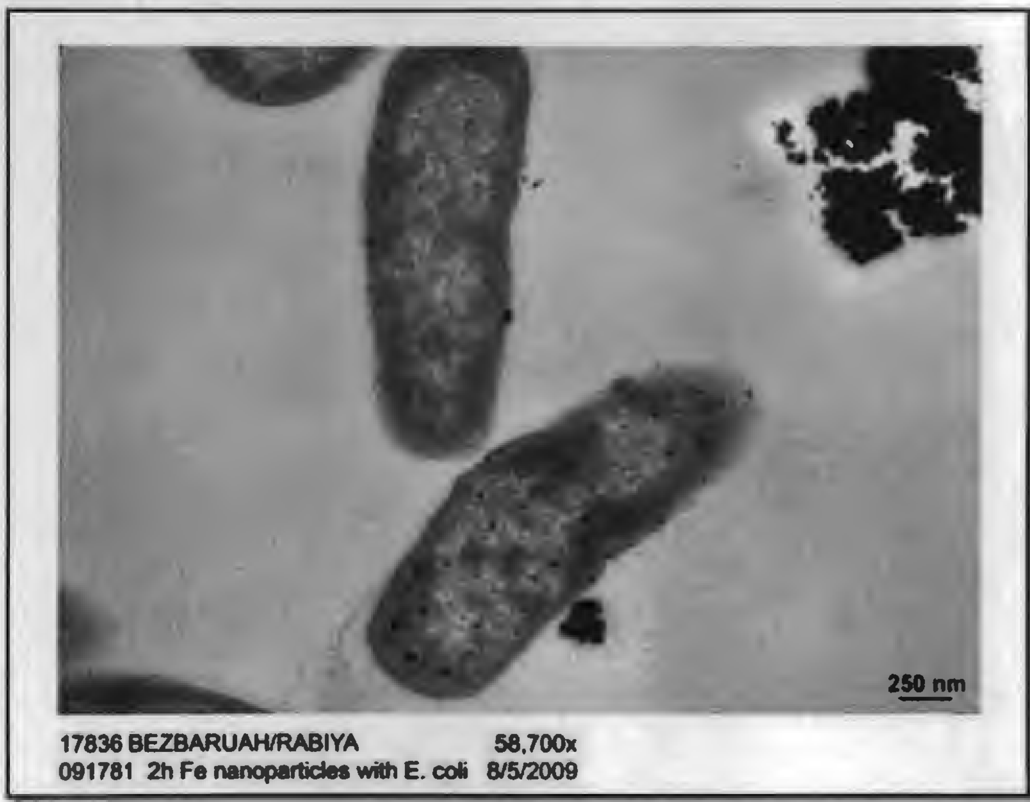


Figure 3.16: TEM images do not give clear idea about the location of NZVI. While the cell on the bottom shows that particles are inside. However, these are two dimensional sections and it is difficult to infer whether the particles are outside or inside the cells.

Figure 3.17: Magnified TEM image of a part of an *E. coli* cell. There is a prominent bulge (circled) which indicates that NZVI might have penetrated the cell membrane and got deposited between the cell wall and the cytoplasm

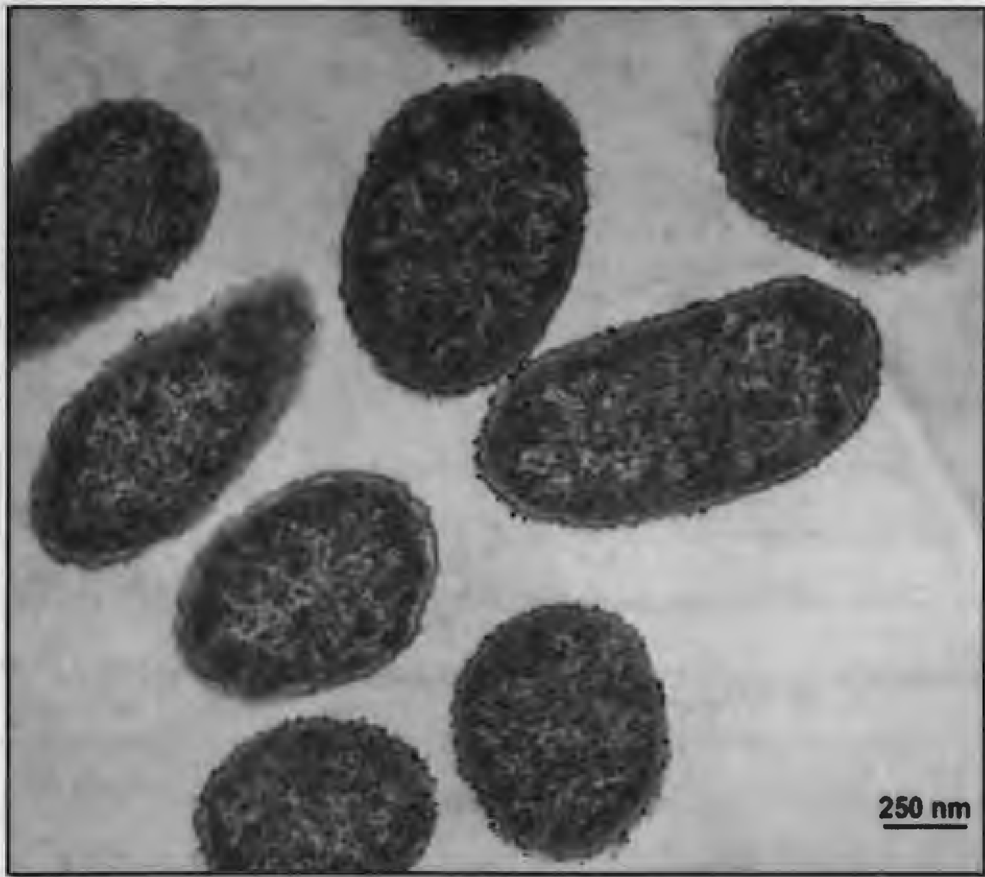


Figure 3.18: TEM image *E. coli* cells collected from a control (no NZVI) unit. There is no distinct difference between the TEM images of the *E. coli* cells where there was no NZVI with the TEM images of cells which were exposed to NZVI particles.

CHAPTER 4. CONCLUSIONS AND FUTURE WORK

4.1 Conclusions

This research investigated nanoscale zerovalent iron (NZVI) and microorganism interactions in environmentally relevant conditions. Microorganisms were exposed to various concentrations of NZVI in buffer solutions and nutrient media under stirring and shaking conditions.

NZVI (0.09- 1.0 mg/mL) showed bactericidal effects on all the three strains (*E. coli* 8739, JM 109, and P. *putida* F1) in buffer solution under stirring (800 rpm) condition. The bacterial cells showed better viability in a buffer solution when stirring (800 rpm) was replaced by shaking (150 rpm). Stirring condition does not represent any real environmental situation while shaking possibly is the closest representation of real world situation. So, it can be concluded that microorganism may survive for some time when exposed to NZVI under nutrient deficient (buffer solution) conditions.

The growth studies for *Pseudomonas putida* F1, *E. coli* JM 109 and *E. coli* 8739 in nutrient media were carried out with different NZVI concentrations (1, 2, 5, 10 mg/mL). All the three strains of bacteria have shown no effect of NZVI with lower NZVI concentrations (1 and 2 mg/mL). In *P. putida* F1 and *E. coli* JM109 were inactivated and shown limited growth, respectively with 5 mg /mL NZVI. However, *E. coli* 8739 shows a remarkable recovery from initial setback (at both 5 and 10 mg/mL NZVI). It can be concluded that NZVI toxicity on microorganisms is dose dependent and microbial species or strain specific. When *E. coli* 8739 was intermittently dosed with 10 mg/mL NZVI, it was observed that NZVI has no effect on microbial growth after certain time lapse. Experiments conducted at sub-optimal temperatures (22°C and 4°C) to study the growth of *E. coli* 8739 indicated that susceptibility of *E. coli* to NZVI

was dependent on the phase of the cell growth. The actively growing (or dividing) cells are not affected by NZVI toxicity.

TEM micrographs could not conclusively show how NZVI interacted with the microorganisms. Most of the images indicated that NZVI particles are deposited on the bacteria (i.e. outside the cell wall). While it appears that some NZVI entered through the cell wall with no disruption of the cell wall observed. In some images, it appears that NZVI has penetrated through the cell wall and got deposited in between cell wall and the cytoplasm. More work is needed to understand how NZVI interactswith bacteria.

Based on this research the following can be concluded:

1. NZVI-microorganism interaction studies should be conducted in nutrient solution such that microorganisms are not deprived of the essential growth nutrients.
2. Toxicity of NZVI on microorganism is NZVI concentration dependent. Higher NZVI concentrations are more toxic to microorganisms.
3. Toxicity of NZVI on microorganisms is microbial strain or species specific.
4. Microorganisms are not affected too adversely by NZVI, if they are in their active growth (or dividing) phase. Microorganisms in their lag phase of growth (i.e. non-dividing) cells are adversely affected by NZVI.

4.2 Future Work

To fully understand the interactions of NZVI- microorganism, further work need to be conducted. Following are a few areas where future research is needed.

1) When bacterial strains were exposed to NZVI, there is generation of oxidants which causes the toxicity or cell damage. In order to gain an insight to this mechanism an oxidative stress test/assay can be performed.

2) Better TEM images are needed to understand the possible mechanism how the nanoparticles have penetrated inside the cell and the interactionsbetween the bacteria and NZVI. The use of high-resolution transmission electronmicroscopy (HRTEM) and high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM) may help in this process. Avoiding the use of heavy metals based compounds such as OsO_4 for staining will give a clear visualization of the bacteria-NZVI images without any background interference.
3) There is a need for sstudy to see whether there isis any adaptation in the strain *E. coli* 8739 in the presence of NZVI or if there is any mutations taking place.
4) Study the interactions of the gram positive bacteria with NZVI. Gram-positive bacteria lack the outer membrane but have a peptidoglycan layer and as such it may behave quite differently than the gram-negative bacteria.

REFERENCES

Aitken, R.J., Chaudhry, M.Q., Boxall, A.B.A., Hull, M. (2006) Manufacture and use of nanomaterials: current status in the UK and global trends. *Occup. Med.* 56: 300-306.

Akerman, M.E., Chan, W.C.W., Laakkonen, P., Bhatia, S.N., Ruoslahti, E. (2002) Nanocrystal targeting in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 99: 12617-12621.

Alowitz, M.J., Scherer, M.M. (2002) Kinetics of Nitrate, Nitrite, and Cr(VI) Reduction by Iron Metal. *Environ. Sci. Technol.* 36: 299-306.

ASTM International (2006) Terminology for Nanotechnology (2006) ASTM E 2456-06 Available at http://www.astm.org. Accessed on November 4, 2010.

Auffan, M., Rose, J., Bottero, J.-Y., Lowry Gregory, V., Jolivet, J.-P., Wiesner Mark, R. (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. *Nat. Nanotechnol.* 4: 634-641.

Auffan, M., Achouak, W., Rose, J., Roncato, M.-A., Chaneac, C., Waite, D.T., Maison, A., Woicik, J.C.,Wiesner, M.R.,Bottero, J.-Y. (2008) Relation between the Redox State of Iron-Based Nanoparticles and Their Cytotoxicity toward Escherichia coli. *Environ. Sci. Technol.* 42: 6730-6735.

Baer, D.R., Amonette, J.E., Engelhard, M.H., Gaspar, D.J., Karakoti, A.S., Kuchibhatla, S. Nachimuthu, P., Nurmi, J. T., Qiang, Y., Sarathy, V.,Seal, S.,Sharma, A.,Tratnyek, P. G.,Wang, C. M. (2008) Characterization challenges for nanomaterials. *Surf. Interface Anal.* 40: 529-537.

Balamurugan, A., Sockalingum, G., Michel, J., Faure, J., Banchet, V., Wortham, L. Bouthors, S., Laurent-Maquin, D., Balossier, G. (2006) Synthesis and characterization of sol gel derived bioactive glass for biomedical applications. *Mater. Lett.* 60: 3752-3757.

Balbus John, M., Florini, K., Denison Richard, A., Walsh Scott, A. (2006) Getting it right the first time: developing nanotechnology while protecting workers, public health, and the environment. *Ann. N Y Acad. Sci.* 1076: 331-342.

Battin, T.J., von der Kammer, F., Weilhartner, A., Ottofuelling, S., Hofmann, T. (2009) Nanostructured TiO_2: Transport Behavior and Effects on Aquatic Microbial Communities under Environmental Conditions. *Environ. Sci. Technol.* 43: 8098-8104.

Bezbaruah Achintya, N., Thompson Jay, M., Chisholm Bret, J. (2009 a) Remediation of alachlor and atrazine contaminated water with zero-valent iron nanoparticles. *J Environ Sci Health B* 44: 518-524.

Bezbaruah, A.N., Krajangpan, S., Chisholm, B.J., Khan, E., Elorza Bermudez, J.J. (2009 b) Entrapment of iron nanoparticles in calcium alginate beads for groundwater remediation applications. *J. Hazard. Mater.* 166: 1339-1343.

Bianco, A., Prato, M. (2003) Can carbon nanotubes be considered useful tools for biological applications? *Adv. Mater.* 15: 1765-1768.

Biswas, P., Wu, C.-Y. (2005) Nanoparticles and the environment. *J. Air Waste Manage.* 55(6): 708-746.

Blowes, D.W., Ptacek, C.J., Jambor, J.L. (1997) In-Situ Remediation of Cr(VI)-Contaminated Groundwater Using Permeable Reactive Walls: Laboratory Studies. *Environ. Sci. Technol.* 31: 3348-3357.

Bosetti, M., Masse, A., Tobin, E., Cannas, M. (2001) Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. *Biomaterials* 23: 887-892.

Bosi, S., Da Ros, T., Spalluto, G., Prato, M. (2003) Fullerene derivatives: an attractive tool for biological applications. *Eur. J. Med. Chem.* 38: 913-923.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M.F., Fievet, F. (2006) Toxicological Impact Studies Based on Escherichia coli Bacteria in Ultrafine ZnO Nanoparticles Colloidal Medium. *Nano Lett.* 6: 866-870.

Buzea, C., Pacheco Ivan, I., Robbie, K. (2007) Nanomaterials and nanoparticles: sources and toxicity. *Biointerphases* 2: MR17-71.

Calvo, P., Gouritin, B., Brigger, I., Lasmezas, C., Deslys, J., Williams, A. Andreux, J. P.Dormont, D. Couvreur, P . (2001) PEGylated polycyanoacrylate nanoparticles as vector for drug delivery in prion diseases. *J Neurosci Methods* 111: 151-155.

Cao, J., Elliott, D., Zhang, W.-x. (2005) Perchlorate reduction by nanoscale iron particles. *J. Nanopart. Res.* 7: 499-506.

Chanana, M., Mao, Z., Wang, D. (2009) Using polymers to make up magnetic nanoparticles for biomedicine. *J. Biomed. Nanotechnol.*, 652-668.

Chen, Y., Dalwadi, G., Benson, H.A.E. (2004) Drug delivery across the blood-brain barrier. *Curr. Drug Delivery* 1: 361-376.

Daniel.M.C., Astruc.D (2004) Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev*: 293-346.

Diao, M., Yao, M. (2009) Use of zero-valent iron nanoparticles in inactivating microbes. In *Water Res.*, 5243-5251.

Elliott, D.W., Zhang, W.-x. (2001) Field Assessment of Nanoscale Bimetallic Particles for Groundwater Treatment. *Environ. Sci. Technol.* 35: 4922-4926.

Elliott, D.W., Zhang, W. (2003) A field assessment of nanoscale bimetallic particles for groundwater treatment. *Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003*: IEC-130.

Farrell, J., Wang, J., O'Day, P., Conklin, M. (2001) Electrochemical and Spectroscopic Study of Arsenate Removal from Water Using Zero-Valent Iron Media. *Environ. Sci. Technol.* 35: 2026-2032.

Gavaskar, A., Tatar, L., Condit, W (2005) Cost and performance report nanoscale zero-valent iron technologies for source remediation. Port Hueneme, CA: Naval Facilities Engineering Command.

Grieger, K.D., Fjordbøge, A., Hartmann, N.B., Eriksson, E., Bjerg, P.L., Baun, A. (2010) Environmental benefits and risks of zero-valent iron nanoparticles (nZVI) for in situ remediation: Risk mitigation or trade-off? *J. Conta. Hydro.* 118: 165-183.

Harris, T.J., Von Maltzahn, G., Derfus, A.M., Ruoslahti, E., Bhatia, S.N. (2006) Proteolytic actuation of nanoparticle self-assembly. *Angew. Chem., Int. Ed.* 45: 3161-3165.

He, F., Zhao, D. (2005) Preparation and Characterization of a New Class of Starch-Stabilized Bimetallic Nanoparticles for Degradation of Chlorinated Hydrocarbons in Water. *Environ. Sci. Technol.* 39: 3314-3320.

He, Y.T., Wan, J., Tokunaga, T. (2008) Kinetic stability of hematite nanoparticles: the effect of particle sizes. *J. Nanopart. Res.* 10: 321-332.

Henn, K.W., Waddill, D.W. (2006) Utilization of Nanoscale Zero-Valent Iron for Source Remediation—A Case Study. 57-77.

Hu, X., Cook, S., Wang, P., Hwang, H.-m. (2009) In vitro evaluation of cytotoxicity of engineered metal oxide nanoparticles. *Sci. Total Environ.* 407: 3070-3072.

Jin, J.L., R. Wang, H. Chen, H. Liang, K., Ma, J. (2007) Magnetic Fe nanoparticle functionalized water-soluble multi-walled carbon nanotubules:Towards the preparation of sorbent for aromatic compounds removal. *Chem.Commun.* 4: 386-388.

Johnston, B.D., Scown, T.M., Moger, J., Cumberland, S.A., Baalousha, M., Linge, K. Jarvis,R., Lead,K., Tyler.J.R., Charles R et al. Bioavailability of Nanoscale Metal Oxides TiO2, CeO2, and ZnO to Fish. *Environ. Sci. Technol.* 44: 1144-1151.

Joo, S.H., Zhao, D. (2008) Destruction of lindane and atrazine using stabilized iron nanoparticles under aerobic and anaerobic conditions: Effects of catalyst and stabilizer. *Chemosphere*, 418-425.

Ju-Nam, Y.,Lead Jamie, R. (2008) Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. *Sci. Total Environ.* 400: 396-414.

Karn, B., Kuiken, T., Otto, M. (2009) Nanotechnology and in situ remediation: a review of the benefits and potential risks. *Environ Health Perspect* 117: 1813-1831.

Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y. Shaily. M., Michael M.J., Jamie R.L. (2008) Nanomaterials in the environment: behavior, fate, bioavailability, and effects. *Environ. Toxicol. Chem.* 27: 1825-1851.

Krajangpan, S., Jarabek, L., Jepperson, J., Chisholm, B.J., Bezbaruah, A. (2008) Polymer modified iron nanoparticles for environmental remediation. *Abstracts of Papers, 235th ACS National Meeting, New Orleans, LA, United States, April 6-10, 2008*, POLY-520.

Kumar, A., Pushparaj, V.L., Murugesan, S., Viswanathan, G., Nalamasu, R., Linhardt, R.J., Omkaram. N., Pulickel A.M. (2006) Synthesis of Silica-Gold Nanocomposites and Their Porous Nanoparticles by an In-Situ Approach. *Langmuir* 22: 8631-8634.

Laosiripojana.N., Sutthisripok.N., Assabumrungrat.S . Synthesis gas production from dry reforming of methane over CeO2 doped Ni/Al2O3: influence of the doping ceria on the resistance toward carbon formation. *Chem Eng J* 112: 13-22.

Lee, C., Kim, J.Y., Lee, W.I., Nelson, K.L., Yoon, J., Sedlak, D.L. (2008) Bactericidal Effect of Zero-Valent Iron Nanoparticles on Escherichia coli. *Environ. Sci. Technol.* 42: 4927-4933.

Li, X.Q., Elliott, D.W., Zhang, W.-x. (2006a) Zero-Valent Iron Nanoparticles for Abatement of Environmental Pollutants: Materials and Engineering Aspects. *Critical Reviews in Solid State and Materials Sciences* 31: 111-122.

Li, X.Q., Brown, D.G., Zhang, W.-x. (2007) Stabilization of biosolids with nanoscale zero-valent iron (nZVI). *J. Nanopart. Res.* 9: 233-243.

Li, Y., Leung, P., Yao, L., Song, Q.W., Newton, E. (2006b) Antimicrobial effect of surgical masks coated with nanoparticles. *J Hosp Infect* 62: 58-63.

Li, Z., Greden, K., Alvarez Pedro, J.J., Gregory Kelvin, B., Lowry Gregory, V. (2010) Adsorbed polymer and NOM limits adhesion and toxicity of nano scale zerovalent iron to E. coli. *Environ. Sci. Technol.* 44: 3462-3467.

Liu, W.-T. (2006) Nanoparticles and their biological and environmental applications. *J. Biosci. Bioeng.* 102: 1-7.

Liu, Y., Lowry, G.V. (2006) Effect of Particle Age (Fe0 Content) and Solution pH On NZVI Reactivity: H_2 Evolution and TCE Dechlorination. *Environ. Sci. Technol.* 40: 6085-6090.

Liu, Y., Phenrat, T., Lowry, G.V. (2007) Effect of TCE Concentration and Dissolved Groundwater Solutes on NZVI-Promoted TCE Dechlorination and H_2 Evolution. *Environ. Sci. Technol.* 41: 7881-7887.

Liu, Y., Majetich, S.A., Tilton, R.D., Sholl, D.S., Lowry, G.V. (2005) TCE Dechlorination Rates, Pathways, and Efficiency of Nanoscale Iron Particles with Different Properties. *Environ. Sci. Technol.* 39: 1338-1345.

Lovern, S.B., Strickler, J.R., Klaper, R. (2007) Behavioral and Physiological Changes in Daphnia magna when Exposed to Nanoparticle Suspensions (Titanium Dioxide, Nano-C60, and C60HxC70Hx). *Environ. Sci. Technol.* 41: 4465-4470.

Markarian.J (2006) Steady growth for biocides. *Plast Addit Compd* 8: 30-33.

Martin, J.E., Herzing, A.A., Yan, W., Li, X.-q., Koel, B.E., Kiely, C.J., Zhang, W.-x. (2008) Determination of the Oxide Layer Thickness in Core-Shell Zerovalent Iron Nanoparticles. *Langmuir* 24: 4329-4334.

Masciangioli, T.M., Zhang, W. (2003) Environmental technologies at the nanoscale. In *Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003*, IEC-002.

Matheson, L.J., Tratnyek, P.G. (1994) Reductive Dehalogenation of Chlorinated Methanes by Iron Metal. *Environ. Sci. Technol.* 28: 2045-2053.

Mauter, M.S.,Elimelech, M. (2008) Environmental Applications of Carbon-Based Nanomaterials. *Environ. Sci. Technol.* 42: 5843-5859.

Morones, J.R., Elechiguerra, J.L., Camacho, A., Holt, K., Kouri, J.B., Ramirez, J.T., Yacaman, M.J. (2005) The bactericidal effect of silver nanoparticles. *Nanotechnology* 16: 2346-2353.

Murphy, C.J., Gole, A.M., Hunyadi, S.E., Stone, J.W., Sisco, P.N., Alkilany, A., Brian K.E., Patrick, H. (2008) Chemical sensing and imaging with metallic nanorods. *Chem. Commun.*: 544-557.

Nair, R., Varghese, S.H., Nair, B.G., Maekawa, T., Yoshida, Y., Kumar, D.S. Nanoparticulate material delivery to plants. *Plant Sci.* 179: 154-163.

Neal, A.L. (2008) What can be inferred from bacterium-nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? *Ecotoxicology* 17: 362-371.

Nel, A., Xia, T., Maedler, L.,Li, N. (2006) Toxic Potential of Materials at the Nanolevel. *Science* 311: 622-627.

NIOSH (2009) Approaches to Safe Nanotechnology. Managing the Health and Safety Concerns Associated with Engineered Nanomaterials. In. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.http://www.cdc.gov/niosh/docs/2009-125/Accessed on December 1, 2010.

Nowack,B., Buchcli,T.D (2007) Occurrence, behaviour and effects of nanoparticles in the environment. *Environ Pollut* 150: 5-22.

Nurmi, J.T., Tratnyek, P.G., Sarathy, V., Baer, D.R., Amonette, J.E., Pecher, K., Wang, Chongmin. W., John L.C.,Dean M.W., Lee P.R., Michelle.D.D. (2005) Characterization and Properties of Metallic Iron Nanoparticles: Spectroscopy, Electrochemistry, and Kinetics. *Environ. Sci. Technol.* 39: 1221-1230.

Pal, S., Tak, Y.K., Song, J.M. (2007) Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. *Appl. Environ. Microbiol.* 73: 1712-1720.

Panacek, A., Kvitek, L., Prucek, R., Kolar, M., Vecerova, R., Pizurova, N., Virender,S. K., Tat'jana, N., Radek, Z . (2006) Silver Colloid Nanoparticles: Synthesis, Characterization, and Their Antibacterial Activity. *J. Phys. Chem. B* 110: 16248-16253.

Parales, R.E., Ditty, J.L., Harwood, C.S. (2000) Toluene-Degrading Bacteria Are Chemotactic towards the Environmental Pollutants Benzene, Toluene, and Trichloroethylene. *App. Enviro. Microbio.* 66: 4098-4104.

Pena, M.E., Korfiatis, G.P., Patel, M., Lippincott, L., Meng, X. (2005) Adsorption of As(V) and As(III) by nanocrystalline titanium dioxide. *Water Res.* 39: 2327-2337.

Peng, X.-H., Qian, X., Mao, H., Wang, A.Y., Chen, Z., Nie, S., Shin, D.M. (2008) Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy. *Int. J. Nanomed.* 3: 311-321.

Phenrat, T., Saleh, N., Sirk, K., Tilton, R., Lowry, G.V. (2007) Aggregation and Sedimentation of Aqueous Nanoiron Dispersions. *Environ. Sci. Technol.* 41: 284-290.

Ponder, S.M., Darab, J.G., Mallouk, T.E. (2000) Remediation of Cr(VI) and Pb(II) aqueous solutions using supported, nanoscale zero-valent iron. *Environ. Sci.Technol.* 34: 2564-2569.

Prato, M. (1999) Fullerene materials. *Top. Curr. Chem.* 199: 173-187.

Quan, X., Yang, S., Ruan, X., Zhao, H. (2005) Preparation of Titania Nanotubes and Their Environmental Applications as Electrode. *Environ. Sci. Technol.* 39: 3770-3775.

Reijnders, L (2006) Cleaner nanotechnology and hazard reduction of manufactured nanoparticles. *Journal of Cleaner Production* 14: 124-133.

Robinson, R.D., Spanier, J.E., Zhang, F., Chan, S.-W., Herman, I.P. (2002) Visible thermal emission from sub-band-gap laser excited cerium dioxide particles. *J. Appl. Phys.*, 1936-1941.

Rosi, N.L., Giljohann, D.A., Thaxton, C.S., Lytton-Jean, A.K.R., Han, M.S., Mirkin, C.A. (2006) Oligonucleotide-Modified Gold Nanoparticles for Intracellular Gene Regulation. *Science* 312: 1027-1030.

Royal Society, and Royal Academy of Engineering (2004) Nanoscience and nanotechnologies: opportunities and uncertainties. Final Report,UK. Available at http://www.nanotec.org.uk/finalReport.htm Accessed on November 2010.

Salata, O.V. (2004) Applications of nanoparticles in biology and medicine. *J. Nanobiotechnol.* 2: 3

Sarathy, V., Tratnyek, P.G., Nurmi, J.T., Baer, D.R., Amonette, J.E., Chun, C.L., Lee P.R., Eric R.J (2008) Aging of Iron Nanoparticles in Aqueous Solution: Effects on Structure and Reactivity. *J. Phys. Chem. C* 112: 2286-2293.

Schreder, B., Schmidt, T., Ptatschek, V., Spanhel, L., Materny, A., Kiefer, W. (2000) Raman characterization of CdTe/CdS-"core-shell"-clusters in colloids and films. *J. Cryst. Growth* 214/215: 782-786.

Serpone, N., Dondi, D., Albini, A. (2007) Role and efficacy of inorganic and organic UV filters in sunscreens and suncare products. *Inorg. Chim. Acta* 360: 794-802.

Shchukin, D.G., Schattka, J.H., Antonietti, M., Caruso, R.A. (2003) Photocatalytic Properties of Porous Metal Oxide Networks Formed by Nanoparticle Infiltration in a Polymer Gel Template. *J. Phys. Chem. B* 107: 952-957.

Sohn, K., Kang, S.W., Ahn, S., Woo, M., Yang, S.-K. (2006) Fe(0) nanoparticles for nitrate reduction: Stability, reactivity, and transformation. *Environ. Sci. Technol.* 40: 5514-5519.

Sondi, I., Salopek-Sondi, B. (2004) Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* 275: 177-182.

Song, W., Li, G., Grassian, V.H., Larsen, S.C. (2005) Development of Improved Materials for Environmental Applications: Nanocrystalline NaY Zeolites. *Environ. Sci. Technol.* 39: 1214-1220.

Stoimenov, P.K., Klinger, R.L., Marchin, G.L., Klabunde, K.J. (2002) Metal Oxide Nanoparticles as Bactericidal Agents. *Langmuir* 18: 6679-6686.

Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., Flank, A.M. (2006) Cytotoxicity of CeO2 Nanoparticles for Escherichia coli. Physico-Chemical Insight of the Cytotoxicity Mechanism. *Environ. Sci. Technol.* 40: 6151-6156.

Thompson, J.M. (2008) Chlorinated Pesticide Remediation using Zero-valent Iron Nanoparticles. In. North Dakota State University, Fargo, ND, USA.

Thompson J. M., Bezbaruah A. N. (2008) Selected Pesticide Remediation with Iron Nanoparticles Modeling and Barrier Applications, Technical Report No. ND08-04. In: North Dakota Water Resources Research Institute, Fargo, ND.

Thompson, J.M., Chisholm, B.J., Bezbaruah, A.N. (2010) Reductive dechlorination of chloroacetanilide herbicide (alachlor) using zero-valent iron nanoparticles. *Environ. Eng. Sci.* 27: 227-232.

Tratnyek, P.G., Johnson, R.L. (2006) Nanotechnologies for water remediation. *NanoToday* 1.

Tsuang, Y.-H., Sun, J.-S., Huang, Y.-C., Lu, C.-H., Chang, W.H.-S., Wang, C.-C. (2008) Studies of photokilling of bacteria using titanium dioxide nanoparticles. *Artif. Organs* 32: 167-174.

U.S.EPA(1999) An In Situ Permeable Reactive Barrier for the Treatment of Hexavalent Chromium and Trichloroethylene in Groundwater. In *Design and Installation.EPA600-R-99-095a.* *http://www.epa.gov/nrmrl/pubs/600R99095/prbdesign_v1.pdf* Accessed on December 5,2010.

U.S.EPA(2007) Science Policy Council. Nanotechnology White Paper. In *U.S. Environmental protection Agency February 2007. Available at http://www.epa.gov/ncer/nano/publications/whitepaper12022005.pdf. Accessed December 5, 2010.*

U.S.EPA(2007b) Estimation of Relative Bioavailability of Lead in Soil and Soil-like Materials Using In Vivo and In Vitro methods. In *OSWER 9285.7-77. May 2007.Availableat* http://www.epa.gov/superfund/health/contaminants/bioavailability/guidance.htm *Accessed on December 10, 2010.*

U.S.EPA(2009) Nanomaterial Research Strategy, Office of Research and Development.EPA620-K-09-011.Available at: http://www.epa.gov/nanoscience/files/nanotech_research_strategy_final.pdf Accessed on December 10, 2010.

Vogel, T.M., Criddle,C.S, McCarty,P.L (1987) Transformation of halogenated aliphatic compounds. *Environ. Sci. Technol.* 21: 722.

Wiesner, M.R., Lowry, G.V., Jones, K.L., Hochella, M.F., Jr., Di Giulio, R.T., Casman, E., Bernhardt, E.S. (2009) Decreasing Uncertainties in Assessing Environmental Exposure, Risk, and Ecological Implications of Nanomaterials. *Environ. Sci. Technol.* 43: 6458-6462.

Williams, D.N., Ehrman, S.H., Holoman, T.R.P. (2006) Evaluation of the microbial growth response to inorganic nanoparticles. *J. Nanobiotechnol.* 4: 3

Xu, J., Bhattacharyya, D. (2005) Membrane-based bimetallic nanoparticles for environmental remediation: Synthesis and reactive properties. *Environ. Prog.* 24: 358-366.

Xu, Y., Zhang, W.-x. (2000) Subcolloidal Fe/Ag Particles for Reductive Dehalogenation of Chlorinated Benzenes. *Ind. Eng. Chem. Res.* 39: 2238-2244.

Xu, Y., Zhao, D. (2007) Reductive immobilization of chromate in water and soil using stabilized iron nanoparticles. *Water Res.* 41: 2101-2108.

Zhang, W., Wang, C.B. (1997) Rapid and complete dechlorination of TCE and PCBs by nanoscale Fe and Pd/Fe particles. *Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17*: ENVR-002.

Zhang, W.-x. (2003) Nanoscale Iron Particles for Environmental Remediation: An Overview. *J. Nanopart. Res.* 5: 323-332.

Zhang, W.-X., Elliott, D. (2006) Applications of iron nanoparticles for groundwater remediation. *Remediation* 16: 7-21.

Zhang, Y., Frankenberger, W.T., Jr. (2006) Removal of Selenate in River and Drainage Waters by Citrobacter braakii Enhanced with Zero-Valent Iron. *J. Agric. Food Chem.* 54: 152-156.

Zhu, H., Wei, J., Wang, K., Wu, D. (2009) Applications of carbon materials in photovoltaic solar cells. *Sol. Energy Mater. Sol. Cells* 93: 1461-1470.