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Metalloporphyrin-catalyzed Reduction Reactions of Hexavalent Chromium

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METALLOPORPHYRIN-CATALYZED REDUCTION REACTIONS OF HEXAVALENT CHROMIUM

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Environmental Engineering and Science

by
Rong Zhang
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Accepted by:
Dr. Mark A. Schlautman, Committee Chair
Dr. Elizabeth R. Carraway
Dr. Brian A. Powell
ABSTRACT

Previous studies have demonstrated that the reduction of oxidized organic and inorganic contaminants could be catalyzed by electron shuttle systems, which generally were biological organic macrocycle complexes with transition metals. Metalloporphyrins (MPs) and their derivatives are well known electron shuttles for many biogeochemical systems. The objective of this research was to study the catalytic capabilities of selected MPs for the reduction of hexavalent chromium (Cr(VI)) in the presence of reducing agents. Zero valent iron (ZVI) was chosen as the primary electron donor in the experimental systems. Protoporphyrin IX (Proto) and Uroporphyrin I (Uro) are naturally occurring porphyrins produced during heme biosynthesis. MPs were prepared by inserting Co(II) or Fe(II) to the dissolved porphyrins. These four synthesized MPs and Vitamin B\textsubscript{12} (VB\textsubscript{12}) were applied to Cr(VI) reductions by micro-sized ZVI (m-ZVI), nano-sized ZVI (n-ZVI), and n-ZVI immobilized in Ca-alginate gel beads. The kinetic data were analyzed using pseudo-first order models, and the catalytic capability was evaluated by the comparison of these reactions to those without a catalyst.

Different concentrations of MPs or VB\textsubscript{12} were added to catalyze Cr(VI) reduction by 1.7 g/L m-ZVI or 0.1 g/L bare/immobilized n-ZVI at pH 7. No significant catalytic effects were found for Cr(VI) reduction by m-ZVI in the presence of 20 \textmu M Proto-Co or Proto-Fe. At the same concentration, Uro-Co and Uro-Fe slightly accelerated the reaction by approximately 7% and 4%, respectively. VB\textsubscript{12} (20 \textmu M) dramatically increased Cr(VI) reduction by m-ZVI, approximately 20% in 200 min. For Cr(VI) reduction by a more reactive form of m-ZVI, VB\textsubscript{12} catalysis was not obvious in the first and second runs of a
reuse test using it. However, the \( \text{VB}_{12} \) significantly catalyzed \( \text{Cr(VI)} \) reduction in the third reuse cycle. This result indicates that the catalyst (\( \text{VB}_{12} \)) may be more important for long-term remediation when using reactive reductants.

Small amounts of \( \text{VB}_{12} \) (0.1 \( \mu M \)) made \( \text{Cr(VI)} \) reduction by n-ZVI reach completion approximately three times faster than when only using the n-ZVI alone. Encapsulation of n-ZVI in Ca-alginate gel beads hindered the \( \text{Cr(VI)} \) reduction rate by a factor of 8 at pH 6 and a factor of 3 at pH 7. Upon adding 5 \( \mu M \) \( \text{VB}_{12} \) to the reaction at pH 7, the reaction rate was significantly enhanced. \( \text{Cr(VI)} \) (100 \( \mu M \)) was totally reduced in 20 min, which was faster than without \( \text{VB}_{12} \) (150 min), as well as when using bare n-ZVI (50 min). Interestingly, n-ZVI gel beads became more reactive after being kept in the anaerobic chamber three months, which may be due to the enlargement of pore sizes, crack on the beads surface, or \( \text{Fe}^{2+} \) produced by \( \text{Fe}^{0} \) hydrolysis. Furthermore, the n-ZVI gel beads were reused multiple times at pH 6. After four reuse cycles, the beads were nearly completely broken, but they collected a lot of precipitated products. Therefore, using this kind of material for \textit{in-situ} remediation may be beneficial from an aesthetic standpoint.

\( \text{VB}_{12} \) immobilized in sol-gel matrices provided a more moderate catalysis than the free \( \text{VB}_{12} \), but the reductions by m-ZVI or n-ZVI were greatly accelerated compared to those not adding \( \text{VB}_{12} \). The collective results of this research indicate that metalloporphyrins and related compounds can facilitated electron transfer and enhanced \( \text{Cr(VI)} \) reductions by ZVI under certain conditions. \( \text{VB}_{12} \) is the most promising catalysts, followed by Uro-Co and Uro-Fe. The \( \text{VB}_{12} \) immobilized sol-gel only slightly inhibited
the overall VB\textsubscript{12} catalytic capability and still significantly catalyzed Cr(VI) reduction.
DEDICATION

This thesis is dedicated to my Heavenly Father for His wonderful blessings, which enable and encourage me to finish this work very well. Also I would like to dedicate this thesis to my husband Richard Zhu and my parents. I thank them for their unfailing love, and support during these past years.
ACKNOWLEDGMENTS

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CHAPTER ONE
INTRODUCTION

1.1 Chromium contamination

Chromium (Cr) is a first-row transition metal in group VI and has an atomic number of 24. Because Cr exhibits high corrosion resistance and many of its compounds have intense colors, it has been widely used in many industries such as leather, tanning, metallurgy, and pigments (Schlautman and Han, 2001; Han et al. 2000). These various applications together with improper disposal have resulted in Cr contaminated soil and groundwater. Chromium is commonly found at many wastesites, and is considered to be one of the priority pollutants by the U.S. Environmental Protection Agency (Xu et al., 2005; Wielinga et al., 2001).

Chromium can exist in several redox states from -2 to +6. However, the trivalent form (Cr(III)) and hexavalent form (Cr(VI)) are the most stable (Schlautman and Han, 2001; Han et al. 2000). Interestingly, Cr(VI) and Cr(III) have totally different mobilities and toxicities. In environment systems, Cr(VI) exists as an oxyanion, such as chromate (CrO$_4^{2-}$), bichromate (HCrO$_4^-$), and dichromate (Cr$_2$O$_7^{2-}$), which are highly soluble and can pass through aquifers to contaminate groundwater and other sources of drinking water (Puzon et al., 2005). Hexavalent Cr is carcinogenic and mutagenic (Schlautman and Han, 2001; Han et al., 2000), so it is toxic to humans, animals, plants, and microorganisms (Xu, et al., 2005). By comparison, Cr(III) is 10 – 100 times less toxic than Cr(VI) (Wei et al. 1993). Furthermore, Cr(III) forms Cr(OH)$_3$ and Cr-Fe hydroxides, which have low solubilities in water and are readily absorbed to the surfaces of clay
Overall, the reduced species Cr(III) is more preferable in the environment due to its lower toxicity and mobility (Schlautman and Han, 2001; Han et al., 2000). Therefore, one of the important strategies for Cr remediation is to immobilize Cr(VI) by reduction to Cr(III) (Han et al., 2000).

1.2 Cr(VI) remediation

1.2.1 Cr(VI) reduction and remediation technologies

Previous laboratory studies have reported that Cr(VI) can be reduced to Cr(III) by many reductants such as zero-valent iron (Fe$^0$, ZVI), Vitamin C, sodium dithionite (Na$_2$S$_2$O$_4$), ferrous sulfate (FeSO$_4$), reduced aquifer materials, plus many other reduced materials which are readily oxidized by Cr(VI) (Qian et al., 2008; Schlautman and Han, 2001; Xu et al., 2004). In addition, in-situ remediation technologies, such as permeable reactive barrier (PRB) and in-situ redox manipulation (ISRM), have been applied in medium scale laboratory and field studies (Istok et al, 1999; Blowes et al., 1997). Both PRB and ISRM apply reactive material zones in the flow path of contaminant plumes where Cr(VI) then contacts the electron donating materials, and becomes reduced to Cr(III).

1.2.2 Cr(VI) reduction by ZVI

Zero-valent iron is one of the most promising reductants because it is considered to be environmentally friendly and thermodynamically favorable for reducing Cr(VI) to
Cr(III) (Qian et al., 2008). Laboratory and field studies have examined Cr(VI) reduction/remediation using iron wires, micro-sized ZVI (m-ZVI) and nano-sized ZVI (n-ZVI) (Alowitz and Scherer, 2002; Qian et al., 2008; Chang, 2005; Wilkin et al., 2005; Ponder et al., 2000; Xu and Zhao, 2007). Zero-valent iron also is one of the effective materials applied in PRBs for Cr(VI) remediation in groundwater (Blowes et al., 1997; Pratt et al., 1997; Wilkin et al., 2005). Two sequential steps may be involved in Cr(VI) reduction by ZVI: adsorption and reduction (Alowitz and Scherer, 2002; Qian et al., 2008; Xu and Zhao, 2007). The surface area of ZVI is an important factor for Cr(VI) reduction rates and n-ZVI has shown faster and more complete reduction reactions than m-ZVI due to higher reactive surface areas (Qian et al., 2008; Ponder et al., 2000). However, the iron type and geochemical differences may also affect the rate and extent of Cr(VI) reduction (Powell et al., 1995; Liu et al., 2008).

The ZVI nanoparticles are highly reactive. However, because of magnetism and van der Waals force, n-ZVI is prone to agglomerate which tremendously decreases its reactivity and dispersibility (Qian et al., 2008; Ponder et al., 2000; Xu and Zhao, 2007). If they were injected to the aquifers for in-situ remediation of redox active contaminants such as Cr(VI), the decreased mobility and the tendency to settle may inhibit the effectiveness of the n-ZVI (Bezbarauah et al., 2009; Xu and Zhao, 2007). Some researchers have reported that n-ZVI immobilization/stabilization by starch, carboxymethyl cellulose (CMC) or resin limits its agglomeration and effectively keeps high n-ZVI reactivity (Qian et al., 2008; Ponder et al., 2000; Xu and Zhao, 2007). Also, Bezbarauah et al. (2009) reported that n-ZVI could be entrapped in Ca-alginate beads,
which is a nontoxic, biodegradable, and nonimmunogenic matrix. The immobilized n-ZVI caused no adverse effects for nitrate degradation, and solved the mobility and settlement problems of n-ZVI. This Ca-alginate immobilized n-ZVI was tested to nitrate reduction and Ca-alginate matrices caused no adverse effects to n-ZVI reactivity (Bezbaruah et al., 2009). But n-ZVI Ca-alginate beads have not tested for metal redox reactions, which may give totally differently results due to insoluble products. In this research, n-ZVI Ca-alginate gel beads were used to reduce Cr(VI) and obtain a better understanding about the application of n-ZVI gel beads to in-situ remediation of oxidized metals.

1.2.3 Cr(VI) reduction by microorganisms

Bioremediation studies have revealed that reduction of Cr(VI) by microorganisms can energetically favorable when adequate electron donors are present (Lovley and Phillips, 1994; Chen and Hao, 1998). However, some environment maybe lack of microorganisms. In addition, high concentrations of Cr(VI) can inhibit microbial activity and growth, and may even be toxic to bacteria (Chen and Hao, 1998). Therefore, Cr(VI) reduction can be slow due to unfavorable environment conditions and bioremediation may not be effective at many waste sites. On the other hand, laboratory abiotic systems could have faster degradation rates than systems utilizing direct microbial reduction (Schanke and Wackett, 1992; Lovley and Phillips, 1994). For example, cell soluble protein could reduce Cr(VI) faster than membrane-bounded protein (Lovley and Phillips, 1994). Furthermore, Lovley and Phillips (1994) found that biologically-produced
coenzymes (e.g., cytochrome C₃) were essential to Cr(VI) reduction. These coenzymes are generally organic macrocycles complexed with transition metals, which can shuttle electrons from electron donors (reductants) to electron acceptors (contaminants). Collectively, developing biomimetic redox catalysts applicable to abiotic systems is becoming one of the promising approaches to in-situ remediation (Schlautman, 2006). Metalloporphyrinogens are transition-meal macrocycles, and they are promising electron transfer shuttles (Dror and Schlautman, 2003 & 2004).

1.3 Metalloporphyrinogens

1.3.1 Porphyrins and Metalloporphyrinogens

Porphyrs are aromatic tetrapyrrole macrocycles (Figure 1.1 a), containing 22 conjugated π electrons (Huheey et al., 1993). According to [4n+2] rule for the aromaticity (n = 4), 18 of them are incorporated into the delocalization pathway. When one or two of the peripheral bounds of porphyrins undergo(s) addition reactions, porphyrins form corrins and chlorins (Figure 1.1 b & c) without a substantial loss of the macrocyclic aromaticity (Kadish et al., 2000). Therefore, porphyrin structure facilitates electron transfer. The most common naturally-occuring porphyrins include Protoporphyrin, Coproporphyrin, and Uroporphyrin (Figure 1.2), which are involved in the heme biosynthesis pathway (Dolphin, 1978).

Porphyrs complex transition metals such as cobalt, iron, nickel, zinc and copper via chelation by the central nitrogen atoms, forming metalloporphyrins (MPs) (Falk et al., 1975). Metalloporphyrins and their derivatives are metalloporphyrinogens (Kadish et al.,
2000), and they function as pigments, coenzymes, etc. (Huheey et al., 1993). Metalloporphyrinogens exist in many biogeochemical environments, such as living cells, soils, and sediments (Dror and Schlautman, 2003). They can mediate biotic or abiotic redox reactions (Kadish et al., 2000).

![Figure 1.1 Structures of Porphyrin, Chlorin and Corrin rings (a and b were adopted from Dlophin, 1978; c was adopted from Smith, 1975)](image_url)
Figure 1.2 Structures of Protoporphyrin IX, Coproporphyrin III and Uroporphyrin I (adopted from Smith, 1975)
1.3.2 Metalloporphyrinogens and electron shuttle systems

Numerous studies have demonstrated that the reduction of oxidized organic and inorganic contaminants can be enhanced by electron shuttle systems (Larson and Weber, 1994; Scheffold et al., 1987). Normally, the electron-transfer mediators are transition-metal macrocycles, which can shuttle electrons from electron donors (reductants) to electron acceptors (oxidized contaminants, Dror and Schlautman, 2003 & 2004). For example, bacterial coenzyme VB$_{12}$ and several synthesized metalloporphyrins (from tetraphenyl porphyrins) could catalyze reductive dechlorination of tetrachloroethylene (PCE), trichloroethylene (TCE), and carbon tetrachloride (CT) (Dror and Schlautman, 2003 & 2004, Dror et al., 2005). Another example is that cytochrome C$_3$ (bacterial coenzyme) functioned as a reductase to Cr(VI) and U(VI) reductions by Desulfovibrio vulgaris (Lovley and Phillips, 1994; Lovley et al., 1993). However, the catalytic capabilities of many naturally-occurring MPs have not been studied or tested, such as MPs from Protoporphyrin, Uroporphyrin, etc. Also the target contaminants should be varied to include organic compounds and metals. But metalloporphyrinogens have not been commonly used to catalyze metal redox reactions. Therefore, to explore biomimetic compounds to facilitate redox reactions in abiotic systems of in-situ remediation, more metalloporphyrinogens need to be studied, and more contaminants should be tested.

1.3.3 Metalloporphyrinogens immobilization

Many researchers have studied immobilization of catalytic macrocycles for the following reasons. First, the treatment approaches of using solutions (homogeneous
systems) may be impractical, because the catalyst could not be separated from the treated solutions for reusage (Burris et al., 1996). However, an immobilized catalysts could be kept in the reactors for reuse. Second, the immobilization may enhance stability of catalyst, such as MPs (Cummingham et al., 2002), which may increase the catalytic capability. Third, the immobilization may extend service life of the catalysts, and facilitate their delivery (Dror et al., 2005).

In previous studies, metalloporphyrinogens have been immobilized in agarose (Burris et al., 1996), resins (Habeck and Sublette, 1995), talc (Matheson, 1994), hectorite, fluorohectorite, amorphous silica surfaces, layered double hydroxide surface (Ukrainczyk et al., 1995), and sol-gel matrices (Dror et al., 2005). These immobilized-catalysts were tested for reductive dechlorination of chloro-organic contaminants (COC), and the results were promising. For instance, PCE reduction by titanium(III) citrate catalyzed by VB₁₂ in homogeneous and heterogeneous (bound to agarose) systems were comparable, and no decrease of PCE dechlorination was observed to immobilized-VB₁₂, which had been reused four times (Burris et al., 1996). In addition, Ukrainczyk et al. (1995) found that in the short-term experiments (2 hr), cobalt tetrakis (1-methyl-4-pyridiniumyl) porphyrin (Co-TMPyP) was more reactive homogeneously than heterogeneously. But in the long-term experiments (3 days), the silica-immobilized Co-TMPyP degraded more CCl₄ than dissolved Co-TMPyP. The reason was that the immobilization in silica made the catalyst (Co-TMPyP) stable. Similarly, Dror et al. (2005) reported that the catalytic capability of sol-gel immobilized-VB₁₂ was slightly slower compared to dissolved VB₁₂ for reduction dechlorination of PCE, TCE, and CT. But the lower reduction rates were compensated by
reusing the immobilized catalysts. The immobilized VB\textsubscript{12} had been reused for 12 cycles (24 hours each) to CT reduction in the presence of titanium citrate (Dror et al., 2005). However, there were some conflicting results reviewed by Burris et al. (1996): TCE was not reactive to dithiothreitol in the presence of immobilized cobalamins; also the reuse of talc immobilized VB\textsubscript{12} decreased the reduction rates of chlorinated methanes and PCE was significantly adsorbed to resin (Duolite S-761). Therefore, the types of metalloporphyrinogens, immobilization matrices and immobilization method may all affect the catalytic capability of immobilized catalysts.

Sol-gel matrices are one of the promising materials to immobilize metalloporphyrinogens. Sol-gel matrices are porous thin layers of ceramic oxides, formed by polymeric or colloidal routes (Klotz et al., 1999). Sol-gel technology allows the encapsulation of organic molecules (e.g., metalloporphyrinogens) inside an inorganic network (silicone oxides) (Mark et al., 1995), which results in hybrid (organic-inorganic) micro- or nano – composites (Sacco et al., 2001). The effects of immobilized catalysts were related to the characteristics of sol-gel matrices, including porosity, pore size, and microstructures. (Zada et al., 2009). These sol-gel characteristics could be modified by varying the synthesis parameter, such as pH, temperature, and the ratio of alkoxides and solvents (Klotz et al., 1999; He et al., 2009; Asomoza et al., 1997; Galarneau et al., 2007, Zada et al., 2009). As discussed above, promising results have been observed from the immobilized tetraphenyl MPs in sol-gel used to COC reductions (Dror et al., 2005). To have further understanding on the behavior of immobilized catalyst, sol-gel technology
should be applied to immobilize more metalloporphyrinogens. Also, the immobilized catalysts need to be tested to other types of contaminants, such as Cr(VI).

1.4 Research objectives

The overall objective of this research was to study whether selected MPs could catalyze Cr(VI) reduction. First of all, Protoporphyrin IX and Uroporphyrin I with Co(II) or Fe(II) as core metal were synthesized and applied to Cr(VI) reductions. The catalytic capabilities of MPs were compared to Vitamin B\textsubscript{12} (VB\textsubscript{12}), which has shown to be a highly active catalyst (Dror and Schlautman, 2004a; Scheffold et al., 1987). Then the roles of different kinds of porphyrins, and core metals on the catalytic reactivity of the MPs were compared. A secondary objective was to test if immobilization of MPs and VB\textsubscript{12} could be performed without causing adverse effects. Therefore, sol-gel encapsulated MPs were prepared and the intrinsic properties of the sol-gel matrices (such as morphology, pore diameters, bulk strength and friability) were studied by scanning electron microscopy. The immobilized MPs were then tested for Cr(VI) reduction by ZVI. A third objective was to examine if metalloporphyrinogens (e.g., VB\textsubscript{12}) could be immobilized in in Ca-alginate gel beads. Finally, the fourth objective was to test how n-ZVI immobilized in Ca-alginate gel beads would affect Cr(VI) reduction compared to bare n-ZVI.
CHAPTER TWO

LITERATURE REVIEW

2.1 Chromium

2.1.1 Hexavalent chromium speciation

Chromium (Cr) has been ranked the third most common contaminant and the second most common inorganic pollutant at waste sites in the United States (Xu et al., 2004). In addition, hexavalent chromium (Cr(VI)) has been consistently ranked among the most frequently encountered or highest-priority metals present in groundwater and soils at DOE facilities (National Research Council, 1999; Lee et al., 2000). As discussed in Section 1.1, Cr(VI) forms as oxyanions, including bichromate (HCrO$_4^-$) and chromate (CrO$_4^{2-}$). Hexavalent chromium exists as typical multiprotic acids and undergoes a multistep deprotonation with pH increasing. The speciation distribution with pH was modeled by Visual Minteq and plotted as Figure 2.1.

2.1.2 Chromium toxicology

As discussed in Section 1.1, Cr(VI) and Cr(III) are the most common oxidation states of Cr in the environment, but they have quite different mobilities and toxicities. Hexavalent Cr can be toxic to humans, animals, plants and microorganisms (Lippard and Berg, 1994; Lee et al., 2000) and can cause organ damage, dermatitis, and respiratory impairment (Xu, et al., 2005). Furthermore, Cr(VI) is carcinogenic, mutagenic, and teratogenic (Han et al. 2000; Leita et al., 2009; Wittbrodt and Palmer 1996; Xu and Zhao
However, Cr(III) is not carcinogenic, and is approximately 10-100 times less toxic than Cr(VI) (Wei et al., 1993; Lippard and Berg, 1994)

![Figure 2.1 Distribution of Cr(VI) species with pH ([CrO$_4^{2-}$] = 100 µM, I = 0.001 M, at 25°C, modeled by Visual Minteq).](image)

Lippard and Berg (1994) explained the mechanism of Cr toxicity. The important factor is if a particular species of Cr can cross cell membranes and enter into cells. Hexavalent Cr, generally existing as anions (CrO$_4^{2-}$ and HCrO$_4^-$), can be carried into cells by anion transport systems. Because ascorbic acid (Vitamin C, Vc) is present in considerable concentrations in normal cells, Vc intracellularly reduces Cr(VI) to Cr(III). The reduced species, Cr(III) and its complexes, cannot cross cell membranes or diffuse back into plasma due to low solubilities and large molecular sizes. Therefore, Cr(III)
forms complexes with cellular components, which causes various adverse biological effects such as lipid peroxidation, cytotoxicity and DNA cleavage (Sreedhara et al., 1997). In addition, Cr(VI) can react with glutathione (GSH) in cytoplasm, forming Cr-S bonds and reducing Cr(VI) to Cr(V) and Cr(IV). Then, Cr(V) and Cr(IV) can bind to DNA and ultimately be reduced to Cr(III)-DNA. The reduction products Cr(III)-DNA disrupt gene function, which makes Cr(VI) carcinogenic (Lippard and Berg, 1994). However, Cr(III) cannot enter cell membranes (Wei et al. 1993; Lippard and Berg, 1994). Therefore, Cr(III) is more preferable to the environment for its lower toxicity and less mobility. One of the most important strategies for chromium remediation is chemical or biological reducing Cr(VI) to Cr(III) (Han et al., 2000; Zhou et al., 2008; Chang, 2005; Xu et al., 2004 & 2005).

2.1.3 Cr(VI) reduction

The reduction of Cr(VI) to Cr(III) can immobilize Cr and decrease its toxicity (Qian et al., 2008; Schlautman and Han, 2001). Many reductants have been applied to Cr(VI) reduction, such as Vitamin C (Xu et al., 2004 & 2005), ferrous iron (Fe²⁺) (Lee et al., 2000; Schlautman and Han, 2001; Hwang et al., 2002; Ludwig et al., 2007), sodium dithionite (Na₂S₂O₄) (Istok et al., 1999; Ludwig et al., 2007), soil humic acids (Leita et al., 2009; Wittbrodt and Palmer, 1996), citric acid (Din and Hartani, 2000; Chen et al., 2007), and L-cysteine (Lay and Levina, 1996). Zero valent iron (Fe⁰, ZVI) is one of the most promising reductants for Cr(VI) remediation because the reaction is relatively rapid (Qian et al., 2008; Zhou et al., 2008), and iron is considered to be benign. In addition,
ZVI is the most common reductant used in PRB for in-situ remediation of Cr(VI) (Ponder et al., 2000; Alowitz and Scherer, 2002; Chang, 2005).

2.1.3.1 Cr(VI) reduction by ZVI

Previous research has shown that the removal of Cr(VI) from water by ZVI is not merely due to its adsorption by oxyhydroxides (Powell et al., 1995), but instead involves a combination of adsorption and reduction by the Fe(0) surface (Zhou et al., 2008; Chang, 2005; Qian et al., 2008). The standard redox potentials for Fe(II)/Fe(0), Fe(III)/Fe(II) and Cr(VI)/Cr(III) are -0.44 V, 0.77 V and 1.33 V respectively (Qian et al., 2008). However, the standard redox potential for Cr(III)/Cr(0) is -0.74 V, which is lower than \( E^{\text{\theta}}_{\text{Fe(II)/Fe(0)}} \) and \( E^{\text{\theta}}_{\text{Fe(III)/Fe(II)}} \). Therefore, ZVI can serve as an electron donor for Cr(VI) reduction to Cr(III), but not to Cr(0) (Chang, 2005; Ponder et al., 2000; Aloitz and Scherer, 2002). The predominant reductive products should be Cr(III) and Fe(III) hydroxides (Eqns. 1 & 2, Qian et al., 2008; Chang, 2005). Electrochemical analyses showed that the products were Cr(OH)₃, and Feₙ₋ₓCrₓ(OH)ₙ(s) (Qian et al., 2008).

\[
\begin{align*}
\text{CrO}_4^{2-} + \text{Fe}^0 + 8\text{H}^+ & \rightarrow \text{Fe}^{3+} + \text{Cr}^{3+} + 4\text{H}_2\text{O} \quad (1) \\
(1-X)\text{Fe}^{3+} + (X)\text{Cr}^{3+} + 2\text{H}_2\text{O} & \rightarrow \text{Fe}_{(1-x)}\text{Cr}_x\text{OH}^{(s)} + 3\text{H}^+ \quad (2)
\end{align*}
\]

An initial high rate of reduction by ZVI is commonly observed regardless of contaminants, but then the reactions slow down because the fouled reactive sites of ZVI particles limit mass transfer (Ponder et al., 2000). Moreover, Cr(VI) reduction by ZVI was enhanced by the existence of complex reagents ethylene diamine tetraacetic acid (EDTA) or NaF in the reaction system because both reagents can complex with Cr(III),
Fe(III), and Cr$_x$Fe$_{1-x}$(OH)$_{y}$, thereby alleviating ZVI surface passivation (Zhou et al., 2008). In addition, Fe(II) can be an intermediate product formed by the oxidation of ZVI and can itself serve as a reductant for Cr(VI) (Eqns. 3, 4 & 5, Ponder et al., 2000; Chang, 2005; Qian et al., 2008; Zhou et al., 2008).

\[
2\text{CrO}_4^{2-} + 3\text{Fe}^0 + 10 \text{H}^+ \rightarrow 2\text{Cr(OH)}_3 + 3\text{Fe}^{2+} + 2\text{H}_2\text{O} \quad (3)
\]

\[
\text{Fe}^0 + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{H}_2\text{O}_2 + 2\text{OH}^- \quad (4)
\]

\[
2\text{Fe}^0 + \text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{Fe}^{2+} + 4\text{OH}^- \quad (5)
\]

Because Fe(II) is capable of reducing Cr(VI) (Eqn. 6; Chang, 2005), it is not surprising that the Fe(II) binding agent 1,10-phenanthroline decreased Cr(VI) reduction (Zhou et al., 2008).

\[
3\text{Fe}^{2+} + \text{Cr}^{6+} \rightarrow 3\text{Fe}^{3+} + \text{Cr}^{3+} \quad (6)
\]

The reactivity of different types of ZVI was compared, including Fe$^0$ filings, Fe$^0$ powder, Fe$^0$ nanoparticles and starch-stabilized Fe$^0$ nanoparticles. The results demonstrated that two factors played important roles in this process. First of all, the surface area significantly affects the reduction rate. Three types of m-ZVI showed similar surface area normalized rate constants (K$_{SA}$) for Cr(VI) reduction (Alowitz and Scherer, 2002). ZVI nanoparticles with higher surface area exhibited higher efficiency in Cr(VI) removal than m-ZVI, but n-ZVI could not keep high reactivity long because of agglomeration (Qian et al., 2008; Ponder et al., 2000; Alowitz and Scherer, 2002; Powell et al., 1995). Starch or resin stabilization may minimize nanoparticles agglomeration, and thereby make them reactive longer (Qian et al., 2008; Ponder et al., 2000). Second, ZVI purity significantly influences Cr(VI) reduction rates (Powell et al., 1995). For example,
Powell et al. (1995) reported that two types of ZVI (pure and impure) demonstrated different reactivity, and the impure ZVI was more reactive. Although their finding might partly be due to surface area differences, the primary hypothesis was that the impure ZVI carried redox couples (Fe\(^{2+}/\text{Fe}, \ O_2/\text{O}^2\)) to initiate the corrosion process, which facilitated electron transfer. But the pure ZVI did not, so that Cr(VI) reduction was slower even after the aquifer material was added.

Cr(VI) reduction by ZVI is pH-dependent, with the rate generally increasing as pH decreases (Qian et al., 2008; Zhou et al., 2008; Chang 2005). This might be because at low pH the reacted corrosion made more active sites for Cr(VI) adsorption and reduction (Chang, 2005; Powell et al., 1995). Also the pH effect may not only depend on H\(^+\) consumption (Eqn. 3), but also because precipitation of Cr(III) hydroxides and mixed Cr(III)/Fe(III) hydroxides are hindered at low pH (Qian et al., 2008). When pH is higher than 8.0, Cr(VI) reduction by ZVI is extremely slow (Chang, 2005; Qian et al., 2008). In addition, Cr(VI) reductions by ZVI can be accelerated by adding more iron, which increases the reactive Fe site concentration (Powell et al., 1995; Qian et al., 2008). Last, increasing the temperature may significantly enhance the reaction rate (Qian et al., 2008). For example, when the temperature was increased from 288 K to 298k, to 308 K, the rate constant for Cr(VI) reduction by Fe\(^0\) was 0.0056, 0.0100, and 0.0132 min\(^{-1}\), respectively.
2.1.3.2 Cr(VI) reduction by ascorbic acid

Ascorbic acid (Vitamin C, Vc) is a natural reductant which exists in animals and humans (Lippard and Berg, 1994; Xu et al., 2004 and 2005). It can exist as different species according to the pH values, and speciation distribution was model by Visual Minteq shown in Figure 2.2. Vc is non-toxic and its application to the rate of Cr(VI) reduction has been studied by Xu et al. (Eqn.7, 2004 and 2005).

\[
\text{Cr}_2\text{O}_7^{2-} + 3\text{C}_6\text{H}_8\text{O}_6 + 8\text{H}^+ \rightarrow 2\text{Cr}^{3+} + 3\text{C}_6\text{H}_6\text{O}_6 + 7\text{H}_2\text{O} \quad (7)
\]

Their results showed that Cr(VI) reduction by Vc was concentration dependent, and that two times of the stoichiometric amount of Vc was able to reduce approximate 90% Cr(VI) in 30 min at pH 5.0. Also, they showed that pH significantly affected the reaction rate with the reaction being faster as pH decreased. When using 20 times the stoichiometric amount of Vc, the reaction went extremely fast in acidic to neutral pH solutions: 100 µM of Cr(VI) was totally reduced in 5, 10, 20 or 400 s at pH 2, 4, 6 and 7, respectively. Although Cr(VI) was also reduced at pH 9, the rate constant was 35 times smaller than the one at pH 7. In addition, the ionic strength (KCl, 0.01 to 1 M), temperature (25 to 40 °C), or irradiation did not affect Cr(VI) reduction, leading them to conclude that Vc was a promising way to clean up soil and groundwater containing Cr(VI) (Xu et al., 2004 & 2005).
2.1.3.3 Cr(VI) reduction by sodium dithionite

Sodium dithionite is a strong reductant but unstable in aqueous solution at pH values of 7 and less (Fruchter et al., 2000) where it undergoes a number of homogeneous and/or heterogeneous disproportionation reactions that ultimately reduce the concentration of dithionite. Its stability is greatly enhanced at high pH. However, the dithionite decomposition products (e.g., sulfites and thiosulfates) may also play a significant role in the long-term treatment of dissolved phase Cr (VI) (Ludwig et al., 2007). A field study of Cr (VI) remediation in groundwater was applied by injection a mixture of ferrous sulfate and sodium dithionite, which was observed over 1020 days and more than 100 m of
linear groundwater flow through the treatment zone (Ludwig et al., 2007). The monitoring data indicated sustainable treatment of dissolved Cr (VI) from initial concentration between 4 and 8 mg/L to less than 0.015 mg/L.

2.1.3.4 Cr(VI) reduction by microorganisms

Biological reduction of Cr(VI) has been studied in laboratories and at field sites but the reactions can be slow due to unfavorable environment conditions (Chen and Hao, 1998). High concentrations of Cr(VI) inhibit microbial activity and growth, and can be toxic to microorganisms (Chen and Hao, 1998). Therefore, biological remediation is not effective at many waste sites. However, laboratory abiotic systems using effective components (e.g., cytochrome c₃) could have faster degradation rates than systems utilizing direct biological reduction reactions (Lovley et al., 1993; Lovley and Phillips, 1994). Also other naturally-occurring metalloporphyrinogens have been studied as catalysts, including VB₁₂, coenzyme F₄₃₀, hematin etc. (Kim and Carraway, 2002; Gantzer and Wackett, 1991; Marks et al., 1989). Therefore, some naturally-occurring metalloporphyrins were chosen to be tested in Cr(VI) reduction by ZVI (both m-ZVI and n-ZVI), sodium dithionite, or Vc in this research. The results may give a better understanding of the catalytic capabilities of metalloporphyrinogens and help to develop biomimetic redox catalysts applicable to abiotic systems for in-situ remediation.
2.2 Cr(VI) \textit{in-situ} remediation

2.2.1 ZVI-based permeable reactive barrier

The most widely applied technology for groundwater remediation is pump-and-treat, which means that the ground water is pumped to the surface and then treated and disposed (Blowes et al., 1997). Pump and treat requires removal of a great amount of groundwater for a long period of time, resulting in high costs (Naftz et al., 2002). Moreover, this procedure leads to a high mix of the extracted contaminants with the aquifer components, which makes pump-and-treat less effective for achieving projected levels of cleanup needed in many cases (Blowes et al., 1997; Naftz et al., 2002). Therefore, alternative technologies such as the permeable reactive barrier (PRB) have been widely studied (Naftz et al., 2002; Blowes et al., 1997; Wilkin et al., 2005). PRB technology involves placing a reactive material zone in the flow path of an aquifer, so that contaminants in groundwater can pass through the zone and be treated (Naftz et al., 2002). PRB technology requires little to no above ground constructions and leads to less exposure of contaminants to workers, resulting in lower treatment costs (Naftz et al., 2002).

The reactive materials used in PRBs should be both reactive to the contaminants requiring treatment and insoluble so that they remain in the treatment zone for a long lifespan (Naftz et al., 2002). Zero-valent iron (ZVI) is the most common reactive media used in PRBs for the \textit{in-situ} remediation of redox-sensitive organic pollutants and metals in groundwater (Lo et al., 2005; Naftz et al., 2002). The mechanisms of Cr(VI) subsurface remediation by ZVI were proposed by Powell et al. (1995). The reduction
processes were hypothesized that Cr(VI) reduction was either coupled by electron transfer between anodic and cathodic regions formed in a corrosion domain or by direct electron transfer on the iron surface. Iron surface analyses by SEM, EDX and XANES have shown that Cr(VI) was reduced to Cr(III) by ZVI in PRB, as illustrated by Equations 1 & 2 in Chapter 1 (Wilkin et al., 2005; Pratt et al., 1997; Astrup et al., 2000). Therefore, the main factors in the performance of PRBs are the surface area and the degree of contact determined by flow velocity (Blowes et al., 1997). Larger surface areas of materials and lower velocities would enhance PRB performance and should be considered in the design of PRB (Blowes et al., 1997).

Many laboratory and field studies discussed Cr(VI) remediation by PRB (Wilkin et al., 2005; Blowes et al., 1997; Pratt et al., 1997). Blowes et al. (1997) simulated Cr(VI) remediation in contaminated groundwater using dynamic columns (ID = 6.5 cm, L = 15 - 20 cm) filled with 10 cm depth of 50% iron filings and 50% quartz. The influent contained 18 mg/L potassium chromate and no Cr(VI) was observed in the effluent (detection limit of 0.05 mg/L) for 150 pore volumes at a water velocity of 10 m/yr (represents a typical pore-water velocity in a shallow aquifer). However, upon increasing the hydraulic velocity to 40 m/yr, Cr(VI) was absent in the effluent for only 80 pore volumes. Another column experiment (ID = 8 cm, L = 6 - 19 cm) using iron filings for Cr(VI) remediation was conducted at high pH (pH = 9 ± 0.5) and high salinity levels by Astrup et al. (2000). They reported that 25 mg/L Cr(VI) was immobilized in the column filled with iron filings, bentonite, and sand (mass ratio of 1:1:18), and no Cr(VI) broke through the column in 60 pore volumes at flow rates 10m/y (for first two months) and 25
m/y (for three years). Meanwhile, the time-of-flight secondary ion mass spectrometry (TOF-SIMS) images done in this research showed that clay particle surfaces severed as precipitation sites. But the precipitation (from the reaction or oxide coating on iron surface by $O_2$ and $H_2O$) did not inhibit the reaction progress (Blowes et al., 1997). All results above indicate that Cr(VI) reduction by ZVI is fast enough for normal dynamic aquifer flowrates and that the PRB performance is enhanced by decreasing hydraulic conductivity and increasing residence time (Blowes et al., 1997; Astrup et al., 2000).

The longevity of PRBs is a concern for its application, because it can be reduced by the passivation of ZVI surface decreasing permeability due to precipitation/clogging of the porous media (Naftz et al., 2002). But one field study demonstrated that a PRB, set up in 1996 at the U.S. Coast Guard Support Center near Elizabeth City (NC), had been used for eight years (Wilkin et al., 2005) and was still effective at reducing Cr(VI) in groundwater from 1.5 mg/L to less than 1 µg/L. Furthermore, Naftz et al. (2002) reported that a ZVI-based PRB at the Borden site had been operated for four years and PRBs at Sunnyvale (CA) had been operating for approximately seven years from 1995. The collective results suggest that ZVI-based PRB is promising and applicable technology to in-situ Cr(VI) remediation.

However, Cr(VI) removal was decreased by approximately 40% in the presence of TCE when compared to the same experiment without TCE (Lo et al., 2005). In addition, Cr(VI) reduction rate constants were hindered 7 - 9% by humic acid and by 10 - 12% by humic acid and hardness ($Ca^{2+}/Mg^{2+}$) together (Liu et al., 2008). Therefore, Cr(VI) removal could be inhibited under common environmental conditions. One of the
objectives in this work was to test if Cr(VI) reduction by ZVI would be faster upon addition of effective catalysts such as MPs. The promising results from this work may be a solution to Cr(VI) *in-situ* remediation, when the environment is not favorable. Or the promising results maybe provide solutions to improve PRB performance and lifetime.

2.2.2 ZVI nanoparticles for *in-situ* remediation

As discussed above, ZVI nanoparticles react quickly with Cr(VI) but tend to agglomerate and lose their reactivity (Qian et al., 2008; Xu and Zhao, 2007). However, n-ZVI stabilization prevented ZVI nanoparticles from agglomeration and kept their high reactivity to Cr(VI) (Qian et al., 2008; Xu and Zhao, 2007). No field study using stabilized n-ZVI has been reported, but a laboratory study investigated the feasibility of applying carboxymethyl cellulose (CMC) stabilized n-ZVI to Cr(VI) *in-situ* remediation in water and soil (Xu and Zhao, 2007). In their research, the batch reductions showed that 34 mg/L Cr(VI) was removed 90% in 48 hr by 0.12 g/L stabilized n-ZVI. Moreover, a column tested revealed that when 5 g Cr(VI)-loaded soil samples (83 mg Cr(VI)/kg soil) was washed by 0.06 g/L stabilized n-ZVI at pH 5.6, no Cr(VI) was found in the effluent in 5.7 bed volumes. Also, Xu and Zhao (2007) observed that the CMC stabilized n-ZVI was highly mobile in soil, and 81% of n-ZVI added broke through the soil bed in less than one bed volume. Although the n-ZVI concentration in the effluent decreased after 3 bed volumes, but CMC is degradable and n-ZVI would convert to iron minerals completely under the subsurface environment, which may contribute to sorption of toxic contaminants like Cr(VI) (Xu and Zhao, 2007). Therefore, the collective results
suggested that the high potential of using CMC stabilized n-ZVI for in-situ remediation of Cr(VI) in water and soil.

2.2.3 In-situ redox manipulation (ISRM)

ISRM is another novel technology for in-situ remediation, where excavation is not needed and permeable redox zones are created by injecting sodium dithionite (NaS$_2$O$_4$), potassium carbonate and potassium bicarbonate into the pathway of Cr(VI) plume (Fruchter et al., 2000; Istok et al., 1999). First of all, S$_2$O$_4^{2-}$ (aq) reduced Fe(III) contained in iron minerals in the aquifer, and then Fe(II) produced was to react with redox sensitive contaminants including Cr(VI) (Fruchter et al., 2000; Istok et al., 1999; Naftz et al., 2002). This method was evaluated by a medium scale laboratory study, imitating DOE Hanford waste site by using the aquifer from that site (Istok et al., 1999). It was about 7 m long in transport distant, and monitored for 72 hours. Injecting approximately one pore volume of 0.1 M NaS$_2$O$_4$ to the aquifer was able to remove 2 mg/L Cr(VI) up to 100 column pore volumes, indicating that this method could be applied to treat Cr(VI)-contaminated groundwater at Hanford waste site (Istok et al., 1999).

In addition, sodium dithionite may be injected with ferrous sulfate in ISRM, where sodium dithionite prevented Fe(II) oxidation, aquifer clogging, and preserved hydraulic conductivity (Ludwig et al., 2007). Ludwig et al. (2007) pumped 0.2 M ferrous sulfate and 0.2 M sodium dithionite into a Cr(VI) plume pathway at a ferrochrome alloy production site in Charleston, SC. Cr(VI) at initial concentrations of 4 to 8 mg/L were
treated to be less than 0.015 mg/L by passing through the redox zone more than 100 m (Ludwig et al., 2007).

However, dithionite is only stable at high pH, not at neutral or lower pH (Ludwig et al., 2007; Fruchter et al., 2000; Istok et al., 1999). Therefore, $K_2CO_3/KHCO_3$ buffer solution (pH ~ 11) has been used together with sodium dithionite, because of less cost and low possibility of clay mobilization/flocculation than sodium-based buffer (Fruchter et al., 2000; Istok et al., 1999). But the pH may drop to below 9.5 quickly after injections, due to the acidity produced during the oxidation sulfur species (Fruchter et al., 2000). However dithionite decomposition products (e.g., sulfites and thiosulfates) can reduce Cr(VI) as well (Eqn. 8; Ludwig et al., 2007), which was beneficial for Cr(VI) treatment, especially for the long term (Ludwig et al., 2007).

$$6H^+ + 2HCrO_4^- + 3HSO_3^- \rightarrow 2Cr^{3+} + 2SO_4^{2-} + S_2O_6^{2-} + 6H_2O \ (8)$$

Since m-ZVI, n-ZVI and sodium dithionite have been applied to Cr(VI) in-situ remediation, all of them were screening as electron donors to Cr(VI) reduction catalyzed by metalloporphyrinogens in this research. The results may give practical information on Cr(VI) in-situ remediation.
2.3 Metalloporphyrinogens

2.3.1 Porphyrin

Porphyrins are naturally occurring macrocycles and exist in many biogeochemical environments, including living cells, soils, sediments, coal, oil shale, petroleum and other types of deposits rich in organic matter (Dror and Schlautman, 2003). Porphyrins contain four pyrrole rings joined by methylidene bridges (Figure 1.1 a; Dolphin, 1978), which makes them have near planar structures (Huheey et al., 1993). Porphyrins are composed of 22 conjugated π electrons, and due to [4n + 2] rule for aromaticity 18 electrons (n = 4) are incorporated into an extensively delocalized pathway (Kadish et al., 2000). If one or two double bonds on porphyrin ring involve additional reactions due to uptake or release of electrons, porphyrins could form corrins and chlorins (Figure 1.1 b & c; Smith, 1975). The processes only cause minimal structural changes to tetrapyrrole macrocycles, and porphyrins do not lose their macrocyclic aromaticity (Dror and Schlautman, 2003). In a word, porphyrins can facilitate electron transfer by narrowing π frontier orbital gaps (Kadish et al., 2000).

2.3.2 Metalloporphyrinogens

Porphyrins have the tendency to complex transition metals by the central nitrogen atoms and thus form metalloporphyrins (MPs) (Kadish et al., 2000). Common redox active transition metals include Fe, Co, Ni, and Zn (Kadish et al., 2000). Thus, MPs can mediate metal-centered reduction and oxidation reactions in many biological and abiotic reactions, and can be applied as biomimetic redox catalysts (Kadish et al., 2000).
Metalloporphyrinogens include MPs and many other porphyrin-type molecules, such as corroles, corrins and corrinoid macrocycles (Dror et al., 2005). They play many roles in natural systems, such as pigments and redox catalysts in electron transport (Huheey et al., 1993). For instance, [meso-tetrakis (4-sulfonatophenyl) porphyrinato] Zn(II), [(TPPS)Zn], can work an electron shuttle in photocatalytic system as shown in Figure 2.3. Na$_2$S$_2$O$_3$ is an electron source, quinolinium-3-carboxamide as an electron acceptor and (TPPS)Zn is a photocatalyst.

The most commonly studied metalloporphyrinogens are bacterial transition-metal coenzymes, naturally-occurring MPs and other biomimetic macrocycles (Lovely and Phillips, 1994; Dror and Schlautman, 2003 & 2004; Kim and Carraway, 2002). For example, Vitamin B$_{12}$ consists of a corrin ring, having cobalt as the core metal, and various attached side groups (Figure 2.4). “VB$_{12}$ is often present in a variety of near-surface natural environment, and it is produced by soil microorganisms, or linked with root exudates and fertilization with organic plant/animal wastes.” (reviewed by Schlautman, 2006). Heme is a group of iron-porphyrins, which shows various biological functions in electron transfer and the transportation of chemical catalysis (Smith, 1975). The cytochrome P450 and c families of proteins are examples of bacterial transition-metal coenzymes with a heme iron center (Gantzer and Wackett, 1991). Both coenzyme F430 and cytochrome c$_3$ were used as electron transfer mediators (Lovley and Phillips, 1994; Lovley et al., 1993).
Figure 2.3 The photocatalytic system of (TPPS)Zn as an electron shuttle. (Adapted from Kaddish et al., 2000)
In an unpublished work, Nielson (2006) studied the kinetics of Fe(II) complexation with Coproporphyrin III and Uroporphyrin III at pH 5.5 and room temperature. The results showed that Fe(II) was inserted to Coproporphyrin III and Uroporphyrin III, but that the reaction could be slow. It took 150 hours and 25 hours to reach equilibrium for Coproporphyrin III and Uroporphyrin III, respectively. Faster MPs preparation methods were reported by Dror and Schlautman (2003), including TPP, TP-OHP, T(methoxy)P, T(p-tolyl)P, T(benzoic)P, T(pyridyl)P, and TMPyP with cobalt, nickel, or iron as the core.
metal. Reflux made the synthesis faster and allowed the synthesis to be finished in 1 hour or 30 minutes.

2.3.3 Metalloporphyrinogens catalyses on reduction reactions

2.3.3.1 Dechlorination of chloro-organic compounds

Previous studies have reported that metalloporphyrinogens can catalyze the reductive dechlorinations of chloro-organic pollutants (e.g., tetrachloroethylene (PCE), trichloroethylene (TCE), carbon tetrachloride (CT), etc.) in the presence of reducing agents. VB$_{12}$ (5% of the target contaminant) was able to catalyze reduction of 20 mg/L PCE by 0.5 g Fe$^0$, and 100 mg/L TCE by 0.5 g Fe$^0$ or Zn$^0$ (Kim and Carraway, 2002). In their study, the surface area normalized first order rate constants were increased approximately 7.7 times by Zn and 12 times by Fe$^0$ for PCE, and 560 times by Zn$^0$ for TCE. Meanwhile, Kim and Carraway (2002) calculated the activation energies (Ea) for PCE dechlorination by both metals and found that Ea was lowered 40 - 60 kJ/mol in the existence of VB$_{12}$. In addition, Gantzer and Wackett (1991) reported that VB$_{12}$ catalyzed the reductive dechlorination of CT, PCE, hexachlorobenzene, pentachlorobenzene, and pentachlorophenol by Ti(III) citrate. Moreover, coenzyme F$_{430}$ (Ni) was able to catalyze the dechlorination of CT, PCE, hexachlorobenzene as well (Gantzer and Wackett, 1991). Also, hematin catalyzed the reductive dechlorination of CT, chloroform, PCE and 1,1,1-TCE to lower chlorinated compounds (Klecka and Gonsior, 1984; Gantzer and Wackett, 1991). What is more, Hemoglobin, hemin, hematin, and chlorophyll(a) showed catalytic
capabilities to the dehalogenation of lindane in the presence of dithiothreitol (Marks et al., 1989).

However, the dissolved porphyrin could not catalyze the reactions. For example, Tetrakis (N-methly-4-pyridiniumyl) porphyrin (TMPyP) was nonreactive to PCE dechlorination (Dror and Schlautman, 2003), and Hematoporphyrin (HP), Protoporphyrin (PP), Coproporphyrin (CP), and Uroporphyrin (UP) were inactive to the dechlorination of hexachlorocyclohexane (Lindane) (Marks et al., 1989). But dechlorination reactions were observed after Ni or Co was complexed with TMPyP, or Co, Fe, Mg, Mo, Ni, or V was inserted into HP, PP, CP, and UP (Dror and Schlautman, 2003; Marks et al., 1989). Therefore, the core metal facilitated electron transfer, and it is essential to the catalytic capability of metalloporphyrinogens.

Using Van del Waal space-filling models of VB_{12} and PCE, Dror and Schlautman (2004) discussed about the catalytic mechanism. They found that the cavities to the core metal of VB_{12} was very small (< 2 Å), but a chlorine atom itself was larger than 3.3 Å, and the whole PCE molecular was even larger. Therefore, the electron transfer might happen through both the core metal and the conjugated macrocycle. Furthermore, Dror and Schlautman (2003) compared 3-D molecular models of VB_{12} with Co-TMPyP. They found that VB_{12} was bulky and had more steric limitations than Co-TMPyP, and the PCE reduction results revealed that Co-TMPyP led to a faster reaction (2 hr) than VB_{12} (48 hr). Therefore, steric effect may also play an important role in the catalytic processes of metalloporphyrinogens. Last, but not least, MPs abilities to transfer electrons and catalyze reduction might significantly relate to their solubility (Dror and Schlautman,
By adjusting pH or adding co-solvent (DFM), Co-TP-OHP, Co-T(benzoic)P, and Ni-TPP were soluble in the reaction systems, and PCE dechlorinations were dramatically enhanced by them.

Overall, these researches suggested that metalloporphyrinogens can catalyze reductive dechlorination by shuttling electrons from electron donors to chlorinated organic contaminants. The entire macromolecule should be one integral system, where electron transfer was not only by the core metal, but also through other locations (Dror and Schlautman, 2003). The steric effect and solubility of metalloporphyrinogens are also important to their catalyses.

2.3.3.2 Reduction of oxidized metals

No MP has been tested for catalyzing metal redox reactions, but previous studies reported that cytochrome C3 from Desulfovibrio vulgaris as a reductase was essential to U(VI) and Cr(VI) reductions (Lovley and Phillips, 1994; Lovley et al., 1993). The soluble protein fractions from the cell could reduce Cr(VI) faster than the membrane-bound fractions (Lovley and Phillips, 1994), which indicated that laboratory abiotic systems could have faster degradation rates than systems utilizing direct biological reduction reactions (Schanke and Wackett, 1992). Although no other published research reported using bacterial transition metal coenzymes, and naturally occurring MPs to catalyze metal reduction reactions, metalloporphyrinogens are promising mediators to this kind of reductions. In this research, MPs (Protoporphyrin IX and Uroporphyrin I with Fe(II) or Co(II) as the core metal) were tested for Cr(VI) reduction in the presence of
ZVI. The results from this work may give us a better understanding on how metalloporphyrinogens catalyze metal redox reactions.

2.4 Metalloporphyrinogens immobilization

2.4.1 Metalloporphyrin immobilization

As mentioned in Section 1.3.3, metalloporphyrinogens have been immobilized in layered minerals (e.g. hectorite, fluorohectorite, and double-layer hydroxides) or inorganic networks (e.g., sol-gel), adsorbed onto mineral surfaces (e.g., amorphous silica, talc) and resin beads, or bound to agarose by covalent bonds (Ukrainczyk et al., 1995; Burris et al., 1996; Dror et al., 2005; Cunningham et al., 2002). The catalytic capabilities of these immobilized transition metal macrocycles were tested to reductive dechlorination of chloro-organic contaminants (COC). Some found that the immobilized cobalamins and porphyrins were not reactive, or the reuse of immobilized catalyst decreased the reduction rates (Burris et al., 1996). However, others observed that the immobilized catalysts may be less reactive in the short-term experiments, but they were more reactive than homogeneous catalysts in the long-term experiments, or the lower rates were compensated by reusing the immobilized catalyst for many cycles (Ukrainczyk et al., 1995; Dror et al., 2005). In addition, it was shown in many studies that the heterogeneous reactions were comparable to their homogeneous reactions (Burris et al., 1996; Ukrainczyk et al., 1995; Dror et al., 2005). Therefore, the catalytic capabilities of immobilized catalysts are related to immobilization material, immobilization method, and
the target contaminants, etc. These factors may play together to affect the activities of matrices-encapsulated catalysts in electron transfer processes.

2.4.2 Sol-gel technology

Sol-gel matrices have been applied to immobilize metalloporphyrinogens. Sol-gel formation is based on polymerization of molecular precursors (e.g., metal alkoxides): the hydrolysis and condensation of alkoxides lead to the formation of metal oxopolymers (Eqns. 9 & 10; Asomoza et al., 1997).

\[
\begin{align*}
\text{Si(OEt)}_4 + 4\text{H}_2\text{O} & \rightarrow \text{Si(OH)}_x \text{(OEt)}_y + \text{EtOH} \quad (9) \\
\text{Si(OH)}_x \text{(OEt)}_y + \text{Si(OH)}_x \text{(OEt)}_y & \rightarrow \text{Si-O-Si} + \text{HOH} + \text{EtOH} \quad (10)
\end{align*}
\]

Because the primary components of sol-gel are silica and water, they are environmentally friendly and safe for remediation purposes (Zada et al., 2009). Sol-gel polymers display glass characteristics, and they can encapsulate organic molecules and larger nano-sized materials inside the inorganic network. Therefore, sol-gel matrices lead to hybrid organic-inorganic nano-composites (Sacco et al., 2001).

Tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS) are the most common precursors (Klotz et al., 1999). But TEOS was the most studied alkoxide because it is easily available and inexpensive. Also TEOS is less toxic than TMOS due to the organic solvent used (Sacco et al., 2001). Because ethanol was normally used to TEOS, methanol to TMOS and ethanol is less toxic than methanol. The preparation method studies have revealed that the water/TMOS or TEOS ratio, organic solvents, surfactants, pH, and aging temperatures were the main factors for sol-gel preparation (He et al., 2009;
Asomoza et al., 1997; Zada et al., 2009). First, the organic solvent (e.g., ethanol, methanol) is essential in the sol-gel recipe because water and alkoxide (e.g., TEOS, TMOS) are immiscible (Asomoza et al., 1997). Second, the hydrolysis of TMOS or TEOS was accelerated “in a slightly acidic medium”, which made the process of sol-gel formation faster (Zada et al., 2009). However, gelation at lower pH might make the sol-gel “nonporous” (Asomoza et al., 1997). Asomoza et al. (1997) compared three batches of sol-gel prepared at the same ratio of TEOS: ethanol: water (1:4:2.5), but at different pH (pH = 3, 7 or 9). They found that sol-gel-1 (prepared at pH 7) had the largest surface area (813 m²/g) among the three, sol-gel-3 (prepared at pH 9) only had the surface area 55 m²/g, but sol-gel-2 (prepared at pH 2) did not adsorb N₂ due to its texture characteristic. Third, temperature and pressure of the aging and drying processes may also play important roles for the characteristics of sol-gel. He et al. (2009) showed that supercritical drying process could prevent the pore collapse during solvents’ evaporation. This research group tried two-step process to make sol-gel: (1) mixing and autoclave at 100°C for aging; (2) supercritical CO₂ drying. Their sol-gels had large pore sizes and high pore volumes, and the surface areas were 1084 and 983 m²/g for two batches. In addition, Fe(III)-porphyrins were entrapped successfully in sol-gel matrices by Sacco et al. (2001), and porous structures of sol-gel were made at high gelation temperature (120°C).

The immobilized macromolecules in sol-gel matrices, which has been reported, include lecithins pheromone, metalloporphyrinogens, etc. (Galarneau et al., 2007; Zada et al., 2009; Sacco et al., 2001; Dror et al., 2005). Peach twig borer pheromone in sol-gels
prepared by various formulations was released relative constantly, which were approximately 14 – 45 µg/day for 28 days (Zada et al., 2009). The researchers suspected that the various pore sizes maybe responsible for the difference. Also the structures of precursors may be affected by the crosslink in the polymerization process, resulting in different cavities in sol-gel (Zada et al., 2009). Zada et al. hypothesized that sol-gel pore sizes could be optimized through the modified immobilization method, which may achieve an ideal release rate for a specific molecular. In addition, a serial of tetraphenyl metalloporphyrins were immobilized in sol-gel matrices, and the reductive dechlorination of PCE, TCE and CT catalyzed by those metalloporphyrins was examined heterogeneously and homogeneously (Dror et al., 2005). The reduction activities of sol-gel immobilized catalysts were similar or lower compared to the reactions in homogeneous systems. However, Vitamin B<sub>12</sub> immobilized by sol-gel could be reused at least twelve cycles (24 hr/cycle). Therefore, sol-gel matrices preserved the catalytic capabilities of metalloporphyrinogens, which compensated the lower reduction rates. However, the immobilized transition-metal macrocycles in sol-gel or other materials have not been applied to metal redox reactions, which is important to waste treatment. Because the metal redox reactions may produce precipitations as the metal oxidation states change, and this is different with COC dechlorination. Therefore, the immobilized catalyst may have different effects on metal redox reactions. In this study, sol-gel immobilized metalloporphyrinogens were prepared and tested for Cr(VI) reduction in the presence of reducing agent.
2.5 Calcium alginate hydrogel

Another promising matrix for the immobilization of catalysts or solid reductants is Ca-alginate. Alginate is a natural polysaccharide, a linear block copolymer of α-D-mannuronic acid and α-L-guluronic acid (Foil et al., 1995). Its carboxylate groups can bind divalent cations in the interchain cavities producing cross-linked complexes, and then forming porous polymeric hydrogel (Grant et al., 1973; Velings and Mestdagh 1995; Foil et al., 1995). Ca-alginate is nontoxic, biodegradable, and nonimmunogenic, so that it has been widely applied as absorption or immobilization matrices in food industries, drug deliveries, and environmental remediation applications (Bezbaruah et al., 2009).

Ca-alginate gel is water insoluble, thermally irreversible, so its gel beads were prepared by dropping sodium alginate solution into a divalent cations solution (e.g., Ca$^{2+}$) (Velings and Mestdagh 1995, Ouwerx et al., 1998). The affinity between Ca$^{2+}$ and alginate is moderate, which allows Ca$^{2+}$ cation to continuously diffuse into the beads during maturation (Velings and Mestdagh, 1995). Therefore, the surface of Ca-alginate beads was more smooth and uniform. But copper-alginate affinity is about ten times stronger than calcium-alginate, so that Cu-alginate beads could not complex uniformly and had rough surfaces (Velings and Mestdagh, 1995). Moreover, the pore sizes of the Ca-alginate gel beads were reported to be 3.17 – 5.07 nm (Benerjee et al., 2007). Therefore, macromolecules (up to MW = 20,000) were capable of diffusing into Ca-alginate gel beads (Tanaka et al., 1984).

Alginate was applied to immobilize microbial cells, so that they could survive in the environment of having high concentrations of contaminants, and could remove the
contaminants effectively (reviewed by Chapatwala et al., 1993). The same research group investigated inorganic cyanides degradation by free cells, alginate-, agar- and carrageenan-immobilized cells. Alginate-immobilized cells exhibited a greater degradation capacity to NaCN than other bio-beads and free cells, which indicated that the alginate-immobilized cells were highly stable, and there were “the rapid exchanges of substrates and products through the alginate matrix” (Chapatwala et al., 1993). Moreover, a mixture of Fe(III) and Ni(II) (hydr)oxides were successfully immobilized in Ca-alginate beads, which enhanced the absorption of As(III) and As(V) in waste-water at pH 6-9 (Escudero et al., 2009). As(III) species (mainly \( H_3AsO_3 \)) interacted with the free hydroxyls on the sorbent, while As(V) (mainly \( H_2AsO_4^- \)) combined with the positively charged group (\(-\text{OH}_2^+\)) through electrostatic attraction, or by specific coordination to produce low solubility compounds with metal hydroxides (\(-\text{MOH}_2^+\)). Escudero et al. (2009) analyzed the sorption data using Langmuir model, and the maximum amount of arsenic sorbed per mass unit of hydroxides \( (q_{\text{max}}, \text{mg/g}) \) was increased 60% by entrapment of iron oxyhydroxide in Ca-alginate beads. But the role of gel matrix on the additional arsenic sorption needs to be clarified in the further study (Escudero et al., 2009).

Another study entrapped iron nanoparticles in Ca-alginate beads, and evaluated the immobilized nanoparticles by testing them for nitrate reduction (Bezbaruah et al., 2009). In this research, SEM and TEM images revealed that the surface of gel beads was undulate, and the pore sizes were not uniform. Also the n-ZVI in the gel matrix was not distributed uniformly, and more n-ZVI was immobilized in the place where Ca-alginate
formed densely. Nitrate at three different concentrations were reduced by bare n-ZVI and n-ZVI gel beads. In 2 hours, the encapsulated n-ZVI displayed similar reactivity with bare n-ZVI for all three experiments. These results indicated that this entrapment was able to make n-ZVI particles relatively stationary in aqueous media, reducing the mobility and sedimentation problems of the n-ZVI particles. Therefore, this technology is promising to be applied to in PRBs for groundwater remediation (Bezbaruah et al., 2009). However, this kind of n-ZVI gel beads has not been tested for metal redox reduction (e.g. Cr(VI) reduction), which may produce low solubility metal hydroxides (Cr(III)/Fe(III) hydroxides). Therefore, the products may affect substrate exchange through Ca-alginate matrix, and reduce the reactivity of n-ZVI immobilized in gel beads. These questions were addressed in this study, which n-ZVI entrapped in Ca-alginate beads were tested to Cr(VI) reduction.
CHAPTER THREE

EXPERIMENTAL SECTION

3.1 Chemicals & Instruments

Chemicals used in this study were potassium chromate (99.0%, ACS, Alfa Aesar), iron (III) chloride hexahydrate (97%, Aldrich), sodium borohydride (>98%, Acros), cobalt(II) acetate tetrahydrate (≥99%, Fluka), iron(II) acetate (95%, Acros Organics), tetrathyethyl orthosilicate (98%, Acros Organics), sodium alginate (Spectrum), calcium chloride (GR, EM Science), sodium hydroxide (>98%, Sigma-Aldrich) and 1,5-diphenylcarbazide (99%, Alfa Aesar). All chemicals were used as received. Commercial zero-valent iron powder (100 mesh) was purchased from Mallinckrodt, and they were pretreated before use. Protoporphyrin IX (>97%, Figure 1.2 a) and Uroporphyrin I dihydrochloride (>97%, Figure 1.2 c) were purchased from Frontier Scientific.

Goods buffers used in this research include 3-n-morpholino propanesulfonic acid (MOPS, EMD), Bis(2-hydroxyethyl)amino-tris(hydroxymethyl) methane (BIS-TRIS, > 98%, Sigma), and 2-amino-2-(hydroxymethyl)-1,3-propanediol (Trizma-base, ≥ 99.9% (titration), Sigma). Cyanocobalamin (Vitamin B<sub>12</sub>, 99%, Figure 2.1) was obtained from Sigma. Other reagents were acetone (ACS, BDH), ethanol (Reagent, BDH), ethyl acetate (AR, ACS, Mallinckrodt), sulfuric acid (AR, ACS, Mallinckrodt), hydrochloric acid (ACS, BDH), glacial acetic acid (>99%, Sigma), and nitric acid (Aristar Ultra, BDH) for the preparation of ICP-MS (Inductively coupled plasma mass spectrometry) samples.
Distilled de-ionized (DDI) water was purified by a Milli-Q water system (18 MΩ·cm). Deoxygenated DDI water was prepared by purging with nitrogen for 12 hrs, and then storing in an anaerobic chamber (COY Laboratory Products INC.). The oxygen concentration in the chamber was monitored by an oxygen meter (COY Laboratory Products INC). The pH values were measured with a 420A model pH meter (Orion) using an Accumet combination pH electrode (Cole-Parmer). Other instruments used in this study were a Varian 50 Bio UV-Visible spectrophotometer, a rotary evaporator (RE47) with a water bath (BM100) (Yamato Scientific Co. Ltd), an ASAP 2010 surface area analyzer, an Inductively coupled plasma mass spectrometry (ICP-MS, X Series II, Thermo), a scanning electron microscope (SEM 3400 S-3400N), a field emission scanning electron microscope (FESEM-Hitachi S4800), an energy dispersive X-ray spectrometer (EDS, with FESEM-Hitachi S4800) for surface elemental analysis, and a particle size analyzer (Brookhaven Instruments Corp., 90 Plus particle sizing software Ver. 4.14). A freeze dryer used for drying the sol-gel samples was from VirTis (BenchTop 6K).

3.2 Micro-sized zero valent iron pretreatment

The purchased m-ZVI was pretreated by washing with acid and acetone according to Qian et al. (2007). A pH 2 sulfuric acid solution was prepared using DDI water. Then 10 g m-ZVI (>100 mesh, <150 µm) was mixed with 100 mL of the acid wash solution by stirring with a glass rod mixer for 2 to 3 minutes. The process was followed by washing with 100 mL acetone. Then, m-ZVI was rinsed with DDI water several times until the pH
was around 6.5. The washed m-ZVI was dried in an oven at 105°C for 1.0 hour under a nitrogen atmosphere. A subsample was weighed and degassed at room temperature and the surface area determined by nitrogen absorption on the ASAP 2010 surface area analyzer.

Three batches were prepared following the procedures above. Batch A was the purchased m-ZVI particles used without sieving, which was washed and placed in an Erlenmeyer flask for drying in the oven. Then, the Batch A m-ZVI was stored on the bench top in a screw capped vial. Batch B was pretreated the same way as Batch A, except that it was then stored in the anaerobic chamber. Batch C m-ZVI was first sieved (120 - 140 mesh, or 105 - 125 µm) and then pretreated. Batch C was dried in the oven using a bottle sealed by an open-top screw cap with PTFE/silicone septa with N₂ purging through the hole in the septa.

3.3 Nano-sized zero valent iron synthesis

n-ZVI was synthesized by sodium borohydride (3.6 M, 50 mL) reduction of ferric chloride (1.2 M, 50 mL), adopted from the method reported by Song and Carraway (2005). The reaction proceeds as follows (Eqn. 11, Song, 2003):

$$\text{Fe(H}_2\text{O)}_6^{3+} + 3\text{BH}_4^- + 3\text{H}_2\text{O} \rightarrow \text{Fe}^0 \downarrow + 3\text{B(OH)}_3 + 10.5 \text{H}_2 \text{ (11)}$$

All reagent solutions were prepared in the anaerobic chamber using deoxygenated DDI water, and then the solutions were moved to a fume hood. The NaBH₄ solution was pumped into FeCl₃ solution using a peristaltic pump at a delivery rate of 1.25 mL/min. The FeCl₃ solution was stirred vigorously using a magnetic stirrer, and the n-ZVI was
formed as black precipitate immediately after the two solutions were mixed. The reaction was preceded with N₂ flowing through the reactor, which continued until the reaction was completed.

The reactor was moved to the anaerobic chamber after the reaction was completed. The final pH of the reacted solutions was adjusted to 2.5 (Batch I) or 4.0 (Batch II) by adding 0.1 mol/L HCl to dissolve iron oxides formed during the synthesis process. Then, the n-ZVI was washed with deoxygenated DDI-water followed by washing with acetone. The iron was dried in the oven under N₂ atmosphere at 105°C for 4 hours, transferred to the anaerobic chamber, pulverized and stored in vials.

A small amount of n-ZVI was taken and added to DDI water. The mixture was sonicated 15 min, and then the particle sizes were analyzed by a particle size analyzer. In addition, the n-ZVI particles were observed by FESEM and STEM. For the observation under FESEM mode, the n-ZVI dry power was stuck on the carbon tape of an aluminum holder. For the observation under STEM mode, the n-ZVI particles were sonicated in 100% ethanol solution for 15 min, and the solution mixture was dropped to a cupper screen. The purity of the synthesized n-ZVI was determined by FESEM-EDS and ICP-MS. The sample preparation method for FESEM-EDS was the same as for FESEM. For ICP-MS measurement, the n-ZVI was dissolved in 2% HNO₃ solution, and then diluted to proper concentration (10-100 ppb) using 2% HNO₃ solution.
3.4 Metalloporphyrin synthesis

A reflux process (Dror and Schlautman, 2003) was applied to synthesize MPs due to slow complexation in water (Nielsen, 2006). A 50% ethanol solution was prepared by diluting 500 mL anhydrous ethanol to 1000 mL with DDI water. Co(II) or Fe(II) acetate stock solutions (0.004 mol/L) were prepared by adding about 100 mg Co(II) acetate tetrahydrate or 70 mg Fe(II) acetate to 100 mL DDI water. A weighed amount (5.6 mg nominal) of Protoporphyrin IX was dissolved in 200 mL 50% ethanol, and the spiked with 5 mL Co (II) or Fe (II) acetate stock solution. The pH was adjusted to 2.5 – 3.0 by adding glacial acetic acid. The mixture was sonicated for 5 minutes, and then refluxed 4 hrs for Co(II) and 2 hours for Fe(II). Similarly, a weighed amount (9.3 mg nominal) of Uroporphyrin I dihydrochloride was dissolved in 200 mL 50% ethanol, and spiked with 5 mL Co(II) or Fe(II) acetate stock solution. The pH was adjusted to approximate 3.0 by adding glacial acetic acid. Then the mixture was sonicated for 5 minutes and then was refluxed 4 hours for Co(II) and 2 hours for Fe(II).

After reflux, the solutions were cooled to room temperature. Then, an aliquot was taken and scanned on the UV-Vis spectrophotometer. The absorption curve was compared to the one before reflux to verify MP formation. To further confirm completion of the synthesis, liquid/liquid separation and solid phase extraction were applied to obtain the free cation for further quantification. First, all solvents present in the solutions were removed using the rotary evaporator. Then, 50 mL ethyl acetate was added to dissolve the MPs, and 50 mL DDI was added to extract the free cation from the organic phase. This was repeated three times. To remove the trace amount of MPs from the aqueous phase,
the aqueous solutions were pass through high performance extraction disks (3M Empore) in a cartridge. Finally, the aqueous solutions were diluted and the concentrations of free metals were quantified by ICP-MS. The MP yields were calculated by dividing the lost metal concentration (total mass of metal added - the amount detected) by the porphryin concentration.

3.5 MPs immobilization in sol-gel matrices

Sol-gel immobilized MPs were prepared through a gelation and drying process, which was modified from the method reported by Dror et al. (2005). A 45 mL mixture of TEOS: Ethanol: MPs (1mM) aqueous solution (7.5: 10: 5, volume ratio) was mixed vigorously using a magnetic stir bar. The gelation took approximate 10 hours to complete at room temperature. Then the gel was poured into a Petri dish to dry in a fume hood until it achieved a constant weight, which took around a week. Another batch of VB_{12} sol-gel was prepared using the same method but after gelation the sol-gel sample was dried using a freeze dryer for 60 hours (55 millitorr, -78 °C). Blank sol-gels were prepared using water instead of MPs (1mM) aqueous solution following the same procedures (dried at room temperature or by freeze drying). Then the sol-gel samples were ground and sieved through a screen (100 mesh). The particles were stuck to the carbon tape on the aluminum holder and observed by FESEM.
3.6 MPs immobilization in Ca-alginate beads

Following the method reported by Bezbaruah et al. (2009), MP immobilization in Ca-alginate beads was modified as below. Ca-alginate beads with immobilized MPs were prepared by dripping VB\(_{12}\) containing gel into CaCl\(_2\). A VB\(_{12}\) 50 mL 50 µM aqueous solution (using oxygen free DDI water) was mixed with 1 g sodium alginate in an Erlenmeyer flask. Then the flask was put into a 40°C water bath for about 3 hours until the gel was totally dissolved to a uniform and transparent state. The gel solution was transferred to the anaerobic chamber, and cooled down for about 0.5 hrs until almost no air bubbles were visible. A 3.5% (g/volume) CaCl\(_2\) aqueous solution was prepared using oxygen free DDI water in the anaerobic chamber. The gel containing VB\(_{12}\) was withdrawn into a 30 mL syringe and added to 500 mL CaCl\(_2\) aqueous solution as single drops. The gel drops were formed as beads in the solution while stirring. The beads were hardened in the solution overnight (about 12 hours), then filtered, and then dried in the chamber for 48 hours. Blank beads were prepared using water instead of VB\(_{12}\) through the same method.

3.7 n-ZVI immobilization in Ca-alginate beads (n-ZVI beads)

A similar process as preparing Ca-alginate beads with immobilized MPs was employed to incorporate n-ZVI into Ca-alginate beads. Oxygen free DDI water 50 mL was mixed with 1 g sodium alginate in an Erlenmeyer flask. Then the flask was put into the 40°C water bath for about 5 hours, until the gel was totally dissolved to form a uniform and transparent state. Then the gel solution was transferred to the anaerobic
chamber, and cooled down for about 0.5 hour until almost no air bubbles were left. The n-ZVI (Batch II) 250 mg was added to the gel solution to mix under sonication for 30 min. The n-ZVI-containing gel was withdrawn into a 30 mL syringe and added as single drops to 500 mL 3.5% (g/volume) CaCl$_2$ aqueous solution, which was prepared in the anaerobic chamber. The gel drops were formed as beads in the solution while stirring. The n-ZVI immobilized beads were hardened in the solution for at least 6 hours, then filtered, and dried in the chamber for 48 hours. The maturation is the crosslinking reaction happened during the gel formation, which took about 6 hours (Velings and Mestdagh, 1995).

3.8 Cr(VI) reduction experiments

Cr(VI) reduction by sodium dithionite, Vc, or m-ZVI was screened to find the proper reaction system to test the catalytic capacity of MPs and VB$_{12}$. Then, the dissolved MPs, VB$_{12}$, and VB$_{12}$ encapsulated in sol-gel were used to catalyze Cr(VI) reductions by m-ZVI. Also, reduction of Cr(VI) was observed in VB$_{12}$ solutions at the presence of n-ZVI and n-ZVI immobilized in Ca-alginate beads. The overall experiments are listed in Table 3.1.

All the reactions were conducted at room temperature. The reduction by sodium dithionite was on the bench top and using an Erlenmeyer flask as a reactor. Vc solution was prepared by O$_2$ free DDI water and the reaction was also on the bench top, but with N$_2$ purging to keep from O$_2$. The reacting solutions were mixed by a magnetic stirrer. Conversely, m-ZVI, n-ZVI, and n-ZVI Ca-alginate beads were added to the reactors.
(amber glass bottles) in the anaerobic chamber. Reactions were initiated by adding chromate solutions (prepared with oxygen free DDI water). Then all reactors were sealed in the anaerobic chamber by polypropylene open-top screw caps with PTFE/silicone Septa, before they were moved to the end-over-end tumbler (50 rpm). The aliquots were taken by a syringe going through the septa, which still could keep the anaerobic environment inside the reactor.

Table 3.1 List of Cr(VI) batch reductions in the presence of reducing agent with/without metalloporphyrinogens

<table>
<thead>
<tr>
<th>Reductant</th>
<th>Amount of reductant</th>
<th>pH</th>
<th>metalloporphyrinogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂S₂O₄</td>
<td>35 µM (0.9 stoi.)</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>40 µM (1 stoi.)</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>m-ZVI</td>
<td>5.0 g/L (Batch A)</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3.4 g/L (Batch A)</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.5 g/L (Batch A)</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch A)</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch A)</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch A)</td>
<td>7.0</td>
<td>10,20, or 30 µM VB₁₂</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch A)</td>
<td>8.0</td>
<td>30 µM VB₁₂</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch A)</td>
<td>7.0</td>
<td>20 µM VB₁₂</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch B)</td>
<td>7.0</td>
<td>20 µM Proto-Co/Proto-Fe</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch B)</td>
<td>7.0</td>
<td>20 µM Uro-Co/Uro-Fe</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L (Batch B)</td>
<td>7.0</td>
<td>20 µM VB₁₂ sol-gel</td>
</tr>
<tr>
<td></td>
<td>1.7 g/L reuse (Batch C)</td>
<td>7.0</td>
<td>with or without 20 µM VB₁₂</td>
</tr>
<tr>
<td>n-ZVI</td>
<td>0.1 g/L (Batch II)</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.1 g/L (Batch I &amp; II)</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.1 g/L (Batch II)</td>
<td>7.0</td>
<td>0.1, 0.5 µM VB₁₂</td>
</tr>
<tr>
<td></td>
<td>0.1 g/L (Batch II)</td>
<td>7.0</td>
<td>0.1, 0.5 µM VB₁₂ sol-gel</td>
</tr>
<tr>
<td>n-ZVI (Batch II) immobilized in Ca-alginate beads</td>
<td>0.1 g/L immobilized</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.1 g/L immobilized reuse</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.1 g/L immobilized</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.1 g/L immobilized</td>
<td>7.0</td>
<td>5 µM VB₁₂</td>
</tr>
</tbody>
</table>

Notes: The initial concentration of Cr(VI) solutions was approximately 25 µM (100 mL) for the reductions by Na₂S₂O₄ and Vc, and 100 µM 100 mL for the others.
3.8.1 Electron donor screening

Sodium dithionite, Vitamin C, and m-ZVI were chosen as electron donors. All of them were screened to Cr(VI) reduction to find the proper reduction system to test MPs. Potassium chromate stock solution was 250 µM, prepared by dissolving 25 mg (nominal) potassium chromate to 50 mL DDI water, and diluting 10 times. Sodium dithionite 10 mg (nominal) was weighed and dissolved in 50 mL DDI water. Then 10 mL potassium chromate stock solution was diluted to 50 mL, and 3 mL sodium dithionite stock solution was diluted to 50 mL. The reaction was on the bench top, initiated by mixing these two solutions (approximate 25 µM chromate and 0.9 stoichiometric amount of dithionite) to a reactor. The aliquots (1 mL) were taken at chosen time, and the pH was detected using a pH probe.

Because Vc is readily oxidized by oxygen, metals, light and heat, Cr(VI) reduction by Vc was conducted under N₂ atmosphere (N₂ purging to the reactor by a frit). The reactor was covered with foil to minimize light exposure and the mouth was covered with parafilm. All solutions were prepared using oxygen free DDI water. A Vc stock solution (approximate 1 mM) was prepared before use by adding 6.8 mg Vc to 10 mL DDI water. Then, 2 mL of the potassium chromate stock solution (2.5 mM) was diluted to 100 mL and 2 mL of the Vc stock solution was diluted to 100 mL. A VB₁₂ stock solution was prepared by adding 10 mg VB₁₂ to 20 mL oxygen free DDI water. Similarly, the reaction was initiated by mixing these two solutions (approximate 26 µM chromate and one stoichiometric amount of Vc) at initial pH 5. Then, pH effects to Cr(VI) reduction by Vc were tested by mixing approximate 26 µM chromate and one
stoichiometric amount of Vc. The initial pH was adjusted by adding KOH to approximately 8 or 10. VB_{12} stock solution 10 mL was spiked to K_{2}CrO_{4} solution, which resulted in 20 µM VB_{12} in the reaction system. VB_{12} at this concentration was used to catalyze the reaction at pH 8 and 10. The aliquots (5 mL) were taken from the reactor at chosen time, and the pH was monitored by a pH probe.

The m-ZVI was pretreated using acid and acetone as mentioned in Section 3.2 (Batch A). Cr(VI) reaction with m-ZVI was proceeded at a pH 7 aqueous solution buffered by 0.05 M MOPS. The MOPS solution (0.05 M, pH 7) was prepared by dissolving MOPS (10.50 g) to 1 L oxygen free DDI water, and the pH was adjusted by adding 1 N NaOH. Cr(VI) stock solution (2 mM) was prepared by adding 19.4 mg K_{2}CrO_{4} to 50 mL buffer solution (pH 7). Then 25 mL Cr(VI) stock solution was spiked and diluted to 500 mL, resulting in 100 µM Cr(VI) solution. The m-ZVI was weighed approximate 170 mg to the reactor in the anaerobic chamber. Then the reaction was initiated by adding Cr(VI) solution (100 µM, 100 mL) to the reactor. The aliquots (5 mL) were taken from the reactor at chosen time.

3.8.2 Preliminary experiments using m-ZVI

In preliminary experiments, the proper amounts of m-ZVI and pH were examined. Cr(VI) (100 µM, total volume 100 mL) reacted with 200, 300, 400 or 600 stoichiometric amounts of m-ZVI (Batch A) at pH 7, which were 170 mg (1.7 g/L), 250 mg (2.5 g/L), 340 mg (3.4 g/L), 500 mg (5.0 g/L) m-ZVI respectively. Moreover, the same amount of
Cr(VI) reduced by 1.7 g/L m-ZVI at pH 8 was studied. Cr(VI) concentrations with time were monitored for all experiments.

3.8.3 MPs-catalyzed Cr(VI) reduction by m-ZVI

Based on the results from the experiments described above, appropriate reaction conditions were chosen for further studies to test MP and VB$_{12}$ catalysis on Cr(VI) reduction by m-ZVI (Batch B). Different concentrations of dissolved VB$_{12}$ were tested first. Then dissolved MPs and sol-gel immobilized VB$_{12}$ with comparable concentrations were used to catalyze Cr(VI) reduction. Finally, m-ZVI (Batch C) was reused with and without VB$_{12}$ in the system.

When VB$_{12}$ was used to catalyze the reaction, 1 mM VB$_{12}$ stock solution was made first by dissolving 13.55 mg VB$_{12}$ in 10 mL oxygen free DDI water. Then it was stored in an amber vial in the anaerobic chamber. Various amount of VB$_{12}$ stock solution was spiked to 100 µM Cr(VI) buffered solution, and well mixed by a magnetic stirrer before reacting with the reductant. For MPs, synthesized MPs 0.01 mmol was dissolved in 500 mL buffered solution to produce 20 µM MPs solutions. Then Cr(VI) stock solution 5 mL was spiked to 100 mL MPs solution. The mixture was totally mixed uniformly, and added to the reactor. Decreases in Cr(VI) concentration were measured with time. Then Cr(VI) removal (%) and pseudo-first order rate constants for the reduction kinetics were calculated.
3.8.4 MPs-catalyzed Cr(VI) reduction by n-ZVI

Because n-ZVI is much more reactive than m-ZVI, less of it (0.1 g/L) was needed to reduce 100 µM Cr(VI). The n-ZVI (Batch II) reacted with Cr(VI) at pH 6 and 7. Dissolved VB_{12} (0.1 or 0.5 µM) and VB_{12} immobilized in sol-gel were added to catalyze the reaction at pH 7 (Batch II n-ZVI). Also Ca-alginate encapsulated n-ZVI (Batch II) was used to Cr(VI) reductions at pH 6 and 7. The reaction at pH 7 was catalyzed by dissolved VB_{12}. What is more, the gel beads were reused multiple times at pH 6, in order to evaluate the application potential of this n-ZVI gel beads.

3.9 Analytical method of Cr(VI)

According to the standard analytical method for Cr(VI) (APHA et al., 2005), a modified diphenyl carbizide (DPC) technique was applied in this research to monitor the reduction of Cr (VI). The detection principle is that aqueous Cr(VI) reacts with DPC in an acid solution (pH = 2 ± 0.5), and produces a red-violet colored complex, which has strong UV/Vis absorption at 542 nm, but the Cr (III) does not form this complex.

The acid solution was made of 10% sulfuric acid and phosphoric acid (1:1, v/v). DPC 50 mg was dissolved in 10 mL acetone. A drop of acid solution and 100 µL DPC acetone solution were added to a 10 mL Cr(VI) solution. The pH of the solution was adjust to 2.0 - 2.5, and Cr(VI) formed the complex with DPC. The samples were stabilized for 5 to 10 minutes, and then analyzed by Varian 50 spectrophotometer at 542 nm. A series of standard Cr (VI) solutions from 0.7 µM to 77µM were prepared to obtain a calibration curve. The lower detection limit of this method was approximately 0.7 µM.
The aliquots taken from the reactors were filtered through syringe filters (0.2 µm, Nylon membrane, VWR). As described above, a drop of acid solution and 100 µL DPC acetone solutions were added to the filtered samples. After 5 to 10 minutes, the samples were determined at 542 nm. The Cr(VI) concentrations were calculated according to Cr(VI) calibration curve (absorption Vs. concentration). Because of the colors of MPs and VB_{12}, and their absorption at 542 nm, the background absorption (only MPs/VB_{12} and chromate) was subtracted from the samples.

3.10 Data Analysis

The Cr(VI) concentrations vs. time were plotted for all the reactions. The Cr(VI) removal (%) in 200 min was calculated to the reactions in the presence of m-ZVI. In addition, the rate constant was calculated using the pseudo-first order model. Because rapid drops were observed at the beginning for these reactions, the kinetic data were modeled as two time periods or not including the rapid drop. There were linear relationships between Ln[Cr(VI)] and reaction time (min), the slopes of which were reaction rate constants. All rate constants and Cr(VI) removals for the different experimental conditions were compared by ANOVA (Minitab). Fisher’s least significance difference test was used to identify significant difference among the different results. The parameters were set as one-way multiple comparisons, and Fisher’s individual error rate was equal to 0.05.
CHAPTER FOUR
MATERIAL CHARACTERIZATION

4.1 m-ZVI characterization

The m-ZVI (<100 mesh) was washed by acid and acetone as described in 3.2. This pretreatment removed iron oxides and oil from the surface of the iron powder, which makes it more reactive (Qian et al., 2007). A subsample of the cleaned m-ZVI (Batch B) was weighed and degassed at room temperature. The surface area was measured by N₂ adsorption. According to the BET method, the m-ZVI used in this research has a surface area of 0.6742 ± 0.0023 m²/g (surface area ± model fitting error). This result is close to values previously reported in the literature, namely, that m-ZVI has a surface area approximately equal to 1 m²/g (Bezbarah et al., 2009).

4.2 n-ZVI characterization

The n-ZVI was prepared following to the method reported by Song (2003). The dried and pulverized particles (<0.5 mg) were added to a 1-cm cuvette, followed by adding DDI water, and sonicating for 10 min. Then, the particle sizes were determined by a particle size analyzer, and the results are shown in Figure 4.1. Most of the n-ZVI particles were in the range 200 – 300 nm according to the number-weighted distribution. Because iron particles settled quickly after sonication due to their high density and magnetic interactions, what the particle size analyzer detected were the sizes of agglomerated particles. However, field emission scanning electron microscopy (FESEM)
revealed that the primary particle size was in the range 30-50 nm (Figure 4.2 a & b). The FESEM technology enabled us to see single particles, and read the sizes by the scale. The images showed that n-ZVI particles were agglomerated, even for the STEM sample that was sonicated (in Section 3.3).

Figure 4.1 particle size distribution of n-ZVI (Batch II) by particle size analyzer

![Particle Size Distribution](image)

Figure 4.2 FESEM and STEM images of n-ZVI (Batch II)

a. n-ZVI image in FESEM mode  

b. n-ZVI image in STEM mode
Furthermore, the purity of n-ZVI particles was determined. First of all, the surface elements of n-ZVI were analyzed by the energy dispersive X-ray spectroscopy (EDS) with FESEM. For Batch II, iron accounted for 92.3%, oxygen was about 7.7%, and no boron was detected (Figure B-1 & Table B-1 in Appendix B). The n-ZVI surface may have been contaminated by oxygen during the sample preparation for FESEM (in Section 3.3), which resulted in high oxygen concentration. Secondly, n-ZVI (Batch I & II) was weighed approximate 1 mg, and diluted to 10 mL using 2% HNO₃ solution (high purity acid). Then, the samples were diluted 1000 times and detected on ICP-MS. The results showed that $^{56}$Fe was about 99.31 ± 0.26%, and boron was about 0.031 ± 0.004% (Table 4.1). The boron concentration was semi-quantitative, because the background counts were high due to helium as the carrier gas. However, the overall results demonstrated that the n-ZVI was essentially pure with only a trace amount of boron. All these are close to 92.0 ± 0.4%, and 7.0 ± 0.4% for iron and boron respectively, reported by Song (2003).

Surface area was measured by N₂ adsorption, and the n-ZVI had a BET surface area of 26.93 ± 3.40 m²/g (mean ± standard error for Batch I & II, Table A-1 in Appendix A), which is about 40 times higher than the m-ZVI used in this work. The n-ZVI has a surface area close to that reported by Song (2003) 27.88 ± 1.69 m²/g (mean ± standard deviation), and by Bezbaruah et al. (2009) 25-54 m²/g. Batch I and II did show similar characteristics mentioned above, except yield and reactivity (reactivity shown in Section 5.3.1). The Batch I yielded approximately 1 g n-ZVI, but Batch II yielded approximately 4 g n-ZVI.
Table 4.1 Element analysis of n-ZVI by ICP-MS

<table>
<thead>
<tr>
<th>Sample</th>
<th>$^{56}\text{Fe} %$</th>
<th>$^{11}\text{B} %$</th>
</tr>
</thead>
<tbody>
<tr>
<td>050509$^a$</td>
<td>98.86</td>
<td>0.039</td>
</tr>
<tr>
<td>051709$^b$</td>
<td>99.76</td>
<td>0.024</td>
</tr>
<tr>
<td>061909$^b$</td>
<td>99.30</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Mean ± Standard error 99.31 ± 0.26 0.031 ± 0.004

Notes: a. n-ZVI was prepared using the method described as Batch I.

b. n-ZVI was prepared using the method described as Batch II.

4.3 n-ZVI Ca-alginate beads characterization

The n-ZVI beads were prepared according to the method reported by Bezbaruah et al. (2009). After maturing in a CaCl$_2$ solution overnight, the beads had diameters of about 4 mm, and were elastic. Upon drying, the beads shrank (~ 3 mm) and hardened. An FESEM graph (Figure 4.3) shows the size and general surface morphology of the dried Ca-alginate beads. The surface was rough and cracked.

Bezbaruah et al. (2009) observed heterogeneous pore sizes because of a non-uniform crosslink between Ca$^{2+}$ and alginate, revealed by their high resolution SEM images. n-ZVI agglomeration in their beads was also observed in their TEM images. These characterizations were not done in the present study.
4.4 Synthesized metalloporphyrins

Four MPs were synthesized by refluxing two different free-base porphyrins (Protoporphyrin and Uroporphyrin) to incorporate two different metals, Co(II) or Fe(II). The MP complexes formed were Protoporphyrin-Co(II)/Fe(II) (Proto-Co/Fe) and Uroporphyrin-Co(II)/Fe(II) (Uro-Co/Fe). Complete metal incorporation was indicated visually by the color change and spectroscopically with UV-Vis spectra. The Proto-Co solution started as a bole color that was similar to the color of the trunk of a tree, and after reflux became a deep chestnut color that was similar to the dark red skin of a chestnut. The Proto-Fe solution had a chocolate color before reflux and turned into a rich maroon after reflux. Uro-Co had a terra cotta color before reflux and became Persian red.
when the reaction was finished. Uro-Fe was bronze and became a rust color (red-orange) that was similar to the color of iron oxide.

In addition, the solution mixture was scanned from 200 - 800 nm by UV-Vis spectrophotometer. The spectra of normalized absorption vs. wavelength were shown as Figure 4.4, 4.5, 4.6 and 4.7. It was observed that the Soret bands of Proto-Co and Proto-Fe hypo-shifted, from 406 nm to 395nm, and from 405 nm to 396 nm, respectively (Figure 4.4 & 4.5). The Q band absorption increased in the range of 450 – 600 nm. Figure 4.6 shows the maximum absorption peak of Uro-Co hyper-shifted from 400 nm to 410 nm, but the Soret peak of Uro-Fe was hypo-shifted from 415 nm to 400 nm, shown in Figure 4.7. The evident changes happened in the Q bands (450 – 600 nm) as well.
Figure 4.4 Spectra of Protoporphyrin IX and Co(acetate)$_2$ in 50% Ethanol. The ratio of Protoporphyrin IX to Co(II) is 1:2.
Figure 4.5 Spectra of Protoporphyrin IX and Fe(acetate)$_2$ in 50% Ethanol. The ratio of Protoporphyrin IX to Fe(II) is 1:2.
Figure 4.6 Spectra of Uroporphyrin I and Co(acetate)$_2$ in 50% Ethanol. The ratio of Uroporphyrin I to Co(II) is 1:2.
The ratio of Uroporphyrin I to Fe(II) is 1:2.

When the reaction was completed, the concentration of free metal cation (Co$^{2+}$ or Fe$^{2+}$) in the aqueous phase was measured by ICP-MS to calculate the yield of MPs from porphyrins. Because ICP-MS can detect both free irons and the core metals in the MPs complexes, separation processes were required. The procedures were described in Section 3.4, and the results are listed in Tables 4.2 and 4.3. Three blanks were prepared the same way as MP refluxed samples. The recoveries of Co and Fe for the liquid/liquid separation were 92.37 ± 0.22% and 94.39 ± 1.44% respectively. These indicated that this extraction method was reliable, and ethyl acetate could keep the majority of free ions in the aqueous
phase. Also the recoveries of free metal for solid phase extraction were reasonable, which was 94.44 ± 0.09% for Co and 93.26 ± 0.46% for Fe. The collective results suggested that the two-step separation was reliable. The MP reflux samples were separated using the same methods. The MP yield was calculated as [MP] (= [complexed metal]) / [virgin porphyrin]. As listed in Table 4.3, the yields were 98.55± 1.75% for Proto-Co, 100.64 ± 3.59% for Proto-Fe, 98.93 ± 2.86% for Uro-Co and 101.19 ± 2.42% for Uro-Fe. The data reported here are the averages and standard errors of two measurements. The blank recovery losses were not used in yield calculation. But the numbers are higher than the blank (Table 4.2), the reason maybe because the metal concentration in the blank solution was higher (no metal was complexed) and made recovery lower. Overall, MPs were synthesized by refluxing porphyrins and acetate, and the completion was indicated by UV-Vis spectra and the concentration of free metal cation decrease.

Table 4.2 $^{59}$Co & $^{56}$Fe extraction blank by ethyl acetate and C$_{18}$ extraction disk

<table>
<thead>
<tr>
<th>Sample names (n=3)</th>
<th>L/L separation blank</th>
<th>SPE blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{59}$Co Recovery (%)</td>
<td>92.37 ± 0.22</td>
<td>94.44 ± 0.09</td>
</tr>
<tr>
<td>$^{56}$Fe Recovery (%)</td>
<td>94.39 ± 1.44</td>
<td>93.26 ± 0.46</td>
</tr>
</tbody>
</table>

Notes: a. Liquid/liquid separation by ethyl acetate  
b. Solid phase extraction by C$_{18}$ extraction disk  
c. Data shown are mean ± standard error
Table 4.3 MPs synthetic yield calculated by measuring $^{59}$Co & $^{56}$Fe remained concentrations after reflux using ICP-MS

<table>
<thead>
<tr>
<th>Sample names (n=2)</th>
<th>MPs yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoporphyrin-Co</td>
<td>98.55 ± 1.75</td>
</tr>
<tr>
<td>Protoporphyrin-Fe</td>
<td>100.64 ± 3.59</td>
</tr>
<tr>
<td>Uroporphyrin-Co</td>
<td>98.93 ± 2.86</td>
</tr>
<tr>
<td>Uroporphyrin-Fe</td>
<td>101.19 ± 2.42</td>
</tr>
</tbody>
</table>

Notes: Data shown are mean ± standard error.

4.5 Metalloporphyrinogen Sol – gel

Figure 4.8 shows MP sol-gels, VB$_{12}$ sol-gel and one blank sol-gel in glass Petri dishes of 8.8 cm inner diameter. All were in powder forms after grinding, and had concentrations of catalysts (mass catalyst/mass sol-gel): VB$_{12}$ 2.75 mg/g; Proto-Co and Proto-Fe, 1.81 mg/g and 1.60 mg/g, respectively; Uro-Co and Uro-Fe, 2.75 mg/g and 2.24 mg/g, respectively (assuming that Co/Fe complexed with two acetates forming octahedral geometry). The Proto-Co and Proto-Fe sol-gels had a dark chocolate color. The Uro-Co and Uro-Fe sol-gels had a chocolate color. The VB$_{12}$ sol-gel was red and the blank was white. These sol-gels were ground into particles small enough to pass a 100-mesh screen that had 150 µm openings. The particle surfaces of these sol-gels were examined under FESEM and shown in Figure 4.9. Their surfaces were flat in general, with tiny islands dispersed. There were no evident pores on the surface. The surface area
was measured by N\textsubscript{2} adsorption on an ASAP 2010 surface area analyzer. The results are shown in Table 4.4. The surface areas of all samples were around 15 - 80 m\textsuperscript{2}/g. The results of pore size distribution also showed that these sol-gels have few mesopores.

However, the freeze dried gel samples had a large surface area (Table 4.4). The reason may be that freeze drying removed solvents at low temperature and pressure, not causing capillary pressure. Therefore, the pore could be produced, but would not collapse during the drying process (He et al., 2009). The adsorption isotherms of blank and VB\textsubscript{12} sol-gel are similar, and both of them have micropores and a lot of mesopores according to the isotherm plateau. But the desorption curves showed that VB\textsubscript{12} sol-gel sample had more hysteresis than blank sol-gel, which means that VB\textsubscript{12} sol-gel had more mesopores.

Figure 4.8 Picture of MPs, VB\textsubscript{12} and blank sol-gels
Figure 4.9 FESEM images of sol-gels: a. Proto-Co, b. Uro-Co, c. VB$_{12}$, d. Proto-Fe, e. Uro-Fe.

Table 4.4 BET surface areas of sol-gel samples by N$_2$ adsorption

<table>
<thead>
<tr>
<th>Sol-gel samples</th>
<th>Surface area (m$^2$/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>14.54 ± 0.24</td>
</tr>
<tr>
<td>VB$_{12}$</td>
<td>30.06 ± 0.46</td>
</tr>
<tr>
<td>Proto-Co</td>
<td>78.16 ± 0.57</td>
</tr>
<tr>
<td>Proto-Fe</td>
<td>79.79 ± 0.51</td>
</tr>
<tr>
<td>Uro-Co</td>
<td>48.54 ± 0.37</td>
</tr>
<tr>
<td>Uro-Fe</td>
<td>49.79 ± 0.51</td>
</tr>
<tr>
<td>Blank (freeze dried)</td>
<td>540.53 ± 5.93</td>
</tr>
<tr>
<td>VB$_{12}$ (freeze dried)</td>
<td>536.59 ± 4.52</td>
</tr>
</tbody>
</table>

Notes: surface area data are mean ± model fitting error.
CHAPTER FIVE
RESULTS AND DISCUSSION

5.1 Electron screening results

As a strong reductant, Na$_2$S$_2$O$_4$ can reduce Cr(VI) very fast (Figure 5.1). Approximately 90% Cr(VI) was reduced by 0.9 stoichiometric amount of Na$_2$S$_2$O$_4$ in 30 min. Then, the reaction continued and only 3% Cr(VI) remained after 120 min. The reason of more than 90% Cr(VI) was reduced by 0.9 stoichiometric amount of reductant maybe because dithionite decomposition products also were involved in the reduction of Cr(VI) (Ludwig et al., 2007). The reaction system was unbuffered, but the pH was kept in the range of 6.0 – 6.2. The Na$_2$S$_2$O$_4$ is not stable at pH 7 or less (Fruchter et al., 2000), and it was observed that Na$_2$S$_2$O$_4$ stock solution was cloudy at the beginning due to dithionite decomposition. Overall, Cr(VI) reduction by Na$_2$S$_2$O$_4$ was fast even thought not at its effective pH value (approximate 9).

Vc rapidly reduced Cr(VI) as well at the initial pH of 5 (Figure 5.1). About 75% Cr(VI) was removed in 30 min, and 90% was reduced in 120 min. However, high pH significantly made Cr(VI) reduction rate slow down (Figure 5.2, pH change ≤ 1 from the initial pH). Only approximate 30% Cr(VI) was reduced in one minute for initial pH 8 and 10, and then these reductions did not go any further. In order to increase the reaction at pH 8 and 10, VB$_{12}$ (20 µM) was added to the reaction systems. VB$_{12}$ enhanced Cr(VI) removal by 6% at pH 8 and 5% at pH 10 in 300 min (Figure 5.2). However, in most natural environments the pH is neutral or less, and Cr(VI) reduction by Vc at low pH was
too fast to follow, and this reaction system is not appropriate for testing metallocoporphyrinogens.

Figure 5.1 Cr(VI) reduction by Sodium dithionite or Vc. ([Cr(VI)] = 25 µM and [Na$_2$S$_2$O$_4$] = 35 µM 0.9 stoichiometric amount of Na$_2$S$_2$O$_4$, or [Cr(VI)] = 26 µM and [Vc] = 40 µM one stoichiometric amount of Vc, both of them reacted at unbuffered systems.) Error bars are standard errors based on replicated tests (n = 2).
Figure 5.2 Effects of initial pH and VB$_{12}$ on Cr(VI) reduction by Vc at high pH. ([Cr(VI)] = 26 µM, [Vc] = 40 µM, one stoichiometric amount of Vc, unbuffered system.) Error bars are standard errors based on replicated tests (n = 2).

5.2 Cr(VI) reduction by m-ZVI

5.2.1 No catalysts

Reduction of Cr(VI) (100 µM) at pH 7 was tested for different amounts of m-ZVI (Batch A). The masses of m-ZVI used were 1.7, 2.5, 3.4 and 5.0 g/L, which corresponded to 200, 300, 400 and 600 stoichiometric amounts to total Cr(VI) in the system, respectively. In all cases, the Cr(VI) concentration decreases with time as shown in Figure 5.3. The reduction reaction rate increased with increasing amount of the reductant added. The lowest amount (1.7 g/L) of m-ZVI added caused about 46% of the original Cr(VI) to be reduced in 200 min. But, adding 300 stoichiometric amounts of m-ZVI (2.5
g/L) dramatically accelerated the Cr(VI) reduction rate, and Cr(VI) was totally reduced in 70 min. Furthermore, the completion of Cr(VI) reduction was observed at 45 min for 3.4 g/L m-ZVI and less than 20 min for 5.0 g/L m-ZVI.

Figure 5.3 Effects of m-ZVI concentration on Cr(VI) reduction. ([Cr(VI)] = 100 µM and the concentration of m-ZVI (Batch A) correspond to 200, 300, 400, and 600 stoichiometric amounts, pH = 7.0 buffered by 0.05 M MOPS.) Error bars are standard errors based on replicated tests (n = 2).

Cr(VI) removals over 0-20 min by different amount of m-ZVI were compared in Figure C-1 of Appendix C. They were 19%, 39%, 81% and 100% by 1.7 g/L, 2.5 g/L, 3.4g/L and 5.0 g/L m-ZVI, respectively. They were significantly different according to the analytical results of ANOVA. In addition, Pseudo-first order reaction rate constants (k_{obs}) were calculated as a function of mass concentration of m-ZVI added (Figure 5.4).
How the data fit the pseudo-first order model is shown in Figure C-2 of Appendix C. ANOVA tests lead to the conclusion that the rate constants ($k_{obs}$) were significantly different for the four different m-ZVI loadings. It is interesting that when the m-ZVI concentration doubled from 1.7 g/L to 3.4 g/L, the reaction rate constant was increased approximately 52 times. However, from 2.5 g/L to 5.0 g/L m-ZVI added the reaction rate was increased approximately 3.5 times. Therefore, surface area played a role in the reaction rate including the mass concentration of m-ZVI. The $\log k_{obs} \sim \log[Fe]$ was plotted as Figure C-3 (Appendix C). The trend showed changes in reaction order, which may be due to surface area effect.

The m-ZVI at 1.7 g/L created a moderate reduction system, which was suitable for the further study of the pH effects and MPs catalytic capacities. The results of 100 µM Cr(VI) reduction by 1.7 g/L m-ZVI at pH 7 and pH 8 are shown in Figure 5.5. As previously studies have reported (Section 2.1.2.1), an increase in pH leads to decreased Cr(VI) reduction. For example, in 200 min 46% Cr(VI) was reduced at pH 7 but only 16% Cr(VI) was removed at pH 8 in the same period of time. The pH significantly affected Cr(VI) reduction by m-ZVI, and the reaction rate at pH 7 was appropriate to test MP catalysis.
Figure 5.4 ANOVA test for Cr(VI) reduction rate constants by different amount of m-ZVI according to pseudo-first order model. ([Cr(VI)] = 100 µM, the loads of m-ZVI (Batch A) were 200, 300, 400 and 600 stoichiometric amounts, pH = 7 buffered by 0.05 M MOPS. Letters on the top of each column are significant letters. Error bars represent standard errors based on replicated tests (n = 2). P < 0.05 is individual error rate, family error rate is 0.1.)
Figure 5.5 pH effect on Cr(VI) reduction. (1.7 g/L m-ZVI (Batch A), [Cr(VI)] = 100 µM, pH = 7.0 buffered by 0.05 M MOPS, pH = 8.0 buffered by 0.05 M Trizma-base). Error bars are standard errors based on replicated tests (n = 2).
5.2.2 Addition of catalysts

5.2.2.1 VB_{12}

Different concentrations of VB_{12} were added to catalyze 100 \mu M Cr(VI) reductions by 1.7 g/L m-ZVI (Batch A) at pH 7. The results are shown in Figure 5.6. Because the core metal of VB_{12} exists as the oxidized form (Co(III)), VB_{12} does not reduce Cr(VI) in the absence of reductant ZVI. A higher concentration of VB_{12} present resulted in faster disappearance of Cr(VI). Of the original Cr(VI) present, 49% was reduced in 200 min when using 10 \mu M VB_{12}. This result, however, was not significantly different from the reduction in the absence of VB_{12}. Upon adding 20 \mu M VB_{12} to the reduction system, Cr(VI) removal reached 60% after the same period of time. Furthermore, 30 \mu M VB_{12} helped to achieve 80% Cr(VI) decrease in 200 min. Therefore, Cr(VI) removal was enhanced approximately 14% and 34% by 20 \mu M and 30 \mu M VB_{12}, respectively. An ANOVA test showed that both of the higher VB_{12} concentrations significantly increased Cr(VI) reduction (Figure 5.7).

Cr(VI) reduction at pH 8 with the two higher concentrations of VB_{12} were also tested. Due to the slow kinetics, the reactions were monitored for 45 hours (Figure 5.8). However, neither 30 \mu M nor 40 \mu M VB_{12} could greatly speed up the reduction of Cr(VI). Removals of Cr(VI) were 21\%, 26\%, 35\% for VB_{12} concentrations of 0, 30 \mu M and 40 \mu M, respectively. Although they were statistically different (Figure D-1 in Appendix D), the reaction rates were quite slow.

In summary, VB_{12} catalysis depended on its concentration in the reactors. Also pH appears to play important role in the catalytic capability of VB_{12}, which may be
linked to the pH effect on Cr(VI) reduction by m-ZVI. Regardless, the same concentration of VB\textsubscript{12} was less effective at catalyzing Cr(VI) reduction at the higher pH value. Therefore, pH 7 and 20 µM of MPs/VB\textsubscript{12} were chosen for further experiments to test catalytic capabilities.

Figure 5.6 Effects of VB\textsubscript{12} concentration on Cr(VI) reduction at pH 7. ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch A), pH = 7.0 buffered by 0.05 M MOPS.) Error bars are standard errors based on replicated tests (n = 2).
Figure 5.7 Cr(VI) removals of reaction catalyzed by different concentration of VB$_{12}$ in 200 min. ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch A), pH = 7.0 buffered by 0.05 M MOPS. Error bars are standard errors based on replicated tests ($n = 2$). $P < 0.05$ is individual error rate, family error rate is 0.15.) Notes: (1) The number on the top of each column represents the mean of Cr(VI) removal ± standard errors. (2) The letter on the top of each column represents “Significance Letter”, which is the result from ANOVA test. They indicated the significant difference between those percentages of concentration decrease.
Figure 5.8 Effects of VB$_{12}$ concentration on Cr(VI) reduction at pH 8. ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch A), pH = 8.0 buffered by 0.05 M Trizma-base.) Error bars are standard errors based on replicated tests (n = 2).

It was deduced that the catalytic effect was caused by VB$_{12}$ working as an electron shuttle (Kim and Carraway, 2002; Dror and Schlautman, 2003). This was shown by the spectra shift before and after mixing with m-ZVI (Figure 5.9). A 20 µM VB$_{12}$ aqueous solution was prepared with deoxygenated DDI water, and then the solution was scanned from 200 to 800 nm on the UV-Vis spectrophotometer. Due to Co(III) as the core metal of VB$_{12}$, the absorbance of the Soret band was at 360 nm and the Q bands were between 450 to 600 nm. But the spectra were hypochromatically shifted after being mixed with 1.7 g/L m-ZVI and kept in the anaerobic chamber with a solid cap on for 24 hours. The maximum adsorption was shifted to 310 nm, and Q bands were at 400 to 500
nm. They demonstrated that Co(III) was reduced to Co(II), and the similar result was reported by Kim and Carraway (2002). Finally the mixture was exposed to oxygen for 2 hours, the graph of UV-Vis absorption showed a red shift, which was because Co(II) was oxidized to Co(III) by oxygen. This experiment was not done for other MPs, and they maybe give similar results.

Figure 5.9 VB$_{12}$ spectra before and after mixing with m-ZVI. [20 µM VB$_{12}$ aqueous solution ($\lambda_{\text{max}} = 360$ nm), after mixing with 1.7g/L m-ZVI 24 hours ($\lambda_{\text{max}} = 310$ nm), and the solution exposed to air for 1 hour ($\lambda_{\text{max}} = 350$ nm).]
5.2.2.2 Metalloporphyrinogens

As shown in Section 5.2.2.1, pH 7 was an appropriate condition to test the catalytic effects of metalloporphyrinogens and, at least for VB\(_{12}\), 20 \( \mu \text{M} \) was the lowest concentration to make a significant difference. Therefore, each synthesized metalloporphyrin (Proto-Co, Proto-Fe, Uro-Co and Uro-Fe) and VB\(_{12}\) 20 \( \mu \text{M} \) was tested in Cr(VI) reduction systems by 1.7 g/L m-ZVI (Batch B) at pH 7. The Batch B m-ZVI was newly prepared and stored in the anaerobic chamber. It was applied to all the reactions in this section, including the reactions with/without a catalyst. Decreases in Cr(VI) concentration with time are shown in Figure 5.10 & 5.11, and Cr(VI) removal (%) over 200 min are shown in Figure 5.12 with the significance letters from ANOVA test.

Without a catalyst present, Cr(VI) was reduced to approximately 45% of its initial concentration (i.e., 55% removal) by m-ZVI. With the Batch B m-ZVI, neither Proto-Co nor Proto-Fe demonstrated significant catalytic capabilities (52% and 51% Cr(VI) removals, respectively; Figure 5.11). However, Uro-Co and Uro-Fe were slightly promising catalysts. In 200 min, 61% Cr(VI) removal was achieved by adding 20 \( \mu \text{M} \) Uro-Co and 59% by adding 20 \( \mu \text{M} \) Uro-Fe, although these results were not statistically different from m-ZVI alone (Figure 5.11). Conversely, 20 \( \mu \text{M} \) VB\(_{12}\) significantly accelerated Cr(VI) reduction (76% Cr(VI) removal) in the same period of time.
Figure 5.10 Effects of 20 µM Proto-Co/Proto-Fe/VB12 on Cr(VI) reduction by m-ZVI. ([Cr(VI)] = 100 µM, m-ZVI 1.7 g/L (Batch B), pH = 7.0 buffered by 0.05 M MOPS.) Error bars are standard errors based on replicated tests (n = 4 for m-ZVI, n = 2 for Proto-Co, n = 3 for all others). The inset shows Cr(VI) concentrations at early times (0 – 20 min).
Figure 5.11 Effects of 20 µM Uro-Co/Uro-Fe/VB$_{12}$ on Cr(VI) reduction by m-ZVI. ([Cr(VI)] = 100 µM, m-ZVI 1.7 g/L (Batch B), pH = 7.0 buffered by 0.05 M MOPS.) Error bars are standard errors based on replicated tests (n = 4 for m-ZVI, n = 2 for Uro-Fe, n = 3 for all others).
Figure 5.12 ANOVA test results for Cr(VI) removal after 200 min reaction time with m-ZVI (Batch B). Values shown on the top of each bar represent the mean Cr(VI) removal ± standard errors (n = 4 for m-ZVI, n = 2 for Proto-Co and Uro-Fe, n = 3 for all others). Different letters above each bar signify significantly different results based on ANOVA and Fisher’s post hoc test (P < 0.05 individual error rate, P < 0.38 family error rate).
It is important to note that the previous analysis over the entire 200 minutes time range may not be appropriate for testing the effects of different catalysts on Cr(VI) reduction by m-ZVI. For example, a rapid drop in Cr(VI) concentration was typically observed in the first 10 min, followed by generally slower changes in Cr(VI) concentrations. Presumably, this initial rapid decrease might reflect Cr(VI) adsorption to m-ZVI rather than true reduction. Therefore, Cr(VI) concentrations were analyzed over two separate time periods: 0 - 10 min and 10 - 200 min (Figure 5.13 a & b). In the first 10 min, Cr(VI) decrease was 29% when using m-ZVI only. But adding catalysts actually inhibited Cr(VI) removal in the first 10 min (removals of 22% for VB<sub>12</sub>, 15% for Proto-Co, 21% for Proto-Fe, 9% for Uro-Co and 12% for Uro-Fe). In all cases, the presence of catalysts (20 µM) resulted in statistically slower decreases in Cr(VI) concentration versus the m-ZVI alone (Figure 5.13 a). However, over the 10 – 200 min reaction time period, VB<sub>12</sub>, Uro-Co, and Uro-Fe all demonstrated significant catalytic effects (Figure 5.13 b), resulting in Cr(VI) removals of 54%, 53% and 47%, respectively, versus the 26% removal by m-ZVI only. Addition of Proto-Co and Proto-Fe also resulted in higher Cr(VI) removals over this same time period (10 – 200 min), although the effect was not statistically significant.
Figure 5.13 Statistic analyses of Cr(VI) concentration decrease (%): a. 0-10 min; b. 10-200 min. (P < 0.05 is individual error rate, family error rates are the same 0.38. Mean ± standard errors, significant letter, and test numbers are the same as explained in Figure 5.12.)
In addition to looking at the overall change in Cr(VI) concentrations, reaction kinetics data were also analyzed using a pseudo-first order model for all reactions in Figure 5.10 & 5.11. Rate constants for the disappearance of Cr(VI) again were calculated separately for 0 – 10 min and 10 – 200 min. All concentration data were fit reasonably well by the model well, as indicated by the linear relationships (R² > 0.97, except Proto-Fe R² = 0.92; Figure D-2 in Appendix D). The fitted rate constants are listed in Table 5.1 as k_fast (0-10 min) and k_slow (10-200 min). Furthermore, these rate constants were compared by ANOVA (Figure 5.14). Cr(VI) removal by m-ZVI was the fastest in the first 10 min (k_fast = 3.44E-02 ± 4.32E-03 min⁻¹), and was significantly faster than any other treatment (Figure 5.14 a). Over 10 to 200 min, Cr(VI) reduction catalyzed by VB_{12} had the fastest rate constant (k_slow = 5.87E-03 ± 3.28E-04 min⁻¹). Uro-Co and Uro-Fe also demonstrated significant catalytic effects on Cr(VI) reduction, resulting in the rate constants of 4.17E-03 ± 1.45E-04 min⁻¹ and 4.00E-03 ± 1.00E-03 min⁻¹, respectively. Compared to m-ZVI alone (k_slow = 2.38E-03 ± 5.92E-04 min⁻¹), VB_{12}, Uro-Co and Uro-Fe significantly increased Cr(VI) reduction rate, which indicated by the statistical tests (Figure 5.14 b). However, neither Proto-Co nor Proto-Fe exhibited significant catalytic capability at the concentration used in these Cr(VI) reduction experiments.
<table>
<thead>
<tr>
<th>Sample</th>
<th>$k_{\text{fast}}$ (min$^{-1}$)*</th>
<th>$k_{\text{slow}}$ (min$^{-1}$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>8.90E-03 ± 1.78E-04</td>
<td>2.43E-05 ± 1.42E-05</td>
</tr>
<tr>
<td>m-ZVI only</td>
<td>3.44E-02 ± 4.32E-03</td>
<td>2.38E-03 ± 5.92E-04</td>
</tr>
<tr>
<td>VB$_{12}$</td>
<td>2.54E-02 ± 5.57E-04</td>
<td>5.87E-03 ± 3.28E-04</td>
</tr>
<tr>
<td>Proto-Co</td>
<td>1.66E-02 ± 4.15E-03</td>
<td>2.80E-03 ± 4.00E-04</td>
</tr>
<tr>
<td>Proto-Fe</td>
<td>2.32E-02 ± 6.11E-04</td>
<td>2.37E-03 ± 1.20E-04</td>
</tr>
<tr>
<td>Uro-Co</td>
<td>9.03E-03 ± 2.63E-03</td>
<td>4.17E-03 ± 1.45E-04</td>
</tr>
<tr>
<td>Uro-Fe</td>
<td>5.30E-03 ± 3.00E-04</td>
<td>4.00E-03 ± 1.00E-03</td>
</tr>
</tbody>
</table>

*Notes: Values shown are mean ± standard error, with the standard errors based on replicated tests: m-ZVI only (n = 4); Proto-Co and Uro-Fe (n = 2); VB12, Proto-Fe and Uro-Co (n = 3).
Figure 5.14 Statistic analyses of Cr(VI) removal first-order rate constants: a. 0-10 min; b. 10-200 min. (P < 0.05 is individual error rate, family error rates are the same 0.38. Mean ± standard error, significance letter, and test numbers are the same as explained in Figure 5.12.)
In general, the removal percentages of Cr(VI) and the first-order rate constants for Cr(VI) removal showed similar trends over the 0-10 and 10-200 min time periods. The presence of metalloporphyrinogens in the reaction system resulted in lower Cr(VI) removal over the first 10 min, with the trend being no catalyst > VB$_{12}$ > Proto-Fe ≈ Proto-Co > Uro-Co ≈ Uro-Fe. We can speculate that the catalysts blocked Cr(VI) adsorption sites on the m-ZVI surface, in effect acting as competitors against Cr(VI) adsorption on m-ZVI. Catalyst solubility may have played an important role in this process. For example, VB$_{12}$ is the most soluble of the five catalysts tested, and the least inhibition was found for it. The other catalysts have moderate solubility, and floccules may have found and covered the m-ZVI surface. An alternative hypothesis is that the presence of carboxyl groups, which have a strong affinity for ZVI, competed with Cr(VI) for surface adsorption sites. Because Uroporphyrin I has eight carboxyl groups, Uro-Co and Uro-Fe tremendously hindered Cr(VI) adsorption by m-ZVI the most. However, the more reactive catalysts (VB$_{12}$, Uro-Co and Uro-Fe) did enhance Cr(VI) reduction over the 10-200 min time period.

The collective results above indicated that the catalytic capacities to enhance Cr(VI) reduction by m-ZVI were VB$_{12}$ > Uro-Co > Uro-Fe > Proto-Co > Proto-Fe. Both solubility and core metal are important factors in the catalytic effects. VB$_{12}$ is very soluble due to hydrophilic groups, such as amino acid and phosphate group. However, all synthesized MPs have moderate solubility, and a small amount of MPs were aggregated, which were visible on the filter when the aliquots were filtered. Because Uro-Co and Uro-Fe were made from Uroporphyrin I containing eight carboxyl groups, and Proto-Co
and Proto-Fe were made from Protoporphyrin IX including two carboxyl groups. Therefore, Uro-Co and Uro-Fe are more soluble than Proto-Co and Proto-Fe, and VB\textsubscript{12} and Uro-Co/Fe demonstrated higher catalyses than Proto-Co/Fe. Also, it was observed that metalloporphyrinogens containing Co were more reactive than the one having Fe. The similar results were observed to other MPs by Dror and Schlautman (2003). The difference from the results of this work was not statistically significant, which may need to be further studied.

5.2.3 m-ZVI pretreatment and reuse

One batch of m-ZVI (Batch C), pretreated as described in the experimental Section 3.2, turned out to be more reactive than the Batch A and Batch B m-ZVI used before. The rate constants of three batches m-ZVI showed that Batch A and B m-ZVI have similar reactivity, but Batch C m-ZVI was approximately one magnitude reactive than Batch A and B (Table 5.2). Presumably this enhanced reactivity was due to being well protected from oxygen during drying process.

The Batch C m-ZVI was used sequentially three times for Cr(VI) reduction to test its reuse with or without 20 µM VB\textsubscript{12} present. When using m-ZVI only, Cr(VI) was totally reduced in 100 min for the first run, and within 300 min for the second reuse cycle (Figure 5.15 a). Addition of 20 µM VB\textsubscript{12} to the reaction system only accelerate Cr(VI) removal slightly: a total Cr(VI) reduction was achieved in 80 min for the first run and 270 min for the second time. Interestingly, VB\textsubscript{12} did not significantly catalyze the reaction in the first and second reuse cycles. But for the third time of reusing m-ZVI, Cr(VI)
remained 36.7% of its initial concentration after 65 hr when no catalyst was added. By comparison, the complete reduction was observed in 41 hr with 20 µM VB_{12} in the reaction system (Figure 5.15 b).

<table>
<thead>
<tr>
<th>Sample</th>
<th>K_{slow} \text{(min}^{-1})^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch A</td>
<td>2.95E-03 ± 1.50E-04</td>
</tr>
<tr>
<td>Batch B</td>
<td>2.38E-03 ± 5.92E-04</td>
</tr>
<tr>
<td>Batch C</td>
<td>3.93E-02 ± 2.25E-03 (1st)</td>
</tr>
</tbody>
</table>

*Notes: Values shown are mean ± standard error, with the standard errors based on replicated tests (n = 2 for Batch A, n = 4 for Batch B, n = 3 for Batch C). The rapid drop at the first 10 min was not included.

The rate constants data were calculated using Pseudo-first order model and analyzed by ANVOA (Figure 5.16). The data fittings were shown in Figure D-3 (Appendix D). It was obvious that Cr(VI) reduction rate decreased with reuse cycles increasing. No significant difference was shown for VB_{12} catalytic effect to Cr(VI) reductions of the first and second reuse cycles. The rate constants of two first reuse tests were 3.93E-02 ± 2.25E-03 \text{ min}^{-1} for m-ZVI alone and 4.54E-02 ± 2.87E-03 \text{ min}^{-1} for m-ZVI with 20 µM VB_{12}, which were approximately 10 times faster than two second reuse cycles with rate constants of 5.53E-03 ± 2.60E-04 \text{ min}^{-1} for m-ZVI alone and 6.77E-03 ±
2.96E-04 min⁻¹ for m-ZVI with 20 µM VB₁₂. Cr(VI) reductions were much slower in the third cycles. However, VB₁₂ significantly enhance the reaction, resulting in the rate constant 1.14E-03 ± 2.31E-04 min⁻¹ compared to 1.61E-04 ± 9.88E-06 min⁻¹ by using m-ZVI alone. According to the rate constants, Batch C m-ZVI was more reactive in the first and second reuse cycles than Batch A and B m-ZVI, but slower in the third cycle. It would appear that the catalyst only helps when ZVI reaction slows down enough.

These results indicated that VB₁₂ did not show a great catalytic effect in a reactive m-ZVI system, but accelerated the reduction later when m-ZVI had been consumed and fouled (not being as reactive as at the beginning). This suggests that a catalyst may not be necessary at the beginning when the reductant is still very reactive (e.g. new ZVI in PRB). However, a catalyst would be helpful in the long-term when the reductant has been consumed and reused multiple times.
Figure 5.15 m-ZVI (Batch C) reuse for Cr(VI) reduction: a. 1\textsuperscript{st} and 2\textsuperscript{nd} cycles; b. 3\textsuperscript{rd} cycle. ([Cr(VI)] = 100 µM, 1.7 g/L Batch C m-ZVI, VB\textsubscript{12} = 20 µM, pH = 7.0 buffered by 0.05 M MOPS.) Error bars represent standard errors based on replicated tests (n = 3). The dash lines indicate when fresh chromate was spiked into the reactors for the second cycle.
Figure 5.16 ANOVA test results for the rate constants of Cr(VI) reduction by m-ZVI reuse experiments at pH 7 with/without VB$_{12}$. ([Cr(VI)] = 100 µM, 1.7 g/L Batch C m-ZVI, VB$_{12}$ = 20 µM, pH = 7.0 buffered by 0.05 M MOPS.) Values shown on the top of each bar represent the mean Cr(VI) removal ± standard errors (n = 3). Different letters above each bar signify significantly different results based on ANOVA and Fisher’s post hoc test (P < 0.05 individual error rate, P < 0.31 family error rate).
5.3 Cr(VI) reduction by n-ZVI

5.3.1 No catalyst

The n-ZVI was synthesized and then used to reduce Cr(VI). Because n-ZVI has a much smaller particle size and higher surface area than m-ZVI, a mass loading of 0.1 g/L n-ZVI was applied in all the experiments. Cr(VI) was reduced rapidly by n-ZVI at pH 7, and reactions were completed in less than 50 min (Figure 5.17). Two batches of n-ZVI demonstrated slightly different reactivity because of acid treatment at different pH after n-ZVI synthesis. The results show that a lower pH acid treatment made n-ZVI (Batch I) more reactive. However, to balance reactivity and recovery, pH 4.0 was appropriate pH for acid treatment. Therefore Batch II n-ZVI was selected for the further studies.

Then, Cr(VI) was reduced at pH 6 (Figure 5.18). A total reduction happened in 6 min at pH 6, compared to 50 min at pH 7. The reaction kinetic data were analyzed as two time periods to compare the rapid drops: 0-1 min and 2-5 min for pH 6, 0-10 min and 10-50 min for pH 7. The data were listed in Table 5.3, and data fitting to the pseudo-first order model were shown in Figure E-1 (Appendix E). The rate constants were approximately 10 times larger at pH 6 than those at pH 7. In addition, ANOVA test revealed that $k_{fast}$ was significantly different from $k_{slow}$ at pH 6, but not at pH 7. However, the difference was much smaller than the difference between $k_{fast}$ and $k_{slow}$ of m-ZVI. These results indicate that the pH played a very important role in Cr(VI) reduction by n-ZVI as well. Only the reaction at pH 7 was chosen to study the catalytic capability of VB$_{12}$. 
According to the surface areas of m-ZVI and n-ZVI reported in Section 4.1 and 4.2, the mass concentrations based on the surface area for Cr(VI) reduction were 1.15 m$^2$/L for m-ZVI and 2.69 m$^2$/L for n-ZVI. Therefore, n-ZVI had about twice the surface area of m-ZVI (Batch A or B). However, n-ZVI was 10 times more reactive than m-ZVI (Batch A or B) by comparing the rate constants at pH 7 listed in Table 5.2 and 5.3. It is very interesting that Batch C gave a high rate constant and was only about 1.5 times lower than n-ZVI. Although the surface area of Batch C m-ZVI was unknown, it had similar particle size with Batch A and B m-ZVIs. Such a difference in reactivity may be because m-ZVI was different (having different lot number) or again Batch C m-ZVI was well protected from being oxidized during the drying process.

![Graph showing Cr(VI) reduction by n-ZVI compared to m-ZVI (Batch C).]([Cr(VI)] = 100 µM, n-ZVI 0.1 g/L, m-ZVI (Batch C) 1.7 g/L, pH 7.0 buffered by 0.05 M MOPS.)

Error bars represent standard errors based on the replicated tests (n = 3).
Figure 5.18 pH Effect on Cr(VI) reduction by n-ZVI. ([Cr(VI)] = 100 µM, n-ZVI (Batch II) 0.1 g/L, pH = 7.0 buffered by 0.05 M MOPS, pH = 6.0 buffered by 0.05 M Bis-tris.) Error bars represent standard errors based on replicated tests (n = 3).
Table 5.3 n-ZVI reactivity at different pH

<table>
<thead>
<tr>
<th>pH</th>
<th>$k_{\text{fast}}$ (min$^{-1}$)*</th>
<th>Sig. let. of $k_{\text{fast}}$</th>
<th>$k_{\text{slow}}$ (min$^{-1}$)*</th>
<th>Sig. let. of $k_{\text{slow}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>5.13E-01 ± 2.68E-02</td>
<td>A</td>
<td>4.00E-01 ± 1.06E-02</td>
<td>B</td>
</tr>
<tr>
<td>7.0</td>
<td>6.57E-02 ± 4.93E-03</td>
<td>C</td>
<td>5.92E-02 ± 2.21E-03</td>
<td>C</td>
</tr>
</tbody>
</table>

*Notes: Values shown are mean ± standard error, with the standard errors based on replicated tests ($n = 3$). The rapid drops were at the first 1 min for Cr(VI) reduction by n-ZVI at pH 6 and at the first 10 min for the reaction at pH 7. Different letters signify significantly different results based on ANOVA and Fisher’s post hoc test ($P < 0.05$ individual error rate, $P < 0.18$ family error rate).

5.3.2 VB$_{12}$

Small amounts of VB$_{12}$ (5 or 3 µM) were added to Cr(VI) reduction system at pH 7, but both of them made the reaction finished within 6 min (data not shown here). Therefore, smaller amounts (0.1 and 0.5 µM VB$_{12}$) were tested to catalyze the reaction. A total reduction was finished in 15 min and 12 min, when being catalyzed by 0.1 and 0.5 µM VB$_{12}$, respectively (Figure 5.19). Amazingly, VB$_{12}$ at such low loading levels showed significant effects on increasing Cr(VI) reduction, compared to the one using no catalyst. The higher loading of VB$_{12}$ (0.5 µM) showed a slight improvement over the lower loading (0.1 µM), indicating that probably 0.1 µM VB$_{12}$ was effective enough. The rate constants were calculated (not including the first rapid drop, Figure F-1 showing the data.
fitting) and analyzed by ANOVA test (Figure 5.20). The results showed that both 0.1 µM VB₁₂ and 0.5 µM VB₁₂ significantly catalyzed Cr(VI) reduction. The rate constant was increased more than 2.5 times by 0.1 µM VB₁₂ and more 4 times by 0.5 µM VB₁₂.

![Graph showing VB₁₂-catalyzed Cr(VI) reduction by n-ZVI. ([Cr(VI)] = 108 µM, n-ZVI (Batch II) 0.1 g/L, VB₁₂ 0.1 or 0.5 µM, pH = 7.0 buffered by 0.05 M MOPS.) Error bars represent standard errors based on replicated tests (n = 3 for n-ZVI alone, n=2 for n-ZVI with 0.1 or 0.5 µM VB₁₂).]
Figure 5.20 ANOVA test results for the rate constants of Cr(VI) reduction catalyzed by VB$_{12}$ at pH 7. ([Cr(VI)] = 100 µM, 0.1 g/L Batch II n-ZVI, VB$_{12}$ = 0.1 or 0.5 µM, pH = 7.0 buffered by 0.05 M MOPS.) Values shown on the top of each bar represent the mean Cr(VI) removal ± standard errors (n = 3 for no VB$_{12}$, n = 2 for 0.1 or 0.5 µM VB$_{12}$). Different letters above each bar signify significantly different results based on ANOVA and Fisher’s post hoc test (P < 0.05 individual error rate, P < 0.10 family error rate).
5.4 Catalytic capability of immobilized VB₁₂

5.4.1 Sol-gel immobilized VB₁₂ for Cr(VI) reduction by m-ZVI (Batch B)

VB₁₂-immobilized sol-gel was applied to a Cr(VI) and m-ZVI reduction system, and the catalytic capability was compared to dissolved VB₁₂. The concentration of VB₁₂ immobilized in sol-gel was calculated according to the total amount of VB₁₂ added and the dried sol-gel samples prepared. The color of DDI water (100 mL) containing 20 µM VB₁₂-immobilized sol-gel, which was mixed on the tumbler, was monitored for 48 hr. The solution was colorless (no absorbance on UV-Vis spectrophotometer), which means that no VB₁₂ was leached out from sol-gel matrix to the solution in 48 hr. In addition, VB₁₂-immobilized sol-gel was mixed with 100 µM Cr(VI) in the reactor in the absence of m-ZVI, the aliquots were taken at the selective time. The results showed that VB₁₂ sol-gel did not absorb or reduce Cr(VI) without m-ZVI in 200 min (Figure 5.21).

When the same amount of VB₁₂ sol-gel was added to the reduction systems, approximately 70 % Cr(VI) was reduced in 200 min, compared to 76 % Cr(VI) removal in 200 min when VB₁₂ was added homogenously (Figure 5.21). VB₁₂-immobilized sol-gel enhanced Cr(VI) reduction by m-ZVI alone approximately 16%, although the statistic test did not showed significant difference (Table 5.4). It may be because the data set of Cr(VI) by m-ZVI alone have high standard error. The rate constants were calculated by Pseudo-first order model as k_fast (0-10 min) and k_slow (10-200 min) (Table 5.4, data fitting shown in Figure G-1). The k_fast was approximate 5 times larger than k_slow from the reaction using VB₁₂ sol-gel. No significant difference was found with k_fast between the reactions with and without the catalyst. However, the slow rate constants showed
significant differences between the two using dissolved or immobilized VB\textsubscript{12} and the one without the catalyst. Therefore, the immobilization of VB\textsubscript{12} in sol-gel made VB\textsubscript{12} slightly less effective than the dissolved VB\textsubscript{12}, but VB\textsubscript{12} sol-gel still greatly enhanced Cr(VI) reduction reaction with no catalyst.

Through the sol-gel characterization in Chapter 4 (Section 4.5), we know that this VB\textsubscript{12} sol-gel had few pores. The reason may be because the sol-gel was dried at room temperature, and some water and organic solvents could not come out of the tight sol-gel matrix and make pores. However, VB\textsubscript{12} sol-gel did catalyze Cr(VI) reduction dramatically. These promising results bring forth some questions, which need to be addressed in the future. For example, how was VB\textsubscript{12} encapsulated in the matrix? And would the VB\textsubscript{12} sol-gel with more pores be a more effective catalyst? In addition, because VB\textsubscript{12} was the most promising catalyst in this study, only VB\textsubscript{12}-immobilized sol-gel was tested on Cr(VI) reductions.
Figure 5.21 Catalysis of VB\textsubscript{12}-immobilized sol-gel on Cr(VI) reduction by m-ZVI. ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch B), dissolved or immobilized VB\textsubscript{12} 20 µM, pH = 7.0 buffered by 0.05 M MOPS.) Error bars represent standard errors based on replicated tests (n = 2).
Table 5.4 Catalytic effects of VB$_{12}$-immobilized in sol-gel to Cr(VI) reduction by m-ZVI

<table>
<thead>
<tr>
<th>sample</th>
<th>$k_{\text{fast}}$ (min$^{-1}$)*</th>
<th>sig. let.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No VB$_{12}$</td>
<td>3.44E-02 ± 8.65E-03</td>
<td>A</td>
</tr>
<tr>
<td>Dissolved VB$_{12}$</td>
<td>2.54E-02 ± 9.64E-04</td>
<td>A</td>
</tr>
<tr>
<td>VB$_{12}$ sol-gel</td>
<td>2.56E-02 ± 5.00E-05</td>
<td>A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>sample</th>
<th>$k_{\text{slow}}$ (min$^{-1}$)*</th>
<th>sig. let.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No VB$_{12}$</td>
<td>2.38E-03 ± 1.18E-03</td>
<td>A</td>
</tr>
<tr>
<td>Dissolved VB$_{12}$</td>
<td>5.87E-03 ± 5.69E-04</td>
<td>B</td>
</tr>
<tr>
<td>VB$_{12}$ sol-gel</td>
<td>4.90E-03 ± 1.00E-04</td>
<td>B</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>sample</th>
<th>removal (%)*</th>
<th>sig. let.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No VB$_{12}$</td>
<td>54.74 ± 6.06</td>
<td>A</td>
</tr>
<tr>
<td>Dissolved VB$_{12}$</td>
<td>76.33 ± 1.50</td>
<td>B</td>
</tr>
<tr>
<td>VB$_{12}$ sol-gel</td>
<td>70.67 ± 0.52</td>
<td>AB</td>
</tr>
</tbody>
</table>

*Notes: Values shown are mean ± standard error, with the standard errors based on replicated tests ($n=4$ for no VB$_{12}$, $n=3$ for dissolved VB$_{12}$, $n=2$ for VB$_{12}$ sol-gel). The $k_{\text{fast}}$ was the rate constant over 0-10 min, the $k_{\text{slow}}$ was over 10-200 min, and the removal of Cr(VI) was in 200 min. Different letters signify significantly different results based on ANOVA and Fisher’s post hoc test ($P < 0.05$ individual error rate, $P < 0.11$ family error rates).
5.4.2 Sol-gel immobilized VB₁₂ for Cr(VI) reduction by n-ZVI (Batch II)

VB₁₂-immobilized sol-gel (0.1 and 0.5 µM) were used to catalyze Cr(VI) reduction heterogeneously at pH 7. The total reductions were achieved in 25 min and 17.5 min for 0.1 and 0.5 µM VB₁₂-immobilized in sol-gel, respectively (Figure 5.22 & 5.23). Compared to a total reduction over 15 min by adding 0.1 µM dissolved VB₁₂, 12.5 min by adding 0.5 µM dissolved VB₁₂, and 50 min without VB₁₂ in the reaction system, VB₁₂-immobilized sol-gel significantly catalyzed the Cr(VI) reduction, although the reaction rate was not as fast as the one adding dissolved VB₁₂ (Table 5.5, rate constant data fitting shown in Figure G-2). The blank sol-gel (no MPs or VB₁₂) seems to absorb Cr(VI) at the beginning, and reach the most at 60 min (Figure 5.22). However, Cr(VI) concentration had been monitored for 20 hr, and it was increased later to approximately 100% of its original concentration.
Figure 5.22 Catalysis of 0.5 µM VB$_{12}$-immobilized sol-gel on Cr(VI) reduction by n-ZVI. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI (Batch II), immobilized VB$_{12}$ 0.5 µM, pH = 7.0 buffered by 0.05 M MOPS. Blank sol-gel experiment was conducted by adding the same amount of blank sol-gel and 100 µM Cr(VI).) Error bars represent standard errors based on replicated tests (n = 1 for blank sol-gel, n=3 for no VB$_{12}$ (n-ZVI alone), n=2 for all others).
Figure 5.23 Catalysis of 0.1 µM VB$_{12}$-immobilized sol-gel on Cr(VI) reduction by n-ZVI. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI (Batch II), immobilized VB$_{12}$ 0.1 µM, pH = 7.0 buffered by 0.05 M MOPS.) Error bars represent standard errors based on replicated tests (n=3 for no VB$_{12}$ (n-ZVI alone), n=2 for all others).
Table 5.5 Catalytic effects of VB$_{12}$-immobilized in sol-gel to Cr(VI) reduction by n-ZVI

<table>
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<th>$k$ (min$^{-1}$)</th>
<th>sig. let.</th>
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<tbody>
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<td>A</td>
</tr>
<tr>
<td>0.1 µM VB$_{12}$</td>
<td>1.60E-01 ± 1.12E-02</td>
<td>B</td>
</tr>
<tr>
<td>0.1 µM VB$_{12}$ in sol-gel</td>
<td>1.04E-01 ± 1.20E-03</td>
<td>C</td>
</tr>
<tr>
<td>0.5 µM VB$_{12}$</td>
<td>2.59E-01 ± 1.05E-02</td>
<td>D</td>
</tr>
<tr>
<td>0.5 µM VB$_{12}$ in sol-gel</td>
<td>1.33E-01 ± 5.50E-04</td>
<td>E</td>
</tr>
</tbody>
</table>

Notes: Values shown are mean ± standard error, with the standard errors based on replicated tests ($n = 3$ for no VB$_{12}$, $n = 2$ for all others). The rapid drop was not obvious, so that the rate constant was calculated from $t = 0$min. Different letters signify significantly different results based on ANOVA and Fisher’s post hoc test ($P < 0.05$ individual error rate, $P < 0.22$ family error rate).
5.4.3 VB$_{12}$ immobilization in Ca-alginate beads

VB$_{12}$ was successfully encapsulated in Ca-alginate beads by dropping alginate gel mixture into the CaCl$_2$ solution containing VB$_{12}$ at the same concentration. However, after being added to DDI water, the VB$_{12}$ was totally leached out from the alginate beads over 75 min (Figure 5.24). According to the previous study by Benerjee et al. (2007), the pore size of their Ca-alginate beads was about 3.17 – 5.07 nm. But a VB$_{12}$ molecule is about 1.0 – 1.5 nm, which is smaller than the pores of Ca-alginate gel beads. Although VB$_{12}$ has big and complicated side groups, they could not prevent VB$_{12}$ from leaching out. Therefore, VB$_{12}$-immobilized Ca-alginate beads will not be feasible for most remediation.

![Figure 5.24 Spectra of the DDI water containing VB$_{12}$-immobilized Ca-alginate beads with time. (50 µM VB$_{12}$ was immobilized, but was totally leached out in 75 minutes.)](image)

<table>
<thead>
<tr>
<th>t</th>
<th>Abs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>10 min</td>
<td>0.2</td>
</tr>
<tr>
<td>20 min</td>
<td>0.15</td>
</tr>
<tr>
<td>30 min</td>
<td>0.1</td>
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<tr>
<td>45 min</td>
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<td>60 min</td>
<td>0.0</td>
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<tr>
<td>75 min</td>
<td>0.0</td>
</tr>
<tr>
<td>90 min</td>
<td>0.0</td>
</tr>
<tr>
<td>wavelength (nm)</td>
<td></td>
</tr>
</tbody>
</table>
5.5 Cr(VI) reduction by n-ZVI (Batch II) immobilized in Ca-alginate beads

5.5.1 No catalyst

The n-ZVI was encapsulated in Ca-alginate beads, and then 0.1 g/L encapsulated n-ZVI was used to reduce 100 µM Cr(VI) in pH 6 and 7 buffered systems. Complete reductions were obtained at 50 min for pH 6 and 150 min for pH 7 (Figure 5.25 & 5.26). Obviously, pH plays an important role in Cr(VI) reduction by n-ZVI immobilized in Ca-alginate beads. By comparing with the complete reduction obtained in 6 min for pH 6, and 50 min for pH 7, n-ZVI immobilized in Ca-alginate did decrease Cr(VI) reduction by the polymer matrix at both pH values. The rate constants were calculated by Pseudo-first order model (Table 5.6). For the reaction at pH 6 by n-ZVI gel beads, k was calculated from t = 0 min and Ln[Cr(VI)] − t showed linear relation (Figure H-3 in Appendix H). However, the reaction at pH 7 by n-ZVI gel beads k was calculated as two time periods 0-100 min and 100-150 min. The reaction went slowly at the beginning (0-100 min), which may be due to the diffusion process to reach n-ZVI inside the beads. These data showed that the reaction by n-ZVI gel beads was slowed down about 10 times at pH 6. At pH 7, Cr(VI) reduction was hindered by Ca-alginate matrix at first, then it was increased and comparable to the reaction by bare n-ZVI.

But the n-ZVI immobilized gel beads became more reactive after being kept in an anaerobic chamber for three months. The same reactions as above were twice as fast at pH 6 (finished in 25 min), and 3.75 times faster at pH 7 (finished in 40 min) than the reactions using newly prepared n-ZVI beads (data shown in Figure H-1 & H-2 of Appendix H). Therefore, the result of Cr(VI) reduction by those more dried n-ZVI Ca-
alginate beads was comparable to the one reduced by bare n-ZVI at pH 7. The hypotheses for the difference are as follow. First, it may be due to the enlargement of pores in the beads, when Ca-alginate became so dried after being kept in the chamber (in a screw cap vial). Second, the beads were cracked after extended storage (shown in Figure 4.3). All these might make Cr(VI) diffusion into the Ca-alginate gel matrix to react with n-ZVI easier. In addition, the difference may be due to n-ZVI oxidation by H₂O in the anaerobic chamber, which produced reactive Fe²⁺ (Eqn. 12).

\[
\text{Fe}^0 + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^- \quad (12)
\]

Although n-ZVI immobilization in Ca-alginate solves the mobility and settling problems associated with n-ZVI (Benerjee et al., 2007), Ca-alginate beads inhibited Cr(VI) reduction at pH 6 and 7, but no adverse effect was observed for the reduction by more dried n-ZVI gel beads at pH 7. Therefore, it may be promising to use dried n-ZVI Ca-alginate beads at neutral pH.
Figure 5.25 Cr(VI) reduction by n-ZVI (Batch II) immobilized Ca-alginate beads at pH 6.0. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI immobilized, pH = 6.0 buffered by 0.05 M Bis-tris. The Ca-alginate beads were used after being dried 48 hr in the anaerobic chamber.) Error bars represent standard errors based on the replicated tests (n = 3 for dissolved n-ZVI, n = 1 for blank gel beads and n-ZVI immobilized gel beads).
Figure 5.26 Cr(VI) reduction by n-ZVI (Batch II) immobilized Ca-alginate beads at pH 7.0. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI immobilized, pH = 7.0 buffered by 0.05 M MOPS. The Ca-alginate beads were used after being dried 48 hr in the anaerobic chamber.) Error bars represent standard errors based on the replicated tests (n = 2 for dissolved n-ZVI, n = 1 for blank gel beads and n-ZVI immobilized gel beads).

5.5.2 VB₁₂

A small amount of free/dissolved VB₁₂ was added to catalyze Cr(VI) reduction by newly prepared n-ZVI Ca-alginate beads at pH 7. VB₁₂ (5 µM) increased the reduction rate, which gave a complete reduction in 18 min (Figure 5.27). Compared with 150 min without VB₁₂, the reaction completion was sped up about eight times. The rate constant was increase more than 3 times by adding VB₁₂ to the reaction system (Table 5.6). Also the reaction went faster than the one using more dried Ca-alginate beads (approximate 40 min). Through this experiment, we found that small amount of VB₁₂ could effectively
catalyze Cr(VI) reduction by immobilized n-ZVI. Therefore, the decreased reaction rate by using encapsulated n-ZVI could be sped up by adding a small amount of VB$_{12}$. The reason maybe because VB$_{12}$ can diffuse to Ca-alginate matrices and mediate electron transfer between immobilized n-ZVI and Cr(VI). VB$_{12}$ or other MPs could be promising to be used to Cr(VI) in-situ remediation by encapsulated n-ZVI.

Figure 5.27 VB$_{12}$-catalyzed Cr(VI) reduction by n-ZVI immobilized Ca-alginate beads at pH 7.0. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI immobilized, pH = 7.0 buffered by 0.05 M MOPS. The Ca-alginate beads were used after being dried 48 hr in the anaerobic chamber. Error bars represent standard errors based on the replicated tests: n = 2 for dissolved n-ZVI, n = 1 for the other experiments.)
Table 5.6 Rate constants of Cr(VI) reduction by n-ZVI immobilized in Ca-alginate beads with/without VB12

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>k (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare n-ZVI</td>
<td>6</td>
<td>4.10E-01 ± 1.26E-02</td>
</tr>
<tr>
<td>n-ZVI in beads</td>
<td>6</td>
<td>5.30E-02</td>
</tr>
<tr>
<td>Bare n-ZVI</td>
<td>7</td>
<td>6.25E-02 ± 3.57E-03</td>
</tr>
<tr>
<td>n-ZVI in beads</td>
<td>7</td>
<td>1.51E-02, 4.37E-02</td>
</tr>
<tr>
<td>n-ZVI in beads with VB12</td>
<td>7</td>
<td>2.28E-01</td>
</tr>
</tbody>
</table>

Notes: Values shown are mean ± standard error, with the standard errors based on replicated tests (n = 3). The rate constants of Cr(VI) reduction by n-ZVI gel beads at pH 6 and pH 7 with VB12 were calculated from t = 0. For the reaction at pH 7 by n-ZVI gel beads, k was calculated as two time periods 0-100 min and 100-150 min.

5.5.3 n-ZVI Ca-alginate beads reuse

The n-ZVI Ca-alginate beads were reused to Cr(VI) reduction at pH 6. Similar to previous experiments, n-ZVI gel beads inhibited Cr(VI) reduction, which made the reaction three times slower than the one using bare n-ZVI in four reuse cycles (Figure 5.28). The beads were broken after four cycles and it was observed that the beads collected a lot of precipitated reduction byproducts, which resulted in clearer solutions than those using bare n-ZVI. Therefore, the application of these kinds of beads to in-situ remediation would be beneficial from an aesthetic standpoint. In addition, the reduced
Cr(VI), Cr(III) was kept in the beads so that Cr can be easily removed from the particular environment.

Figure 5.28 Reusability of bare n-ZVI (a) and n-ZVI Ca-alginate beads (b). ([Cr(VI)] = 100 μM, 0.1 g/L n-ZVI bare or immobilized, pH = 6.0 buffered by 0.05 M Bis-tris.) Error bars represent standard errors based on the replicated tests (n = 2 for bare n-ZVI, n = 3 for n-ZVI beads).
CHAPTER SIX
CONCLUSIONS & RECOMMENDATION

6.1 Conclusions

6.1.1 Cr(VI) reduction by ZVI

The reactivity of ZVI is related to the pretreatment of m-ZVI and the acid treatment of synthesized n-ZVI. The pretreatment made m-ZVI reactive by removing oil and iron oxides on its particle surface. During the drying process, a closed vial with N₂ purging was extremely effective in protecting the iron from being oxidized (Batch C). Therefore, it made Cr(VI) reduction much faster than those using m-ZVI dried in an open mouth Erlenmeyer (Batch A & B). In fact, Batch B was a little bit more reactive than Batch A, due to being stored in the anaerobic chamber (less exposure to oxygen) and weighed inside the chamber. But Batch A m-ZVI was stored and weighed on the bench-top, and then was taken into the chamber to initiate the reaction. It was observed that 55% Cr(VI) removal by Batch B and 46% by Batch A at the same amount and pH 7. In addition, the pH for acid treatment (pH 4 or 2.5) for n-ZVI after synthesis slightly affected its reactivity. The lower pH led to more reactive n-ZVI, but much less yield.

As previous studies reported, Cr(VI) reductions by bare m-ZVI or n-ZVI included adsorption and reduction (in Section 2.1.2.1). Therefore, the surface area plays a significant role to the reaction rate, and a higher surface area makes the reduction faster. A rapid drop at the beginning was observed when using m-ZVI or n-ZVI in this work. The surface area of n-ZVI was about 40 times higher than m-ZVI, which led to a
complete reduction in 50 min when using 0.1 g/L n-ZVI, compared with a 55% Cr(VI) decrease in 200 min when using 1.7 g/L m-ZVI (Batch B, 100 µM Cr(VI) and pH 7 for all reactions). Another important factor for Cr(VI) reduction reaction by ZVI is the pH. The reduction was significantly increased with pH decrease for both m-ZVI and n-ZVI. Cr(VI) removals were 46% and 16% by 1.7 g/L m-ZVI (Batch A) at pH 7 and 8 respectively. In comparison, n-ZVI 0.1 g/L made Cr(VI) reduction completed in 6 min at pH 6, but in 50 min at pH 7.

6.1.2 n-ZVI immobilization

The n-ZVI was successfully immobilized in Ca-alginate as reported by Bezbaruah et al. (2009). Although the gel beads caused no adverse effect on nitrate reduction compared to bare n-ZVI (Bezbaruah et al., 2009), the gel matrix inhibited Cr(VI) reduction. The n-ZVI immobilized in newly prepared Ca-alginate beads made the reduction completion 10 times later at pH 6 and 3 times later at pH 7, compared to using bare n-ZVI. But no significant difference was shown when using extendedly stored n-ZVI Ca-alginate beads (3 months in the anaerobic chamber) at pH 7. The reason may be because of enlarged pores sized, crack on the beads’ surface, or Fe$^{2+}$ produced by Fe$^{0}$ hydrolysis in the anaerobic chamber, which made it easier for Cr(VI) diffusion into the Ca-alginate gel matrix and reacting with n-ZVI. Therefore, complete drying may be necessary for n-ZVI Ca-alginate beads before application. Less hindrance would occur to Cr(VI) reduction at pH 7 or higher pH. What is more, the n-ZVI gel beads were able to be reused multiple times. After four reuse cycles, the beads were almost broken. Also it was
observed that the beads collected a lot of precipitated products. This is a difference between nitrate reduction and oxidized metal reduction, and maybe the reason why the reaction rate was decreased. However, this phenomenon also demonstrated that the \textit{in-situ} remediation using n-ZVI Ca-alginate beads could be beneficial from aesthetics point of view. Overall, nontoxic and biodegradable Ca-alginate can be used to immobilize n-ZVI, which is promising in environmental remediation for many reasons.

6.1.3 Metalloporphyrinogen-catalyzed Cr(VI) reduction by ZVI

6.1.3.1 m-ZVI

After electron donor screening, metalloporphyrinogens were used to Cr(VI) reduction by ZVI. First of all, Proto-Co/Fe and Uro-Co/Fe were synthesized successfully by refluxing dissolved porphyrins and Co/Fe acetate in 50\% ethanol at approximate pH 3. The synthesis completion was indicated by UV-Vis spectra and by measuring dissolved Co or Fe remained after reflux. Cr(VI) (100 µM) reduction by 200 stoichiometric amount of m-ZVI (1.7 g/L) at pH 7 was considered as the appropriate reduction system to test metalloporphyrinogens’ catalyses. In the preliminary experiments, it was observed that catalytic effect of VB$_{12}$ was increased with concentration increasing. Also the catalytic effect was more obvious at pH 7 than pH 8. VB$_{12}$ (20 µM) significantly catalyzed Cr(VI) reduction at pH 7.

The four MPs and VB$_{12}$ demonstrated different catalytic capabilities for Cr(VI) reduction by m-ZVI. Proto-Co and Proto-Fe could not accelerate Cr(VI) reduction in 200 min, compared to the reduction using m-ZVI only. However, VB$_{12}$, Uro-Co, and Uro-Fe
showed catalytic effects. The ANOVA test showed that VB12 made a significant
difference. The reduction rate decreases over the first 10 min were observed at the
presence of a catalyst. The reason may be the existence of electron shuttle moderated the
reaction rate by transferring electrons or moderate solubility of MPs.

Interestingly, when VB12 was applied to a more reactive reduction system of m-
ZVI (Batch C), it did not show great catalytic effect at the first two reuse cycles. But
VB12 dramatically increased the reduction at the third time when the reductant was
consumed and less reactive. This suggested that a catalyst may not be so important at the
beginning when the reductant is reactive, and would catalyze the reduction when the
reductant has been consumed and fouled.

In addition, VB12 working as electron shuttles was visualized by UV-Vis spectra
before and after mixing with m-ZVI. VB12 was the most promising catalyst in this study,
and the four synthesized MPs were less effective. The suspicion is that solubility and core
metal play important roles in the catalytic capacity. The more soluble the
metalloporphyrinogens are, the more reactive they are. Also it seems that having Co(II)
as the core metal made the metalloporphyrinogens more effective than the one with
Fe(II), which need to be further studied.

6.1.3.2 n-ZVI

A small amount of VB12 could effectively catalyze Cr(VI) reduction by n-ZVI at
pH 7.0. VB12 (0.1 µM) made the reduction completed about 3.3 times earlier than the one
using n-ZVI only, meanwhile a higher loading of VB12 (0.5, 3 or 5 µM) only slightly
improved the reduction catalyzed by 0.1 µM VB$_{12}$. This result indicated that the lower VB$_{12}$ concentration was effective enough.

6.1.3.3 n-ZVI Ca-alginate beads

Dissolved VB$_{12}$ (5 µM) significantly catalyzed Cr(VI) reduction by n-ZVI immobilized in Ca-alginate beads at pH 7. The reaction was completed 8 times earlier than the one without VB$_{12}$, and 2.6 times earlier than the one using bare n-ZVI only. Lower concentration of VB$_{12}$ was not tested. But it was obvious that VB$_{12}$ at low concentrations can significantly catalyze Cr(VI) reduction by the immobilized n-ZVI, resulting in even faster reduction rate than using bare n-ZVI. Therefore, n-ZVI Ca-alginate beads are promising to be used to Cr(VI) in-situ remediation. The decreased reduction rate could be compensated by the less mobility and settling of n-ZVI, or sped up by metalloporphyrinogens added or in the environment.

6.1.4 Metalloporphyrinogen immobilization

Metalloporphyrinogens were successfully immobilized in the sol-gel matrix. Those dried at room temperature were glass-like, having few pores observed by SEM and the surface area measurement. Because the dissolved VB$_{12}$ was the best catalyst to Cr(VI) among the five being studied in this research. Therefore, only VB$_{12}$-immobilized sol-gel was tested to Cr(VI) reduction by ZVI. Although the immobilized VB$_{12}$ was less effective than dissolved VB$_{12}$, the results showed that the immobilized VB$_{12}$ significantly catalyzed reductions by m-ZVI and n-ZVI. Later, VB$_{12}$-immobilized sol-gel was made
porous having a high surface area through drying by a freeze dryer. More pores should make the immobilized MPs more reachable, and therefore more reactive. But, this VB$_{12}$ sol-gel has not been tested yet. Furthermore, VB$_{12}$ was able to be encapsulated in Ca-alginate beads, but the catalyst totally leached out when applied to an aqueous solution due to the high solubility of VB$_{12}$ and the big pore size of Ca-alginate gel beads. Therefore, the Ca-alginate beads containing VB$_{12}$ are not feasible for most of environmental remediation schemes.

6.2 Future work

As discussed above, several issues can be addressed in the near future. Some are highlighted in this section. First of all, the catalytic effects of synthesized MPs need to be further studied. The catalytic capability of Proto-Co and Proto-Fe was not obvious. Uro-Co and Uro-Fe are very promising revealed by the experiment results in 5.2.2.2, but the effects were not significant. Also it is still not clear from the results that the role of the core metal in the catalytic effect, because no significant difference was found in Cr(VI) removals between Uro-Co and Uro-Fe, between Proto-Co and Proto-Fe. Therefore MPs with higher concentrations would need to be tested.

Secondly, it is essential to test these MPs on other contaminants, except Cr(VI). Many oxidized pollutants have been released to the environment. For example, hexavalent uranium (U(VI)), nitrate, Pb(II), or chlorinated organic compounds. Each of them can be detoxified by reducing to a lower oxidation state. U(VI) is one of the co-
contaminants with Cr(VI), which is often found at DOE waste site. From a practical point view, it is vital to test the four MPs on U(VI) reduction.

Thirdly, the porous VB$_{12}$ sol-gel should be tested to Cr(VI) reduction. It is very promising, and should be more reactive due to VB$_{12}$ being easy to be reached through more pores. Its effects would be compared to dissolved VB$_{12}$ and VB$_{12}$ sol-gel prepared at room temperature. If this kind of sol-gel could make immobilized catalyst reactive, the four synthesized MPs would be immobilized in sol-gel using freeze drying method and tested to Cr(VI) reduction. Also the reuse experiments should be done to these sol-gel samples.

Last, but not least, it is necessary to optimize MPs-catalyzed reductions. It would be very helpful to know how MPs catalytic effects would be affected by other ligands, ionic strength, etc. Because Cr(VI) or other contaminants may form complexes with cations or anions, which would affect reduction rate. Also the ionic concentration may have an impact on electron transfer process. The collective objectives are to have a better understanding on catalytic capability of metalloporphyrinogens, and simulate one best-way to apply them to in-situ remediation.
APPENDICES
Appendix A

n-ZVI surface area analysis

Table A-1: n-ZVI surface area analysis by N\(_2\) adsorption

<table>
<thead>
<tr>
<th>Samples</th>
<th>BET surface area(^a) (m(^2)/g)</th>
<th>Adsorption average pore diameter (4V/A by BET, Å)</th>
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</thead>
<tbody>
<tr>
<td>050519(^b)</td>
<td>22.54</td>
<td>179.69</td>
</tr>
<tr>
<td>051709(^c)</td>
<td>33.64</td>
<td>173.09</td>
</tr>
<tr>
<td>061909(^c)</td>
<td>24.62</td>
<td>167.35</td>
</tr>
</tbody>
</table>

Mean ± Standard error\(^d\) 26.93 ± 3.41 173.38 ± 3.56

Notes: a. Method and Instrument: N\(_2\) adsorption BET method; b. n-ZVI has acid treatment at pH 2.5 (as Batch I); c. n-ZVI has acid treatment at pH 4 (as Batch II); d. The standard error was calculated based on the three batches of n-ZVI.
Appendix B

n-ZVI surface element analysis by FESEM-EDS

Figure B-1: n-ZVI surface element analysis by FESEM-EDS.

Table B-1: n-ZVI surface element analysis by FESEM-EDS

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight%</th>
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</thead>
<tbody>
<tr>
<td>O</td>
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</tr>
<tr>
<td>Fe</td>
<td>92.3</td>
</tr>
<tr>
<td>Totals</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Appendix C

Cr(VI) reduction by m-ZVI

Figure C-1: ANOVA test for Cr(VI) removals at 20 min by different amount of m-ZVI. ([Cr(VI)] = 100 µM, the loads of m-ZVI (Batch A) were 200, 300, 400 and 600 stoichiometric amounts, pH = 7 buffered by 0.05 M MOPS. Letters on the top of each column are significant letters. Error bars represent standard errors based on replicated tests (n = 2). P< 0.05 is individual error rate, family error rate is 0.15.)
1.7 g/L m-ZVI

2.5 g/L m-ZVI
Figure C-2: Data fitting of Cr(VI) reduction by m-ZVI to pseudo-first order model. ([Cr(VI)] = 100 µM, the loads of m-ZVI (Batch A) were 200, 300 and 400 stoichiometric amounts responded to 1.7 g/L, 2.5 g/L and 3.4 g/L, pH = 7 buffered by 0.05 M MOPS.) The rapid drops at the beginning were not included.
Figure C-3: Pseudo-first order Cr(VI) reduction rate constants vs. the concentration of m-ZVI added. ([Cr(VI)] = 100 µM, the loads of m-ZVI (Batch A) were 200, 300, 400 and 600 stoichiometric amounts, pH = 7.0 buffered by 0.05 M MOPS.)
Appendix D

Metalloporphyrinogen-catalyzed Cr(VI) reduction by m-ZVI

Figure D-1: ANOVA test for Cr(VI) removals of VB_{12}-catalyzed reduction at 45 hr and pH 8. ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch A), pH = 8.0 buffered by 0.05 M Bis-tris. Error bars represent standard errors based on replicated tests (n = 2). P< 0.05 is individual error rate, family error rate is 0.1.)
Figure D-2: Data fitting of catalyzed-Cr(VI) reduction by VB$_{12}$ and MPs to Pseudo-first order model (10-200 min). ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch B), 20 µM Proto-Co/Fe, Uro-Co/Fe or VB12, pH = 7.0 buffered by 0.05 M MOPS.)
The image contains two graphs showing the relationship between time (min) and ln[Cr(VI)] for different conditions.

Graph 1: m-ZVI-2\textsuperscript{cd}
- Time (min) range: 0 to 300
- ln[Cr(VI)] range: 3 to 5

Graph 2: VB\textsubscript{12}-2\textsuperscript{cd}
- Time (min) range: 0 to 300
- ln[Cr(VI)] range: 3 to 5

Both graphs display linear trends with data points and regression lines indicating the decay of Cr(VI) over time.
Figure D-3: Data fitting of m-ZVI reuse tests to Pseudo-first order model with/without VB$_{12}$. ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch C), 20 µM VB$_{12}$, pH = 7.0 buffered by 0.05 M MOPS.) The rapid drops at the beginning were included.
Appendix E

Cr(VI) reduction by n-ZVI

Figure E-1: Data fitting of Cr(VI) reduction by n-ZVI to Pseudo-first order model. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI (Batch II), pH = 6.0 buffered by 0.05 M Bis-tris, pH = 7.0 buffered by 0.05 M MOPS.) The rapid drop was included for both pH values.
Appendix F

VB$_{12}$-catalyzed Cr(VI) reduction by n-ZVI

Figure F-1: Data fitting of catalyzed-Cr(VI) reduction by VB$_{12}$ to Pseudo-first order model. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI (Batch II), the reaction was catalyzed by 0.1 or 0.5 µM VB$_{12}$, pH = 7.0 buffered by 0.05 M MOPS.)
Appendix G

Cr(VI) reduction catalyzed by immobilized VB_{12}

Figure G-1: Data fitting of catalyzed-Cr(VI) reduction by immobilized-VB_{12} in the presence of m-ZVI (10-200 min). ([Cr(VI)] = 100 µM, 1.7 g/L m-ZVI (Batch B), the reaction was catalyzed by 20 µM VB_{12}, pH = 7.0 buffered by 0.05 M MOPS.)
Figure G-2: Data fitting of catalyzed-Cr(VI) reduction by immobilized-VB$_{12}$ in the presence of n-ZVI. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI (Batch II), the reaction was catalyzed by 0.1 or 0.5 µM VB$_{12}$, pH = 7.0 buffered by 0.05 M MOPS.)
Appendix H

Cr(VI) reduction by n-ZVI immobilized in Ca-alginate beads

Figure H-1: Cr(VI) reduction by n-ZVI immobilized Ca-alginate beads at pH 6. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI immobilized, pH = 6.0 buffered by 0.05 M Bis-tris, n = 1, new beads were used after being dried 48 hr, other beads were used after being stored approximate 3 months in the anaerobic chamber.)
Figure H-2: Cr(VI) reduction by n-ZVI immobilized Ca-alginate beads at pH 7. ([Cr(VI)] = 100 µM, 0.1 g/L n-ZVI immobilized, pH = 7.0 buffered by 0.05 M MOPS, new beads were used after being dried 48 hr (n = 1), other beads were used after being stored approximate 3 months in the anaerobic chamber (n = 3). Error bars represent standard errors based on replicated tests.)
Figure H-3: Data fitting of Cr(VI) reduction by n-ZVI immobilized in Ca-alginate beads. ([Cr(VI)] = 100 μM, 0.1 g/L n-ZVI immobilized in Ca-alginate beads (newly prepared), pH = 6.0 buffered by 0.05 M Bis-tris, pH = 7.0 buffered by 0.05 M MOPS.) The rate constants for pH 7 were calculated in two time periods: 0-100 min and 100-150 min.
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