MONTANA STATE UNIVERSITY

Transport and activity of dissimilatory metal-reducing bacteria in porous media for the remediation of heavy metals and chlorinated hydrocarbons
by Robin Gerlach

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Engineering
Montana State University
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Abstract:
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The reduction of chromium [Cr(VI)], a compound of significant environmental concern at many Department of Energy, Department of Defense, and Environmental Protection Agency Superfund sites, can be facilitated by DMRB. Starved S. algae BrY cells can be resuscitated into an actively metabolizing state in the presence of ferric iron [Fe(III)], which is abundant in the environment. Active S. algae BrY cells can either directly reduce Cr(VI) or produce surface reactive ferrous iron [Fe(II)]. Fe(II) chemically reduces and precipitates Cr(VI) eliminating it from contaminated water. S. algae BrY cells can also potentially contribute towards the long term reactivity of zero valent iron subsurface barriers. The microbial reduction of Fe(III) allows for the removal or activation of surface associated corrosion products and results in increased transformation rates of carbon tetrachloride, another widespread environmental contaminant.

The results summarized in this dissertation indicate that the injection of starved DMRB is a promising technology for the remediation of subsurface environments contaminated with heavy metals and chlorinated hydrocarbons.

TRANSPORT AND ACTIVITY OF DISSIMILATORY METAL-REDUCING BACTERIA IN POROUS

MEDIA FOR THE REMEDIATION OF HEAVY METALS

AND CHLORINATED HYDROCARBONS

by

Robin Gerlach

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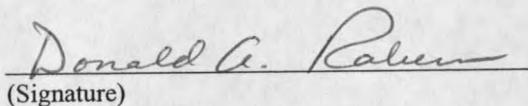
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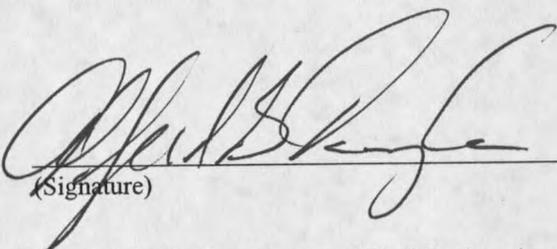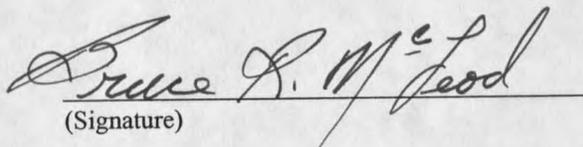
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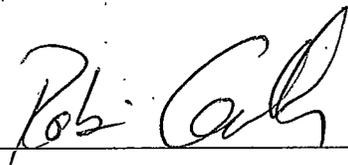
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ABSTRACT

Dissimilatory metal-reducing bacteria (DMRB) are capable of reducing a wide range of metals and the injection of DMRB is considered for the cleanup of contaminated soil and groundwater. The success of such an approach, among other factors, relies upon the bacterial transport through porous media and the contaminant transformation by DMRB.

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CHAPTER 1

INTRODUCTION

Every year, large quantities of organic and inorganic compounds are released into the environment as the result of human activities. These releases can be deliberate or accidental. Due to their toxicity and recalcitrance in the environment, many of these compounds can accumulate to unacceptable levels and pose a severe risk to humans and the environment. Davis (1972) was one of the first to suggest the application of bacteria in the remediation of contaminated groundwater. The use of indigenous or exogenous bacteria for the cleanup of contaminated groundwater has since become a widely applied approach.

In situ bioremediation has become one of the preferred technologies for the cleanup of contaminated sites since it avoids the need for excavation and subsequent disposal of hazardous waste. While the bacterial degradation of fuel hydrocarbons and polyaromatic hydrocarbons is well documented (Alexander, 1999; Crawford and Crawford, 1996), the use of bacteria for the cleanup of soils and groundwater contaminated with chlorinated hydrocarbons (U.S.EPA, 2000) and metals (Evanko and Dzombak, 1997; McCullough et al., 1999) remains the focus of much research.

The increased need and interest for biological metal and radionuclide remediation is partially based on more than 50 years of using nuclear energy for both peaceful and military purposes. Many Department of Energy (DOE) and Department of Defense (DOD) sites are contaminated with hazardous and radioactive waste. These sites create a legacy that is estimated to cost the DOE alone more than US $ 60 billion over the next 10 years (McCullough et al., 1999). The contamination at many DOE facilities is spread over large areas and deep below the surface (McCullough et al., 1999). Conventional treatment strategies, such as pump and treat or excavation with subsequent treatment, might not be practical, economical, or advisable due to the long term liability that might be created. Thus, the development of alternative strategies for the treatment of DOE sites can potentially save billions of dollars.

Two subsurface remediation strategies have received much attention over the recent years and have demonstrated potential for the development of economically viable, long term, low maintenance cleanup strategies.

1. The use of permeable reactive barriers (PRBs). PRBs established downstream of contaminated sites have proven to effectively prevent off-site migration of chlorinated hydrocarbons, metals, and radionuclides (Scherer et al., 2000; U.S.EPA, 1998; Yuyun and Allen, 1999).

2. The metabolism of dissimilatory metal-reducing bacteria (DMRB). DMRB have demonstrated potential for the remediation of groundwater contaminated with chlorinated hydrocarbons, heavy metals, and radionuclides (Amonette et al., 2000; McCullough et al., 1999; Workman et al., 1997).

Dissimilatory metal-reducing bacteria (DMRB) gain energy for growth by coupling the oxidation of organic compounds or hydrogen to the dissimilatory reduction of ferric iron [$Fe(III)$] and other oxidized metals. DMRB can enzymatically reduce large amounts of a wide range of metal ions, including $Fe(III)$, $Cr(VI)$ and $U(VI)$ (Harding, 1997; Lovley, 1993; Lovley, 1995b). Although the direct enzymatic reduction of heavy metals by DMRB potentially allows for the development of remediation strategies, the indirect reduction of soluble contaminants such as $Cr(VI)$, $U(VI)$, and carbon tetrachloride might be more important and feasible in subsurface environments (Fredrickson and Gorby, 1996a; Harding, 1997; McCullough et al., 1999). DMRB can reduce a wide range of ferric iron minerals to produce redox reactive ferrous iron [$Fe(II)$] (Caccavo, Jr. et al., 1992). Ferrous iron can chemically react with soluble contaminants including $Cr(VI)$, $U(VI)$, and carbon tetrachloride (Amonette et al., 2000; Eary and Rai, 1988; McCullough et al., 1999).

Over the last decade, it has become apparent that the biological and geochemical processes described above can govern the fate of metals and radionuclides in the subsurface. Consequently, a number of governmental agencies have begun to support research addressing the fate of heavy metals and radionuclides in the subsurface. The DOE, for instance, established the Natural and Accelerated

Bioremediation Research (NABIR) program in 1997. The NABIR program focuses on the development of a strong theoretical foundation for the assessment and remediation of DOE sites contaminated with mixtures of metals and radionuclides. The high interest in the biogeochemical processes involved also recently led to a five day symposium on "Chemical-Biological Interactions in Contaminant Fate", organized by the Division of Environmental Chemistry at the 220th American Chemical Society National Meeting in Washington D.C. (August 20-24, 2000). The majority of the presentations focused on the influence of iron minerals and dissimilatory metal-reducing bacteria on the fate of chlorinated hydrocarbons, heavy metals, and radionuclides. The presentations clearly demonstrated the importance of metal-reducing bacteria in contaminated subsurface environments.

The idea of utilizing DMRB to establish and maintain permeable reactive barriers (PRB) in the subsurface is intriguing. DMRB, present in the subsurface or injected into the subsurface, could be stimulated to produce redox reactive ferrous iron from indigenous or injected ferric iron. The produced ferrous iron would chemically react with chlorinated hydrocarbons, Cr(VI), U(VI), and other contaminants. However, reports of field trials evaluating the use of DMRB for the establishment of redox reactive subsurface barriers are lacking. Thus, this research project is designed to improve the basis for future field demonstrations involving the use of DMRB for environmental cleanup.

The major research goals of this dissertation are:

1. The development of a strategy for the enhancement of bacterial transport through porous media, and

2. The assessment of using dissimilatory metal-reducing bacteria for the *in situ* bioremediation of heavy metals and chlorinated hydrocarbons.

These two main research goals are reflected in the structure of this dissertation. The first part of the introduction reviews the processes and parameters governing bacterial transport through porous media. This review is the basis for Chapters 2 and 3, which evaluate bacterial starvation as a promising strategy for the bacterial transport enhancement (Chapter 2), and document the changes in transport-related bacterial cell properties during long term starvation (Chapter 3). The second part of the introduction provides an

overview of the current literature on permeable reactive subsurface barriers and microbial metal-reduction. This overview provides the background for the Chapters 4 and 5, which describe the potential use of DMRB to establish and maintain redox reactive zones in the subsurface capable of the continuous precipitation of Cr(VI) (Chapter 4) and the potential of DMRB to influence the performance of zero valent iron redox reactive subsurface barriers (Chapter 5).

Bacterial Transport Through Porous Media for Bioaugmentation (Research Goal 1)

The transformation rates achievable by stimulation of the indigenous microflora at contaminated subsurface sites are often insufficient to develop economically viable remediation technologies. An insufficient number of indigenous microorganisms capable of degrading the compounds of interest or the inability to effectively stimulate the indigenous microflora are commonly encountered reasons for low transformation rates (Vogel, 1996; Walter, 1997). Therefore, bioaugmentation, the injection of microorganisms which can biotransform environmental contaminants, has been proposed and implemented (Dybas et al., 1998; Ellis et al., 2000; McCullough et al., 1999; Salanitro et al., 2000; Steffan et al., 1999). Unfortunately, the injection of previously cultivated bacteria into subsurface formations can cause excessive biofilm growth in injection wells. The resulting clogging of well screens poses a major problem in the development of subsurface bioaugmentation strategies (Molz et al., 1986; Nelson et al., 1985, Malusis et al., 1997; Maxwell and Baqai, 1995). Thus, for the successful development of subsurface bioaugmentation strategies, the ability to avoid excessive adhesion, limit biofilm growth, and to facilitate bacterial transport through porous media needs to be improved. Much research has focused on the development of strategies for the enhancement of bacterial transport through porous media. However, a promising technology applicable to a wide range of bacterial strains and to different hydrogeological conditions has yet to be developed.

A bacterial strain or consortium chosen for subsurface bioaugmentation should have the following characteristics. The microorganisms should easily obtain regulatory approval for injection. The microorganisms should be easily transported to avoid the plugging of injection wells and readily distributed

over large areas. The cells should yet be adhesive enough to distribute themselves along the flowpath. They should be compatible with the indigenous microflora, i.e. they should not negatively influence the existing microflora, but should be able to survive and actively metabolize the compounds of interest. Bacterial strains or consortia for bioaugmentation should be inexpensive to obtain and easy to culture.

The following literature review on bacterial transport through porous media will justify the choice of bacterial starvation as a means for enhancing bacterial transport through porous media.

Parameters Influencing Bacterial Transport Through Porous Media

Bacterial transport is typically governed by aqueous phase advective movement coupled with retardation by adhesion onto surfaces and straining or trapping in interstitial pores. Bouwer et al. (2000), Mills (1997), Lawrence and Hendry (1996), and Harvey (1991) provide excellent reviews of the factors influencing bacterial transport through porous media. The numerous parameters influencing bacterial transport and adhesion in porous media can be grouped into three major categories, 1) solution chemistry, 2) porous media characteristics and hydraulic conditions, and 3) properties of the bacterial cells. These categories are discussed in the following paragraphs.

Influence of Solution Chemistry on Bacterial Transport Through Porous Media. A wide range of solute characteristics has been reported to influence bacterial adhesion to surfaces and transport through porous media. An increase in ionic strength has been correlated with increasing attachment (Fontes et al., 1991; Gannon et al., 1991b; Jewett et al., 1995; Mills et al., 1994; Scholl et al., 1990; Tan et al., 1994) and is usually attributed to the compression of the electrostatic double layer in the presence of high ion concentrations (Das and Caccavo Jr., 2000; Deshpande and Shonnard, 1999; Mills et al., 1994). Scholl and Harvey (1992), Kinoshita et al. (1993), and Jewett et al. (1995) report the effect of changes in pH values on bacterial transport and attachment, however, no uniform results are found. Increased attachment with decreasing pH is reported by Scholl and Harvey (1995) and Kinoshita et al. (1993), while Jewett et al. (1995) state that changes in pH from 5.5 to 7 do not affect bacterial transport through porous media

columns. Temperature has also been shown to influence bacterial transport, however the effect seems to be case dependent. While Bellamy et al. (1985) describe higher cell removal at higher temperatures, Sarkar et al. (1994) describe lower removal at higher temperatures. Johnson and Logan (1996) investigated the influence of dissolved and sediment organic matter (DOM, SOM) on bacterial transport through porous media columns and describe an increase in travel distance in the presence of SOM. This increase in travel distance is attributed to an increase in negative surface charge of the iron-coated quartz sand through the addition of SOM. Like SOM, surfactants or dispersants can also result in decreased attachment and therefore facilitate the transport of bacteria through porous media. This is most likely due to decreased cell porous media interactions in the presence of surface active compounds. However, the activity or viability of the bacteria may be influenced negatively by the addition of such compounds (Goldberg et al., 1990; Gross and Logan, 1995b; Jackson et al., 1994; Paul and Jeffrey, 1984; Sarkar et al., 1994).

Influence of Porous Media Properties and Hydraulic Conditions on Bacterial Transport Through Porous Media. A number of porous media characteristics and hydraulic conditions have been reported to influence bacterial adhesion and transport through porous media. For instance, the pore size, grain size, and their distributions can influence bacterial transport through porous media (Fontes et al., 1991; Marlow et al., 1991; Sharma and McInerney, 1994; Smith et al., 1985; Wood and Ehrlich, 1978). Sharma and McInerney (1994) report that penetration rates of non-motile strains increase linearly with the size of the glass beads used, however, penetration rates of motile strains become independent with bead diameters of 398 μm or larger. Presumably, the lower specific surface area provided by larger grains and the higher hydraulic conductivity of the coarse grained porous medium allow for increased transport (Fontes et al., 1991; Marlow et al., 1991). Wood and Ehrlich (1978) and Smith et al. (1985) attribute enhanced bacterial transport to preferred flowpaths, which are provided by fractures or macropores present in the native material. The soil mineralogy (e.g. presence of Fe-minerals) and the organic matter content can influence the porous media surface charge and surface hydrophobicity, respectively. Both, the surface charge and the surface hydrophobicity, can influence bacterial adhesion to surfaces and transport through porous media.

The presence of positively charged surfaces like Fe-minerals in soils is reported to lead to increased attachment of bacterial cells, which carry a net negative charge at neutral pH values (Johnson and Logan, 1996; McCaulou et al., 1994; Mills et al., 1994; Scholl and Harvey, 1992; Scholl et al., 1990). Hydrophobic surfaces have been described to increase the number of cells adhering in batch and column systems (Absolom et al., 1983; Fletcher and Loeb, 1979; McCaulou et al., 1994) however, hydrophilic cells might show decreased attachment to hydrophobic surfaces (Paul and Jeffrey, 1985).

In addition to the surface chemistry, the physical characteristics, such as surface roughness, can significantly influence attachment regardless of the prevailing chemistry (Geesey and Costerton, 1979; Mueller, 1996). Thus, it is not surprising that hydrodynamic conditions have also been found to play a very important role in bacterial adhesion to surfaces. Rijnaarts et al. (1993) and Scheuerman et al. (1998) suggest that the hydrodynamics can become much more important in view of bacterial attachment than the physicochemical conditions.

The interstitial fluid velocity can vary widely when cells are actively injected into the subsurface. The interstitial fluid velocity depends on the pumping rates during injection and the radial distance from the point of injection. Most authors report an increase in breakthrough and dispersal of bacteria at higher interstitial fluid velocities (Gannon et al., 1991b; Marlow et al., 1991; Sarkar et al., 1994; Tan et al., 1994), although Camesano and Logan (1998) report higher dispersal at low pumping velocities and Gross et al. (1995) observe no influence of fluid velocity on bacterial transport through porous media.

Influence of Cell Properties on Bacterial Transport through Porous Media. A number of bacterial cell properties have an influence on bacterial adhesion and bacterial transport through porous media. The cell surface charge, often measured as zeta potential, has been shown to influence bacterial adhesion to surfaces. Cell adhesion has been correlated mostly inversely but also directly with surface net electrostatic charge (Gilbert et al., 1991; Sharma et al., 1985; VanLoosdrecht et al., 1987a). Cell hydrophobicity has been reported to influence bacterial adhesion to surfaces and transport through porous media (Caccavo, Jr. et al., 1997; DeFlaun et al., 1999; Fletcher and Marshall, 1982; VanLoosdrecht et al., 1987b) and separate

mechanisms have been found for the bacterial adhesion to hydrophilic and hydrophobic surfaces (Paul and Jeffrey, 1985). Both, the hydrophobicity and net electrostatic charge, are influenced by the type of surface molecules present on the outside of the cells. Proteins and (lipo)polysaccharides are believed to influence bacterial adhesion to surfaces and transport through porous media but it cannot yet be predicted how certain surface molecules influence bacterial transport and adhesion (Abbott et al., 1983; Caccavo, Jr., 1999; Caccavo, Jr. et al., 1997; Costerton et al., 1978; Fletcher, 1976; Fletcher and Floodgate, 1973; Rijnaarts et al., 1996; Williams and Fletcher, 1996).

Cell motility and chemotaxis can have an influence on transport through porous media. While the influence of bacterial motility on bacterial transport through porous media seems to be mostly negligible at flow velocities typically encountered in aquifers (Barton and Ford, 1995; Barton and Ford, 1997; Camper et al., 1993), bacterial motility is suggested to result in increased dispersal of cells (Camesano and Logan, 1998) and in increased penetration under static conditions (Jenneman et al., 1985; Reynolds et al., 1989). Cell appendages, such as flagella and pili, have also been shown to become important during initial bacterial attachment to surfaces. This influence is attributed to an increase in collisions due to the greater effective diameter, an increase in effective diffusivity, and to the ability of the bacterial appendages to reach across the boundary layer between the liquid and the solid phase (Busscher et al., 1990a; Busscher et al., 1990b; Busscher et al., 1992; Mueller, 1996; O'Toole and Kolter, 1998a; O'Toole and Kolter, 1998b; Piette and Idziak, 1991). Das and Caccavo suggest that the flagellum acts as a motility-independent adhesin (Das and Caccavo, unpublished results).

Other bacterial cell properties correlated with transport through porous media include the cell size and cell shape. Smaller and more spherical cells are commonly thought to be transported better through porous media than larger and elongated cells (Fontes et al., 1991; Gannon et al., 1991a; Weiss et al., 1995). Harvey et al. (1997) show that the buoyant density of bacterial cells can influence the sedimentation rates of bacteria in porous media environments, but it is considered negligible at ambient groundwater flow velocities (Corapcioglu and Haridas, 1984).

Many of the cell properties listed above are influenced by the physiological state of the bacteria and can be significantly different for the same bacterium depending on the environmental conditions (Grasso et al., 1996; VanLoosdrecht et al., 1987a). Thus, controlling the physiology of a bacterial strain before injection offers great potential for the development of effective bacterial transport strategies.

Implications for Bioaugmentation Strategies

The review of the current literature implies that bacterial transport through porous media is influenced by a combination of numerous parameters. The importance of any single parameter is specific to any given situation and cannot be predicted easily. Camper et al. (1993) and Harvey (1997) state that bacterial transport experiments should be performed with the aquifer material of interest and as close as possible to the expected conditions in the field since the determination of individual characteristics of cells or the porous medium will not allow an accurate prediction. A good overview on how to design and standardize bacterial transport experiments is given by Harvey (1997).

The examination of the parameters influencing bacterial adhesion to surfaces and transport through porous media makes evident that only a very limited number of parameters can be effectively manipulated in field scale applications. Attempts to change the solution chemistry or the physicochemical properties of the porous medium are likely to become an economical and technological challenge. Thus, manipulating parameters falling into these two categories might be extremely difficult or not advisable. In addition to the economical and technological challenges, lowering the ionic strength or changing the pH of groundwater could lead to undesirable changes in the groundwater chemistry potentially resulting in increased contaminant mobility. Manipulating the physicochemical properties of the aquifer by for instance hydraulically or pneumatically fracturing the aquifer is difficult to control over large areas and might result in increased contaminant transport through zones with high hydraulic conductivity. *Manipulating and controlling the bacterial inoculum and the interstitial fluid velocity appear to have the greatest potential of economical and technological success in field scale subsurface bioaugmentation strategies.*

Bacteria are commonly cultured in large amounts before injection in bioaugmentation projects. The cultivation step allows for the manipulation of the inoculum in many different ways. Strains with a decreased tendency to adhere to surfaces can be selected or bacteria can be harvested at a specific growth rate. Procedures on how to select for such strains have been described in Caccavo et al. (1997) and DeFlaun et al. (1990, 1999). Unfortunately, these procedures can be time consuming and might not be successful for every bacterial strain or situation. Transport enhancement strategies which are potentially applicable to any bacterial strain and consortium are more desirable. The following paragraph will indicate that a potentially applicable strategy involves the use of starved bacteria.

Bacterial Starvation as a Transport Enhancement Strategy

Starvation is believed to be a common survival mechanism for bacteria which cannot form spores or cysts (Hood and MacDonell, 1987; Kjelleberg et al., 1993b; Tabor et al., 1981). Starvation of bacteria can result in radical size reduction and a rapid decrease in metabolic activity until the bacteria approach complete dormancy (Kjelleberg, 1993a). Starved bacteria can survive for years in the absence of nutrients (Amy and Haldeman, 1997; Kjelleberg, 1993a) and the improved transport of metabolically dormant cells is believed to contribute towards the presence of bacteria in the deep subsurface (Fredrickson and Onstott, 1996b; Lappin-Scott and Costerton, 1990). Starved bacteria can be resuscitated relatively rapidly by the addition of suitable nutrients (Amy and Morita, 1983; Cunningham et al., 1997; Kjelleberg, 1993a; Novitsky and Morita, 1978), making bacterial starvation a potentially effective means of facilitating transport of through porous media for a number of engineering applications (Bouwer et al., 2000; Cunningham et al., 1997; Cusack et al., 1992; Gerlach et al., 1998; Lappin-Scott and Costerton, 1992; Lappin-Scott et al., 1988a; Lappin-Scott et al., 1988b; MacLeod et al., 1988; Sharp et al., 1999).

The injection, resuscitation, and subsequent plugging of high permeability zones in oil bearing formations using starved bacteria is demonstrated in the literature (Cusack et al., 1992; Lappin-Scott and Costerton, 1990; Lappin-Scott et al., 1988a; Shaw et al., 1985). The use of starved bacteria results in significant improvements in secondary oil recovery since the bacteria travel deeper into the high

permeability zones and form bacterial plugs upon resuscitation, which force the injected water through the low permeability zones to recover residual oil more efficiently.

Starved bacteria have also been used in a series of laboratory and field scale experiments demonstrating the possibility to form hydraulic groundwater barriers. Starved cells derived from bacteria known to produce large amounts of extracellular polysaccharides (EPS) were injected into porous media and resuscitated. The bacteria are reported to form thick biofilms upon resuscitation and to decrease the porous media permeability dramatically (Cunningham, 2000; Cunningham et al., 1997).

It is well known that a number of changes occur during long term starvation of non spore-forming bacteria. A large amount of RNA and protein appear to be degraded rapidly at the onset of starvation (Kaplan and Apirion, 1975b; Pine, 1965), however synthesis of new proteins appears to be required for long term survival of starving cells (Groat et al., 1986; Hengge-Aronis, 1993; Kaplan and Apirion, 1975a; Kjelleberg et al., 1993b; Reeve et al., 1984). These proteins are believed to be part of a general stress response, often involving RpoS, and allow bacteria to become more stress resistant and more efficient scavengers (Cappelier et al., 2000; Fischer et al., 1998; Foster and Spector, 1995; Matin, 1991; Matin et al., 1989; Spector, 1998; Spector et al., 1999; Svensater et al., 2000; Teich et al., 1999; Zinser and Kolter, 1999; Zinser and Kolter, 2000). Increased stress resistance is verified by the observation that starved cells survived better under a range of stress conditions than their vegetative counterparts (Albertson et al., 1990; Hartke et al., 1994; Nystrom et al., 1988; Nystrom et al., 1990; Nystrom et al., 1992) and that slow growing cells are better adapted for starvation stress survival than their fast growing counterparts (Muller and Babel, 1996).

In the section "Influence of Cell Properties on Bacterial Transport through Porous Media" cell surface properties were discussed, which are known to influence bacterial attachment to surfaces and transport through porous media. It was discussed that the growth state of bacteria and the presence of nutrients can influence attachment. Thus, it is not surprising that bacterial starvation can significantly influence the attachment of bacteria to surfaces. Short term starvation of bacteria can result in an increased tendency to attach to surfaces (Dawson et al., 1981; Kjelleberg, 1984) whereas long term starvation (weeks

to months) may decrease bacterial attachment and enhance bacterial transport through porous media (Bouwer et al., 2000; Cusack et al., 1992; Gerlach et al., 1998; Lappin-Scott and Costerton, 1992; Lappin-Scott et al., 1988a; Lappin-Scott et al., 1988b; MacLeod et al., 1988; Sharp et al., 1999).

It is one goal of this research to develop and evaluate strategies for the enhancement of bacterial transport through porous media, which are potentially applicable to field relevant scales, a wide range of bacterial strains, and different hydrogeological conditions. Ideally, such a strategy should allow to culture a large number of bacteria inexpensively, which are mobile enough to transport over large distances and yet adhesive enough to attach to soil particles. This would allow establishing a sessile population capable of transforming dissolved and sorbed contaminants. Long term nutrient starvation appears to provide such a widely applicable strategy for the enhancement of bacterial transport through porous media.

The motivation for using the DMRB, *Shewanella algae* BrY, for these studies is explained in the following section.

Permeable Reactive Subsurface Barriers and Microbial Metal-Reduction (Research Goal 2)

The use of permeable subsurface treatment zones (or barriers) established downstream of contaminated areas provides potential for preventing the off-site migration of contaminants without severely influencing the natural groundwater flow. Permeable reactive barriers (PRBs) can provide long term control of a contamination problem, if their reactivity is maintained over extended periods of time. PRBs are commonly constructed by excavating trenches perpendicular to the groundwater flow direction and refilling these trenches with the reactive material of choice. These systems represent semi-passive approaches, which minimize the exposure of operating personnel to potentially hazardous compounds.

Permeable reactive barriers can be made in a number of different designs and consist of many different materials (Figure 1.1 and Figure 1.2, Gavaskar et al., 1998; Sacre, 1997; Scherer et al., 2000; U.S.EPA, 1997; U.S.EPA, 1998; Yuyun and Allen, 1999).

(a) Continuous reactive barrier configuration

(b) Funnel-and-gate configuration

(c) Multiple caisson gates

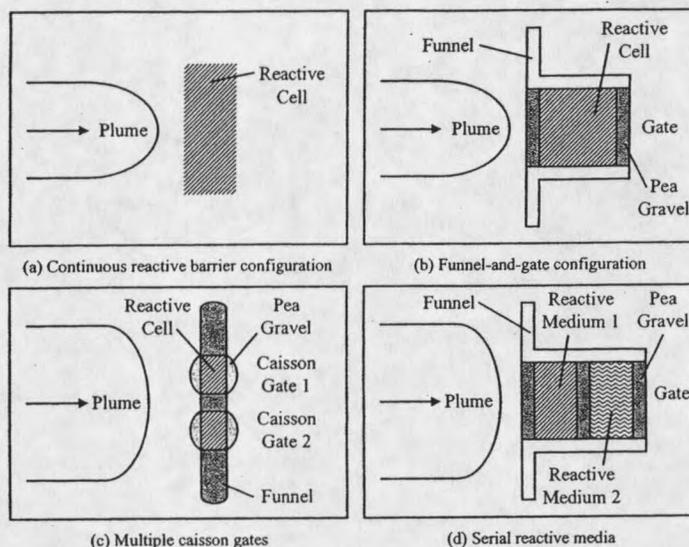(d) Serial reactive media

Figure 1.1.   Examples of permeable reactive barrier designs (from Gavaskar et al., 1998).

The most commonly used material is zero valent iron (Fe(0)). Fe(0) has been employed in permeable reactive subsurface barriers which are capable of remediating a wide range of contaminants including chlorinated organics (R-Cl, Eykholt and Davenport, 1998, Gatpagar et al., 1997, Gillham and O'Hannesin, 1994, Helland et al., 1995, Johnson et al., 1996, Johnson et al., 1998, Roberts et al., 1996, Sayles et al., 1997, Siantar et al., 1996), heavy metal and radionuclide oxyanions, such as chromate (CrO_4^{2-}, Gould, 1982, Cantrell et al., 1995) and oxycations, such as uranyl (UO_2^{2+}) (Fiedor et al., 1998), nitroaromatics (R-NO$_2$, Cao et al., 1999, Agrawal and Tratnyek, 1996), and nitrate (NO_3^-, Huang et al., 1998, Till et al., 1998, Siantar et al., 1996). The corrosion of Fe(0) provides the electrons necessary for the reduction of these contaminants according to the following half reactions.

$$Fe(0) \rightarrow Fe^{2+} + 2\ e^- \qquad \text{(Eq. 1.1)}$$

$$Fe^{2+} \rightarrow Fe^{3+} + e^- \qquad \text{(Eq. 1.2)}$$
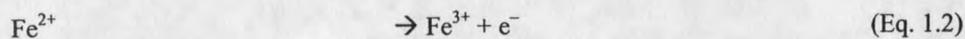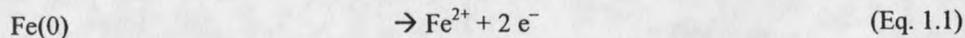
The electrons provided react with the different contaminants according to following half reactions.

$$R\text{-}Cl + 2\ e^- + H^+ \rightarrow R\text{-}H + Cl^- \qquad \text{(Eq. 1.3)}$$

$$CrO_4^{2-} + 3\ e^- + 8\ H^+ \rightarrow Cr^{3+} + 4\ H_2O \qquad \text{(Eq. 1.4)}$$

$$UO_2^{2+} + 2\ e^- \rightarrow UO_2 \qquad \text{(Eq. 1.5)}$$