Quantifying bacteria-contaminant interactions (Bacillus subtilis).

Lachlan Charles Wandlass. MacLean

University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

Recommended Citation
https://scholar.uwindsor.ca/etd/3253

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.
QUANTIFYING BACTERIA – CONTAMINANT INTERACTIONS

By

Lachlan Charles Wandlass MacLean

A Thesis Submitted to the Faculty of Graduate Studies and Research through the Department of Earth Sciences in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario
Canada
2003

© 2003 Lachlan Charles Wandlass MacLean
The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L’auteur conserve la propriété du droit d’auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-85150-8
ABSTRACT

Experiments were conducted to probe for the existence of positive sites on the cell wall of the Gram-positive bacterium Bacillus subtilis through a combination of electrophoretic mobility measurements and anion adsorption experiments with iodide. Iodide adsorption onto Bacillus subtilis was measured as a function of pH, ionic strength, solid:solute ratio, and time. The experimental data were interpreted using a surface complexation approach. The I\textsuperscript{-} adsorption data were best fit with a single surface site reaction, with the iodide ion forming a surface complex with the positively-charged amino functional group located on the bacterial cell wall: R – NH\textsubscript{3}\textsuperscript{+} + I\textsuperscript{-} \rightleftharpoons R – NH\textsubscript{3} – I\textsuperscript{-} (log K = 8.9 ±0.2). Electrophoretic mobility measurements, conducted as a function of pH and electrolyte ionic strength, support the presence of positively-charged surface functionalities at low pH under experimental conditions. Amino-anion stability constants may be incorporated into surface complexation models in order to accurately predict the bioavailability and exposure risk of radioiodine in the environment.

Additional anion adsorption experiments in metal-anion-bacteria ternary systems were conducted to determine the effect of multi-valent cations on the electrokinetic properties of the cell wall and its ability to adsorb anions. Adsorption results demonstrated that both anions and (oxy)anions have a pH-dependent affinity for the
cell wall of *B. subtilis* below pH 5. Increased adsorption of anions at higher pH values (>pH 4) was observed in the presence of 1 mM Ca. In contrast, no significant little adsorption was observed at similar pH values in the presence of both 0.1 mM and 1 mM La. The presence of positively-charged ions at the bacteria – solute interface can influence the mobility of anionic contaminants and further refines the surface complex approach.
CO-AUTHORSHIP STATEMENT

The following thesis contains material from a manuscript that has been submitted to the Environmental Science and Technology.

1. Environmental Science and Technology

The manuscript titled, "Experimental studies of bacteria – iodide interactions: extending the bacterial surface complex model", is co-authored by L.C.W. MacLean and D.A. Fowle. Laboratory work presented in this manuscript was performed by the author. The submitted version of this manuscript appears in Chapter 2.
FOR MY DAD
ACKNOWLEDGEMENTS

First, I would like to express my sincere gratitude to David Fowle, my supervisor, for having the patience and guidance to allow me the opportunity to alter my career path, thank you.

I would also like to thank my committee members, Dr. Brian Fryer and Dr. Doug Haffner, for their help and advice throughout my two years. Thanks to all my co-workers and grad students who I have met in the Biogeochemistry lab, Earth Sciences, and at GLIER. Especially to my office mates: Melissa, Hongxia, and Karlis for putting up with my madness.

My time here in Windsor wouldn’t have been half as satisfying without some great friends I was fortunate enough to meet: Johari Pannalal, Erin Bennett, Tom Collins, Scott MacLellan, Mark Cooke, and Lis Sabo. A special thanks to Sean Crowe, a truly great friend, for his wisdom and support throughout my two years here.

Finally to my family who have been my foundation throughout my life. My sisters and brother: Christy, Mairi, Jon Duart, and Katie Jane. To Sheila, who wasn’t here in person but always in spirit. Lastly, I want to thank my parents, Jon and Vivienne, for their constant source of inspiration and unwavering support throughout my life. I love you all.
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................ III

CO-AUTHORSHIP STATEMENT ................................................................. V

DEDICATION ............................................................................................... VI

ACKNOWLEDGEMENTS .......................................................................... VII

TABLE OF CONTENTS ........................................................................... VIII

LIST OF FIGURES ................................................................................... XI

LIST OF TABLES ...................................................................................... XII

LIST OF NOMENCLATURE....................................................................... XIII

I. MICROBIAL RETENTION OF CONTAMINANTS – A REVIEW .... 1

1.1 INTRODUCTION .................................................................................. 1

1.2 BACTERIAL CELL WALL ................................................................... 3

1.3 ADSORPTION .................................................................................... 5

1.3.1 Metal Adsorption ........................................................................ 6

1.3.2 Anion Adsorption ....................................................................... 6

1.4 SURFACE COMPLEX MODELING ..................................................... 7

1.5 RESEARCH OBJECTIVES ................................................................... 8

1.5.1 Anion Adsorption Experiments .................................................. 8

1.5.2 Ternary Complexation Studies ................................................... 9

1.6 ANION GEOCHEMISTRY ................................................................ 10

1.6.1 Iodine ......................................................................................... 10
1.6.2 Chromium ................................................................. 11
1.6.3 Selenium ................................................................. 12
1.6.4 Cations ................................................................. 12
1.7 REFERENCES ................................................................. 13

II. EXPERIMENTAL STUDIES OF BACTERIA - IODIDE INTERACTIONS: EXTENDING THE BACTERIAL SURFACE COMPLEX MODEL ................................................................. 18

2.1 INTRODUCTION ................................................................. 18
2.2 MATERIALS AND METHODS ............................................. 23
2.3 RESULTS AND DISCUSSION ........................................... 26
2.3.1 Surface Complexation Model ...................................... 29
2.4 ACKNOWLEDGEMENTS ................................................ 32
2.5 REFERENCES ................................................................. 33

III. TERNARY COMPLEXATION AT THE BACTERIA-SOLUTE INTERFACE: IMPLICATIONS FOR ANION CONTAMINANT TRANSPORT ................................................................. 44

3.1 INTRODUCTION ................................................................. 44
3.2 BACTERIA CELL WALLS ................................................ 45
3.3 TERNARY INTERACTIONS .............................................. 46
3.4 METHODS AND MATERIALS ......................................... 48
3.5 RESULTS ................................................................. 52
3.5.1 Control Experiments .............................................. 52
3.5.2 Anion Adsorption ................................................................. 53
3.5.3 Calcium Ternary Adsorption ................................................. 53
3.5.4 Lanthanum Ternary Adsorption ............................................. 54
3.5.5 Cation adsorption .............................................................. 55
3.5.6 Surface Charge ................................................................. 55
3.6 DISCUSSION ........................................................................... 57
3.7 CONCLUSIONS ..................................................................... 60
3.8 REFERENCES ......................................................................... 62

IV. CONCLUSIONS ........................................................................ 76

VITA AUCTORIS ........................................................................... 78
LIST OF FIGURES

Figure 2.1a ................................................................. 37
Figure 2.1b ................................................................. 38
Figure 2.1c ................................................................. 39
Figure 2.2 ................................................................. 40
Figure 2.3 ................................................................. 41
Figure 2.4 ................................................................. 42
Figure 3.1 ................................................................. 66
Figure 3.2a .............................................................. 67
Figure 3.2b .............................................................. 68
Figure 3.2c .............................................................. 69
Figure 3.3 ................................................................. 70
Figure 3.4 ................................................................. 71
Figure 3.5 ................................................................. 72
Figure 3.6 ................................................................. 73
Figure 3.7a .............................................................. 74
Figure 3.7b .............................................................. 75
LIST OF TABLES

Table 2.1.............................................................................................................43
# LIST OF NOMENCLATURE

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>AuCN</td>
<td>Gold Cyanide</td>
</tr>
<tr>
<td>C</td>
<td>Capacitance</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>Calcium Nitrate</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>CO₃</td>
<td>Carbonate</td>
</tr>
<tr>
<td>Cr</td>
<td>Chromium</td>
</tr>
<tr>
<td>°C</td>
<td>Celsius</td>
</tr>
<tr>
<td>EDL</td>
<td>Electric Double Layer</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>EM</td>
<td>Electrophoretic Mobility</td>
</tr>
<tr>
<td>F</td>
<td>Faraday’s Constant</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>H</td>
<td>Hour</td>
</tr>
<tr>
<td>HCrO₄⁻</td>
<td>Chromate</td>
</tr>
<tr>
<td>HNO₃</td>
<td>Nitric Acid</td>
</tr>
<tr>
<td>I</td>
<td>Iodine</td>
</tr>
<tr>
<td>IC</td>
<td>Ion Chromatography</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>Ion Coupled Plasma Optical Emission Spectroscopy</td>
</tr>
<tr>
<td>K</td>
<td>Stability Constant</td>
</tr>
<tr>
<td>K_app</td>
<td>Apparent Stability Constant</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>$K_{\text{int}}$</td>
<td>Intrinsic Stability Constant</td>
</tr>
<tr>
<td>$K_{2}\text{CrO}_4$</td>
<td>Potassium Chromate</td>
</tr>
<tr>
<td>$\text{KI}$</td>
<td>Potassium Iodide</td>
</tr>
<tr>
<td>$\text{KNO}_3$</td>
<td>Potassium Nitrate</td>
</tr>
<tr>
<td>$\text{La}_2\text{O}_3$</td>
<td>Lanthanum Oxide</td>
</tr>
<tr>
<td>$L$</td>
<td>Litre</td>
</tr>
<tr>
<td>$M$</td>
<td>Molal</td>
</tr>
<tr>
<td>$\text{mM}$</td>
<td>Milli-Molal</td>
</tr>
<tr>
<td>$\text{min}$</td>
<td>Minute</td>
</tr>
<tr>
<td>$M\Omega$</td>
<td>Milli-Ohms</td>
</tr>
<tr>
<td>$\text{Na}_2\text{SeO}_4$</td>
<td>Disodium Selenate</td>
</tr>
<tr>
<td>$\text{NaNO}_3$</td>
<td>Sodium Nitrate</td>
</tr>
<tr>
<td>$\text{NaOH}$</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>$\text{Pb}$</td>
<td>Lead</td>
</tr>
<tr>
<td>pH</td>
<td>$-\log[H]$</td>
</tr>
<tr>
<td>$\text{pK}_a$</td>
<td>$-\log K$</td>
</tr>
<tr>
<td>$R$</td>
<td>Gas Constant</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions Per Minute</td>
</tr>
<tr>
<td>SCM</td>
<td>Surface Complexation Model</td>
</tr>
<tr>
<td>Se</td>
<td>Selenium</td>
</tr>
<tr>
<td>$\text{SeO}_4^{2-}$</td>
<td>Selenate</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>TCP</td>
<td>2,4,6-trichlorophenol</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>μm</td>
<td>micro-metre</td>
</tr>
<tr>
<td>V(Y)</td>
<td>Output Variance Parameter</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
</tr>
<tr>
<td>ΔZ</td>
<td>Change in Charge of Surface Species</td>
</tr>
<tr>
<td>φ</td>
<td>Electric Field Potential</td>
</tr>
<tr>
<td>σ</td>
<td>Surface Charge</td>
</tr>
</tbody>
</table>
I. MICROBIAL RETENTION OF CONTAMINANTS – A REVIEW

1.1 Introduction

The solid-solute interface regulates contaminant mobility in near-surface hydrogeochemical systems (Stumm and Morgan, 1996). Mineral surfaces have been the main focus of attempts to quantify contaminant - surface interactions (Davis and Kent, 1990; Koretsky, 2000). However, there is growing recognition for the roles of microorganisms in these contaminated environments. Bacteria are ubiquitous in near surface aqueous environments as both free flowing solids and/or attached to mineral surfaces in the form of biofilms (Little et al., 1997). Bacteria surfaces represent upwards of 40% of reactive surfaces associated with metal adsorption in low temperature geologic systems (Ledin et al., 1996) and possess highly-reactive cell wall surfaces that can significantly influence geochemical processes (Nealson and Stahl, 1997). Numerous studies have demonstrated the ability of various bacteria to influence the mobility of contaminants in experimental aqueous systems (Beveridge and Koval, 1981; Fein et al., 1997; Fowle and Fein, 2000; Wightman and Fein, 2001) through adsorption to functional groups associated with the bacterial cell wall. Thus the presence of bacteria must be considered in any quantitative mass transport model of contaminants in near-surface hydrogeochemical systems.
Several chemical equilibria models have been developed to quantify contaminant interactions for use in reactive contaminant transport models (Davis and Kent, 1990; Stumm and Morgan, 1996; Fein, 2000). Yet the majority of these models, including the $K_D$ and Langmuir bulk partitioning approaches, are ill equipped to cope with the complex geochemical interactions within an aqueous system (Fein et al., 1997; Koretsky, 2000). These bulk partitioning models are applicable only to the conditions (pH, electrolyte chemistry, mineralogical compositions) in which they were determined (Fein, 2000). Conversely, thermodynamically-based surface complexation models (SCM) explicitly represent the chemical structure of the solid–water interface, treating the sorbed solute as separate reactions (Dzombak and Morel, 1990). Moreover, equilibrium constants ($K_{int}$) derived from SCMs are invariant with respect to the parameters which affect partition coefficients and thus are we are able to apply coefficients to systems other then those at which they were determined. Recently, a number of workers have successfully incorporated SCMs to quantify bacteria – metal interactions in controlled laboratory experiments (Plette et al., 1995; Fein et al., 1997; Fowle and Fein, 2000; Martinez and Ferris, 2001). Yet in order to develop a complete surface complex model to quantify bacteria – contaminant reactions we must have a better understanding of the
surface mechanisms involved in both anion binding and ternary complexes.

This thesis summarizes aqueous geochemical experiments which have been conducted to investigate interactions between a common Gram-positive soil bacterium, *Bacillus subtilis*, and various anions. We probe for the existence of positive sites on the cell wall of the Gram-positive bacterium *Bacillus subtilis* through a combination of electrophoretic mobility measurements and anion adsorption experiments with iodide. We quantify these chemical interactions utilizing a thermodynamically driven bacterial surface complexation model. Additional adsorption experiments were conducted in anion – metal – bacteria systems to determine the influence of multivalent cations on bacteria cell wall charge and subsequently anion adsorption. This work is critical for quantifying the mobility and geochemical reactions of common and toxic (oxy)anions such as chromate and selenate as well as radioactive isotopes of iodine distributed in and around nuclear waste depositories and power plants.

### 1.2 Bacterial Cell Wall

The bacterial cell wall provides a rigid, protective and selectively porous barrier between the cell and its environment. Although a few
microbes such as the mycoplasmas and certain archaea lack cell walls, the vast majority of bacteria (eubacteria) do exhibit cell wall structures (Heritage et al., 1996). Most bacteria are divided into two groups depending upon their ability or inability to retain a crystal violet-iodine complex when exposed to an organic solvent such as acetone or alcohol. Those that retain the complex are called Gram-positive; those that cannot are said to be Gram-negative (Heritage et al., 1996). Both Gram-positive and Gram-negative bacteria cell walls contain a variety of surface organic functional groups which display electrostatic, chemical, and hydrophobic affinities for aqueous metals, dissolved organic molecules, and mineral surfaces (Beveridge and Murray, 1980). The most common organic functional groups displayed on the bacterial cell wall surfaces are the anionic carboxylate, phosphoryl and hydroxylate sites and the positively-charged amine groups although bacteria differ in the amount and source of these functional groups. For example, Gram-positive bacteria have the carboxyl functional group as its most common moiety due to the presence of peptidoglycan which makes up a significant proportion of the cell wall. Conversely, the peptidoglycan found in Gram-negative cell walls is fundamentally the same structure as that found in Gram-positive cells but thinner, consisting of only one or two layers of peptidoglycan in their cell wall structure (Beveridge, 1989). It is thought that lipopolysaccharides, which make
up the outer membrane of Gram-negative bacteria play an essential role in metal binding abilities of the cell surface (Langley and Beveridge, 1999; Haas et al., 2001). The bacterial cell surface charge exhibits similar characteristics to that of many mineral surfaces whereby the net charge of the cell surface becomes increasingly negative with increasing pH (due to functional groups deprotonation).

1.3 Adsorption

The fate of many contaminants is controlled by the reactions of solutes with solid surfaces (Stumm, 1992). The ability of solute to bind to the surface of the bacteria cell wall can be attributed to either a chemical or physical interaction with the cell surface. Chemical adsorption occurs by the formation of coordinative bonds formed between the solute and surface. Physical adsorption includes electrostatic, hydrophobic and Van der Waals attraction. Electrostatic adsorption occurs between a solute and surface with opposite charge. Hydrophobic interactions occur as water is expelled from hydrophobic surfaces, thus attracting the nearby solutes. Van der Waals force is a constant attractive force between atoms and molecules caused by the oscillating charges of neutral molecules or atoms which produces synchronized dipoles that attract one another (Stumm and Morgan, 1996).
1.3.1 Metal Adsorption

Previous laboratory investigations have demonstrated effective adsorption of metal cations to bacterial cell walls (Beveridge and Murray, 1976, 1980; Daughney and Fein, 1998; Fowle and Fein, 1999, Fowle et al., 2000). Bacterial cell walls become negatively charged above pH 3.0 due to deprotonation of the surface ionizable surface functional groups. The majority of trace and heavy metals display a positive charge in solution and are attracted to the negatively charged cell wall where covalent and/or electrostatic reactions occur.

1.3.2 Anion Adsorption

Under low pH conditions the cell wall is fully protonated which will enable anions to interact with localized positive moieties associated with the peptidoglycan layer and proteins within the cell wall (Niu and Volesky, 1999; Haas et al., 2001). Most studies have focused on the adsorption of negatively charged organics such as 2,4,6-Trichlorophenol (TCP) and Humic acid to the bacteria cell wall. Daughney and Fein (1998) and Fein et al. (1999) demonstrated that both TCP and Humic acid, respectively, are significantly adsorbed to the cell surface of *B. subtilis* below a pH of 6. At higher pH values the negative charge of the bacteria cell walls repulsed both negatively-
charged organics. Furthermore, Wightman and Fein (2001) determined that the presence of humic acid in Cd - bacteria interactions diminished the amount of Cd that adsorbed to the cell wall at high pH by forming aqueous Cd-humate complexes. Conversely, the presence of Cd at low pH in humic acid - bacteria interactions had no effect on adsorption of humic acid to the cell wall, indicating that no competition exists between Cd and humic acid for bacteria surface adsorption sites at low pH.

1.4 Surface Complex Modeling

Experimental sorption data have been described by various empirical means, including partition coefficients, isotherm equations, and conditional binding constants (Davis and Kent, 1990). Because different functional groups become active under different geochemical conditions, these models which utilize binding constant values that change as a function of pH, ionic strength, mineralogy, aqueous solute speciation, temperature, pressure, and solute:surface area ratio are not ideal for calculating metal speciation in systems and conditions other than those studied (Fein, 2000).

Surface complexation models (SCM) are a specific type of chemical equilibrium approach used to quantify the extent of
adsorption. SCMs are the standard method for quantifying solute adsorption onto mineral surfaces by explicitly describing the chemical reactions that occur between the solute and specific sites on the surface of interest, thereby accounting for surface and aqueous speciation changes as a function of pH and solution composition (Stumm and Morgan, 1996; Fein, 2000). The underlying principle is that experimental examinations of isolated chemical reactions can be incorporated into geochemical models that estimate the distribution of dissolved mass in systems not studied in the laboratory (Fein et al., 1999). Therefore surface complexation models require a detailed understanding of the surfaces involved, the adsorption-desorption mechanisms present and the aqueous solution composition (Fein, 2000).

1.5 Research Objectives

1.5.1 Anion Adsorption Experiments

The bacteria cell wall is neutrally charged at low pH and becomes increasingly negatively charged at higher pH (Fein et al., 1999). Therefore the association of anions with the bacteria cell wall is expected to occur at low pH (2.5 – 3.5) when the cell wall is fully or partially protonated. Decreasing adsorption is expected at higher pH
as surface functional groups deprotonate, increasing the negative charge on the cell wall. This work will determine the ability of both Gram-positive and Gram-negative bacteria cell walls to sorb I⁻ from aqueous solutions as a function of pH and ionic strength. Reversibility measurements will determine the reversibility of any I⁻-bacteria adsorption reactions. The ability of the bacteria surface to fully desorb I⁻ would indicate a reversible reaction which would therefore enable the use of mass balance equations in our surface complexation model, thereby explicitly accounting for the effects of pH-dependent surface speciation and surface charge on the extent of adsorption (Yee et al., 2000). Reaction kinetics were measured as part of the surface complexation modeling approach.

1.5.2 Ternary Complexation Studies

Because bacterial surface functional groups are generally deprotonated at mid to high pH values (e.g. >5) contaminant anions such as hexavalent chromium and iodide are electrostatically repelled from the bacterial surface at pH conditions found in many natural groundwater environments. However, the presence of counterions (cations) in solution can enhance anion adsorption at mid to high pH values by shielding the bacterial negative charge and/or providing positively charged sites for anion interaction. Care must be taken
when modeling the influence of bacteria surfaces on contaminant mobility reactive transport models based only on ion-bacteria adsorption studies as most subsurface environments contain multiple counter-ions which influence contaminant – bacteria interactions. In this study, the metal cations lanthanum (III) and calcium (II) were used to determine the effect that these counterions have on the adsorptive and surface charge properties of bacteria surfaces. Chemical alterations on the bacterial cell wall were observed by electrophoretic mobility measurements.

1.6 Anion Geochemistry

1.6.1 Iodine

Iodine occurs most commonly as iodide (I⁻) in naturally oxygenated ground waters (Fuge and Johnson, 1986; Kaplan et al., 2000; Koch-Steindl et al., 2001). Because radio-iodine will occur predominantly as I⁻ in natural systems it is unlikely to form insoluble salts in natural waters and exhibits low amounts of sorption to common soil minerals. Combined, these properties make iodide one of the most mobile of the radioactive isotopes (Lefèvre et al., 1999; AECL, 1994)) in the environment. For example, ¹²⁹I is a product of nuclear power plants, reprocessing plants, waste disposal, and nuclear
weapon production (Balsley et al., 1996; Lefèvre et al., 1999; Pollner and Zagvai, 1999). Because $^{129}$I forms no insoluble salts in natural waters, exhibits low sorption to common soil minerals because of its anionic nature and has a long half-life ($1.6 \times 10^7$ years), it is potentially the most mobile of the radioiodine isotopes (Lefèvre et al., 1999). Most releases of $^{129}$I occur during the reprocessing of spent nuclear fuel and as a result of failures in containment tanks used to store processed fuel.

1.6.2 Chromium

Chromium is used in many industrial processes such as plating, alloying, tanning of animal hides, and as a water corrosion inhibitor. Chromium exists in nature in 2 valence states: trivalent chromium (III) and hexavalent chromium (VI). Cr(VI) is toxic to both plants and animals in concentrations as low as 0.01 mM in solution and 0.1 mM in soils (Turner and Rust, 1971; Fendorf, 1995). Cr(VI) is water-soluble, neutral or negatively charged under most pH conditions (Fein et al., 2002), and therefore very mobile in subsurface fluids and readily available to biota due to the high solubility of minerals containing it. Therefore knowledge of the chemical reactions that will influence its fate is essential in order to better predict its
movement in near-surface aqueous systems, including at the solid/water interface.

1.6.3 Selenium

Selenium is an essential trace element for animals and humans but can be toxic and lead to Se poisoning (selenosis) in humans and animals (Tinggi, 2003). Selenium occurs in oxidizing solutions as the selenite (SeO$_3^{2-}$) or selenate (SeO$_4^{2-}$) ionic species.

1.6.4 Cations

Calcium (Ca) and lanthanum (La) were utilized as the divalent and trivalent cations, respectively. Ca$^{2+}$ is a common component of most groundwater systems (Langmuir, 1997). Fowle and Fein (1999) quantified the adsorption of Ca$^{2+}$ to the cell wall of _B. subtilis_. La$^{3+}$ is a rare earth element (REE) commonly present in nuclear fuel wastewater streams (Texier et al., 1999) and has a stable valence of +3 in solutions (Figure 1). Adsorption of La$^{3+}$ to _Pseudomonas aeruginosa_ has been observed by Texier et al., (1999, 2000).
1.7 References


II. EXPERIMENTAL STUDIES OF BACTERIA - IODIDE INTERACTIONS: EXTENDING THE BACTERIAL SURFACE COMPLEX MODEL

2.1 Introduction

It is well established that reactive surfaces of minerals and organic matter are an important control on contaminant mobility in aqueous systems and a significant amount of effort has been expended to characterize important solid surfaces with respect to functional groups and sorptive capacities (Davis and Kent, 1990; Brown et al., 1999). It is increasingly recognized that bacteria, abundant in aqueous environments, also possess geochemically reactive surfaces (Beveridge and Murray, 1980; Fein et al., 1997; Fowle and Fein, 1999; Haas et al., 2001). Bacteria cell walls contain a variety of surface organic functional groups, including amino, carboxylic, hydroxyl, and phosphate sites (Beveridge and Murray, 1980).

---

1 A version of this chapter has been submitted to Environmental Science and Technology as:

L.C.W. MacLean and D.A. Fowle, April, 2003. Experimental Studies of Bacteria – Iodide Interactions: Extending the Bacterial Surface Complex Model.
and recent work has focused on classifying these surface structural moieties and their respective metal cation and proton binding capacities (Beveridge and Murray, 1980; Cox et al., 1999; Poortinga et al., 2002). Few studies have demonstrated or quantified the extent and processes for anion adsorption onto bacterial surfaces. In general the lack of anion adsorption on bacterial surfaces has been attributed to the abundance of deprotonated carboxyl and phosphate groups at pH values greater then pH 4, which in turn leads to repulsion between the surface sites and the anion of interest.

Recent potentiometric studies (Martinez et al., 2002; Fein, pers. comm.) provide indirect evidence for the presence of a surface functional site with a pK_a value ranging 2-3.2 which we propose to be associated with the first proton disassociation of a surface amino group (see Eq 2.1). If this functional group is in fact an amino site then it will be the only major surface functionality with a positive charge and in turn may influence the mobility of anionic contaminants at low pH values. We test for the existence of positive sites on the cell wall of the Gram-positive bacterium *Bacillus subtilis* through a combination of electrophoretic mobility measurements and anion adsorption experiments.

We utilized iodide as our anion probe for three reasons: 1) it remains a stable anion (I\(^-\)) throughout our experimental conditions; 2)
it demonstrates little or no hydrophobicity, i.e. any adsorption is considered chemical or electrostatic in nature; and 3) because of the interest in the geochemical fate and toxicity of its radioactive isotopes (Fuge and Johnson, 1986; Balsley et al., 1996; Yu et al., 1996). Unlike most radionuclides produced during bomb testing, which have returned to near pre-nuclear levels, the amount of radioactive iodine in the atmosphere and in surface waters has continued to increase due to releases from nuclear fuel reprocessing facilities (Moran et al., 1999). Iodine occurs most commonly as iodide (I⁻) in naturally oxygenated ground waters (Kaplan et al., 2000; Koch-Steindl et al., 2001). Because radio-iodine will occur predominantly as I⁻ in natural systems it is unlikely to form insoluble salts in natural waters and exhibits low amounts of sorption to common soil minerals. Combined these properties make iodide one of the most mobile of the radioactive isotopes (AECL, 1994; Lefèvre et al., 1999) in the environment. Its transport from constructed repositories into the biosphere is predicted to be very rapid (Balsley et al., 1996) and is commonly among the largest contributors to the calculated health risks associated with long-term nuclear waste disposal in the subsurface (AECL, 1994).

In conjunction with the adsorption experiments we will test whether the site-specific surface complexation model (SCM) of Fein et al. (1997) can be used to quantify I⁻ adsorption onto the Gram-positive bacterium *B. subtilis*. The SCM for *B. subtilis* describes specific
adsorption reactions between the functional groups of the bacterial cell wall and the species in solution through mass action laws that are governed by thermodynamic stability constants. The bacterial surface deprotonation reactions for the carboxyl, phosphoryl, hydroxyl, and the proposed amino functional groups are characterized by the following reactions from Fein, (pers. comm.):

\[
\begin{align*}
    R - \text{NH}_3^+ &\leftrightarrow R - \text{NH}_2^0 + H^+ \quad pK_a = 3.2 \\
    R - \text{COO}^- + H^0 &\leftrightarrow R - \text{COO}^- + H^+ \quad pK_a = 4.6 \\
    R - \text{O}^- + H^0 &\leftrightarrow R - \text{O}^- + H^+ \quad pK_a = 8.8 \\
    R - \text{PO}_3^- + H^0 &\leftrightarrow R - \text{PO}_3^- + H^+ \quad pK_a = 6.7
\end{align*}
\]

(2.1) (2.2) (2.3) (2.4)

where \( R \) represents the bacterium to which each functional group is attached. The site densities and surface area utilized for this model have been determined by Fein et al. (1997). Protonation of the cell wall amino functional groups creates a positively charged surface site for anion adsorption according to reaction 1. Protonation of this site will in turn lead to the development of localized areas of positive charge within the bacterial cell wall. Clearly this positive charge will in turn affect the interactions of ions with the bacterial surface sites. We can account for these effects on surface acidity constants and ion stability constants through the following relationship:
where $\Delta Z$ is the change in the charge of the surface species for the reaction under consideration; $F$ and $R$ are Faraday's constant and the gas constant, respectively; $T$ is absolute temperature; $K_{\text{intrinsic}}$ represents the equilibrium constant referenced to zero surface charge; and $\phi_0$ is the electric field potential of the bacterial surface (Fowle et al., 2000). We relate the surface electrical potential to surface charge $(\sigma)$ by a constant capacitance double-layer model:

$$C = \sigma/\psi$$

where $C$ is the integral capacitance of the $B. subtilis$ – aqueous electrolyte interface (8.0 F/m²) (20, 21). With this approach, equilibrium constant values can be used to quantitatively predict the extent of ion adsorption onto specific bacterial surface sites over a wide range of pH, ion concentration, and surface site concentration conditions (Fein et al., 1997; Fowle et al., 2000; Daughney and Fein, 1998; Daughney et al., 1998).

The objective of this study is to probe for the presence of a positively charged functional site on the bacteria surface at low pH through adsorption reactions with iodide and by the electrophoretic measurements of the cell wall under varying pH and ionic strength.
conditions. In addition we will document the interactions between \textit{B. subtilis} and iodide, specifically the kinetics and extent of adsorption of iodide onto the cell wall and incorporate these results into a site-specific SCM.

\section*{2.2 Materials and Methods}

The bacterial species \textit{Bacillus subtilis} were cultured following the procedure outlined in Fowle and Fein (2000). The bacteria cell walls were rinsed 5X in the experimental electrolyte (0.1-0.001 M KNO$_3$) and soaked for 1 hour in the electrolyte prior to the experiments to remove any remnants of compounds contained in the culture medium. Integrity of the cell walls after the wash procedure was monitored using microscopy and Molecular Probes - LIVE/DEAD \textit{BacLight} bacterial viability kit. All solutions in this study were prepared with reverse osmosis deionized (18M\(\Omega\)) water. Prior to each experiment, the bacteria were pelleted by centrifugation at 6500 rpm for 60 min and the pellet mass measured in order to determine the concentration of surface functional groups in each experiment. Therefore the weight of bacteria used in each experiment is not reported as a dried weight but as a wet weight. In these experiments the wet weight was 9.8 \(\pm\) 1.1 times the dry weight.
All I⁻ - bacteria sorption experiments were conducted in 0.001 M KNO₃ electrolyte solutions, except those experiments that involved the varying background electrolyte ionic strength. Batch experiments were conducted at 25 ±1 °C in the dark as a function of pH, electrolyte ionic strength, and equilibration time. Bacteria were suspended in 0.001 M KNO₃ electrolyte, and aqueous I⁻ was added to the bacteria-electrolyte solution to create a homogeneous parent solution of known bacterial (1 g/L – 100 g/L) and I⁻ (10⁻⁵ m) concentrations. Aliquots of the parent solution were transferred to the reaction vessels (acid-washed polypropylene) and the suspension in each vessel was adjusted to the desired pH value using small volumes (less than 1.5% of total experimental volume) of standardized acid or base. A pH interval of 2.5 – 7.5 was chosen to ensure a considerable range of pH was represented while avoiding large ionic strength effects below pH values of 2.5. The reaction vessels were placed on a rotating rack and provided (25 rpm) end-over-end agitation. The final equilibrium pH was recorded, and the suspension was filtered through a 0.45µm nylon filter. Samples were analyzed immediately for I⁻ concentrations by a Dionex DX600 Ion Chromatography (IC) system with conductivity detection. The IC procedure included a Dionex IonPac AS16 4mm column, with a NaOH eluent solution. The concentration of I⁻ remaining in solution was considered to be that proportion of ion not
associated with the bacteria wall. Control experiments followed each experimental procedure exactly with the absence of bacteria.

Kinetic studies were conducted within a homogeneous parent solution of bacteria + iodide + electrolyte prepared as the adsorption experiment described above. The solution was stirred and maintained at pH 3.1 with an automatic titrator (Man-tech PC-Titrator) set to pH 3.1 ± 0.05. Aliquots were retrieved with a systematic sampling regime for 500 minutes. Samples collected were filtered as above with the filtrate covered and stored at 4° C prior to IC analysis.

Desorption experiments were conducted to determine the reversibility of I⁻ - bacteria adsorption reactions. A homogeneous parent solution of bacteria + iodide + electrolyte was prepared as the adsorption experiment described above. The parent solution was adjusted to pH 2.6 and placed on a rotating rack and provided (25 rpm) end-over-end agitation for 2 h. Two hours was considered enough time for maximum adsorption based on results of the kinetic experiments. Aliquots from this parent solution were taken and adjusted to sequentially higher pH values (2.5 – 8.0) and allowed to equilibrate for 2 h on a rotating rack and sampled for I⁻ content as described above.

The electrophoretic mobility of the surface of B. subtilis was measured in a homogenous bacteria – electrolyte solution as a function of ionic strength and pH. The electrophoretic mobility of a
particle is proportional to the applied field of the shear plane or zone where ions are no longer dragged along by a moving particle (Stumm and Morgan, 1996; Malvern Instruments, 2000). The magnitude of this potential would demonstrate the charge associated with the bacterial cell. The charge of the particle would dictate its direction and velocity in the applied electric field which was measured using laser Doppler electrophoresis. The electrophoretic mobility of the bacteria cell walls were measured using a Malvern Instruments Zetasizer 3000HSA. A more detailed review of electrophoretic measurements can be found in Wilson et al. (Wilson et al., 2001).

2.3 Results and Discussion

The cell walls of B. subtilis display a strong pH dependent affinity for I− (Figure 2.1a). The concentration of I− bound to the bacterium is strongly dependent on the solution pH and the solid:solute ratio. Adsorption increases with decreasing pH presumably due to the protonation of cell wall functional groups at low pH. At low bacterial surface concentrations (1g/L) little adsorption of I− onto the cell wall of B. subtilis is observed. However, increasing the concentration of bacteria caused a significant increase in the concentration of I− adsorbed to the cell wall. A maximum I− adsorption
of 87% was observed at pH 2.5 (10^{-8} M of I⁻ and 100g of bacteria/L). This increase in adsorption with increasing bacteria concentrations is attributable to the increase in reactive sites available for surface reaction in our experimental system. The control experiments conducted without bacteria revealed insignificant loss throughout the experimental controls, indicating no loss of I⁻ onto the reaction vessels, through precipitation or oxidation. Therefore, we assume that the difference between the starting I⁻ concentrations and the measured final concentration in each sample is due entirely to adsorption, and we represent the data in terms of percentage of the original concentration that is adsorbed.

Results of the adsorption kinetics experiments are depicted in Figure 2.1b and indicate that adsorption proceeds rapidly as equilibrium is reached in under 2 h with no significant changes to the extent of adsorption after 2h. Desorption experiments (Figure 2.1c) are in good agreement with adsorption experiments indicating a reversible chemical reaction which therefore enables use of mass balance equations in our surface complexation model. Furthermore, the relatively rapid kinetics and reversibility of I⁻ binding demonstrate that the loss of I⁻ from the aqueous phase in these under-saturated systems is likely through sorption to the bacteria surface.

Figure 2.2 shows the inverse relationship between the ionic strength of the solution and the association of I⁻ onto the bacterial
surface. In a homogeneous solution of 0.01 M ionic strength little adsorption is observed, however, as the ionic strength is lowered first to 0.005 M and then 0.001 M a significant increase in adsorption is observed at each subsequent ionic strength. These results are indirect evidence of an outer-sphere complex which is strongly dependent on ionic strength (Stumm, 1992; Stumm and Morgan, 1996).

Mobility measurements of the cell wall (Figure 2.3) provide clear evidence that at low pH (<3) the surface of *B. subtilis* is positively charged in low ionic strength solutions (0.001 M and 0.01 M) and neutral or negatively charged under high ionic strength (0.1 M) conditions. The presence of counter-ions (i.e. NO$_3^-$) at high concentrations (0.1 M) shields the positively-charged functional groups localized on the cell wall and presents a negative charge or an un-charge at the shear plane. As the ionic strength of the background electrolyte is reduced and NO$_3^-$ concentrations are too low to fully shield the bacterial surface, a positively charged wall is expressed. The ionic strength dependent adsorption experiments described previously in Figure 2.2 support this model. As the ionic strength of the electrolyte decreases there is an increase in the amount of I$^-$ associated with the cell wall. These results are in agreement with earlier work by Niu and Volesky (1999) who describe AuCN$_2^-$ bound to the cell wall of *B. subtilis* at low pH.
2.3.1 Surface Complexation Model

The experimental data were used to calculate stability constants for the I⁻ functional group adsorption reactions. We tested each model coupling I⁻ adsorption onto either protonated amino, carboxyl, phosphate, or hydroxyl sites. We used the program FITEQL 3.1 (Herbelin and Westall, 1994) to compare models involving different I⁻ adsorption reaction stoichiometries and to determine the model that most accurately describes our data. The results of these calculations, for each model and bacteria concentration are compiled in Table 2.1. The misfit of each model is quantified using the $V(Y)$ variance function in FITEQL:

$$V(Y) = \frac{\sum (Y_{\text{calc}} - Y_{\exp})}{s_{\exp}^{n_p n_{\text{II}} - n_u}}$$

(2.7)

where $Y_{\text{calc}}$ and $Y_{\exp}$ are the calculated and the experimental data, $s_{\exp}$ is the error associated with the experimental data (default FITEQL 3.1), $n_p$ is the number of data, $n_{\text{II}}$ is the number of group II components (total and free concentrations are known), $n_u$ is the number of adjustable parameters, and $V(Y)$ is the variance in $Y$. The
V(Y) value provides a quantitative measure of the goodness of fit of each model, and we use this parameter to determine the best-fitting model. The following reaction stoichiometry best represents our experimental data:

\[ \text{I}^- + \text{R} - \text{NH}_3^+ \leftrightarrow \text{R} - \text{NH}_3 - \text{I}^0 \]  

(2.8)

where R - NH$_3^+$ represents the protonated amine surface functional group. The calculation of the stability constants for the surface complexation reactions uses the equilibrium constants from Fein et al. (1997) for the acid/base properties of *B. subtilis*. The surface complexation modeling (Figure 2.4) shows the best fit is with a protonated amino group.

Because structural and metabolic characteristics of bacteria cell walls vary between species and environmental systems additional research is required to elucidate how bacteria can influence the geochemical movement of iodide under more natural conditions. For example, bacteria can display various surface structures such as s-layers, proteins, enzymes, and porins that could affect the interaction between the cell wall and iodide. It has been reported that bacteria surfaces covered by proteinaceous structures can have a zero point charge exceeding pH 4 (Poortinga et al., 2002) which would shift the iodide adsorption edge to higher pH values (Stumm, 1992).
Metabolically-active bacteria can create a proton motive force (pmf) during ATP synthesis in which protons are pumped across the cell membrane to create a proton gradient (McKane and Kandel, 1996), in the process creating a protonated surface (Calamita et al., 2001) which could neutralize the negatively-charged functional sites on the cell wall. This would enable closer interaction between the cell surface and negatively-charged contaminants such as iodide in high pH environments. Furthermore, since contaminated sites often contain both metals and anions, the presence of multi-valent cations in aqueous environments could create a “bridging” effect that may increase anion adsorption at higher pH (Wightman and Fein, 2001).

Our experiments demonstrate that: 1) the interaction between the bacteria surface and I⁻ is dependent on pH, adsorbent concentration, and ionic strength; 2) ionic strength experiments demonstrate that the iodide – bacteria association is dominantly an electrostatic reaction which suggests the presence of a positively charged cell wall under these conditions; and 3) electrophoretic mobility measurements support the adsorption data for a positive surface charge. The positive charge associated with the cell wall is likely due to the presence of positively charged amino functional groups in the peptidoglycan proteins associated with the cell wall (Campbell, 1991). Our results enhance the current model of the bacteria surface and will have an effect on the model interaction
between metal cations and the bacteria surface under low pH conditions (Beveridge and Murray, 1980). These results indicate that the presence of bacteria may compromise the containment measures implemented at nuclear waste disposal sites. Numerous studies have demonstrated the uptake of I- onto the surfaces of minerals (Balsley et al., 1996; Yu et al., 1996; Lefèvre et al., 1999) which have been proposed as fill material around radioactive waste tank nests. However, the presence of bacteria in these systems may hinder the uptake of radioiodine onto the mineral surfaces at circum-neutral pH and enhance its mobility away from these disposal sites rather than retard it. Further research is required to quantify these mineral – bacteria – iodide interactions to accurately determine the fate of radioiodine in these proposed systems.

2.4 Acknowledgements

This work was supported by Natural Sciences and Engineering Research Council (NSERC) of Canada, Canadian Foundation of Innovation (CFI) and Ontario Innovation Trust (OIT) awards to DAF. LCWM would like to thank Sean Crowe for helpful suggestions during preparation of this manuscript.
2.5 References


Malvern Instruments: Zeta Potential Measurements, Manual number MAN0150; 2000


Figure 2.1a. Percent adsorption of I\textsuperscript{-} by \textit{B. subtilis} as a function of pH and bacteria concentration. Experiments were conducted in 0.001 M KNO\textsubscript{3} with 10\textsuperscript{-5.0} M I\textsuperscript{-} and with 1.0, 10.0, 40.0, or 100.0 g of bacteria/L.
Figure 2.1b. Percent adsorption of I\(^-\) by *B. subtilis* as a function of time. Experiments were conducted in 0.001 M KNO\(_3\) at pH 3.1 with 10\(^{-5.0}\) M I\(^-\) and with 10 g of bacteria/L.
Figure 2.1c. The percent of I\textsuperscript{-} associated with the bacterial surface after adsorption (circles) and desorption (squares) as a function of pH in 0.001 M KNO\textsubscript{3} with 10\textsuperscript{-5.0} M I\textsuperscript{-} and a bacteria concentration of 10 g/L. Desorption began after a 2-h period of adsorption at pH 6.5. pH was then lowered to between 2.5 and 6.5.
Figure 2.2. Percent adsorption of I⁻ by *B. subtilis* as a function of pH and ionic strength. Experiments were conducted in 0.01 M (triangles), 0.005 M (squares), and 0.001 M (circles) KNO₃ with 10⁻⁵.0 M I⁻ and with 10 g of bacteria/L.
Figure 2.3. Electrophoretic mobility measurements of *B. subtilis* as a function of pH and ionic strength. Experiments were conducted in 0.1 M (triangles), 0.005 M (squares), 0.001 M (circles) KNO₃ with 10 g of bacteria/L.
Figure 2.4. I⁻ adsorption onto *B. subtilis* as a function of pH. Experimental values were obtained from experiments conducted in 0.001 M KNO₃ with 10⁻⁵.₀ M I⁻. The curves represent the best-fitting surface complexation model for each bacterial concentration, calculated using the weighted average values of the equilibrium constants of the adsorption reactions.
<table>
<thead>
<tr>
<th>Bacterial conc.</th>
<th>Model</th>
<th>$^b$pK$_a$</th>
<th>Log K</th>
<th>V(Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 g of bacteria/L</td>
<td>R-NH$_3$-I$^0$</td>
<td>3.2</td>
<td>9.33</td>
<td>4.91</td>
</tr>
<tr>
<td>10 g of bacteria/L</td>
<td>R-NH$_3$-I$^0$</td>
<td>3.2</td>
<td>9.07</td>
<td>1.43</td>
</tr>
<tr>
<td>40 g of bacteria/L</td>
<td>R-NH$_3$-I$^0$</td>
<td>3.2</td>
<td>8.69</td>
<td>1.38</td>
</tr>
<tr>
<td>100 g of bacteria/L</td>
<td>R-NH$_3$-I$^0$</td>
<td>3.2</td>
<td>8.35</td>
<td>1.45</td>
</tr>
</tbody>
</table>

$^a$Best-fitting model

$^b$Based on data from Fein (9).

Table 2.1. Comparison of I - B. subtilis Adsorption Models with variance, V(Y), as calculated by FITEQL. $^a$Choice of best fitting model is based upon fit of weighted average log K values and overall variance as calculated by FITEQL. $^b$Based on data from Fein (9).
III. TERNARY COMPLEXATION AT THE BACTERIA-SOLUTE INTERFACE: IMPLICATIONS FOR ANION CONTAMINANT TRANSPORT

3.1 Introduction

Bacteria are found in most fluid-rock systems, including fresh and saline surface waters (Geeser et al., 1977; Harvery et al., 1982), groundwaters (Mahmood and Rama, 1993; Corapcioglu and Kim, 1995), deep-sea hydrothermal systems (Mandernack and Tebo, 1993; Baker et al, 1994), and deep sedimentary basins (Ghiorsche and Wobber, 1989; Yakimov et al., 1995). For example, near-surface soil systems contain, on average, $10^6$ to $10^8$ cells per gram of sediment (Chapelle, 1993). Because of their ubiquitous existence, bacteria are clearly linked to cycling of elements in near-surface aqueous systems (Ledin, 2000). Additionally, the high surface area to volume ratio possessed by bacteria provides a large reactive surface that regulates the fate of various contaminants in near-surface hydrogeochemical systems. Numerous studies have investigated the chemical interaction of metals (Beveridge and Murray, 1980; Fein et al., 1997; Daughney and Fein, 1998; Fowle and Fein, 1999; Fowle et al., 2000), organics (Daughney and Fein, 1998; Fein et al., 1999), and anions (Niu and Volesky, 1999; MacLean and Fowle, 2003) onto bacterial surfaces. However, most experiments were conducted in model
systems of one cation or one anion and a known mass of bacteria. In contrast, many contaminated sites contain both metal cations and anionic or (oxy)anionic ligands which may form ternary surface complexes, thereby influencing the adsorptive properties of each individual solute (Fein, 2002). Pelette et al. (1996) observed a reversal of surface charge on the Gram-positive Rhodococcus erythropolis in the presence of high concentrations of Cd\(^{2+}\) and Zn\(^{2+}\). Wasserman and Felmy (1998) observed in their model that at an ionic strength of 0.001 M, 65% of the negative surface charge of B. brevis was reduced in the presence of 10\(^{-6}\) M of a trivalent cation. Clearly we must consider the possibility of synergistic geochemical reactions with bacterial surfaces in our models of ion transport.

3.2 Bacteria Cell Walls

The bacteria cell walls are comprised of a series of complex polymers that include both positively and negatively charged moieties that control the interaction between cell walls and ions in aqueous solutions. The cell wall of most Gram-positive bacteria includes large amounts of peptidoglycan and teichoic or teichuronic acids. Anionic sites include carboxylate (from peptidoglycan) and phosphate (from teichoic acid). Positively charged sites are exclusively ammonium,
from D-alanine (teichoic acid), amino sugar (glycan), and dianaminopimelic acid (peptide portion of peptidoglycan) (Doyle, 1989). The more numerous anionic sites produce a negatively-charged cell wall above pH 3.5 for most Gram-positive bacteria. However, in the presence of multivalent cations the negatively-charged cell wall and the structure of the surrounding electric double layer (EDL) can be shielded or potentially undergo charge reversal (Doelman, 1979; Collins and Stotsky, 1992; Wasserman and Felmy, 1998; Poortinga et al., 2000). For example, Doelman (1979) measured the electrophoretic mobility of Gram-negative bacteria in solutions containing varying concentrations of Pb^{2+}. At low Pb concentrations (<0.05 mM), a negative mobility was observed. Increasing lead concentrations led to a progressive shielding of the negative charge, eventually becoming positive at Pb concentrations above 0.3 mM.

3.3 Ternary Interactions

Schindler (1990) identifies two groups of ternary surface complexes that are likely to form in complex aquatic settings: Type A complexes in which a metal cation bridges the negative charges of both the reactive surface and ligand present in the system; and Type B complexes in which a ligand bridges the positive charges of the
reactive surface and a metal cation. The focus of this study is on Type A complexes as they are the probable complex in our anion based experiments. Type A ternary complexes tend to involve polyvalent cations and form as the result of excess charge density of the adsorbed cation that is only partially satisfied during its association with the surface functional group. This leaves a surplus of positive charge available for further adsorption of negatively-charged ligands. Fein (2002) provides a generic reaction that illustrates the formation of Type A ternary surface complexes:

\[ \equiv \text{SO}^- + M^{m+} + L^{y-} \leftrightarrow \equiv \text{SOML}^{(m-y-1)+} \]  

where \( \text{SO}^- \) is the negatively-charged surface, \( M \) is the metal cation with \( m \) charge, and \( L \) is the anion with \( y \) charge.

However, both Schindler (1990) and Fein (2002) describe mineral – metal – organic interactions and do not discuss the potential of bacteria populations to provide reactive surfaces for ternary complexation. Few studies have been reported on ternary interactions involving bacteria surfaces. The presence of humic acid was observed to diminish Cd adsorption onto \textit{B. subtilis} by Wightman and Fein (2001) and Fein and Delea (1999) demonstrated that aqueous EDTA can significantly diminish Cd adsorption onto \textit{B. subtilis}. However, both studies involved an aqueous organic species
out competing the cell wall for the aqueous Cd and fail to produce stability constants for ternary complexes. Consequently, we seek to illustrate via our experimental design the importance of ternary complexation on bacteria-contaminant interactions.

In this paper we compare the adsorption of iodide, chromate, and selenate anions to the surface of *B. subtilis* in a binary system and in the presence of the multivalent cations Ca$^{2+}$ and La$^{3+}$. Additionally we utilize electrophoretic mobility measurements to indirectly observe the influence of the multivalent cations on the surface charge of the bacterial cell wall.

### 3.4 Methods and Materials

*B. subtilis* cells were obtained from T.J. Beveridge (University of Guelph, Ontario, Canada). We used *B. subtilis* in these experiments because it represents a common soil bacteria and its wall structure has been studied exhaustively through bioassay studies (Beveridge and Murray, 1980) and its thermodynamic characteristics of cell wall surface sites have been determined by Fein et al. (1997). *Bacillus subtilis* was prepared and cultured following the procedure outlined in Fowle and Fein (2000) with the exception that the bacteria was soaked for 1hr and rinsed 5X in the background electrolyte of each
experiment. All solutions in this study were prepared with 18 MΩ water. The wash protocol ensures that the bacteria cell walls are stripped of competing metals and anions from the growth medium. Without a thorough wash, the total concentration of metals and anions in the system would be unknown, and the equilibrium states could not be calculated because the mass balance constraints would be unknown. Integrity of the cell walls after the wash procedure was monitored using microscopy, Molecular Probes – LIVE/DEAD BacLight bacterial viability kit, and the DOC measurements. Prior to each experiment, the bacteria were pelleted by centrifugation at 7500 rpm for 60 min. The mass of the pellet was measured in order to determine the concentration of surface functional groups in each experiment (See Fein et al., 1997 for a detailed explanation). Note that the weight of bacteria used in each experiment is not reported as a dried weight, but as a wet weight after centrifugation (ca. 10:1 wet:dry). The experiments were conducted under nutrient-absent conditions and therefore metabolic bacterial processes were minimized or negligible. All solutions in this study were prepared with RO deionized (18 MΩ) water.

All anion sorption experiments with B. subtilis were studied in 0.001 M KNO₃ or NaNO₃ electrolyte solutions. Batch experiments were conducted at 25 ± 1 °C as a function of pH, solid/solute ratio, and equilibration time. Bacteria were suspended in 0.001 M of the
experimental electrolyte, and a known amount of anion was added to the bacteria-electrolyte solution to create a homogeneous parent solution of known bacterial (10 g/L) and anion concentrations (10^{-4} – 10^{-5} M). Aliquots of the parent solution were transferred to the reaction vessels (acid-washed polypropylene), and the pH of the suspension in each vessel was adjusted to the desired pH value using small volumes (less than 1% of total experimental volume) of standardized HNO_3 or NaOH. The pH interval of 2.5 – 8.5 was chosen to ensure a considerable range of pH was represented while minimizing the strong changes in ionic strength below pH 2.5. The reaction vessels were placed on a rotating rack and provided (25 rpm) end-over-end agitation. The final equilibrium pH was recorded, and the suspension was filtered through a 0.45μm nylon filter. Immediately following sampling HNO_3 was added to each sample and all samples were stored at 4°C prior to analysis. Each sample was analyzed for both the anion and cation of interest by ICP-AES except the iodide, which was analyzed using ion chromatography utilizing a Dionex IonPac AS16 4mm column, with a NaOH eluent solution. The bacteria do not lyse, sporulate, or multiply during our experiments; therefore, cell concentrations or surface area changes do not affect our results. Control experiments were conducted that followed each experimental procedure exactly with bacteria absent. Batch
adsorption studies were conducted using $K_2CrO_4$ (Ricca), $Na_2SeO_4$ (EM Science), KI (Fisher), $La_2O_3$ (Alfa Aesar), and $Ca(NO_3)_2$ (Fisher).

Ternary experiments followed the same experimental procedures as the anion - bacteria adsorption experiments outlined above. The only exception is that $Ca^{2+}$ or $La^{3+}$ was added simultaneously with the anion and bacteria to the electrolyte. Aliquots from this parent solution were taken and adjusted to pH values from 2.5 - 8. The reaction vessels were placed on a rotating rack and provided (25 rpm) end-over-end agitation. The reaction vessels equilibrated for 2 h and were sampled for both anion and cation as described above. Filtrates were stored for later analysis as described above for adsorption experiments.

The concentrations of $10^{-3}$ M $Ca^{2+}$ and $10^{-4}$ M $La^{3+}$ used are all into average ranges found in natural near-surface groundwater environments (USEPA, 1983). The electrophoretic mobility (EM) of the surface of $B. subtilis$ in the presence of either $10^{-3}$ M $Ca^{2+}$ or $10^{-4}$ M $La^{3+}$ was measured in a homogenous metal - bacteria - electrolyte solution as a function of ionic strength and pH. The electrophoretic mobility of a particle is proportional to the applied field of the shear plane or zone where ions are no longer dragged along by a moving particle (Stumm and Morgan, 1996; Malvern Instruments, 2000). The magnitude of this potential is related to the charge associated with the bacterial cell. For example, the charge of the particle would dictate its
direction and velocity in the applied electric field which was measured using laser Doppler electrophoresis. The electrophoretic mobility of the bacteria cell walls were measured using a Malvern Instruments Zetasizer 3000HSA. A more detailed review of electrophoretic measurements can be found in Wilson et al. (2001).

3.5 Results

3.5.1 Control Experiments

Experiments conducted in the absence of bacteria (data not shown) demonstrated insignificant loss throughout the experimental controls indicating no loss onto the reaction vessels, through precipitation or oxidation. The exception was the ternary complexation reactions involving 1 mM La which displayed an increased amount of Cr and Se removal from solution in both adsorption and control experiments, indicating an uptake by La precipitation independent of bacteria surfaces. All adsorption data are presented in terms of the percentage of the original concentration of the anion in question that is removed from solution.
3.5.2 Anion Adsorption

Results of the anion – bacteria adsorption experiments are shown in Figure 3.1. Initial iodide adsorption datum from MacLean and Fowle (2003, Chapter 2). HCrO$_4^-$ bacteria experiments demonstrated significant adsorption under low pH conditions with decreasing adsorption with increasing pH. A maximum adsorption of 41% was observed at pH 2.9. The adsorption decreased with increasing pH down to 0% adsorbed at pH 7.0. Se – bacteria experiments show a significant adsorption of SeO$_4^{2-}$ under low pH conditions with decreasing adsorption with increasing pH. A maximum adsorption of 45% was observed at pH 2.6. The adsorption decreased with increasing pH down to 0% adsorbed at pH 4.8.

3.5.3 Calcium Ternary Adsorption

Ternary I$^-$ - Ca adsorption experiments are shown in Figure 3.2a along with initial iodide – bacteria adsorption data from MacLean and Fowle (2003, Chapter 2). An increased amount of adsorbed I$^-$ at higher pH values (>3.5) is observed in the presence of 1 mM Ca$^{2+}$ and a shift in the adsorption edge is clearly demonstrated. Similar to I$^-$, HCrO$_4^-$ adsorption in the presence of 1 mM Ca is observed to increase by 5 to 20 percent between pH values of 3.5 – 7.5 compared to the HCrO$_4^-$ - bacteria binary system (Figure 3.2b). However, the presence
of 0.1 mM divalent Ca²⁺ in the SeO₄⁻ system did not exhibit any effective adsorption increases (Figure 3.2c).

### 3.5.4 Lanthanum Ternary Adsorption

The results of the lanthanum – iodide ternary experiment show significantly less adsorption above pH 3.5 compared with the Ca²⁺ - I⁻ experiments (Figure 3.3). At low pH, where iodide is significantly adsorbed in La-free solutions, a significant decrease is observed in the presence of La³⁺. The presence of La³⁺ decreased the amount of HCrO₄⁻ adsorption from 41% to 34% at pH 2.9 (Figure 3.3) but no significant adsorption was observed up to a pH of 7. In similar fashion, SeO₄⁻ in La³⁺ ternary solutions displayed little adsorption above pH 4.0 compared with the ternary Ca²⁺ - SeO₄⁻ system (Figure 3.3). In the binary SeO₄⁻ - bacteria system a decrease in adsorption from 45% at pH 2.6 to 26% at the same pH 2.6 occurs in the presence of La³⁺.

HCrO₄⁻ and SeO₄⁻ adsorption experiments were conducted in the presence of 1 mM La³⁺ to determine if the lack of increased adsorption at higher pH values was a result of a low concentration of La³⁺. Significant increase in both HCrO₄⁻ and SeO₄⁻ adsorption above pH 6.5 is observed (Figure 3.4). However, the control experiments also display this anion removal which indicates that adsorption of
HCrO$_4^-$ and SeO$_4^-$ in the presence of 1 mM La$^{3+}$ is likely a precipitation effect.

3.5.5 Cation adsorption

Under low pH conditions (pH < 2.5) little adsorption of either cation is observed (Figure 3.5). However, as pH rises above 3 there is a significant increase in adsorption of both La$^{3+}$ and Ca$^{2+}$. At a concentration of 0.1 mM, La$^{3+}$ increases in adsorption from 9% at pH 2.6 to 98% at pH 5.5. Ca$^{2+}$, at a concentration of 1 mM, increases in adsorption from 5% at pH 2.5 to 41% at pH 7.2.

La$^{3+}$ adsorption at a concentration of 0.1 mM, in the presence of HCrO$_4^-$ and SeO$_4^-$ was measured to determine the influence of anion adsorption on La$^{3+}$ uptake by the bacteria. The results (Figure 3.6) showed no change in La$^{3+}$ adsorption in the presence of 0.01 mM SeO$_4^-$ and 0.1 mM HCrO$_4^-$.

3.5.6 Surface Charge

Electrophoretic mobility measurements demonstrated that the presence of the divalent Ca cation significantly reduced the negative mobility of the bacteria cell wall. Above a pH of 3.0, up to 65% reduction was observed at a Ca$^{2+}$ concentration of 1 mM and up to
80% reduction occurred at a Ca$^{2+}$ concentration of 10 mM (Figure 3.7a), compared to the baseline NaNO$_3$ background electrolyte in the absence of Ca$^{2+}$. A slight increase was observed at both Ca$^{2+}$ concentrations below pH 3.0. These results demonstrate the ability of the divalent Ca$^{2+}$ cation to dampen the negative charge of the B. subtilis cell wall above pH 3.0.

The mobility experiments performed in the presence of the trivalent La$^{3+}$ cation showed a more pronounced result on the electrophoretic mobility of the B. subtilis cell wall than the divalent experiments (Figure 3.7b). In the presence of no La$^{3+}$, the bacteria – electrolyte solution displayed a negative mobility above pH 2.5. When La$^{3+}$ was introduced into the solution the negative mobility was observed to decrease with increasing La$^{3+}$ concentrations. A charge reversal was observed both at 0.08 and 0.1 mM concentrations. Above pH 6.0 the charge reversal is likely the result of a La$^{3+}$ hydroxide precipitate forming on the bacteria surface. At higher La$^{3+}$ concentrations (0.1 mM) the charge reversal is observed across the entire pH range which is likely both a specific adsorption effect and a precipitation effect.
3.6 Discussion

The results of our adsorption experiments illustrate significant adsorption of chromate and selenate to the cell wall of B. subtilis at low pH (<pH 3.0). Outer-sphere complexation with a protonated amino surface site (R-NH₃⁺) has been previously proposed as a mechanism for anion adsorption (MacLean and Fowle, 2003). We used the experimental adsorption data to calculate stability constants for the anion – amine functional group adsorption reactions. The program FITEQL 3.1 (Herbelin and Westall, 1994) was used to compare models involving different anion adsorption reaction stoichiometries and determine the model that most accurately describes our data. The equilibrium constants from Fein et al. (2003; pers comm.) for the acid/base properties of B. subtilis were used to calculate the stability constants for the surface complexation reactions. The surface complexation modeling of iodide and selenate (Figure 3.1a) show that the best fit between the surface functional groups and the anions is with a protonated amino group. In contrast the model does not fit the Cr adsorption experiments. This is not unexpected as Fein et al. 2002 demonstrated significant reduction of Cr(VI) to Cr(III) at these pH values. Therefore Cr(VI) adsorption to B. subtilis is clearly not an equilibrium process and can not be modeled without further experiments that inhibit reduction.
The results of the ternary solution experiments display contrasting and at first appraisal unexpected results between the La-ternary system and the Ca-ternary system. Both iodide and chromate increased in adsorption to the cell surface in the presence of calcium but displayed little affinity for the cell wall in the La-ternary systems. Selenate was not drastically affected by either cation, however, its lack of adsorption at higher pH values in the presence of Ca can be attributed to the lower concentration of Ca used (0.1 mM compared to 1 mM used in the Cr and I experiments). Hard Soft Acid Base theory (HSAB) (Langmuir, 1997) predicts that La and the (oxy)anions as hard acids and hard bases respectively should have a high affinity to form aqueous complexes. However, no interaction was observed in our results - anions are not taken up by the adsorbed La\(^{3+}\) nor are La-anion complexes formed in solution since no decrease of La\(^{3+}\) adsorption is observed in the presence of the anions.

There is considerable evidence in the literature that multivalent metal cations can reduce the overall negative charge of various bacteria surfaces at circum-neutral pH (ex. Doelman, 1979; Collins and Stotzky, 1992; Chen and Ting, 1995; Wasserman and Felmy, 1998). Our electrophoretic mobility experiments also demonstrate that the presence of multivalent cations in our experimental aqueous systems can shield or even reverse the negative charge associated with the deprotonated cell wall of *B. subtilis*. This shielding effect is a
function of both the surface charge density of the metal and its
cooncentration in the experiment. Collins and Stotzky (1992) utilized
EM measurements to demonstrate that various divalent cations in
solution decreased the negative mobility on the cell wall of *B. subtilis*
in the same way as our EM results. Wasserman and Felmy, (1998)
modeled an increased surface potential on the cell wall of *Bacillus*
brevis in the presence of divalent and trivalent cations, with the larger
effect shown by the trivalent cation, consistent with our EM
measurements.

The presence 1 mM Ca$^{2+}$ significantly dampens the negative
potential at the cell wall and an increase in adsorption at higher pH is
observed in the Ca$^{2+}$ ternary systems. Conversely, the presence of 0.1
mM La$^{3+}$ leads to an actual charge reversal at the cell wall yet no
increase in adsorption at higher pH is observed. Clearly there is a
discrepancy between the positive mobility at the shear plane and
actual adsorption of anions. We suggest the possibility that the
anions are bound outer-spherically or in the diffuse layer of the
bacteria cell and may not be close enough to interact with the
specifically-adsorbed La cation. In this scenario the NO$_3^-$ anions from
the bulk solution congregate at the shear plane and shield the anions
of interest from the positive charged surface. A similar model but
different scenario can be used to explain the increased adsorption in
the presence of Ca$^{2+}$. Since Ca$^{2+}$ likely adsorbs in the diffuse layer of
the cell wall (Fowle and Fein, 2000) the distance between the Ca\(^{2+}\) cations and the anions of interest is much smaller and enables a ternary effect to be observed. A third possibility is the increasing presence of CO\(_3^-\) ions out-compete the anions of interest for the positive sites at higher pH (>6).

A decrease of anion adsorption is observed at low pH in the presence of La\(^{3+}\). It is likely that steric effects are responsible. La\(^{3+}\) begins to adsorb to the cell wall at pH 2.5 and may block access to some positive sites for anion adsorption. As HCrO\(_4^-\) is a combination of adsorption and reducing mechanisms we see smaller effects than both I\(^-\) and SeO\(_4^-\) which are both adsorbed to the cell wall. If the cause was a protonation effect we would expect to observe similar reduction of HCrO\(_4^-\) adsorption as the other two anions.

3.7 Conclusions

The experiments from this study demonstrate that both anions and (oxy)anions have an affinity for the cell wall of *B. subtilis* below pH 5. Increased adsorption of anions at higher pH values (>pH 4) was observed in the presence of 1 mM Ca. In contrast, no significant adsorption was observed at similar pH values in the presence of both 0.1 mM and 1 mM La. The lack of adsorption in the ternary La\(^{3+}\)
experiments contrasts the charge reversal observed on the bacteria surface in the presence of La$^{3+}$. We propose that a difference in distance from the plane of shear is a plausible explanation lack of adsorption at higher pH.

As many contaminated sites contain both cations and anions, the adsorption at circum-neutral pH of anions such as I$^-$, and (oxy)anions Cr and Se, can be greatly enhanced by the bridging effect of calcium present in the outer-sphere and diffuse region of bacteria surfaces. Therefore the presence of multivalent cations will influence bacteria – anion interactions in near-surface hydrogeochemical environments and need to be considered in accurate mass transport modeling.

Further work is planned to determine the mechanisms that prevent adsorption of anions in the presence of La$^{3+}$. Ternary adsorption experiments conducted in aqueous solutions containing only La$^{3+}$ and our anions of interest will demonstrate the effects of NO$_3^-$ in our adsorption results. Furthermore, future adsorption experiments will be conducted under a nitrogen atmosphere to reduce the amount of CO$_3^{2-}$ in aqueous solution during the adsorption phase. Additionally, we will conduct adsorption experiments in the presence of a divalent cation that is known to adsorb inner-spherically with the cell wall, such as Cd$^{2+}$. If the adsorption trend is similar to the La$^{3+}$
experiments we could conclude that the results are likely due to the presence of inner-spherically bound cations.

3.8 References


Malvern Instruments: Zeta Potential Measurements, Manual number MAN0150; 2000


Figure 3.1. Percent anion adsorption by *B. subtilis* as a function of pH. Experiments were conducted in 0.001 m NaNO$_3$ or KNO$_3$ with 0.01 mM I$^-$, 0.01 mM Se, and 0.1 mM Cr and with 10.0 g of bacteria/L. Iodide adsorption data from MacLean and Fowle (2003, Chapter 2)
Figure 3.2a. Percent adsorption of $I^-$ by *B. subtilis* as a function of pH in the presence of 1 mM Ca$^{2+}$. Ternary $I^-$ - Ca adsorption experiments are shown in Figure 3.2a along with initial adsorption MacLean and Fowle (2003, Chapter 2). Experiments were conducted in 1 mM KNO$_3$ with 0.01 mM I$^-$ and with 10 g of bacteria/L.
Figure 3.2b. Percent adsorption of HCrO$_3^-$ by *B. subtilis* as a function of pH in the presence of 1 mM Ca$^{2+}$. Experiments were conducted in 1 mM KNO$_3$ with 0.1 mM HCrO$_3^-$ and with 10 g of bacteria/L.
Figure 3.2c. Percent adsorption of $\text{SeO}_3^-$ by $B. \text{subtilis}$ as a function of pH in the presence of 0.1 mM Ca$^{2+}$. Experiments were conducted in 1 mM NaNO$_3$ with 0.01 mM SeO$_3^-$ and with 10 g of bacteria/L.
Figure 3.3. Percent anion adsorption by *B. subtilis* as a function of pH in the presence of 0.1 mM La$^{3+}$. Hollow symbols represent adsorption in the absence of trivalent La. Experiments were conducted in 1 mM NaNO$_3$ or KNO$_3$ with 0.01 mM I$^-$, 0.01 mM Se, and 0.1 mM Cr and with 10.0 g of bacteria/L.
Figure 3.4. Percent HCrO$_3^-$ and SeO$_3^-$ adsorption by B. subtilis as a function of pH in the presence of 1 mM La$^{3+}$. Hollow symbols represent control experimental results. Experiments were conducted in 1 mM NaNO$_3$ or KNO$_3$ with 0.01 mM Se, and 0.1 mM Cr and with 10.0 g of bacteria/L.
Figure 3.5. Percent cation adsorption by *B. subtilis* as a function of pH. Experiments were conducted in 1 mM NaNO$_3$ or KNO$_3$ with 1 mM Ca$^{2+}$ or 0.1 mM La$^{3+}$ and with 10.0 g of bacteria/L.
Figure 3.6. Percent La$^{3+}$ adsorption by B. subtilis as a function of pH in the presence of 0.1 mM HCrO$_5^-$ and 0.01 mM SeO$_3^{2-}$ and with 10.0 g of bacteria/L.
Figure 3.7a. Comparison of *B. subtilis* electrophoretic mobility as a function of pH in the presence of 1 and 10 mM Ca\(^{2+}\) and 1 mM NaNO\(_3\).
Figure 3.7b. Comparison of \textit{B. subtilis} electrophoretic mobility as a function of pH in the presence of 0.01, 0.08, and 0.1 mM La$^{3+}$ and 1mM NaNO$_3$. 
IV. CONCLUSIONS

There is growing recognition that bacteria possess highly-reactive cell wall surfaces that can significantly influence contaminant mobility in near-surface hydrogeochemical systems. Thus the presence of bacteria must be considered in any quantitative mass transport model of contaminants in near-surface hydrogeochemical systems.

Our experiments with the Gram-positive Bacillus subtilis demonstrate the presence of a positively charged cell wall in low ionic strength (<0.1 M) solutions, supported by electrophoretic mobility measurements. Furthermore, adsorption experiments demonstrate that both anions and (oxy)anions have a pH-dependent affinity for the cell wall of B. subtilis below pH 5. Increased adsorption of anions at higher pH values (>pH 4) was observed in the presence of 1 mM Ca. In contrast, no significant little adsorption was observed at similar pH values in the presence of both 0.1 mM and 1 mM La. This work enhances and builds on the current models of the bacteria surface.

As many contaminated sites contain both cations and anions the adsorption at circum-neutral pH of anions such as I⁻, and (oxy)anions Cr and Se, can be greatly enhanced by the bridging effect of calcium present in the outer-sphere and diffuse region of bacteria surfaces. Clearly the presence of Ca²⁺ cations influence bacteria –
anion interactions in near-surface hydrogeochemical environments and need to be considered in accurate mass transport modeling.
VITA AUCTORIS

Lachlan Charles Wandless MacLean

DATE AND PLACE OF BIRTH

CITIZENSHIP
Canadian

EDUCATION

M.Sc. Earth Sciences
University of Windsor, Windsor, Ontario
Canada
Research: Quantifying bacteria – contaminant interactions

B.Sc. Geology/Biology
Acadia University, Wolfville, Nova Scotia
Canada

Diploma in Hydrogeological Engineering Technology
Northern Alberta Institute of Technology, Edmonton, Alberta
Canada

REFERENCES


MacLean, L.C.W. and D.A. Fowle. Experimental studies of bacteria - iodide interactions: extending the bacterial surface complex model. Submitted to Environmental Science and Technology