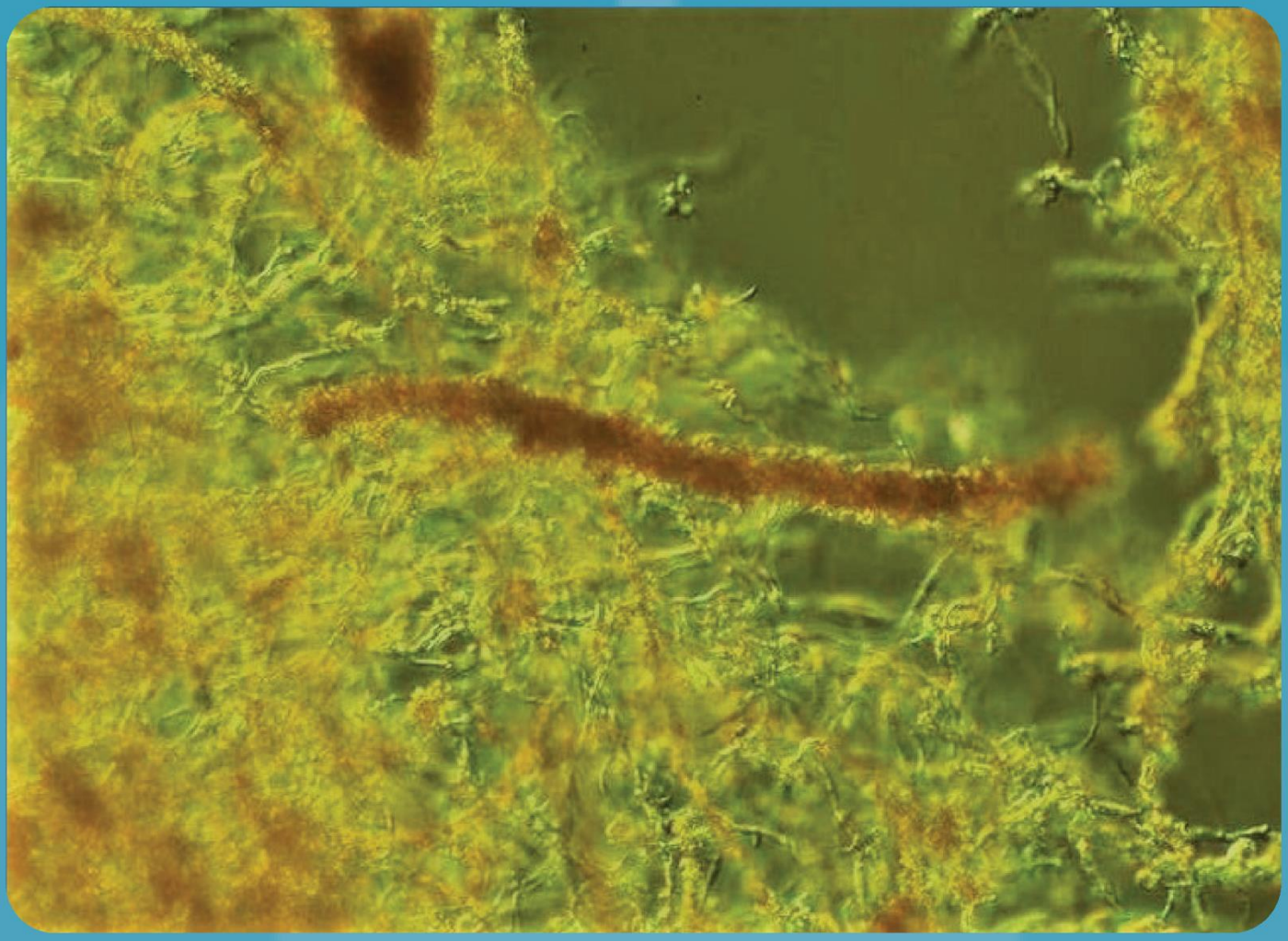

Groundwater Microbiology

F. Grant Ferris, Natalie Szponar and Brock A. Edwards

THE GROUNDWATER PROJECT

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The Groundwater Project
Guelph, Ontario, Canada

Published by the Groundwater Project, Guelph, Ontario, Canada, 2021.

Ferris, F. Grant.

Groundwater Microbiology / F. Grant Ferris, Natavlie Szponar, and Brock A. Edwards - Guelph, Ontario, Canada, 2021.

66 p.

ISBN: 978-1-77470-005-1

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Citation: Ferris, F. Grant, Natalie Szponar, and Brock A. Edwards, 2021, Groundwater Microbiology↗. The Groundwater Project, Guelph, Ontario, Canada.

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The Groundwater Project Foreword

The United Nations theme for World Water Day on March 22, 2022, is "Groundwater: making the invisible visible." This aligns with the essence of the Groundwater Project (GW-Project), which is aimed at raising groundwater consciousness and strengthening groundwater expertise worldwide, and is being accomplished by publishing books and supporting materials about "all-things-groundwater".

The GW-Project, a non-profit organization registered in Canada in 2019, is committed to contribute to advancement in education and brings a new approach to the creation and dissemination of knowledge for understanding and problem solving. The GW-Project operates the website https://gw-project.org as a global platform for the democratization of groundwater knowledge and is founded on the principle that:

"Knowledge should be free and the best knowledge should be free knowledge." Anonymous

The mission of the GW-Project is to provide accessible, engaging, high-quality, educational materials, free-of-charge online in many languages, to all who want to learn about groundwater and understand how groundwater relates to and sustains ecological systems and humanity. This is a new type of global educational endeavor in that it is based on volunteerism of professionals from different disciplines and includes academics, consultants and retirees. The GW-Project involves many hundreds of volunteers associated with more than 200 organizations from over 14 countries and six continents, with growing participation.

The GW-Project, which began publishing books in August 2020, is an ongoing endeavor and will continue with hundreds of books being published online over the coming years, first in English and then in other languages, for downloading wherever the Internet is available. The GW-Project publications also include supporting materials such as videos, lectures, laboratory demonstrations, and learning tools in addition to providing, or linking to, public domain software for various groundwater applications supporting the educational process.

The GW-Project is a living entity, so subsequent editions of the books will be published from time to time. Users are invited to propose revisions.

We thank you for being part of the GW-Project community. We hope to hear from you about your experience with using the books and related materials. We welcome ideas and volunteers!

The GW-Project Steering Committee

July, 2021

Foreword

Groundwater microbiology is the study of the microscopic organisms, mostly bacteria, that inhabit groundwater systems. Of the topics that make up groundwater science, groundwater microbiology has undergone immense advances in the past 40 years. Microbes live nearly everywhere in the subsurface, even under harsh conditions of high temperature and salinity at great depth. Microbes influence and often control geochemical processes and hence they are important to the natural quality of groundwater such as the concentrations of iron, manganese and arsenic, and, along with oxidation-reduction potential and pH, determine the odor and color of well water. Microbes determine when well clogging is a problem, and often they determine whether or not anthropogenic chemicals (contaminants) disappear or persist in groundwater.

Commonly, microbes have resided in a geological stratum over geologic time and are activated when conditions change. Like human beings, they require energy and nutrients (e.g., food) to survive but microbes can persist for millennia with little of these life essentials. However, when conditions provide more of their life sustaining needs, the microbes multiply and change the hydrochemistry. Changes can also occur in response to human alterations of the landscape and hydrology. As groundwater moves along its flow path from recharge to discharge areas, it may pass through strata that do not contain microbes and then enter a zone where conditions are more favorable to microbes that change the groundwater markedly. Groundwater chemistry may be governed by the minerals in contact with the water along its flow paths and or the microbes may control the chemistry. Zones of substantial microbial activity may occupy only a small portion of the groundwater system yet be the dominant influence on chemical composition of the water in much of the system. What groundwater transports into a zone, what happens in the zone and what is transported away from the zone are important. This book introduces the principles of groundwater microbiology, including microbe cell structure and growth, the bioenergetics and metabolism of microorganisms, the geochemical and physical influences on microbes with emphasis on natural water quality but also considering the role of microbes in contaminated groundwater.

This is the first Groundwater Project book written by a professor in collaboration with graduate students. The GW-P encourages this type of team effort. Dr. Grant Ferris is a senior professor in the Department of Earth Sciences at the University of Toronto, Canada, and conducts research in environmental microbiology at many locations around the globe. That department is also the academic home of Natalie Sponzar where, after a decade as a consulting hydrogeologist, she is a Doctor of Philosophy candidate focused on aqueous and isotope geochemistry. Brock Edwards, whose research is focused on gas emissions from active volcanoes, is a Doctor of Philosophy candidate at the Centre for Earth Observation Science at the University of Manitoba, Canada.

John Cherry, The Groundwater Project Leader

Guelph, Ontario, Canada, July 2021

Preface

Groundwater microbiology is the study of the microscopic organisms that inhabit subsurface groundwater systems. For many years, it was generally assumed that the geological materials beneath our feet were repositories of long-dead organisms rather than habitats for living ones. Recent discoveries over the last few decades have demonstrated the existence of a diverse world of microorganisms living in the subsurface, independent of sunlight and oxygen and often thriving under extreme physical environmental conditions and nutrient limitations.

Groundwater systems are particularly favorable environments for microorganisms, with relatively stable temperatures, nutrients supplied by flowing groundwater, and abundant mineral and fracture surfaces to colonize. Any consideration of groundwater systems must therefore address the microorganisms that live there – for they are not passive inhabitants. Rather, the unique metabolic capabilities of microbes implicate them as key agents in the flow of energy and the processing of organic and inorganic compounds in subsurface environments, affecting the chemical composition of groundwater as well as the physical properties of the sediments and rock formations through which these hidden waters move.

This book introduces the principals of groundwater microbiology, from aspects of cell structure and growth, to the bioenergetics and metabolism of subsurface microorganisms, to the geochemical and physical influences of widespread microbial activity on groundwater systems and water quality. As summarized in the later sections of the book, the coupling of microorganisms with their geological surroundings – a bond established billions of years ago as life took hold on our planet – is of particular value in bioremediation and microbially induced mineral precipitation applications, such as the clean-up of contaminated groundwater systems and reduction of aquifer permeability.

As revealed in this book, microbiology has a central role in determining the patterns of groundwater chemistry. Studies of groundwater contamination and its remediation that do not incorporate microbiological insight are likely to overlook opportunities to understand the important processes governing conditions at the site and thus, fail to recognize alternative treatment options. Therefore, we recommend inclusion of a team member with microbiology expertise in every major investigation of groundwater contamination and remediation.

Acknowledgements

We deeply appreciate the thorough and useful reviews of and contributions to this book by the following individuals:

- Francis Chapelle, United States Geological Survey, United States of America;
- Bruce Rittman, Arizona State University, United States of America;
- Doug Mackay, University of California Davis, United States of America;
- Jim Barker, University of Waterloo, Canada;
- Jim Spain, Georgia Institute of Technology, United States of America;
- Dave McWhorter, Colorado State University, United States of America;
- Everton de Oliveira, Hidroplan, Brazil; and,
- Hugh Whiteley, University of Guelph, Canada.

We are grateful for Amanda Sills' oversight of this book as well as Juliana Apolonio's copyediting, both of the Groundwater Project, Guelph, Ontario, Canada. We thank Eileen Poeter (Colorado School of Mines, Golden, Colorado, USA) for technical editing, layout editing and production of this book.

1 Introduction

As an integral part of the hydrologic cycle, groundwater systems are influenced by a host of physical, environmental, hydraulic, and biogeochemical processes. These include seasonal changes in climate and precipitation, local to regional variations in the porosity and permeability of subsurface materials, the formation and dissolution of minerals along groundwater flow paths, as well as reactive mass transport and cycling of chemical substances by microorganisms. With the understanding that groundwater is a critical global resource of highly variable quantity and quality, the role of microbiology in biogeochemical processes has emerged as an essential topic in hydrogeology.

For many years, it was commonly thought that the subsurface of the Earth was mostly devoid of life. The presence of living organisms was believed to be limited mainly to soil and rhizosphere (plant root) environments. However, discoveries over the last few decades revealed a vast and diverse microbial biosphere extending several kilometers below the Earth's surface (Kallmeyer et al., 2012; Bar-On et al., 2018; Magnabosco et al., 2018). These developments have emerged out of the growing realization that the ingredients considered necessary for life above ground – sunlight, oxygen, abundant organic carbon – are immaterial to many microorganisms. A wide variety of "unconventional" energy sources are utilized by different microbes, including a tremendous assortment of inorganic compounds as well as some organic compounds that are toxic to other organisms. These unique metabolic capabilities implicate microorganisms as key agents in the flow of energy and turnover of condensed matter (i.e., crystalline and amorphous solids and liquids, including liquid crystals, glasses, polymers, and gels) in reactive transport processes that link groundwater systems to surface environments in the hydrologic cycle (Falkowski et al., 2008; Lin et al., 2012).

As an exclusive habitat for microorganisms, the subsurface is characterized by a total absence of light, relatively constant temperatures, and a scarcity of nutrients including organic carbon. Microbial metabolism under such uninviting conditions may be far slower than on the Earth's surface, calibrated more to the length and timescales of hydrogeological processes than daily and seasonal sunlight-dominated cycles. But the relatively stable conditions of the subsurface mean that populations of microorganisms can survive or even thrive over long periods of time, and in doing so exert a significant biogeochemical influence on their surroundings. This is especially important in a societal context because microbial activity directly affects the chemical composition and quality of groundwater, which constitutes a critical drinking water resource for much of the world's population. The presence of microorganisms may be beneficial, for example by contributing to the degradation of toxic substances, or detrimental as in the case of aquifer contamination by pathogenic microbes from sewage wastes.

This book delves into the essentials of groundwater microbiology, beginning with the characteristic features of microorganisms and their overwhelming predominance in subsurface environments on Earth. Microbial cell structure, growth, ecology, and bioenergetics are introduced to build some familiarity with the expansive subject of microbiology. The concept of groundwater systems as a habitat for microorganisms is discussed in terms of physical limitations and the depth distribution of life in subsurface environments. Aspects of dissolved inorganic carbon equilibria and pH, oxidation-reduction (redox) processes, mineral precipitation-dissolution reactions, and the behavior of microbial cells as geochemically reactive solids are considered in an examination of the influence of microbial activity on chemical processes in groundwater. Final sections of the book address the transport and movement of microorganisms through groundwater systems, as well as some well-known and potential roles of microorganisms in contaminant hydrogeology and remediation.

2 Microbiology

Microbiology is the study of extremely small life forms that cannot be seen without magnification by a magnifying lens or microscope (Heim et al., 2017). This operational definition applies to unicellular organisms that are smaller than about 10^{-4} m (0.1 mm or 100 μm) in size (Figure 1). The microscopic world also includes viruses, which are the smallest biological entities in nature (diameter of ~10^{-7} m) containing protein and genetic material, either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). They are infectious agents that depend on host cell metabolic processes to reproduce, so there is some debate as to whether viruses are really alive.

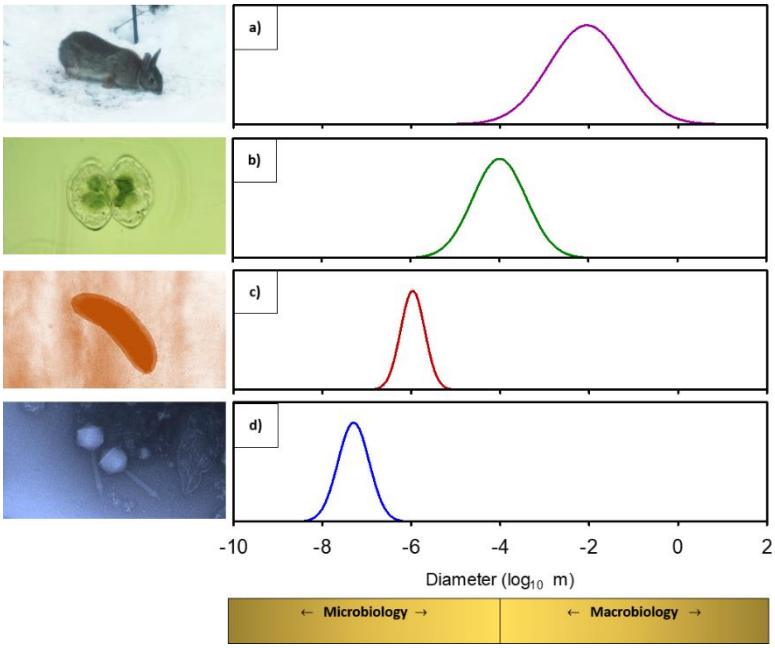

Figure 1 - Size distributions of a) macro-eukaryotes, b) micro-eukaryotes, c) prokaryotes, and d) viruses.

Beyond size, the scope of microbiology is wide ranging. It spans across all three domains of life with different microorganisms classified as *Bacteria*, *Archaea*, or *Eukarya*. All members of the first two domains are prokaryotic microorganisms that lack membranous nuclei. The nickname "bacteria" is often used, as it is in this book, to refer to prokaryotes in general because *Bacteria* were once classified as *Eubacteria*, while *Archaea* were classified as *Archaebacteria*. The third domain, *Eukarya*, are eukaryotes that have true membrane-bound nuclei. This defining characteristic is common not only to plants and animals but also a variety of microorganisms including fungi, protozoa, and algae.

The classification of organisms has been revolutionized by extraordinary advances in molecular biology that permit sequencing of entire chromosomes. Among prokaryotes, these methods have exposed an overwhelming amount of genetic diversity that is difficult to reconcile within the traditional hierarchy of taxonomic categories. A major part of the problem is that sexual reproduction as a defining characteristic at the taxonomic level of a species does not apply to prokaryotes, which usually multiply by asexual binary fission. Another intriguing part of this dilemma is that it is now possible to identify microorganisms in nature that cannot be isolated and grown in pure laboratory cultures. Such efforts suggest that unculturable microorganisms account for more than 90 percent of the total diversity of most prokaryotic microbial populations.

In comparison to eukaryotes, prokaryotes are among the most abundant, widespread, and functionally diverse organisms on Earth. There are typically 105 to 106 cells in a milliliter of freshwater and 10^8 to 10^9 cells in a gram of soil. Global inventories indicate nearly 10^{30} cells of bacteria inhabit Earth and include as many as 10^{12} (i.e., a trillion) different species, according to some ecological models (Mora et al., 2011; Locey and Lennon, 2016; Louca et al., 2019). This is several orders of magnitude greater than the 10^8 to 10^9 number of species forecast for eukaryotes. When it comes to total biomass, global estimates are on the order of 500 to 700 GtC (giga metric tons of Carbon), with 1 GtC = 10^{12} kg of carbon. Overall, prokaryotes are suggested to account for anywhere from 15 to 48 percent (81 to 327 Gt C) of the total carbon mass on Earth, with as much as 90 percent of prokaryotic biomass residing in terrestrial groundwater systems and below the seafloor (Kallmeyer et al., 2012; Bar-On et al., 2018; Magnabosco et al., 2018).

2.1 Prokaryote Cell Structure

Prokaryotes are living entities with a characteristic size and distinct cell structure. They are surrounded by complex multilayered envelopes that serve as a protective boundary between the inside and outside of individual cells (Konhauser, 2007; Kleanthous and Armitage, 2015). The cell envelope also provides physical strength and shape to bacteria, supports the generation of energy for growth and division, permits selective passage of nutrients from the outside and waste products from the inside, facilitates motility, and allows cells to interact with their surroundings. The cell envelope of almost

all bacteria consists of two principal layers: an inner cytoplasmic membrane (also known as the plasma membrane) and an external cell wall.

The cytoplasmic membrane consists of a bilayer of lipids, as well as proteins that contribute to active solute transport, metabolic processes, and communication between the cell and the environment. Most of the lipids are phospholipids composed of fatty acids that are attached to a glycerol phosphate backbone by ester bonds in *Bacteria* and ether bonds in *Archaea*. Small polar molecules can diffuse through the lipid bilayer, but the passage of ions and large polar molecules is restricted and depends on specific transport proteins embedded in the membrane. The viability of prokaryotic cells depends critically on the physical integrity of the cytoplasmic membrane because structural failure results in cell death.

Two basic types of prokaryotic cells walls are distinguished by their response to the Gram stain, a long-standing test for the classification of bacteria by light microscopy (Figure 2). The cell walls of bacteria staining Gram-positive consist of peptidoglycan (a meshwork of mucopolysaccharides cross-linked in three dimensions by peptide bridges) and a variety of secondary polymers (teichoic or teichuronic acids and proteins). In contrast, Gram-negative walls contain lipopolysaccharides, phospholipids, and proteins arranged in a membrane bilayer (the outer membrane). Sandwiched between the outer membrane and cytoplasmic membrane is a thin layer of peptidoglycan. Some bacteria have cell walls that are neither Gram-positive nor Gram-negative. The cell walls of *Archaea* lack the kind of peptidoglycan that is found in *Bacteria* and instead contain either pseudopeptidoglycan, glycoproteins, or proteins alone. Moreover, prokaryotic cell walls are different from those of eukaryotic plants and fungi, which are made from cellulose and chitin, respectively.

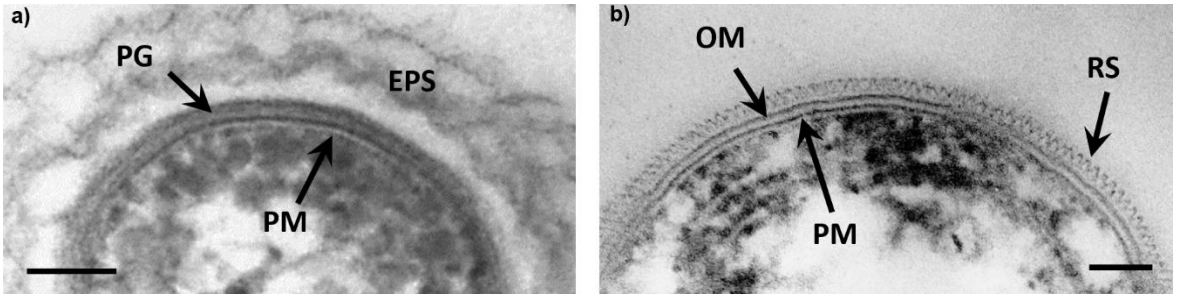

Figure 2 - Thin-section transmission electron micrographs showing examples of Gram-positive a) and Gram-negative b) bacterial cells. The cells are enclosed by a plasma membrane (PM). A peptidoglycan cell wall (PG) surrounds the Gram-positive cell, whereas the Gram-negative cell wall has an outer membrane (OM); sandwiched between the outer membrane and cytoplasmic membrane is a thin layer of peptidoglycan. Extracellular polymeric substances (EPS), which are produced by many types of bacteria, are evident on the Gram-positive cell. A proteinaceous regularly structured surface layer (RS) exists on the Gram-negative bacteria cell. Scale bars = 80 nm.

The cell wall is responsible for providing mechanical strength and shape to prokaryotic cells. If agents such as lysozymes or antibiotics damage the stress-bearing peptidoglycan layer, cell lysis (break down of the membrane of a cell) will occur owing to

the turgor pressure of the cytoplasm. A good analogy for turgor pressure is the force pushing outwards in a balloon filled with water. When it comes to cell shape, most bacteria display one of three basic morphologies: spherical coccus, rod-shaped bacilli, or curved varieties that range from comma-shaped vibrio to elongated spirals. While many exist as solitary cells, some remain linked together in pairs, cuboidal tetrads, chains, or random clusters depending on the geometry of cell division (Figure 3).

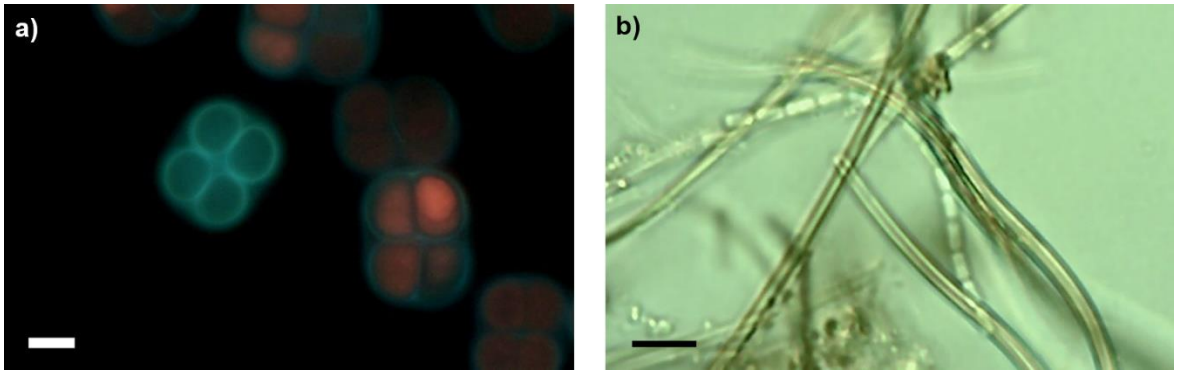

Figure 3 - a) An epifluorescence photomicrograph of coccoid-shaped bacterial cells growing in tetrads. b) A differential interference contrast photomicrograph of rod-shaped bacteria growing in a chain among empty filamentous bacterial sheaths. Scale bars = 4.0 μm.

Extracellular polymeric substances (EPS) composed mainly of acidic polysaccharides (carbohydrates of bonded sugar molecules) and proteins are often secreted in copious amounts by prokaryotes to form external hydrated coatings around cells (Figure 2a). These EPS layers display a high degree of structural variability, ranging from diffuse slime layers to highly organized capsules and sheaths. Two physicochemical properties of EPS contribute to their functions. First, these cell surface coatings are highly hydrated and protect against moisture deficits and water loss. Second, deprotonation (the transfer of a proton in an acid-base reaction) of acidic functional groups fosters the development of a net negative surface charge. This allows EPS to work like an ion exchange resin (sorbent) for the capture of dissolved nutrients and protection against toxic chemical agents. EPS serves additional important functions, which include assisting in cell recognition, attachment to surfaces, and formation of biofilms.

Many prokaryotes possess proteinaceous regularly structured surface arrays (RS or S-layers) on the outside of their cell walls (Figure 2b). These are assemblies of protein subunits arranged into either linear, square, tetragonal, or hexagonal packing formats. Pores are located between the protein subunits that extend completely through the array, providing open channels that are 2 to 3 nm in diameter to the underlying cell wall. This allows RS-layers to function as a molecular sieve, allowing passage of small molecules while excluding large deleterious agents such as wall-degrading enzymes, as well as protecting cells from attack by bacteriophage viruses or predatory bacteria such as *Bdellovibrio*.

Among *Bacteria*, flagella are helical protein filaments about 20 nm in diameter and up to 20 μm in length that are responsible for swimming motility (Figure 4a). They can be located at either one or both ends of a cell or be arranged in a uniform (peritrichous) manner about a cell. The basal bodies of flagella are anchored in the cytoplasmic membrane and consist of ring structures that act as a miniature electric motor. Flagellar rotation is driven by energy obtained from the active transport of ions across the cytoplasmic membrane. When flagella rotate in a clockwise direction, cells swim in a forward direction, whereas counter-clockwise rotation produces a tumbling motion.

Pili (sometimes called fimbriae) are fine filamentous protein appendages that are 2 to 10 nm in diameter and up to several micrometers in length (Figure 4b). They extend outwards from the cytoplasmic membrane through the cell wall, but do not have complex anchoring structures analogous to flagellar basal bodies. Some pili play a role in facilitating prokaryotic attachment to surfaces, while others allow for the transfer of genetic material between cells in a process called conjugation. Another group, referred to as type IV pili, are capable of contraction and cause a twitching motion that is sometimes exhibited by cells attached to surfaces. Additional evidence suggests the gliding motility of some bacteria over solid surfaces is at least partially dependent on the production of type IV pili. Further indications suggest the flagella of *Archaea* resemble type IV pili, in contrast to those of *Bacteria*.

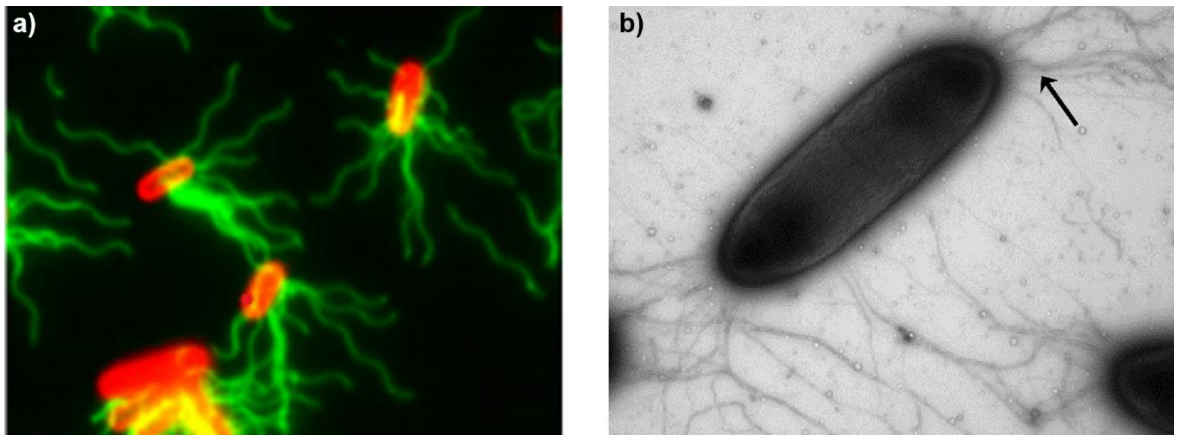

Figure 4 - a) An epifluorescence photomicrograph of ***Bacillus subtilis*** cells with peritrichous flagella labeled with a green fluorescent stain (reproduced under the terms of Creative Commons Attribution 3.0 license from Wang et al., 2017). b) A transmission electron micrograph negative stain of type IV pili (arrow) on ***Pseudomonas aeruginosa*** (reproduced under the terms of Creative Commons Attribution 3.0 license from Chanyi and Koval, 2014). Scale bars = 500 nm.

To survive periods of environmental stress, some genera of *Bacteria* (such as *Bacillus* and *Clostridium*) form dormant, resistant, non-reproductive structures called endospores. *Archaea* are not known to form endospores. Individual cells that undergo sporulation produce a single endospore internally within the cytoplasm. Once assembled, an endospore contains a core of DNA and ribosomes surrounded by a protective coat with multiple layers of peptidoglycan and proteins. Endospores are inactive and show no

detectable metabolism. They can survive extreme physical and chemical stresses such as ultraviolet radiation, extreme temperatures, disinfectants, and desiccation. In this inanimate state, endospores may remain viable for hundreds to thousands, perhaps even millions, of years.

2.2 Cell Growth and Environment

While prokaryotes are abundant and widely distributed in nature, their growth rates and metabolic functions are sensitive to environmental conditions. This includes physical and chemical parameters such as temperature, pressure, pH, oxidation-reduction (redox) potential, ionic strength, and nutrient availability. For some environments with extreme conditions, such as high temperature or low pH, prokaryotes are the only form of life capable of survival and growth.

Of all environmental properties, temperature exerts a particularly strong effect on prokaryotic growth. This is because chemical and metabolic reaction rates increase with temperature following the Arrhenius relationship as shown in Equation 1.

$$k = A\, e^{-E_a/RT} \tag{1}$$

where:

k = reaction rate coefficient (1/T)

A = collision frequency factor (1/T)

E_a = activation energy of the reaction (ML^2)/(T^2 mol)

R = universal gas constant (ML^2)/(T^2 °K mol)

T = absolute temperature (°K)

Prokaryotes typically grow best near a characteristic optimal temperature. The optimal growth temperature of psychrophilic (cold-loving) prokaryotes is around 10°C, whereas thermophiles (heat-loving) prefer temperatures above 40°C. Mesophilic prokaryotes grow well at midrange temperatures between 20 and 40°C. At the extremes, prokaryotes survive at temperatures as low as −20°C and as high as 120°C, provided water is maintained in a liquid state by dissolved salts and high pressure, respectively.

Exposure to high pressures tends to impede the growth of prokaryotes that are accustomed to atmospheric pressure. But among those that live in high pressure deep-ocean and subsurface environments, microbes classified as both barotolerant (able to tolerate high pressures) and barophilic (requiring high pressures to function) have been found. These prokaryotes are able to stabilize their cytoplasmic membranes by altering the fatty acid composition of the phospholipids to compensate for extreme pressure gradients between the inside and outside of the cells.

Defined as the negative logarithm of the molar proton (hydrogen ion, H^+) concentration, pH is regarded as a master variable in chemistry and biology. pH is defined in Equation 2.

$$\mathrm{pH} = -\log[H^+] \tag{2}$$

This is because protons are involved in almost all types of chemical and metabolic reactions, including acid-base, aqueous complexation, surface adsorption, and redox reactions. The vast majority of prokaryotes are quite comfortable growing in the circumneutral pH range of most natural waters, from around 5.7 for pristine meteoric water to about 8.0 for seawater. Acidophiles are adapted to grow at pH < 3.0, for example in acid drainage from mines and acidic hot springs. At the other end of the pH spectrum, alkaliphiles occur at pH > 10 in saline alkaline lakes and calcareous alkali soils.

Environmental redox potentials measured with a platinum electrode are often discussed in terms of the presence (aerobic, oxic) or absence (anaerobic, anoxic) of oxygen. This is an oversimplification of the electrochemical meaning of redox potential (Eh), which is defined relative to the standard hydrogen electrode for a generic half-cell reaction involving oxidant *ox* and conjugate reductant *red* (Equation 3) by the Nernst equation (Equation 4).

$$ox + nH^+ + ne^- = red \tag{3}$$

$$Eh = Eh^0 + \frac{2.303RT}{nF}\log\frac{[ox][H^+]^n}{[red]} \tag{4}$$

Based on the Nernst equation, high redox potentials (Eh) are indicative of oxidizing conditions (greater abundance of oxidized chemical species), whereas lower Eh values correspond to reducing conditions (reduced chemical species dominate). Moreover, Eh tends to decrease with increasing pH because of the logarithmic dependence on proton concentration. When the concentrations of *ox* and *red* are equal at pH = 0 (so-called standard conditions), then Eh equals the standard half-cell potential Eh^0.

The use of platinum electrodes to measure environmental redox potentials is historically based on the work of C.E. Zobell with marine sediments (Zobell, 1946). However, sediments and groundwater systems are seldom at thermodynamic equilibrium with respect to oxidation-reduction reactions. In addition, many important oxidants (such as molecular oxygen) and reductants (such as organic carbon) do not react reversibly on platinum electrodes. For these reasons, Eh measurements have not proven to be quantitatively meaningful in aqueous environments, including groundwater systems (Lindberg and Runnells, 1984).

When it comes to prokaryotic growth, the distinction between aerobic (molecular oxygen present) versus anaerobic (molecular oxygen low or absent) conditions is more important than whether a specific environment is oxidizing or reducing. Many prokaryotes, and nearly all eukaryotes, require oxygen for survival and growth. The lower

limit for these strict aerobes is approximately 1 percent (referred to as the Pasteur Point) of the atmospheric oxygen concentration (pO_2 = 0.21 atm); however, a vast number of other prokaryotes can grow in the absence of oxygen as either facultative or strict anaerobes (Stolper et al., 2010). This is the main reason why complex prokaryotic communities thrive in environments isolated from direct contact with the atmosphere, such as within sediments and groundwater systems.

The ionic strength of natural waters has multiple implications for prokaryotic growth, especially in terms of water activity and osmotic balance. As a measure of the concentration of ions in solution, molar ionic strength *I* (mole/L) is calculated for *n* ionic species as shown in Equation 5.

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 \tag{5}$$

where:

c_i = molar concentration of ion species *i* (mol/L²)

z_i = ion charge (dimensionless)

Because it is not always possible (or feasible) to obtain a complete chemical analysis of all dissolved ions in solution, a convenient approximation for molar ionic strength as a function of total dissolved solids (*TDS* in mg/L) is given by Equation 6.

$$I = (2.5 \times 10^{-5})\, TDS \tag{6}$$

An increase in ionic strength causes the solution to shift away from ideal behavior, making ion and molecular interactions more dependent on activities (dimensionless effective concentrations, a_i) rather than absolute concentrations. Considering the concentration of chemical species *i* in solution relative to a standard concentration c_p (taken as unity for a pure phase) and the corresponding activity coefficient γ_i (which decreases as a function of increasing ionic strength), activity is calculated as shown in Equation 7.

$$a_i = \gamma_i \frac{c_i}{c_p} \tag{7}$$

For prokaryotes, higher ionic strengths and lower solute activities may impede growth by slowing down rates of chemical and metabolic reactions (Small et al., 2001). A far more serious consequence is the decrease in water activity that accompanies an increase in *TDS* and ionic strength. In fact, few prokaryotes can tolerate water activities much below 0.98, which approximately corresponds to the salinity of seawater. At lower water activities, water is drawn out of cells by osmosis thereby disrupting normal cellular growth. Nevertheless, some prokaryotes (halophiles) manage to grow in brines (> 20 percent *NaCl* by weight) at extremely low water activity levels (down to about 0.80).

The growth of prokaryotes generally implies an increase in the number of individual cells (Allan and Waclaw, 2019). Under ideal laboratory conditions with an unlimited supply of nutrients, the differential rate of increase in cell numbers with respect

to time (t) depends on the frequency of cell division, specified by the growth rate constant (μ), and the number of prokaryotes that are growing (N) as shown by Equation 8.

$$\frac{dN}{dt} = \mu N \tag{8}$$

Integration yields the familiar exponential growth equation (Equation 9), that is the progressive increase in prokaryotic cell numbers over time (Figure 5).

$$N_t = N_0\, e^{\mu t} \tag{9}$$

with a characteristic doubling time $T = \ln(2)/\mu = 0.693/\mu$ (Allan and Waclaw, 2019).

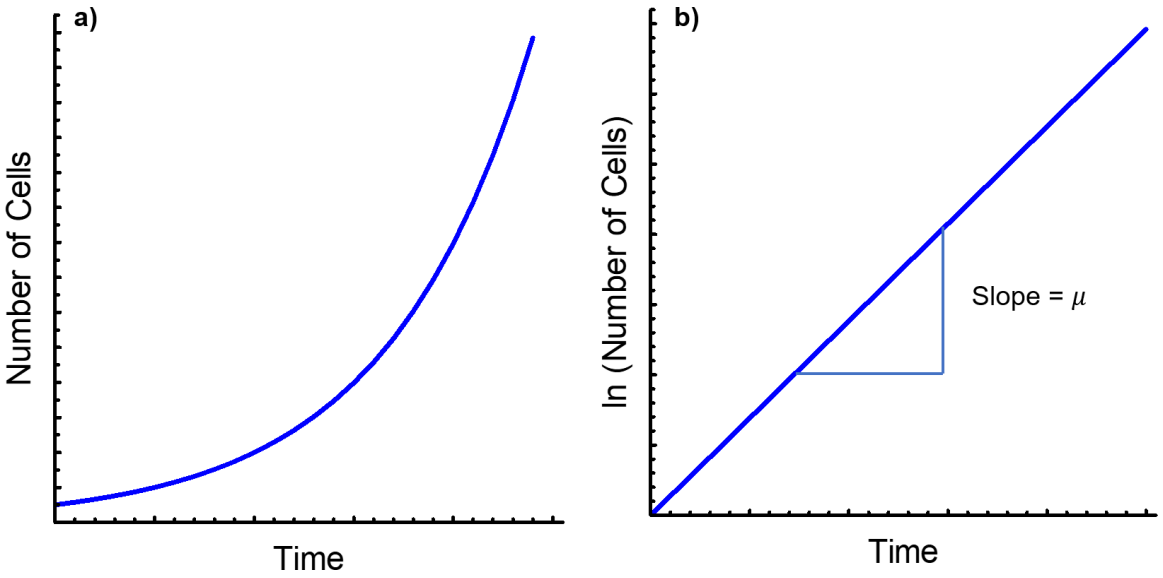

Figure 5 - a) Illustration of microbial growth and the exponential increase in cell numbers as a function of time. b) A plot of the natural logarithm of cell numbers as a function of time, where the growth rate constant μ is the slope of the line.

A major limitation of the exponential growth model is that it does not take into account the influence of nutrient availability on prokaryotes growth rate. This situation is described by the extended Monod equation as shown in Equation 10.

$$\mu = \frac{(\mu_{max} + m)\, S}{(K_s + S)} - m \tag{10}$$

where:

μ_{max} = maximum growth rate constant (1/T)

S = concentration of a single limiting nutrient concentration (i.e., where other nutrients are in excess) (M/L^3)

K_S = nutrient concentration that corresponds to one half of μ_{max} (M/L^3)

m = maintenance energy coefficient of metabolic processes that keep cells alive (1/T) (Figure 6)

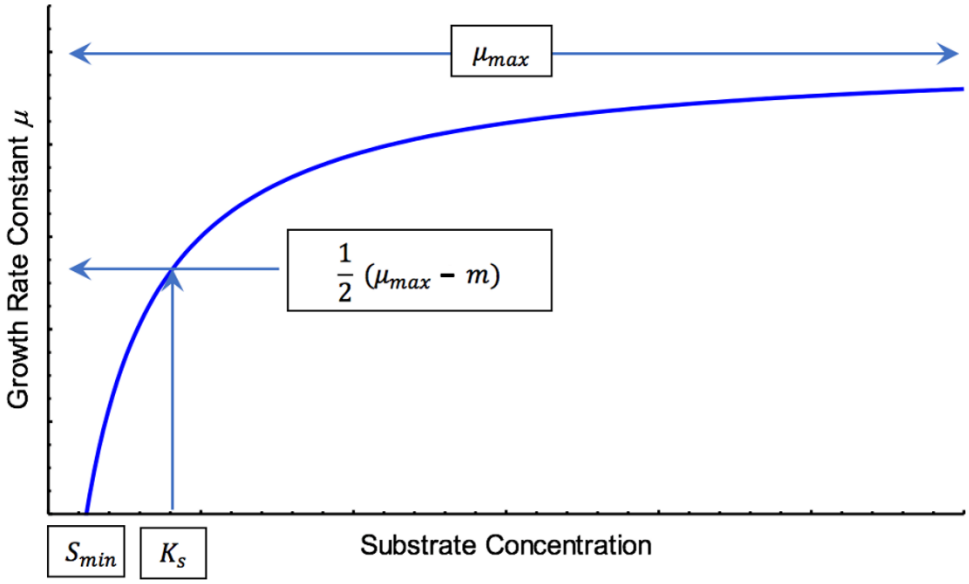

Figure 6 - The dependence of growth rate constant μ as a function of substrate concentration according to the extended Monod equation. As the substrate concentration increases, the value of μ asymptotically approaches the maximum growth rate constant μmax. The substrate concentration, equal to K_S, corresponds to the point at which μ is equal to one half of ($\mu max - m$). The minimum substrate concentration required for maintenance energy is equivalent to S_{min}.

The Monod equation indicates an increasing supply of nutrients promotes a higher frequency of cell division and faster growth rates; however, there is a finite metabolic limit to how fast cells can grow (μ_{max}) beyond which growth rates become independent of nutrient availability. There is also a minimum substrate concentration (S_{min}) imposed by maintenance energy requirements. At S_{min}, cell growth comes to a stop ($\mu = 0$). Rearrangement of Equation 10 for cells staying alive but not growing yields Equation 11.

$$S_{min} = \frac{mK_s}{\mu_{max}} \tag{11}$$

In natural environments, particularly groundwater systems, nutrient concentrations are normally much closer to S_{min} than K_S. Under such near starvation conditions, prokaryotes grow slowly if at all (Hoehler and Jorgensen, 2013; LaRowe and Amend, 2015, 2019). Cell numbers are typically low and tend to remain constant over time in a balance between cell growth and loss of cells owing to death or physical removal from the system. In this situation, the change in cell numbers over time takes the form of Equation 12.

$$\frac{dN}{dt} = \mu N - k_d N = (\mu - k_d)N \tag{12}$$

A decay constant k_d is added to account for the rate at which cell numbers are lost (Allan and Waclaw, 2019). If the rates of growth and loss are equal, cell numbers will not change over time. This situation is representative of a steady-state condition. When the

growth rate is greater than the loss rate, cell numbers will increase. On the other hand, cell numbers will decrease if the growth rate is less than the rate at which cells are lost.

Although the Monod relationship takes nutrient *supply* into account, it does not address the issue of nutrient *quality*. Essential nutrients required for cell growth include sources of carbon, nitrogen, phosphorus, sulfur, potassium, magnesium, calcium, oxygen, and iron. Trace nutrients include elements such as manganese, copper, cobalt, zinc, and molybdenum. All of these nutrients can be obtained to meet the specific growth requirements of different prokaryotic microorganisms from a vast assortment of organic and inorganic materials. But availability alone does not make one nutrient compound any better than another. Other factors come into play including molecular size and structure, solubility, and ionic charge. For instance, the nutritional value of large insoluble materials is generally lower than that of smaller soluble molecules. Similarly, branching and ring structures in the carbon backbone of complex organic compounds are restrictive to metabolic processing. When it comes to uptake of ionic nutrients, cells must rely on active transport because ions cannot diffuse freely across the cytoplasmic membrane.

Prokaryotic cells are mostly water (80 percent), so their bulk density is nearly the same as the density of water. This makes prokaryotes rather buoyant, which permits free and unconfined planktonic growth in aqueous suspension (Figure 7a). It also makes prokaryotes susceptible to advective transport and dispersion as suspended particles in moving water. Some prokaryotes are well adapted to planktonic growth and can survive long periods of starvation under the dilute nutrient-poor conditions that prevail in most natural waters; however, the reality is that most prokaryotes grow in biofilms attached to surfaces (McDougald et al., 2012; Marshall, 2013). These adherent prokaryotic communities consist mainly of EPS and other biopolymers that immobilize living cells on surfaces (Figure 7b). In comparison to their planktonic counterparts, prokaryotes growing in biofilms benefit directly from solid-liquid interfacial forces, such as surface tension and ion adsorption, which contribute to the accumulation and increased metabolic accessibility of nutrients.

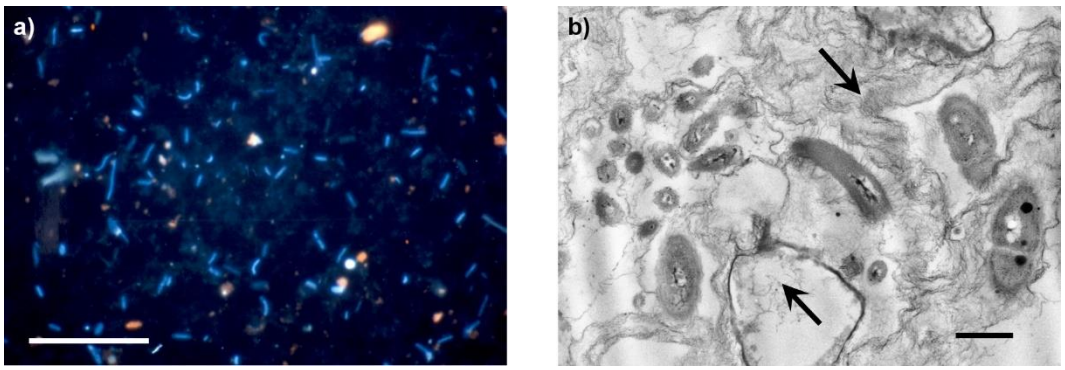

Figure 7 - a) Epifluorescence photomicrograph of planktonic bacteria from a freshwater sample. Scale bar = 20 μm. b) Thin-section transmission electron micrograph showing microcolonies of epilithic biofilm bacteria (indicated by arrows) surrounded by large amounts of fibrous extracellular polymeric substances. Scale bar = 1.0 μm.

As a general rule, the numerical abundance of living organisms increases with decreasing size. The smallest and most abundant biological entities in nature are viruses, followed by prokaryotes, then eukaryotes. On average, the number of viruses suspended in a typical sample of surface or shallow groundwater approaches 10^7 per mL, followed by 10^5 prokaryotes per mL and 10^2 single cell eukaryotic organisms per mL (Kyle et al., 2008). Total numbers are even higher in soils and sediment samples, which can contain up to 10^{10} viruses, 10^9 prokaryotes, and 10^5 eukaryotes per gram. This implies that a great majority of the microorganisms in subsurface environments grow in adherent biofilms on mineral surfaces (McDougald et al., 2012; Marshall, 2013).

2.3 Mass Transport and Bioenergetic Considerations

Recognition of the abundance and enormous genetic diversity among prokaryotes is balanced by unavoidable limitations imposed on life by physics and chemistry. The main physical processes that impinge on prokaryotic life relate to fluid dynamics and mass transport of solutes, including essential nutrients and metabolic waste products. At the same time, there is no way for any living organism to escape the laws of chemical thermodynamics that govern the spontaneity and progress of metabolic processes, including catabolic energy yielding and anabolic biosynthetic reactions.

Because of their small size, prokaryotic cells exist under conditions where viscous forces dominate over inertial forces. The dimensionless ratio of inertial to viscous forces corresponds to Reynold's number (R_e), which is used in fluid dynamics to predict different flow patterns as a function of fluid density (ρ), relative flow velocity (u), characteristic linear length scale (L), and dynamic viscosity (μ) as shown in Equation 13.

$$R_e = \frac{\rho u L}{\mu} \tag{13}$$

At low Reynold's numbers characteristic of prokaryotes in the 10^{-6} m size range ($R_e << 1$), water flows in a direction parallel to cell surfaces in a smooth laminar fashion. In effect this isolates prokaryotic cells inside a viscous boundary layer of water, even in the presence of high groundwater flow velocities (> 100 m/d) and turbulence that occurs in some karst aquifers (Shoemaker et al., 2008).

Dissolved solutes are carried along by advection at the same relative velocity and in the same direction as water in the laminar flow boundary layer around cells. Because the flow of water runs parallel to the surface of cells, direct access to essential nutrients and dispersal of metabolic waste products by means of advective mass transport is not possible. To overcome this limitation, prokaryotes rely on molecular diffusion to mediate the lateral mass transport of solutes towards and away from cells.

In accordance with Fick's first law, the diffusive flux (F_x; moles/m^2·s) in a direction perpendicular to the surface of a cell depends on the diffusion coefficient (D; m^2/s) and

change in concentration of a solute with respect to distance from the cell surface (dC/dx; moles/m^4) as indicated by Equation 14.

$$F_x = D \cdot \frac{dC}{dx} \tag{14}$$

This relationship implies that higher (steeper) concentration gradients will increase diffusional mass transfer rates for prokaryotic microorganisms.

Metabolic rates of nutrient uptake and waste excretion play an instrumental role in the development and maintenance of concentration gradients around cells. Specifically, nutrient uptake will tend to decrease cell surface solute concentrations relative to surrounding mainstream concentrations, thereby establishing a concentration gradient towards the cell. Waste excretion will have the opposite effect on solute concentrations, resulting in a concentration gradient that extends away from the cell. On the other hand, higher mainstream nutrient solute concentrations or lower waste concentrations relative to cell surface concentrations will serve to increase concentration gradients and diffusional mass transfer to the benefit of microbial cells. Conversely, a decrease in mainstream nutrient concentrations or increase in waste concentrations will have the opposite effect on concentration gradients, resulting in a cutback on diffusional mass transfer and possible metabolic malnutrition.

Adoption of a larger cell size with a greater surface area seems like it would be helpful strategy to mitigate diffusion limitations. However, the diffusion distance to the middle of spheroidal coccoid bacterial cell (the cell radius) is greater than for a rod-shaped cell of the same volume (Figure 8). Diffusion time (t) furthermore varies in proportion to the square of diffusion distance (l) and molecular diffusion coefficient (D) as shown by Equation 15.

$$t = \frac{l^2}{2D} \tag{15}$$

For a doubling in size (volume) of a coccoid cell with an initial radius of 1.0 μm, the respective diffusion distance will increase by 30 percent to 1.3 μm and the corresponding solute diffusion time will increase by almost 70 percent. Conversely, diffusion distances and times remain unchanged in a fixed radius rod-shaped cell that doubles in volume by elongation instead of expanding outwards.

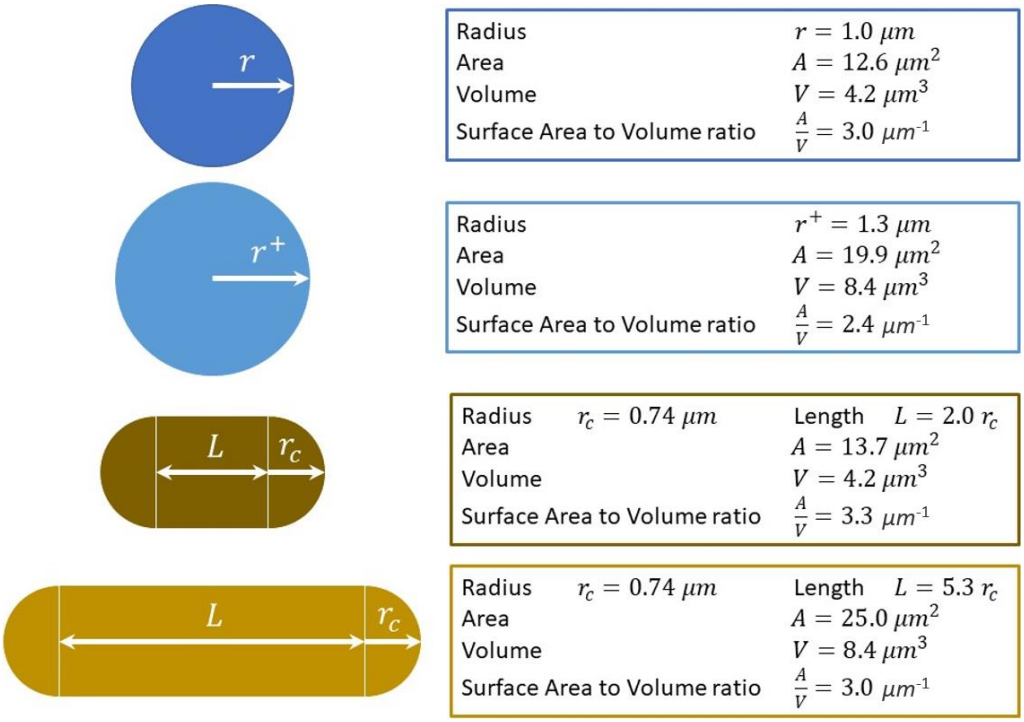

Figure 8 - Relationships between the characteristic length scales (radius) of spheroidal coccoid and rod-shaped bacterial cells with corresponding cell surface areas, cellular volumes, and surface area to volume ratios.

The issue of thermodynamics boils down to how different kinds of microorganisms generate and conserve energy needed for growth and reproduction by cellular division (Bethke et al., 2011; Bird et al., 2011). All forms of life, including microorganisms, depend strictly on oxidation reactions for energy generation. In these reactions, a reduced chemical substance undergoes oxidization with the transfer of electrons to another, more oxidized, chemical substance. Such reactions are spontaneous and exergonic, corresponding to a negative Gibbs energy of reaction (ΔG_r). The amount of energy that is released is directly proportional to the difference in redox potential (ΔEh) between half-cell reactions of the reduced electron donor and oxidized electron acceptor as shown in Equation 16.

$$\Delta G_r = -nF\Delta Eh = -nF\left(Eh_{electron\ acceptor} - Eh_{electron\ donor}\right) \quad (16)$$

where:

n = number of electrons transferred in the reaction

F = Faraday constant (C/mol)

The Eh values of the electron acceptor and electron donor are given by the Nernst equation (Equation 4).

The two main types of energy-generating pathways are respiration and fermentation. Respiration involves the transfer of electrons through a chain of metabolic intermediates, ultimately ending with a terminal electron acceptor. At various steps through the electron transport chain, released energy is captured in biochemical form as adenosine triphosphate (ATP). In fermentation, ATP is formed when a mixed oxidation state chemical compound is split in two, with one part being oxidized and the other

reduced. Compared to respiration, the energy yield of fermentation tends to be lower. For this reason, respiration ranks as the preferred energy generation pathway of most organisms on Earth.

Aerobic respiration using molecular oxygen as a terminal electron acceptor yields the greatest amount of energy. This is because of the high standard redox potential (Eh = 1.23 V) of the oxygen-water half-cell reaction relative to that of chemical substances that may serve as electron donors (Table 1). But what really sets microorganisms (especially prokaryotes) apart from other forms of life is the vast array of other terminal electron acceptors that are used when oxygen is not available. This is called anaerobic respiration.

Table 1 - Standard potentials of some electron acceptor and electron donor half-cell reactions.

Electron Acceptors	Eh^0 (V)
$1/4\,O_2 + H^+ + e^- = 1/2\,H_2O$	1.23
$1/5\,NO_3^- + 6/5\,H^+ + e^- = 1/10\,N_2 + 3/5\,H_2O$	1.24
$1/2\,MnO_2 + 2H^+ + e^- = 1/2\,Mn^{2+} + H_2O$	1.22
$Fe(OH)_3 + 3H^+ + e^- = Fe^{2+} + 3H_2O$	1.07
$1/8SO_4^{2-} + 9/8\,H^+ + e^- = 1/8\,HS^- + 1/2\,H_2O$	0.25
$1/8CO_2 + H^+ + e^- = 1/8\,CH_4 + 1/4\,H_2O$ (methane)	0.17
Electron Donors	Eh^0 (V)
$H^+ + e^- = 1/2\,H_2$	0
$1/6\,CO_2 + H^+ + e^- = 1/6\,CH_3OH + 1/6\,H2O$ (methanol)	0.03
$1/4CO_2 + H^+ + e^- = 1/4CH_2O + 1/4\,H_2O$ (formaldehyde)	-0.07
$1/2\,CO_2 + H^+ + e^- = 1/2\,HCOOH$ (formic acid)	-0.20
$CO_2 + H^+ + e^- = 1/2\,C_2H_2O_4$ (oxalic acid)	-0.48
$1/6\,CO_2 + H^+ + e^- = 1/12C_2H_4 + 1/3\,H_2O$ (ethylene)	0.07
$1/6\,CO_2 + H^+ + e^- = 1/12C_2H_5OH + 1/4\,H_2O$ (ethanol)	0.09
$1/8\,CO_2 + H^+ + e^- = 1/8CH_3COOH + 1/4\,H_2O$ (acetic acid)	0.12
$1/4\ CO_2 + H^+ + e^- = 1/24C_6H_{12}O_6 + 1/4\,H_2O$ (glucose)	-0.01

The conventional way of explaining anaerobic respiration uses the iconic imagery of steps down an energy ladder, where each downwards interval corresponds to a terminal electron acceptor half-cell reaction with a lower standard redox potential. This gives the

often cited – and well worth memorizing – sequence of terminal electron acceptors for anaerobic respiration as nitrate, followed by Mn(IV), Fe(III), sulfate, and finally carbon dioxide. The corresponding conjugate reductants of the terminal electron acceptors are nitrogen, Fe(II) and Mn(II), sulfide, and methane/acetic acid.

At the same time, the image of a redox ladder for anaerobic respiration in several ways does not do justice to what happens in nature. First, the ladder is more like a slide, or continuum, because ΔG_r does not depend on Eh^0 alone (Figure 9); instead, Eh is a critical variable as determined by actual concentrations of oxidants and conjugate reductants based on the Nernst equation (Equation 4). Second, the conventional list of terminal electron acceptors does not fully reflect the vast pool of potential oxidants available for anaerobic respiration in natural environments. These include various inorganic and organic substances, some of which are toxic to other organisms. As a third point, energy harvesting may occur simultaneously at different "rungs" of the redox ladder within a given environmental setting, by different groups of microorganisms or by a single microbial community shifting between energy sources.

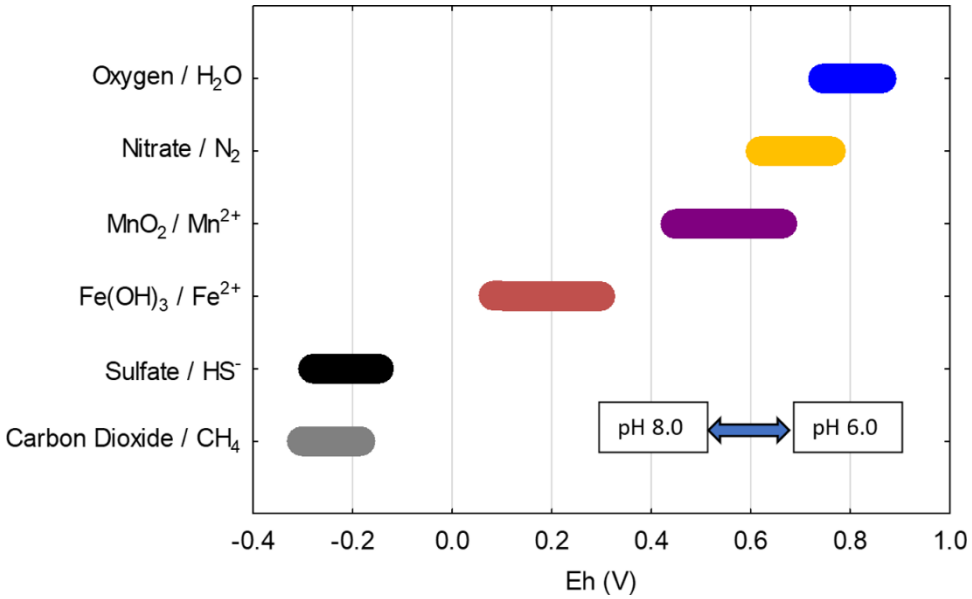

Figure 9 - Range of oxidation-reduction (Eh) potentials for common terminal electron acceptors from pH 6.0 (upper values) to pH 8.0 (lower values). The Eh values were calculated using the Nernst equation (Equation 4) on the basis of half-cell reactions listed in Table 1. A concentration of 10^{-5} M was assumed for all dissolved chemical species other than H^+, and unity activity for solid mineral phases and water with atmospheric $pCO_2 = 10^{-3.50}$, $pO_2 = 10^{-0.68}$, and $pN_2 = 10^{-0.11}$ atm.

2.4 Metabolic Groups

Classification of prokaryotes in terms of metabolic capacity and physiology is widely used as a framework to describe specific roles of microbes in ecosystem function (Amend and Teske, 2005; Anderson et al., 2006; Shirokova and Ferris, 2013). Groups defined in this way may include microorganisms that are unrelated by phylogenetic criteria

used in taxonomy. For example, anaerobic respiration by sulfate reduction is carried out by prokaryotic microorganisms from several different phylogenetic lines, including various phyla and genera in the domain of *Bacteria* as well as some species of *Archaea*. Despite their major phylogenetic differences, sulfate-reducing prokaryotes represent a distinct metabolic (phenotypic) group that plays an important ecological role in the biogeochemical cycling of sulfur and carbon. Apart from ecophysiological categorization based on terminal electron acceptors, two additional criteria are used to define metabolic groups of prokaryotes: the source of energy used to fuel cell metabolism and the process used to acquire carbon to support cell growth.

Prokaryotic microorganisms capture energy from either light-driven reactions (phototrophs) or the oxidation of reduced chemical compounds (chemotrophs), as shown in Figure 10. While the availability of light energy from the sun restricts phototrophs to surface environments, chemotrophs thrive even in the complete darkness found in deep ocean and underground habitats. Among chemotrophs, those that oxidize reduced organic substances are known as organotrophs, whereas lithotrophs oxidize inorganics such as ammonium (NH_4^+), sulfide (S^{2-}), or ferrous iron (Fe^{2+}). When it comes to the acquisition of carbon, autotrophs rely on the reduction of carbon dioxide to produce cellular organic material. In contrast, heterotrophs (often taken to be synonymous with organotrophs) utilize pre-existing organic molecules to foster cell growth and division. Photoheterotrophs rely on light to generate energy and organic matter as a source of carbon. These various terms give practical ecophysiological definitions for the different metabolic groups of prokaryotic microorganisms found in natural systems.

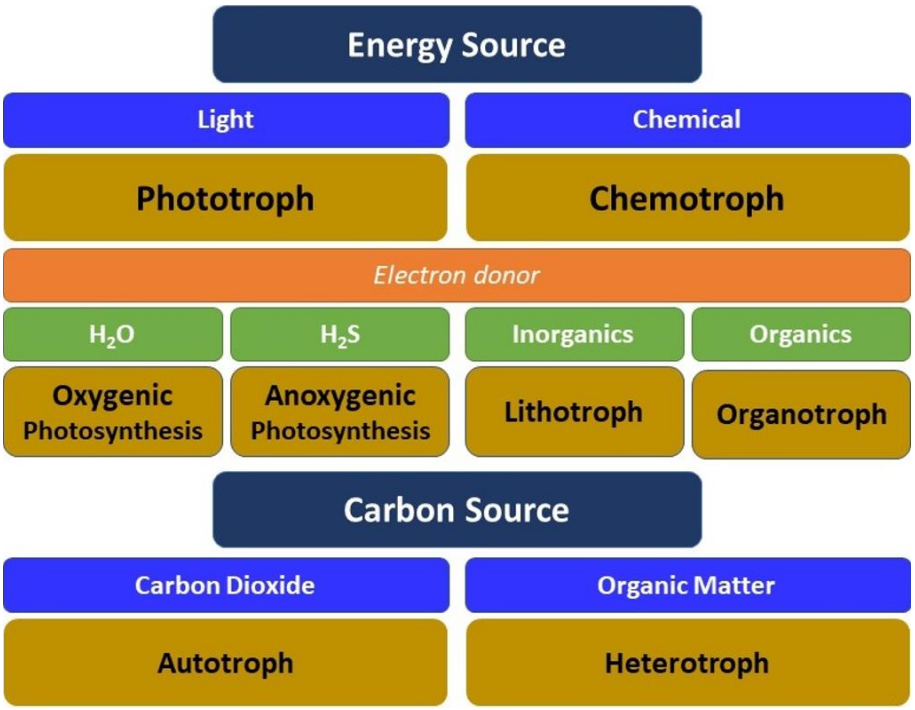

Figure 10 - Functional groups of microorganisms based on energy and carbon sources.

3 Groundwater Systems as Habitats for Microbial Life

The types of places in which living organisms grow and multiply are often described as habitats. Each habitat is characterized by a set of physical and chemical features that are essential for sustaining life. In the context of hydrogeology, one of the most critical physical parameters for describing different habitats is by magnitude of the length scale. In groundwater systems, length scales extend over large distances of 10^3 m (1 km) all the way down to distances as small as 10^{-6} m (1 μm), depending on how habitat size is defined. Length scale is critical because it defines other important physical properties for habitability such as surface areas and relative volumes of solids, water, air, and other fluids. These factors determine not only where it is possible for life to take refuge but also influence water movement (with links to Darcy's law through hydraulic conductivity), groundwater chemistry, and reactive mass transport processes (with links to advection, dispersion and reaction relationships).

On a global scale, the vast amount of water that exists underground is difficult to imagine with volume estimates soaring into, and above, tens of millions of cubic kilometers (Gleeson et al., 2015). This is almost as much water as there is in some ocean basins. But, remarkably, all of that groundwater is mostly invisible to macroscopic ($> 10^{-4}$ m) observation because it is hidden away in microscopic ($< 10^{-4}$ m) interstitial pore spaces between mineral grains in sediments, as well as along joints and fracture planes in bedrock. As a consequence, the mineral surface area to water volume ratio in groundwater systems far exceeds that of surface environments. Nevertheless, when it comes to actual living space, most subsurface environments are only accessible to microorganisms.

To get an idea of the limitations of physical space in subsurface environments, Figure 11 compares the size range of different life forms with pore diameters in unconsolidated clay and sands, along with corresponding values for shale, sandstone, dolomite, and aperture widths in fractured/jointed rocks. Apart from some wider fracture and joint apertures that occur in rocks, macro-eukaryotes are just too big to live in groundwater environments. Even the fit for micro-eukaryotes is tight as their size range is similar to pore diameters in sands, meaning there is very little room to grow and divide. On the other hand, the smaller size of prokaryotes allows them to live in comfort within most porous unconsolidated sands. While planktonic growth and movement between pores is possible, most cells grow in biofilms attached to mineral grains or rock surfaces. Overall, attached microorganisms dominate groundwater systems in terms of biomass and activity; however, there is a dynamic equilibrium between attachment and detachment processes as bacteria transition between planktonic and attached modes of growth in response to changes in environmental conditions such as nutrient availability or fluctuations in oxidation-reduction potential. In the tighter confines of pores in clays and consolidated sedimentary rocks such as shale, space begins to become a limiting factor not

only for prokaryotic cells but also for individual virus particles (Rebata-Landa and Santamarina, 2006).

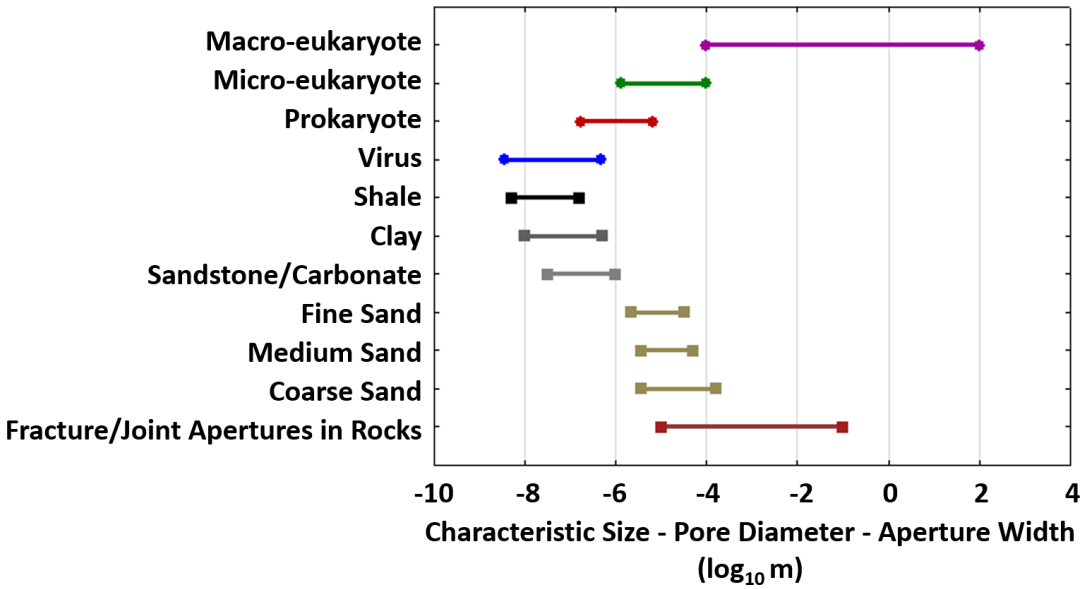

Figure 11 - Comparison of characteristic pore diameters in sediments and fracture/joint aperture widths in rocks with the size range of viruses, prokaryotes, micro-eukaryotes, and macro-eukaryotes. The blue dashed line represents the upper size limit of all microorganisms, whereas the red dashed line defines the lower size limit of prokaryotes.

The steady decline of microbial cell concentrations with increasing depth underground is well documented. In near-surface groundwater systems at depths less than 100 m, cell numbers average 10^7/g in cores and around 10^5/mL in groundwater samples. These cellular concentrations decrease to respective mean values of about 10^4/g and 10^3/mL at depths of > 1000 m. Increasing ionic strength with depth has been identified as a particularly important factor contributing to lower cell numbers in the deep subsurface. Within the soil zone, declining microbial numbers over a depth interval from 1 to 2 m typically correlate with decreasing organic carbon concentration. This is because aerobic heterotrophs degrade and consume cellulose-rich organic matter derived from plants. At greater depths, the relationship between microbial cell concentrations and organic carbon weakens, implying an enhanced role of lithoautotrophic microorganisms in deep subsurface carbon cycling.

Another important physical parameter that defines habitats in groundwater systems is temperature (Amend and Teske, 2005; Taylor and Stefan, 2009; Bonte et al., 2013a,b). The reason temperature is so critical is that it controls when and where water exists in a liquid state, which is an absolute life requirement. For pure water, this is from 0 to 100°C at a standard pressure of 1 atm. Rates of metabolic processes and chemical reactions, as well as the stability of biomolecules, are all dependent on temperature. The density and viscosity of water are also sensitive to temperature, as is the transport of metabolites and other chemical substances by molecular diffusion.

In contrast to seasonal changes in climate above ground, the high specific heat capacity of water, insulating properties of geological materials, and lack of solar irradiance keep shallow groundwaters close to the mean annual air temperature (Menberg et al., 2014; Benz et al., 2017). With the exception of polar regions, this means that most shallow (no more than 60 m depth) groundwater systems around the world fall well inside the required habitat temperature range of microorganisms (Figure 12).

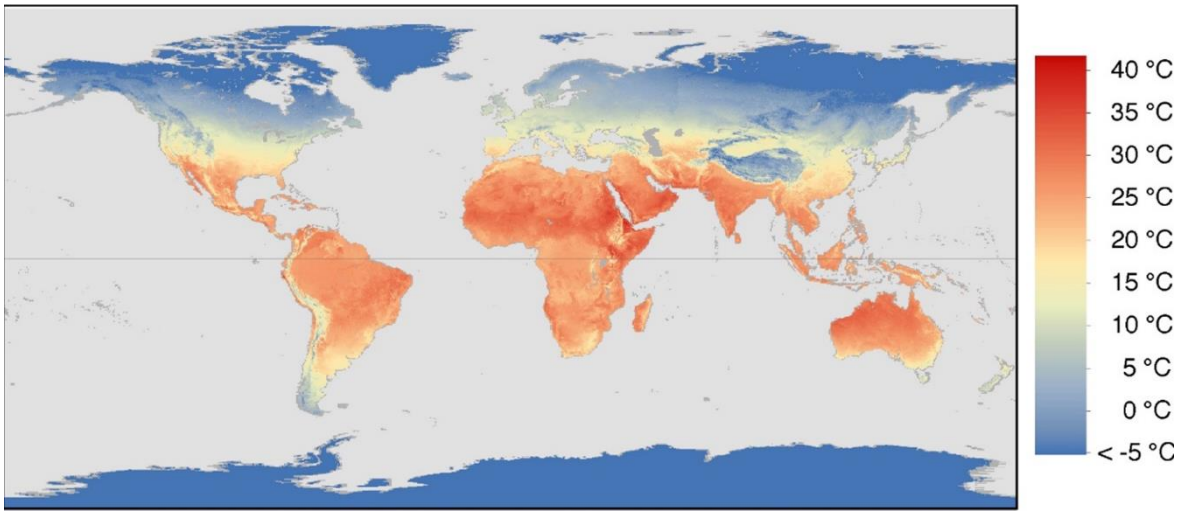

Figure 12 - Global temperature estimates for shallow (< 60 m) groundwater (Reproduced under the terms of Creative Commons Attribution 3.0 license from Benz et al., 2017).

Temperature tends to increase with depth underground because of multiple heat sources inside the Earth, such as radioactive decay and latent heat from core crystallization. Although variable, especially in hot volcanic regions, the typical geothermal gradient in most areas of the world is about 25 to 30°C/km. This means that temperatures will approach 100°C at depths of 3 to 4 km, which gives a rough idea of the depth to which microbial life can exist in groundwater systems (Colwell and D'Hondt, 2013).

Numerous chemical properties are relevant to the characterization of microbial habitats in groundwater systems, including pH (Equation 2), *Eh* (redox) potential (Equation 4), and ionic strength (Equation 5). In addition, concentrations of different solid materials, gases, and dissolved solutes help define overall chemical conditions of a habitat, particularly in terms of nutrient availability and energy supply. These concentrations are often reported in different units, for example as percentages, parts per million, or molarity. Similarly, for gases, parts per million by volume and partial pressure are often used interchangeably. It is therefore important to be able to convert from one unit to another in any quantitative investigation of these parameters within groundwater systems.

4 Influence of Microorganisms on Groundwater Chemistry

The chemical composition of groundwater is regulated by heterogeneous interactions between the solid, liquid, and gas phase (Glynn and Plummer, 2005). These reactive systems feature multiple contributions from acid-base, surface and aqueous complexation, and oxidation-reduction reactions, as well as frequent involvement of prokaryotic microorganisms. Mass transport processes such as advection and dispersion also come into play, which give rise to changes in groundwater chemistry over a wide range of time scales and flow path lengths.

Prokaryotes contribute to chemical reactions in groundwater systems in two ways. First, metabolic enzyme activity can speed up (catalyze) slow reactions and force corresponding reaction quotients to shift rapidly towards or away from equilibrium. Second, prokaryotic cells behave as microscopic reactive solids owing to the chemical reactivity of functional groups, such as carboxyl or phosphoryl substituents, in the macromolecular components of cell walls, external sheaths, and EPS. The metabolic intervention of microbes in chemical reactions affects many aspects of groundwater chemistry, including pH, redox conditions, mineral dissolution and precipitation processes, and the chemical speciation of solutes (Chapelle, 2000). As reactive solids, bacteria not only contribute to the sorption (surface complexation) of dissolved ions but also serve as heterogenous nucleation templates for mineral precipitation.

4.1 Chemical Equilibria and Reaction Rates in Groundwater

Descriptions of chemical processes in groundwater systems are usually formulated in terms of mass action and mass balance considerations, as well as reaction kinetics. The application of these basic aspects of physical chemistry and thermodynamics provides quantitative insight into the extent, direction, and rate of chemical reactions, including those involving microorganisms. A general reaction is shown in Equation 17.

$$aA + bB = cC + dD \tag{17}$$

The corresponding mass action equilibrium constant K is calculated as shown in Equation 18.

$$K = \frac{\{C\}^c\{D\}^d}{\{A\}^a\{B\}^b} = e^{\frac{-\Delta G^0}{RT}};\ \Delta G^0 = -RT\ln K \tag{18}$$

where:

A,B = activities of reactants (dimensionless)

C,D = activities of products (C,D) *at equilibrium* with stoichiometric coefficients given in lower case letters (dimensionless)

R = universal gas constant (ML^2)/(T^2 °K mol)

T = temperature (°K)

ΔG^0 = standard Gibbs energy of reaction Δ $(ML^2)/(T^2\,mol)$

For a reversible reaction at equilibrium, the concentrations of reactants and products remain constant. This condition requires the rates of the forward (R_f) and reverse (R_r) reactions to be equal as shown in Equation 19 such that the equilibrium constant can be expressed as Equation 20 with corresponding forward (k_f) and reverse (k_r) rate coefficients.

$$R_f = k_f\{A\}^a\{B\}^b = R_r = k_r\{C\}^c\{D\}^d \tag{19}$$

$$K = \frac{k_f}{k_r} = \frac{\{C\}^c\{D\}^d}{\{A\}^a\{B\}^b} \tag{20}$$

The dependence of the rate coefficients on temperature and reaction activation energy is evident from the Arrhenius relationship (Equation 1).

A far more interesting condition, especially for groundwater systems, is when a reaction is not at equilibrium. Here, the Gibbs energy for a reaction (ΔG_r) to occur is described by Equation 21.

$$\Delta G_r = \Delta G^0 + RT \ln \frac{\{C\}^c\{D\}^d}{\{A\}^a\{B\}^b} \tag{21}$$

For reactant and product activities observed away from equilibrium, defining the reaction quotient of products to reactants as Q, gives Equation 22.

$$\Delta G_r = \Delta G^0 + RT \ln Q = RT \ln \frac{Q}{K} \tag{22}$$

From this expression, one finds that at equilibrium Q is the same as K, so $\Delta G_r = 0$. If Q is less than K, ΔG_r is negative. This means the reaction is spontaneous. Conversely, $\Delta G_r = 0$ is positive when Q is greater than K, indicating the reaction is not possible (unless energy is supplied from another spontaneous reaction). The capacity to use energy-yielding spontaneous reactions to drive energetically unfavorable reactions is a defining characteristic of biosynthetic processes in microbial metabolism.

Comparison of reaction quotients to equilibrium constants is widely applied to the study of mineral dissolution and precipitation reactions; however, subtle differences in terminology and interpretation exist. For dissolution reactions, the equilibrium constant is known as the solubility product constant (K_{sp}) and the reaction quotient is referred to as the ion activity product (IAP). The saturation index (SI) is defined by Equation 23.

$$SI = \log_{10} \frac{IAP}{K_{sp}} \tag{23}$$

When the IAP is equal to K_{sp}, $SI = 0$ and the solution is said to be at equilibrium with respect to the mineral under consideration. Should IAP be greater than K_{sp}, the SI will be positive, which indicates the solution is oversaturated and mineral precipitation is possible.

If the *IAP* is less than K_{sp}, the *SI* will be negative, which indicates the solution is undersaturated and mineral dissolution is possible.

4.2 Carbonate Equilibria and Groundwater pH

In groundwater systems, pH is initially established as meteoric water comes into equilibrium with atmospheric carbon dioxide (Langmuir, 1997). This results in the formation of diprotic carbonic acid (a diprotic acid yields two H^+ ions per acid molecule), which subsequently undergoes dissociation to form first bicarbonate and then carbonate. As the first deprotonation product of carbonic acid, the amphoteric behavior of bicarbonate (i.e., its ability to react as either a monoprotic acid or base) is particularly important for groundwater pH. The interactions between dissolved inorganic species are intricate; however, the impact of carbonate equilibria on pH can be explored by considering the ion charge balance that would be expected in pristine meteoric water as shown in Equation 24.

$$[H^+] = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] \quad (24)$$

Over the pH range of most natural waters, the concentrations of carbonate and hydroxyl ions are much smaller than the concentration of bicarbonate. This simplifies the charge balance to Equation 25.

$$[H^+] = [HCO_3^-] \quad (25)$$

Combining the charge balance with the mass action equilibrium constants for the formation of carbonic acid (*KH* = 10-1.47) and dissociation into bicarbonate (*K1* = 10-6.35) gives the proton concentration as a function of carbon dioxide partial pressure (*pCO2* = 10-3.5) as shown in Equation 26.

$$[H^+] = \sqrt{K_1 K_H pCO_2} \quad (26)$$

This relationship yields a pH of 5.7 (corresponding to -log[*H*+]; Equation 2). The equation also shows that an increase in the partial pressure of carbon dioxide will tend to decrease pH, whereas a decrease in carbon dioxide partial pressure promotes an increase in pH.

In groundwater systems, carbon dioxide is produced from the degradation of organic matter by heterotrophic microorganisms, particularly as meteoric water infiltrates through soil and the vadose zone. As a consequence, subsurface carbon dioxide partial pressures are often higher than in the open atmosphere. This serves to increase the formation of carbonic acid, which is the main source of protons for mineral weathering and dissolution reactions in groundwater systems (Kump et al., 2000; Wilson, 2004).

While heterotrophic microbial activity tends to increase carbon dioxide partial pressure, autotrophs have the opposite effect, as they rely on the uptake and reduction of carbon dioxide to produce cellular organic material. If the metabolic demand for carbon is sufficiently high, a drawdown in the partial pressure of carbon dioxide may occur. To compensate, pH increases as protons recombine with bicarbonate to compensate for decreases in carbonic acid concentrations. In the subsurface, the influence of autotrophs on

carbonate equilibria and pH is usually not as pronounced as that of heterotrophs, particularly in systems buffered by higher concentrations of dissolved inorganic carbon.

4.3 Redox Conditions

Microbially mediated oxidation-reduction reactions play a major role in regulating redox conditions in groundwater systems (Kuma and Riyazuddin, 2012; Liebensteiner et al., 2014; Tesoriero et al., 2015; Enright and Ferris, 2016; Enright et al., 2019). This is because microorganisms rely on the transfer of electrons from reduced electron donors to oxidized terminal electron acceptors as a source of energy for cell growth and division. The overall Gibbs energy of such reactions is determined by the difference between oxidant (electron acceptor) and reductant (electron donor) half-cell redox potentials (Equation 16). Those reactions that provide the maximum amount of energy are favored by microorganisms and typically dominate over competing reactions. The most common electron donors in groundwater systems are dissolved and particulate organic carbon (Shen et al., 2015). In some cases, reduced forms of nitrogen, iron, and sulfur are important. Among commonly available electron acceptors, oxygen yields more energy than any other oxidant in aerobic respiration. When oxygen is depleted, the next most energetically favorable electron acceptor in anaerobic respiration is nitrate, followed by manganese, ferric iron, sulfate, and finally carbon dioxide.

The sequential nature of electron acceptor utilization by microorganisms, which was introduced in Section 3 as the "redox ladder," tends to promote the development of increasingly reducing conditions along groundwater flow paths depending on the availability of electron donors (Figure 13). As redox conditions change, documenting the sequential depletion of oxidants and corresponding production of distinctive conjugate reductants can be used to identify specific zones where microbial activity is dominated by a single electron-accepting process (Groffman and Crossey, 1999; McMahon and Chapelle, 2008). Characteristic threshold concentrations that have been suggested for the identification of redox zones in groundwater systems are given in Table 2. It is important to keep in mind that some care should be exercised when interpreting these threshold concentrations to account for factors such as differences in microbial community composition, availability of electron donors, and scale at which groundwater sampling occurs.

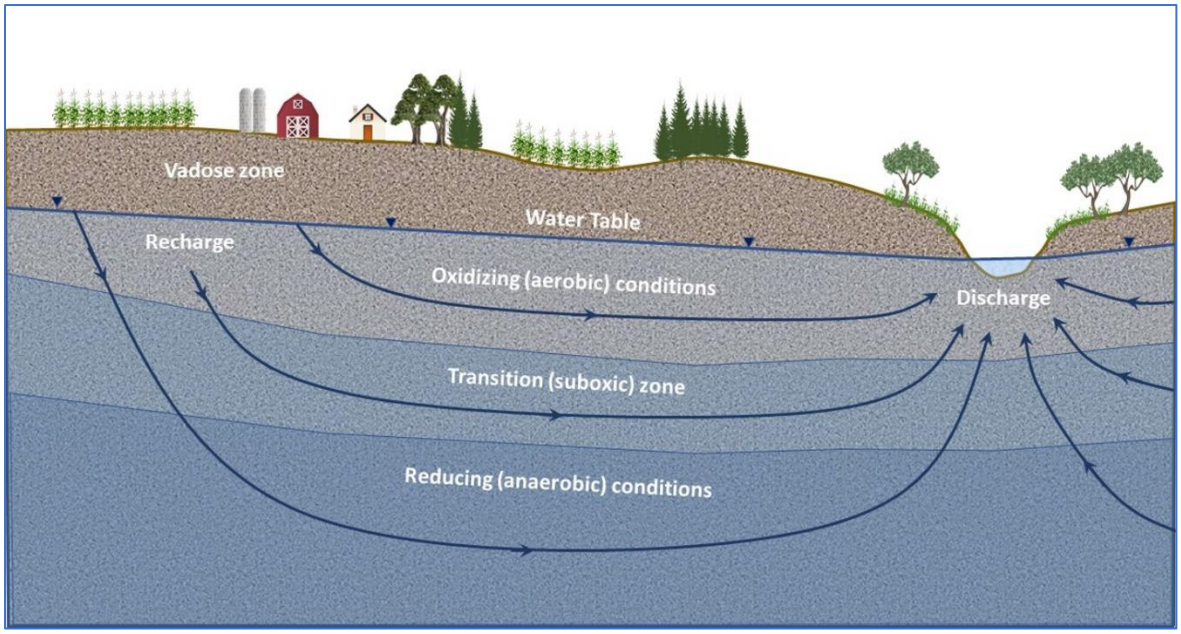

Figure 13 - Evolution of redox conditions along groundwater flow paths. Typically, redox conditions tend to become more reducing with increasing travel distance and residence time of water underground. Redox conditions in discharge areas tend to be variable owing to mixing of groundwater and surface water.

Table 2 - Threshold concentrations suggested for identifying microbial redox processes in groundwater systems (McMahon and Chapelle, 2008).

	Concentration mg/L				
Redox Process	O_2	NO_3^-	Mn^{2+}	Fe^{2+}	SO_4^{2-}
Aerobic (oxic)					
O_2 Reduction	$\geq$ 0.5		< 0.05	< 0.1	
Anaerobic (anoxic)					
NO_3^- reduction	< 0.5	$\geq$ 2.2	< 0.05	< 0.1	
$Mn(IV)$ reduction	< 0.5	< 2.2	$\geq$ 0.05	< 0.1	
$Fe(III)/SO_4^{2-}$ reduction	< 0.5	< 2.2		$\geq$ 0.1	$\geq$ 0.5
CO_2 reduction	< 0.5	< 2.2		> 0.1	< 0.5

The first electron acceptor consumed along groundwater flow paths is oxygen. The transition from aerobic to anaerobic respiration is thought to take place when oxygen concentrations fall below a level of about 0.5 mg/L. For the onset of nitrate reduction (denitrification), the threshold oxygen concentration might be as high as 2.0 mg/L, implying a moderate degree of overlap with aerobic respiration. This is consistent with the growth of some heterotrophic microorganisms as facultative anaerobes, which utilize both aerobic and anaerobic respiration. The reduction of nitrate is also used by various lithotrophs in the oxidation of inorganic electron donors such as sulfide or ferrous iron. Sources of nitrate in groundwater include agricultural fertilizers, as well as lithotrophic microbial oxidation of ammonium released from the decomposition of nitrogen-rich organic substances by microorganisms.

In groundwater systems, Mn(IV) and Fe(III) are relatively abundant but typically occur as insoluble oxide mineral precipitates instead of dissolved ionic species. Because of this, elevated concentrations of dissolved Mn^{2+} and Fe^{2+} produced from the reductive dissolution of iron and manganese oxides are used as proxies to identify zones of active microbial Mn(IV) and Fe(III) reduction. The susceptibility of Mn^{2+} and Fe^{2+} to chemical and microbial oxidation, mineral precipitation, and solid-phase sorption processes add another layer of biogeochemical complexity that could result in an underestimation of the extent of Mn(IV) and Fe(III) reduction.

After oxygen and nitrate are exhausted, it is sometimes difficult to distinguish between zones of active Fe(III) and sulfate reduction. This relates to the energetic dependence of Fe(III) reduction on the crystalline form of iron oxides present in a groundwater system, as well as the natural variability in dissolved sulfate concentrations. As a general rule of thumb, poorly ordered hydrous ferric oxides are more easily reduced by microorganisms than crystalline varieties such as goethite or hematite (Roden et al., 2004; Langley et al., 2009a). These low-complexity ferric oxides can be thought of as more bioavailable, in the same way that simple monosaccharide sugars are more suited to yeast microorganisms than long-chain polysaccharides. Additionally, higher sulfate concentrations (≥ 0.5 mg/L) are energetically more favorable to sulfate reduction. Elevated concentrations of sulfide can also be used as a proxy for sulfate reduction, subject to the same caveats that apply to Mn^{2+} and Fe^{2+} proxies for Mn(IV) and Fe(III) reduction, respectively.

The redox conditions under which carbon dioxide reduction becomes the predominant anaerobic respiratory process are typically highly reducing owing to depleted concentrations of more strongly oxidizing electron acceptors such as sulfate (< 0.5 mg/L). It is tempting to use the presence of methane as a proxy for microbial carbon dioxide reduction; however, this can be misleading as some methanogenic microbes produce methane from the fermentation of acetic acid instead of the reduction of carbon dioxide. Other microorganisms produce acetic acid from carbon dioxide reduction instead of methane reduction.

An additional way to follow the shift in redox conditions along a groundwater flow path is to follow the change in microbial community composition from the unsaturated vadose zone to the saturated zone immediately below the water table. This can be accomplished using molecular biological techniques to identify and quantify different metabolic groups of microorganisms. A study on a shallow pristine sand aquifer found the relative abundance of microorganisms using oxygen as an electron acceptor decreases as water infiltrates through the vadose zone and becomes increasingly isolated from the atmosphere (Figure 14). The apparent preference for nitrate as an electron acceptor can be traced to the ability of some microorganisms to grow as facultative anaerobes. Below the water table, utilization of oxygen as an electron acceptor decreases while nitrite, Fe(III), and

sulfate become more important. When it comes to electron donors (the energy source), organic matter and heterotrophic processes dominate above and below the water table. With the exception of Fe(II)-oxidizing microorganisms in the unsaturated zone, the relative abundance of lithotrophs using inorganic electron donors is lower than for their heterotrophic counterparts. Reduced Fe(II)-bearing minerals such as biotite and amphiboles are candidates as possible sources of Fe(II) to support lithotrophic microbial growth, particularly under relatively oxidizing conditions above the water table.

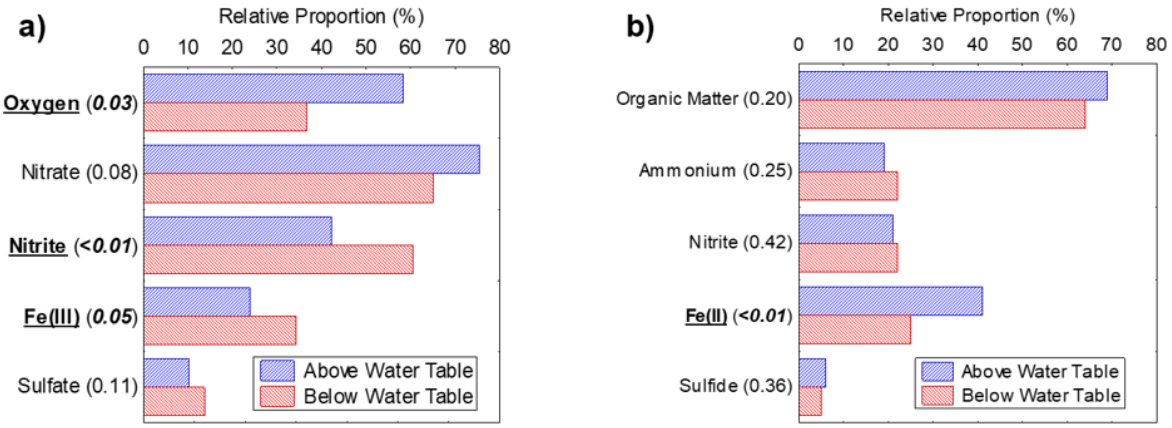

Figure 14 - Relative proportion of potential a) electron acceptor and b) electron donor use among bacteria in core samples obtained from above and below the water table in a shallow pristine sand aquifer on the Canadian Shield. The p-values of *t*-tests for the difference in average relative proportions of bacteria above and below the water table are given in brackets; significant values ($p < 0.05$) are italicized and corresponding metabolic groups are underlined in bold (adapted from Shirokova and Ferris, 2013).

Microbial contributions to redox transformations in groundwater systems are not strictly confined to the common electron acceptors that dominate in anaerobic respiration. In fact, possible oxidants for metabolic processes under reducing conditions include a wide variety of inorganic and organic substances, many of which are environmental pollutants. The conjugate reductants arising from the use of these atypical electron acceptors by microorganisms often display different physical, chemical, and biological properties than the parent compounds. Using chromium as an example, microbial reduction of Cr(VI) in the form of dissolved chromate (CrO_4^{2-}) to Cr(III) produces the Cr^{3+} ion, which is highly insoluble. An important difference between the Cr(VI) and Cr(III) oxidation states is that hexavalent chromium is highly toxic, whereas trivalent chromium is an important trace nutrient. Arsenate (AsO_4^{3-}) is another noteworthy electron acceptor in groundwaters because arsenic poisoning is a risk to human health (Gorra et al., 2012). Among organic contaminants in groundwater, highly oxidized chlorinated solvents such as tetrachloroethene (PCE) and trichloroethene (TCE) are known to behave as electron acceptors and undergo stepwise reductive dechlorination in microbial metabolism. While this contributes to the attenuation of PCE and TCE, an undesirable outcome in some settings is the production and accumulation of the carcinogenic intermediate, vinyl chloride (VC).

4.4 Behavior of Bacteria as Geochemically Reactive Solids

The solid phase reactivity of microbial cells is related to the presence of acid functional groups, for example carboxyl and phosphoryl substituents, in the structural polymers of their cell walls, external sheaths, and extracellular polysaccharides (Kulczycki et al., 2002, 2005; Kennedy et al., 2011; Hao et al., 2013). Deprotonation of these various functional groups contributes to the development of surface charge on cells and provides discrete complexation sites for the electrostatic sorption of dissolved counter ions (Martinez and Ferris 2001; Smith and Ferris, 2001; Sokolov et al., 2001; Phoenix et al., 2007). These reactions are especially important for the solid phase partitioning of dissolved ions that are present in groundwater at a concentration below that imposed by the solubility of any mineral in which they might occur.

A general competitive sorption reaction between a proton (H^+) and a metal cation (Me^{z+}) at a protonated surface carboxyl binding site on a microbial cell ($B \cdot COOH$) can be written as shown in Equation 27.

$$B \cdot COOH + Me^{z+} = B \cdot COOMe^{z-1} + H^+ \quad (27)$$

The release of protons and sorption of metal cations is described by apparent rate constant (K_{app}) and pH-conditional (K_{pH}) sorption constant (Equation 28).

$$K_{pH} = \frac{K_{app}}{[H^+]} = \frac{[B \cdot COOMe^{z-1}]}{[B \cdot COOH][Me^{z+}]} \quad (28)$$

The equilibrium mass action expression emphasizes that ion sorption by microorganisms depends not only on proton (pH) and dissolved metal cation concentrations but also on the number of reactive chemical groups per cell.

The pH dependence of ion sorption reactions in an important intrinsic feature of solid phase sorbents, including microorganisms. For those metals that predominantly exist as cations in solution, sorption is significantly enhanced as pH increases and surface groups deprotonate. Conversely, oxyanions of metals and metalloids sorb better at low pH values as surface groups become protonated. The chemical properties of the sorbate ions will also influence sorption behavior. A particularly important factor is ionic potential, or the ratio of electric charge to radius of an ion. Sorption of smaller, highly charged ions is favored over larger, weakly charged ions. A change in ionic charge arising from aqueous complexation reactions has a similar effect as sorption strength tends to decrease when ions are complexed.

A plot that describes the amount of a dissolved chemical species that is sorbed by a sorbent as a function of increasing concentration, measured at constant temperature, is called a sorption isotherm. Three main types of sorption isotherms have been used to quantify sorption reactions involving bacteria and other sorbent solids. Considering a metal cation as the sorbate, the first type of sorption isotherm is represented by a linear distribution (partition) coefficient (K_d) as illustrated by Equation 29.

$$[B \cdot Me^{z-1}] = K_d[Me^{z+}] \quad (29)$$

A major limitation of the linear distribution isotherm (Figure 15a) is that it does not consider the finite number of sorption sites that exist on a solid phase sorbent. Instead of constantly increasing with increasing sorbate concentrations, the amount that is sorbed will tend to progressively decrease as sorption sites are occupied. This can lead to an overestimation of the amount of a chemical species that is sorbed, particularly at high sorbate concentrations. The deviation from linear behavior is captured to some extent in the empirical Freundlich sorption isotherm that is shown as Equation 30.

$$[B \cdot Me^{z-1}] = K_F[Me^{z+}]^{1/n} \quad (30)$$

where:

K_F = Freundlich sorption coefficient (L^3/M)

$1/n$ = exponent of non-linearity (Figure 15b)

When $n = 1$, the Freundlich relationship reduces to the linear distribution isotherm. The Freundlich isotherm is limited in that it does not include an explicit account for the number of available sorption sites, nor does it allow for variations in pH and ionic strength. This makes it difficult to compare interpretations of sorption data between locations.

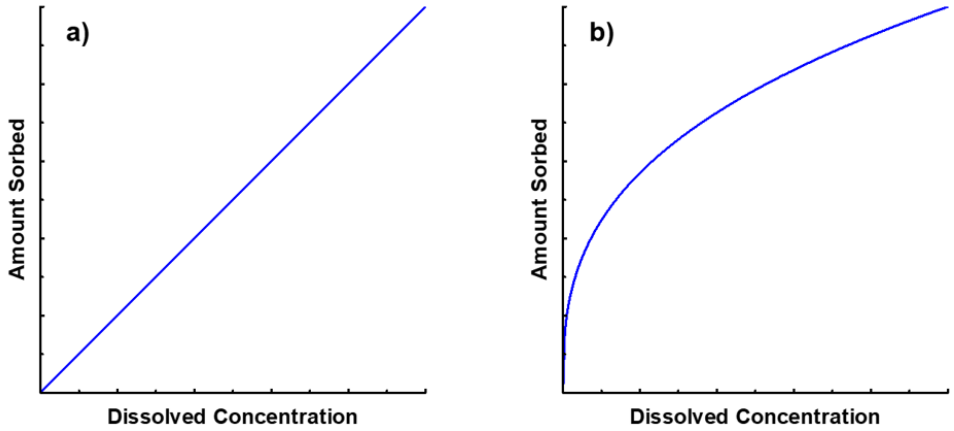

Figure 15 - Comparison of the amount sorbed as a function of increasing equilibrium dissolved sorbate concentration predicted by the a) linear distribution and b) Freundlich sorption isotherms.

The Langmuir sorption isotherm is the third type of sorption isotherm (Figure 16). Derived from mass action and mass balance considerations, the Langmuir isotherm is extensively used in environmental and microbial geochemistry. From the total number of available sorption sites (B_{Total}), the number of unoccupied sites is expressed by Equation 31.

$$[B \cdot COOH] = B_{Total} - [B \cdot Me^{z-1}] \quad (31)$$

Substitution of Equation 31 into the mass action expression (Equation 27) and rearrangement results in Equation 32.

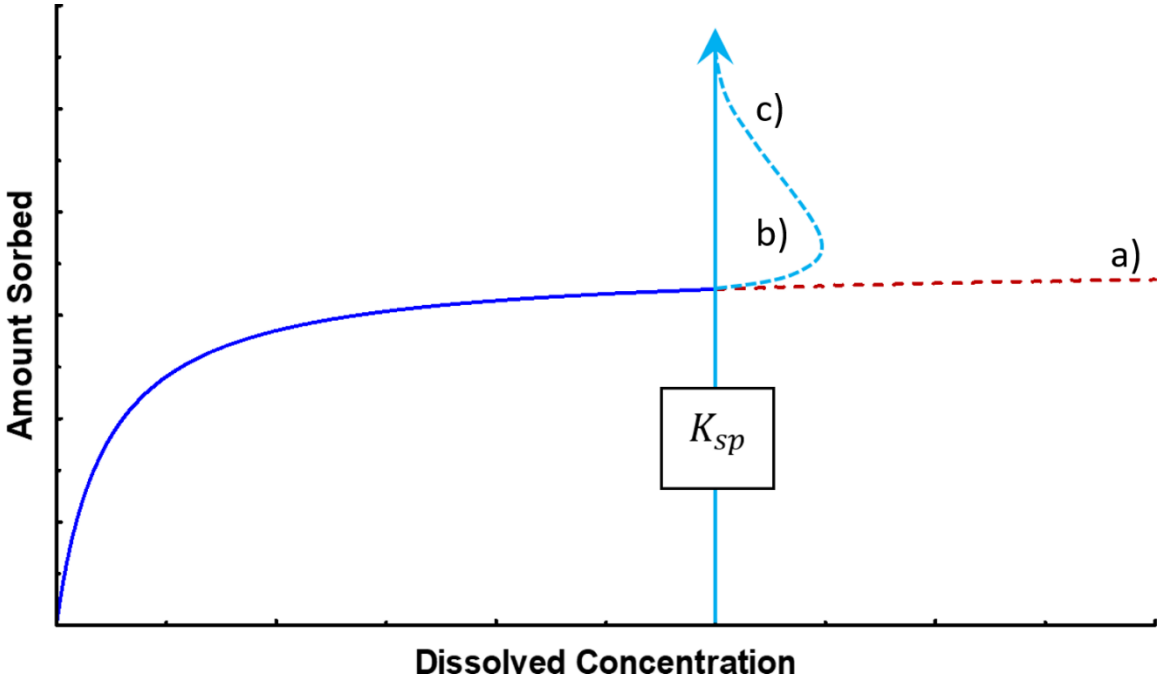

Figure 16 - Illustration of the Langmuir sorption isotherm. a) The amount sorbed asymptotically increases with equilibrium dissolved sorbate concentration until all of the sorption sites are filled and saturated. b) If the dissolved sorbate concentration exceeds the solubility limit of a mineral phase (K_{sp}), surface precipitation may occur and result in an apparent increase in the amount sorbed. c) If the initial surface precipitates are small and more soluble than larger mature crystals, dissolved concentrations may increase beyond the K_{sp} limit before decreasing as surface mineral precipitates grow in size.

$$[B \cdot Me^{z-1}] = \frac{B_{Total} K_{pH} [Me^{z+}]}{(1 + K_{pH}[Me^{z+}])} = \frac{B_{Total} K_{app} [Me^{z+}][H^+]^{-1}}{(1 + K_{app} B_{Total} K_{app} [Me^{z+}][H^+]^{-1})} \quad (32)$$

A key feature of the Langmuir sorption isotherm is that, at high concentrations of sorbate ions (and high pH in the case of cation sorption), the amount sorbed from solution asymptotically approaches (saturates) the total number of available sorption sites. Once all of the sorption sites are filled, no further sorption will occur. If dissolved ion concentrations continue to increase, the solution may eventually become oversaturated with respect to a mineral phase of some kind. This can trigger heterogeneous nucleation and surface precipitation of minerals on sorbents such as bacterial cells. The transition between sorption and surface precipitation is representative of a reactive continuum of solid phase partitioning reactions for dissolved ions in both pristine and contaminated systems (Warren and Ferris, 1998).

4.5 Mineral Dissolution and Precipitation

Groundwater is typically in simultaneous contact with mixed assemblages of different solid minerals. At shallow depths, infiltration of dilute undersaturated meteoric water typically promotes mineral dissolution. The dissolution of minerals not only contributes to the acquisition of solutes by groundwater but also fosters the development of secondary porosity and increased hydraulic conductivity. This is especially pronounced in karst systems that occur in areas with carbonate bedrock. With increasing depth and

residence time underground, groundwater gradually approaches equilibrium with respect to the minerals that are present. At the same time, chemical and microbiological reactions may cause groundwater to become over- or undersaturated and bring about mineral precipitation or dissolution, respectively. In contrast to dissolution reactions, mineral precipitation processes in groundwater systems promote the formation of coatings on mineral grain and fracture aperture surfaces. This can lead to cementation and closure of pore throats with a coincident decrease in porosity and hydraulic conductivity.

Mineral dissolution processes are classified as either congruent or incongruent reactions. Congruent dissolution refers to minerals that dissolve completely into their constituent ions, whereas incongruent dissolution applies to minerals that partially dissolve and leave behind a residual solid weathering product. Both types of mineral dissolution processes tend to consume protons as reactants, which forces the release of sorbed cations into solution to conserve electroneutrality.

The most common source of protons in mineral dissolution reactions is carbonic acid, which is generated from the degradation of organic matter by heterotrophic microbial activity. Other inorganic and organic acids are produced by microorganisms as well. These include sulfuric acid from the oxidation of sulfide minerals, as well as a wide variety of carboxylic acids such as acetic acid and oxalic acid. Among these, organic acids are known to contribute to ligand-promoted mineral dissolution reactions by complexing released cations. In these reactions, the base (L^-) of an acid (HL) is the metal-complexing ligand for the metal cation (Me^{z+}) as shown in Equation 33.

$$Me^{z+} + HL = H^+ + MeL^{z-1} \quad (33)$$

In ligand-promoted dissolution, the formation of the metal-ligand complex essentially removes the free metal cation (product of the dissolution reaction) from solution. This causes a shift away from equilibrium and sustains further dissolution in accordance with Le Chatelier's principle. The same shift in the equilibrium solubility of a mineral occurs when a change in oxidation state of a dissolution product occurs. For example, the oxidation of Fe^{2+} to Fe^{3+} from the dissolution of Fe(II)-bearing silicate minerals by lithotrophic Fe(II)-oxidizing bacteria promotes the dissolution reaction (Shelobolina et al., 2012; Shirokova et al., 2016).

Of the various microorganisms active under anaerobic conditions, dissimilatory iron and manganese reducers are notable as active agents in the reductive dissolution of oxide minerals. The hydrous iron and manganese oxides utilized by these microorganisms as source electron acceptors for anaerobic respiration typically occur as thin coatings on other mineral grains, as well as particulate organic materials. Dissolution of these coatings by microbial reduction under low oxygen conditions frequently results in gleyic (gray-blue-green) color characteristics, such as those evident in hand samples of borehole cuttings and cores from Mn(IV)- and Fe(III)-reduction zones in groundwater systems (Figure 17).

Figure 17 - An excavation exposing gray-blue-green gleyic discoloration produced at the base of a forested slope (bottom profile) in response to downwards infiltration of water and microbial reduction of red-brown iron oxide minerals (upper two profiles) under anaerobic conditions. The distance along the excavation is approximately 2.0 m and the depth of the lower gleyed profile is about 0.6 m. (Reproduced under the terms of Creative Commons Attribution 3.0 license from Schwarz et al., 2018).

Microorganisms contribute to the precipitation of a wide variety of minerals including oxides, phosphates, carbonates, sulfides, and silicates as shown in Figure 18 (Fortin et al., 1997; Ferris et al., 2000). The mechanisms of microbial mineral precipitation are diverse, but generally involve two distinct phases: nucleation and crystal growth. Nucleation is the most critical stage for mineral precipitation and occurs either homogeneously or heterogeneously. In homogeneous reactions, mineral nuclei are formed by the random collision of ions in solution. Conversely, heterogeneous nucleation involves

the formation of crystal nuclei on the surfaces of homologous (similar crystallographic mineral surfaces) or foreign solids such as microbial cells. Of these two nucleation processes, heterogenous nucleation dominates in groundwater systems. Once a stable nucleus has formed, crystal growth can spontaneously proceed provided that the concentrations of ions in solution continue to exceed the solubility product of the solid mineral phase (the solution must be oversaturated).

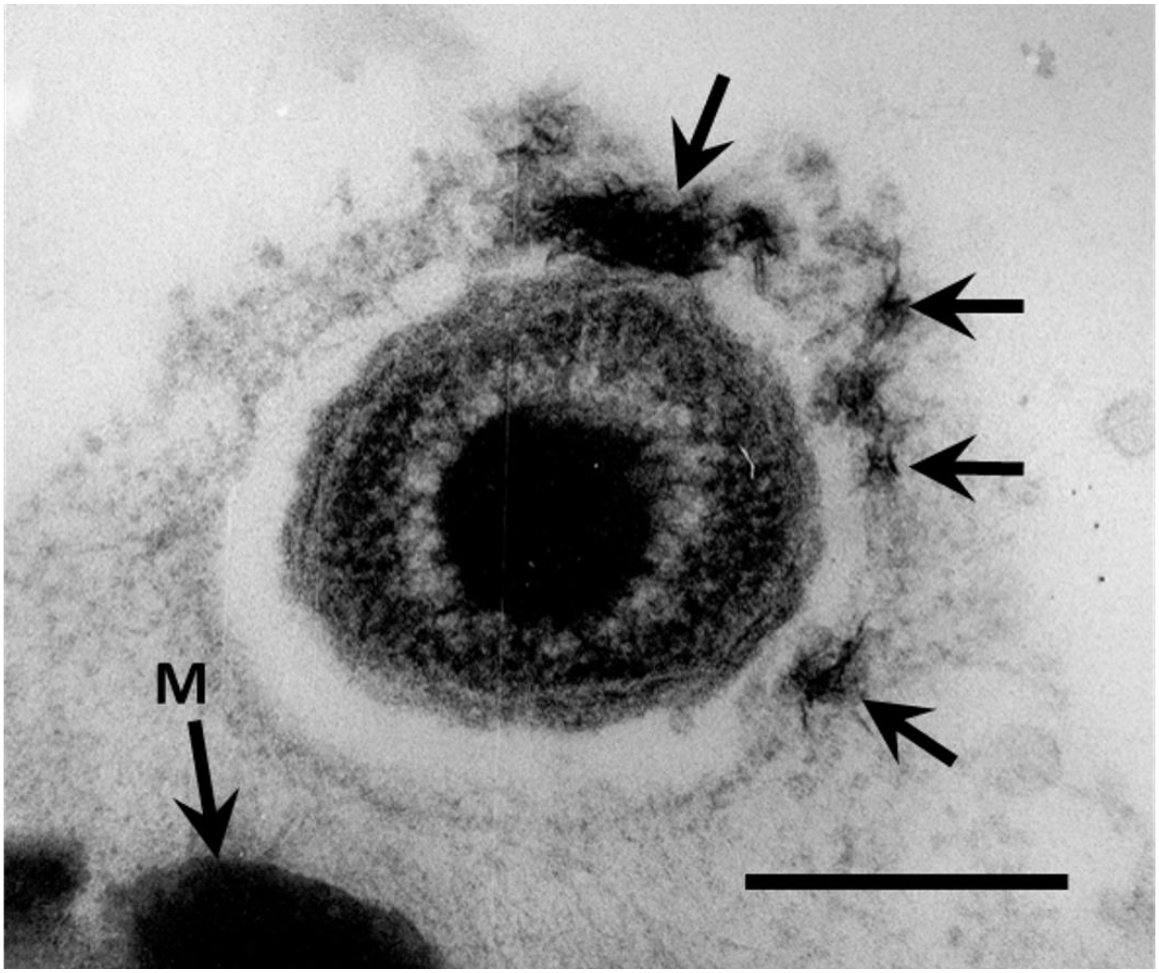

Figure 18 - A thin-section transmission electron micrograph showing heterogenous nucleation and precipitation of a solid mineral phase (indicated by arrows) on a bacterial cell attached to a mineral grain (M). Scale bar = 360 nm.

The Gibbs energy for crystal nucleation is constrained by the bulk free energy of the solution (ΔG_{bulk}) and the interfacial free energy of the corresponding solid phase ($\Delta G_{interface}$) as shown in Equation 34.

$$\Delta G_r = \Delta G_{bulk} + \Delta G_{interface} \tag{34}$$

The bulk free energy term is a function of the degree to which a solution is oversaturated (Equation 21) as expressed by Equation 35,

$$\Delta G_{bulk} = -RT \ln \frac{IAP}{K_{so}} = -2.303RT \log_{10} \frac{IAP}{K_{so}} = -2.303RT(SI) \tag{35}$$

whereas the interfacial free energy depends on interfacial surface tension of the mineral phase (γ) and molar surface area of the nucleus in contact with water (A_{cw}) as described by Equation 36.

$$\Delta G_{interface} = A_{cw}\gamma \quad (36)$$

The interfacial free energy term represents the work that must be done to create a new mineral surface. Together, these relationships provide a useful model to better understand microbial contributions to mineral precipitation.

Microbial activity will often trigger a change in solution chemistry that leads to oversaturation and a higher SI value. For example, bacterial Fe(II) oxidation often gives rise to dissolved Fe^{3+} concentrations that far exceed the solubility of iron oxides (Emerson et al., 2010; Edwards et al., 2018). This alone can induce mineral precipitation by lowering the bulk free energy term for both homogeneous and heterogeneous nucleation reactions. However, chemically reactive sites on microbial cells that facilitate ion sorption at nucleation sites will tend to reduce the mean interfacial surface energy of the solid phase and decrease the surface of the nucleus in contact with the bulk solution. The expected result is a reduction in the overall interfacial free energy, which is conducive to heterogeneous nucleation and precipitation.

5 Transport of Microbes in Groundwater

Microorganisms in groundwater systems can be classified based on their origin and degree of isolation from surface environments. Autochthonous microbes are those that are permanent long-term subsurface residents, whereas allochthonous species come from other environments such as surface waters or the soil zone. The physical isolation and adaptation of autochthonous microorganisms to life underground is a stark example of allopatric (non-overlapping) speciation and evolution over long periods of time, perhaps billions of years in the case of some deep groundwater environments (> 1000 m depth; Magnabosco et al., 2018). Allochthonous microorganisms are transported into the subsurface most often with recharge through downward surface water percolation. Over time, allochthonous microorganisms may become part of the autochthonous microbial community as they adapt to living conditions underground.

In groundwater systems, free-floating planktonic microorganisms undergo advective transport as suspended particulate species that move along with the pore water. Their transport velocity is governed by the hydraulic pressure gradient, porosity, and permeability distribution in accordance with Darcy's law. Groundwater transport of microorganisms is also subject to the effects of diffusion and hydrodynamic dispersion. The movement of dissolved nutrients and electron acceptors are coupled to the same processes, which are described by the advection-dispersion equation (Equation 37) for the rate of mass transport (Brun and Engesgaard, 2002; Steefel et al., 2005; Tufenkji, 2007).

$$\frac{\partial C}{\partial t} = \left[D_x \frac{\partial^2 C}{\partial x^2} + D_y \frac{\partial^2 C}{\partial y^2} + D_z \frac{\partial^2 C}{\partial z^2}\right] - \left[v_x \frac{\partial C}{\partial x} + v_y \frac{\partial C}{\partial y} + v_z \frac{\partial C}{\partial z}\right] \quad (37)$$

where:

x, y, z = principle directions of transport in three dimensions (L)

C = concentration of dissolved solute or suspended particulate (M/L^3)

D = hydrodynamic dispersion coefficients (L^2/T)

v = average linear velocity (L/T)

t = time (T)

Microorganisms are subject to a transport phenomenon known as size exclusion. When this happens, transported suspended particles appear to move faster and experience less dispersion than conservative (non-reacting) solutes. Size exclusion in the transport of microorganisms is evident in breakthrough curves from a smaller range of normalized concentrations (C/C_0) and shorter retention times of microbes compared to a dissolved tracer (Figure 19). Field experiments indicate microbial cells may be transported at velocities as much as 70 percent greater than the average linear velocity of pore water.

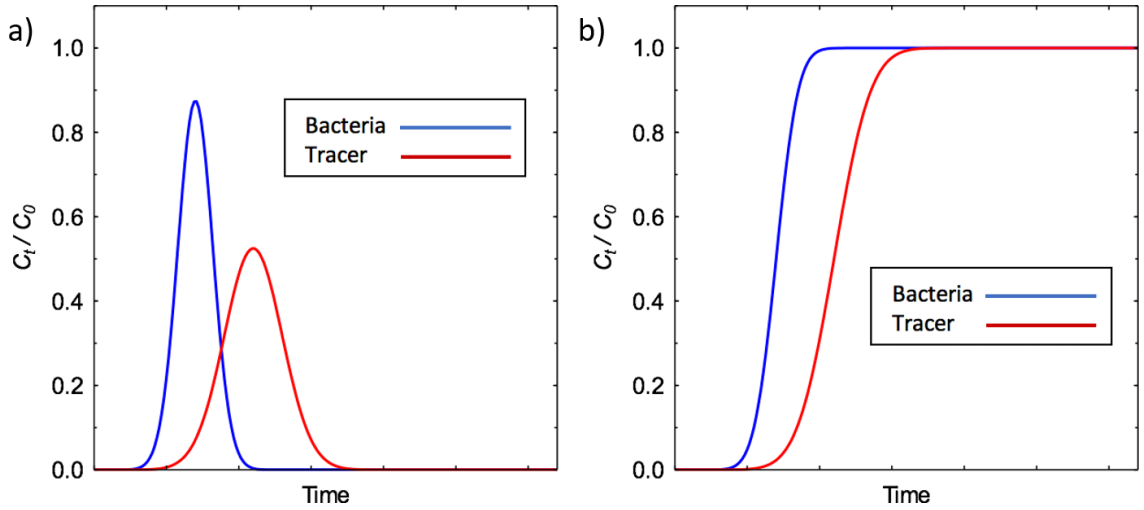

Figure 19 - Breakthrough curves of normalized concentrations over time: a) after a slug injection and: b) during continuous injection (right) of a bacterial suspension with a dissolved tracer.

The reason for faster transport of microorganisms as a result of size exclusion relates to the hyperbolic distribution of water velocities inside pores. Maximum velocity occurs along the centerline, whereas friction and other forces reduce water velocity at the pore walls to zero. On the molecular scale of dissolved solutes, the full distribution of water velocities is sampled in transport processes. Conversely, by virtue of their larger size, microbes and other suspended particulates experience higher velocities near the centerline of pores, leading to an average velocity that is faster than that of a dissolved tracer.

The removal of suspended bacteria from groundwater is mediated by straining and filtration processes. Straining involves the trapping of microorganisms in pore throats or

facture apertures that are too small to allow passage. This process is a not only a function of porosity but also depends on pore geometry and the tortuosity of groundwater flow paths. Physical filtration refers to the removal of suspended bacteria from groundwater by collision and deposition on pore wall surfaces. The probability of resuspension after filtration depends on the interplay between hydrodynamic shear and adhesive interfacial forces (sorption affinity). After deposition, microbial cells may secrete large amounts of EPS to increase adherence and initiate biofilm formation.

As suspended bacterial cells are transported in groundwater, they can sorb dissolved substances from solution and carry them along, in effect masquerading as particulate solids. This piggyback process is known as facilitated transport. If the sorbed chemical species happens to come from a point source such as a contaminant spill, size exclusion processes in bacterial transport may decrease the time and increase the distance over which a contaminant moves. In the same way, facilitated transport has considerable potential to aid in the transfer of nutrients from a nutrient-rich area to a nutrient-poor area, thereby stimulating microbial activity over longer groundwater flow paths.

A significant fraction of waterborne disease worldwide is caused by the introduction and transport of allochthonous pathogenic microorganisms (protozoa, bacteria, viruses) in groundwater systems. Many, if not most, of the microbial pathogens in groundwater are contaminants derived from human and animal fecal waste. Primary sources of these disease-causing microbes include failed septic tanks, seepage from waste lagoons, leaky sewer lines, and old or improperly sealed landfills (Macler and Merkle, 2000). Shallow unconfined aquifers are particularly at risk because of their closer proximity to surface sources of microbial contamination (Jin and Flury, 2002; Pandey et al., 2014).

The large size of pathogenic protozoa such as *Giardia* and *Cryptosporidium* is a feature that contributes to straining, which limits transport to short distances through groundwater systems (Figure 20). For this reason, the presence of protozoa in deeper aquifers implies a direct introduction of surface water from downward flow through fractured rock or karst with limited unconsolidated overlying soil layers (Jin and Flury, 2002). On the other hand, smaller microbial pathogens such as bacteria and viruses are more likely to experience size exclusion and be transported over greater distances in groundwater than protozoa (Figure 20; Taylor et al., 2004; Tufenkji, 2007).

Straining, filtration, and size exclusion processes emphasize the importance of considering the nature and relative pore space of geological media when evaluating the fate and transport of pathogens and the vulnerability of groundwater. Additional factors such as solution chemistry, virus and cell surface characteristics, soil properties, and temperature influence the survival, transport, and sorption of microbial pathogens in porous media (Jin and Flury, 2002; United States Environmental Protection Agency [USEPA], 2002; Pang et al., 2004). These considerations are particularly relevant when assessing the setback distance of septic tanks from source water wells and shorelines. More

information regarding management of septic systems to prevent contamination of groundwater is available on the USEPA website and from Pang et al. (2004).

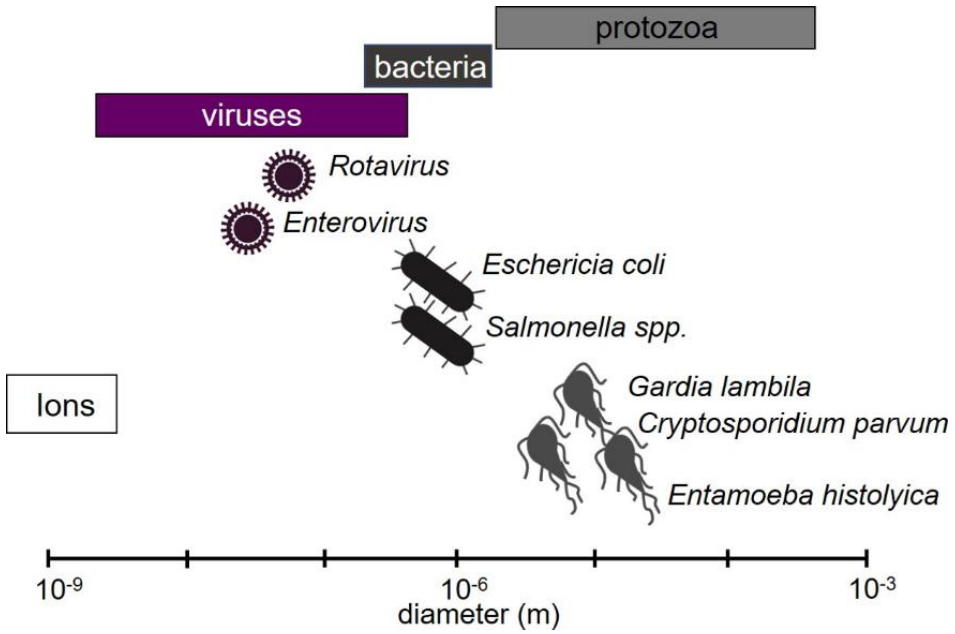

Figure 20 - Examples of microbial pathogens found in surface and groundwater that are of concern for human and ecological health. Because of their large size and susceptibility to straining, protozoa are general indicators of surface water contamination. Alternatively, smaller bacteria and viruses can be transported to groundwater (McKay et al., 1993; Taylor et al., 2004). The presence of microbial pathogens in groundwater is often inferred by the detection of fecal indicator bacteria including total coliform bacteria, *Escherichia coli*, *Enterococci*, and coliphage (viruses infecting coliform bacteria). These indicator microorganisms, similar to other pathogens, generally do not grow outside their natural environments in groundwater. Their ability to survive in groundwater environments is limited by conditions such as temperature, competition with other bacteria, predation by other organisms, and entrapment in pore spaces (Macler and Merkle, 2000; Jin and Flury, 2002). Therefore, finding fecal indicator bacteria in groundwater in measurable numbers means there is an increased likelihood of pathogens being present as well.

6 Applied Groundwater Microbiology

Groundwater microbiology is applied in a wide variety of processes that have been developed not only for *in situ* bioremediation of organic and inorganic contaminants but also for the control and management of groundwater flow. The success of these applications stems from the tremendous metabolic flexibility that exists among different microorganisms and their influence on groundwater geochemistry, including heterogenous solid phase adsorption and mineral precipitation reactions. Another important factor that is often taken for granted, but is worth reemphasizing, is the small size of microbial cells. This physical attribute is what allows microorganisms to gain access to, and reside within, subsurface habitats that are either suffering from contamination or are targets for management of groundwater flow.

A primary consideration for *in situ* bioremediation is oxidation state, which determines whether contaminants can be metabolized by microorganisms as an electron donor (reductant), an electron acceptor (oxidant), or not at all (as is the case with elements

that lack multiple oxidation states such as strontium or cadmium). If a contaminant is subject to microbial metabolism, it can be removed from groundwater by transformation (oxidative or reductive degradation) into inert products. Options for contaminants that cannot be metabolized include capture and immobilization by adsorption or coprecipitation in minerals precipitated in response to microbial metabolic activity.

6.1 Microbial Bioremediation and Removal of Groundwater Contaminants

Microbial bioremediation has become a widely used technology that is viewed as a more sustainable and economical approach compared to other treatment methods (e.g., pump and treat systems). It has been successfully implemented in many cleanup operations involving groundwater contaminated with organic pollutants such as petroleum hydrocarbons and chlorinated organic solvents as well as management and control of leachate pollution plumes from landfills (USEPA, 1998, 2002, 2013). Petroleum hydrocarbons contaminants span a wide range of reduced organic chemical substances, including BTEX compounds (benzene, toluene, ethyl benzene, and xylene) that are typically used as electron donors in microbial metabolism. The opposite is true of chlorinated solvents, such as carbon tetrachloride (PCE) and trichloroethene (TCE), which are highly oxidized and metabolized as electron acceptors.

Three different strategies are used in groundwater bioremediation. These include:

- *natural attenuation* – where the autochthonous (natural) microbial community is left to eliminate the target contaminant without human intervention, relying on physical, chemical, and microbiological processes that occur naturally within a contaminant plume;
- *biostimulation* – where the natural microbial community is stimulated to eliminate the target contaminant by the addition of essential nutrients; and
- *bioaugmentation* – where, in addition to nutrients, select strains of bacteria may be injected into the subsurface to promote the elimination of the target contaminant.

The success of these strategies depends on the availability of appropriate electron acceptors and electron donors, whether the target contaminant is reduced (e.g., petroleum hydrocarbons) or oxidized (e.g., chlorinated solvents), and groundwater flow rates are slow enough to allow for degradation to occur (Christensen et al., 2000).

Aerobic biodegradation of reduced organic contaminants takes place in the presence of oxygen (Haritash and Kaushik, 2009; Bamforth and Singleton, 2005). This means the success of aerobic bioremediation is directly dependent on the availability of oxygen. If oxygen becomes limiting, as it often does in groundwater systems, it can be supplied directly to the subsurface by air sparging or through injection of a chemical oxidant (e.g., hydrogen peroxide) that decomposes to release oxygen.

In the absence of oxygen, *anaerobic biodegradation* may proceed using electron acceptors such as nitrate or sulfate for metabolic oxidation of a contaminant. This approach is widely employed at petroleum hydrocarbon contaminated sites where oxygen has been depleted (Chandra et al., 2013; Meckenstock et al., 2016; Varjani and Upasani, 2017). To stimulate anaerobic biodegradation, an amendment that contains an electron acceptor such as sulfate may be added to promote microbial degradation of petroleum hydrocarbons (USEPA, 2013).

Anaerobic conditions are also needed for the initial dechlorination of highly chlorinated organic solvents such as PCE and TCE, which serve as electron acceptors instead of electron donors (Hopkins et al., 1993; Meckenstock et al., 2015). As these compounds are metabolized, chloride atoms are removed and replaced by hydrogen atoms to form products (conjugate reductants) that are less oxidized than the original chlorinated compound. In some cases, the products are not sufficiently oxidized to serve as electron acceptors and can only be degraded further as electron donors in aerobic respiration. For example, PCE and TCE are highly oxidized and only undergo partial reductive dechlorination to less oxidized dichloroethane (DCE), vinyl chloride (VC), and chloroethane (CE) (Mohn and Tiedje, 1992; Kielhorn et al., 2000). Subsequent oxidation of DCE, VC, and CE to non-toxic forms ethylene, ethane, or ethanol requires aerobic conditions (e.g., Semprini et al., 1990; Semprini and McCarty, 1991; Hopkins et al., 1993).

Microorganisms can be used to remove inorganic contaminants such as nitrogenous nutrient compounds (e.g., nitrate or ammonia) that exist in groundwater in amounts above regulatory guidelines, as well as redox active toxic metals and metalloids. However, unlike organic contaminants that microbes can degrade by oxidation to carbon dioxide or nutrients that can be taken up and assimilated during metabolism, detoxification of metal and metalloid contaminants is accomplished through dissimilatory metabolic processes that remove or provide electrons for cellular energetics (Lovely and Coates, 1997; USEPA, 2013). Common applications include microbial reduction of Cr(VI), U(VI), and Se(VI) to more insoluble oxidation states: Cr(III), U(IV), and elemental Se, respectively. Other metals, such as Fe(II) or Mn(II), are subject to microbial oxidation and precipitation of insoluble oxides (Figure 21).

Figure 21 - Extensive precipitation of orange-colored insoluble hydrous ferric oxides by Fe(II)-oxidizing bacteria in a groundwater discharge zone near Deep River, Ontario, Canada.

6.2 Applications of Microbially Induced Mineral Precipitation

The precipitation of minerals by groundwater microorganisms can be applied in several different ways, depending foremost on the chemical reactivity and physical properties of the mineral precipitates. Hydrous ferric oxides, sulfide, and carbonate minerals are frequently targeted for use because of their capacity to immobilize inorganic contaminants through surface adsorption and co-precipitation reactions. Carbonate minerals are also known to be effective cementing agents that will adhere to and bind unconsolidated mineral grains together.

As products of lithotrophic bacterial Fe(II)-oxidation, hydrous ferric oxides are recognized as potent adsorbent solids that have a high chemical affinity for dissolved ions (Langmuir, 1997). This reactivity contributes to the removal of metal cations such as Sr^{2+}, Cd^{2+}, Pb^{2+}, and UO_2^{2+} from solution. Anionic solutes such as arsenate (AsO_4^{3-}), chromate (CrO_4^{2-}), phosphate (PO_4^{3-}), and iodide (I^-) are also adsorbed by hydrous ferric oxides (Katsoyiannis and Zouboulis, 2006). The ubiquitous environmental distribution of Fe(II)-oxidizing bacteria makes bacteriogenic hydrous ferric oxide precipitation a strong candidate for natural attention of inorganic contaminants in groundwater systems.

Sulfide is produced as a conjugate reductant in anaerobic microbial respiration with sulfate as the terminal electron acceptor. This can trigger oversaturation and precipitation of sulfide mineral phases incorporating contaminants such as Cd^{2+}, Pb^{2+}, Hg^{2+}, and As^{3+}. The availability of organic matter as an electron donor for bacterial sulfate reduction is essential for sulfide production. If organic matter (or sulfate) concentrations are too low, biostimulation involving injection of one or both limiting nutrients can be used to induce sulfide mineral precipitation.

A major distinction between applications of microbial precipitation of hydrous ferric oxides and metal sulfides relates to differences in groundwater redox conditions. Bacterial oxidation of Fe(II) and precipitation of hydrous ferric oxides requires relatively oxidizing conditions using oxygen or nitrate as electron acceptors, whereas reducing conditions are needed for metal sulfide precipitation by microorganisms that use sulfate as an electron acceptor. In the intermediate redox zone, metabolic production of Fe(II) in response to microbial Fe(III)-reduction contributes to the transformation of hydrous ferric oxides into Fe(II)/Fe(III) "green rust" minerals (Figure 22). These mixed oxidation state mineral phases not only retain the adsorptive properties of hydrous ferric oxides (Parmar et al., 2001; Perez et al., 2021) but also behave as solid phase reductants for contaminants such as nitrate, chromate, selenate (SeO_4^{2-}), and carbon tetrachloride (Erbs et al., 1999; Genin et al., 2001).

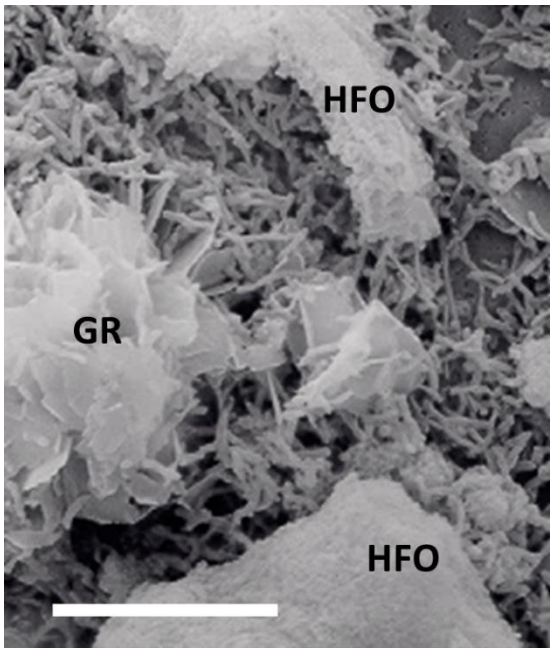

Figure 22 - Scanning electron micrograph showing platy crystals of green rust (GR) that have formed from the reduction of hydrous ferric oxide (HFO) in a culture of the Fe(III)-reducing bacterium *Shewanella algae* strain BrY. The bacteria are visible as an unorganized mass of elongated rods between the mineral precipitates. Scale bar = 10 μm.

Applications of microbially induced calcium carbonate precipitation commonly rely on the activity of ureolytic bacteria (Ferris and Stehmeier, 1992; Ferris et al., 1996; Fujita et al., 2000). Both aerobic and anaerobic bacterial species catalyze the hydrolysis of urea using the urease enzyme to produce ammonium ions and dissolved inorganic carbon (DIC), which give rise to an increase in pH (Ferris et al., 2003). In the presence of dissolved calcium, which is often injected together with urea, the higher DIC concentrations and pH lead to oversaturation and precipitation of calcium carbonate minerals (Figure 23). Denitrification, ammonification, sulfate reduction, and methane oxidation have also been implicated in microbially induced carbonate mineral precipitation (Anbu et al., 2016; Zhu and Dittrich, 2016; Eltarahony et al., 2020).

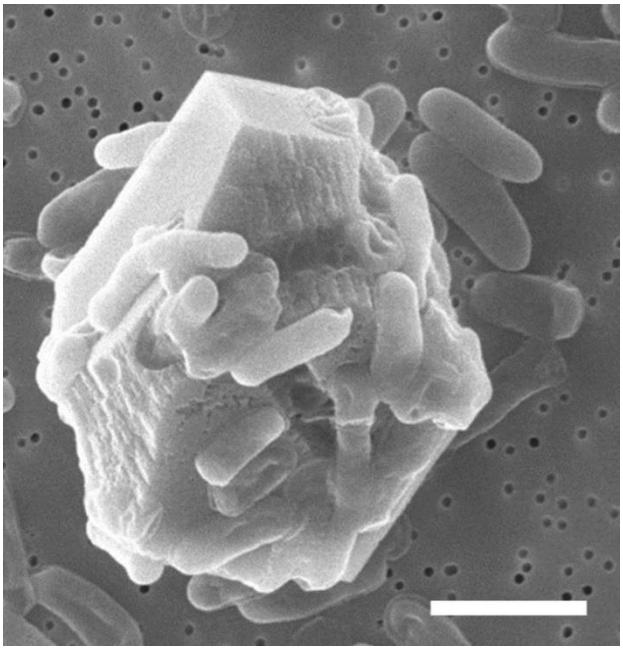

Figure 23 - Scanning electron micrograph of a growing calcite crystal precipitated in artificial groundwater by the ureolytic bacterium *Sporoscarcina ureae*. The bacteria appear as rod-shaped cells that are surrounding and adhering to the surface of the calcite crystal. Scale bar = 3.0 μm.

The precipitation of carbonate minerals by microorganisms is advantageous for the capture and immobilization of contaminants with an ionic radius similar to Ca^{2+}. This reactivity extends from coprecipitation and isomorphic replacement of Ca^{2+} during crystal growth (Langmuir, 1997; Mitchell and Ferris, 2005). Groundwater contaminants identified as candidates for mitigation by microbially induced mineral precipitation include Cd^{2+}, Pb^{2+}, Zn^{2+}, and Hg^{2+} as well as radionuclides ^{90}Sr and ^{60}Co (Mitchell and Ferris 2005; Eltarahony et al., 2020).

Another useful aspect of microbial carbonate mineral precipitation is the effectiveness of the process in cementing unconsolidated sediments (biocementation/bioconsolidation) and infilling of empty pore spaces (biomineral

plugging/grouting). Practical applications in geomechanics include improvement of shear strength and stiffness of loose (uncompacted) deposits of alluvium, whereas permeability reduction is the primary goal in hydrogeological engineering to reduce groundwater invasion into tunnels, oil field production operations, and other underground works (Jack et al., 1991, 1993; Ferris and Stehmeier, 1992; Ferris et al., 1996; Anbu et al., 2016; Minto et al., 2016).

In comparison to traditional chemical-based grouts, the precipitation of carbonate minerals by microorganisms through biostimulation or bioaugmentation not only requires lower injection pressures but also penetrates deeper into smaller pores and fracture apertures (Minto et al., 2016). This is because the nutrient and mineralizing solutions needed to induce microbial carbonate mineral precipitation have a viscosity close to that of water (near $1.0\ mPa \cdot s$), whereas the viscosity of chemical cements tends to be much higher (approximately $50\ mPa \cdot s$).

7 Closing

Understanding basic microbiology and chemistry supports the work of groundwater professionals because the interaction of microorganisms and chemical compounds in groundwater flow systems can be used to modify subsurface conditions. Such modification can mitigate contamination in groundwater systems, enhance or decrease the transmissibility of groundwater, and strengthen or weaken the subsurface structure. Relying on microbial activity for these modifications is generally a cost-effective engineering approach to managing the subsurface. The simplest, least expensive option, is to allow natural processes to accomplish the work, but when those processes progress slower than is acceptable for the problem at hand, the natural pace and type of microbial activity can be increased by adding nutrients or additional microbial species along with the nutrients. Research with the aim of improving microbiologic solutions to groundwater problems continues through efforts to genetically engineer microorganisms with improved catabolic activity (Janssen and Stucki, 2020).

8 Exercises

Exercise 1

What is the most important difference between prokaryotic and eukaryotic microorganisms?

Click for solution to exercise 1

Exercise 2

Describe the differences between prokaryotic cell walls that are distinguished by their response to the Gram stain.

Click for solution to exercise 2

Exercise 3

What is produced by prokaryotic cells that allows them to not only adhere to mineral surfaces, protect against dehydration, and form biofilms but also contribute to the adsorption of dissolved ionic chemical substances from solution?

Click for solution to exercise 3

Exercise 4

What is the doubling time for a microorganism with an exponential growth rate constant of 0.005 s^{-1}?

Click for solution to exercise 4

Exercise 5

Why is the magnitude of length scale such an important physical property for describing microbial habitats in groundwater systems?

Click for solution to exercise 5

Exercise 6

Explain why shallow groundwater systems occurring in similar climates have the same average temperature.

Click for solution to exercise 6

Exercise 7

Assuming a geothermal thermal gradient of 25 to 30°C per km, how would the temperature preference of microorganisms living at a depth of 2000 m be described?

Click for solution to exercise 7

Exercise 8

You take a core sample from 20 m depth and break off a 3.5 g subsample of soil to analyze for microbial community composition. Based on the average cell density in subsurface materials at this depth, what is the approximate population of microorganisms contained in your subsample?

Click for solution to exercise 8

Exercise 9

Place the following terminal electron acceptors for microbial respiration in order of decreasing potential metabolic energy yield. What are the corresponding conjugate reductants of the electron acceptors?

a) Carbon dioxide
b) Sulfate
c) Fe(III)/Mn(IV)
d) Oxygen
e) Nitrate

Click for solution to exercise 9

Exercise 10

What is the metabolic classification of subsurface prokaryotic microorganisms that use chemical compounds as a source of energy with *inorganic* substances serving as electron donors and carbon dioxide being fixed to make cellular biomass?

Click for solution to exercise 10

Exercise 11

What is the metabolic classification of subsurface prokaryotic microorganisms that use chemical compounds as a source of energy with *organic* substances serving as both electron donors and a carbon source to make cellular biomass?

Click for solution to exercise 11

Exercise 12

Why are shales and clay not especially good habitats for prokaryotic microorganisms?

Click for solution to exercise 12

Exercise 13

As part of your hunt for evidence of micro-eukaryotes and prokaryotes at a field site, you take a core sample of carbonate rock. Would you expect to find both types of your target microorganisms, just one type, or neither? Explain.

Click for solution to exercise 13

Exercise 14

Describe the ways in which prokaryotic microorganisms contribute to chemical reactions in groundwater systems.

Click for solution to exercise 14

Exercise 15

Briefly explain the difference in pH sorption behavior between positively and negatively charged ions on sorbent solids in groundwater systems, including prokaryotic microbial cells surrounded by extracellular polymeric substances.

Click for solution to exercise 15

Exercise 16

What role do microbes play in the cleanup of groundwater pollutants?

Click for solution to exercise 16

Exercise 17

You are working as a hydrogeologist on a site where groundwater is contaminated with BTEX. You have been asked to provide a rationale for the use of bioremediation to remove the BTEX from the groundwater and provide and describe three possible bioremediation strategies to eliminate the target contaminants. Explain what groundwater conditions would enhance microbial remediation of the target contaminants and why? Assume that microorganisms that can degrade hydrocarbons are ubiquitous at your site.

Click for solution to exercise 17

Exercise 18

In what way do groundwater microorganisms contribute to mineral dissolution reactions?

Click for solution to exercise 18

Exercise 19

Explain why dissimilatory Fe(III)- and Mn(IV)-reducing bacteria are important for the reductive dissolution of oxide minerals in groundwater.

Click for solution to exercise 19

Exercise 20

What are some applications of microbial carbonate mineral precipitation?

Click for solution to exercise 20

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10 Exercise Solutions

Solution Exercise 1

Prokaryotic microorganisms lack membranous nuclei, whereas eukaryotes have membrane-bound nuclei.

Return to Exercise 1

Solution Exercise 2

The cell walls of prokaryotes staining Gram-positive consist of peptidoglycan, which is a meshwork of mucopolysaccharides cross-linked in three dimensions by peptide bridges, and a variety of secondary polymers (teichoic or teichuronic acids and proteins). On the other hand, the cell walls of prokaryotes that stain Gram-negative contain lipopolysaccharides, phospholipids, and proteins arranged in a membrane bilayer (the outer membrane). Sandwiched between the outer membrane and cytoplasmic membrane is a thin layer of peptidoglycan.

Bonus - Some bacteria with cell walls are neither Gram-positive nor Gram-negative according to the Gram-stain. The cell walls of *Archaea* lack the kind of peptidoglycan that is found in prokaryotes and instead contain either pseudopeptidoglycan, glycoproteins, or proteins alone.

Return to Exercise 2

Solution Exercise 3

Extracellular polymeric substances (EPS).

Return to Exercise 3

Solution Exercise 4

Doubling time for exponential microbial growth is given by the equation $T = ln(2)/\mu = 0.693/\mu$ in which the frequency of cell division is specified by the exponential growth rate constant μ. This gives a doubling time of 138.6 s, which is equivalent to 2.3 min.

Return to Exercise 4

Solution Exercise 5

In groundwater systems, length scales extend over large distances of 10^3 m (km) all the way down to 10^{-6} m (μm) levels, depending on how habitat size is defined. Length scale is critical because it defines other important physical properties for habitability such as surface areas and relative volumes of solids, water, air, and other fluids. These factors not only determine where it is possible for microbial life to take refuge but also influence water movement, groundwater chemistry, and reactive mass transport processes.

Return to Exercise 5

Solution Exercise 6

Being below the surface, groundwater is insulated from the daily cycles of solar irradiance and seasonal climate changes above ground. The insulating properties of subsurface material and high specific heat capacity of water result in shallow groundwater retaining a fairly constant temperature, roughly equal to the mean annual air temperature of the location. Deeper groundwater is warmed by the geothermal gradient of the region.

Return to Exercise 6

Solution Exercise 7

The expected temperature in a deep groundwater system at 2000 m depth would be around 50 to 60°C higher than the temperature at the surface which averages about 15°C, which corresponds to the temperature range of thermophilic microorganisms.

Return to Exercise 7

Solution Exercise 8

~35 million cells. In near-surface groundwater systems, prokaryotic microbial cell numbers average 10^7 cells/g. Therefore, a 3.5 g sample would contain 10^7 cells/g × 3.5 g = 3.5 × 10^7 (35 million) cells.

Return to Exercise 8

Solution Exercise 9

1. Oxygen – water
2. Nitrate – nitrogen
3. Fe(III)/Mn(IV) – Fe(II)/Mn(II)
4. Sulfate – sulfide
5. Carbon dioxide – methane

Return to Exercise 9

Solution Exercise 10

Autotrophs rely on chemical compounds of inorganic (i.e., lithic) substances as their source of energy for reduction of carbon dioxide, so they are classed as Chemolithoautotrophs.

Return to Exercise 10

Solution Exercise 11

Heterotrophs (often synonymous with organotrophs) rely on organic molecules that already exist in the system to promote their growth, so they are classed as Chemoorganoheterotrophs.

Return to Exercise 11

Solution Exercise 12

The pore size diameters in shales and clays (typically < 10^{-7} m) are too small to provide enough room to accommodate prokaryotic microorganisms with cell diameters greater than 10^{-7} m.

Return to Exercise 12

Solution Exercise 13

Only one (prokaryotes). Life in the subsurface is limited by pore diameter. The smaller size of prokaryotes allows them to live within the pore diameter of carbonate rocks.

Return to Exercise 13

Solution Exercise 14

Prokaryotic microorganisms contribute to chemical reactions in groundwater systems in two ways. First, metabolic enzyme activity can speed up (catalyze) slow reactions and force corresponding reaction quotients to shift rapidly towards or away from equilibrium. This affects many aspects of groundwater chemistry including pH, redox conditions, mineral dissolution and precipitation processes, and the chemical speciation of solutes. Second, prokaryotic cells behave as microscopic reactive solids owing to the chemical reactivity of functional groups, such as carboxyl or phosphoryl substituents, in the macromolecular components of cell walls, external sheaths, and EPS. As reactive solids, bacteria not only contribute to the sorption of dissolved ions but also serve as heterogeneous nucleation templates for mineral precipitation.

Return to Exercise 14

Solution Exercise 15

As pH increases, sorbent solids tend to develop a more negative surface charge that favors increased (positively charged) cation sorption and decreased (negatively charged) anion sorption.

Return to Exercise 15

Solution Exercise 16

Microbes are used to clean up groundwater pollutants in processes known as 'bioremediation'. Bioremediation uses microorganisms to reduce pollution through the biological degradation of pollutants (e.g., petroleum hydrocarbons and chlorinated compounds) into non-toxic substances. The pollutants can act as either electron donors (e.g., BTEX) or electron acceptors (e.g., TCE) in microbial metabolism.

Return to Exercise 16

Solution Exercise 17

Rationale: Microorganisms can use BTEX compounds as electron donors in their metabolism. As long as there are available nutrients and electron acceptors in the groundwater, microorganisms can metabolize and therefore degrade target contaminants.

Three possible strategies include:

1. natural attenuation - natural microbial community is left to eliminate the target contaminant without human intervention;
2. biostimulation - essential nutrients are added to stimulate the natural microbial community to eliminate the target contaminant; and
3. bioaugmentation - nutrients and select strains of bacteria are injected into the subsurface to promote the elimination of the target contaminant.

Among commonly available electron acceptors, oxygen yields more energy than any other oxidant in aerobic respiration. To enhance microbial degradation of BTEX compounds, you could increase the availability of oxygen in groundwater. Oxygen can be added to groundwater either directly to the subsurface by air sparging or through injection of a chemical oxidant (e.g., hydrogen peroxide) that decomposes to release oxygen.

Return to Exercise 17

Solution Exercise 18

Mineral dissolution processes tend to consume protons as reactants, which forces a release of sorbed cations into solution to conserve electroneutrality. The most common source of protons in mineral dissolution reactions is carbonic acid, which is generated from the degradation of organic matter by heterotrophic microbial activity. Other inorganic and organic acids are produced by microorganisms as well. These include sulfuric acid from the oxidation of sulfide minerals, as well as a wide variety of carboxylic acids such as acetic acid and oxalic acid.

Return to Exercise 18

Solution Exercise 19

Hydrous iron and manganese oxides are utilized by dissimilatory Fe(III)- and Mn(IV)-reducing bacteria as solid-phase electron acceptors for anaerobic respiration. These oxide minerals typically occur as thin coatings on other mineral grains, as well as particulate organic materials. Dissolution of these coatings by microbial reduction under low oxygen conditions frequently results in gleyic (gray-blue-green) color characteristics, evident in hand samples of borehole cuttings and cores from Mn(IV)- and Fe(III)-reduction zones in groundwater systems.

Return to Exercise 19

Solution Exercise 20

Microbial carbonate mineral precipitation can be used in cementing unconsolidated sediments and infilling of empty pore spaces to improve shear strength and stiffness of loose deposits and/or reduce permeability to control groundwater flow.

Return to Exercise 20

11 About the Authors

F. Grant Ferris is a Professor in the Department of Earth Sciences at the University of Toronto. For the past 30 years, he has been bridging the worlds of microbial geochemistry research and industry applications. With a formal training in microbiology attained during his B.Sc. and PhD degrees at the University of Guelph, Grant went on to complete a National Aeronautics and Space Administration (NASA) postdoctoral fellowship at Scripps Institution of Oceanography in California, and a Natural Science and Engineering Research Council postdoctoral fellowship at Western University in Ontario. Following his fellowships, Grant joined the private sector where he worked in the oil and gas industry on pipeline corrosion, enhanced oil recovery and cleaning-up refinery wastes. During this time, he designed and patented a biomineral plugging process for reducing the porosity and permeability of subsurface geological formations. After several years working in industry, Grant joined the University of Toronto and established one of the first microbial geochemistry laboratories in Canada.

Over the course of his career, Grant has contributed an immense body of knowledge to the area of microbial geochemistry. His research group has explored some of the most remote and inaccessible environments on Earth. Study sites have ranged from the high altitude of the Atacama Desert in Chile to the abyssal depths of the North Pacific Ocean; hydrothermal fields in Yellowstone National Park to geysers in Iceland and New Zealand; underground nuclear waste disposal facilities in Sweden to ephemeral saline alkaline lakes in British Columbia; and blood red waters of the Rio Tinto in Spain to Ellesmere Island in the Canadian High Arctic. Grant has also served as an editor for several world-renown scientific journals and held executive positions with the American Society for Microbiology and International Society for Environmental Biogeochemistry. He was the only non-American on the Space Studies Board of the National Academy of Science (U.S.A) committee on Assessment of Planetary Protection Requirements for Mars Sample Return Missions, and an early member of the Astrobiology Working Group of the Canadian Space Agency.

Grant's contribution to the scientific field is complimented by his dedication to teaching and mentoring of students and postdoctoral fellows. Recognizing Grant's contributions, in 2012, he was inducted into the Royal Society of Canada.

Natalie Szponar is a multidisciplinary environmental scientist who uses microbial, geochemical, and hydrogeological tools to understand different geological and environmental processes. Natalie received her Bachelor of Science in aqueous geochemistry at McMaster University and Master of Science in stable isotope geochemistry at Memorial University of Newfoundland. For her graduate research, she worked to understand the role of microbes in the cycling of carbon in contaminated and undisturbed aqueous environments. At Memorial University she also completed an internship with the Astrobiology Working Group of the Canadian Space Agency, working to understand the life-supporting potential of non-Earth planets. After completing her Masters, Natalie began working as a hydrogeologist and environmental consultant at a private consulting firm where she worked for more than nine years. As part of her consulting work, Natalie travelled across Canada to work on various environmental assessment and remediation programs. In 2016, she returned to academia to pursue a doctoral degree in Earth Sciences, joining the Trace Metal and Metal Isotope Laboratory Group at the University of Toronto. During her doctoral studies, Natalie largely worked in Peru, building capacity for research and sampling in areas impacted by artisanal and small-scale gold mining and assisting in the Peru's efforts to understand the effects of these mining operations on the environment.

Brock A. Edwards is a third-year PhD student at the University of Manitoba researching the role of volcanic gas emissions in the global mercury cycle. Along with colleagues at the Icelandic Meteorological Office and the University of Iceland, he recently studied early-eruption emissions of mercury and other gases from the ongoing Fagradalsfjall eruption in southwest Iceland. His previous research as an undergraduate and Master's student at the University of Toronto focused on the biogeochemical cycling of iron in freshwater environments and the influence of microorganisms on water quality and contaminant mobility.

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