

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

In the early days of the nuclear technology, nuclear waste management was thought to be an easy task that is modest and readily amenable to solutions. The underground deposition of nuclear and uranium waste materials in sealed steel canisters was identified as one of the key strategies that can ensure the protection of people and the environment, now and in the future. However, sixty years into the nuclear age, radioactive waste disposal in subsurface nuclear repositories has become an increasingly intractable problem (Crowley, 1997). Currently, designing efficient nuclear waste isolation canisters is not the only problem faced by the nuclear industry; there are still unresolved problems of possible groundwater pollution due to the leakage of radionuclides from sites of their disposal. In particular, the presence of radioisotope fission products, even at low concentrations, in the environment is of more concern (Francis, 1998). This is due to their high radiological decay rates, their decay-heat production, and their potential biological hazards to lower and higher living organisms. Among these, cesium (Cs) and strontium (Sr), which are produced by the fission of uranium or plutonium in relatively high yields, are among the most hazardous radiotoxic contaminants for the environment (Watson et al., 1989). Sr-90 is among the radionuclides of concern because of its long half-life (28 years), and most importantly high retention in the body due to its chemical similarity with calcium. The radioisotopes of Cs (Cs-137 and Cs-135) also pose a serious radiation hazard due to their long half-life (2×10^6 and 30 years, respectively), and high solubilities, mobilities and bioavailability in aqueous systems (Chicote et al., 2004).

In the past, contaminated groundwater sources have been treated using the pump and treat method. However, this method is neither cost effective nor reliable as it can not guarantee complete remediation of the contaminated site. On the other hand, the use of conventional adsorbents, such as zeolites and synthetic organic ion exchangers, for radionuclide clean-up is their unsuitability at high pH, high sodium concentrations, and in irradiated environments (Chaalal and Islam, 2001). Therefore, these findings advocate an urgent ideological shift from the current conventional nuclear waste management practices to new or existing paradigms to ensure sustainability of the nuclear technology. The basic concepts of these paradigm shifts include; the use of environmentally friendly waste management methods, the

development of cost-effective methods for *in situ* containment and stabilization of contaminants, and maintaining retrievability of the disposed waste (National Research Council, 1996; North, 1999). The present study is a detailed investigation on the potential role and applicability of biological remediation (bioremediation) methods as an alternative for the selective uptake of metallic fission products from contaminated water sources. This study is an effort towards elucidation of the crucial microbial mechanisms during metal bioremediation as an initial step towards the successful development and proper design of biological containment barrier technologies at radionuclide-contaminated aquifers.

1.2 AIM AND OBJECTIVES

The main aim of this study is to investigate the potential role of natural microbial processes, as means for controlling radionuclide dispersion in the environment through biological containment. The specific objectives for this study are:

- To investigate the kinetics of bacterial growth and inhibition, as well as culture diversity changes during Sr^{2+} , Co^{2+} and Cs^+ bioremediation in a batch anaerobic bioreactor
- To evaluate the biosorption kinetics and identify mechanisms of Sr^{2+} , Cs^+ and Co^{2+} biosorption in a batch SRB bioreactor under bacterial growth and non-growth conditions.
- To identify the key bacterial surface reactive sites that effect metal adsorption, and determine the stability of the metal complexes formed during microbial metal immobilization.
- To evaluate the effect of ionic strength and temperature on the bacterial adsorptive properties, as well as the stability of Sr, Co and Cs complexes on the bacterial cell surface.

1.3 SCOPE OF THE STUDY

The present study investigates the potential application of microbial technology (or bioremediation) for controlling radionuclide dispersion as a strategy of safeguarding against long term environmental pollution. In essence, this study addresses three sets of questions; (1) can these microorganisms survive and perform satisfactorily under conditions of variable (low to high) metal concentrations?, (2) What are the main mechanism(s) for metal immobilization in the bioreactor under bacterial growth and non-growth conditions, (3) What

are the effect(s) of varying pH, ionic strength and temperature on the biosorptive properties of the bacteria, and stability of the immobile metal complexes formed? Currently, several studies focusing on the application of *in situ* bioprecipitation (ISBP) in the presence of sulphate reducing bacteria (SRB), as an effective method of metal removal from contaminated groundwater have been undertaken. However, there are limited studies on the immobilization of metal pollutants, mainly through biosorption, in the presence of SRB. Thus, this thesis establishes the applicability and performance of different bioremediation techniques for metal removal in an artificial contaminated aqueous system under anaerobic conditions. This study also enhances the scope of metal/radionuclide chemistry, speciation and the stability of the metal complexes in contaminated aqueous systems, under variable environmental conditions. It also considers the basic requirements for microorganism survival and performance in these conditions. This thesis can be also considered among the few to investigate the SRB cell-metal interactions with prospects of controlling subsurface radionuclide decontamination under high metal, ionic strength and anaerobic conditions.

1.4 METHODOLOGY

This study includes four basic parts; (1) an introduction to the present study, and a review of the relevant literature necessary for tackling the subject at hand, (2) an overview of the approach used to conduct the research, and (3) a thorough discussion of the results obtained and implications for addressing the problem, and lastly (4) conclusions based on the results obtained, as well recommendations. Experiments are conducted under conditions that closely represent radionuclide contaminated aqueous system, which is anoxic and of variable pH, temperature and ionic strength. Prior to metal removal experiments, SRB growth was stimulated by a series of enrichment procedures. Functional bench-scale batch anaerobic bioreactors (2L) were used for evaluating the bacterial population and diversity changes, sulphate consumption, pH changes and metal removal over the treatment period. By monitoring the SRB community responses, the potential use of metal-specific SRB cultures as the reactive component in PRBs for metal immobilization, were revealed. Further batch equilibrium metal adsorption experiments were conducted in rubber-sealed anaerobic serum bottles (100 mL) under non-growth conditions, to investigate the complexation of metal ions onto bacterial cell surfaces. Results from these experiments, were able to provide vital information on the stability of the metal complexes, and the effect of pH, ionic strength and temperature on the passive adsorption of the metals by the bacterial culture.

1.5 SIGNIFICANCE OF THE STUDY

The value of the present study is that it presents a different perspective on a topic that has raised concern but has not been systematically addressed due to lack of information. This study presents a conceptual framework for the applicability of bioremediation as means of controlling radionuclide mobility in the subsurface environment. The biological and chemical reaction mechanisms that facilitate metal uptake, stability of the metal ion-bacteria complexes formed and other considerations regarding microbial metal immobilization processes are presented. The well researched scientific ideas, analytical information and findings contained in this thesis can help to improve the scientific basis for the performance assessment of microbial technologies for radionuclide immobilization. At the same time, it also prepares other researchers for the challenges and conditions to be met for undertaking such study. Taken in combination with other research findings, this study should considerably progress the development of a rational strategy to the vexing challenge of radionuclide mobility and migration in underground nuclear waste repositories, and a useful contribution towards implementation of national policy towards radioactive waste management.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND

Due to the impact of the rapidly growing demand for energy, as well as the concerns about global warming, there has been a resurgence of interest in nuclear energy. Nuclear energy is one of the few economically viable base-load electricity generation technologies, which could help reduce the current output of CO₂ emissions into the atmosphere by approximately 8% (Mourogov et al., 2002). The world's nuclear generating capacity currently stands at about 372 GWe, with the United States of America and France as the major producers, 27 and 17 percent, respectively (Figure 2.1). Electricity consumption in South Africa has been steadily increasing since the 1980s and it is predicted that by the year 2025 electricity demand will exceed supply (Musango et al., 2009). Therefore, in order to be able to meet future domestic and industrial electricity demands, there has been a renewed interest in the production of nuclear power. While there has been an improved public perception of the nuclear technology, some of the leading problems associated with this technology still remain. Up to date, no suitable alternative route of radioactive waste treatment has yet been formulated; while in the interim huge amounts of spent fuel are discharged globally (Lior, 2008).

The primary waste form resulting from nuclear energy production is spent nuclear fuel (SNF). The presence and toxicity of long-lived actinides and their fission products in spent fuel is of primary concern because of their potential for migration from the waste repositories and contaminated sites to the environment (Francis, 1998). Currently, two spent fuel management paths are being pursued; reprocessing and direct disposal. In some countries, reprocessing has been thought to be a better option; while in some there is broad scientific agreement that deep geologic disposal using a system of engineered and natural barriers to isolate the waste is the best. However, about 40 years after geological disposal has been recommended, neither the United States of America nor any other country has succeeded in developing a safe repository for their high level waste (HLW) (North, 1998).

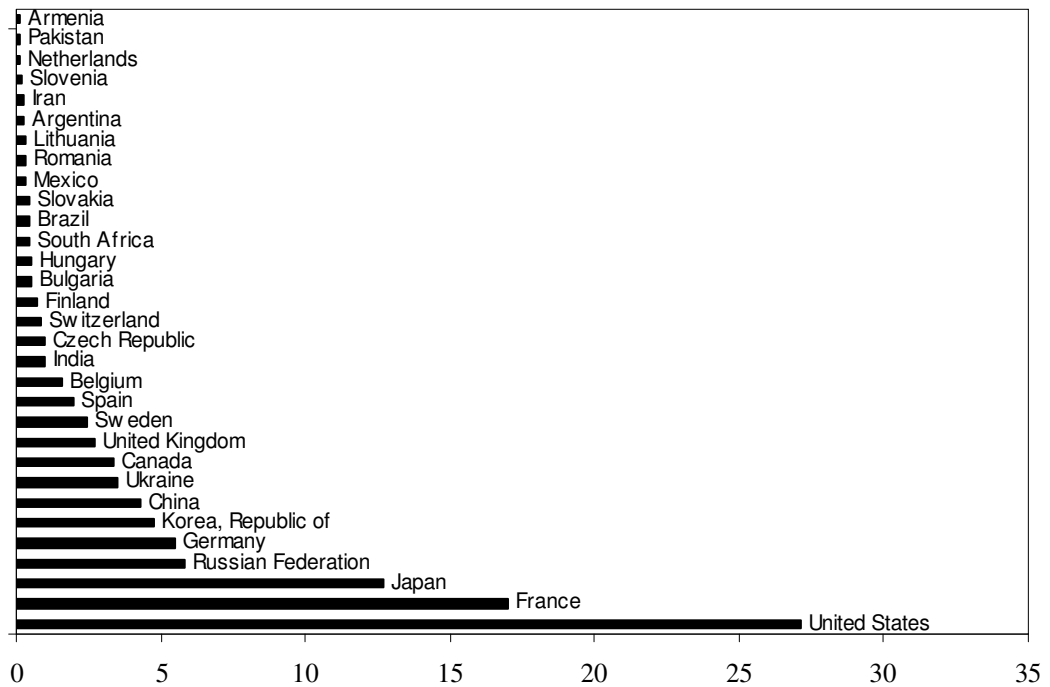


Figure 2.1: Global nuclear power generating capacity (%) per country (IAEA, 2009).

2.2 SPENT NUCLEAR FUEL MANAGEMENT

2.2.1 Structure and Composition of Spent Nuclear Fuel

The most predominant type of fuel used in most nuclear reactors is UO_2 , and in some cases metallic U or a combination of UO_2 and PuO_2 (Kleykamp, 1985). The UO_2 fuel contains radionuclides, which can be separated into a number of distinct categories; (1) fission products which migrate to grain boundaries in the fuel and occur as finely dispersed fission gas bubbles, such as Xe and Kr; (2) metallic fission products, such as Mo, Tc, Ru, Rh, and Pd, which occur in the grain boundaries as immiscible, micron to nanometre sized metallic precipitates (ϵ -particles); (3) fission products that occur as oxide precipitates of Rb, Cs, Ba, and Zr, and (4) fission products that form solid solutions with the UO_2 fuel matrix, such as Sr, Zr, Nb, and the rare earth elements (Figure 2.2) (Kleykamp, 1985; Shoesmith et al., 2000; Buck et al., 2004; Bruno and Ewing, 2006).

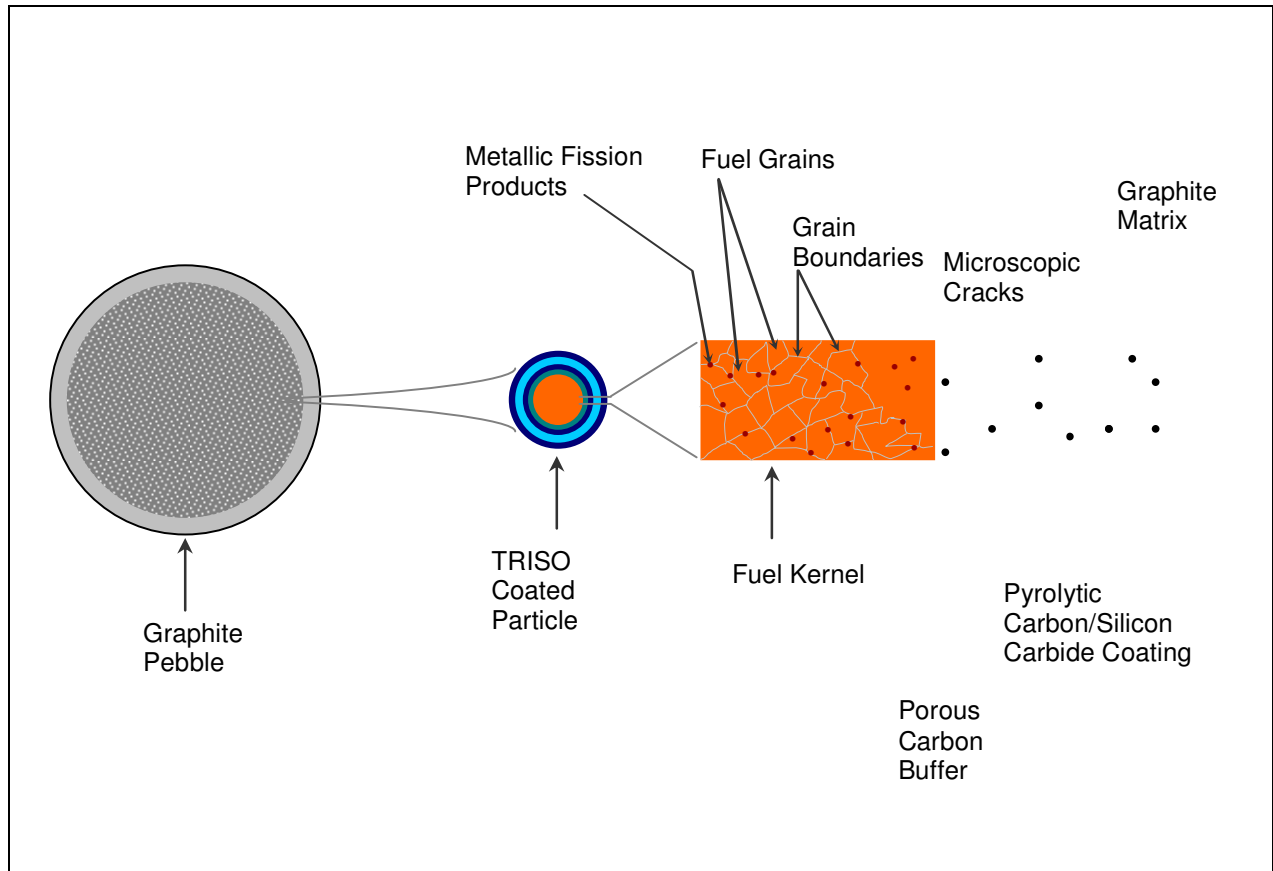


Figure 2.2: Schematic representation of spent nuclear fuel structure and propagation of fission products and impurities (Adapted from Chirwa, 2010).

2.2.2 Spent Nuclear Fuel Reprocessing

Currently, spent fuel reprocessing is the most widely used method, and different countries employ different reprocessing techniques, depending on the type of waste, level of activity and their state (gas, liquid or solid) (Figure 2.3). Commercial reprocessing methods that have been developed for use include variants of the PUREX (plutonium/uranium extraction) process, and UREX+ reprocessing with transmutation (Lagus, 2005). During the PUREX process plutonium and uranium are each separated from the other spent fuel products which are then vitrified and stored in a geological repository. The UREX+ technology with accelerated transmutation of waste (ATW) improves on the conventional PUREX technology by mixing the plutonium with minor actinides, making the process more proliferation-resistant (Lagus, 2005). Apart from the

high costs involved, reprocessing results in the generation of huge amounts of radioactive solid and liquid wastes. Solid radioactive waste such as fuel element cladding hulls, hardware, and other insoluble residues are generated during fuel dissolution. They may contain activation products, as well as some undissolved fission products, uranium and plutonium (Buck et al., 2004). Therefore, proliferation risks are and will be the major detriments to reprocessing. Although new technologies, such as transmutation, have been demonstrated to help curb proliferation risks, much research is still needed to formalize their industrial application (Bunn et al., 2003).

CLASSIFICATION		TREATMENT			CONDITIONING
LLW	Liquid	LIQUID WASTE	SOLID WASTE	GASEOUS WASTE	Cementation
		Chemical treatment	Compaction	Scrubbing	
ILW	Solid	Ion exchange	Incineration	Adsorption	Polymerization
		Reverse osmosis	Size fragmentation	Prefiltration	
HLW	Gas	Evaporation	Repackaging	High efficiency filtration	Bituminisation

Figure 2.3: Common nuclear waste management practices. LLW= low level waste, ILW=intermediate level waste and HLW=high level waste (Adapted from Raj et al., 2006).

2.2.3 Spent Nuclear Fuel Disposal

The predominant form of high level nuclear waste available for disposal is the spent fuel bundle discharged from reactor. The radioactivity, chemistry and thermal output are the main factors that are considered for proper spent nuclear fuel disposal (Long and Ewing, 2004). The fundamental concept of nuclear waste disposal sites is the isolation of the high level waste in deep geologic settings; and that the geologic medium itself coupled to the waste form and other engineered barriers should isolate the waste from the biosphere without active monitoring or control by future societies. Geologic disposal relies on the concept of a series of multiple barriers, each designed to limit radionuclide release, and these include the natural barriers (the

groundwater flow system and the rocks/clays that constitute the geologic setting) and the engineered barriers (such as the waste form, canisters, and backfill). For high-level waste (HLW) including spent nuclear fuel, massive engineered barriers have been designed which, where in an appropriate geological setting, should provide the required assurance of safety. However, numerous recent studies have shown that waste repositories are sites of enhanced ecological danger mainly due to canister corrosion problems resulting in the release of the waste contents into the environment (Kosareva et al., 2006; Bazansak et al., 1999). The main factors that have been found to exacerbate canister corrosion problems include; presence of water and oxygen, high temperatures, and most importantly the presence of microorganisms, causing the so called 'microbially-induced corrosion' (Bennett and Gens, 2008; Johnson and King, 2008; Bruhn et al., 2009). In several cases, spent fuel repositories have been compromised, leading to contamination of huge volumes of soil and groundwater. For example, at the Hanford site through leakage from surface disposal sites and tank leakage at the near-surface transuranic storage facilities at the Savannah River site. Buried nuclear wastes consist of concentrated forms of various radioactive chemical compounds, which if exposed to the general population, may cause serious life-threatening disorders (Bazansak et al., 1999).

2.3 ENVIRONMENTAL FISSION PRODUCT CONTAMINATION

2.3.1 Speciation and Chemistry of Fission Products

Migration of radionuclide contaminants depends on, among others, the radio element itself, speciation and the nature of the exchanging medium. Particularly, it has been suggested that aqueous speciation of some radionuclides (e.g., Co-60, Cs-137, Am-241, Pu-239, and Cm-244) plays an important role in sorption, hence retardation of contaminants in aquifers (Caron and Mankarios, 2004). The redox chemistry of fission products differs from other radionuclides because of the limited oxidation states. For example, both Cs and Sr exist in only one oxidation state, (Cs^+ and Sr^{2+} , respectively) and their environmental behaviour is therefore not dependent on any redox chemistry. Their oxidation states account for their high bioavailability and mobility in the environment (Siminoff et al., 2007). Cs^+ is a very soft, ductile, alkali metal that is liquid at 28.4°C . It is the most electropositive and reactive of the alkali metals and forms compounds with a variety of anions and alloys with the other alkali metals and with gold. The predominant aqueous species in groundwater is the uncomplexed Cs^+ ion (Lujaniene et al., 2006). Sr^{2+} , like

other alkaline earth metals, forms complexes and insoluble precipitates. Technetium (Tc) is known to exist in all oxidation states from +7 to -1. However, there are only two environmentally stable oxidation states, Tc(VII), as the anion TcO_4^- and Tc(IV). The dominant species in natural aqueous solutions in equilibrium with the atmosphere is the pertechnetate (TcO_4^-), which has a high geochemical mobility and bioavailability (Lloyd et al., 2002; Lloyd and Renshaw, 2005). One other specific radionuclide of concern is Co-60 due to its strong potential for offsite migration. In the presence of organic chelating agents, such as EDTA, Co-60 forms stable complexes, thus increasing its aqueous solubility and potential for transport offsite (Means et al., 1978). Cobalt isotopes are also important contaminants, and they are activation products in metallic structures of nuclear power plants. Co only exists in two oxidation states; Co(III) and Co(II), with the latter/former, being the most predominant in aqueous ecosystems (Simonoff et al., 2007).

2.3.2 Mechanisms of Release into the Environment

One of the key processes here is the dissolution of the spent nuclear fuel matrix in groundwater, liberating radioactive fission products and actinides (Smellie and Karlsson, 1999). In subsurface granitic groundwaters (500-700 m deep), the conditions are reducing and UO_2 has very low solubility. However, the ionizing radiation emitted from the spent nuclear fuel induces radiolysis of the groundwater resulting in the production of both oxidants (OH^\bullet , H_2O_2 , HO_2^\bullet , and O_2) and reductants (e^-_{aq} , H^\bullet and H_2) at the fuel interface. The oxidants produced can result in the oxidation of the insoluble U(IV) in the fuel matrix to the significantly more soluble U(VI), and thereby enhance matrix dissolution. Microbial gas production has also been reported to result in a local increase in pressure in the repository, allowing the development of fissures, and consequently the discharge of radionuclides into the adjacent environment (West and McKinley, 2001; Jonsson et al., 2007). Bioleaching of the waste form due to microbially produced ligands is another most likely mechanism by which radionuclides could be mobilized and distributed to the biosphere (Brainard et al., 1992). Microbially-induced corrosion (MIC) has also been reported to facilitate the release of radionuclides to the biosphere (Little et al., 1991; King and Stroes-Gascoyne, 1995; Parmar et al., 2000; Johnson and King, 2008; Bruhn et al., 2009).

2.3.3 Biological Hazards of Fission Products

The presence of fission products in the environment is of particular concern because of their high radiological decay rates, their decay-heat production, and their potential biological hazards. Fission products such as cesium (Cs-137) and strontium (Sr-90), which are produced by the fission of uranium or plutonium in relatively high yield, are the most hazardous radiotoxic contaminants for the environment. These products pose a serious radiation hazard to the health of man and the environment. Due to its chemical similarity with calcium, Sr-90 is easily incorporated into bone and continues to irradiate localized tissues with the eventual development of bone sarcoma and leukemia (Kossman and Weiss, 2000; Noshchenko et al., 2001; Dewiere et al., 2004; Greve et al., 2007). The radioisotopes of Cs (Cs-137 and Cs-135) are also hazardous due to their long half-lives (2×10^6 and 30 years, respectively), and high solubilities, mobilities and bioavailability (Lujanienė et al., 2006). Cs is analogous to K^+ , and enters cells via pathways for K^+ , and the specific mechanisms by which Cs^+ is transported into the cell have been reviewed by Lloyd and Macaskie (2000). The activation product, Co, is another radionuclide of concern, particularly in radiation protection as it is a key γ -ray source. The conventional approach for radiation protection is based on the International Commission of Radiological Protection (ICRP) model, the linear no threshold (LNT) model of radiation carcinogenesis, which implies that ionizing radiation is always harmful, no matter how small the dose (Chen et al., 2007).

2.3.4 Fission Product Treatment Options

Common methods used for the removal of Sr-90, Cs-137 and Co-60 from storage pool water are *ex situ* removal technologies, which include; adsorption on zeolites and synthetic organic ion exchangers (Ebner et al., 2001; Paterson-Beedle et al., 2006; Smiciklas et al., 2006). However, the main disadvantages with the above methods is their unsuitability at high pH, high sodium concentrations, and in irradiated environments (Chaalal and Islam, 2001). The efficient removal and recovery of other fission products, particularly radioiodine, technetium, using physical and chemical methods are somewhat more difficult, and are still under development. In Japan, the use of inorganic sorbents for fission product removal is under investigation, while the use of extractants such as crown ethers, cobalt dicarbollides and calix-crown ethers have also been studied with some success in the United States of America, Czech Republic and France, respectively.

Apart from the high costs involved, physical and chemical methods are well known to be aggressive and invasive treatment strategies that can have negative impacts on biodiversity and can even result in increased dispersion of radioactive materials (Bazansak et al., 1999; Lloyd and Renshaw, 2005). Given the technical limitations of physico-chemical approaches, there has been an unprecedented attention on the application of innovative techniques for fission products waste treatment. Among the most successful and widely used innovative technologies for cleanup of sites contaminated with hazardous chemicals is bioremediation (the use of microorganisms or microbial processes to treat environmental metal/radionuclide contamination) (Kumar et al., 2007). Bioremediation exploits the metabolic reactions of microorganisms to destroy contaminants or to transform them into species whose mobility is controlled (Banazsak et al 1999). Bioremediation is relatively cost effective, as target compounds are stabilized and/or detoxified by the biomass, eliminating the need for expensive chemical additions to achieve the desired treatment goals (Mulligan et al., 2001). Given the potential environmental and financial benefits of bioremediation, the microbial immobilization of long-lived fission products under geologic repository settings, surprisingly, has received less attention.

The main treatment goals for nuclear wastes by biological approaches include, for example, the selective accumulation of radionuclides for possible recovery or ultimate disposal, the immobilization of radionuclides in a subsurface aquifer to prevent migration into water supplies, or the selective leaching of radionuclides from contaminated soil (Lloyd and Lovley, 2001). Ideally subsurface bioremediation strategies should require less or no maintenance; and for this reason there has been a widespread investigation of passive biological remediation techniques (Hedin et al., 1994; Younger et al., 2002). On the other hand, active *in situ* bioremediation treatment depends on the viability and activity of the microorganisms, which might not always be the case under high metal and radioactive stress conditions. Active bioremediation treatment requires regular monitoring of the inhabiting microbial communities. Nonetheless, both techniques rely on microbial processes that effect the retardation of fission products mobility in subsurface environments, through bioprecipitation, bioaccumulation, biosorption, and biotransformation (Lloyd and Macaskie 2000; Lloyd et al., 2002; Lloyd and Renshaw, 2005).

2.4 METAL IONS UPTAKE BY BACTERIA

2.4.1 Microbial Mechanisms of Metal Uptake

Microorganisms possess mechanisms by which metal ions can be taken up and accumulated from their environment. An understanding of these mechanisms is vital for the optimization of the bioremediation process. In addition, this understanding is essential in order to fully utilize the selectivity and efficiency of the process and to overcome ionic competition and interference effects by other ionic species which exist along with the targeted element in the ionic matrix of the contact solution (Tsezos and Volesky, 1982; Lovley and Phillips, 1992; Avery and Tobin, 1992; Beveridge and Murray, 1980). Based on cell metabolism, the uptake of metals can be classified into two classes; metabolism dependent and non-metabolism dependent metal uptake. The process of metabolism dependent metal uptake is strictly dependent on cell metabolism and involves active translocation of metals into the cell, which results in the accumulation of the metal inside the cell (Gadd, 1989). On the other hand, during non-metabolism dependent, physico-chemical interactions, such as; physical adsorption, ion exchange and chemical sorption; between the metal and microbial cell surface facilitate metal uptake (Kuyucak and Volesky, 1988). The four well known microbial processes that effect metal uptake; bioprecipitation, bioaccumulation, biotransformation and biosorption, are briefly presented below, and are used as illustration of the wide variety of physical-chemical phenomena which are involved during metal uptake.

Bioprecipitation

Bioprecipitation of metals by microorganisms may be either metabolism dependent or independent. During metabolism-dependent precipitation or dissimilatory reduction, the transformation of the metal is unrelated to its intake by the microbial catalyst. Consequently, the reduced metal is precipitated in the extracellular medium, as a defense system (Valls and de Lorenzo, 2002). For example, Cd removal from solution by *Arthrobacter* and *Pseudomonas* (Scott and Palmer, 1990) and the enzymatic precipitation of various heavy metals including; U(VI), Cr(VI), Tc(VI) and As(V) by SRB (Lloyd, 2003). In the case where precipitation is independent of cellular metabolism, it is normally a result of the chemical interaction between the metal and a biogenic ligand (Veglio and Beolchini, 1997). Biogenic ligands are generated during cell metabolism and they include; phosphates, carbonates and sulphides, which can result

in the precipitation of metals and radionuclides (stable or radioactive) ions, and hence their immobilization. Examples include; the formation of less soluble metal sulphides in the presence of SRB (White et al., 1997), the precipitation of strontium carbonate, by carbonates produced by *Ralstonia eutropha* and *Pseudomonas fluorescens* (Anderson and Appanna, 1994), and precipitation of soluble U(VI) compounds to insoluble U(IV) hydroxide or carbonate at neutral pH (Phillips et al., 1995). In other microorganisms metal precipitation is mediated by the liberation of inorganic compounds, such as; oxalates, phosphates or hydroxides from organic donor molecules (Tucker et al., 1998). A well documented example involves the precipitation of metals as by *Citrobacter sp.* This bacterium was found to be tolerant to high concentrations of U, Ni, and Zr through the formation of highly insoluble metal phosphates (Macaskie et al., 2000).

Bioaccumulation

Bioaccumulation is an energy dependent process of metal uptake against a concentration gradient. During bioaccumulation metals are transported from the outside of the microbial cell, across the cellular membrane, and into the cell cytoplasm, where the metal is sequestered and therefore immobilized. Examples include the intracellular accumulation of Hg, Pb, Ag and Cd by *Escherichia coli* using an energy dependent transport system (Gadd, 1988). In the case of radionuclides, they may enter microbial cells through a classical transport system, as would an ion normally involved in the physiology of the cell, for example; Cs⁺ instead of K⁺ or Sr²⁺ and Ra²⁺ instead of Ca²⁺ (Simonoff et al., 2007; Greve et al., 2007). Once in the cell, radionuclide is sequestered by cysteine-rich metallothioneins (Turner and Robinson, 1995).

Biotransformation

Radionuclides, including U(VI), Pu(VI), Tc(VII), Co(III), have been subjected to enzymatic biotransformation by microorganisms from soluble mobile forms to reduced and less soluble forms (Francis, 1998; Gorby et al., 1998; Istok et al., 2004). Bioreduction can result in the precipitation of solid radionuclides through enzymatic reduction, or biomethylation (production of volatile derivatives). The extracellular enzymatic transformation of radionuclides by microbial isolates, such as, *Desulfovibrio desulfuricans*, *Geobacter sulfurreducens*, *Geothrix fermentans*, *Deltaproteobacteria*, and *Clostridium*, is well documented (Lloyd et al., 2003; Suzuki et al., 2003; Brodie et al., 2006). Biomethylation can produce volatile methyl derivatives, as for Se, Tc,

Hg, I, and so decrease the concentration of soluble contaminants in water or soil. Biodegradation of associated organic compounds has been observed with citrate, which forms highly soluble radionuclide citrate complexes. These complexes can then be degraded by microorganisms resulting in the precipitation of the radionuclides as observed with *Pseudomonas aeruginosa* and *P. putida* (Thomas et al., 2000).

Biosorption

Biosorption, the passive sequestration of metals by interactions with live or dead biological materials, is a practical and rather widely used approach for the bioremediation metal and radionuclide contamination (Barkay and Schaefer, 2001). Mechanisms responsible for biosorption, may be one or a combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and microprecipitation (Veglio and Beolchini, 1997; Vijayaraghavan and Yun, 2008; Wang and Chen, 2006). The surface of cells carries a net negative charge due to the presence of carboxyl, hydroxyl, phosphate and sulphhydryl groups, and so during biosorption the metal cations are strongly bound (Beveridge, 1989). In the laboratory, the process of metal biosorption can be performed using several approaches; in batch and continuous modes of operation. Most industrial applications prefer a continuous mode of operation, however, batch bioreactor experiments have been found to be useful in evaluating required fundamental information, such as biosorbent efficiency, optimum experimental conditions, biosorption rate and possibility of biomass regeneration (Veglio and Beolchini, 1997; Gadd, 2009). Over the years, metal adsorption onto bacteria has acquired great importance in existing efforts towards environmental bioremediation, and there is still ongoing research on the adsorption of metal ions under varying conditions (Avery and Tobin, 1992; Lloyd and Macaskie, 2000; Parmar et al., 2000; Borrok and Fein, 2005).

2.4.2 Genetic Diversity of Bacterial Biosorbents

In the last decade or so, a significant amount of research has been conducted on the genetic diversity of microorganisms capable of accumulating metal ions from solution (Volesky and Holan, 1995; Gadd, 2000; Valls and de Lorenzo, 2002; Zouboulis et al., 2004). Among these, bacteria have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and abundance (Wang and Chen, 2009). In addition, bacteria are

capable of growing under controlled conditions and are resilient to a wide range of environmental situations, including radionuclide polluted sites (Urrutia, 1997). Since then, a wide array of bacterial species has been discovered as excellent biosorbents for the removal of different metal and radionuclide ions (Table 2.1). One of the most successful and well documented bacterial bioremediation strategies involves sulphate reducing bacteria (SRB). Maximum SRB growth occurs around slightly acidic to slightly alkaline pH (between pH 6-8), with a few exceptional strains that are acid-tolerant and can grow in the pH range 3-4 (White et al., 1997). Most SRB are mesophilic, attaining optimum growth at a temperature range of 25°C-30°C. Some, however, are able to grow at temperatures below 5°C while spore forming thermophilic species grow at temperatures ranging from 65°C to 85°C. SRB are important members of microbial communities with economic, environmental and biotechnological interest. They have been investigated for the removal of a wide variety of metals and radionuclides, such as As, Cr, Fe, Hg, Pd, Pt, Mn, Mo, Rh, Se, Te, Tc, U and Zn (Lovley and Phillips, 1992; Lloyd et al., 1998; Tebo and Obratzsova, 1998; Tucker et al., 1998; Smith and Gadd, 2000; Lloyd et al., 2001; Rashamuse, 2003; Ngwenya and Whitely, 2006; Jin et al., 2007).

Table 2.1 Genetic diversity of bacterial biosorbents

SPECIES	METAL ION	REFERENCES
<i>Bacillus sp.</i>	Al, Cd, Cr, Co, Cu, Fe, Ni, Pb, Sr	Fein et al., 1997; Zouboulis et al., 2004; Philip and Venkobachar, 2001; Srinath et al., 2003
<i>Citrobacter sp.</i>	Ag, Pb, Cd, Th, U, Zn	Macaskie et al., 1987; Jeong et al., 1997; Yong and Macaskie, 1997; Puranik and Paknikar, 1999; Xie et al., 2008
Sulphate reducing bacteria <i>sp.</i>	Au, Cr, Cu, Mo, Pd, Se, Tc, U, Zn	Chen et al., 2000; Creamer et al., 2006; Lovley and Phillips, 1992; Lloyd et al., 1999; Tucker et al., 1998
<i>Escherichia coli</i>	Ag, Au, Cd, Cr, Cu, Fe, Hg, Ni	Churchill et al., 1995; Weon et al., 2003; Ansaria and Malik, 2007; Deplanche and Macaskie, 2008; Quintelas et al., 2009
<i>Pseudomonas sp</i>	Cd, Cr, Co, Cu, Mn, Ni, U, Zn	Pardo et al., 2003; Vullo et al., 2008; Choi et al., 2009; Ziagova et al., 2007
<i>Thiobacillus sp</i>	Cu, Zn	Liu et al., 2004
<i>Geobacillus sp</i>	Cd, Cu, Mn, Ni and Zn	Ozdemir et al., 2009
<i>Enterobacter sp</i>	Cu, Cd, Pb	Lu et al., 2006
<i>Streptomyces sp</i>	Cd, Fe, Ni	Puranik et al., 1995, Selatnia et al., 2004

They are obligate anaerobes that decompose simple organic compounds using sulphate as the terminal electron acceptor. The result is the production of sulphide that may be given off as H₂S gas or react with metals to form less soluble metal sulphides (White and Gadd, 1996; White et al., 1995). The advantages of using SRB for bioremediation is the knowledge that they (i) possess a high metal-sequestering ability due to the presence of extracellular polymeric substances (EPS) that play a major role in the sorption of metals (Beech and Cheung, 1995; Braissant et al., 2007), (ii) are suitable for the treatment of high volume and low concentration complex wastewaters, (iii) are well recognised as principal inhabitants of deep geologic repositories, and have been shown to survive in such extreme ecosystems, which are anoxic and irradiated (Pederson et al., 2000; Haveman and Pederson, 2002; Chicote et al., 2004; Bruhn et al., 2009), (iv) metal removal by SRB is not limited to *ex situ* treatment in sulphidogenic bioreactors (Goncalves et al., 2007), but can also be applied *in situ* passive systems, such as artificial wetlands and, more recently, permeable reactive barriers (PRB) (Costa et al., 2008).

Metal ions uptake by biomass mainly depends on the components on the cell surface and the spatial structure of the cell wall. Peptidoglycan, teichoic acids and lipoteichoic acids are all important chemical components of bacterial surface structures. Some functional groups have also been found to bind metal ions, especially carboxyl group. In other studies, it has been shown that the O-, N-, S-, or P-containing groups also participate directly in the binding a certain metals (Darnall et al., 1986). Some active sites involved in the metal uptake are determined by using techniques of titration, infra-red and Raman spectroscopy, electron dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), electron microscopy (scanning and/or transmission), nuclear magnetic resonance (NMR), X-ray diffraction analysis (XRD), and XAFS (X-ray absorption fine structure spectroscopy) (Volesky, 2007).

2.5 METAL ADSORPTION BY BACTERIA

2.5.1 Factors Influencing Metal Ions Adsorption by Bacteria

Metal ions adsorption by bacterial biomass mainly depends on the metal involved and ionic charges of metal ion, the chemical composition of the metal ion solution and other external environmental factors such as solution pH, temperature, ionic strength, bacteria dosage, initial solute concentration and agitation rate (Gadd, 1986; Volesky, 1990; Vijayaraghavan and Yun,

2008). These factors do not only have an effect on the adsorptive properties of the bacterial surface reactive sites, but might also influence the stability of metal complexes (Benner et al., 1999). Therefore, an investigation of the effect of these factors on metal adsorption onto the microbial biomass is essential for the industrial application of biosorption, as it gives information about the performance of the process which is necessary for process design (Gadd, 2009).

Effect of pH

Solution pH seems to be the most important parameter in the adsorption of metals, as it affects the solution chemistry of the metals, the activity of the functional groups on the biomass cell surface and the competition of metallic ions in solution (Friis and Myers-Keith, 1986; Wang and Chen, 2006). The dependence of metal adsorption on solution pH has been reported in almost all biosorption systems examined, where at lower pH values, the biosorption capacity of metals is often reduced due to competition between cations and protons (H^+) for binding sites (Vijayaraghavan and Yun, 2008). In general, a pH range of 3-6 has been suggested as favourable for metal ion adsorption onto microbial biomass. This is because at higher pH values (>6), the formation of metal complexes due to chemical precipitation reactions may complicate the biosorption process. The increased adsorption capacity of various metals such as; Cd, Cu, Cr, Ni, Pb and Zn with increasing pH (3-6) has been reported. The observed metal ion adsorption increase with increasing pH has been attributed to the overall electronegative charge of the bacterial surfaces, which facilitate the complexation of metal ions onto the bacterial cell surface (Beveridge, 1981; Fein et al., 1997; Ngwenya et al., 2003; Yee et al., 2004; Johnson et al., 2007; Vasquez et al., 2007; Bueno et al., 2008; Calfa and Torem, 2008). However, there are exceptional cases, for example, the biosorption of some metal ions; for example, Ag^+ , Hg^{2+} and $AuCl_4^-$, has been shown to be pH-independent and which is explained by the formation of covalent complexes with N and S-containing ligands (Darnall et al., 1986).

Effect of temperature

Temperature is an important parameter for the equilibrium and kinetic adsorption of metals onto microbial biomass, as it can have an effect on the stability of the metal complexes and the ionization of the bacterial cell wall moieties (Sag and Kutsal, 1995). In literature, both an increase in equilibrium metal adsorption with increasing temperature, and temperature

independent metal adsorption onto bacterial cell surfaces has been reported. For example, in a study by Zouboulis and co workers (2004), an increase in the equilibrium adsorption capacity for Cr onto *Bacillus licheniformis* was observed with increasing temperature (25-50°C), while the adsorption of Cd on the same bacterial biomass was not significantly affected when the temperature was increased. In a study by Ginn and Fein (2009), almost similar Cd and Pb adsorption trends as a function of temperature (5-80°C) were observed for the bacterial species; *Bacillus subtilis* and *Pseudomonas mendocina*. The effect of temperature on Cd adsorption capacity, speciation and thermodynamic stability of the important Cd surface complexes was negligible. Similarly, minimal temperature effects were also observed for the extent of Pb adsorption onto bacteria at above pH 5. However, increasing the temperature had a significant effect on speciation of the important Pb surface complexes, whereby stability constants increased with increasing temperature. Based on the studies cited above, it is apparent that the effect of temperature on metal ion adsorption onto bacterial surfaces is inconclusive, and varies with both the metal ions involved, bacterial species of interest and other environmental conditions such as pH.

Effect of ionic strength

Numerous authors have investigated the effect of ionic strength on the adsorption of different metals such as Cd, Cs, Eu, Hg, Pb and Zn onto different bacterial species, where the extent of metal adsorption decreased with increasing ionic strength. The observed decrease in the extent of metal adsorption at higher ionic strength solutions, has been ascribed to: (1) the competition between the metal ions and the electrolyte cations for the adsorption sites on the bacterial cell surface; (2) the decrease of activities of the metal ions due to the increase of ionic strength; (3) the formation of ionic pairs or chelating compounds, and (4) the presence of salts may compress the electric double layer surrounding negatively charged surfaces, resulting in the release of the adsorbed metal ions species back into solution (Ledin et al., 1997; Daughney and Fein, 1998; Cox et al., 1999; Small et al., 2001; Yee et al., 2004; Borrok and Fein, 2005; Beolchini et al., 2006). On the contrary, the ionic strength independent adsorption of metals onto bacterial surfaces has also been reported. For example, in a study by Haas and Dichristina (2001), no variations in the extent of U adsorption onto the Gram negative bacterium, *Shewanella putrefaciens*, were observed over the ionic strength range of 0.02 to 0.1 M. In this case, the

independent adsorption of metal ions with increasing background electrolyte concentration has been interpreted to indicate that the sorption process is primarily non-electrostatic in nature (Stumm and Morgan, 1996). From these observations, it is apparent that the extent of metal adsorption at different ionic strengths varies with the metal adsorbed, and that the magnitude of electrostatic effects is not dependent on the bacterial species involved. However, more research still needs to be done to draw any valid conclusions on the effect of ionic strength on the adsorption of metal ions onto bacterial biomass.

Effect of biomass to metal ion concentration ratio

The effect of metal to biomass ratio on the adsorption kinetics has been studied before. Generally, it has been suggested that metal adsorption increases with increasing bacteria-to-metal concentration ratio. However, the adsorbed metal ion quantity per unit weight of biosorbent (specific metal uptake) decreases with increasing the biosorbent concentration, and has been reported in a number of studies (Gadd et al., 1988; Fourest and Roux, 1992; Daughney et al., 1998; Esposito et al., 2001; Daughney et al., 2001). These results may be explained by the fact that at low metal concentrations and high biomass concentrations the ratio of the biomass sorptive surface to total metal available is high; hence, all metal may be interacted with biosorbent and removed.

2.5.2 Modelling the Kinetics of Metal Adsorption by Bacteria

In order to investigate the mechanism of biosorption and potential rate controlling step such as mass transport and chemical reaction processes, kinetic models have been used to test batch sorption experimental data (Sarkar et al., 2003). Kinetics studies of sorption in wastewater treatment are significant since (i) the data can be used for determining the residence time of a sorbate at the solid-solution interface, (ii) the rate of sorption can be used to deduce predictive models for column experiments, and (iii) these studies can be used in understanding the mechanisms and effect of different environmental factors on the sorption process. In the past decades, several mathematical models have been proposed to describe sorption data, which can generally be classified as sorption reaction models and diffusion models. Diffusion-based kinetic models are always constructed on the basis of three consecutive steps; (1) diffusion across the liquid film surrounding the adsorbent particles, i.e., external diffusion or film

diffusion; (2) diffusion in the liquid contained in the pores and/or along the pore walls, which is so-called internal diffusion or intra-particle diffusion; and (3) adsorption and desorption between the adsorbate and active sites, i.e., mass action (Lazaridis and Asouhidou, 2003). On the other hand, reaction-based kinetic models originating from chemical reaction kinetics are based on the whole sorption process without considering the above mentioned steps.

Reaction-based kinetic models

Numerous authors have applied reaction-based kinetic models to describe the reaction order of adsorption systems based on the sorption capacity of the sorbent (McKay and Ho, 1999; Ho and McKay, 2000; Aksu, 2001; Chen et al., 2008; Chegrouche et al., 2009). Kinetic models, such as the Lagergren's pseudo first order equation (Lagergren, 1898), and the pseudo second order equation (Ho and McKay, 1999), are among the most widely used kinetic models to describe the biosorption process, and they are listed in Table 2.2. The pseudo first-order rate expression of Lagergren is based on solid capacity, and requires that the equilibrium sorption capacity, q_{eq} , be known. In many cases q_{eq} is unknown and as adsorption tends to become unmeasurably slow, the amount sorbed is still significantly smaller than the equilibrium amount. For this reason it is necessary to obtain the real equilibrium sorption capacity, q_{eq} , by extrapolating the experimental data using a trial and error method. Furthermore in most cases the Lagergren first order equation does not fit well for the whole range of contact time and is generally applicable over the initial 20–30 minutes of the sorption process (Lagergren, 1898).

Table 2.2 Expressions for the most frequently used reaction rate models

Model	Equation	Parameter description	Reference
Lagergren first order	$\frac{dq_t}{dt} = k_1(q_e - q_t)$	q_t = concentration of ion species in the sorbent at time t (mg g^{-1}), q_{eq} = equilibrium concentration of adsorbed ionic species in the sorbent (mg g^{-1}) and k_1 = biosorption constant of pseudo-first-order Lagergren equation (min^{-1})	Lagergren, 1898
Pseudo second order	$\frac{dq_t}{dt} = k_2(q_e - q_t)^2$	q_t = concentration of ion species in the sorbent at time t (mg g^{-1}), q_{eq} = equilibrium concentration of adsorbed ionic species in the sorbent (mg g^{-1}), and k_2 = biosorption constant of pseudo-second-order Lagergren equation ($\text{min}^{-1} \text{g mg}^{-1}$).	Ho and McKay, 1999

The pseudo second-order equation is also based on the sorption capacity of the solid phase. Contrary to the other model it predicts the behaviour over the whole range of adsorption and is in agreement with an adsorption mechanism being the rate controlling step. In addition, this model assumes that the sorption follows the Langmuir equation (Ho and McKay, 1999).

Diffusion-based models

It is well known that a typical liquid/solid adsorption involves film diffusion, intraparticle diffusion, and mass action. For physical adsorption, mass action is a very rapid process and is normally negligible for kinetic studies. Thus, for a solid-liquid sorption process, the solute transfer is usually characterized by either external mass transfer (boundary layer diffusion) or intraparticle diffusion or both (McKay, 1984). If external diffusion of metal cations (within the diffuse layers outside the sorbent) is the rate-limiting step then the external mass transfer model can be fitted into sorption data with some success. However, when intraparticle diffusion (transfer of metal cations from the adsorbent surface to the internal active binding sites) is the rate-limiting step, then sorption data can be described by the intraparticle diffusion model (Table 2.3).

Table 2.3 Expressions of the diffusion-based kinetic biosorption models

Model	Equation	Parameter description	Reference
External mass transfer model	$\ln \frac{C_t}{C_0} = -k_f \frac{A}{V} t$	C_0 = initial metal concentration (mg L^{-1}), C_t = metal concentration at time t (mg L^{-1}), A =external sorption area (m^2g^{-1}), V = total solution volume (L), and k_f = the external diffusion coefficient (cm s^{-1}).	Mathews and Weber, 1976
Intraparticle model	$q_t = k_i t^{0.5}$	q_t = concentration of ion species in the sorbent at time t (mg.g^{-1}) and k_i = the intraparticle diffusion rate ($\text{mg.g.min}^{0.5}$).	Weber and Morris, 1963

2.5.3 Modelling Equilibrium Batch Biosorption: Isotherm models

Equilibrium isotherm models are simple mathematical equations, which give a good description of the experimental behavior of a biosorbent over a limited range of operating conditions (Gadd et al., 1988; Kratochvil and Volesky, 1998; Gadd, 2009). Traditionally, isotherm models have been used to describe the adsorptive patterns of trace metals onto soils and sediments, but they have also been used to evaluate the sorption performance of different biosorbents. The main

limitation of these models is their basis on constant temperature and pH conditions, and their inability to predict spatial variations (Fein, 2000). Table 2.4 shows some of the single-component isotherm models that have been frequently used. Among these, the Langmuir model and the Freundlich model are the most widely accepted and well documented. The basic assumption of the Langmuir sorption isotherm is that sorption takes place at specific homogeneous sites within the sorbent. It is then assumed that once a metal ion occupies a site, no further sorption can take place at that site (Langmuir, 1918). The Langmuir isotherm model incorporates two easily interpretable constants: q_{max} and b . The parameter q_{max} , provides essential information on the maximum metal uptake capacity of the sorbent, and is useful for comparing the differences in metal uptake capacities between various biosorbent and metal species (Kapoor and Viraraghavan 1995; Pagnanelli et al., 2002; Volesky and Holan 1995). Low values of b are reflected in the steep initial slope of a sorption isotherm, indicating a desirable high affinity. Thus, for good sorbents in general, one is looking for a high q_{max} and a low b value (Kratochvil and Volesky, 1998).

Table 2.4 Some of the frequently used single component isotherm models.

Isotherm	Equation	Parameter description	Reference
Langmuir	$q = \frac{bq_{max}C_e}{1 + bC_e}$	q is equilibrium metal sorption capacity; C_e is the equilibrium metal concentration in solution; q_{max} and b are Langmuir constants related to maximum sorption capacity (monolayer capacity) and bonding energy of adsorption, respectively.	Langmuir, 1918
Freundlich	$q = kC_e^{1/n}$	k is a biosorption equilibrium constant, representative of the sorption capacity; and n is a constant indicative of biosorption intensity.	Freundlich, 1906
Combination Langmuir-Freundlich	$q = \frac{bq_{max}C_e^{1/n}}{1 + bC_e^{1/n}}$	Parameters same as above. Assuming that the surface is homogeneous, but that the sorption is a cooperative process due to adsorbate-adsorbate interactions.	Sips, 1948
Redlich-Peterson	$q = \frac{aC_e}{1 + bC_e^n}$	a , b , and n are the Redlich-Peterson parameters. The exponent n lies between 0 and 1. For $n=1$ the model converts to the Langmuir form.	Redlich and Peterson, 1959
Brunauer-Emmett-Teller (BET)	$q = \frac{BCQ^0}{(C_s - C) \left[1 + (B-1)C/C_s \right]}$	C_s is the saturation concentration of the adsorbed component; B a constant indicating the energy of interaction between the solute and the adsorbent surface, and Q^0 is a constant indicating the amount of solute adsorbed forming a complete monolayer	Brunauer et al., 1938

The Freundlich isotherm model assumes that the uptake of metal ions occurs on a heterogeneous surface by multilayer adsorption and that the amount of sorbate adsorbed increases infinitely as the concentration increases (Freundlich 1906). Both these models represent the theoretical model for monolayer adsorption. However, these adsorption isotherms may exhibit an irregular pattern due to the complex nature of both the sorbent material and its varied multiple active sites, as well as the complex solution chemistry of some metallic compounds (Volesky and Holan, 1995). The Redlich–Peterson isotherm incorporates the features of both the Langmuir and the Freundlich isotherms. The Redlich–Peterson isotherm has a linear dependence on concentration in the numerator and an exponential function in the denominator (Redlich and Peterson, 1959). The BET model describes the multi-layer adsorption at the adsorbent surface and assumes that the Langmuir isotherm applies to each layer (Brunauer et al., 1938). A practical consideration to the problem of metal pollution is the presence of more than one metal pollutant rather than monometal situation. In such a scenario it becomes essential to study the effect of the presence of co-cations on the biosorption capacity of the biosorbent (Schiewer and Volesky, 2000; Veglio et al., 2003). Therefore, to describe the biosorption of two or multi-metal ions within a system, various multi-component isotherm models have been developed, and examples are shown in Table 2.5.

Table 2.5 Examples of multi-component adsorption models

Isotherm	Equation	Parameter description	Reference
Competitive Langmuir	$q_i = \frac{b_i q_{\max i} C_{e_i}}{1 + \sum_{i=1}^N b_i C_{e_i}}$	C_{e_i} and q_i are the unadsorbed concentration of each component at equilibrium and the adsorbed quantity of each component per g of biomass at equilibrium, respectively. b_i and $q_{\max i}$ are derived from the corresponding individual Langmuir isotherm equations.	Langmuir, 1918
Combination Langmuir-Freundlich	$q = \frac{b_i q_{\max i} C_{e_i}^{1/n_i}}{1 + \sum_{i=1}^N b_i C_{e_i}^{1/n_i}}$	Parameters same as above.	Sips, 1948
Competitive Redlich-Peterson	$q = \frac{a_i C_{e_i}}{1 + \sum_{i=1}^N b_i C_{e_i}^{n_i}}$	a , b , and n are the Redlich–Peterson parameters. The exponent n lies between 0 and 1. For $n = 1$ the model converts to the Langmuir form.	Bellot and Condoret, 1993
IAST: Ideal Adsorbed Solution Theory	$\frac{1}{q_t} = \sum \frac{Y_i}{q_i^0}$	Y_i is the solute concentration of component i in the solid phase. q_i^0 is the phase concentration of a single adsorbed component in equations with C_i^0	Radke and Prausnitz, 1972

2.5.4 Surface Complexation Modelling

Surface complexation models (SCM) are based on the concept of surface charge generated from the amphoteric surface sites are capable of explaining the reaction with sorbing cationic or anionic species to form surface complexes (Volesky, 2003). In the past, SCMs were exclusively used for describing metal ion adsorption on pure mineral materials (Stumm et al., 1970; Davis and Kent, 1990; Dzombak and Morel, 1990; Stumm and Morgan, 1996). However, in recent years, surface complexation modeling has also been successfully applied to predict trends of ion sorption at the bacteria-water interface (Fein et al., 1997; Cox et al., 1999; Fowle and Fein, 1999; Daughney et al., 2001; Haas et al., 2001; Yee and Fein, 2001; Wightman et al., 2001; Ngwenya et al., 2003; Borrok and Fein, 2005; Ginn and Fein, 2009). Generally, models of bacterial surface protonation serve two purposes: firstly, to clarify the molecular-scale adsorption mechanisms that occur between aqueous solutes and the bacterial surface, and secondly, to enable quantitative geochemical modeling of mass transport in bacteria-bearing systems. However, the lack of agreement for a universal model accounting metal ion adsorption onto bacterial cell surfaces has hindered progress towards achieving both purposes (Borrok et al., 2005). SCMs can be broadly classified into two groups: electrostatic models, which take into account electrostatic effects, and models that neglect the electrostatic effects (non-electrostatic model – NEM). Numerous authors have demonstrated that either of the two classes of SCMs can be successfully used to describe the adsorption behavior of protons and metal ions onto bacterial cell surfaces (Brassard et al., 1990; Plette et al., 1995; Fein et al., 1997; Volesky and Yang, 1999; Cox et al., 1999; Martinez et al., 2002; Ngwenya et al., 2003; Borrok and Fein, 2005; Johnson et al., 2007, Ginn and Fein, 2009).

Electrostatic Models

Electrostatic models (e.g., (e.g., the constant capacitance model, diffuse layer model, and the triple layer model) all contain at least one coulombic correction factor to account for the effect of surface charge on surface complexation. These coulombic correction factors take the form of electrostatic potential terms, $e^{-F\psi_i/RT}$, where ψ_i is the surface potential in the i^{th} surface plane in the intrinsic conditional surface complexation constant expressions (Goldberg, 1995). Numerous authors have suggested that the application of electrostatic models to account for the hydrophobic and electrostatic interactions that occur between the bacterial surface and the

aqueous metal ions play an important role in proton-binding (Plette et al., 1995; van der Wal et al., 1997; Daughney and Fein, 1998; Daughney et al., 2002; Martinez et al., 2002). For example, numerous authors have successfully used the CCM approach, relating surface charge to surface potential using an arbitrarily assigned capacitance value, to describe the electrostatic effects of metal adsorption onto bacterial surfaces (Fein et al., 1997; Yee and Fein, 2001, Ngwenya et al., 2003, Haas, 2004, Ojeda et al., 2008; Ngwenya et al., 2009). The assumptions in the CCM are: (i) all surface complexes are inner-sphere complexes; (ii) the constant ionic medium reference state determines the activity of the aqueous species and therefore no surface complexes are formed with ions from the background electrolyte; (iii) one plane of charge represents the surface; (iv) the surface potential, ψ_0 , is related linearly to the surface charge density, σ_0 , (where o represents the surface plane), by the capacitance of the bacterial surface (Equation 2.1). This linear relationship is limited to high ionic strength conditions and generally cannot be extended to dilute electrolyte solutions. In the CCM, the intrinsic discrete acidity constant of a site is shifted, and its pH range of influence broadened, by an electrostatic potential, ψ , at the so-called bacteria/water interface as shown in Equation 2.2

$$\sigma_0 = \frac{CSa}{F} \psi_0 \quad [2.1]$$

$$K_{\text{int}} = K \exp^{(-ZF\psi/RT)} \quad [2.2]$$

Where C is the capacitance density ($F \text{ m}^{-2}$), S is the surface area ($\text{m}^2 \text{ g}^{-1}$), a is the suspension density (g L^{-1}), F is the Faraday constant (C mol_c^{-1}), σ_0 has units of $\text{mol}_c \text{ L}^{-1}$ and ψ_0 , has units of V , K_{int} is the intrinsic stability constant referenced to zero surface charge and zero surface coverage, Z refers to the charge of the adsorbing ion, R the molar gas constant, and T the absolute temperature.

One other electrostatic model that has been frequently used account for bacteria surface double layer interactions is the Donnan electrostatic model (Ohshima and Kondo, 1990; Plette et al., 1995; Wasserman and Felmy, 1998; Martinez et al., 2002; Yee et al., 2004; Burnett et al., 2006; Hetzer et al., 2006; Heinrich et al., 2008). This model invokes the assumption that the cell wall is

an ion-penetrable volume composed of homogeneous cross-linked ionizable functional groups. In addition, this model assumes that all counter ions necessary to balance the bacterial surface charge are present within the hydrated bacterial cell wall volume and the Donnan volume can be estimated, directly measured, or empirically-derived (Marinsky and Ephraim, 1986). The deprotonation of these functional groups forms an electrical potential which extends across the membrane to the interface between the cell wall surface and bulk solution. However, the extent to which Donnan potential theory can describe metal sorption data remains poorly understood. Moreover, due to the large uncertainties associated with the estimation of bacterial cell geometry parameters, particularly bacteria consortia, the applicability of this model in describing metal adsorption data remain questionable (Borrok and Fein, 2005).

Non-Electrostatic Model (NEM)

The non-electrostatic model (NEM) excludes explicit electrostatic terms from the mass law equations for surface equilibria, such that all chemical and electrostatic interactions are implicitly included in the equilibrium constant for the reaction (i.e, the free energy of sorption). This approach treats surface functional groups in the same manner as dissolved ligands or complexing metal ions but without a separate activity coefficient correction for the surface complex (Davis and Kent, 1990). In addition, because of neglect of the electrostatic effects, the results generated are conditional on ionic strength and the stability constants determined represent apparent constants, not intrinsic constants (Cox et al., 1999). A number of researchers have successfully used the NEM approach to describe the protonation and metal ion adsorption reactions at the bacterial cell surface and water interface (Cox et al., 1999; Borrok et al., 2004; Borrok et al., 2005; Fein et al., 2005; Pagnanelli et al., 2006; Johnson et al., 2007; Tourney et al., 2008; Ginn and Fein, 2009), where experiments were conducted at constant ionic strength conditions; and neglecting the electrostatic effects on the bacterial cell surface. However, in another study, Borrok and Fein (2005) used the NEM approach in comparison to electrostatic models to demonstrate that the ionic strength effect on bacterial surface proton binding reactions (over the range of 0.01 M to 0.5 M) is often relatively small and not significantly greater than experimental and modeling uncertainties. It was concluded in this study that the use of electrostatic corrections that rely on curve-fitting procedures with no external verification of some of the input parameters, e.g., capacitance or Donnan values, may not be justifiable.

2.5.5 Metal Desorption

Desorption of loaded biomass enables re-use of the biomass, and recovery and/or containment of sorbed materials, although it is desirable that the desorbing agent does not significantly damage or degrade the biomass. While the regeneration of the biosorbent may be accomplished by washing the metal-laden biosorbent with an appropriate desorbent solution, including dilute mineral acids, alkalis, and complexing agents, however, the type and strength of this solution depend on the extent of binding of the deposited metal (Puranik and Paknikar, 1997). One commonly used procedure that is designed to partitioning different trace metals based on their chemical nature is sequential extraction (Tessier et al., 1979). Separating these fractions using sequential extraction methods makes it possible to study the speciation and possible association between metals and sorbent. Traditionally, sequential extraction has been widely used in soil and sediment studies; however, recently, it has also been used to chemically leach metals out of sludge samples, and various sorbents (De Rome and Gadd, 1987; Mattuschka and Straube, 1993; Pagnanelli et al. 2002).

Generally, single extractants may broadly be divided into three main classes: (i) weak replacement of ion salts ($MgCl_2$, $CaCl_2$, NH_4NO_3), (ii) dilute solutions of either weak acids (acetic acid) or strong acids (HCl, HNO_3) and (iii) chelating agents (DTPA, EDTA). The weak replacement ion salts are able to release into solution metals which are associated with the exchange sites on the solid-phase and hence can be considered as bioavailable. The chelating agents, such as DTPA and EDTA, form complexes with free metal ions in solution and thus reduce the activities of the free metal ions in solution. The use of strong or dilute mineral acids is probably the most common method of metal desorption, and has been widely demonstrated. For example, Chandrashekar et al. (1998) conducted experiments to investigate the recovery of Ca, Fe and Ni ions from the loaded waste fungal biomass of *Aspergillus species* as a function of HCl concentration. The results revealed that with increase in HCl concentrations, metal desorption increased and at 5M HCl, complete removal of Ca and Fe was achieved while about 78% Ni was desorbed. Generally, this technique is easy to apply, inexpensive, and require little data analysis.

2.6 APPLICATION OF MICROBIAL TECHNOLOGY IN NUCLEAR WASTE MANAGEMENT

2.6.1 Scientific Framework of Technology

The distribution and diversity of microorganisms inhabiting contaminated sites and of the genes that code for phenotypes facilitating metal–microbe interactions are critical elements in metal and radionuclide bioremediation (Bazansak et al., 1999). Both a familiarity with the physiology of the active microbial populations and an understanding of how the relevant microbial processes can be manipulated *in situ* are crucial for the implementation of successful remedial strategies (Barkay and Schaefer, 2001). Recently, numerous authors have reported the presence of a wide range of indigenous microorganisms including; anaerobic denitrifiers, fermenters, sulphate reducers and methanogens from irradiated ecosystems (Bruhn et al., 1999; Kovacova et al., 2002; Chicote et al., 2004). In recent studies, the biostimulation and bioaugmentation concepts have been successfully implemented in laboratory scale investigations to promote the *in situ* immobilization of U, Tc, Cr, and other harmful metals generated from the nuclear power industry (Kumar et al., 2007). Viable microbial populations have been found in the waters of spent nuclear fuel pools, walls of a pool storing nuclear materials at a Spanish nuclear power plant; (Chicote et al., 2004) and in an underground granitic rock nuclear waste repository in Canada, where a range of heterotrophic aerobic and anaerobic bacteria ranging from 10^1 to 10^6 cells/g dry weight buffer were found. Among these were approximately 10^2 sulphate reducing bacteria (SRB) cells and methanogenes per gram of dry weight buffer, in spite of the oligotrophic and radioactive environment (Kovacova et al., 2002; Sarro et al., 2005). The survival of these microorganisms in such conditions is due to their resistance to gamma radiation, UV, H_2O_2 , and desiccation. These microorganisms have also been found to be capable of the selective accumulation of a range of metals and radionuclides including; Mn, Zn, Cs, U, and Co (Romanovskaia et al., 2002; Gadd, 2004).

Since then there has been ongoing research on the use of microorganisms, to treat radioactive waste of different radiation levels and origin. The SRB metal remediation concept is not new, however their occurrence in radioactive environments has aroused a renewed interest in their application for subsurface radioactive waste management, particularly, groundwater bioremediation. The main concept behind the bioremediation of contaminated groundwater is

through the *in situ* emplacement of an adsorptive coating with SRB as bio-adsorbents/detoxifying agents on the aquifer matrix to control radionuclides dispersion to the biosphere (Figure 2.4). The operational conditions and design of this subsurface microbial treatment technology is very similar to biological permeable reactive barrier (PRB) systems. A PRB is an engineered zone of reactive material placed in an aquifer that allows passage of groundwater while retaining or degrading the contaminants (Morrison et al., 2002). These techniques employ a wall of biological reactive media, installed in the path of a contaminated groundwater flow, to remove or treat groundwater contaminants *in situ*. The reactive media is permeable to the groundwater flow and as it moves horizontally through the barrier, driven by natural hydraulic gradients, a variety of natural microbiological processes remove the contaminants from the water. These processes include precipitation, biosorption, enzymatic reduction, or biodegradation. Details on the design and configurations for subsurface PRBs have been reviewed by numerous authors (Gibert et al., 2002; Amos and Younger, 2003).

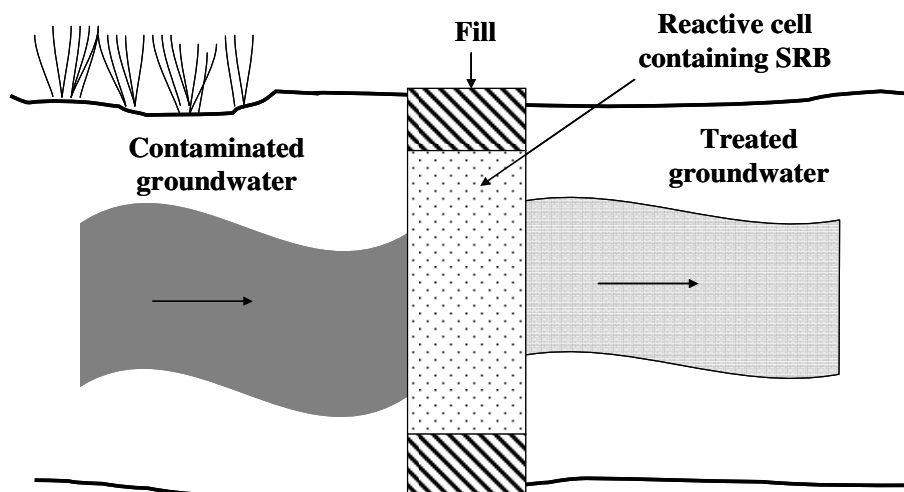


Figure 2.4: Elevated view of a permeable reactive barrier configuration for groundwater treatment. (Adapted from Gavaskar, 1999).

2.6.2 Prospects of Radionuclide Bioremediation in Passive Microbial Bioreactors

Passive bioreactors are preferred to traditional water treatment plant technologies because they allow higher metal removal at low pH and generate more stable sludge with lower operation costs and minimal energy consumption. By far, the remediation of groundwater contaminated as a result of radionuclide migration from repository sources remains one of the most intractable problems of environmental restoration, and as such one of the major challenges faced by the nuclear energy industry. Recently, innovative technologies, such as bioremediation, have been considered as one of the most promising solutions for the remediation of subsurface radionuclide contamination (Costa et al., 2008). The sustainability of the bioremediation system depends on a number of factors, which include; organic substrate sources and their degradation, effluent chemistry, microbiological diversity and activities and reactor configuration and hydraulics (Neculita et al., 2007). The mechanisms of metal removal for the passive bioreactor treatment system are very similar to biological permeable reactive barriers (PRBs). Therefore, in the context of PRB operation, natural biogeochemical processes form an integral basis for the successful operation of the bioremediation technology. Treatment in PRBs can be both abiotic and biotic; abiotic PRB treatment involves the use of neutralizing agents (such as lime), adsorbents and zero-valent iron (ZVI), which can be used as filling materials for metal sorption. On the other hand, in the biotic PRB system, natural microbial processes of SRB are exploited (Blowes et al., 2000; Gibert et al., 2002).

In microbial bioreactors, a wide range of reactions takes place which can remove metallic contaminants from water, and they include; precipitation in the form of sulphides, hydroxides, and carbonates; adsorption, surface precipitation, and polymerization on inorganic support, solid organic matter, bacteria, and metal precipitates are sorption mechanisms that can also occur. Adsorption of dissolved metals onto organic sites in the substrate material is an important process upon the start of a passive bioreactor (Pagnanelli et al., 2010). Over the years, metal adsorption onto microbial substrates (mainly, bacteria) has acquired great importance in existing efforts towards environmental bioremediation, therefore it should also be taken in account for future BPT and PRB designs to cater for a wider spectrum of metal/radionuclides, particularly those that cannot be precipitated into insoluble precipitates. Currently, this technology has been successfully used for the treatment of chlorinated aliphatic hydrocarbons, remediation of

chromium VI and the treatment of acidic mine drainage. Efforts for the adoption of this technique for subsurface radioactive waste management are still in the research phase.

2.6.3 Challenges and Future Perspectives

For *in situ* bioremediation to be successful, it is vital that the right microbes are in the right place with the right environmental factors for degradation to occur. In some cases, microbial metabolism of contaminants may produce toxic metabolites, therefore resulting in further environmental pollution. Consequently, bioremediation as a scientifically intensive procedure must be tailored to the site-specific conditions, which means that there, has to be feasibility studies on a small-scale before the actual clean-up of contaminated sites. However, demonstrated long term benefits of this technology compensate its limitations, and as such have paved the way for switching from the aggressive conventional spent fuel management practices into environmentally friendly and cost effective approaches. Development of novel and more efficient commercial bioremediation methods for spent fuel management are underway. In some countries, for example United States of America, these technologies are already in the testing phase and when integrated into existing waste management processes, they will greatly increase the efficiency and application of biological approaches in spent fuel management (Skeen et al., 2006).

The next generation bioremediation technologies include controlling and/or manipulating the subsurface microbiology via engineered bioremediation, relying on intrinsic bioremediation to prevent migration, and using *ex situ* bioprocesses in the treatment and separation of radionuclides from organic-metal waste mixtures. These may lead to the discovery of new physical mutagens that are more effective or have unique properties. These methods have the potential to reduce the cost of operations by several magnitudes, and simplify the overall process of SNF mgt. Some of the promising strategies for *in situ* metal bioremediation are biostimulation and bioaugmentation, and it has been reported that the addition of appropriate carbons sources to the contaminated aquifer effectively stimulates the growth of bacteria, responsible for controlling the migration of radionuclides *in situ* (Ortiz-Bernad et al., 2004).