CHAPTER 5

KINETIC AND EQUILIBRIUM STUDIES FOR Sr^{2+}, Co^{2+} and Cs^{+} UPTAKE ONTO SRB CELLS

5.1 BACKGROUND

Theoretically, metal sorption by bacteria is assumed to be a function of: (i) cell surface properties, such as charge and orientation of the functional groups on the cell surface; (ii) metal speciation in the aqueous phase; and (iii) release of metal-complexing exudates (Volesky and Holan, 1995). In addition, metal sorption also depends on the metal involved and the composition of the system, for instance, pH, ionic strength, temperature, metal to bacteria ratio (Gadd, 1986; Volesky, 1990; Daughney and Fein, 1998; Wang and Chen, 2007; Vijayaraghavan and Yun, 2008). However, up till now, metal sorption by bacteria (under constant or different experimental conditions) is still poorly understood (Vijayaraghavan and Yun, 2008). To enhance our understanding on biosorption, knowledge of the thermodynamic and kinetic aspects of the sorption process should be established so to know more about its performance and mechanisms. From a mechanistic point of view, the prediction of the sorption mechanism or rate-limiting step is crucial for interpretation of the experimental data. Biosorption kinetics are significant as the data can be used for determining the equilibration time of the biosorption process, as well as in understanding the mechanisms and effect of different environmental factors on the biosorption process (Sarkar et al., 2003). In an attempt to clarify the mechanism of Sr^{2+} , Cs^{+} and Co^{2+} removal from aqueous solution by SRB cells, and identify the main factors controlling sorption rate such as; mass transport, pore diffusion and chemical reaction processes, the pseudo-first-order, pseudo-second-order (Lagergren, 1898), and external and intraparticle diffusion (Weber and Morris, 1963) kinetic models were evaluated. On the other hand, the classical Langmuir (Langmuir, 1918) and Freundlich (Freundlich, 1907) equilibrium sorption isotherms were used to evaluate the overall biosorption performance of the bacterial biosorbent.

In order to clarify the molecular-scale adsorption reactions that occur between aqueous solutes (protons and Sr^{2+}, Cs^{+} and Co^{2+}) and the bacterial surface, and quantify metal adsorption onto the bacterial cell surfaces, a surface complexation modeling approach was used. Prior to SCM

development, preliminary biomass characterization studies were carried out using a combination of Gram-staining, SEM, phylogenetic analysis, potentiometric titrations and FTIR analysis. Biomass characterization results on Gram-staining result, morphological and phylogenetic nature of the present culture were discussed in the previous Chapter. Forward and reverse titration curves of the bacteria showed that the present SRB consortium displayed a significant buffering behaviour over the pH range 3.5-10.3 (Figure 3, Appendix 2). The significant buffering may be due to the presence of different functional groups on the bacterial surface consuming the added base by donating protons, as there was no evidence of saturation observed. FT-IR spectroscopy provides chemical information about the biomolecular composition of whole bacterial cells. Since this technique probes the whole cell, so that the structures uniquely associated with the cell wall cannot be assessed in isolation of all of the cytoplasm, results obtained are for bacterial characterization purposes due to the high specificity of the obtained spectra. The FTIR spectrum obtained indicated the presence of different functional groups, including organic sulphates, hydroxyl, aromatic phosphates and phosphodiester, primary and secondary amines and carboxylic groups (Table 2, Appendix 2). These results serve as a guidance for the identification of the different possible mechanisms (physical adsorption, complexation, ionic exchange, surface micro-precipitation) operating at the cell surface-metal solution interface, and aid the identification of the active sites on bacterial cell wall(Haas, 2004).

5.2 KINETIC STUDIES OF Sr^{2+}, Cs^{+} AND Co^{2+} BIOSORPTION

5.2.1 Effect of Initial Concentration

The effect of initial concentration on the kinetics of Sr^{2+}, Cs^{+} and Co^{2+} biosorption from aqueous solution was investigated at a concentration range of 25-500 mg/L. The results obtained from experimental controls, which consisted of SRB cells suspended in aqueous solution without metal and aqueous metal solution but without bacterial inoculum (abiotic control), showed that metal losses due to abiotic processes was insignificant, and that the SRB cells did not release any metal ions into solution. Therefore, the observed metal removal was due to uptake by the bacterial cells. When the experimental data for Sr^{2+}, Cs^{+} and Co^{2+} sorption at different initial concentrations were analysed with the Lagergren pseudo-first-order kinetic model, poor model fits were obtained (Table 5.1). This is because this model fails to account for the slower phase of metal biosorption (Ho and McKay, 1998; Ho and McKay, 2000; Aksu, 2001).

Table 5.1 Pseudo-first order model parameters for the effect of initial concentration on the kinetics of Sr^{2+}, Cs^{+} and Co^{2+} removal in single metal solutions.

C_o	Sr^{2+}			Co^{2+}			Cs^{+}		
(mg/L)	q_{eq}	$K_1(\times10^{-2})$	R^2	q_{eq}	$K_1(\times10^{-2})$	R^2	q_{eq}	$K_1(\times10^{-2})$	R^2
25	12.7	1.22	0.780	16.4	1.08	0.964	3.32	0.88	0.525
75	35.9	1.64	0.835	36.0	1.49	0.946	33.1	1.66	0.819
100	30.0	1.51	0.823	27.1	1.13	0.949	35.5	1.08	0.962
300	190.6	1.83	0.958	58.6	1.50	0.938	81.5	1.66	0.939
500	330.3	2.11	0.950	192.5	1.97	0.917	9.67	1.20	0.689

However, good conformity between experimental data and model results was observed upon analysis with the pseudo-second-order kinetic model, suggesting that the adsorption is the rate controlling step (Ho and Mckay, 1998). Generally, the equilibrium metal uptake capacity (q_{eq}) of the bacteria increased with increasing initial concentration, clearly demonstrating that the initial concentration was the driving force for the mass transfer of Sr^{2+}, Cs^{+} and Co^{2+} onto the bacteria (Table 5.2). The adsorption rate constant (k_2) for Sr^{2+} and Cs^{+} decreased with increasing initial concentration. These observations imply that the biosorption of these metal ions is concentration dependent, where at higher initial concentrations; the ratio of available adsorption sites to metal ions is less and as the binding sites saturates the rate of biosorption declines. Similar results have been reported elsewhere in literature (Aksu, 2001; Shaukat et al., 2005; Chen et al., 2008; Chegrouche et al., 2009; Ahmadpour et al., 2010). The experimental and model representations of the data for removal of Sr^{2+}, Cs^{+} and Co^{2+} by SRB at different initial concentration are shown in Figure 5.1.

Table 5.2 Pseudo-second order model parameters for the effect of initial concentration on the kinetics of Sr^{2+}, Cs^{+} and Co^{2+} removal in single metal solutions.

C_o	Sr^{2+}			Co^{2+}			**Cs^{+}**		
(mg/L)	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2
25	40.3	33.2	0.998	28.3	10.0	0.994	13.0	82.8	0.999
75	135.1	11.4	0.999	78.4	9.38	0.999	77.6	10.1	0.999
100	147.1	1.34	0.999	78.1	13.3	0.999	92.3	9.39	0.998
300	256.4	1.65	0.997	93.7	5.01	0.994	128.4	3.15	0.994
500	370.3	0.798	0.995	188.9	1.13	0.979	66.7	10.4	0.999

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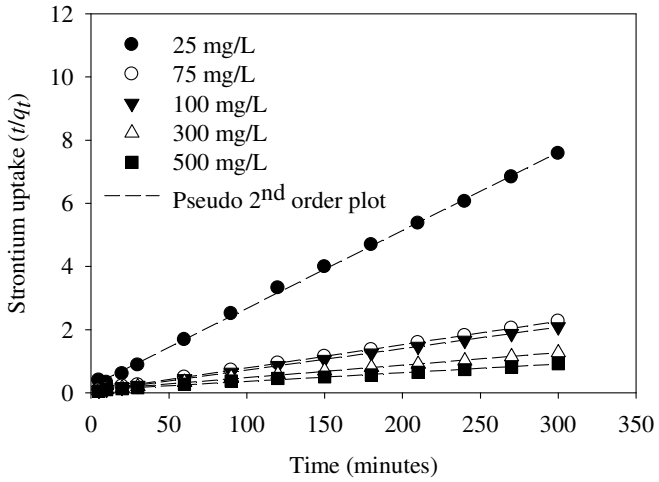

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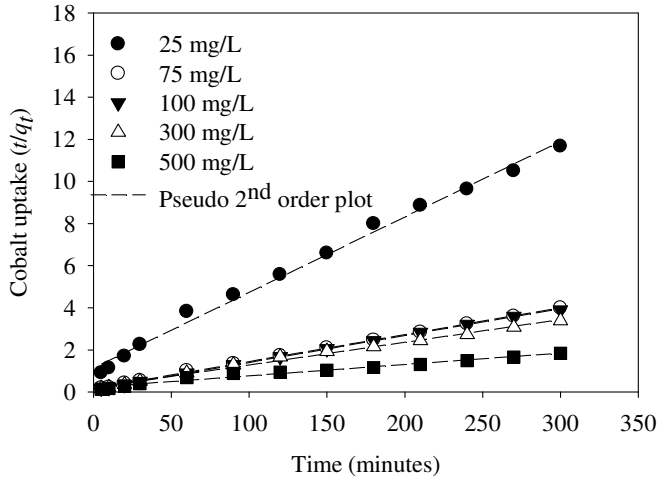

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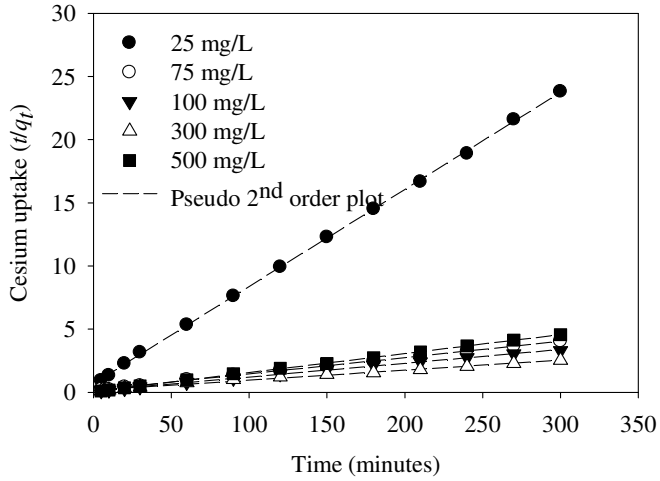

Figure 5.1: Pseudo-second-order model plot for the effect of different initial metal concentrations on the removal of Sr^{2+} (A), Co^{2+} (B) and Cs^{+} (C) from solution by SRB.

The conformity of the data to the second-order rate model suggests that the present biosorption process occurred through electrostatic electron exchange between the bacterial sorbent and the metal. Generally, the uptake of metals by SRB was observed in the order Cs<Co<Sr. The obtained results suggest that the present metal removal process is a second-order reaction. It can therefore be assumed that the rate limiting step of the metal removal process is chemisorption. When the data was analysed with the diffusion (external and intraparticle) models, it was apparent that the sorption of Sr^{2+}, Cs^{+} and Co^{2+} occurred only on the external surface of the SRB cells. The external diffusion model shows excellent correlation with the experimental sorption data, with high correlation coefficients obtained as shown in Table 5.3. This suggests that metal sorption is probably a surface process occurring on the exterior of the sorbent particle. These results are in agreement with earlier observations by Ho and McKay (2000) and Mullen et al. (1989), where chemical sorption was determined to be the underlying mechanism for metal removal from solution. The external diffusion coefficient (k_f) values decreased with an increase in the initial metal concentration. This may be due to the lower competition for the sorption surface sites at lower concentration. At higher concentrations, the competition for the surface active sites will be high and consequently lower sorption rates are obtained.

Table 5.3 External diffusion parameters for the effect of initial concentration on the kinetics of Sr^{2+}, Cs^{+} and Co^{2+} removal in single metal solutions.

C_o	Sr^{2+}		Co^{2+}		Cs^{+}	
(mg/L)	k_f ($\times 10^{-4}$)	R^2	k_f($\times 10^{-4}$)	R^2	k_f($\times 10^{-4}$)	R^2
25	5.91	0.971	2.00	0.982	1.18	0.923
75	5.36	0.985	2.18	0.972	2.09	0.955
100	4.09	0.988	1.91	0.942	2.09	0.979
300	1.55	0.974	0.727	0.962	0.727	0.973
500	1.00	0.911	0.455	0.922	0.364	0.964

5.2.2 Effect of pH

Solution pH is a measure of the concentration of H+ in solution, and H+ can compete with the cations in aqueous solution for sorption sites on the cells. Hence lower pH (high H+ concentration) leads to less adsorption of other cations. In addition, pH also affects the surface charge of the adsorbent, degree of the ionization, and speciation of the adsorbate (Choudhary and Sar, 2009). Speciation of Sr^{2+}, Cs^{+} and Co^{2+} at pH 2-9 was determined using the chemical speciation model MINTEQ (Allison et al., 1990). At an initial concentration of 75 mg/L and pH range 2-9, results obtained showed that most of Sr (99.8%) and Cs (99.96%) species in solution were present in their highly dissociated forms (Sr^{2+} and Cs^{+}, respectively). Only about 0.2% and 0.04% existed as $SrCl^{+}$ and CsCl, respectively. This observation strongly suggests that both cations undergo limited hydrolysis, which is in agreement with earlier findings by Baes and Mesmer (1976).

With regard to cobalt speciation, both initial concentration and pH played a significant role in the speciation of Co^{2+} in solution. Generally, an increase in pH resulted in the decrease in the highly dissociated Co species. At low pH (pH 2-4), Co species were present as 99.7% Co^{2+} and 0.3% $CoNO_3^{+}$. At near neutral pH (pH 5-7) slight precipitation occurred as Co species were distributed as follows: 99.68% Co^{2+}, 0.3% $CoNO_3^{+}$ and 0.016% $CoOH^{+}$. Increasing the pH to 8 resulted in Co^{2+} precipitation due to the formation of $Co(OH)_2$ (0.12%), whereas the rest of the Co species were distributed as follows: ~97% Co^{2+} and 2% $CoOH^{+}$. Further increase in pH to 9, resulted in increased precipitation with approximately 10% of the initial concentration present as $Co(OH)_2$, 77% as Co^{2+}, 0.3% as $CoNO_3^{+}$, 13% $CoOH^{+}$ and 0.1% as $Co_4(OH)_4^{4+}$. These findings are also in agreement with earlier studies on Co^{2+} hydrolysis reported in literature (Baes and Mesmer, 1976).

The pseudo-second-order model provided the best fit for effect of pH on Sr^{2+}, Cs^{+}, and Co^{2+} removal by SRB, and the obtained parameters are shown in Table 5.4. Higher removal capacity was coupled to lower rate constants for Sr^{2+}. Similarly, the fit of the data to the pseudo-second-order model is in agreement with a mechanism where adsorption is the main rate controlling step. Comparisons between experimental and model data for removal of Sr^{2+}, Cs^{+} and Co^{2+} by

SRB at different pHs (2-9) is shown in Figure 5.2. Sr^{2+} sorption by SRB was pH independent as there were minimal differences in the uptake capacities at different pH ranges. The low initial Sr^{2+}concentrations resulted in an almost similar removal capacity at the different pH values. The present SRB culture has demonstrated a unique high Sr^{2+} binding at the different pH values. Cs^{+} removal, on the other hand was dependent on the solution pH. High Cs^{+} removal was observed between pH 2 and 6, and thereafter an increase in pH resulted in decreased uptake. This is a common feature whenever sorption is pH dependent as functional groups with low pKa values such as a carboxyl (pKa 3.0–4.0) with other side chain carboxyl groups (pKa 4.0–4.5) become deprotonated with increasing pH. The deprotonation of bacterial surface sites generates a net negative charge at the surface, which favours the binding of cationic species (Fein et al., 1997; Douglas and Beveridge, 1998). Similarly, for Cs^{+}, the pH dependent uptake can be attributed to the deprotonation of cell wall functional groups that occurs with increasing pH, progressively resulting in increased Cs^{+} uptake capacity until all the binding sites are saturated. Solution pH thus influences the main mechanism of removal of cations from solution, and consequently the sorption capacity of the cationic species from solution.

Table 5.4: Pseudo-second order model parameters for the effect of pH on the kinetics of Sr^{2+}, Cs^{+} and Co^{2+} removal in single metal solutions.

pH	Sr^{2+}			Co^{2+}			Cs^{+}		
	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2
2	134.6	6.374	0.998	78.6	9.202	0.999	76.4	10.9	0.999
4	132.5	6.191	0.997	78.7	9.076	0.999	75.5	9.81	0.999
6	137.1	4.845	0.996	82.3	8.846	0.998	77.0	10.3	0.999
7	137.8	5.044	0.996	85.8	14.7	0.999	76.3	8.77	0.998
8	138.9	4.751	0.996	91.1	8.26	0.998	73.4	11.0	0.999
9	139.8	4.873	0.996	119.1	4.247	0.996	65.5	9.42	0.999

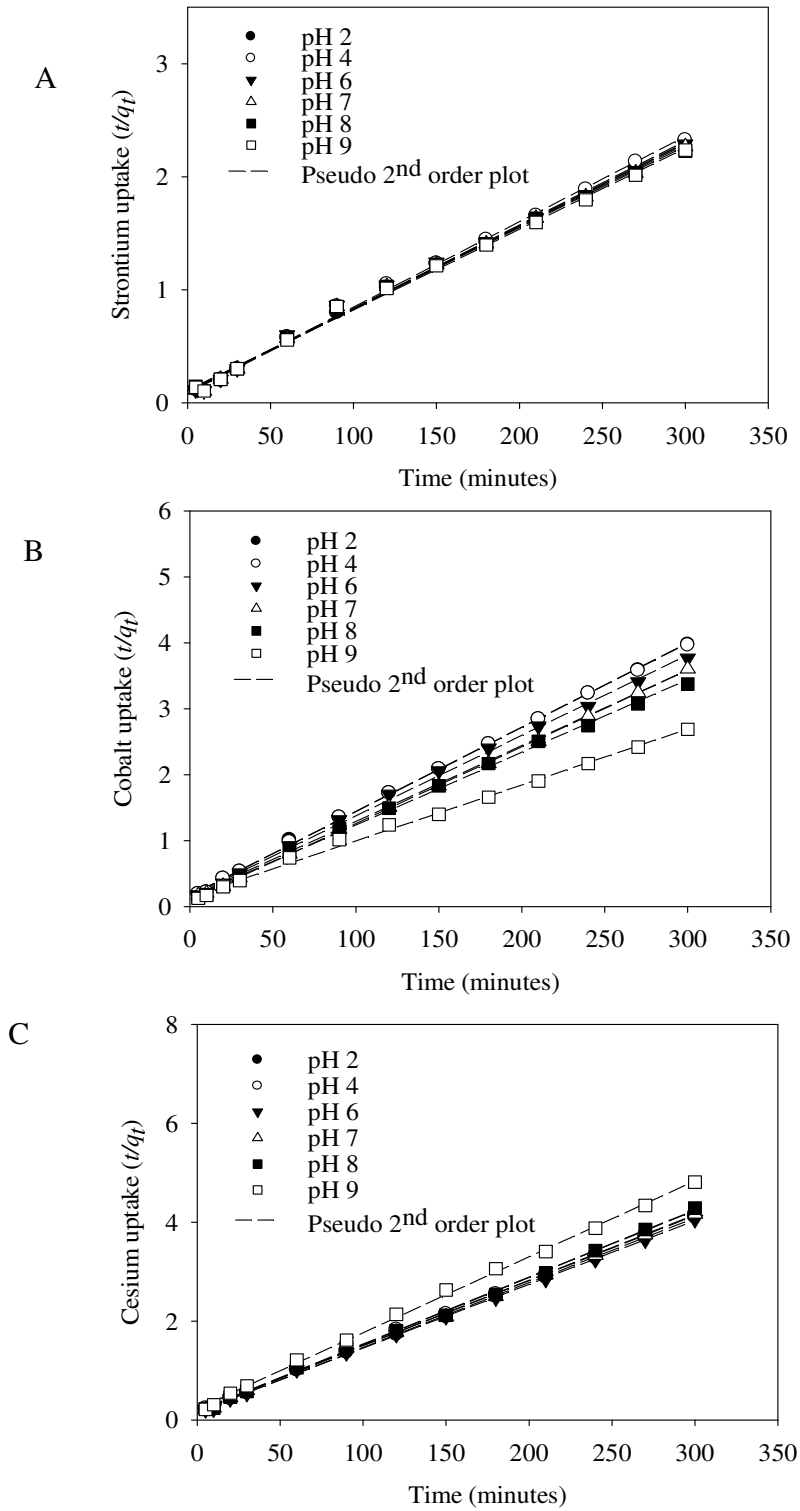

Figure 5.2: Pseudo-second-order model plot for the effect of pH on the removal of Sr^{2+} (A), Co^{2+} (B) and Cs^{+} (C) from solution by SRB.

5.2.3 Effect of Sorbent Dose

The 'best fit' of the experimental data obtained was again obtained when the data was analysed using the pseudo-second order model. Table 5.5 is a summary of the parameters obtained for the effect of sorbent dose on the sorption kinetics of Sr^{2+}, Cs^{+} and Co^{2+} from aqueous solutions at a fixed initial concentration of 75 mg/L and pH 4.

Table 5.5 Pseudo-second order model parameters for the effect of sorbent dose on the kinetics of Sr^{2+}, Cs^{+} and Co^{2+} removal in single metal solutions.

Dose (gL^{-1})	Sr^{2+}			Co^{2+}			Cs^{+}		
	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2
0.5	10.7.7	23.8	0.999	78.3	9.76	0.999	80.0	7.01	0.999
1.0	57.6	24.0	0.999	78.4	9.93	0.999	77.6	10.2	0.999
2.0	29.9	32.4	0.996	82.0	9.52	0.998	91.2	10.7	0.998
3.0	21.5	21.1	0.970	86.0	14.0	0.999	126.4	3.76	0.993

Figure 5.3 shows the comparisons between experimental and model data for removal of Sr^{2+}, Cs^{+} and Co^{2+} by SRB at different initial SRB dosage. Evidently, an increase in the biomass concentration resulted in an increased equilibrium metal uptake, and similar results have been reported by a number of authors, including; Gadd et al. (1988); Fourest and Roux (1992); Daughney et al. (1998); Esposito et al. (2001); Daughney et al. (2001); Burnett et al. (2007) and Ahmadpour et al. (2010). This can be attributed to the increased surface area, which in turn increases the number of binding sites (Esposito et al., 2001). A higher cell concentration is also expected to be accompanied by an increased accumulation of cellular metabolites, which further facilitate metal ion precipitation. However, in order to achieve a cost effective sorption process without drastically reducing the metal uptake efficiency, a lower biomass could be used. Additionally, in some cases increasing the biomass concentration does not necessarily result in remarkably increased metal uptake as larger amounts of biomass have been found to interfere with metal binding sites as they tend to agglomerate (Gadd et al., 1988).

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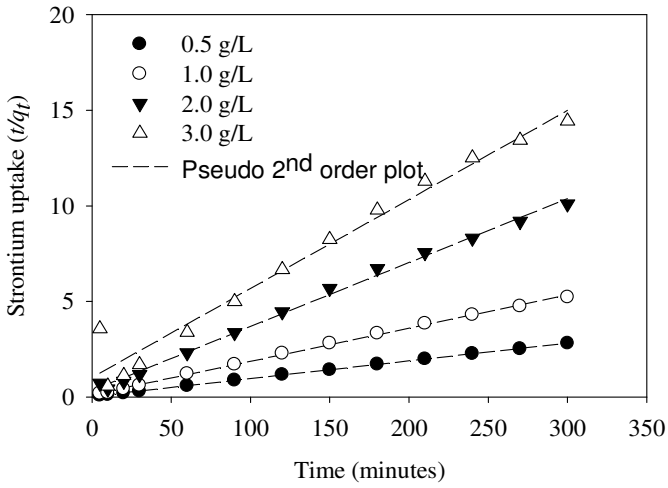

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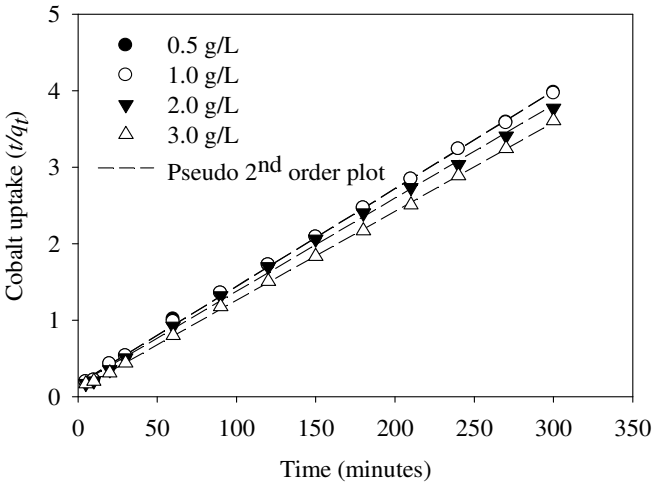

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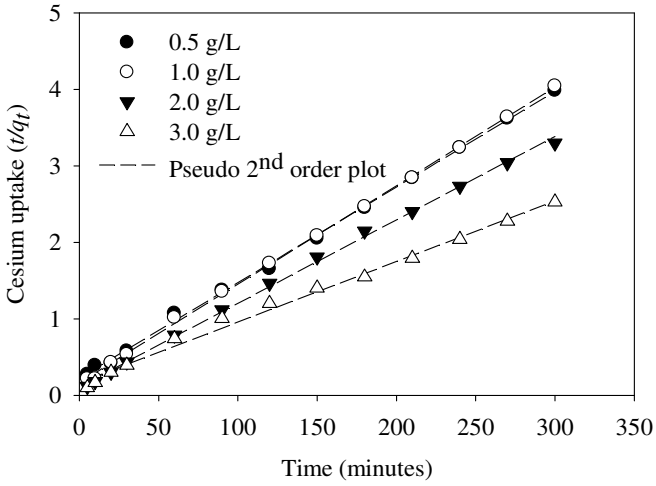

Figure 5.3: Pseudo-second-order model plot for the effect of sorbent dose on the removal of Sr^{2+} (A), Co^{2+} (B) and Cs^{+} (C) from solution by SRB.

5.2.4 Effect of Metabolic State

To determine the effect of metabolic state on Sr^{2+}, Cs^{+} and Co^{2+} uptake, each SRB biomass type (at a biomass density of 1 g/L) was exposed to an aqueous solution containing the target metal at an initial concentration of 75 mg/L. Kinetic parameters obtained after analysis of the data by the pseudo-second order model are shown in Table 5.6. The results obtained suggest that the effect of metabolic state on Sr^{2+}, Cs^{+} and Co^{2+} biosorption also follows a pseudo-second order process, since there was a precise match between the experimental and the calculated q_{eq} values, and higher correlation coefficient values. These results support an earlier suggestion that chemical sorption is the main rate-controlling step.

Table 5.6 Pseudo-second order model parameters for the effect of metabolic state on the kinetics of Sr^{2+}, Cs^{+} and Co^{2+} removal in single metal solutions.

Metabolic State	Sr^{2+}			Co^{2+}			Cs^{+}		
	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2	q_{eq}	$k_2(\times10^{-4})$	R^2
Heat-killed	72.1	9.5	0.999	36.9	26.0	0.998	47.6	14.9	0.999
Growing	130.9	7.37	0.997	76.3	6.16	0.990	55.6	13.7	0.996
Non-growing	114.4	14.3	0.998	73.7	10.8	0.998	68.6	11.8	0.996

At the end of the incubation period, a higher Sr^{2+} uptake efficiency (86%) was achieved with growing SRB cells, followed closely by resting cells (77%), and lastly heat-killed SRB cells (48%). There was a close match between the experimental data and model data (Figure 5.4), and the similarity in the sorption capacities (q_t) by growing and resting SRB biomass for the first 90 minutes of incubation suggest that during the initial phases of sorption living SRB cells employ almost similar sorption mechanisms for metal ions uptake. The additional sorption observed thereafter in the growing cells can be attributed to other metabolism-related Sr^{2+} precipitation strategies discussed in the previous chapter. For instance, as part of their metabolism actively growing SRB cells produce biogenic ligands which are responsible for further precipitation of metal ions into insoluble forms. The sorption process by dead cells was characterized by a rapid initial uptake in the first 30 minutes of incubation, and thereafter a slow phase until equilibrium was reached, a common phenomenon also reported in other studies (Choi and Yun, 2004; Lu et al., 2006). Evidently, live cells have consistently displayed high metal removal capacities, whereas non-viable bacteria cells performed poorly (Parmar et al., 2000).

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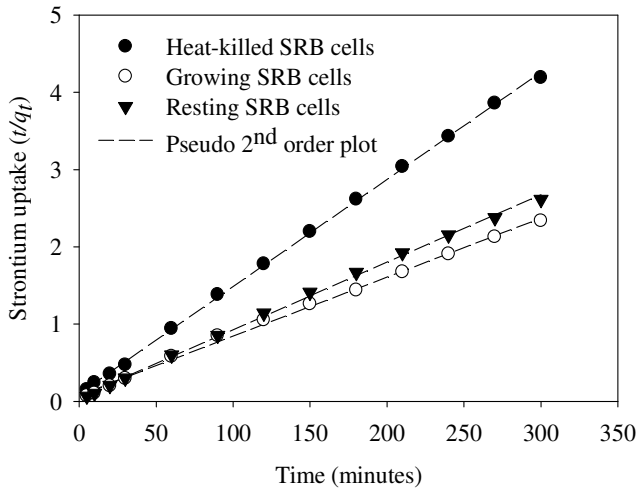

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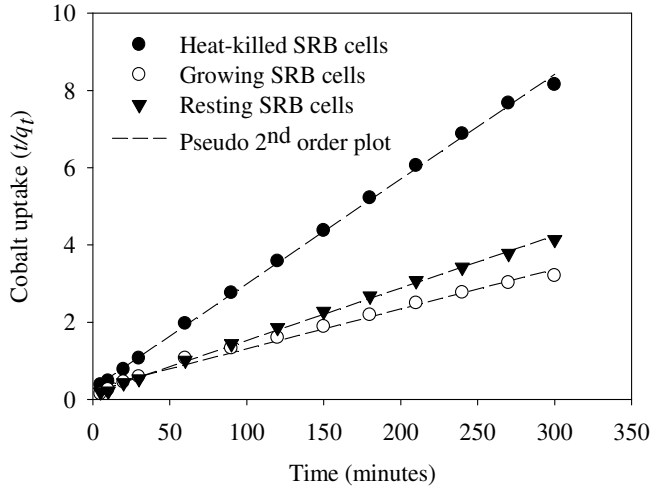

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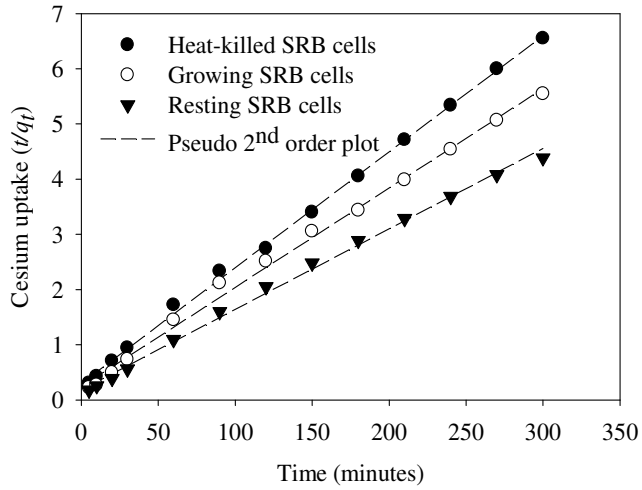

Figure 5.4: Pseudo-second-order model plot for the effect of metabolic state on the removal of Sr^{2+}(A), Co^{2+} (B) and Cs^{+} (C) from solution by SRB.

5.3 EQUILIBRIUM Sr^{2+}, Co^{2+} and Cs^{+} BIOSORPTION

5.3.1 Sr^{2+}, Co^{2+} and Cs^{+} Biosorption from Single Metal Solutions

Equilibrium sorption isotherms are empirical models that provide basic information on a given system and are useful for comparing different biosorbents and affinities of different metal ions for the same biosorbent (Gadd, 2009). The Langmuir isotherm model provided the best fit for the equilibrium data for Sr^{2+}, Co^{2+}, and Cs^{+} removal from single metal systems by SRB as shown by the higher R^2 values than in the Freundlich model (Table 5.7). However, the parameters obtained from experimental data analysis with the Freundlich give an indication of the non-heterogenecity of the sites involved in the sorption process, as n>1, suggesting a monolayer sorption process.

Table 5.7 Langmuir and Freundlich model parameters for the removal of Sr^{2+}, Cs^{+} and Co^{2+} in single metal solutions.

Metal ion	Langmuir model			Freundlich model		
	q_{max} (mg g^{-1})	b ($\times 10^{-2}$)	R^2	k	n	R^2
Sr^{2+}	405.5	1.95	0.974	52.3	3.28	0.955
Cs^{+}	192.9	1.55	0.995	13.0	2.19	0.981
Co^{2+}	203.3	1.19	0.961	16.2	2.57	0.882

The Langmuir model allows the determination of a very important parameter, the maximum sorption capacity (q_{max}), which is useful for comparison of sorption capabilities. Consequently, the results for the equilibrium sorption process for Sr^{2+}, Co^{2+}, and Cs^{+} in single metal systems are discussed based on the parameters obtained from the Langmuir model plots. The maximum removal capacity (q_{max}) was almost double for Sr^{2+} compared to Cs^{+} and Co^{2+}. Similarly, the binding affinity coefficient (b) was higher for Sr^{2+} compared to Cs^{+} and Co^{2+}. The SRB culture in this study exhibited a superior Sr^{2+} binding capacity, compared to other studies in literature as shown in Table 5.8. Comparisons between the experimental data and the Langmuir and Freundlich isotherm model plots are shown in Figure 5.5. The isotherm model plots obtained for the removal of Sr^{2+}, Co^{2+} and Cs^{+} from single metal systems by SRB suggest that the sorption process conforms to a monolayer binding process, since the best fit was obtained with the Langmuir model (Kratochvil and Volesky, 1998).

Table 5.8 Equilibrium sorption performances of various sorbents for Sr^{2+}, Co^{2+} and Cs^{+} uptake from aqueous solution.

Metal ion	q_{max} (mg g^{-1})	Sorbent	Reference
Sr^{2+}	**405.5**	**SRB consortium**	**This study**
	140.0	Pakistani coal	Shaukat et al., 2005
	55.0	*Bacillus sp.*	Tajer et al., 2007
	26.67	*Cystoseira indica*	Dabbagh et al., 2007
	12.89	*Amaranthus spinosus*	Chen, 1997
Cs^{+}	**192.9**	**SRB consortium**	**This study**
	14.5-71.9	Marine algae species	Jalali-Rad et al., 2004
	28.6	*Bacillus polymyxa*	Shevchuk and Klimenko, 2009
Co^{2+}	468.75	*Oscillatoria sp*	Ahuja et al., 1999
	203.3	**SRB consortium**	**This study**
	190	*Rhizopus sp*	Suhasini et al., 1999
	8.42	Sludge	Van Hullebusch et al., 2004

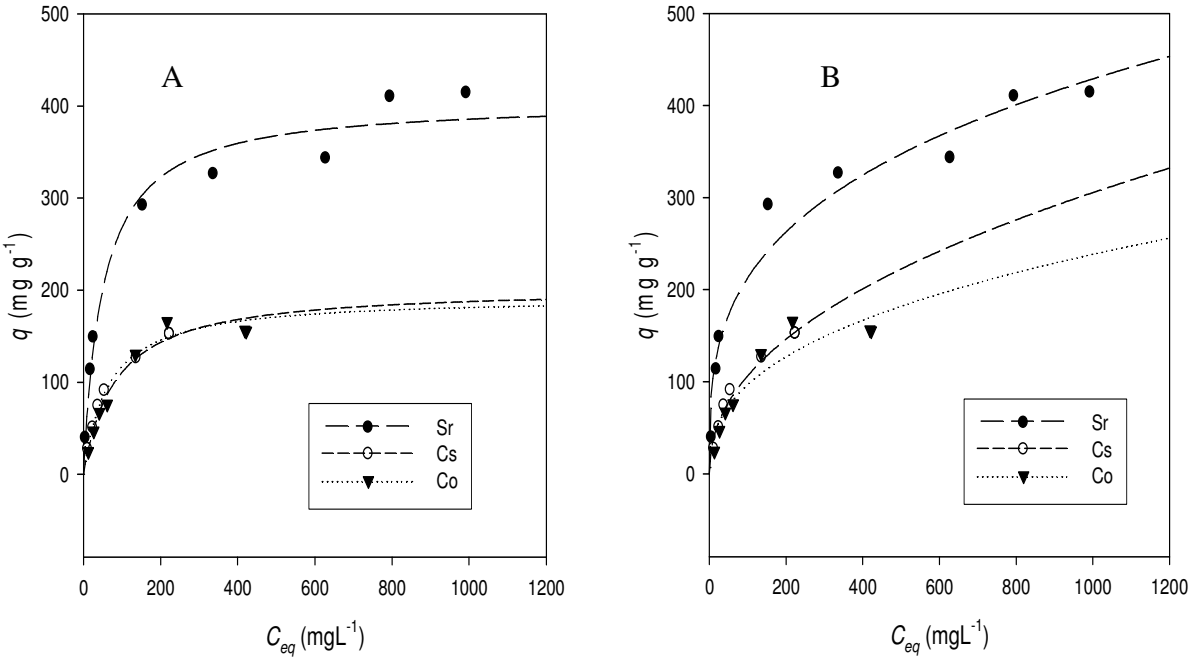

Figure 5.5: Langmuir (A) and Freundlich (B) isotherm plots for Sr^{2+}, Co^{2+} and Cs^{+} sorption by SRB at a biomass density of 0.5 g/L. (pH = 4 and temperature = 25°C).

5.3.2 Competitive Binding of Sr, Cs And Co in Binary Metal Solutions

The effect of a secondary metal ion on metal uptake by SRB was investigated in binary metal solutions to investigate the specificity of the uptake process. The Langmuir isotherm model was used to interpret the binary-component equilibrium sorption data, and the parameters obtained are shown in Table 5.9. The results show a 33% and 34% loss in the Sr^{2+}removal capacity (q_{max}) in the presence of Cs^{+} and Co^{2+}, respectively. In the presence of Co^{2+} and Sr^{2+}, the Cs^{+} removal capacity was lowered by 55% and 45%, respectively. Among the binary systems investigated, Co^{2+} uptake was the most sensitive to the presence of Cs^{+}, where a 76% reduction of the sorption capacity (q_{max}) was observed. However, in the presence of Sr^{2+}, only a 9% loss of removal capacity was observed. With regard to the results, the following inferences can be made: (1) the present SRB culture demonstrated a high Sr^{2+} affinity. This is evident in all the binary metal solutions investigated in this study, (2) specialized binding-sites/processes specifically for divalent metals (Sr^{2+} and Co^{2+}) removal may exist. This is supported by minimal inhibitory effects by the presence of a second cation, with the exception of the Co-Cs system, (3) No specific binding sites for Cs^{+} may exist. This is indicated by the inhibition of Cs^{+} removal, in the presence of other cations. A similar phenomenon of competitive biosorption has been reported for the binary adsorption of Pb^{2+} and Cu^{2+} onto *Aspergillus flavus*, where it was shown that the biosorption capacities of the metal ions in the binary metal mixture were lower than that of noncompetitive conditions (Akar and Tunali, 2006). Similarly, the same observations were made for the competitive adsorption of Cd^{2+} and Pb^{2+} (Fan et al., 2008). The results clearly demonstrate the antagonistic effect of multiple metal ions in a biosorption system, which is a result of the competition for adsorption sites on the cell surfaces and/or the screening effect by the competing metal ions.

Table 5.9 Equilibrium sorption parameters for Sr^{2+}, Cs^{+} and Co^{2+} uptake from binary systems.

Metal system	Component	Langmuir			Freundlich		
		q_{max} (mg g^{-1})	$b(\times 10^{-2})$	R^2	k	n	R^2
Sr-Cs	Sr^{2+}	277.8	5.72	0.985	42.7	3.20	0.871
	Cs^{+}	107.5	3.06	0.938	18.0	3.34	0.670
Cs-Co	Cs^{+}	131.6	2.52	0.958	15.6	2.76	0.794
	Co^{2+}	49.3	3.40	0.986	7.55	2.89	0.847
Co-Sr	Co^{2+}	185.2	2.19	0.950	27.9	4.09	0.601
	Sr^{2+}	274.8	7.41	0.974	44.8	3.16	0.889

5.4 ADSORPTION OF PROTONS AND Sr^{2+}, Co^{2+} AND Cs^{+} ONTO SRB CELL SURFACES

5.4.1 Surface Complexation Modelling Approach

A number of acid-base surface complexation models (SCM) have been applied to predict the identities and concentrations of functional groups involved in metal adsorption on the bacterial surfaces (Fein et al., 1997; Daughney et al., 2001; Haas et al., 2001; Yee and Fein, 2001; Ngwenya et al., 2003).In this study, the titration experimental data was analysed with the nonelectrostatic model (NEM). This model neglects the effects of the bacterial surface electric field, and has been successfully used to invoke up to four discrete sites on bacteria surfaces of individual and mixed bacterial cultures (Borrok and Fein, 2005; Fein et al., 2005; Pagnanelli et al., 2006; Johnson et al., 2007). Although, our experiments were conducted at different ionic strengths, the rationale of using the NEM approach is based on the findings by Borrok and Fein (2005). In their study, it was concluded that the effect of ionic strength (over the range of 0.01 M to 0.5 M) on the apparent proton binding reactions is relatively small and not significantly greater than experimental and modelling uncertainties of using electrostatic models. Therefore, application of the NEM approach is an initial step to define the type, and quantify and compare the magnitude of ionic strength effects on the SRB cell surfaces to those of other bacterial species/consortia that have been studied before. Experimental titration data was reported as H^{+} added per gram (wet weight) bacteria, calculated using Equation 3.15. An example of an input file for computation of the number of apparent proton binding constants (pK_a) and their concentrations on the surfaces of the bacterial consortium is shown in Appendix B1. All apparent proton binding constants (pK_a) reported in this study were corrected for ionic strength and temperature effects using the Davies equation (Appendix 4b). Apparent stability constants for the adsorption of Sr^{2+}, Co^{2+} and Cs^{+} onto the surfaces of the bacterial consortium were computed from the experimental data obtained, which was entered into the FITMOD program as shown in Appendix B3.

5.4.2 Modelling the Acid-base Properties of SRB

Results from acid–base titrations of bacterial suspensions allow the determination of the absolute concentrations and deprotonation constants of specific proton-active surface sites on the cell walls (Daughney and Fein, 1998). The titration curves obtained in this study indicate that the

present SRB consortium displayed a significant buffering behaviour over the pH range (pH 3.5-10.3) studied due to the presence of different functional groups on the bacterial surface consuming the added base by donating protons (Figure 5.6). There was no evidence of saturation observed with respect to proton adsorption. The experimental potentiometric titration curves obtained are similar in shape and position to each other as well as to those determined previously for a number of individual bacterial and mixed bacterial species (Haas et al., 2001; Yee and Fein, 2001; Ngwenya et al., 2003; Haas, 2004; Borrok et al., 2004; Borrok and Fein, 2005). The positions of the titration curves of the bacterial consortium also changed slightly as a function of ionic strength from 0.01 to 0.5 M. These results are in agreement with findings by other authors for the titration of bacteria consortia (Borrok et al., 2004; Borrok and Fein, 2005; Johnson et al., 2007). Regardless of the ionic strength of the medium, a four-site non-electrostatic model (NEM) yielded an excellent fit to the experimental titration data compared to fewer discrete sites fit of the same model (Table 5.10).

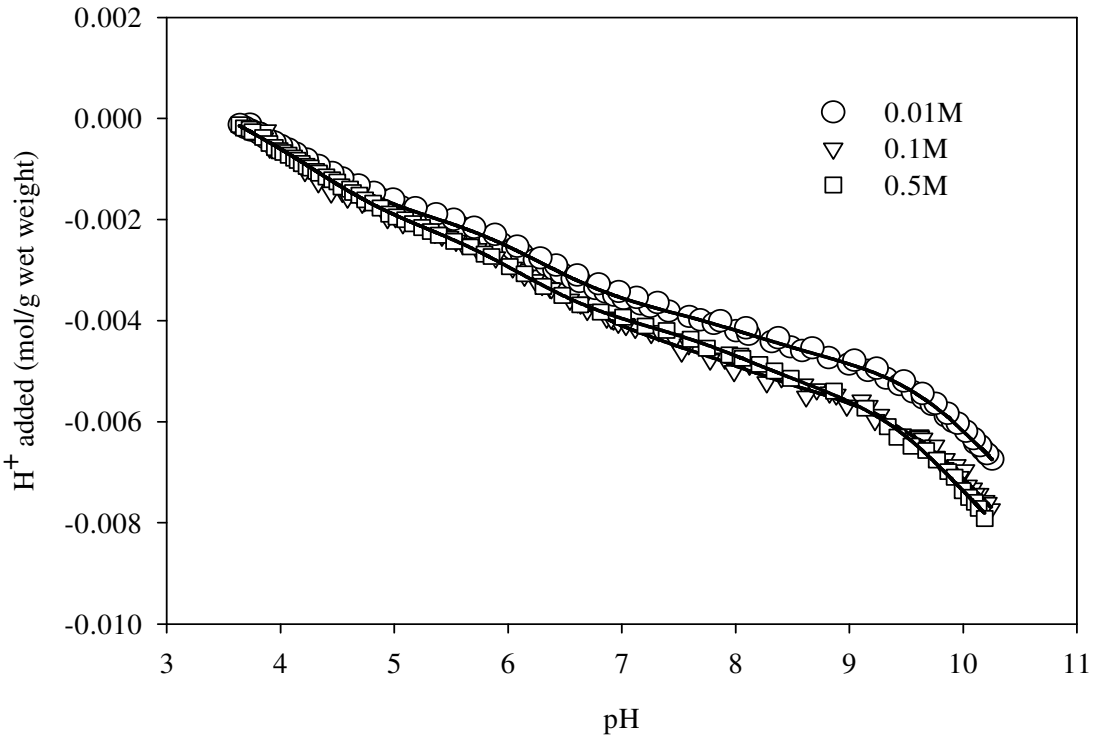

Figure 5.6: Potentiometric titration data of a viable SRB consortium reported as H+ added per gram (wet weight) in 0.01 M, 0.1 M and 0.5 M $NaNO_3$ and 25°C. Data shown are averages of three replicates performed at each ionic strength.

Since the NEM neglects the electrostatic effects on the bacterial cell surface, therefore, the proton binding constants reported are conditional on the ionic strength conditions in which the experiments were conducted. The apparent proton binding constants (pK_a) for the four NEM sites were each assigned to a corresponding functional group according to published literature. Site 1 corresponds to carboxylic acid functional groups (pK$_a$ = 3-5). The near-neutral site 2 was assigned to phosphates (pK_a = 6-7), and site 3 and 4 (pK$_a$ = 8-12) are basic sites, that correspond to either hydroxyl or amine groups (Cox et al., 1999). Generally, the apparent proton binding constants obtained in this study were almost similar at the different ionic strengths, except the pK_a values for site 2, which increased slightly with increasing ionic strength, in agreement with results by Borrok and Fein (2005). However, the apparent total site concentration showed a slight increase with increasing ionic strength; 8.33×10^{-3}, 9.17×10^{-3} and 9.54×10^{-3} mol/L for 0.01 M, 0.1 M and 0.5 M, respectively. Such observations have been reported by a number of researchers (Daughney and Fein, 1998; Cox et al., 1999; Martinez et al., 2002; Borrok and Fein, 2005). These observations suggest that the ionic strength effects on bacterial surface reactions are universal, despite the modeling approach adopted. Similar to earlier observations by Borrok and Fein (2005), insignificant differences were observed between the apparent proton binding constants and total site concentrations (about 12%) obtained over the ionic strength range of 0.01 to 0.5M.

Table 5.10 Compilation of SRB deprotonation constants, surface site densities and variance at 0.01 M, 0.1 M and 0.5 M $NaNO_3$ and 25°C as calculated by FITMOD.

Ionic	*pK_a values*				*Site concentrations ($\times10^{-3}$ mol/L)*				**V(*Y*)**
strength	1	2	3	4	C_1	C_2	C_3	C_4	
0.01 M	5.03				3.739				585.8
	4.55	7.31			2.362	2.985			73.0
	4.43	6.60	9.64		2.041	2.187	2.759		4.06
	4.40	6.39	8.22	10.27	1.966	1.893	1.002	3.473	1.17
0.1 M	5.20				4.597				601.3
	4.68	7.55			2.844	3.415			85.4
	4.55	6.81	9.73		2.451	2.525	2.982		14.7
	4.52	6.58	8.25	10.30	2.362	2.124	1.104	3.576	12.2
0.5 M	5.19				4.345				543.7
	4.84	8.18			2.928	3.970			71.2
	4.65	6.69	9.69		2.305	2.404	3.653		4.51
	4.62	6.43	8.35	10.24	2.201	2.016	1.362	3.964	1.77

[a] apparent pK$_a$ values corrected for ionic strength and temperature effects, [b] Overall variance computed by FITMOD

Such observations have been reported to demonstrate the insignificant effect of ionic strength on proton reactions on the bacterial surface are insignificant (Borrok and Fein, 2005). Results obtained in this study showed that the hydroxyl/amine sites were the most abundant binding sites (accounting for about 40% of the total concentration of binding sites), which is comparable to other studies reported in literature (Daughney et al., 1998; Haas et al., 2001; Ngwenya et al., 2003). Although the effects of ionic strength on the bacterial cell surface reactions are well documented, few studies report the effect of temperature on metal adsorption by bacteria (Ginn and Fein, 2009). With regards to varying temperature, the apparent proton binding constants of the consortium were assumed not to vary significantly from those obtained at 25°C. This assumption is based on results obtained a study by Wrightman and coworkers (2001), where it was concluded that a single set of pre-determined proton binding constants and site concentrations can be used to estimate proton adsorption over different experimental temperature ranges. Therefore, the apparent proton binding constants and site concentrations obtained for the bacterial consortium at 25°C and 0.1M $NaNO_3$ were used for determining the effect of temperature on proton and metal adsorption. A similar approach was followed by Borrok and Fein (2005) and Ginn and Fein (2009).

5.4.3 Determination of Apparent Stability Constants for Metal-bacteria Complexes

In this study, individual or combinations of the deprotonated form of the first three sites were considered for Sr^{2+}, Co^{2+} and Cs^{+} adsorption onto bacterial surfaces. The fourth site was not considered because metal adsorption at a higher pH (>8) was either insignificant, or resulted in significant chemical precipitation of the metal (as is the case with Co). Apparent stability constants for the formation of significant metal-bacteria complexes were estimated using the NEM model. Therefore, the constants reported are conditional on the ionic strength conditions in which the experiments were conducted. Sr^{2+}, Co^{2+} and Cs^{+} adsorption data obtained from experimental studies was used to provide constraints on the adsorption sites and apparent thermodynamic stabilities of the relevant metal–bacterial surface complexes.

Effect of Ionic Strength

The impact of ionic strength on the adsorption of metal cations onto bacterial surfaces has been studied 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). The adsorption behaviour of metal ions onto a sorbent under varying ionic strength conditions is assumed to be an indication of whether the metal is sorbed as an inner-sphere or outer sphere complex. Particularly, outer-sphere complexes are intrinsically more sensitive to variation in ionic strength than inner-sphere complexes, as the availability of free sorbent binding sites tends to decrease with increasing electrolyte concentrations (Stumm and Morgan, 1996). In this study, varying degrees of ionic strength dependence of Sr^{2+}, Co^{2+} and Cs^{+} adsorption onto the SRB biomass were observed in the present study. Figure 5.7 evidently shows that Sr^{2+} adsorption onto the SRB biomass was least affected under increasing ionic strength conditions, as minimal differences was observed even with a ten-fold (from 0.01 to 0.1M) increase in ionic strength. An almost similar observation was reported for the adsorption of U onto *Shewanella putrefaciens* by Haas and Dichristina (2001). However, increasing the ionic strength to 0.5M resulted in a slight decrease (about 10%) in Sr^{2+} adsorption by the SRB biomass. The observed slight sensitivity of Sr^{2+}, adsorption at higher ionic strengths is in agreement with earlier reports suggesting that adsorption occurs through an outer-sphere electrostatic complexation reactions, reported earlier (this study; Small et al., 2001; Yee et al., 2004; Borrok and Fein, 2005).

On the contrary, the adsorption of Co^{2+} and Cs^{+} onto the SRB biomass decreased with increasing ionic strength concentration. Since a non-electrostatic model was adopted for the present study, the contribution of the electrostatic effects on the bacterial cell surface cannot be confirmed to any degree of certainty. However, the observed reduction in adsorption capacity of these metal ions is a clear demonstration of the competition between the metal ions and the electrolyte cations for the adsorption sites on the bacterial cell surface. These results are in agreement with earlier observation in this study (Section 5.3.2), where a 76% reduction in Co^{2+} adsorption capacity was observed in the presence of a monovalent cation (Cs^{+}). Similarly, the decreased uptake of Cs^{+} ions by biosorbents in the presence of excess monovalent (electrolyte) cations has been reported before (Harjula and Lehto, 1986; Solecki, 2006). This phenomenon has been attributed to a number of factors, including; competition for deprotonated binding sites between the metal ion and the background electrolyte cation (Na^{+}), and changes in the activity of both the metal binding functional group sites and aqueous metal ions as a function of ionic strength (Borrok and Fein, 2005).

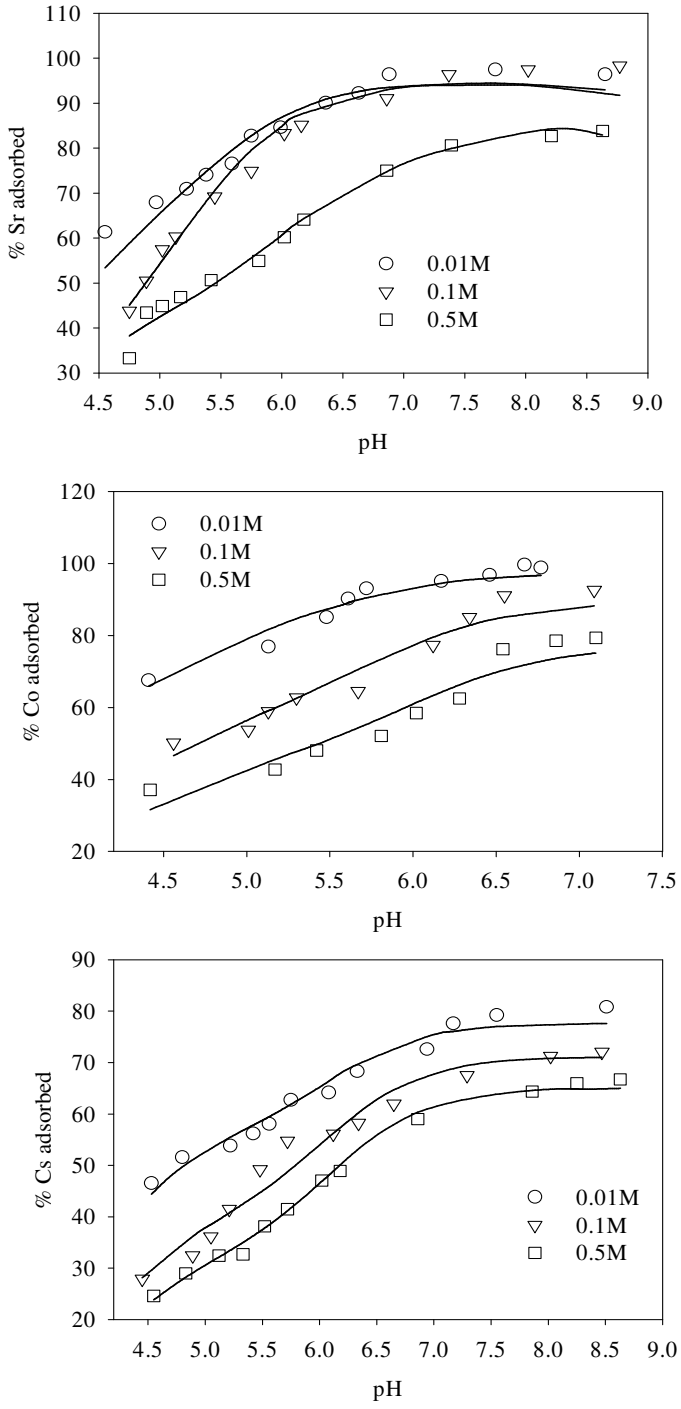

Figure 5.7: Ionic strength dependence on the adsorption behaviour of Sr^{2+}, Co^{2+} and Cs^{+} onto a viable SRB consortium at 25°C. Initial concentrations = 2.85×10^{-4}M, 8.85×10^{-5}M and 3.76×10^{-5}M for Sr^{2+}, Co^{2+} and Cs^{+} , respectively and bacterial biomass = 21.6 g/L (wet weight).

However, regardless of the ionic strength concentration, an adsorption capacity in the range 60-100% was observed for all the metal ions (Sr^{2+}, Co^{2+} and Cs^{+}) at pH ≥ 6.5, suggesting the presence of high affinity sites on the SRB cell surface that bind the metal ions as inner-sphere complexes. As mentioned before (Section 5.4.3), to exclude chemical precipitation reactions, only the first three sites were considered for Sr^{2+}, Co^{2+} and Cs^{+} adsorption. From Table 5.11, it is evident that a 2 site metal adsorption NEM, involving site 1 (carboxylic) and 2 (phosphates) yielded an excellent fit to the experimental Sr^{2+}, Co^{2+} and Cs^{+} adsorption data. All the apparent stability constants obtained were in the range 1-4, in agreement with results obtained by other researchers, which might suggest earlier reports that bacteria exhibit universal proton and metal adsorption behaviour (Borrok et al., 2004; Johnson et al., 2007). The apparent stability constants obtained in this study slightly increased with increasing ionic strength, further confirming the complexation of Sr^{2+}, Co^{2+} and Cs^{+} as outer-sphere complexes. However, further comparisons of the current results with earlier studies cannot be made with any certainty, as different bacteria species, were used, and the electrostatic effects on the bacterial cell surface were not considered.

Table 5.11 Compilation of Sr^{2+}, Co^{2+} and Cs^{+} apparent stability constants at different ionic strengths (0.01 M, 0.1 M and 0.5 M) and 25°C as calculated by FITMOD using the NEM.

Metal ion	Ionic strength (M)	Stability constants [a]			V(Y) [b]
		1	2	3	
Sr^{2+}	0.01	1.53	2.40	NA	2.70
	0.1	1.96	2.41	NA	4.66
	0.5	1.86	3.00	NA	21.5
Co^{2+}	0.01	1.42	2.29	NA	1.07
	0.1	2.04	3.21	NA	3.00
	0.5	2.47	3.93	NA	5.31
Cs^{+}	0.01	1.60	3.35	NA	0.29
	0.1	1.88	3.44	NA	0.71
	0.5	2.02	3.55	NA	0.11

[a] stability constants corrected for both ionic strength and temperature effects, [b] Overall variance computed by FITMOD, NA = not applicable

Effect of Temperature

Over the years very there have been limited studies on the effect of temperature on the adsorption of aqueous metal cations onto bacterial surfaces. Results from the few available

studies indicate that, bacteria demonstrate similar metal adsorption behaviors as a function of temperature. However, it has been hypothesized that under higher pH conditions, the effect of temperature on adsorption depends both on the metal and on the bacterial species of interest (Borrok and Fein, 2005; Ginn and Fein, 2009). In the present study, best-fit models for metal adsorption were generated using up to 3 sites for Sr^{2+} and Cs^{+}, while for Co^{2+} a combination of only site 1 and 2 yielded the best fit (Table 5.12). Generally, there were no marked differences in the apparent stability constants for Co^{2+} and Cs^{+} adsorption over the studied temperature range. As reported earlier (Section 5.2.2), Cs^{+} undergoes limited hydrolysis over the studied pH range, therefore, it is not surprising that the adsorption behaviour of these cations and apparent stability constants are not affected by varying temperature conditions.

Table 5.12 Compilation of Sr^{2+}, Co^{2+} and Cs^{+} apparent stability constants and variance at different temperatures and 0.1M as calculated by FITMOD using the nonelectrostatic model.

Metal ion	Temperature (°C)	Stability constants [a]			V(*Y*)[b]
		1	2	3	
Sr^{2+}	5	1.54	2.48	3.37	0.65
	50	1.69	2.20	3.34	1.33
	75	1.91	2.07	4.06	1.09
Co^{2+}	5	2.07	3.49	NA	5.89
	50	2.06	3.26	NA	2.91
	75	1.99	3.31	NA	5.31
Cs^{+}	5	1.58	3.44	4.78	0.19
	50	1.56	3.47	4.85	0.15
	75	1.58	3.43	5.03	0.08

[a] = apparent constants, [b]= calculated by FITMOD, and NA = not applicable

On the contrary, Co^{2+} readily undergoes hydrolysis with increasing pH, however, since our studies were constrained between a pH range of 2-7 (to exclude precipitation reaction), results obtained suggest that temperature has no effect on the availability of the Co^{2+} species critical for complexation. Similarly, no significant differences were observed for the adsorption of Cd onto the bacteria *Bacillus licheniforms* and *Pseudomonas mendocina* when the temperature was increased from 5 to 80°C (Zouboulis et al., 2004; Ginn and Fein, 2009). However, the observed misfit between the experimental and model Co^{2+} adsorption data at higher pH can be attributed to the contribution of precipitation reactions (hydroxides) at pH>6 (Figure 5.8).

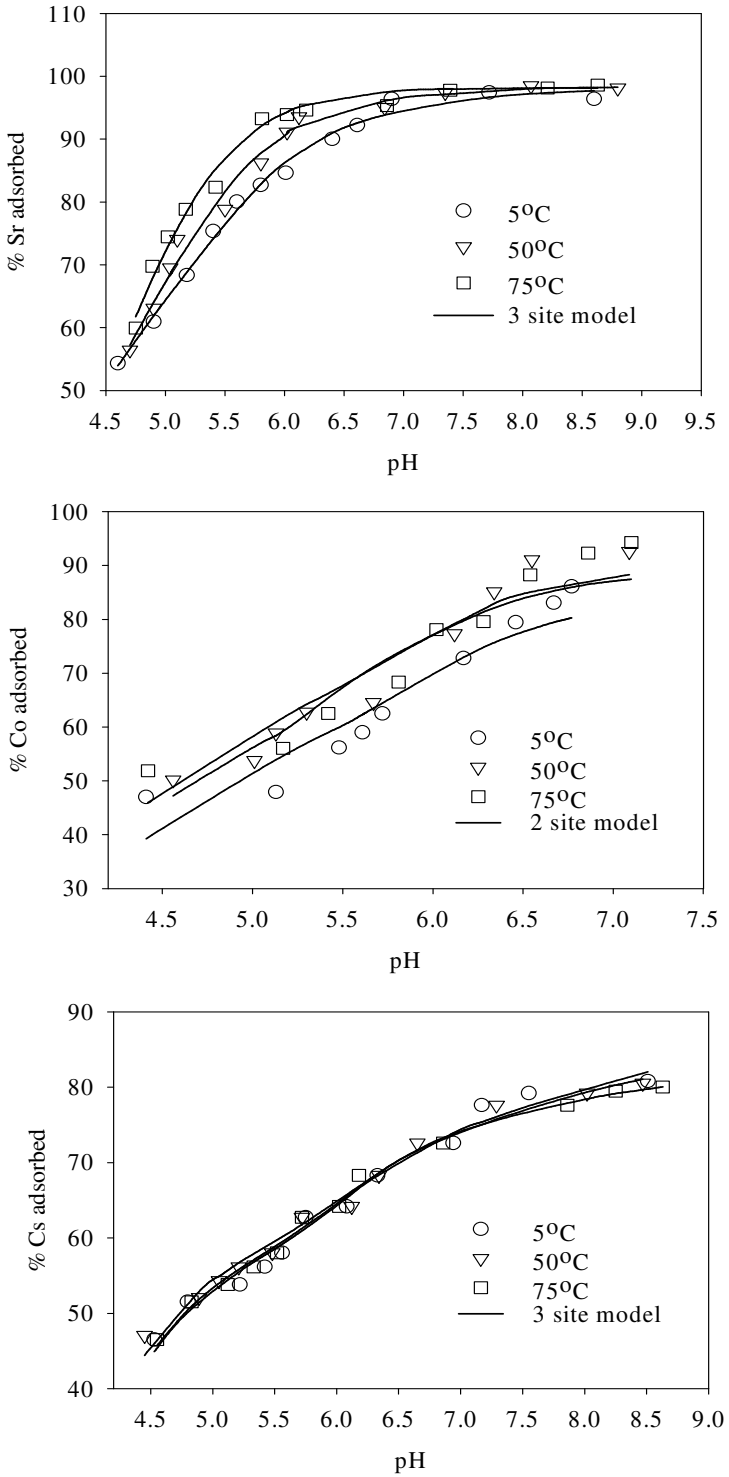

Figure 5.8: Temperature dependence on the adsorption behaviour of Sr^{2+}, Co^{2+} and Cs^{+} onto a viable SRB consortium at 0.1 M $NaNO_3$.

With regards to Sr^{2+} adsorption, an increase in temperature resulted in an increase in the apparent stability constants. It has been suggested that for some metal ions, increasing the temperature influences the speciation of the important metal-surface complexes, whereby the stability constants increase with increasing temperature (Ginn and Fein, 2009). Based on the results obtained in this study, it is apparent that the effect of temperature on metal ion adsorption onto bacterial surfaces varies with the metal ions involved and pH conditions. In summary, results obtained in this study showed that the formation of stable metal-ligand complexes involved the carboxylic sites, phosphates and a third basic site for Sr^{2+} and Cs^{+}, whereas for Co^{2+}, only the first two were involved.

5.5 SUMMARY

The results further suggest that metal binding by bacterial cells is a complex phenomenon, and cannot be fully explained by simple empirical models such as the Langmuir and Freundlich isotherms alone. The preliminary data on the kinetics of fission products removal by SRB in single metal systems and equilibrium binary component systems only serves as the basis for further research on the sorption behaviour of a range of metals onto bacterial sorbents. The preferential order of the SRB consortium for equilibrium metal ion uptake efficiency in single metal systems was observed in the order; Sr>Co>Cs, with maximum biosorption capacities of 405.5, 203.3 and 192.2 mg/g, respectively. In line with the high Sr^{2+} tolerance demonstrated by the present SRB consortium, a superior Sr^{2+} binding capacity was also observed. This value is higher that values reported for sorbents that have been used in other studies (Shaukat et al., 2005; Dabbagh et al., 2007, Chegrouche et al., 2009). Additionally, the SRB consortium also demonstrated a specialized Sr^{2+} binding, as its removal was least sensitive to the presence of other fission products. Initial metal concentration and solution pH are important factors in determining metal removal kinetics. Chemisorption is the main rate limiting step for the removal of Sr^{2+}, Co^{2+} and Cs^{+} from solution at different initial concentrations and pH values.

Results obtained from potentiometric titration studies of the bacterial consortium gave a qualitative measure of the acid-base properties of the bacterial cell surface. Results from this study indicate that the surfaces of the present SRB consortium can be adequately defined by a four-site nonelectrostatic model as the effect of ionic strength on the bacterial surface reactions

was insignificant. Site 1 corresponds to carboxylic acid functional groups (pK_a = 4-5). The near-neutral site 2 was assigned to phosphates (pK_a =6-7), site 3 and 4 are basic sites, and corresponds to phenolic sites (pK_a = 8-12). The most abundant apparent proton binding site belongs to the site 4 (hydroxyl/amine group) and accounts for about 40% of the total concentration of binding sites for the consortium. The apparent total site concentration (C_{tot}) slightly increased with increasing ionic strength, 8.33×10^{-3}, 9.17×10^{-3} and 9.54×10^{-3} mol/L for 0.01M, 0.1M and 0.5M, respectively. Previous studies on other selected bacteria as described by different models yielded lesser total site concentrations of reactive sites (in decreasing order); 3.31×10^{-3} mol/L, 2.24×10^{-3} mol/L, 1.42×10^{-3} mol/L, 1.46×10^{-3} mol/L, 1.27×10^{-3} mol/L, and 7.8×10^{-5} mol/L, for *Pseudomonas aeruginosa*, *Escherichia coli*, *Aquabacterium commune, Calothrix sp, Enterobacteriaceae sp*,and *Shewanella putrefaciens*, respectively (Yee and Fein, 2001; Haas et al., 2001; Ngwenya et al., 2003; Yee et al., 2004; Ojeda et al., 2008). Therefore, the high buffering capacity and metal ion adsorption capacity demonstrated by the mixed SRB culture can be directly attributed to the high total concentration of apparent reactive sites.

The effect of ionic strength on the adsorption of Co^{2+} and Cs^{+} was evident, where at higher ionic strengths decreased metal adsorption was observed. The observed decrease in adsorption capacity was attributed to the presence of excessive amounts of electrolyte cations, thereby competing with Co^{2+} and Cs^{+} for binding sites on the bacterial surface. However, Sr^{2+} adsorption was insensitive to changes in ionic strength in the range 0.01-0.1M. However, further increasing the ionic strength to 0.5M resulted in a slight decrease (about 10%) in Sr^{2+} adsorption by the SRB biomass. Co^{2+} and Cs^{+} adsorption was insensitive to changes in temperature, whereas changes in temperature, however, had a slight effect on the adsorption and immobilization of Sr^{2+} species. In summary, findings from this study strongly suggest that the biological adsorption and stability of the metal ions is influenced by; surface properties of the consortium (including the orientation of the surface functional groups), and metal speciation in the aqueous phase, due to changes in pH and ionic strength.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The main aim of the present study was to investigate the potential role of natural microbial processes, and their applicability in controlling metal and radionuclide pollution in the environment. A sulphidogenic bioreactor was used as a model for simulating conditions that closely mimic a groundwater system contaminated with variable metal concentrations, under oxygen-depleted (anoxic) and reducing conditions. Results from this study have demonstrated that the presence of specific metal-tolerant and binding bacterial cultures, lack of oxidizing of agents within the system, coupled with low metal solubility at variable pH and ionic strength conditions renders the immobilized metal complexes in the system stable. The stability of the immobilized metal complexes under variable environmental conditions is critical as it determines the overall applicability and long term performance of a microbial remediation system, particularly during *in situ* bioremediation (Satyawali et al., 2010). Through this project, the multifaceted interrelationships between microbial metal (Sr^{2+}, Co^{2+} and Cs^{+}) immobilization, and cell viability and activity were established. For the first time, our work has demonstrated the unique tolerance of individual SRB microorganisms, belonging to *Citrobacter, Paenibacillus* and *Stenotrophomonas* genera, towards Sr^{2+}, Co^{2+} and Cs^{+}, respectively.

Our work has demonstrated that lower initial Sr^{2+}, Co^{2+} and Cs^{+} concentrations (≤100 mg/L) have minimal effects on the growth and metabolism (biological sulphate reduction) of the present mixed SRB culture. These observations imply that at sufficiently low inhibitory concentrations, the growing SRB biomass can be effectively used for the *in situ* bioremediation of metals and radionuclides provided the energy and electron acceptor sources requirement is satisfied. The mixed SRB culture was less sensitive to the presence of Cs than Sr or Co, which eliminates one potential problem in the use of live cells for its removal. The results obtained indicate that Sr^{2+}, Co^{2+} and Cs^{+} removal from solution in the sulphidogenic bioreactor occurred through one or a combination of complex interaction mechanisms including; biosorption, chemical and biological precipitation, bioreduction, and bioaccumulation. Further analyses

revealed that cell viability is not a critical factor that determines the mechanism of microbial Sr^{2+} and Cs^{+} removal from solution, as removal occurred mainly through biosorption. On the other hand, Co^{2+} uptake from solution was dependent on cell viability, where under viable SRB conditions, metal removal through a combination of biosorption, bioprecipitation and bioaccumulation. Results from batch kinetic metal removal studies further confirmed that the removal efficiency of the metal ions (Sr^{2+}, Co^{2+} and Cs^{+}) was dependent on the initial metal concentration, solution pH and cell viability. Live bacterial cells consistently demonstrated a superior metal biosorption capacity, compared to heat-killed bacterial cells. The metal uptake efficiency of the SRB biomass increased up till the threshold limit of 405, 203 and 192 mg/g was reached for Sr^{2+}, Co^{2+} and Cs^{+}, respectively.

So far, the present study is the first to study the surface characteristics and proton and Sr^{2+}, Co^{2+} and Cs^{+} adsorption behaviour of an SRB consortium under anaerobic conditions. A combination of potentiometric titrations and FTIR spectroscopy eased the identification of the four discrete functional groups, carboxylic (pK_a = 3-5), phosphate (pK_a = 6-7), and 2 basic sites (pK_a = 8-12), belonging to either and hydroxyl or amine groups, which were unique to this culture. The high binding capacity of the bacterial consortium was attributed to the high concentration of reactive surface sites (C_{tot} = 9.17×10^{-3} mol/L). Whether, culture growth conditions, that is, aerobic or anaerobic and type of medium, have a direct influence on the observed results remains unclear, but can only be determined through further research. Results from this study suggest that a simple 2 site model (involving carboxylic and phosphoryl groups) is adequate to account for metal adsorption onto bacterial surfaces. The adsorption capacities of the metals decreased with increasing ionic strength, whereas variable temperature conditions had no effect on the sorptive properties of the bacterial cell surface. Consequently, taking into account the observed results, the following conclusions can be drawn;

- Exposure of the initial SRB consortium to Sr^{2+}, Co^{2+} and Cs^{+} resulted in the emergence of metal-specific and -tolerant novel SRB microorganisms, belonging *Citrobacter, Paenibacillus* and *Stenotrophomonas* genera, respectively.

- The toxic and inhibitory effects of the metal ions (Sr^{2+}, Co^{2+} and Cs^{+}) on the SRB culture were unique for each metal ion, due to the differences in their chemical and physical properties, thereby determining cell viability and activity. For example, in a heavily contaminated bioreactor system (≥300 mg/L metal), the growth of SRB and ability for sulphate reduction was retarded.
- The removal capacity of the metals in the bioreactors was dependent on biomass concentration, where high metal removal rates (up to 100%) were observed at less toxic and inhibitory metal concentrations.
- Metal removal by the biomass mainly occurred through non-specific electrostatic reactions by the formation of outer-sphere metal-bacteria complexes. The present SRB culture demonstrated superior Sr^{2+}, Co^{2+} and Cs^{+} binding capacities ($q_{max)}$ of 405, 203 and 192 mg/g for Sr^{2+}, Co^{2+} and Cs^{+}, respectively.
- The SRB surface is characterized by the presence of four discrete functional groups; carboxylic (pK_a = 3-5), phosphate (pK_a = 6-7), and 2 basic sites (pK_a = 8-12), belonging to either and hydroxyl or amine groups, which were unique to this culture. Compared to other bacteria, the high metal adsorption capacity demonstrated by this culture can be attributed to the high concentration of apparent reactive sites ($C_{tot} = 9.17\times10^{-3}$ mol/L).
- The adsorption of metal ions onto the bacterial surface was highly sensitive to changing ionic strength and pH conditions, and less sensitive to changes in temperature.

These findings suggest that indigenous anaerobic microorganisms, such as SRB, might hold a promise towards their use for *in situ* bioremediation processes due to their outstanding metal-tolerance and sequestering abilities. In particular, evaluation of the biomass population biology kinetic parameters forms an important aspect towards the development of effective radionuclide retardation strategies utilizing growing microorganisms. Taking all results together they contribute to a more realistic description of the influence of microbial actions on the migration behavior of radionuclide contaminants in the environment and are helpful for an improved risk assessment for instance for potential underground nuclear waste repositories. Further studies related to this subject should focus on two main directions. The first one should be concentrated on direct interactions of other high priority radionuclides with different microorgansisms,

including those isolated from potential nuclear waste repository areas. Prior to metal uptake studies, it is important that the phylogeny, radiotoxicity and metal tolerance of the cultures is established. Results obtained from this study suggested that the observed differences in metal tolerance and removal amongst the different microorganisms might be due to the inherent characteristics of the microorganisms as well as the metal itself. Therefore, future studies should focus on the application of rigorous screening procedures involving the use of autoradiography, which has great potential for isolation of microorganisms with particularly high affinities for different metal ions. Alternatively, manipulation of the physiological status of microorganisms can dramatically alter the transport and mobility of metal ions, particularly, monovalent cations, which are difficult to remove from solution. Particularly, osmotic shock procedures have so far proved to be the most successful treatments for stimulating Cs removal and recovery by microorganisms. Other manipulations, at both the cellular and molecular level, which are can influence metal uptake by bacterial biomass have yet to be characterized and investigated. Secondly, future research should also focus on the less studied indirect interaction between radionuclides and microbially produced bioligands. In addition, one important goal to be achieved in future studies will be the determination of the intrinsic stability constants and the structure of the formed metal-complexes species. These constants will be used directly in risk assessment programs.