Characterisation of surface uptake and biosorption of cationic nuclear fission products by sulphate-reducing bacteria

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ABSTRACT

The treatment of radioactive fission products – ⁹⁰Sr²⁺, ⁶⁰Co²⁺ and ¹³⁷Cs⁺ – from simulated nuclear wastewater was evaluated using locally-isolated sulphate-reducing organisms. In this study, sulphate-reducing bacteria (SRB) were used as biosorbents for removal of the cationic fission products. The cultures achieved 90%, 100%, and 80% removal of Sr²⁺, Co²⁺ and Cs⁺, respectively, for a 75 mg×ℓ⁻¹ solution of each metal under a low ionic strength of 0.01 M. Increasing the ionic strength of the solution to 0.5 M resulted in a decreased metal uptake to 80%, 65% and 70% for Sr²⁺, Co²⁺ and Cs⁺, respectively. Approximately 68% of the adsorbed fraction on cell surfaces was exchangeable (i.e., was desorbed under acidic conditions). Using surface complexation models and equilibrium modelling analysis, reaction sites on the cell walls of the cultures were determined to belong to the –COOH and –H₂PO₄ groups (pK_a = 4–5 and 7–8, respectively). The distribution of the isoelectric equilibrium points for cell surfaces was consistent with the composition and characteristics of the identified microbial species in the culture which was dominated by the Gram(+-ve) Bacilli – Lysinibacillus boronitolerans AB199591 – and biofloc-forming Gram(-ve) SRBs, such as Desulfomonile tiedgei AF418162, Syntrophobacter wolinii X70995, and Desulfokalicella vacuolata L42613. The high exchangeable fraction on the cells and the higher removal rates under lower ionic strength indicates that metal binding was non-electrostatic which was consistent with outer-sphere complexation behaviour.

Keywords: radionuclide recovery, surface complexation, sulphate-reducing bacteria, biosorption kinetics, bioremediation.

INTRODUCTION

The radioactivity in wastewater and sludge coming from nuclear fuel and radiochemical processing is mainly detected as a result of the presence of metallic fission products and transuranic elements in the wastewater stream. Among the troublesome elements are the lightweight fission products caesium (Ce-137), strontium (Sr-90) and cobalt (Co-60). These elements are characterised by very high mobility in water, high radiological decay rates and short half-lives. For example, strontium is highly mobile in both soils and groundwater systems (Dewiere et al., 2011) and has a half-life of 28 years (Chirwa, 2011). Although these elements are released in very small amounts compared to other waste compounds such as radiocarbon-14 (C-14), sodium and nitrate, their presence raises the radiation level enough for the whole bulk of the wastewater to be classified as low to medium level nuclear waste (LLW, MLW) (Greve et al., 2007). Such classification increases the cost of treatment and requires the disposal of the wastewater in specialized ponds with limited capacity.

The cationic fission products are easily taken up by plants and eventually enter the ecosystem through the food chain and mineral recycling (Ajilouni, 2007). Fission products are notorious for being chemically identical to benign and essential minerals such as potassium (K⁺), magnesium (Mg²⁺) and iron (Fe²⁺). For example, the radioactive divalent cation strontium-90 (⁹⁰Sr²⁺) is easily absorbed by vertebrates because, chemically, it resembles calcium (Ca²⁺), which is a critical component of the mammalian diet. After integrating into the bone matrix, Sr-90 continues to irradiate surrounding bone and muscle tissue resulting in bone sarcoma and leukaemia (Wohl et al., 2013). Due to such hazards, it is desirable to prevent the migration of water contaminated with nuclear radioisotopes into surface water and groundwater resources used as water supply sources.

Extensive research has been conducted on subsurface microbial interactions that relate to the chemistry and physical dynamics of radionuclide migration and remediation in geological systems (Kumar et al., 2007). Sulphate-reducing organisms (SROs) – also referred to as sulphate-reducing bacteria (SRB) – thrive in the deep aquifer environments typical of deep mines and geological nuclear waste repositories (Greve et al., 2007). Coincidentally, many sulphate-reducing organisms have been shown to possess unique properties on their cell walls that allow them to adsorb and retain metallic elements. The cells can thus be utilised in the adsorption of metallic fission products from radioactive wastewater (Chen et al., 2005; Ngwenya and Chirwa, 2010). The uptake of metallic species onto the surfaces of SROs can involve metabolic processes in living cells requiring energy dispersion or can occur as pseudo physical-chemical processes on the surfaces of dead biomass (Dewiere et al., 2004; Gadd, 2010).

During previous investigations, other researchers linked the adsorptive processes on bacterial cell walls to the presence of reactive functional groups on the bacterial cell surfaces (Vijayaraghavan and Yun, 2008; Zouboulis et al., 2004; Pagnanelli et al., 2006). Although attempts have been made to characterise the adsorption of metallic species on bacterial cell surfaces, there is still limited information on the actual role of the detected functional groups in facilitating metal uptake.

So far, adsorption of metallic species onto cell surfaces can be classified either as electrostatic (influenced by the charges of the adsorbent) or non-electrostatic, in which no charges are...
involved. Electrostatic models (e.g. the constant capacitance model (CCM), diffuse layer model (DLM), and the triple layer model (TLMI)) 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, $e^{-(E \cdot z)/RT}$, where $E$ = charge of the adsorbing ion, $F$ = Faraday constant, $z$ = valence of the adsorbing ion, $R$ = molar gas constant (J mol$^{-1}$K$^{-1}$), and $T$ = absolute temperature (K) (Goldberg, 1995). The voltage potential ($V$) is calculated as a charge density per gram-mole per joule of energy spent (C m$^{-2}$) (Goldberg, 1995). The electrostatic model (EM) has been used successfully in relating surface charge to surface potential using arbitrarily assigned capacitance values to describe the electrostatic effect of metal adsorption onto bacterial surfaces (Ojeda et al., 2008).

In the CCM, the intrinsic discrete acidity constant of the reaction site is shifted, and its pH range of influence broadened, by the electrostatic potential, $V_i$, at the so-called bacteria/water surface plane in the intrinsic conditional surface complexation constant expression, $K_i = Z = molar gas constant (1 \text{gmol}^{-1} \times \text{K}^{-1})$, and $T$ = absolute temperature (K) (Goldberg, 1995). The voltage potential ($V$) is calculated as a charge density per gram-mole per joule of energy spent (C m$^{-2}$) (Goldberg, 1995). The electrostatic model (EM) has been used successfully in relating surface charge to surface potential using arbitrarily assigned capacitance values to describe the electrostatic effect of metal adsorption onto bacterial surfaces (Ojeda et al., 2008).

Colonies were developed on 9 mℓ solid medium prepared from Medium A converted to solid medium by adding 2% universal agar. 9 mℓ of the medium at 40°C was mixed with 1 mℓ of sample in a petri dish and allowed to solidify at room temperature. The plates were then incubated at 30°C in an anaerobic jar containing an Anaero Pack O$_2$ Absorbing/CO$_2$ Generating System (Mitsubishi Gas Chemical Company, Inc., New York, New York, USA). After 24 h, individual colonies were picked and cultured in a corresponding growth medium. The process of isolation was repeated 3 times to achieve a high degree of purity in the colonies. Transfers to petri dishes and all other plate procedures were conducted in anaerobic glove bags purged with 99.9% pure N$_2$ gas. Genomic DNA was extracted from individual colonies according to the protocol described for the Wizard Genomic DNA purification kit (Promega). Individual species were identified by 16S rRNA fingerprinting using the method published earlier (Ngwenya, 2011).

**SRB cell surface characterisation**

Cells for cell surface characterisation experiments were harvested at Day 5 from cultures grown in Postgate medium (Medium B). Acid/base properties of SRB cells with regard to H$^+$ and OH$^-$ ion binding were studied by potentiometric titrations of solutions containing the harvested cells. Titrations were performed using an automated titration system comprising of a burette system, glass electrode and a pH meter (Metrohm 718 STAT-Titrino model, Metrohm, UK). Prior to titration, 0.3 g (wt weight) SRB cells were suspended in 25 mℓ of the electrolyte solution which had been purged with 99.9% pure N$_2$ for 60 min to eliminate CO$_2$. The suspension was immediately placed into a sealed titration vessel with continuous stirring at 140 r.min$^{-1}$ under N$_2$. Titrations were carried out by the gradual addition of pre-set small volumes of 0.1 M NaOH or 0.1 M HCl titrant (standardized against reagent grade KC$_2$H$_3$O$_2$ and Na$_2$CO$_3$, respectively). The titrator was set to add successive base or acid after a stability of 0.1 mV.s$^{-1}$ was attained. Three sets of duplicate SRB suspensions were first titrated with 0.1 M HCl to pH 4 and then titrated to high pH (pH 10) with 0.1 M NaOH. Blank titrations devoid of SRB cells were also performed which served as controls. Reversibility of the SRB acid/base behaviour was established by performing reverse titrations.

**Materials and Methods**

**Culture and medium**

An inoculum culture of SRB was supplied by a member of the Council for Scientific and Industrial Research (CSIR) (Pretoria, South Africa). The inoculum culture was originally collected from a deep coal mine and was maintained in the laboratory-scale semi-fused batch reactor under sulphate-reducing conditions. The original culture was assumed to be acclimated to sulphate and accumulation of sulphide in solution. 110–170 μg/l in coal mine water. The feasibility of an engineering process for removal and recovery of sulphate and accumulation of sulphide in solution.

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Sr$^2+$, Co$^{2+}$ and Cs$^+$ adsorption experiments

Sr$^2+$, Co$^{2+}$ and Cs$^+$ adsorption experiments were conducted under anaerobic conditions in batches with different pH, ionic strength, and temperature conditions. Uptake of each metal was evaluated in a bacterial suspension consisting of approximately 1 g dry weight in basal mineral medium (BMM). The bacterial suspension in BMM was supplemented with the experimental metal at 100 mg x$^{-1}$ prepared in 0.1 M NaNO$_3$. The initial values of Sr$^2+$, Co$^{2+}$ and Cs$^+$ concentration were prepared from high precision weight measurements using a Sartorius Cubis MSA524S100DI Analytical Balance (precision of 0.0001 g). The measured values were compared with optimised values derived from simulation of the whole dataset using the programme MINTEQA2 (Allison et al., 1991). The initial values presented in Tables are nominal values from the weighed salts and prepared solutions from the measured values. After recording the initial pH, the suspension was divided into a series of 100-mℓ serum bottles, and the pH in each bottle was adjusted to the desired value by adding drops of concentrated HNO$_3$ or NaOH solution. The serum bottles were immediately sealed with airtight rubber stoppers, and incubated in a Labcon SPL-MP 15 Orbital Shaker (Labcon Laboratory Services, South Africa) at 100 r·min$^{-1}$, and allowed to equilibrate for 3 h at 25±0.5°C. At the end of the equilibration period, samples were collected by sterile needle and syringe followed by centrifugation to remove suspended solids and precipitates. The clear supernatant was then acidified to 1% v/v HNO$_3$ and stored at 4°C, after immediately sealing with airtight rubber stoppers, and incubated in a bacterial suspension consisting of approximately 1 g dry weight in basal mineral medium (BMM). The bacterial suspension in BMM was supplemented with the experimental metal at 2 mg x$^{-1}$ in all samples including a final concentration of 2 mg x$^{-1}$ K$^+$ in all samples including a final concentration of mild acid concentration (0.1 N HCl) for 5 h under continuous shaking. All experiments were performed in triplicate.

Metal analysis

Strontium concentration in liquid samples was determined using the A Analyst 400 Perkin Elmer AAS (Perkin Elmer, Shelton, USA) at a wavelength of 460.7 nm in a nitrous oxide-acetylene flame. Strontium ionization in the flame was suppressed by the addition of a potassium chloride solution to give a final concentration of 2 mg x$^{-1}$ K$^+$ in all samples including the standards and blank. Cobalt and caesium were measured similarly at wavelengths of 240.7 and 852.1 nm, respectively.

Surface complexation modelling approach

Several researchers have applied different acid-base surface complexation models (SCM) to predict the identities of functional groups involved in the adsorption on the bacterial cell surface (Fein et al., 2005; Ngwenya et al., 2003; Tourney et al., 2008). In this study, the programme FITMOD, which is a modified version of the computer program FITEQL 2.0 by Westall (1982), was used to construct geochemical models describing proton interaction with the bacteria. A proton balance approach was utilized to optimize protonation constants of the various functional groups on the bacterial surface. The goodness-of-fit of the different models to the titration data was determined by the overall variance, $V(Y)$, which is calculated by FITMOD as follows:

$$V(Y) = \frac{\sum (Y - \bar{Y})^2}{n - \bar{Y}}$$

where: $V(Y) =$ overall variance, $Y =$ error in the mass balance calculations, $S_y =$ default experimental error calculated by FITMOD, $n =$ number of data points, $n_y =$ number of chemical components for which total and free concentrations are known, $P =$ data point location (count), and $n_0 =$ number of adjustable parameters. In Eq. 2, the value of $Y$ is updated by $\lambda_i$ to optimise $V(Y)$. Values of $V(Y)$ between 1 and 20 generally indicate an acceptable fit to the data (Daughney et al., 2004). FITMOD incorporates a number of models; ranging from non-electrostatic single layer models (NEM-SLMs) to electrostatic double-layer models (EM-DLMs). For comparison purposes, the titration experimental data obtained in this study were analysed to determine which model best represents the complexation behaviour on SRB cell surfaces. The titration data were plotted in terms of the concentration of deprotonated sites per mass of SRB (mol g$^{-1}$) as shown in Eq. 3.

$$[H^+]_{\text{added/released}} = \left( C_{a_n} - C_{a_k} - [H^+] + [OH^-] \right)/m_p$$

where: $C_a$, $C_{a_k}$, [H$^+$] and [OH$^-$] are the molar concentrations of acid, base, H$^+$ and OH$^-$ species, respectively, and $m_p =$ the concentration of SRB cells (mg x$^{-1}$) in the suspension.

The mass law relationship, in conjunction with the mole balance expressions, was used to define the adsorptive process. A 1:1 metal/surface site stoichiometry was used for all model calculations, and equilibrium constants for aqueous metal hydrolysis were obtained from simulated profiles following the method established by Baes and Mesmer (1976). This information was used to account for Sr, Co and Cs adsorption onto SRB cell surfaces according to Eq. 4.

$$M^+ + R^- A^- \leftrightarrow R^- A^- M$$


Under equilibrium conditions partitioning between the solid surface and the aqueous phase is therefore quantified by the following mass law:

$$K_w = \frac{[R^- A^- M]}{[M^+][R^- A^-]}$$

where: $K_w =$ stability constant based on the anionic property of the microbial cell wall, and square brackets denote concentrations of the specified species.

EXPERIMENTAL RESULTS

Culture composition and sulphate-reducing activity

The microbial species in the environmental mixed cultures were isolated and characterised to allow further development of more efficient pure cultures in future. The culture was maintained under sulphate-reducing conditions using a sulphate-reducing selective medium described earlier in this article. Individual species grown under the prescribed conditions confirmed the presence of sulphate-reducing organisms, both in stock and enrichment cultures. DNA was extracted and amplified from single colonies for use in the 16S rRNA fingerprinting. For the Gram(+)ve colonies, 1 000 replicates Boot-strap analysis was conducted to assess the reliability of the groupings with Paenibacillus polymyxa X60632 as an outgroup. Gram(+)ve species in samples after 2-day exposure to 75 mg x$^{-1}$ of Sr$^2+$, Co$^{2+}$, and Cs$^+$ were identified as 98% homologs of Lysinibacillus boronitolerans AB599959 (Fig. 1). These
earlier studies on acid mine drainage by Ahmed et al. (2007). Lysinibacillus sphaericus was detected in reducing activity of Lysinibacillus sphaericus reducing species (Misra et al., 2014). The group also contained the sulphate-nents and hydroxyl (–OH) groups from the glycan (sugar) units of SO₄²⁻ in solutions from 3.02 g×ℓ⁻¹ to 1.40 g×ℓ⁻¹, a reduction of 53.4%, in 14.6 days with concomitant accumulation of HS⁻, which correlated with cell production (Fig. 3c). It appears therefore that sulphate reduction was necessary for cell growth in the mixed culture used in this study.

**Partitioning of reactive species on cell surface**

Scanning electron micrographs of SRB biomass in a 75 mg×ℓ⁻¹ Sr²⁺ solution showed white precipitates deposited in the vicinity of cells. Figure 4A shows that precipitates were absent in a heat-killed culture control suggesting that metal ion precipitation may require some metabolic factors to occur. The energy dispersion x-ray (EDX) analysis showed that Sr was not a dominant species on the surface of cells with only 0.5% detected on the surface. Speciation analysis of bacteria-free controls revealed that the solutions were undersaturated with respect to insoluble Sr species. This is consistent with the observation of adsorption as the dominant mechanism for metal removal from solution. The results show significant Sr²⁺ adsorbed to the cells (Fig. 4B). EDX analysis of the cells from the live cultures showed that Sr²⁺ comprised about 68% of the elemental composition at the cell surfaces. Thus strontium was the dominant species on the surface of live cultures which indicates active adsorption of strontium on live culture cells.

A mass balance analysis was conducted to determine the distribution of cell surface functional groups on SRB cells as shown in Fig. 5a, b. The analysis was conducted from solutions containing 75 mg×ℓ⁻¹ of Sr²⁺, Co²⁺ and Cs⁺, i.e., 75 mg×ℓ⁻¹ of each species. The mass balance on the adsorbed and desorbed species shows that most of the metallic species were localised on the surface of the cells. The desorption analysis showed that up to 70% of Sr²⁺, 43% of Co²⁺ and 55% of Ca²⁺ adsorbed on live cells (44% Sr²⁺, 50% Co²⁺ and 65% Ca²⁺ adsorbed on heat-killed cells) was exchangeable. This suggests that the majority of desorbed species were bound to the cells by weak electrostatic forces.
interactions that were easily disrupted by a change in the ionic orientation of the medium solution such as an increase in H⁺.

The proportions of fission products Sr²⁺ and Cs⁺ covalently bonded to the cell surfaces were found to be distributed on carboxylates (18–28%), oxides (8–10%), and sulphides (3–5%). The residue contained less than 0.3% of Sr²⁺ and Cs⁺. The distribution of Co²⁺ was significantly different as shown by the grey bars in the graph (Fig. 5a, b). Almost 50% of cobalt was detected in the precipitate residue. Further analysis was conducted on the cell surface characteristics using potentiometric titration to determine the predominant adsorption process. The information was used later in deciding the choice of complexation model during equilibrium modelling using FITMOD.

Distribution of cell surface functional groups

Since up to 70% of the metals in solution were exchangeable using a dilute acid regeneration process, a non-electrostatic model (NEM) was attempted first. Analysis of the potentiometric titration data yielded 4 buffering equilibrium points on the cell surface, i.e. – Point 1 (pK_a = 4–5) associated with –COOH...
**Figure 4**
SEM image and EDX analysis of live culture exposed to 75 mg·ℓ⁻¹ Sr²⁺ – elemental analysis on cell surface from (A) 0.2% Sr detected on the surface of cells heat-killed culture (Control) and (B) 68.22% detected on the surface of live cells. The white box indicates area scanned with EDX. (From: Ngwenya and Chirwa, 2011)

**Figure 5**
Partitioning of Sr²⁺, Co²⁺ and Cs⁺ species on available (A) and dead (B) SRB biomass. F1 = exchangeable, F2 = carboxylates, F3 = oxides, F4 = sulphide/organics, and F5 = residential fraction.
functional groups, Point 2 (pK_a = 6–7) associated with –H_2PO_4^- and functional groups, Point 3 (pK_a = 8–9) associated with ammnonium groups, and Equilibrium Point 4 (pK_a = 10) being the protonated amine groups (Table 1). Some of the anticipated functional groups such as hydroxyl –OH groups (pK_a = 17) and amide (pK_a = 15) lie outside the scanned range.

It was observed in all of the tested cases that the ammonium –NH_4^+ (pK_a = 9) and protonated amine –NH_2.H^+ (pK_a = 10) comprised the majority of reactive sites (Table 1). However, the majority of the values in the preferred operational range for the culture pH 4.5–6.8 were a combination of the carboxylic (–COOH) groups with approximately 40% of the reaction sites in the cell wall of the Gram(+ve) component of the culture. 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 ions and the background electrolyte cation (Na^+) (Solecki, 2006), 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).

Table 1
Compilation of SRB deprotonation constants, surface site densities and variance at 0.01 M, 0.10 M and 0.50 M NaNO_3 and 25 °C as calculated by FITMOD

<table>
<thead>
<tr>
<th>Ionic strength</th>
<th>pK_a values*</th>
<th>Site concentrations (×10^{-3}mol·ℓ^{-1})</th>
<th>V(Y)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>0.01 M</td>
<td>4.94</td>
<td>4.46</td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>4.48</td>
<td>4.35</td>
</tr>
<tr>
<td>0.5 M</td>
<td>4.91</td>
<td>4.56</td>
<td>4.37</td>
</tr>
</tbody>
</table>

* pK_a values corrected for ionic strength and temperature effects
a Overall variance computed by FITMOD

Effect of ionic strength on equilibrium states

Solution pH affected the surface charge of the adsorbent by varying the degree of ionization and speciation of the adsorbate. The results from batch experiments conducted under varying pH and constant initial concentration showed an increase in the uptake of divalent species, Sr^{2+} and Co^{2+} due to increased pH, whereby the uptake of the monovalent species Cs^+ was inhibited at high pH. At an initial concentration of 75 mg·ℓ^{-1} and pH range 2–9, results obtained showed that most of the 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^2- and CsCl, respectively. This observation suggests that Sr and Cs cations undergo limited hydrolysis, which is in agreement with earlier findings by Baes and Mesmer (1976). In the case of cobalt, both initial concentration and pH played a significant role in its speciation. Generally, an increase in pH resulted in a decrease in the highly dissociated Co species. At low pH (pH 2–4), 99.7% Co species were present as Co^{3+} and 0.3% as CoNO_3^- (Fig. 7). At near-neutral pH (pH 5–7), slight precipitation occurred as Co species were distributed as follows:

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As mentioned above, Cs⁺ and Co²⁺ were affected by the solution pH in opposite ways. Whereas pH increase enhanced Co²⁺ removal, high pH inhibited Cs⁺ removal. The dependence of Cs⁺ on pH indicates association with the lower $pK_a$ reaction sites such as the carboxylic groups ($pK_a = 4.5$). In the low $pK_a$ groups, the reaction sites are increasingly deprotonated as pH values exceed 6. The deprotonation of carboxylic groups generates a net negative charge at the surface, which favours the binding of monovalent cationic species but these sites will trigger competition with other monovalent ions from the solution such as Na⁺ and K⁺ from the growth medium (Solecki, 2006; Apell and Diller, 2002).
Effect of temperature on equilibrium states

The effect of temperature on the adsorption behaviour was evaluated within the physiologically relevant pH range (pH = 4.5 to 8.5) and a range of temperatures (5, 50 and 70°C) (Table 2, Fig. 7). The pH range evaluated is related to the medium-range reaction sites containing the carboxylate, phosphate, and possibly some phenolic functional groups. The results show that, for Sr$^{2+}$, the adsorption reaction responded to temperature at lower pH values (pH < 7.5), whereas for monovalent species Cs$^+$ the adsorption reaction rate did not respond to the change in temperature (Table 2, Fig. 7). Compared with the results for the effect of ionic strength (Fig. 6), the effect of temperature is shown to be less significant than the effect of ionic strength. However, Fig. 7 further demonstrates that pH change increases the uptake rate with the influence extending from low pH to pH 7.5. At pH values above 7.5, further increase in pH has no effect on the uptake rate for all three of the metal species tested in this study. This may be due to direct precipitation of metallic species by the electronegative anions in the solution.

**DISCUSSION**

A preliminary analysis was conducted to determine the adsorption capacity of SRB cells using the classical Langmuir and Freundlich isotherm models (Ngwenya and Chirwa, 2010). Since the equilibrium data showed a definite saturation characteristic, the Langmuir model was adapted for adsorption capacity. The observed binding capacities for binary and tertiary ion systems was Sr$^{2+}$>Co$^{2+}$>Cs$^+$ (with $q_{\text{max}}=405.5$, 203.3 and 192.2 mg ion/g cells, respectively) (Ngwenya, 2011). However, the aim of this stage of the study was to investigate the features associated with the binding capacities, such as concentration of reaction sites and distribution of functional groups on the cell surfaces. The classical isotherms could not be used to gain the above knowledge since they are based on highly theoretical concepts which do not reflect the actual nature of chemical reactions. An equilibrium modelling approach was therefore followed to evaluate the ion exchange properties, speciation of species, and dissociation factors, over a range of pH, ionic strength and temperature conditions.

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 4-site non-electrostatic model as shown in Table 1. Site 1 corresponds to carboxylic acid functional groups ($pK_a=4.5$). This reaction site was seen to be critical in the binding of monovalent ionic species such as Cs$^+$. Carboxylic groups deprotonate easily as the pH of the solutions rises above 6, thereby exposing reaction sites for the Cs$^+$ binding. The other two divalent species Sr$^{2+}$ and Co$^{2+}$ were heavily influenced by the near-neutral pH reaction sites ($pK_a=6.7$ and $pK_a=8$). The result of this association was the direct opposite on the effect of pH on Cs$^+$ uptake. The implication on the binding of the metals to cell surfaces is significant since pH will play a critical role in determining the ionic strength of the solution.

The most abundant proton binding site on the SRB culture belonged to Site 4 (protonated amide groups), which accounted for about 39% of the total concentration of binding sites for the consortium. The $pK_a$ of this site is very high – in the region of $pK_a=10.0$, which means that binding to this site will be preferred at a relatively high pH. However, the results from Fig. 5 suggest that overall uptake of the metals under higher pH and higher ionic strength actually decreased. This discrepancy is explained by high precipitation rates at the high pH and competition for sites with other ions in solution under high ionic strength. The sum of all the conditions and effects above leads us to conclude that the most critical sites of reaction in the actual operation range would be the carboxylic $pK_a=5$ and phosphate $pK_a$ range 6–8. Over this $pK_a$ range, an optimum amount of deprotonated reaction sites will be available and the ionic strength will be weak enough to avoid precipitation and covalent bonding under high ionic strength conditions. Operation of pH under the Site 2–3 conditions (pH 6–7) will provide conditions for weak electrostatic interactions necessary for optimising the exchangeable fraction (F1) in Fig. 5a.

Estimation of reaction site concentration is important in the evaluation of the quality of SRB cells as a potential biosorbent for actual application. Through this analysis, it is possible to compare the quality of the current culture with that previously reported for other biosorbents. The total concentration of reaction sites ($C_{\text{tot}}$) increased slightly with increasing ionic strength. The observed values were 10.2×$10^{-3}$, 11.3×$10^{-3}$ and 12.2×$10^{-3}$ mol·L$^{-1}$ (average standard error, $\sigma_{\text{a}}=0.15×10^{-3}$) at ion concentrations of 0.01 M, 0.1 M and 0.5 M, respectively. The values observed here are 3 to 4 times higher than values obtained from pure cultures of *Pseudomonas aeruginosa* (3.31×$10^{-4}$ mol·L$^{-1}$), *Escherichia coli* (2.24×$10^{-4}$ mol·L$^{-1}$), *Aquabacterium commune*

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**Table 2**

Compilation of Sr$^{2+}$, Co$^{2+}$ and Cs$^+$ stability constants and variance at different temperatures and 0.1 M and 25°C as calculated by FITMOD using the non-electrostatic model

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Temperature (°C)</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr$^{2+}$</td>
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<td>3.37</td>
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<td>1.69</td>
<td>2.20</td>
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<td>75</td>
<td>1.91</td>
<td>2.07</td>
<td>4.06</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>5</td>
<td>1.65</td>
<td>3.07</td>
<td>--</td>
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<tr>
<td></td>
<td>50</td>
<td>1.61</td>
<td>2.81</td>
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<tr>
<td></td>
<td>75</td>
<td>1.54</td>
<td>2.85</td>
<td>--</td>
</tr>
<tr>
<td>Cs$^+$</td>
<td>5</td>
<td>1.58</td>
<td>3.44</td>
<td>4.78</td>
</tr>
<tr>
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<td>1.56</td>
<td>3.47</td>
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<td></td>
<td>75</td>
<td>1.58</td>
<td>3.43</td>
<td>5.03</td>
</tr>
</tbody>
</table>

*Overall variance computed by FITMOD

Biochem. 39 (8) 909–916.
Enterobacteriaceae sp. (1.27×10⁻³ mol×ℓ⁻¹) (Almaguer-Cantú et al., 2011; Yee and Fein, 2001). The only species reported in the culture from this study was Shewanella putrefaciens (Almaguer-Cantú et al., 2011; Yee and Fein, 2001). The concentration of the surface functional groups (–COOH and –OH).

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 concentration of the surface functional groups), and metal speciation in the aqueous phase. Both the ionic strength and pH play a critical role in achieving optimum efficiency of the system since both affect the availability of exchangeable reaction sites, the extent of stronger associations at higher pH, and the degree of competition with the other cations in solution.

CONCLUSIONS

Cell surfaces of a mixed culture of sulphate-reducing bacteria with a high affinity for cationic nuclear fission products, Sr²⁺, Co²⁺, and Cs⁺, were determined to be predominantly comprised of carboxylate and phosphate sites (about 50% of the total concentration of all binding sites observed). The culture differed from previously studied cultures due to the presence of Gram(+)ve species with a higher concentration of polar functional groups (–COOH) and (–OH) on the cell walls. Approximately 68% of adsorbed metal species were exchangeable, demonstrating the feasibility of recovery of metals from wastewater streams using this culture. This observation is further supported by the observed predominance of weak interactions, which resulted in higher metal uptake at medium to low pH values below 7. The decreased uptake of metals with increasing pH above 7 is attributed to competition for sites, with increasing dissociation of other metal complexes such as Ca²⁺ from sources within the medium resulting in incompetition for sites with Sr²⁺, Co²⁺ and Cs⁺.

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REFERENCES


HARJULA R and LEHTO J (1986) Effect of sodium and potassium ions on cesium absorption from nuclear power plant waste solutions on synthetic zeolites. Nucl. Chem. Waste Manage. 6 (2) 133–137.


**SYMBOLS**

$C_a$ molar concentration of acid

$C_{b}$ molar concentration of base

$C_{tot}$ total site concentration on bacterial cell surface

$F$ Faraday constant (C⋅mol⁻¹)

$k_2$ second-order reaction rate constant (T⁻¹)

$K$ zero charge/zero coverage constant

$K_a$ stability constant

$K_{int}$ intrinsic stability constant

$m_b$ concentration of SRB cells

$M^+$ target metal

$n_{ch}$ number of chemical components

$n_d$ number of data points

$n_p$ number of adjustable parameters

$q_{eq}$ equilibrium coefficient (g metal ion⋅g biomass⁻¹)

$R$ molar gas constant (J⋅gmol⁻¹×K⁻¹)

$R\cdot A^-$ deprotonated bacterial surface site

$R\cdot A^-$ metal site complex

$\sigma_e$ average standard error

$S_\gamma$ default experimental error

$T$ absolute temperature (°K)

$Y$ error in the mass balance calculations (from the program FITMOD)

$\Psi$ surface potential measured in volts (V)

$Z$ charge of the adsorbing ion

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