Biological removal of cationic fission products from nuclear wastewater

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ABSTRACT

Nuclear energy is becoming a preferred energy source amidst rising concerns over the impacts of fossil fuel based energy on global warming and climate change. However, the radioactive waste generated during nuclear power generation contains harmful long-lived fission products such as strontium (Sr). In this study, cationic strontium uptake from solution by microbial cultures obtained from mine wastewater is evaluated. A high strontium removal capacity (q_{max}) with maximum loading of 444 mg/g biomass was achieved by a mixed sulphate reducing bacteria (SRB) culture. Sr removal in SRB was facilitated by cell surface based electrostatic interactions with the formation of weak ionic bonds, as 68% of the adsorbed Sr^{2+} was easily desorbed from the biomass in an ion exchange reaction with $MgCl_2$. To a lesser extent, precipitation reactions were also found to account for the removal of Sr from aqueous solution as about 3% of the sorbed Sr was precipitated due to the presence of chemical ligands while the remainder occurred as an immobile fraction. Further analysis of the Sr-loaded SRB biomass by scanning electron microscopy (SEM) coupled to energy dispersive X-ray (EDX) confirmed extracellular Sr^{2+} precipitation as a result of chemical interaction. In summary, the obtained results demonstrate the prospects of using biological technologies for the remediation of industrial wastewaters contaminated by fission products.

Key words | adsorption, bioprecipitation, strontium, sulphate reducing bacteria, wastewater

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INTRODUCTION

Radioisotope fission products are routinely or accidentally discharged into the environment with wastewaters from various activities, such as mining and milling of nuclear fuel, fall out from nuclear weapon testing or leakage from storage facilities at nuclear installations as well as from industrial and medical facilities (Singh *et al.* 2008). Fission products are considered the most hazardous elements to living organisms in the environment due to their radiotoxicity and longevity. Among these, radiostrontium (Sr-90) is the most abundant radiochemical component of nuclear waste (Watson *et al.* 1989). Strontium is highly mobile in both soils and groundwater systems (Dewiere *et al.* 2004) and it has a half-life of 28 years. Due to its chemical similarity with calcium, it is easily incorporated into bone material in mammals. When incorporated in the organisms in this manner, it continues to irradiate localized tissues with the eventual development of bone sarcoma and leukaemia (Chen 1997). The main disadvantages of using conventional adsorbents, such as zeolites and synthetic organic ion exchangers, for strontium removal from radioactive waste is their unsuitability at high pH, high sodium concentrations, and in irradiated environments (Chaalal & Islam 2001). Microbial adsorbents, on the other hand, have been shown to possess high capacities for the selective uptake of a range of metals and radionuclides from dilute metal-bearing solutions (Beveridge 1989; Mullen *et al.* 1989; Chubar *et al.* 2008).

The use of microorganisms in the treatment of radioactive wastewater is mainly governed by their survival in extreme conditions of heat, desiccation and radiation. In recent studies it has been shown that microorganisms can survive in irradiated environments, such as the walls of a pool storing nuclear materials at a Spanish nuclear power plant and in an underground granitic rock nuclear waste repository in Canada, where a range of heterotrophic aerobic and

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anaerobic bacteria ranging from 10^1 to 10^6 cells/g dry weight buffer were found. Among these were approximately 10^2 sulphate reducing bacteria (SRB) and methanogenes per gram of dry weight buffer (Kováčová *et al.* 2002; Chicote *et al.* 2004). This has sparked interest on the use of these microorganisms, particularly SRB to treat wastewaters of different radiation levels. The sulphate reducing bacteria metal remediation concept is not new, however their occurrence in radioactive environments has renewed interest in their application for radioactive waste remediation. SRB has been shown to catalyze the reduction of toxic forms of metals and radionuclides, such as U(VI), Tc(VII), Pd(II), Cr(VI), As(V) and Mo(VI) to less toxic forms (Lovley & Phillips 1992, 1994; Lovley *et al.* 1993; Lloyd *et al.* 1998, 1999; Tucker *et al.* 1998; Smith & Gadd 2000).

In this study, the performance of a mixed SRB culture for the removal of Sr^{2+} from aqueous solution is evaluated. The reversibility of the interaction between the biomass and metal ion (Sr^{2+}) is used as a basis for predicting the nature of the main metal removal mechanism(s) involved.

MATERIALS AND METHODS

Source and growth of microorganisms

A starter culture of a sulphate reducing bacteria consortium was isolated from a coal mine dumpsite. SRB cells were cultivated in sterile modified Postgate medium C containing (per litre of distilled water); 6 mL sodium lactate (60% solution w/v), 1 g yeast extract, 1 g NH_4Cl, 4.5 g Na_2SO_4, 0.5 g KH_2PO_4, 0.06 g $MgSO_4.7\ H_2O$, 0.1 g sodium citrate. 2 H_2O, 0.06 g $CaCl_2.6\ H_2O$, 0.1 g ascorbic acid and 0.1 g sodium thioglycollate prepared according to published procedures (Postgate 1984). Cells were aseptically transferred to 1 L rubber-sealed flasks and allowed to grow until mid-stationary phase (5–7 days), after which they were harvested by centrifugation (6000 × g, 15 minutes). Cells were then repeatedly washed in deionized water to remove growth medium impurities. Metal cations that maybe present on SRB cell wall surfaces were stripped by soaking in 0.001 M EDTA for 30 minutes, followed by intensive rinsing in deionized water.

Sr^{2+} removal experiments

Sr^{2+} removal experiments were conducted under equilibrium conditions in bench scale anaerobic bioreactors (2 L). All glassware was soaked in 10% HNO_3 and rinsed with distilled water prior to and after use to remove any traces of metal ions. A Sr^{2+} stock solution was prepared from $SrCl_2.6\ H_2O$ (Merck, South Africa), by dissolving the appropriate amount in 10 mL HNO_3/distilled water solution (1:1) and then diluted to 1 L to give a final concentration of 1000 μg mL^{-1}. Working concentrations and standard solutions were prepared by diluting the stock solution with distilled water to give the desired concentration. Experiments were initiated by inoculating each of the bioreactors with a predetermined SRB biomass (dry weight). The initial Sr^{2+} concentration was varied between 75–1000 mg/L, while the SRB biomass density was kept constant at 1 mg/L. The suspensions were agitated for 3 hours and then samples were withdrawn for residual Sr^{2+} concentrations analysis.

Desorption studies

Reversibility of the SRB-metal ion interaction was conducted in order to elucidate the role of the adsorption and precipitation reactions on the removal of Sr^{2+} from solution. Sr^{2+}-loaded SRB biomass was treated with the appropriate desorbing agents to determine the distribution of species in the insoluble fraction. Species distribution was performed by a five-step sequential extraction according to a method by Tessier *et al.* (1979).

Localization and identification of Sr precipitates

SRB biomass previously exposed to 100 mg/L Sr^{2+} was obtained by filtering through a 0.22 μm membrane. The membranes containing the strontium-loaded cells were then folded into a quarter, so as to avoid loss of cells during treatment. The membranes were then prepared for scanning electron microscopy (SEM) by first fixing overnight in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) solution. The fixative solution was decanted off and cells were then washed twice in phosphate buffer (0.1 M, pH 7.0) for 10 minutes. Thereafter, the cells were dehydrated in a series of ethanol solutions, 30%, 50%, 70%, 80%, 90%, and twice in absolute ethanol, and each step lasted for 15 minutes. The samples were then dried in liquid CO_2 for 2 hours at critical point (31.1°C and 73 atmos.). Pieces of the membranes containing dried cells were cut into small squares and then mounted on stubs with double-sided tape, and then gold-coated for 30 minutes in a Large Desk II Cold Sputter Etch Coater. The prepared samples were then observed under a scanning electron microscope (JEOL-JSM-840 SEM) equipped with an Energy Dispersive X-ray (EDX) spectrometer using a Cd^{109} radioisotope source and a Si(Li)

semiconductor detector of resolution 170 eV for 5.9 keV Mn K_α X-ray. SRB cells not exposed to a rhodium solution served as a control.

Analytical procedures

Total strontium (Sr_T), solid-phase strontium (Sr_S) and dissolved Sr^{2+} (Sr_D) species concentrations were determined separately. Prior to Sr_S and Sr_D determination, raw samples were centrifuged at 15000 g × 20 minutes to separate the dissolved and insoluble fractions. To determine Sr_D, 2.5 mL of the supernatant was dispensed into 10 mL acid washed tubes to which 0.5 mL of 30% H_2O_2 and 0.1 mL of 70% trace metal grade HNO_3 were added and incubated at 60°C overnight. The resulting pellet was also subjected to the same acid digestion treatment. Following digestion or extraction, strontium concentrations were determined at a wavelength of 460.7 nm in a nitrous oxide-acetylene flame using an AAnalyst 400 Perkin Elmer AAS (Perkin Elmer, Shelton, USA). Strontium ionization in the flame was suppressed by the addition of a potassium chloride solution to give a final concentration of 2 mg/L K^+ in all samples including the standards and blank.

RESULTS AND DICSUSSION

Performance Evaluation of Sr^{2+} removal

The classical Langmuir and Freundlich isotherm models were used to provide information on the Sr^{2+} sorption capacity of the bacteria. The linearized forms of the equations are:

$$\frac{1}{q} = \frac{1}{q_{max}} + \frac{1}{C_{eq}} \cdot \frac{1}{b\, q_{max}} \quad (1)$$

$$\log(q) = \log(k) + \frac{1}{n}\log(C_{eq}) \quad (2)$$

where: q = sorption uptake (mg/g), q_{max} = maximum sorbate uptake (mg/g), b = coefficient related to the affinity between the sorbent and sorbate, C_{eq} = equilibrium concentration of the sorbate remaining in the solution (mg/L), k = constant corresponding to the binding capacity and n = coefficient related to the affinity between the sorbent and sorbate.

Results obtained for the equilibrium sorption of Sr^{2+} followed the Langmuir model, as indicated by the higher correlation coefficient (Table 1). This suggests that Sr^{2+} removal occurred until equilibrium was reached, as opposed to precipitation reactions where the data cannot be fitted with a simple Langmuir model. Precipitation reactions follow a multi-layer model including surface precipitates. In this study, a Langmuir model is used to provide information on the adsorption capability of the sorbent (SRB biomass). The model represents the overall equilibrium sorption process behaviour. Sulphate reducing bacteria biomass is demonstrated as a potent Sr^{2+} sorbent with a maximum sorption capacity (q_{max}) of 444 mg/g. This value is higher than reported values elsewhere in literature both specifically for Sr^{2+} (Shaukat *et al.* 2005; Dabbagh *et al.* 2007, Chegrouche *et al.* 2009) and for sulphate reducing bacteria (Vijayaraghavan & Yun 2008).

Table 1 | Langmuir and Freundlich model parameters for the equilibrium sorption of Sr^{2+} by a SRB biomass (1 g L^{-1})

Langmuir model			Freundlich model		
q_{max} (mg g^{-1})	b	R^2	k	n	R^2
444	0.011	0.993	17.2	1.95	0.986

Desorption studies

Results obtained in this study showed 68% Sr^{2+} removal through adsorption reactions. Most of the solid phase Sr^{2+} species were easily desorbed from biomass using $MgCl_2$ (Figure 1). Metal species in the desorbed fraction give an indication of the amount of Sr^{2+} that is bound on the biomass surface by relatively weak electrostatic interactions which are easily released by the ion-exchange process (Dahl *et al.* 2008). The elevated concentrations of Sr^{2+} in the desorbed fraction may be due to the release from the complexing agents on the microbial cell surface.

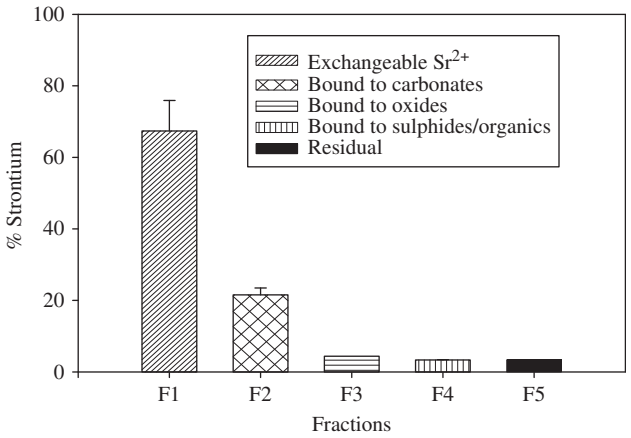

Figure 1 | Partitioning of strontium species in the solid fraction after exposure to an SRB consortium.

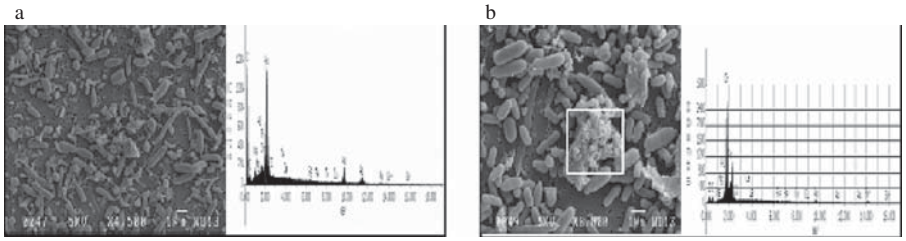

Figure 2 | SEM image and EDX analysis of control (a), and an area of SRB cells that was exposed to 100 mg L^{-1} Sr^{2+} (b). Elemental analysis: Sr = 0%, Mg = 4.58%, Al = 2.62%, Si = 8.44%, S = 18.18%, Ca = 15.52%, Fe = 15.53% and P = 35.12% (a), and Sr = 65.27%, S = 33.45%, Na = 0.34%, Cl = 0.4%, Fe = 0.36% and Ca = 0.18% (b).

Since the bulk of Sr^{2+} is removed from solution through the simple adsorption process, this could lead to the development of an efficient and cost-effective removal process of divalent fission product from the nuclear wastewater stream. Other processes are attributed to the removal of the remainder of the Sr^{2+} that is found in the solid phase.

SEM/EDX analysis of solid-phase strontium precipitates

Scanning electron micrographs of SRB biomass previously exposed to medium containing Sr^{2+} revealed whitish precipitates on the surface of the cells which were not observed in controls, suggesting minor extracellular precipitation of metal ions to bacterial surface (Figure 2a). 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.

For the small part that precipitated, EDX analysis showed that about 65% was Sr^{2+} compounds (Figure 2b). The absence of precipitates and as well as Sr peaks in the control sample after EDX analysis confirms that the observed Sr^{2+} precipitates and peaks are a result of the interaction between the bacteria and the metal ions mainly through adsorption and to a lesser extent precipitation reactions. These findings are in agreement with other reports where functional groups on the cell surface of bacteria facilitated bulk metal binding from aqueous solution (Sherbet 1978). However, further studies still need to be conducted to clarify the observed findings.

CONCLUSION

Most of the strontium removed from solution was bound onto biomass surface mainly through cell surface complexation, ion exchange, adsorption, electrostatic and hydrophobic interactions, and while the rest was through micro-precipitation. These results indicate that sulphate reducing bacteria can play a vital role in the remediation of wastewaters heavily polluted with fission products and radionuclides. In addition, metal desorption studies indicated that recovery of the sorbed metal can be cost-effective exercise since major removal occurs through ion exchange processes. Thus, the sulphate reducing bacteria remediation system can be effectively used for water bodies contaminated by strontium as an *in situ* remediation strategy, and thereafter the biomass recovered for possible re-use. Knowledge of such mechanisms may be useful in engineering the removal of other harmful fission products and recovery of valuable radionuclides from wastewater of nuclear facilities.

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