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Removal and biotransformation of U(VI) and Cr(VI) by aerobically grown mixed microbial granules

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ABSTRACT

We assessed the potential of aerobic granular sludge consisting of mixed species of bacteria to remove and immobilize uranium (VI) and chromium (VI). Microbial granules were cultivated in a laboratory sequencing batch reactor (SBR) by feeding with acetate-containing synthetic media. Microbial granules formed in the SBR exhibited excellent settling characteristics and predominantly consisted of rod/cocci shaped bacteria. The microbial granules immobilized 218 ± 2 mg of U(VI) g^{-1} dry granular biomass. X-ray photoelectron spectroscopy (XPS) showed the association of U(VI) with the microbial granules and transformation of U(VI) to U(IV). Microbial granules reduced Cr(VI) and immobilized to Cr(III) at 0.17 mmoles/d/g under anaerobic conditions. X-ray absorption near edge spectroscopy (XANES) of chromium associated with microbial granules revealed complete conversion of Cr(VI) to Cr(III). Extended X-ray absorption fine structure (EXAFS) analysis of the Cr-laden microbial granules showed similarity to Cr(III)-phosphate. This study demonstrates the biotransformation and immobilization of U(VI) and Cr(VI) by mixed species microbial granules.

Keywords: Aerobic microbial granules; Aerobic granular sludge; Aerobic granules; Biosorption; Biotransformation; Bioremediation; Cr(VI) reduction; Uranium (VI)

1. Introduction

In natural and engineered settings, bacteria predominantly grow as mixed microbial communities (biofilms, bioflocs and microbial granules). Bacteria growing in a community exhibit enhanced resistance and tolerance to toxic metals, compared with those which are growing in a single culture. Development of bioremediation/biological treatment techniques based on transformation and immobilization of metals/radionuclides using bacteria growing in consortia might be advantageous because (i) the cells in a community can tolerate higher concentrations of metals/radionuclides compared to planktonic cells, and, (ii) they allow easy post-treatment separation of the biomass from the treated liquid either through flocculation or granulation. Aerobic granular sludge (aerobic microbial granules) have attracted research interest due to its potential use in the development of new generation wastewater treatment plants [1,2]. In these systems, microbial community is grown as compact and dense microbial granules without using carrier material [3]. Because of high settling velocities,

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aerobic microbial granules eliminate the use of large settling tanks and allow relatively high biomass concentrations in the reactor leading to higher conversion rates (i.e., carbon, nitrogen removal) [4]. Moreover, aerobic microbial granule based system requires only 20% of the surface area needed for a conventional activated sludge system and needs less investment and lower operational cost compared to activated sludge system [4]. Previous work showed that aerobic microbial granules possess tremendous ability for biosorption of heavy metals and uranium (VI) [5,6]. Bacterial transformation of metals/radionuclides to less soluble forms is a promising bioremediation strategy for immobilization of contaminants [7–9]. Studies on the transformation and immobilization of metals/radionuclides by mixed bacterial consortia are limited [10]. The main aim of this work was to determine the biotransformation and immobilization of U(VI) and Cr(VI) by microbial consortia in aerobically grown microbial granules.

2. Materials and methods

2.1. Generation of aerobic microbial granules

Microbial granules were formed in a 3 l working volume column-type sequencing batch reactor (SBR). SBR setup and operation details have been described previously [11]. Briefly, the SBR (diameter: 6.2 cm, height: 120 cm) was inoculated with activated sludge flocs collected from an operating municipal wastewater treatment plant (Kalpakkam, India). The SBR was fed with synthetic wastewater (SWW) prepared in deionized water and consisting of the following: CH_3COONa (63 mM), $MgSO_4{\cdot}7H_20$ (3.6 mM), KCl (4.7 mM), NH_4Cl (35.4 mM), K_2HPO_4 (4.2 mM), KH_2PO_4 (2.1 mM) and 0.1 ml of trace elements mix [12]. Air was supplied at the bottom of the SBR using an oil-free air compressor and the superficial air velocity was maintained at 1.6 cm/sec by controlling aeration rate. The SBR was operated at room temperature (~30°C) with 66% volume exchange ratio and 6 h cycle period. Addition of the media at the bottom of the SBR and the withdrawal of the effluent from a port situated at 34 cm height from the bottom were performed using peristaltic pumps operated using electronic timers. Microbial granules harvested after 2 mon of SBR operation were used in U(VI)/Cr(VI) immobilization experiments.

2.2. U(VI)-immobilization experiments

Experiments were carried out in 100 ml of minimal medium containing U(VI) and aerobic microbial granules (wet weight: 5 g). The minimal medium consisted of the following ingredients (g per l): NH_4Cl, 1 g; $MgSO_4{\cdot}7H_2O$, 0.2 g; CH_3COONa, 3 g; yeast extract, 0.1 g; and 0.1 ml trace elements mix [12]. The pH of the media was adjusted to 5.5. The U(VI) stock was prepared by dissolving uranyl nitrate ($UO_2(NO_3)_2$) (Merck, India) in distilled water. The flasks were incubated in a rotary shaker set at 100 rpm and 30°C. Samples of 0.5 ml aliquots were collected at regular time intervals and analysed for U(VI). At the end of the experiment, microbial granules were lyophilized and analyzed for uranium association and speciation by XPS.

2.3. Cr(VI) immobilization experiments

Cr(VI) reduction experiments were performed under aerobic and anaerobic conditions in acetate minimal medium (AMM) consisting of the following ingredients (g/l): 1.0 NH_4Cl, 0.2 $MgSO_4{\cdot}7H_2O$, 3.02 CH_3COONa, 0.5 KH_2PO_4, and 0.5 yeast extract. The pH of medium was adjusted to 7.0 before autoclaving it. For the aerobic reduction of Cr(VI), 250 ml flasks containing 100 ml of AMM with chromate and the microbial granules were incubated on a rotary shaker at 100 rpm at 30°C. For anaerobic reduction, 100 ml glass bottles containing 100 ml of AMM with chromate and microbial granules were bubbled with nitrogen gas and sealed with rubber stoppers incubated at 30°C. Cr(VI) (0.2 to 3 mM) was introduced prior to the experiment by adding a stock solution of potassium dichromate prepared in sterile filtered distilled water. Samples were collected at regular intervals, and solids were separated and analyzed for Cr(VI) in the supernatant by spectrophotometer. At the end of experiment, microbial granules were collected, lyophilized and analyzed for Cr oxidation state and its association with the biomass by XANES and EXAFS respectively, at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, USA.

2.4. Microscopy

Morphology of the microbial granules was documented with a DP70 camera (Olympus, Japan) connected to a SMZ1000 stereozoom microscope (Nikon, Japan). Particle size and circularity (Circularity values ranges from 0 (infinitely elongated polygon) to 1 (perfect circle)) were determined by using the freeware *ImageJ* v1.43. Microstructure was determined using confocal laser scanning microscope (CLSM) and scanning electron microscope (SEM). For CLSM imaging, microbial granules were stained with *Bac*Light™ bacterial viability kit (Molecular Probes, USA) and imaged using a confocal laser scanning microscope TCS SP2 AOBS (Leica Microsystems, Germany) equipped with an inverted microscope (Leica DMIRE2). A 63x 1.2 NA water immersion objective was used for imaging.

Argon laser (488 nm line) was used for excitation and emission was recorded between 500 and 520 nm for SYTO 9 and between 600 and 680 nm for propidium iodide (PI). For SEM imaging, the microbial granules were fixed overnight with 2.5% glutaraldehyde and dehydrated in a series of ethanol solutions (50%, 70%, 90% and 100%). The dehydrated microbial granules were sputter-coated with gold-palladium and imaged using a scanning electron microscope (Philips XL30 ESEM).

2.5. Analytical procedures

Volatile suspended solids (VSS), sludge volume index (SVI), biomass density and dry weight of biomass were determined according to standard methods [14]. Soluble uranium in the samples was analysed spectrophotometrically using arsenazo III [15]. Uranium associated with the microbial granules was analysed by X-ray photoelectron spectroscopy (XPS). Cr (VI) concentration remaining in the medium was measured using Cr (VI)-specific colorimetric reagent S-diphenylcarbazide (DPC). A pink color was developed when Cr (VI) was present in the samples and the absorbance measured immediately at 540 nm using UV-vis spectrophotometer (Shimazdu, Japan). Microbial granules exposed to Cr(VI) were lyophilized and analysed using X-ray absorption near edge spectroscopy (XANES) and Extended X-ray absorption fine structure (EXAFS) as described elsewhere [13].

3. Results and discussion

3.1. Characteristics of microbial granules

The morphology of microbial granules sampled from the SBR for U(VI) and Cr(VI) immobilization experiments is shown in Fig. 1. The average size of the granules was 1.8 mm, and average circularity was 0.9.

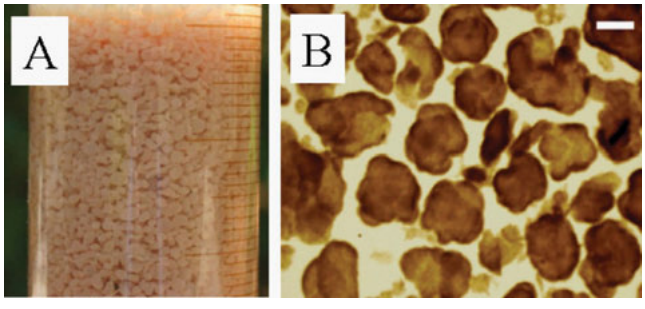

Fig. 1. Morphology of the aerobic microbial granules cultivated in a 3 l sequencing batch reactor. A) Cultivated microbial granules transferred to a measuring cylinder B) stereozoom micrograph. Scale bar in B, 1 mm.

Microbial granules exhibited excellent settling abilities with SVI_{10min} of 30 ml/g and the biomass density was 40 g VSS/l. Settling characteristics of the aerobic microbial granules formed in the SBR were similar to those reported by others [16]. Filamentous microorganisms, predominant in the seed sludge, were not observed in the microbial granules. Staining with general nucleic acid binding fluorophore revealed that the microbial granules consists of several cells clusters with extensive water channels (Fig. 2a). The majority of the cell clusters consisted of closely organized bacterial cells. SEM showed the presence of rod and cocci shaped cells on the outer surface of the aerobic microbial granules (Fig. 2b).

3.2. U(VI) immobilization

Almost complete removal of U(VI) from 6 to 100 mg/l U(VI) solution was observed within one hour of incubation (Fig. 3). The U(VI) remaining after biosorption was found to be less than 1 mg/l. In the case of 140 mg/l U(VI) initial concentration, about 10 mg/l

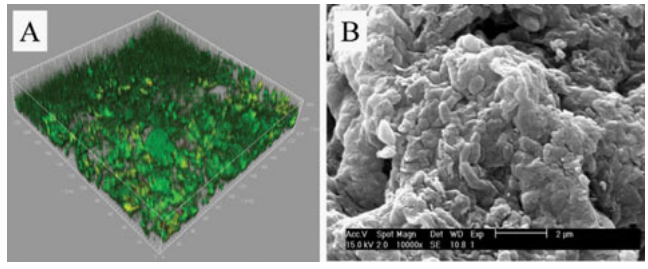

Fig. 2. Microstructure of aerobic microbial granule as shown by confocal microscopy (A) and scanning electron microscopy (B) Confocal image consisted of 28 xy-slices obtained at z-interval of 2 μm.

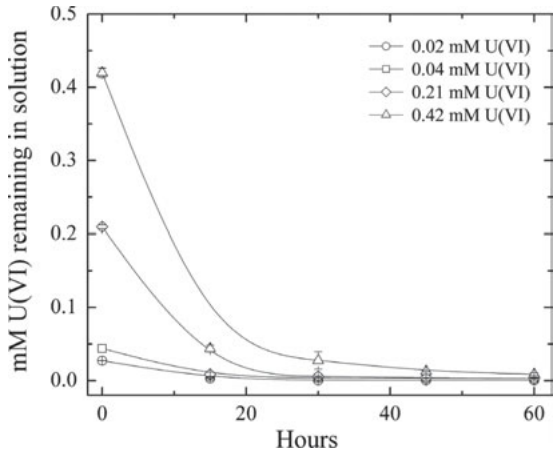

Fig. 3. U(VI) removal by aerobic microbial granules. The initial biomass content was constant (5 g wet weight).

U(VI) was remaining in solution even after 24 h of incubation. The maximum U(VI) removal was 0.93±0.01 mmoles U(VI) g^{-1} granular biomass [6]. Maximum U(VI) removal observed in this study is less than the reported values for *Pseudomonas* strain [17]. However, the U(VI) removal capacity of the bacteria decreased significantly when the bacterial cells were immobilized for practical use [17]. XPS confirmed that U(VI) removed from the media was associated with the microbial granules (Fig. 4). Appearance of peak specific for U(IV) shows the reduction mediated by the microbial granules (Fig. 4). U(VI) removal was primarily driven by the biosorption on to the microbial granules. Appearance of U(IV) indicates that the micro-anaerobic environments present within the macro-scale microbial granules might have facilitated the microbial transformation.

3.3. Cr(VI) immobilization

In order to determine the mechanism of Cr(VI) removal, experiments were performed (i) without the microbial granules (ii) with lyophilized microbial granules (iii) with microbial granules in the absence of electron donor (i.e., acetate) and (iv) with microbial granules with electron donor. No decrease in the concentration of Cr(VI) was observed in the cell-free control. Similarly, no significant change in the Cr(VI) concentration in the media was observed in the absence of an electron donor or when the experiment was performed with lyophilized granular biomass. These results confirm the role of bacterial cell metabolism in Cr(VI) removal process. The Cr(VI) reduction was evident by means of decolorization (yellow to colorless) of the media, and Cr(VI)-measurements by DPC. Although Cr(VI) reduction occurred both under aerobic and anaerobic conditions, it was efficient and complete under anaerobic conditions (Fig. 5). Time required for complete removal of Cr(VI) was dependant on the initial biomass content (Fig. 6).

Stable and sustainable reduction of 0.2 mM of Cr(VI) was confirmed in fed-batch experiments performed using same biomass and replenishing the spent medium with fresh media containing U(VI). However, the exposure to Cr(VI) resulted in an increase in the number of cells stained with propidium iodide, indicating membrane damage. Although the number of cells stained by PI (red) increased markedly during the exposure to Cr (VI), many cell clusters were still stained by Syto9 (green)

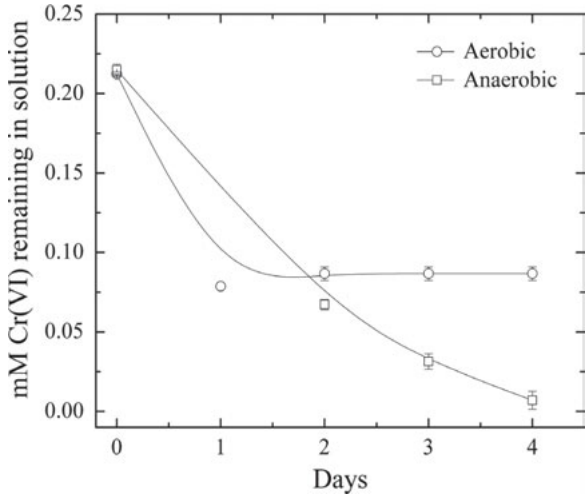

Fig. 5. Cr(VI) reduction under aerobic and anaerobic conditions. Initial biomass and Cr(VI) were 10 g granules and 0.21 mM respectively.

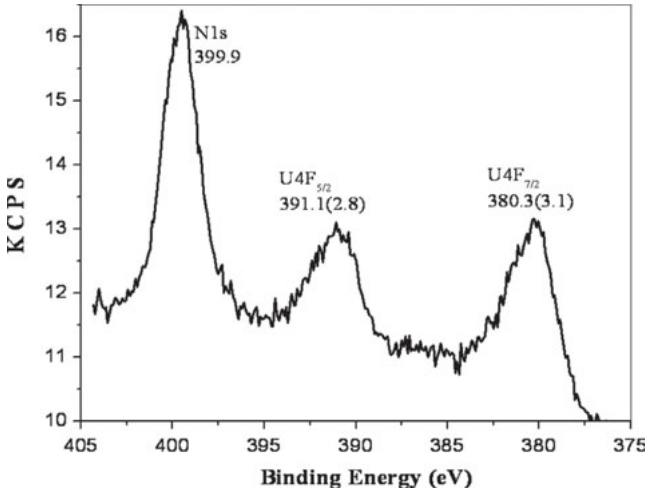

Fig. 4. XPS of uranium associated with the aerobic microbial granules. XPS showed the presence of U(VI) and U(IV).

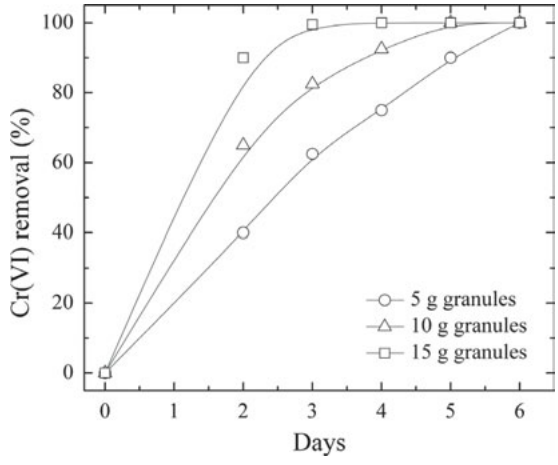

Fig. 6. Cr(VI) removal by microbial granules under anaerobic conditions. Experiments were conducted in 100 ml volume containing 0.2 mM of Cr(VI).

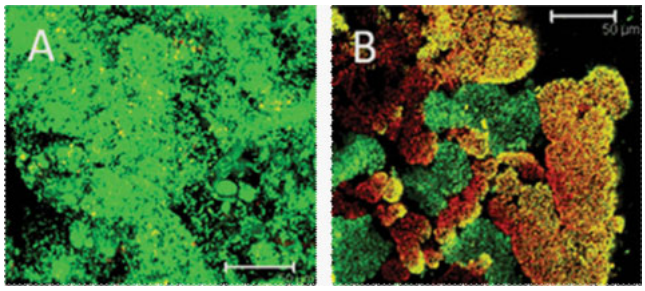

Fig. 7. Distribution of live and dead cells in aerobic granules upon exposure to Cr (VI) for 24 h. A) Microbial granules not exposed to Cr (VI). B) Microbial granules exposed to 10 mg/l Cr (VI) shows the distribution of cells with normal (green) and compromised (red) cell membrane. Scale bar = 50 µm.

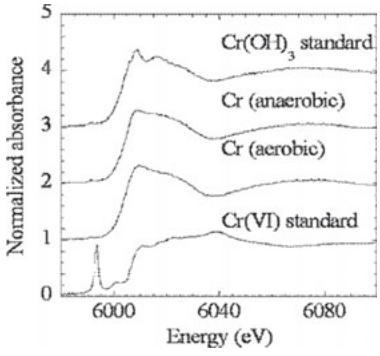

Fig. 8. XANES spectra of Cr speciation in microbial granule samples incubated aerobically and anaerobically [13].

or by both the fluorophores (yellow) (Fig. 7). Analysis of Cr(VI) speciation by XANES further confirmed the biotransformation of Cr(VI) to Cr(III) (Fig. 8). EXAFS analyses revealed that Cr(III) associated with microbial granules was similar to Cr(III)-phosphate (Fig. 9). Interestingly, aerobically grown microbial granules exhibited Cr(VI) reduction without any lag period shows that chromate reducing enzymes are constitutively present in the cells.

The study has implications when the remediation of mixed wastes is desirable. Remediation of mixed hazardous wastes is much more difficult than dealing with individual contaminants. This is because a bacterium capable of degrading one component of the waste may be totally inhibited by another toxic component. This has been the case particularly in radioactive waste containing a mixture of contaminants, organic and metal/radionuclides. Moreover, dilution of concentrated wastes before treatment is not always feasible. For example, in the case of radioactive mixed wastes, dilution presents regulatory and safety concerns. Development of biological treatment processes to remove the components of such concentrated mixed wastes become practical only

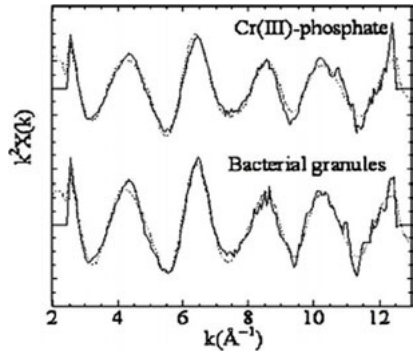

Fig. 9. The fitted k^2-weighted (2.5–12.5Å) showing association of 1 mM Cr(III) with mixed bacterial granules [13]. Experimental data (solid line); fitted data (dotted line).

when the volume is not significantly increased. Under such situations, microbial granules may allow designing compact and high-rate biological treatment systems.

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References

[1] C. Di Iaconi, R. Ramadori, A. Lopez and R. Passino, Aerobic granular sludge systems: the new generation of wastewater treatment technologies, Ind. Eng. Chem. Res., 46 (2007) 6661–6665.
[2] S.S. Adav, D.J. Lee, K.Y. Show and J.H. Tay, Aerobic granular sludge: recent advances, Biotechnol. Adv., 26 (2008) 411–423.
[3] E. Morgenroth, T. Sherden, M.C.M. van Loosdrecht, J.J. Heijnen, and P.A. Wilderer, Aerobic granular sludge in a sequencing batch reactor, Water Res., 31 (1997) 3191–3194.
[4] L.M. de Bruin, M.K. de Kreuk, H.F. van der Roest, C. Uijterlinde and M.C. van Loosdrecht, Aerobic granular technology: an alternative to activated sludge, Water Sci. Technol., 49 (2004) 1–7.
[5] Y. Liu, H. Xu, S.F. Yang and J.H. Tay, A general model for biosorption of Cd^{+2}, Cu^{+2}, and Zn^{+2} by aerobic granules, J. Biotechnol., 102 (2003) 233–239.
[6] Y.V. Nancharaiah, H.M. Joshi, T.V.K. Mohan, V.P. Venugopalan and S.V. Narasimhan, Aerobic granular biomass: a novel biomaterial for efficient uranium removal, Curr. Sci. INDIA, 91 (2006) 503–509.
[7] A.J. Francis, J. Biotransformation of uranium and other actinides in radioactive wastes, J. Alloy. Compd., 271–273 (1998) 78–84.

[8] A.J. Francis, Microbial mobilization and immobilization of plutonium, J. Alloy. Compd., 444–445 (2007) 500–505.
[9] N.S. Bolan, D.C. Adriano, R. Natesan and Bon-Jun Koo, The effects of organic amendments on the reduction and phytoavailability of Cr(VI) in mineral soil, J. Environ. Qual., 32 (2003) 120–128.
[10] W.L. Smith and G.M. Gadd, Reduction and precipitation of chromate by mixed culture sulphate-reducing bacterial biofilms, J. Appl. Microbiol., 88 (2000) 983–991.
[11] Y.V. Nancharaiah, N. Schwarzenbeck, T.V. Mohan, S.V. Narasimhan, P.A. Wilderer and V.P. Venugopalan, Biodegradation of nitrilotriacetic acid (NTA) and ferric-NTA complex by aerobic microbial granules, Water Res., 40 (2006) 1539–1546.
[12] Y.V. Nancharaiah, H.M. Joshi, M. Hausner and V.P. Venugopalan, Bioagumentation of aerobic microbial granules with *Pseudomonas putida* carrying TOL plasmid, Chemosphere, 71 (2008) 30–35.
[13] Y.V. Nancharaiah, C. Dodge, V.P. Venugopalan, S.V. Narasimhan and A.J. Francis, Immobilization of Cr(VI) and its reduction to Cr(III) phosphate by granular biofilms comprising of mixture of microbes, Appl. Environ. Microbiol., 76 (2010) 2433–2438.
[14] APHA, Standard methods for the examination of water and wastewater, 20th edn., American Public Health Association, Washington, DC, 1998.
[15] E. Hosten and H.E. Rohwer, Complexation reactions of uranyl with arsenazo III, Anal. Chim. Acta, 355 (1997) 95–100.
[16] J.J. Beun, A. Hendriks, M.C.M. van Loosdrecht, E. Morgenroth, P.A. Wilderer and J.J. Heijnen, Aerobic granulation in a sequencing batch airlift reactor, Water Res., 33 (1999) 2283–2290.
[17] P. Sar, S.F. Kazy and S.F. D'Souza, Radionuclide remediation using a bacterial biosorbent, Int. Biodeterior. Biodegrad., 54 (2004) 193–202.