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Section: Biofuels and Environmental Biotechnology

Accumulation of zirconium phosphate by a *Serratia* sp.: A benign system for the removal of radionuclides from aqueous flows

Claire Mennan, Marion Paterson-Beedle and Lynne E Macaskie*

School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

*Author for correspondence Fax: +44 (0)121 414 5925; E-mail: l.e.macaskie@bham.ac.uk

Keywords Biofilm, Biomineralisation, Cobalt, *Serratia*, Strontium, Zirconium

Abstract

Metal phosphate deposited enzymatically on *Serratia* sp. has been used successfully for the removal of radionuclides from aqueous flows. Previous studies using biogenic hydrogen uranyl phosphate (HUP) on *Serratia* sp. biofilm showed removal of 100 % of ^{90}Sr , ^{137}Cs , and ^{60}Co via their intercalation into biogenic HUP crystals. Zirconium phosphates (ZrP) offer a potential non-toxic and non-radioactive alternative to HUP for water decontamination. A method was developed for biomanufacturing ZrP. Biogenic ZrP removed ca. 100 % of Sr^{2+} and Co^{2+} (0.5 mM) from solutions to a molar ratio at saturation of ca. 1:0.6 for both Zr:Sr and Zr:Co. The potential for drinking water decontamination via bio-ZrP is discussed with respect to bio-HUP and also other commercially available materials.

Introduction

Nuclear power is enjoying a resurgence since its widespread implementation would reduce atmospheric release of ‘greenhouse gases’; however, public embracement of nuclear technology is still limited by the perceived risks of environmental contamination and the need for effective waste treatment.

Radioactive wastes are of concern as they have no practical purpose and present a threat to the environment through accidental release, improper storage and potential terrorism, such as a “dirty bomb”. Ion exchange methodology used in the chemical, nuclear and waste water treatment industries is highly efficient when compared to equivalent chemical technologies but can be expensive (Stylianou et al. 2007). Biological-based processes could present a viable alternative; however, a bioremediation system should be sufficiently robust to withstand radiation damage and also minimize remobilization of entrapped radionuclides over time. A pioneering approach used a biological system to make hydrogen uranyl phosphate (HUP) which was used as a stable ‘host’ mineral matrix to capture and hold target metals such as ^{60}Co , ^{90}Sr and ^{137}Cs through intercalative ion exchange (Paterson-Beedle et al 2006).

Biogenic HUP is made using a *Serratia* sp. via the activity of a radiotolerant (Jeong, 1992) acid phosphatase enzyme. This liberates inorganic phosphate ligand (HPO_4^{2-}) from a phosphate donor to deposit (with UO_2^{2+}) polycrystalline cell bound $\text{HUO}_2\text{PO}_4 \cdot n\text{H}_2\text{O}$ (Macaskie et al. 1992). Chemically synthesized zirconium (IV) phosphates, most commonly $\alpha\text{-Zr}(\text{HPO}_4)_2 \cdot n\text{H}_2\text{O}$ ($\alpha\text{-ZrP}$), have similar ion exchange properties (Clearfield 1988). Zirconium would be ideal for the remediation of wastes and potable water where non-toxicity is a pre-requisite. The *Serratia* metal phosphate deposition system has been demonstrated for tetravalent metals (Th(IV) , Zr(IV)) (Yong and Macaskie 1998; Basnakova and Macaskie, 1999) although Zr(IV) was deposited poorly (Basnakova and Macaskie, 1999). Therefore the objective of this study was to develop effective biogenic ZrP deposition by the *Serratia* sp., working towards a method for use as an entrapment matrix for removal of metals such as Co^{2+} , Sr^{2+} and Cs^+ . These are convenient surrogates for the nuclides $^{60}\text{Co}^{2+}$, $^{90}\text{Sr}^{2+}$ and $^{137}\text{Cs}^+$, for which application of the HUP-based methodology to real nuclear waste was shown previously (Paterson-Beedle et al. 2006). The potential for this biogenic approach compared to some commercial materials is shown in Table 1.

The aim of this study is to show proof of concept for a portable and effective system for the removal of the radionuclide surrogates Co^{2+} and Sr^{2+} from aqueous solutions using biomanufactured ZrP , which is discussed in the context of the bio-HUP system and commercial comparators shown in Table 1.

Materials and Methods

Microorganism, support and biofilm production

Serratia sp. (NCIMB 40259) was used under license from Isis Innovation, Oxford, UK. Cells were grown as a biofilm on polyurethane reticulated foam (Filtren TM30, cubes: 1 cm³ and discs: diameter = 2 cm and depth = 0.5 cm, 1.57 cm³), supplied by Recticel, Belgium. The biofilm was grown in an air-lift fermenter under carbon-limiting continuous culture with the phosphatase activity of the cells from the fermenter outflow determined as described previously (Finlay et al. 1999, Macaskie et al. 2005; Paterson-Beedle and Macaskie 2004). The steady state specific activity was ~3,800 units (nmol *p*-nitrophenol released from *p*-nitrophenyl phosphate per min per mg protein). To determine the amount of biomass (dry weight) attached to the polyurethane foam, cubes or discs (8) with and without biofilm, were dried at (60 °C; 48 h) and weighed. The amount of dry biomass was calculated by the difference of the average weights. The variations between the weights of foam and foam+biofilm samples were within 7 % and 15 %, respectively.

Preparation of reactor packed-bed systems for Zr bioaccumulation

Two packing systems were used for bioaccumulation of Zr onto biofilm immobilised onto polyurethane foam: (i) quartered cubes (88 per reactor, 0.125 cm³) were packed in cylindrical glass columns (9 cm; i.d. 1.5 cm) and (ii) discs (8 per reactor) were stacked in cylindrical glass columns (5 cm; i.d. 2 cm), the purpose of which was to reduce the channeling in the reactor, which was confirmed by magnetic resonance imaging (Graf von der Schulenburg et al. 2008).

All experiments used a standard solution of ZrOCl₂·8H₂O (1 mM) and glycerol 2-phosphate (G2P, 5 mM), pH 5 (Zr/G2P) unless otherwise stated. Reactors (6 per treatment) with packing system (a) were exposed to a continuous upward flow (ca. 7.8 ml/h, 72.6 h), via an external peristaltic pump (Watson-Marlow, 323), of Zr/G2P with and without sodium citrate buffer (3 mM, pH 5). For the determination of the background adsorption/biosorption of Zr from aqueous flows biofilm-reactors (2) were autoclaved and treated in parallel. Reactors (2) with packing system (b) were exposed to a continuous flow (ca. 8.2 ml/h, 66.6 h) of Zr/G2P alone, or identical reactors (6) were exposed to a flow of Zr/G2P at 14 ml/h for 22.5 h, then at 6.3 ml/h for a further 49.5 h. The outflow solutions of all reactors were analysed for Zr and P (see below). The variations between reactors were within 8 %.

In other experiments, reactors (6) with packing system (b) were exposed to an intermittent flow of Zr/G2P with ethanol (5 M), pH 5, with filter paper (Whatman, qualitative, diameter ca. 2 cm) fitted on top of the disc stack to retain the precipitate ('ZrP-biogel'). The reactors were filled with an upward flow (ca. 25 ml/h), as above, left statically (24 h), drained and filled with fresh solution, daily for 10 days. The outflow solutions were analysed daily for Zr and P. Deposited material on the biofilm ('ZrP-biogel') was estimated by difference. The variations between reactors were within 5 %.

Removal of Co and Sr from aqueous solutions

Bioreactor systems, containing ZrP material, were tested for the removal of Co^{2+} and Sr^{2+} from solution using methods described by Paterson-Beedle et al. (2006). All experiments were carried out twice using separate preparations and the variation between them was within 7 %. To determine the background adsorption/biosorption of metals from aqueous flows, reactors (biofilm immobilised onto polyurethane foam, without supported ZrP-biogel) were treated in parallel.

Spectrophotometric analyses of metals and phosphate

Determination of Zr was as described by Basnakova and Macaskie (1999), using Xylenol Orange, with a slight modification. The calibration was made from stock solution: $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ made to 1 mM in a solution comprising 1 ml conc. HCl in 100 ml of deionised water. For analysis, Zr sample or standard (200 μl , 1-32 μg) was added to a cuvette (1.5 ml), followed by HCl (8 M, 200 μl), deionised water (1.4 ml) and Xylenol Orange (0.05% w/v, 200 μl). The solution was mixed and the absorbance was measured at 535 nm. The inorganic phosphate contents of outflows were measured as described by Yong and Macaskie (1995).

Co^{2+} was estimated using the method described by Onishi (1986), with a slight modification. To sample or standard solution [$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] containing Co^{2+} (30 μl , 1-10 μg) was added citric acid (0.2 M, 250 μl) and 0.3 ml phosphate/boric acid buffer (6.2 g boric acid, 35.6 g Na_2HPO_4 and 500 ml 1 M NaOH, total volume of 1 l, final pH ~8.0. Nitroso-R salt (Fluka, UK) (0.2%, 125 μl) was added while stirring. The samples were covered, heated (100 °C; 1 min), supplemented with HNO_3 (250 μl , 1 min), cooled in the dark, deionised water (325 μl) was then added and Co^{2+} was estimated at A_{420} by reference to the standard.

A method was developed (from Yong et al. 1996), for the determination of strontium, using arsenazo III. To samples or standard ($\text{Sr}(\text{NO}_3)_2$) (30 μl , 1-7 μg), 1.97 ml of 0.1 M borate buffer, pH 9.0 was added.

Strontium was visualised by the addition of 0.1 ml of 0.15 % (w/v) arsenazo III, with estimation of the blue-violet complex (A_{649}) which develops in 25 min at pH 9-10 (Michaylova and Kouleva 1974).

X-ray powder diffraction (XRD) analysis

Biogenic ZrP samples (ZrP-biogel) were prepared by removing ZrP loaded foam discs from reactors into universal bottles (25 ml), adding deionised water and squeezing the foam to remove biomass and bound ZrP. Detached ZrP-biogel material was separated, settled under gravity, washed with deionised water a further three times and left to dry at ambient temperature. For analysis, some ZrP was put between sheets of Magic Tape and examined using a Siemens D5000 diffractometer (transmission mode) equipped with a Ge monochromator to produce Cu $K_{\alpha 1}$ radiation, and a Braun position sensitive detector.

Results and discussion

Bioaccumulation of Zr(IV) by Serratia sp. biofilm immobilised onto polyurethane foam in packed-bed reactor systems

The structure (amorphous, semicrystal or crystal) of ZrP depends on the method used for its synthesis (Bogdanov et al. 1997). However, the stoichiometric P to Zr ratio is 2 (e.g. $Zr(HPO_4)_2$), irrespective of the degree of crystallinity. α -ZrP is usually synthesized by reacting concentrated phosphoric acid with an aqueous solution of $ZrOCl_2$, e.g. Clearfield and Stynes (1964), Trobajo et al. (2000). The gelatinous amorphous precipitate initially obtained slowly crystallizes if the precipitate is heated under reflux in its mother liquor. Such harsh conditions for the synthesis of ZrP are not compatible with an enzymatically-accelerated metal phosphate biomineralization process. Other factors influencing this crystallisation process are the solubility product of the metal phosphate, the nature and concentration of the complexing ligand(s) present and the pH of the solution. Previous studies showed that, despite the low solubility products quoted in the literature, in practice tetravalent metal phosphates tend not to desolubilise readily due to the extensive hydrolysis of the tetravalent ion in solution (Yong and Macaskie, 1998, Basnakova and Macaskie, 1999).

Previous work showed that packed-bed *Serratia* sp. biofilm reactors exposed to uranyl nitrate with G2P accumulated hydrogen uranyl phosphate (Paterson-Beedle et al. 2006) with citrate buffer present to suppress UO_2^{2+} hydrolysis. Under similar conditions (here metal:citrate ratio of 1:3, pH 5: Table 2), the Zr

uptake was lower (by ~70 %) for reactors with citrate; however, the Zr:P molar ratios (1:~0.8) were similar in each case (Table 2). Since, in contrast to UO_2^{2+} , suppression of Zr(IV) hydrolysis resulted in less effective biomineral formation in this study, citrate was omitted from subsequent tests. As a control, the background adsorption/biosorption of Zr(IV) from the flow by heat-denatured biofilm was 3.4 and 4.4 % of the input concentration for systems with and without citrate, respectively, as compared to 21 % and 68 % using live-biofilm (Table 2). The Zr uptake ($\mu\text{g}/\text{h}/\text{ml}$ column volume) of reactors packed with cubes (without citrate) was similar to that of discs (40 and 41 respectively, Table 2). The space velocity (i.e. number of reactor volumes of feed treated in a unit time) was 43 % higher for the reactor packed with discs compared to that with cubes (Table 2). Importantly, the Zr:P molar ratios of ~ 1:1 indicate that α -ZrP ($\text{Zr}(\text{HPO}_4)_2$) was not the main composition of the product retained by the bioreactors (Table 2).

Polyvalent cations, such as Zr(VI), hydrolyse extensively in water (Baes and Mesmer 1986) and as hydroxyl groups accumulate on the cation through hydrolysis or base addition, they polymerise by olation, eventually precipitating (Clearfield 1988). Rapid precipitation in the cold leads to amorphous or poorly crystalline products (Clearfield, 1988) and it is likely that hydrous oxides are also being accumulated in the bioreactor along with ZrP. Examination of the product by environmental scanning electron microscopy showed a gelatinous mass with no visible features, while X-ray powder diffraction analysis gave no interpretable powder pattern and no defined peaks, confirming a poorly crystalline or amorphous material (not shown). The steady state concentrations of Zr(IV) and phosphate removed from solution by reactors (without citrate) were ~0.73 mM and ~0.66 mM (~1.5 mM phosphate removal would be expected for $\text{Zr}(\text{HPO}_4)_2$) respectively, assuming 100 % hydrolysis of the G2P (see below). Hence the material is ‘enriched’ for Zr as compared to the expected ratio of Zr:P of 1:2 for $\text{Zr}(\text{HPO}_4)_2$ (Table 2).

System (ii) (discs) was chosen for further work because it produces less fluid channelling in columns compared to cubes (see Nott et al. 2005 and Graf von der Schulenburg et al. 2008) and the disc system showed higher phosphate uptake and space velocity (see Table 2).

Use of ethanol to promote ZrP formation

Hydration of Zr(IV) ions in solution can be reduced if oxygen-containing additives, e.g. alcohols, polyalcohols such as glycerol, or carboxylic acids, are used during the sol-gel synthesis of ZrP (Wong, 2006). The incorporation of additives in the ZrOCl_2 solution displaces water molecules that are coordinated to Zr ions and bridges Zr ions forming water soluble polymers; the additive should be selected so that it can be displaced from the Zr ions during the reaction with phosphoric acid or phosphate that

forms ZrP (Wong 2006). The typical amount of additive used by Wong (2006) was 20 % (w/v) by weight of ZrOCl_2 , the molar ratio of ZrOCl_2 to H_3PO_4 was $\sim 1:3$ and H_3PO_4 was ~ 4.5 M. However, the present study using 1 mM Zr(IV) showed that citrate (3 mM) and also the glycerol liberated from G2P were ineffective in the biomanufacture of crystalline ZrP, detectable by XRD. Previous mass balance studies showed that at a pH of 4.5 70% of the input G2P was accountable as equimolar glycerol and liberated phosphate (M. Paterson-Beedle, unpublished) and since the phosphatase activity at pH 4 is approx. half that at neutral pH it was assumed that the glycerol concentration in the present study was 5 mM (i.e. complete hydrolysis) although this was not measured.. Ethanol was tested as an alternative additive. Preliminary tests indicated that high concentrations (2.5-5 M ethanol) were optimal in promoting ZrP formation. A semi-continuous process was developed using the packed-bed biofilm reactor (discs) with an intermittent flow of Zr/G2P and ethanol (5 M), pH 5, for 10 days, interspersed with static 24 h periods (see Materials and Methods). Under these conditions (total ZrP loading time was 10 days including reactor refill time) the Zr uptake was increased to 95 % and the Zr:P molar ratio calculated (average of six reactors) was 1:3.4, i.e. the material was now phosphate-enriched as compared to the studies without ethanol and also with respect to the expected $\text{Zr}(\text{HPO}_4)_2$ (1:2 ratio). Subsequent tests showed that the ethanol-derived material contained a crystalline $\text{Na}_2\text{Zr}(\text{PO}_4)_2$ component by XRD analysis (M Paterson-Beedle and J E. Readman unpublished). A full characterisation of the ZrP-biogel material is outside the scope of this study and will be reported in a subsequent publication (J.E. Readman, M. Paterson-Beedle, K.W.Chapman, P.J.Chupas, L.E. Macaskie and J.A. Hriljac in preparation)

Removal of Co and Sr from aqueous solutions using packed-bed reactors containing Zr supported on Serratia sp. biofilm

With deposition of ZrP successfully achieved (via ethanol treatment), two bioreactor systems, with the following Zr:P molar ratios, were tested for the removal of Co^{2+} and Sr^{2+} from solution: (i) 1:0.33 (prepared without ethanol) and (ii) 1:3.5 (prepared with ethanol). The removal of both metals was 100% (system ii shown in Fig. 1). The estimated Zr and P contents per reactor and respective uptakes of Sr^{2+} and Co^{2+} per reactor are shown in Table 3. The uptake of ions (Co^{2+} or Sr^{2+}) was similar for both systems, i.e. ~ 0.02 mmoles per reactor, even though system (i) contained a $\sim$ ten fold higher content of Zr compared to system (ii) (the phosphate contents were similar, Table 3) . The background adsorption/biosorption of Co^{2+} and Sr^{2+} ions from aqueous flows by polyurethane foam coated with *Serratia* sp. biofilm without ZrP was ~ 0.0025 mmoles per reactor (~ 12 % of that containing ZrP).

According to the literature the uptake of cations for ZrO_2 , in the pH range of 5.5 to 6.0, is only 0.05 mmol/g solid; by contrast the ion exchange capacity for $\text{Zr}(\text{HPO}_4)_2 \cdot \text{H}_2\text{O}$ is ~6.64 meq/g (Clearfield, 1988). System (i) contains ten fold more Zr than system (ii), therefore, assuming the total amount of Zr in system (i) as being ZrO_2 , the estimated uptake of Co^{2+} and Sr^{2+} , would be 0.5 and 0.4 mmol/g ZrO_2 , respectively, which is ten fold higher than that reported in the literature for ZrO_2 . Therefore, these results suggest that the biogenic Zr product in system (i) is possibly a mixture of zirconium oxides and phosphates. If it is assumed that the total Zr in system (ii) is α -ZrP, the estimated ion exchange capacity for Co^{2+} and Sr^{2+} would be 4.3 and 4.2 meq/g ZrP, respectively, which is 35% lower than that described in the literature for pure α -ZrP. However, this lower experimentally-determined capacity could be due to the additional presence of Zr oxides, insufficient contact time given for the ion exchange under the dynamic conditions, as well as the pH of the metal solutions (~6.0). Greater uptake occurs at alkaline pH (Clearfield 1988) but a slightly acidic pH was chosen to minimise the Zr(IV) hydrolysis and formation of ZrO_2 . It is known that α -ZrP exhibits sieving behaviour in acid solutions, and ions larger than K^+ are exchanged at an appreciable rate only in alkaline solution (Clearfield, 1988). Under these conditions sufficient protons on the layers are neutralised to allow swelling of the layers to admit larger ions (Clearfield, 1988).

The breakthrough curves for the removal of Co^{2+} and Sr^{2+} on ZrP-biogel from system (ii) is shown in Figure 1. It can be seen that for Co^{2+} 1 % and 50 % breakthrough occurred after passing 20.5 ml (~2.6 column volumes) and 37.4 ml (~4.8 column volumes) of the feed solution, respectively. Similarly, for Sr^{2+} , 1 % and 50 % breakthrough occurred after passing 24.5 ml (~3.1 column volumes) and 39.7 ml (~5.1 column volumes), respectively. Although the total ion uptake was similar for both systems, i.e. ~0.02 mmoles per reactor (Table 3), system (ii) was more efficient than system (i) in terms of quantity of reagents ($\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ and G2P) used to produce ZrP, i.e. ten fold less. However, the potential drawback of system (ii) was the reaction time, i.e. ~3 times longer than system (i).

Although the column breakthroughs (~4 column volumes) *per se* seem low it should be noted that Co^{2+} and Sr^{2+} were supplied at 0.5 mM. A real nuclear waste (e.g. as described by Paterson-Beedle et al. 2006) contains low nuclide concentrations (e.g. ^{137}Cs : 1.781 Bq/ml or 4.08 pM and ^{60}Co : 0.115 Bq/ml or 0.05 pM) and hence the column capacities would not be reached until after substantial flows have passed. It could be argued that at such low concentrations the metals of interest may not be removed against an excess of other ions (e.g. Na^+). However other studies have shown that target nuclides (^{137}Cs , ^{90}Sr , ^{60}Co) are removed in co-challenge with UO_2^{2+} (i.e. co-crystallisation) at the expense of 5 mM sodium glycerol 2-phosphate (i.e. 5 mM Na^+) (M. Paterson-Beedle, unpublished) suggesting a potential selectivity that will warrant further study. The phosphatase enzyme *per se* is radiostable (Jeong, 1992) and it should also be noted that uptake of the radionuclides via ion exchange into pre-existing metal phosphate on the biofilm

did not appear to compromise biofilm integrity for the duration of the experiments since removal of ^{137}Cs and ^{60}Co was constant at 85% and 97% of the input metal (1.78 Bq/ml of $^{137}\text{Cs}^+$ and 0.115 Bq/ml of $^{60}\text{Co}^{2+}$ Paterson-Beedle et al 2006).

In order to calculate ‘real’ potential for waste treatment, taking bio-HUP as an example with, here, a maximum loading of 9 g of U (or 16.6 g HUP) per g of biomass (Macaskie et al. 1992) and a loading of 1.6 g HUP per g biomass (Paterson-Beedle et al. 2006), the HUP biomaterial capacity was compared to several commercially available materials (Table 1). In the present study, assuming a composition of $\text{Zr}(\text{HPO}_4)_2$, the total solid (g) per g biomass would be 0.5 % of that of the maximum load of HUP (16.6 g HUP/g biomass). The hydrous, gel-like nature of the material and its swelling in the column precluded increased loading per column.

The estimated capacity of the ZrP reactor, if a pure solution of Co^{2+} , at a concentration similar to that found in low active waste, i.e. $\sim 3.9 \times 10^{-7} \text{ mg/l}$ (see Table 1) is assumed, would be $\sim 4 \times 10^8$ bed volumes. Similarly, using a pure solution of Sr^{2+} , in a concentration range of $8.3 \times 10^{-6} - 1.4 \times 10^{-8} \text{ mg/l}$ (see Table 1), the capacity would be in the range of $\sim 2.8 \times 10^7 - \sim 1.6 \times 10^{10}$ bed volumes and the capacity (l/kg) would be $\sim 5.1 \times 10^8 - \sim 3.0 \times 10^{11}$ (dry weight). These estimated capacities obtained from ZrP reactors (from Table 3, system ii) are similar to those obtained for HUP (1.6 g per g biomass) and are in the order of ~ 2 -fold greater than for commercial materials at slightly acidic pH. This is to be expected since the commercial material for treating Sr(II) is based on titanium (IV) oxide (Table 1) and the superiority of ZrP as compared to Zr(IV) oxide is discussed above. It should also be noted that the commercial material requires a pH of > 10 for Sr^{2+} removal and between pH 4-8 for Co^{2+} removal whereas the bio-ZrP removes Sr^{2+} comparably to Co^{2+} at slightly acidic pH (Fig. 1) and also comparably to a reference $\text{Zr}(\text{HPO}_4)_2 \cdot n\text{H}_2\text{O}$ material prepared by J.E. Readman (3.9 and 3.1 mequiv Sr^{2+}/g respectively) using a batch contacting system (pH 7) to overcome the problem of gel swelling out of the column (not shown). On the basis of these projections the potential for ZrP-biogel in wastewater decontamination warrants more detailed development of the material, elucidation of its structure and better understanding of the mechanism of Sr^{2+} and Co^{2+} removal in a mixed ZrP/ ZrO_2 system.

From an economic viewpoint, large-scale manufacture of bio-ZrP at the expense of glycerol 2-phosphate is unattractive, even given that a ‘rapid response’ nuclear decontamination operation may be required at a future date. It is also not clear that sufficient G2P could be made available at short notice. These problems could be overcome by the use of phytic acid (inositol phosphate: 6 mol phosphate/mol as compared to 1 mol/mol for G2P). Phytic acid, a major plant phosphate storage material, is produced at large scale as a waste from biodiesel production. Its disposal is problematic and a future use for phytic acid (see Paterson-Beedle et al. 2010) in the production of bio-Zr-based nuclear decontamination materials [and also similar

materials based on bio-hydroxyapatite ('bio-HA'): L.E. Macaskie, S. Handley-Siddhu and J.C.Renshaw, unpublished] should also be explored. Such studies should seek to establish that the biomineral is stable to dissolution over time but preliminary tests using bio-HA have suggested that the biomineral is more stable to dissolution than its 'chemical' counterpart. Future studies would aim to compare bio-HA and bio-ZrP in this respect.

Conclusions

This study shows that packed bed reactors containing *Serratia* sp. biofilm immobilised onto polyurethane reticulated foam, accumulated zirconium phosphate. The ZrP biomaterial produced without ethanol was Zr-enriched (molar ratio Zr:P of 1:1), whilst that produced with ethanol was phosphate-enriched (molar ratio Zr:P of 1:3.4), compared to the 1:2 expected for $\text{Zr}(\text{HPO}_4)_2$, indicating that $\text{Zr}(\text{HPO}_4)_2$ is not the sole composition of the product. The ethanolic biogenic material, supported on foam-immobilised biofilm, removed 100 % of Co^{2+} and Sr^{2+} until the reactor reached saturation. The estimated capacities (l/kg) obtained for the bio-ZrP reactors were similar to those obtained for bio-HUP; this, and bio-ZP are shown to be superior to commercial materials in this respect. These conclusions warrant further development of the ZrP biomaterial.

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Table 1. Comparison between hydrogen uranyl phosphate (HUP) biomaterial and some commercial inorganic materials.

Agent	CsTreat® ^a	SrTreat® ^a	CoTreat® ^a	Bio-HUP
Principle	Hexacyano-ferrate, granular	Ti oxide based, granular	Ti oxide based, granular	HUP on biomass, foam lattice
Bulk density (kg/l)	0.6	0.8	0.8	0.16 (dry weight, dw) ^b 0.71 (wet weight, ww) ^c
Grain size	0.25-0.85 mm	0.3-0.85 mm	0.3-0.85 mm	Nanoscale, surface array
Capacity (bed volumes, BV) ^d	6x10 ⁴ -2.4x10 ⁵	1.2x10 ⁵ -4.0x10 ⁵	2x10 ⁴ -4.0x10 ⁴	3x10 ⁷ -3x10 ¹⁰ (Cs) ^e 5x10 ⁷ -3x10 ¹⁰ (Sr) ^e ~8x10 ⁸ (Co) ^e
Capacity (l/kg)	1x10 ⁵ -4.0x10 ⁵	1.5x10 ⁵ -5x10 ⁵	2.5x10 ⁴ -5x10 ⁴	7.5x10 ⁸ -7.1x10 ¹¹ (dw) ^f 1.6x10 ⁸ -1.6x10 ¹¹ (ww) ^{f,c} 8.0x10 ⁹ -7.6x10 ¹² (dw) ^g 1.8x10 ⁹ -1.7x10 ¹² (ww) ^{g,c}
pH	1-13	>10	4-8	Full range not tested

^aSource: <http://www.fortum.com>. ^bCalculations based on maximum HUP loadings (16.6 g/g biomass) on biofilm immobilized onto polyurethane foam (cubes) packed in reactors as described in Paterson-Beedle et al. (2006). ^cEstimated using 78 % moisture content. ^dIn the bioreactor this is taken as 11 ml, but the interstitial volumes will vary according to how much HUP has been used in the ion exchange layer. ^eCalculations were based on ion exchange capacities of Bio-HUP (1.6 g/g biomass, 148.9 mg HUP/reactor) for ⁸⁵Sr (0.055 mmol), ¹³⁷Cs (0.11 mmol) and ⁶⁰Co (0.055 mmol), as described in Paterson-Beedle et al. (2006). For the estimation of the capacities (BV), typical concentrations (mg/l) of these elements in low active waste (Sr: 8.3 x 10⁻⁶; Cs: 4.1 x 10⁻⁵ and Co: 3.9 x 10⁻⁷) and in fuel rod storage pond stream (Sr: 1.4 x 10⁻⁸ and Cs: 4.3 x 10⁻⁸) (Macaskie 1991), were used. ^fCalculations based on the capacity (BV) in the range of 3x10⁷-3x10¹⁰ and Bio-HUP loading of 1.6 g/g biomass. ^gCalculations based Bio-HUP loading of 16.6 g/g biomass.

Table 2. Comparison of bioreactor systems, packed with *Serratia* sp. biofilm immobilized onto polyurethane foam with different formats, for the uptake of Zr from aqueous solutions, with and without citrate.

Description	With citrate ^a	Without citrate ^a	Without citrate ^b
Format of polyurethane foam	Cube	Cube	Disc
Volume of each foam (cm ³)	0.125	0.125	1.57
Number of foams with biofilm per reactor	88	88	8
Column volume (CV) (ml)	11	11	7.83
Flow-rate (ml/h)	7.8	7.8	8.2
Time (h)	72.6	72.6	66.6
Space velocity (1/h)	0.7	0.7	1.0
Biomass (dry weight) (mg per reactor)	88	88	120
Zr uptake (% of total Zr in inflow)	21	68	65
Total Zr uptake (mg)	9.94	32	21.4
Total Zr uptake (mmol)	0.11	0.35	0.24
Zr uptake (µg/h.ml CV)	12.5	40	41.1
Zr uptake (mg/g biomass)	0.11	0.36	0.18
Total P uptake (mg)	2.74	8.25	9.6
Total P uptake (mmol)	0.09	0.27	0.31
P uptake (µg/h.ml CV)	3.43	10.33	18.42
P uptake (mg/g biomass)	0.03	0.09	0.08
Zr : P (molar ratio)	1 : 0.82	1 : 0.76	1 : 1.32

^aReactors (1.5 cm x 9 cm) packed with cubes and exposed to Zr/G2P with and without citrate buffer (see text), pH 5. ^bReactors (2 cm x 5 cm) packed with discs and exposed to Zr/G2P, pH 5. The disc format was used since this avoided patchy ZrP deposition due to channeling (see text). The ‘ideal’ ratio of Zr:P for Zr(HPO₄)₂ is 1:2 (see text). Representative data are shown; each experiment was replicated as described in Materials and Methods with variations of within 7 % throughout.

Table 3. Comparison of two bioreactor systems, packed with ZrP supported on *Serratia* sp. biofilm immobilized onto polyurethane foam discs, for the uptake of Co^{2+} or Sr^{2+} from aqueous solutions.

Description	Co^{2+}		Sr^{2+}	
	System (i) ^a	System (ii) ^b	System (i) ^a	System (ii) ^b
Zr (mg/reactor) ^c	35.15	3.00	34.03	3.04
Zr (mmol/reactor) ^c	0.39	0.03	0.37	0.03
P (mg/reactor) ^c	4.19	3.65	4.04	3.52
P (mmol/reactor) ^c	0.14	0.12	0.13	0.11
Metal uptake(mg/reactor)^d	1.41	1.18	1.75	1.75
Metal uptake (mmol/reactor)^d	0.02	0.02	0.02	0.02
Zr:Metal (molar ratio)^d	0.06	0.61	0.05	0.60

^aSystem (i): packed-bed reactors containing Zr supported on *Serratia* sp. biofilm immobilized onto discs, exposed to Zr/G2P (14 ml/h for 22.5 h, then at 6.3 ml/h for a further 49.5 h), with a Zr:P molar ratio of 1:0.33 (see text). ^bSystem (ii): packed-bed reactors similar to system (i) but exposed to an intermittent flow Zr/G2P with ethanol, during 10 days, and with a Zr:P molar ratio of 1:3.4 (see text). ^cParameters before $\text{Co}^{2+}/\text{Sr}^{2+}$ addition. ^dUptake of test metals (in bold). Representative data are shown; each experiment was replicated as described in Materials and Methods with variations of within 8 % throughout.

Figure 1. Break through curves for the sorption of Co^{2+} (●) and Sr^{2+} (○) on ZrP-biogel supported onto *Serratia* sp. biofilm immobilised onto polyurethane foam discs from system (ii) and packed in reactors. Reactors were exposed to $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (0.5 mM, pH 6.1) or $\text{Sr}(\text{NO}_3)_2$ (0.5 mM, pH 6.1). C = concentration of metal in the effluent (mg/l) and C_o = concentration of metal in the feed (mg/l) Flow-rate: 4.8 ml/h; column volume (without ZrP supported on biofilm, see Table 2): 7.83 ml and amount of Zr in column: ca. ~3 mg.

Figure 1