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Adsorption of Sr by Immobilized Microorganisms

Scientific Note

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INTRODUCTION

Wastewaters from numerous industrial and laboratory operations can contain toxic or undesirable components such as metal ions, which must be removed before discharge to surface waters. Adsorption processes that have high removal efficiencies are attractive methods for removing such contaminants. For economic operations, it is desirable to have an adsorbent that is selective for the metal contaminant of interest, has high capacity for the contaminant, has rapid adsorption kinetics, can be economically produced, and can be regenerated to a concentrated waste product or decomposed to a low-volume waste. Selected microorganisms are potentially useful adsorbents for these applications because they can be inexpensive, have high selectivities, and have high capacities for adsorption of many heavy metals, which are often problems in a variety of industries.

Some wastewaters in the nuclear industry and laboratories contain low concentrations of radionuclides. Fission products such as Sr and Cs (*1*), along with activated corrosion products, are important contaminants in nuclear reactor coolant waters and nuclear fuel reprocessing wastes.

Wastewaters at the Oak Ridge National Laboratory (ORNL) primarily contain only radioactive Sr and Cs. Low-level radioactive wastewaters at ORNL are typical of those at several other similar facilities, and Sr is the

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Table 1
Composition of Reference ORNL Wastewater

Component	Concentration ppm, mg/L
Ca^{2+}	40
Mg^{2+}	8
Na^{+}	5
K^{+}	2
Si^{3+}	2
Sr^{2+}	0.1
Al^{3+}	0.1
Fe^{2+}	0.1
Zn^{2+}	0.1
HCO_3^{-}	93
SO_4^{2-}	23
Cl^{-}	10
NO_3^{-}	11
CO_3^{2-}	7
F^{-}	1
Major radiochemical components	
^{90}Sr	4000 Bq/L
^{137}Cs	400 Bq/L
Gross beta	6000 Bq/L

primary problem. The composition of an ORNL wastewater of particular interest is given in Table 1. Chemically, this is a relatively pure water stream with small concentrations of Ca, Mg, Na, and K. There are even smaller concentrations of other cations such as Sr, Al, Fe, and Zn. The anions are largely bicarbonate, sulfate, chloride, and nitrate. The 4000 Bq/L from ^{90}Sr corresponds to only approximately 0.001 (wt) ppm of ^{90}Sr; so there is also a significant concentration of nonradioactive Sr. Thus, the effective adsorption operations will have to remove Sr at the concentration of the stable isotope(s).

Previous studies of microbial adsorption have emphasized "heavy" metals that are adsorbed effectively by selected microorganisms (2–4), but Sr removal is a special or new challenge for adsorption by microorganisms. One preliminary study showed that *Citrobacter* sp. can remove limited quantities of Sr from dilute solutions (*5*).

A laboratory-scale packed column containing microbial cells immobilized within a gelatin matrix has been prepared, and its application to removal of Sr from a simulated wastewater is described here. It is necessary to immobilize the microorganism in a packed bed where the liquid dispersion rate is low. Immobilized organisms in gelatin beads make effective biosorbent packing. This permits the high concentration gradients in the

Table 2
Rank of Sr Uptake by 14-d Distribution Coefficient Values

Organism	Distribution coefficient 14 d	2 d	7 d	pH
Rhizopus	26,240	1,470	85,533	7
Micrococcus	9,983	24,943	12,010	7
Anabaena	8,198	5,554	10,304	7
Streptococcus	5,488	297	2,173	7
Bacillus	5,240	62,996	5,085	4
Chlamydomonas	3,750	1,639	1,785	7
Coelastrum	2,993	3,518	1,925	4
Penicillium	2,914	7,181	3,625	7
Scenedesmus	2,175	452	1,215	7
Citrobacter	1,804	771	1,507	4
Zooglea	1,542	493	1,858	7
Ashbya	1,380	81	3,569	4
Escherichia	1,233	967	1,961	7
Paecilomyces	1,084	1,808	656	7
Chlorella	1,069	734	1,915	7
Candida	976	764	1,139	7
Pseudomonas	866	6,321	1,003	7
Saccharomyces	760	917	1,800	4
Streptomyces	744	4,147	3,949	7
Caulobacter	451	619	1,055	7

fluid, which are necessary for large changes in the Sr concentration (from the inlet to the outlet concentration).

EXPERIMENTAL PROCEDURE

Several microorganisms were first tested for their ability to adsorb (take up) Sr. The results are shown in Table 2. From these results, *Micrococcus luteus* (ATCC-4698) was chosen as the first organism for more detailed study. Both batch equilibrations and column adsorption experiments were used. The gel used to immobilize the cells is also a potential adsorbent for Sr, and adsorption by the gel has also been measured. Details of cell culturing, gel preparation, cell immobilization, and biosorption measurements are given in the following.

Organism and Culture Conditions

M. lutcus ATCC 4698 was grown on enriched nutrient broth (composition, in g/L: dehydrated heart infusion broth, 12.5; dehydrated nutrient broth, 5.4; yeast extract, 2.5; dehydrated tryptic soy broth, 10.0; proteose

peptone, 2.0). All dehydrated media were obtained from Difco Laboratories in Detroit, MI. Flasks of broth were inoculated at a density of 10^6 cells/mL and cultured at 30°C. The cells were harvested during the exponential growth phase by centrifugation at 7000 G for 10 min at 4°C. They were then washed three times with deionized water and diluted to the appropriate concentration as indicated.

Equilibrium Binding Studies (Batch System)

Typically, washed *M. luteus* cells were placed in solutions of $SrCl_2$ in deionized distilled water at pH 7 or buffered to pH 4 with 0.01 *M* phosphate buffer and 30°C in shake flasks with 50 rpm agitation for 48 h. The results are shown in Tables 2 and 3. The supernatants in the flasks were sampled at various times, and the Sr remaining in the supernatant was measured by atomic adsorption spectroscopy.

Preparation of the Gel

The particular gel chosen for this study was prepared with a matrix of bone gelatin and propylene glycol alginate (PGA) crosslinked by a dilute caustic solution. The resulting gel beads are stable and require no electrolyte to maintain crosslinkage. The gelatin, itself, is also able to adsorb or exchange metal ions and, thus, it enhances the adsorption capacity of the biosorbent. The data reported here were obtained with a gel made from 15 wt% gelatin, 2 wt% PGA, and 20 wt% dry cells.

Spherical particles are obtained by forming droplets of the gel-cell mixture in a mineral oil. More uniform droplet sizes can usually be obtained forming the droplets on vibrating nozzles using a technique discussed previously (*6*), but as the concentration of cells is increased, the viscosity of the gel mixture increases. This makes it difficult to use small nozzles; however, cell concentrations up to 40 wt% have been used successfully by simply agitating the gelatin-cell mixture in mineral oil. The gel-cell droplets settle through the mineral oil into a 10 wt% NaOH solution for crosslinking. They are washed with water before being used as adsorbents.

Sr Adsorption in Columns with Immobilized Cells

The initial gel particles with 20 wt% cells were approximately 4.08 mm in diameter. Glass columns of different sizes were used during the column loading experiments, with volumes ranging from 9 to more than 50 mL. Since the particles were relatively large when compared with the diameter of the column, the experimental results were affected by wall effects.

The gel beads were placed in glass columns and washed again with demineralized water before the adsorption experiments. Adsorption tests

Table 3
Results of Batch Equilibration of Gel and Micrococcus with 10 ppm (mg/L) Sr Solution

16 mL of gelatin beads with 104 mL of 9.62 ppm Sr		
Time, h	Conc. remaining in solution, ppm	Total Sr on gel, ppm
0	8.96	
4	2.07	277
24	0.26	356
48	0.26	356
4 mL of M. l. pellet with 116 mL of 8.62 ppm Sr		
Time, h	Conc. remaining in solution, ppm	Sr on organisms, ppm
0	0.18	1233
4	0.32	1212
24	1.1	1177
48	1.24	1167
16 mL of gelatin and 4 mL of M. l. pellet with 100 mL of 10 ppm Sr		
Time, h	Conc. remaining in solution, ppm	Sr on solids, ppm
0	0.3	273
4	0.7	260
24	1.26	240
48	0.98	250
16 mL of gelatin containing 4 mL of M. l. with 100 L of 10 ppm Sr		
Time, h	Conc. remaining in solution, ppm	Sr on solid, ppm
0	7.7	20.5
4	0.88	254
24	0.24	276
48	0.24	276

were made with demineralized water containing small concentrations of Sr, and the solutions were pumped through the columns in downflow with peristaltic pumps. The column effluents were collected with a fraction collector and analyzed for Sr by atomic adsorption spectroscopy. The fraction volumes were ~3–10 mL, depending on the size and column used.

RESULTS

Initial Screening of Organisms

Preliminary experiments were made in shake flasks in which microorganisms equivalent to approximately 1 g (dry wt) were suspended in 100 mL of a 100 μg/mL (ppm) solution of $SrCl_2$ (*see* Table 2). The results are given in terms of distribution coefficients (concentrations in the liquid phase). The concentration in the cells was calculated from the decline in the solution concentration resulting from biosorption by the cells, and the concentration in the cells was calculated from the decline in the concentration in the solution. Solution concentrations were measured after 2, 7, and 14 d, and the organisms tested are listed in order of decreasing adsorption after 14 d. This longer time was selected as the basis for principal ordering because the time in which cells would be exposed to Sr solutions in applied waste cleanup systems is likely to be approximately this long, or longer.

Since the measurements were made with only a single Sr concentration, they do not represent a complete description of adsorption equilibrium. These tests were intended only to provide a simple basis for selecting promising organisms for further study. More extensive measurements will be required to establish the complete adsorption capabilities of the most promising organisms and make accurate comparisons.

The quantity of Sr adsorbed (or apparent distribution coefficients) differed for different times of contact with solution, and the order shown in Table 2 would have been slightly different if it had been based on the 2-or 7-d data. The effects of different exposure time could result from differences in the rates of biosorption by the various organisms, but, in other cases, the Sr removal actually peaked and declined after the removal had reached a peak. One organism (*M. luteus*) was selected for more detailed study.

Equilibrium Studies (Batch Equilibrations)

Both *M. luteus* cells and bone gelatin are capable of adsorbing Sr; so it was necessary to determine the contribution of each material when removing Sr. Batch equilibrations were made with approximately 100 mL of solution containing 10 ppm of Sr (as $SrCl_2$) and 16 mL of gelatin beads and/or 4 g (approx 4 mL) of centrifuged *M. luteus* cells. The amount of Sr adsorbed by the cells and/or gelatin was determined by the decrease in the concentration in the supernatant solution. Because many of the results may be transient measurements, only solution and solid concentrations are reported rather than distribution coefficients or other forms of equilibrium relationship.

The first portion of Table 3 shows adsorption of Sr by the bone gelatin alone. Note that the bone gelatin is able to adsorb most of the Sr and re-

duce the concentration in the solution from 9.62 to 0.26 ppm. However, the adsorption process is relatively slow and requires more than a day to approach equilibrium.

The second portion of the table shows the adsorption by 4 g of centrifuged cells alone, without the presence of gelatin. This small quantity of cells removes essentially all of the Sr in less than 30 min (the few minutes required to inject the cells into the solution and subsequently remove a sample of the supernatant). The actual role of Sr removal is too rapid to determine by the experimental procedure used.

After the initial rapid rate of Sr removal by *M. luteus*, the Sr concentration in the solution had fallen to very low values. Then, the concentration began to increase when a substantial fraction of the adsorbed Sr was released back into the solution. This behavior was observed in several tests made with different quantities of cells and the same volume of solution. This may occur because Sr is adsorbed rapidly by some material that is then slowly released from the cells.

The remaining batch equilibration experiments give results that are compatible with the preliminary hypothesis, although they do not "prove" it. The third portion of Table 3 shows the concentration of Sr remaining in the solution after contact with both 16 mL of gelatin and 4 g of cells. The cells were in a separate mass and were not incorporated within the gel. (They were not immobilized.) The concentration of Sr dropped rapidly and then rose slowly as it had in the experiment with cells alone. The concentration in the solution after 48 h was far greater than it would have been if the gelatin had been used alone. The gelatin had little effect on the Sr behavior. Because the rate of adsorption by the cells or cell component is so rapid, there is little time for the gelatin to adsorb significant quantities of Sr. The only additional assumption required to account for the behavior shown in the third portion of Table 3 is that the materials that adsorb the Sr and escape from the cells have molecular weights sufficiently large that they cannot penetrate the gel.

Finally, batch equilibration experiments shown in the fourth part of Table 3, with 16 mL of gelatin and 4 g of cells *with the cells immobilized within the gelatin*, show that the gelatin beads with immobilized cells were able to adsorb the Sr effectively. There was no evidence that the adsorbed Sr was subsequently released. The behavior was similar to that observed in the gelatin without cells, but the short time data suggest that the rate of adsorption may have been enhanced by the presence of the cells. The cells appear to aid Sr adsorption initially (short times) because they can adsorb the Sr so quickly. Adsorption rates are not as rapid as they would have been without the presence of immobilizing gelatin because it is necessary for the Sr to diffuse through the gelatin matrix to reach the cells.

Although the adsorption of Sr by *M. luteus* showed unexpected complex behavior, the results with the immobilized organism remain promising, and there appears to be no problem for immobilized cells of *M. luteus*.

ADSORPTION BY DEEP BEDS OF CELL FILLED GELATIN

The adsorption of Sr from a 10 ppm solution of $SrCl_2$ by a 52 mL column of 4.1 mm gel particles with 20 wt% cells is shown in Fig. 1. The small column-to-particle-diameter ratio caused significant wall effects that are evident in the early breakthrough at very low concentrations. However, the major portion of the breakthrough curve, which occurred after approximately 3600 mL, demonstrates the capacity of the cell-filled gel beads for Sr. This breakthrough occurs after 70 column volumes and indicates a Sr loading of approximately 1500 ppm within the gel, based on wet gel particles that occupy approximately 50% of the column volume. Since the solution concentration was higher in this experiment than the final equilibration concentration observed in the preliminary screening tests, no accurate comparison can be made between the two measurements. The presence of gel also complicates any such comparison. This loading is less than one would expect from the distribution coefficients measured in the preliminary screening tests, but further studies will be required to determine which factor is affecting the loading. It is encouraging to note the relatively steep portion of the loading front does not suggest slow kinetics, an effect of large particle size, or other problems that unduly hinder metal adsorption at this flowrate. The batch equilibration experiments suggest that the immobilized cells assist in maintaining high adsorption rates. Note, however, that the flowrates were relatively slow in this particular experiment and the volume between approx 3500–3800 mL, over which most of the interesting portion of the breakthrough occurred, took a few days. This is encouraging because there is no evidence that very slow kinetics, such as those suggested for gel alone, have hampered the adsorption rate/performance in the existing experiments. Examination of the kinetics of adsorption in columns will require experiments at several higher flowrates.

Elution of the Sr from the gel can be achieved with other electrolytes. Figure 2 shows an elution with a 0.3 *M* KCl solution. The elution is very rapid, and essentially all of the Sr (as estimated by a material balance between the Sr initially loaded on the column and the Sr eluted from the column) is removed in the first 200–300 mL. Again, note that the eluting front probably would have been sharper if the gel particles had been smaller. More accurate material balances will be needed if it appears that there is more than one form of Sr adsorption (such as adsorption on different kinds of binding sites). The Sr can be recovered at much higher concentrations than the feed concentration. Because the wall effects were larger in this particular experiment than one would find in a well-designed operating system, the maximum recoverable concentration should not be estimated from this particular figure.

When the gel contacted the 0.3 *M* KCl eluate solution, the particles contracted considerably, and the bed height decreased to less than one-half its original height. This osmotic effect is similar to the Donnan swell-

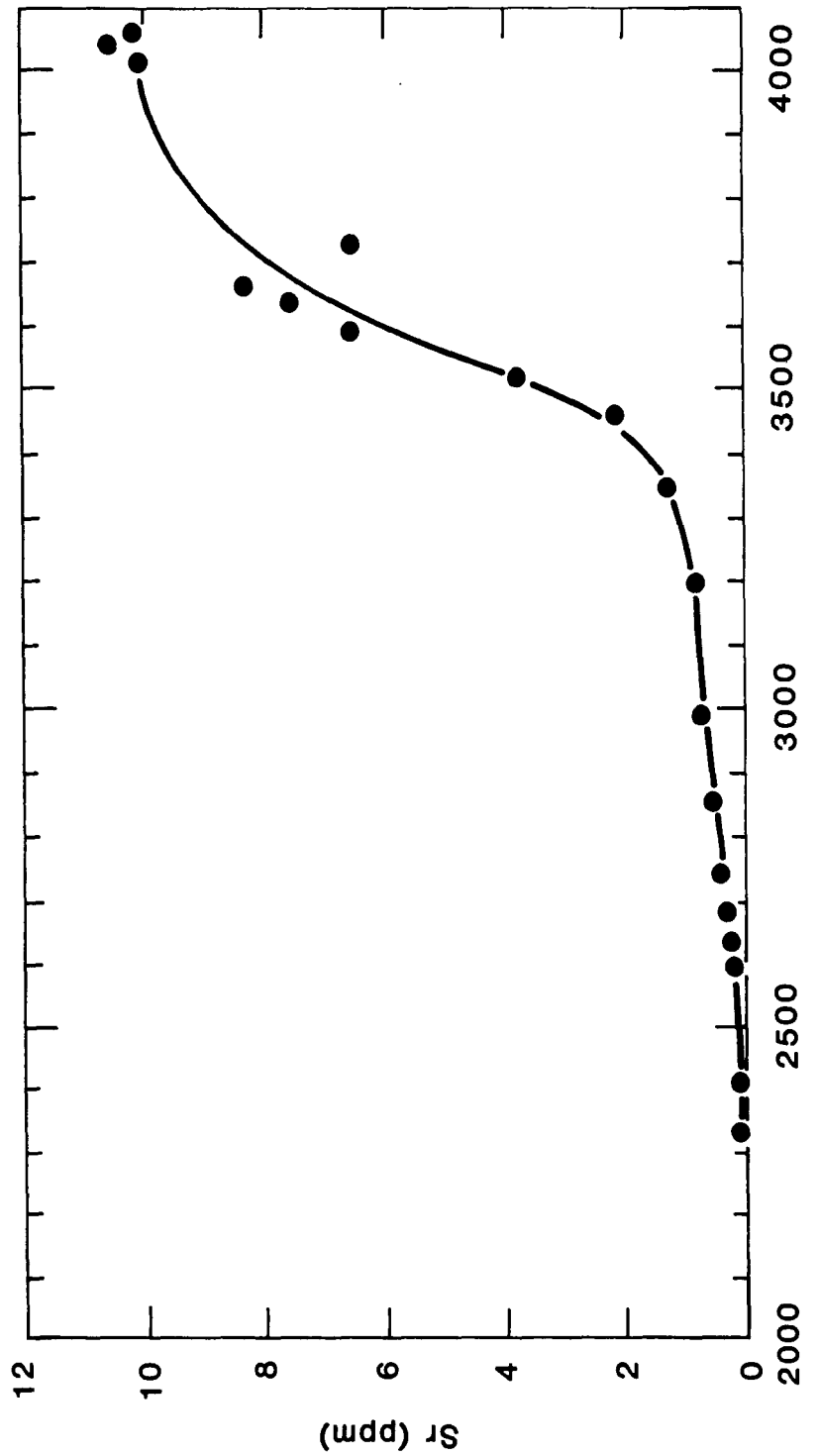

Fig. 1. Loading (breakthrough) curve for 10 ppm Sr solution and a 52 mL column (1.4 cm ID) filled with bone gelatin (20% *M. luteus* cells).

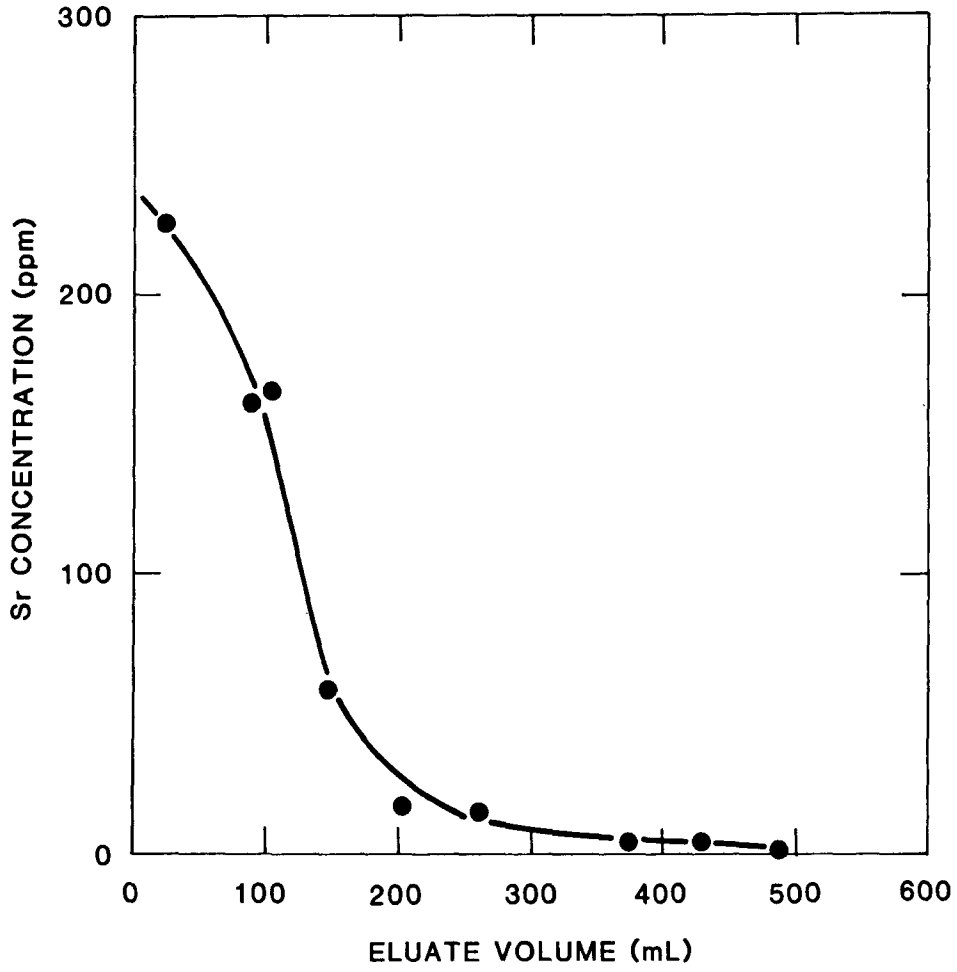

Fig. 2. Elution of 52 mL column of Sr loading bone gelatin (with 20% *M luteus*) using 0.3 *M* KCl solution.

ing/contraction of ion-exchange resins and suggests that the bone gelatin may contain fixed-charge groups neutralized by mobile ions. Exchange of these ions may be responsible for a portion of the Sr adsorption observed. The vendor specified that the Ca plus Mg content of the original bone gelatin was 40 ppm. However, the solidification in NaOH solution could have added Na ions and replaced Ca ions.

CONCLUSIONS

Microbial cells immobilized within bone gel particles can adsorb Sr from dilute solutions. The specific ability to adsorb Sr could have practical applications since it is an important pollutant in wastewaters from indus-

tries and laboratories handling nuclear materials. The adsorption is only partially attributed to the microbial cells; the gel itself is able to adsorb considerable Sr. *M. luteus* cells adsorb Sr very quickly, but the component(s) of the cells that contains the Sr appears to be slowly lost from the cells. The Sr released from the cells is likely to be associated with relatively high molecular weight materials that can neither enter nor escape from the gelatin. Although the apparent release of a Sr-bearing component from the cells complicates Sr adsorption by *M. luteus* cells, it may have little effect on the usefulness of this organism for adsorption operations if the active adsorbing material is retained within immobilizing gels. The presence of other salts would reduce the adsorption of Sr, and moderately high concentrations can even be used to elute Sr from gel particles.

Other commercial adsorbents are also able to remove Sr from such wastewaters. The practical application of Sr adsorption by immobilized cells in the treatment of wastewaters depends on the cost and stability of the gel particles. The potential for applying these adsorbents can be enhanced if improved organisms are found with even greater capacities and selectivities for Sr.

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Session 4
Biological Processing of Fossil Fuels