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MICROBIAL ACCUMULATION OF URANIUM, RADIUM, AND CESIUM*

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Diverse microbial species varied considerably in their ability to accumulate uranium, cesium, and radium. Mechanistic differences in uranium uptake by Saccharomyces cerevisiae and Pseudomonas aeruginosa were indicated. S. cerevisiae exhibited a slow (hours) surface accumulation of uranium which was subject to environmental factors, while P. aeruginosa accumulated uranium rapidly (minutes) as dense intracellular deposits and did not appear to be affected by environmental parameters. Metabolism was not required for uranium uptake by either organism. Cesium and radium were concentrated to a considerably lesser extent than uranium by the several species tested.

Key words: microbial; metal accumulation; uranium; radium; cesium.

1. Introduction

The intent of this report is to summarize our findings concerning some fundamental aspects of radionuclide accumulation by microbial cells, to consider these results with respect to what has been reported about microbial metal biosorption, and to illustrate the complexities attendant to an understanding of the phenomenon. The studies described here were carried out in conjunction with our efforts to develop a process to utilize microbial cells as a biosorbent for the concentration and removal of heavy metals from select aqueous waste streams, in particular, nuclear processing waste waters. We felt from the beginning that an understanding of the mechanism(s) of metal uptake would provide a better rationale for process design and potentially enable the enhancement of metal uptake through environmental or genetic manipulation of the microorganisms being utilized.

Although microorganisms are capable of concentrating a variety of metal species via metabolically mediated and growth associated processes [see 1-3

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for reviews and other papers in these proceedings], the phenomenon of biosorption (i.e., the physical/chemical complexation of metals by cellular components) appeared to have significant advantages for process application [4]. The use of metal biosorption for the treatment of wastewater and recovery of valuable heavy metals had been suggested as early as 1949 by Ruchoft [5]. Other workers have since reported schemes for the biosorptive removal of metal radionuclides from aqueous streams [6-9] and even the biosorptive extraction of uranium from natural waters has been proposed [10-11].

As indicated, biosorption is generally considered as a complexation between metal cations and negatively charged extracellular components. For instance, Ruchoft [5] attributed the ability of activated sludge to remove ^{239}plutonium to a microbial population providing a gelatinous matrix for metal adsorption. Other investigators [8,12-14] have also suggested that microbial extracellular polymers having negatively charged constituents were responsible for metal ion complexation. A detailed investigation by Rothstein and Meier [15] provided evidence that surface associated reactive groups chemically similar to high molecular weight polyphosphates were responsible for uranium uptake by Saccharomyces cerevisiae. Dounce and Flagg [16] concluded that the carboxyl groups of proteins were effective in complexing uranium. Complimentary studies by Beveridge and Murray [17] and Matthews and Doyle [T. H. Matthews and R. J. Doyle Abstr. Annu. Meet. Am. Soc. Microbiol., 1979, K86, p. 159] showed that the carboxyl groups of the peptidoglycans in cell walls of Bacillus subtilis are the primary sites of divalent metal complexation. Tsezos and Volesky [18] have suggested that the primary site of uranium and thorium binding by Rhizopus arrhizus is the chitin component within the cell walls of this fungus. However, their electron micrographs appeared to show considerable thorium accumulation at the outer wall surface.

Our own studies have been directed at a characterization of uranium uptake by S. cerevisiae and Pseudomonas aeruginosa. These organisms were selected for study from an earlier survey [19] because they demonstrated significant uranium uptake, they had both been used in previous metal uptake studies, and many fundamental aspects of their physiology and structure were well documented.

The ability of a variety of microorganisms to accumulate cesium and radium was considered in regard to potential treatment processes for two

radioactively contaminated waste solutions. The results of these studies are briefly discussed to further demonstrate the complexities of the biosorption phenomenon.

2. Methods

The detailed methodology used in our experiments has been described previously [4,19,20]. Essentially, washed cells were resuspended in an aqueous solution containing a known concentration of the radionuclide. At the desired time intervals, cells were removed from aliquots of the primary suspension by centrifugation. The remaining soluble uranium was assayed spectrophotometrically using Arsenazo III reagent [4]. ^{137}Cesium and ^{226}Ra were assayed by γ radiation counting. Cell-free controls were run concurrently.

Growth associated ^{137}Cs and ^{226}Ra accumulation were determined as above after growth of the organisms in an appropriate medium containing the radionuclides.

3. Results and Discussion

3.1 Uranium

Biosorptive complexation of metal cations by definition does not require metabolic activity by the microorganism nor is pre-exposure or adaptation to the metal required. However, it is possible that pre-exposure could result in an increase in biosorption sites if the organism was capable of utilizing the phenomenon as a detoxification mechanism. Conversely, pre-exposure might induce a population with a lesser affinity for potentially toxic metals. In our uranium uptake experiments and those of the other workers previously cited, the cells were cultured in the absence of uranium. Additionally, they were washed free of extraneous nutrients prior to exposure to uranium. Thus the measured uranium uptake was considered to be due to complexation sites normally occurring within the cell structure. Uranium uptake by S. cerevisiae and P. aeruginosa was found to occur rapidly (1-2 h and a few minutes respectively). In fact, using a specially devised ion-exchange procedure [20], we have determined that when P. aeruginosa is exposed to a solution containing 100 g/m^3 uranyl nitrate, approximately 99% of the uranium becomes firmly associated with the cells in less than 10 sec. As a consequence of this rapid uptake rate, the

apparent lack of any effect of the conditions imposed during uranium uptake by this organism may be due to our inability to monitor the time course of reaction within a time frame of several seconds.

Both species have a substantial capacity for uranium in that they can accumulate from 10 to 15% of their original dry cell weight as uranium. This capacity is similar to that obtained for a mixed culture of denitrifying bacteria [21] and R. arrhizus [9].

Electron micrographs of S. cerevisiae cells which had been exposed to uranium revealed that the metal accumulated as needle-like fibrils in a layer approximately 0.2 μm thick on the cell surfaces [20]. Furthermore, only 32% of the cells within a population had uranium deposits. Since only 32% of the cells possessed uranium deposits, the metal concentration on that fraction approaches 50% on a dry weight basis. It was difficult to imagine that there were sufficient binding sites to account for this much uranium. However, Beveridge and Murray [22] observed a non-stoichiometric accumulation of metals on isolated walls of B. subtilis. They suggested that metal ions complex with existing reactive sites and that additional metal crystallizes on these bound molecules.

In contrast to the surface accumulation exhibited by S. cerevisiae, uranium was found as dense intracellular deposits in P. aeruginosa [20]. These deposits did not appear to be associated with a particular structure or physical location within the cells. Intracellular deposition was demonstrated in cells exposed to uranium for 2 h. Electron microscopic examination after shorter exposure times has not been attempted. Although we lack visual evidence of immediate intracellular deposition of uranium, we do know uranium is firmly associated with the cells within a few seconds. As was observed with S. cerevisiae, not all cells of P. aeruginosa possess uranium deposits. Only 40 to 45% of the cells did so.

Our current investigation is being directed at the question of why only a fraction of the cells within a population of either species is capable of accumulating uranium. This has significant process implications for if we can induce all of the cells to accumulate uranium, we can greatly enhance the potential for utilizing these cells as biosorbents. We also feel it is of fundamental importance to understanding the mechanism of metal uptake in these cells. We have found that the rate of uranium uptake and total cellular capacity for the metal by S. cerevisiae is affected by growth conditions, presumably through growth rate induced changes in the cell wall composition

[20-23]. We are currently attempting to correlate cell wall composition with growth and uranium uptake rates and to determine if that fraction of cell population accumulating uranium is altered by growth conditions.

Consistent with the view that metal biosorption occurs by complexation of positively charged metal ions with negatively charged reactive sites (e.g., carboxyl and phosphate groups), uranium uptake by S. cerevisiae was affected by environmental parameters including pH, temperature, and competing cations [20]. Once bound, uranium could not be removed by water washing. Metal complexing agents such as ammonium carbonate, nitric acid, and disodium ethylenediaminetetraacetic acid (all 0.1 M) were only partially effective, removing 83.5, 59.3, and 72.3% respectively, of the bound uranium after 16 h. Any interpretation of the environmental effects observed must take into account the effect of the environment on the reactive metal binding sites as well as the complex solution chemistry of uranium.

While we have not specifically identified the uranium complexing sites on the surface of S. cerevisiae, experiments employing chemical pretreatment of the cells (i.e., formaldehyde) and pure wall components (i.e., amino acids, phosphomannan) suggest that both phosphate and protein moieties could be involved [20].

Uranium uptake by S. cerevisiae and P. aeruginosa was not metabolically mediated [20]. With the caveat that we have been unable to measure the transition from initial to equilibrium states during uranium uptake by P. aeruginosa, none of the conditions and treatments which affect uranium uptake by S. cerevisiae have an effect on P. aeruginosa. We are currently unable to explain the uranium uptake phenomenon in this organism.

3.2 Cesium

The ability of microorganisms to remove ^{137}Cs from solution was considered in regard to a potential treatment process for select high-level radioactively contaminated waste solution, SRSSW. The actual waste solution was simulated for our laboratory studies with a mixture of reagent-grade chemicals. It contained several metal species other than cesium and high concentrations of nitrate. Unlabeled CsCl was added to SRSSW and to the other test solutions to attain the concentrations shown in Table 1; also added was about 50 μCi of $^{137}CsCl$ as a tracer.

As shown in Table 1, the distribution coefficients obtained were relatively low, and in all cases the bulk of the cesium (as evidenced by soluble radioactivity measurements) remained in solution. The denitrifying

Table 1. Uptake of ^{137}Cs by microorganisms

Microorganism	Cell stage	Culture medium	Initial cesium concentration (g/m^3)	Cell concentration (g/100 ml)	Distribution coefficient[a]
Pseudomonas aeruginosa	Rest	SRSSW at pH 4	0.12	0.4	11
		SRSSW at pH 7	0.12	0.4	12
		SRSSW at pH 10.2	0.12	0.4	9
	Growth	Complex organic[b]	0.017	0.2	16
Saccharomyces cerevisiae	Rest	Water at pH ~6	0.006	0.6	26
	Growth	Complex organic[b]	0.017	0.4	37
Mixed culture of denitrifying bacteria	Growth	Denitrifying[c]	0.013	0.002, 0.003	228, 469
	Growth	SRSSW[c]	0.013	0.04	9
			0.013	0.04	15
			0.013	0.006, 0.010	95, 137
			0.013	0.03	21
			0.913	0.05	49

[a] Distribution coefficient: $\frac{\text{cpm } ^{137}Cs \text{ per g cells}}{\text{cpm } ^{137}Cs \text{ per g solution}}$.

[b] Complex organic medium (see ref. 20).

[c] Ethanol (0.5% v/v) as carbon source (see ref. 19).

bacteria grew little, if at all, in the presence of cesium when ethanol was the carbon source and the cultures were incubated anaerobically under a nitrogen atmosphere.

Published reports of microbial cesium accumulation indicate that both growth-related and adsorptive phenomena occur [8,24-29]. The growth stage at which maximum accumulation occurs varies in these reports. Also, it has been found that cesium is not permanently associated with growing cells and that firm binding is more significant in old cells and detritus.

We attempted to compare our results with those in the literature. Notably, it has been shown that cesium accumulation factors or distribution coefficients decrease as the cesium concentration increases. For example, Fisel *et al.* [8] observed that Paecillomyces marquandii exhibits maximum accumulation (34% of added ^{137}Cs) at the lowest cesium concentration tested ($\sim$0.008 mM Cs). With Chlorella pyrenoidosa [27], increasing the cesium concentration from a tracer level (10 µCi ^{137}Cs/L) to 0.5 mM decreases the concentration factor by three fold. Williams [28] also found that a cesium concentration of 0.15 mM significantly reduces the concentration factor in Euglena intermedia. Cesium incorporation studies done by other workers [25,26,28] involved very low ^{137}Cs levels (2 to 20 x 10^{-5} µCi/mL), and relatively high distribution coefficients were obtained.

The results in the literature as well as our own are presented as distribution coefficients or accumulation factors. Microbial cells appear to have a finite capacity for accumulating metal ions by biosorption [4,9,20], when they are exposed to solutions containing increasing concentrations of metal ions. Once they have adsorbed all of the metal possible, soluble metal ions may not inhibit further metal uptake, but merely depress the observed distribution coefficient. We were unable to deduce the loading capacity from the data in the literature, and our own experiments were not designed to obtain this value. However, a very rough estimate from our data indicated a loading capacity of less than 10 µg of Cs per gram of cells. In all cases, the greater portion of cesium remained in solution, which provides evidence that the cells were saturated with cesium under the conditions imposed.

3.3 Radium

^{226}Radium incorporation by microbial cells was considered with regard to another radioactive waste problem, namely contaminated waste storage ponds. In addition to ^{226}Ra, the water contains a variety of other metal species and 1.3% nitrate. Samples of the pond water were treated as indicated in Table 2. ^{226}Radium incorporation was cetermined for resting cells and under growth conditions.

It can be seen that some organisms can accumulate ^{226}Ra under resting cell or growth conditions (Table 2). However, the distribution coefficients are relatively low.

Little is known about the microbial accumulation of radium. Jilek *et al.* [6] observed the removal of ^{226}radium from a waste stream by chemically derivitized mycelia of Penicillium chrysogenum but no distribution coefficients or cellular capacities were reported. P. A. Taylor of the Chemical Process Department, Y-12 Development Division, Nuclear Division, Union Carbide Corp., Oak Ridge, Tennessee (unpublished), found 95% of the ^{226}Ra removed during long-term (months) growth of denitrifying microorganisms in the same pond water we examined. Methanol was used as a carbon source. In contrast, we found very little ^{226}Ra removal either by biosorption or during short-term (weeks) growth experiments. Further experimentation will hopefully resolve these differences.

4. Conclusion

Our studies and the work of others provide adequate evidence that the biosorptive association of metal cations with microbial cells involves varied and complex phenomena. There are significant differences in the extent of accumulation as well as the mechanisms by which accumulation occurs. In a process environment, using pure or defined mixed cultures, it is possible to exercise some degree of control over the phenomenon. However, in a natural environment containing a multitude of microbial and chemical species, it would be difficult to predict, except in a general way, the association of radionuclides with microbial cells.

Table 2. Uptake of ^{226}Ra ($^{345}pCi/L$) from contaminated pond water by growing cultures and resting cells

Microorganisms and conditions		Distribution coefficient[a]
Growing cultures		
Mixed culture of denitrifying bacteria		
Glucose grown (20d)		104, 119
Ethanol grown (20d)		124, 126
Chlorellapyrenoidosa		
Shake culture (17d)		51
Static culture (17d)		41
WS algae[b]		
Shake culture (17d)		46
Static culture (17d)		32
Resting cells		
Pseudomonas aeruginosa	pH 4	<<1[c]
	pH 6.9	<<1[c]
Saccharomyces cerevisiae	pH 4	<<1[c]
		<<1[c]
Ashbya gossypii	pH 7.7	344
Penicillium chrysogenum	pH 7.7	624
Mixed culture of denitrifying bacteria	pH 3.9	7
	pH 5.7	95
	pH 7.7	38

[a]Distribution coefficient: $\frac{\text{pCi } ^{226}Ra/g \text{ cells}}{\text{pCi } ^{226}Ra/g \text{ solution}}$.

[b]Mixed algal, glue-green algal population isolated from pond water.

[c]pCi ^{226}Ra/g cells essentially equivalent to background.

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