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Short Communication

Microbial accumulation of neptunium

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SUMMARY

Resting cells of several species of microorganisms removed neptunium (Np) from an aqueous solution. Concentrations of up to 15 mg Np per g cells (dry weight) were obtained. Maximum uptake by *Micrococcus luteus* occurred in less than 10 min.

INTRODUCTION

Neptunium (Np), an activation product of uranium-238, can occur in wastewaters from nuclear facilities. It was recently proposed that wastewater outfalls be required to meet the 'derived concentration guide' for drinking water (DOE Draft Order 5480.XX, Radiation Protection of Public and Environment). A discharge limit of 3×10^{-5} μCi/l has been proposed for Np. There is concern within the nuclear industry that this requirement could be difficult to meet for Np using existing technologies. Our experience with the microbial uptake of other radionuclides [4,5] prompted a preliminary investigation of whether microorganisms could accommodate Np and be of use in removing this radionuclide from nuclear processing wastewaters.

MATERIALS AND METHODS

Briefly, the Np uptake experiments were conducted as follows. Ten milliliters of aqueous suspensions of washed cells (10 ml water used in place of cells in controls) was added to 40 ml of a solution of ^{237}Np (pH adjusted with 0.1 M NaOH) contained in a stoppered, 500 ml polyethylene Erlenmeyer flask. The flasks were incubated on a reciprocal shaker (2 in. stroke, 180 strokes/min) at room temperature. One milliliter samples were withdrawn at intervals and centrifuged ($\approx$2000 $\times$ *g*, 15 min) to remove the suspended cells. The level of ^{237}Np remaining in solution was determined by liquid

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Table 1

Accumulation of Np by microbial cells

Organism (growth medium)[a]	Time (h)	Np remaining in solution[b] (mg/l)	Cells (mg/l dry wt.)	Sorbed Np (mg/g cells)	Distribution coefficient[c]
Control[d]	1	36	–	–	–
	3.5	35	–	–	–
Saccharomyces cerevisiae NRRL Y2574 (YM)	1	33	1.7	2	40
	3.5	29	1.7	4	140
Pseudomonas aeruginosa NRRL B-4452 (YM)	1	15	1.8	11	710
	3.5	14	1.8	11	820
Streptomyces viridochromogenes ATCC 3356 (YM)	1	13	1.8	13	940
	3.5	10	1.8	15	1540
Micrococcus luteus ATCC 4698 (ENR)	1	9	2.0	14	1560
	3.5	10	2.0	13	1390
Rhizopus oryzae NRRL 395 (YM)	1	28	1.0	8	310
	3.5	30	1.0	6	200
Scenedesmus obliquus (Bold's)	1	31	0.3	16	500
	3.5	32	0.3	14	430
Denitrifying mixed culture[e]	1	22	1.6	8	380
	3.5	22	1.6	8	380

[a] YM = YM broth (Difco, Detroit, MI). ENR = heart infusion broth, 1.25%; nutrient broth, 0.54%; yeast extract, 0.25%; tryptic soy broth, 1%; proteose-peptone, 0.2% (all Difco). Bold's (see Ref. 1). The bacteria and fungi were grown for 48 h at 30°C. *Scenedesmus obliquus* was cultured for 5 days.

[b] [(dpm) (237 mg/mol)]/[(0.9 counting efficiency) (5×10^{-4} l) (3.709×10^{8} dpm/mmol)]; the initial Np concentration was 36 mg/l.

[c] [g Np/g cells (dry wt.)]/[g Np/g solution].

[d] Control contained water in place of cell suspension.

[e] Obtained from a fluidized-bed denitrifying bioreactor (courtesy of J.B. Patton, Westinghouse Material Co. of Ohio, Fernald, OH).

scintillation counting (0.5 ml sample in 10 ml of Insta-Gel scintillation fluid (Packard Instruments Co., Inc., Downers Grove, IL). Additional details are given in the tables.

The dry weight of cells in each flask was calculated from a separate, known volume of the cell suspension dried at 95–100°C for 18 h.

RESULTS AND DISCUSSION

The organisms (or similar strains) used in this study have been previously demonstrated to accumulate a variety of radionuclides [2,3]. As shown in Table 1, all accumulated Np; several accumulated from 11 to 15 mg Np/g cells (dry weight). The dis-

Table 2

Effect of pH on Np accumulation[a]

Organism	pH	Np in solution (mg/l) at time (min):			Sorbed Np (mg/g dry cells) at time (min):	
		0	10	60	10	60
Control	4	37	36	35		
	5.4	33	32	31		
	7	39	38	36		
M. luteus	4	37	11	11	13	12
ATCC 4698	5.4	33	14	12	9	10
	7	39	15	12	12	13
S. viridochromogenes	4	37	16	12	13	16
ATCC 3356	5.4	33	19	12	9	13

[a] See Table 1 for experimental details.

tribution coefficients [(g metal per g cells)/(g metal per g solution)] ranged from a few hundred to ≈ 1500. This is an order of magnitude less than that found for several other metals and microorganisms [3].

The complex solution chemistry of radionuclides and hence biosorption may be significantly affected by pH [4,5]. The results shown in Table 1 were obtained at an initial pH of 4.0. The effect of initial solution pH on Np uptake was examined further as shown in Table 2. Np uptake by *Micrococcus luteus* was greater at pH 4 and 7 than at pH 5.4. A similar decrease in Np uptake at pH 5.4 occurred for *Streptomyces viridochromogenes*. The reason(s) are not understood at this point.

Np uptake was rapid. Maximum uptake (at pH 4) by *M. luteus* occurred within 10 min (Table 2). The rate of uptake by *S. viridochromogenes* was somewhat slower. There appeared to be a slight reduction in the rate of uptake at pH 5.4 compared to pH 4, although the significance of these limited data is uncertain.

Little (if any) accumulation of Np occurred (data not shown) when *M. luteus* and the denitrifying mixed culture were exposed to Np for 3 h at an initial concentration of ≈ 35 μg/l (3×10^{-2} μCi/l). This is approximately 1000 $\times$ the proposed effluent requirement. Thus the utility of these particular species as biosorbents for removing Np from nuclear processing waste streams is not indicated. However, other microorganisms and biomass sources (e.g., sewage sludge) could be tested. If it is further determined that microorganisms in general have a similar low affinity for Np, this information would be useful nevertheless in understanding the distribution and fate of Np in nature and in waste treatment systems.

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