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Disposal and Storage Sites

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Sulfate-reducing bacteria were grown in media containing (1) mercury and (2) a mixture of heavy metals to determine if these bacteria could effectively precipitate heavy metals from an aqueous solution. Previous studies have shown that (1) sulfate-reducing bacteria produce hydrogen sulfide and (2) that hydrogen sulfide reacts with various heavy metals producing insoluble complexes. Data from these studies showed that when bacteria were incubated with heavy metals for 2 to 4 days a marked decrease in the solubility of mercury, lead and cadmium resulted. For example, after incubation with sulfate-reducing bacteria 90% of the mercury, zinc, lead and cadmium, and 60% of the copper and iron were filterable. The results suggest that sulfate-reducing bacteria may be used to remove heavy metal contaminants from aqueous solutions.

Reaction

TABLE 1

The formation of a mercury precipitate in a medium containing sulfate-reducing bacteria and mercuric chloride.

Total Mercury added (ppm, mg 1 liter)	Percent mercury recovered in 3 layers of secrose gradient		
	20% Sucrose (Soluble Hg^{+2})	40% Sucrose (bacteria)	60% Sucrose (Hg precipi- tate)
0.0001	1.3 $\pm .3$	2.4 ± 1.2	96.3 ± 3.1
0.1	0.5 ± 0.5	2.1 ± 0.6	97.4 ± 2.2
1	16.2 ± 5.1	6.3 ± 2.3	77.5 ± 6.8
10	32 ± 4.1	11 ± 2.0	58 ± 7.9
100	61 ± 6.6	15 ± 1.8	24 ± 3.9
- Control	98 ± 1	0	0

Mean $\pm$ SD of four experiments. Sulfate reducing bacteria were grown in a sulfate medium containing $^{203}\text{HgCl}_2$ (100 nCi) and HgCl_2 at the indicated concentration. Incubations were performed at 37°C under aerobic conditions for 7 days. At the end of the incubation an aliquot of the culture was loaded onto a sucrose step gradient (20, 40 and 60%) and centrifuged at 4,500 rpm for 20 minutes. The soluble Hg^{+2} , bacteria, and precipitate (HgS) were found in the 20, 40, and 60% sucrose fractions, respectively. The control was uninoculated and contained 1 ppm mercury.

TABLE 2

The formation of a precipitable cadmium complex in a medium containing sulfate reducing bacteria and cadmium chloride.

Total Cadmium added (ppm, mg 1 liter)	Percent cadmium recovered in 3 layers		
	20% Sucrose (Soluble Cd ⁺²)	40% Sucrose (Bacteria)	60% Sucrose (Cd precipitate)
0.0005	0.4 ± 0.1	2.8 ± 0.6	96.8 ± 3.1
0.5	1.0 ± 0.3	2.6 ± 0.4	96.4 ± 2.6
5	9.8 ± 1.4	4.4 ± 1.0	85.8 ± 4.3
50	37 ± 3.6	6.9 ± 0.9	56.1 ± 10.1
Control	99 ± 1	0	0

Mean ± SD of four experiments. Methods similar to the experiments shown in Table 1 except that cadmium chloride was substituted for mercury chloride.

Table 3

The formation of precipitable heavy metal complexes in a medium containing sulfate-reducing bacteria and several heavy metal compounds

Heavy metal Compounds	Concentration		% Filterable
	Starting	Final (mg/liter)	
CdCl ₂	0.580	.06	89.7
CoCl ₂	4.66	4.79	0
CrCl ₂	0.91	0.90	0
K ₂ Cr ₂ O ₇	6.31	0.90	61.3
CuSO ₄	0.78	0.33	57.7
Fe(NH ₄) ₂ (SO ₄) ₂	3.55	1.58	55.5
MnSO ₄	0.77	0.80	0
KMnO ₄	2.57	1.19	53.7
NiCl ₂	2.23	2.28	0
Pb(CH ₃ COO) ₂	12.25	0.95	92.2
ZnCl ₂	1.0	0.10	90.0

A mixed culture of sulfate-reducing bacteria was inoculated into 10 ml of sulfate broth and incubated at 35°C for 4 days. Samples were filtered through 0.45 μ filter, and the metal content of each filtrate was determined by ICP AA spectroscopy.

Table 4

The induction of an insoluble mercury complex by biological action

Samples	With Gravel Bed concentration		Without Gravel Bed concentration	
	starting ppb (mg/liter)	final	starting ppb (mg/liter)	final
NWA4	9.57	< 0.1	9.57	< 0.1
NHPI ₁			5.58	< 0.1
NHPI ₂			3.24	< 0.1
SEB4	18.57	< 0.1	18.57	< 0.1
NHPI ₃			5.93	< 0.1
NHPI ₄			8.22	< 0.1
SCA5	20.43	< 0.01	20.43	< 0.1

Sulfate-reducing bacteria were grown in flasks containing a sulfate medium and with and without an added gravel bed. The cultures were overlaid with effluents from storm drains or runoffs from Y-12. Cultures were incubated at 35°C for 6 days and 5 to 10 ml aliquots were filtered through 0.45 μ filters, and the mercury content of filtrates determined by AA spectroscopy using the cold vapor technique. [Mercury content in each sample was determined before and after they were filtered through a 0.45 μ filter and differences in the mercury before and after filtration was not observed.] Over 99% of the mercury in uninoculated controls was found in the filtrate.

Figure Removal of Hg from Solution
by Sulfate-reducing Bacteria

A culture of bacteria isolated from soil was inoculated into 100 ml of broth containing (per liter), yeast extract 1.0 g, sodium lactate 5.2 g, MgSO_4 0.2 g, ascorbic acid 0.1 g, K_2HPO_4 0.01 g, NaCl 10 g, and, if indicated, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ 0.1 g, in a bed of sterile aquarium gravel. The gravel bed was overlaid with 500 ml of HgCl_2 solution containing $^{203}\text{HgCl}_2$. Dissolved Hg was measured by filtering samples through a 0.22 micron membrane filter and counting the filtrate with a gamma counter.

Table Solubility product constants
of heavy-metal salts

Metal	Salt		
	S^{-2}	SO_4^{-2}	CO_3^{-2}
Cd	3.6×10^{-29}	2.8×10^{-2}	1×10^{-4}
Hg	3×10^{-50}	7.4×10^{-7}	—
Pb	3.4×10^{-28}	5.6×10^{-2}	3.3×10^{-14}