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Abstract

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Biological waste treatment processes include biosorption, detoxification of toxic wastes by bioconversion, and precipitation of heavy metals and radionuclides as a result of microbial metabolism. We have studied the precipitation of heavy metals by sulfide generated as a result of anaerobic sulfate respiration by sulfate-reducing bacteria. These bacteria are able to remove salts of mercury, lead, cadmium, nickel, and zinc from media that support sulfate respiration. A survey of metal-contaminated soils along East Fork Poplar Creek showed that all samples contained sulfate-reducing bacteria, but none had sufficient concentration of free sulfate to support active sulfate respiration. This situation represents a potential hazard in that sulfate-reducing bacteria can methylate mercury under conditions of low available sulfate, possibly releasing methyl mercury to the groundwater. We have proposed that amendment of soil with sulfate will promote sulfate respiration by bacteria. This would both enhance precipitation of heavy metals and also inhibit the formation of the toxic methylated metal compounds. Studies with soil columns demonstrated the feasibility of surface application of calcium sulfate to soil in providing sufficient concentration of sulfate at depths of at least 40 cm. No leaching of heavy metals has been observed in columns amended with sulfate. We therefore suggest that field studies on stabilization of metal wastes in soil by surface amendment with calcium sulfate would be appropriate.

Introduction

Biological waste degradation is an ancient process, probably having begun shortly after wastes began to be generated by living organisms. Although the importance of decay processes has long been recognized, controlled processes for the biological degradation of waste (for example in sewage treatment) have been developed only recently. A great deal remains to be learned about biological waste treatment, and the potential for application of these systems is both large and relatively poorly exploited.

The Oak Ridge Research Institute is engaged in research and development aimed at establishing microbial systems for treatment of biological and chemical wastes. These systems include the use of microbes as biosorbents to concentrate wastes from solution, biochemical conversions of toxic chemical wastes to less toxic or innocuous forms, and biologically mediated precipitation of wastes, either from solution or in situ.

Biosorption processes range from simple adsorption by biological waste products (e.g. straw), through ion exchange at the cell surface, to active transport and intracellular deposition, and can have different degrees of specificity. In any well-designed system, the biosorbent can be readily regenerated at the cost of a few inexpensive nutrients. Our interests are in the use of microbes to sorb heavy metals and radionuclides from waste streams.

Bioconversion of toxic wastes has been successfully applied in a number of cases in which groundwater has been contaminated by chemical spills. In these treatment systems, nutrients are typically added to the groundwater through injection wells, naturally occurring soil microbes proliferate, and those with the appropriate metabolic capabilities degrade

the pollutants. We pursuing studies on the occurrence of soil microbes in sites contaminated with halogenated hydrocarbons such as perchloroethylene.

We are also currently investigating microbial processes for the removal or stabilization of heavy metals and radionuclides. In these processes, the metals may be precipitated by reaction with products of microbial metabolism, or they may be converted to an insoluble form by reductive enzymes. In the latter system, for example, reductases produced by growth of a strain of Pseudomonas can reduce mercuric, silver, and gold ions to the elemental form. Experiments with this system are currently in progress in our laboratory.

Experimental

This report will focus on precipitation of heavy metals by biological sulfide formation. A ubiquitous group of soil organisms, the sulfate-reducing bacteria, carry out a form of respiration in which sulfate is reduced to sulfide. This process is obligately anaerobic, i.e., it cannot proceed in the presence of oxygen. Naturally occurring organic acids in the soil serve as the carbon and energy source for growth of the organisms. Thus the requirements for sulfate respiration include adequate amounts of both sulfate and organic acids.

We have carried out a series of experiments to characterize the precipitation of heavy metals by sulfate-reducing bacteria. Growth media for these bacteria typically contain lactate and sulfate, plus ferrous ion, which reacts with the sulfide generated by sulfate reduction. The resulting black ferrous sulfide precipitate serves as an indication of sulfide production. Many other metal sulfides are also insoluble, notably those of mercury, cadmium, and lead. Figure 1 demonstrate that soluble

mercury can be removed from a mixed culture containing sulfate-reducing bacteria.. In this experiment, as the bacteria grew, mercuric sulfide precipitated, and the level of soluble mercury was reduced to background, to around 0.05 ppb in one experiment (Figure 1b). Table 1 shows that several other heavy metals can be precipitated in the same way. These organisms can also be used to remove heavy metals from waste water. Waste water from several sources at the Y-12 plant at the Oak Ridge facility. In the experiment shown in Table 2, waste water was mixed with lactate-sulfate broth and inoculated with sulfate-reducing bacteria. The concentration of mercury in each sample was measured before and after growth of the bacteria. In each case mercury levels were reduced below the detection limit (0.1 ppb) of the analytic method.

The sulfides formed are not tightly bound to the cells. Therefore, they do not form deposits that poison the cell or physically block the flow of metabolites into or out of the cells. By use of a density gradient centrifugation technique, we have demonstrated the separation of precipitate from cells (Table 3). This experiment also showed that the sulfate-reducers grow in high concentrations of mercury. A similar result was obtained with cadmium (Table 4).

We have also studied the action of sulfate-reducing bacteria in situ. In the past, the Y-12 plant released quantities of heavy metals into East Fork Poplar Creek, which originates in the DOE reservation and flows through the city of Oak Ridge (Figure 2), in some cases through playing fields and gardens. At some locations along the creek, heavy metals have accumulated in the soil, so that mercury levels as high as 1800 mg/kg soil have been measured. Table 5 shows the distribution of several heavy metals in the soil at the sites indicated on the map in Figure 2. It is clear from these

data that metal pollution problem is extensive, too extensive geographically to make spot clean-up efforts practical.

A major question concerning public health has been to what extent the mercury and other metals are leached into groundwater and are thus mobilized into the food chain or into contact with people. In addition, in the absence of adequate sulfate, sulfate-reducing bacteria metabolize the organic acids, but intermediates are produced which can add methyl groups to mercury and other metals. These methylated metals are water-soluble and highly toxic, adding to the human health hazard.

We have suggested that heavy metals might be more completely immobilized in the soil by precipitating them as sulfides. To do so, we would amend the soil so as to enhance sulfate-reducing activity. To determine whether this approach is indeed feasible, we have been investigating the conditions required to enhance reduction in situ. We sampled soil at several locations along East Fork Poplar Creek for use in these studies. Soil was slurried in water containing iron and various concentrations of sulfate, and the potential of these slurries to cause the formation of ferrous sulfide was assessed. We have found that sulfate-reducing activity can be observed in suspensions of soil from every site sampled. However, no activity was observed unless exogenous sulfate was added to the suspension. This implies that the soils are depleted of sulfate relative to the levels necessary to support sulfate respiration. In these experiments about 300 ppm sulfate was optimal for enhancing sulfate reduction.

These results suggest that enhancement of sulfate respiration in situ might be accomplished by adding sulfate to the soil, preferably in some slowly released form. Bench-scale experiments were necessary to

investigate this possibility. Therefore we constructed columns 10 cm in diameter, with 40 cm of soil taken from a site with about 1000 mg/kg mercury. As the sulfate source we chose CaSO_4 , because of its limited solubility. Water was added to the columns at a rate simulating average rainfall amounts. Water percolating through these columns was collected, and the concentrations of sulfate and heavy metals were measured. In one column, no sulfate was added; in one, CaSO_4 was mixed evenly with the soil at a rate of 500 mg/kg; and in one, the same total amount of CaSO_4 was applied to the soil surface. Figure 3 shows that sulfate applied to the surface of the soil had begun to leach through by about thirty days, whereas sulfate mixed throughout the soil leached out immediately. Measurements of soluble mercury in the leachate (Table 6) showed that leaching from sulfate-amended columns was slightly less than from non-amended columns, indicating that the treatment did not mobilize the mercury present in the soil. Analysis for other metals in the leachate is currently in progress.

Conclusions

We have demonstrated that anaerobic sulfate respiration by sulfate-reducing bacteria can precipitate heavy metals from solution, including from metal-contaminated waste water. We have also shown that these bacteria are present in a wide variety of soil samples, but that their respiratory activity requires the addition of sulfate. Because metal sulfides are generally insoluble and because metabolism by these organisms in the absence of sulfate may lead to methylation of metals, we believe that metal-contaminated soils should be amended with sulfate. Surface addition of CaSO_4 appears to be sufficient to provide adequate sub-surface sulfate levels for sulfate respiration. This activity should help to stabilize metal pollutants in soil.

In addition, we believe that waste treatment systems can be devised in which sulfate-reducing bacteria remove metals from solution as insoluble sulfides. The resulting metal waste would be highly concentrated, and could more easily be disposed of, for example in an appropriate waste storage facility or as a stable component of a glass or grout mixture.

We believe that this and other biological systems have great potential for application to remediation and/or stabilization of metal pollutants and radionuclides in both waste water and soil. The Oak Ridge Research Institute will continue to develop treatment methodologies, and is actively seeking opportunities to develop new applications at other DOE sites.

TABLE 1.

REMOVAL OF MERCURY FROM Y-12 WASTES

Bacterial treatment can remove mercury from wastewater.

Source	Mercury Conc. (ppb)	
	Untreated	Treated
Storm Drain	9.6	0.1
Coal Pile	18-20	0.1
New Hope Pond	3-8	0.1

Wastewater samples obtained from Y-12 were amended with nutrients and inoculated with sulfate-reducing bacteria. Mercury levels in treated and untreated samples were measured after filtration

TABLE 2.

PRECIPITATION OF HEAVY METALS

Biological sulfide precipitation can reduce the level of metals in solution.

Metal	Permit Level (ppb)	Experimental Results (ppb)	
		Starting	Final
Cadmium	3.5	0.5	0.02
		500	5.0
Mercury	2.0	5.0	0.06
		100	0.5
Nickel	3980	10,000	2400
Zinc	300	1000	100
Copper	22	780	330
Iron	1000	10,000	2480
Lead	170	10,000	620

Metal concentrations were measured after filtration.

TABLE 3.

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

Total Mercury added (ppm, mg / liter)	Percent mercury recovered in 3 layers of secrose gradient		
	20% Sucrose (Soluble Hg)	40% Sucrose (bacteria)	60% Sucrose (Hg precipi- tate)
0.0001	1.3 ± .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 ± 4.8
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 ± SD of four experiments. Sulfate reducing bacteria were grown in a sulfate medium containing $^{203}\text{HgCl}_2$ (100 μCi) 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 4

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

Total Cadmium added (ppm, mg / liter)	Percent cadmium recovered in 3 layers		
	20% Sucrose (Soluble Hg)	40% Sucrose (Bacteria)	60% Sucrose (Hg 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 $\pm$ SD of four experiments. Methods similar to the experiments shown in Table 1 except that cadmium chloride was substituted for mercury chloride.

TABLE 5.

The concentration of several elements in soil
from East Fork Poplar Creek

Place	Site Number		PPM Arsenic	PPM Cadmium	PPM Lead	PPM Mercury	PPM Selenium
Gulf House	1	Left	.59	.45	5.65	1.97	129.57
	2	Middle	.73	12.4	9.85	12.19	190.99
	3	Right	5.49	8.176	10.67	507.55	197.05
NCA	4	Left	5.69	92.9	17.29	974.83	271.51
	5	Middle	3.18	2.67	12.48	49.03	231.15
	6	Right	4.32	12.8	12.92	97.57	100.71
K-Mart	7	Left	.58	26.8	5.61	61.06	191.47
	8	Middle	1.32	6.1	7.46	16.16	162.55
	9	Right	2.57	9.5	7.63	48.50	128.98
Bruners	10	Left	6.10	11.5	17.41	1799.36	190.52
	11	Middle	3.40	8.9	7.61	17.91	124.68
	12	Right	6.93	13.2	9.57	987.94	209.11
Sewage Plant	13	Left	4.64	26.1	22.17	43.52	126.16
	14	Middle	2.63	3.3	5.84	33.18	59.86
	15	Right	4.17	35.5	11.29	220.85	69.81
East Fork Turnpike Bldg.	16	Left	2.83	10.7	6.55	78.84	168.90
	17	Middle	.57	12.2	3.86	1.33	90.05
	18	Right	2.11	9.8	5.67	65.43	142.95
Poplar Creek Parameter Road	19	Left	1.79	7.3	3.95	22.79	99.10
	20	Middle	.75	13.6	5.58	12.51	73.00
	21	Right	3.88	7.6	5.82	6.13	93.61
Bear Creek	22	Left	1.32	11.2	2.41	11.23	111.25
	23	Middle	1.90	16.2	6.26	3.47	101.29
	24	Right	2.20	12.3	7.04	34.62	99.27
Bear Creek	25	Left	7.08	1.8	5.07	6.61	100.69
	26	Middle	4.38	5.5	3.78	.77	107.74
	27	Right	2.37	3.8	5.37	2.64	195.09
Bear Creek	28	Left	4.34	1.13	4.43	.59	127.04
	29	Middle	2.53	9.5	4.23	.72	101.78
	30	Right	2.77	1.11	4.48	13.07	122.52

PPM = MILLIGRAM OF ELEMENT / KILOGRAM SOIL

TABLE 6.

Mercury Concentration (ppb)
in Leachate from Soil Columns

Day	CaSO ₄		
	None	Top	Mixed
7	3.9	1.1	1.3
18	-	0.3	0.3
35	0.1	0.1	0.1
42	2.60	0.42	0.22
49	0.1	0.1	0.1
Mean	1.68	0.39	0.40

Figure 1.

Removal of Hg from Solution
by Sulfate-reducing bacteria

A culture of bacteria isolated from soil was inoculated into broth containing yeast-extract and sodium lactate as carbon sources and MgSO_4 and $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ as sulfate sources. A solution containing $^{203}\text{HgCl}_2$ and nonradioactive HgCl_2 at the indicated concentrations was added. Dissolved Hg was measured by filtering samples at the indicated times and counting the filtrate with a gamma counter.

SAMPLE SITE LOCATIONS, EFPC

FIGURE 2.

FIGURE 3. Sulfate Concentration in Effluent of Soil Columns