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Removal of mercury from mercury-contaminated sediments using a combined method of chemical leaching and volatilization of mercury by bacteria

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Abstract

A method for the removal of mercury sulfide from mercury-contaminated sediments was developed, which consists of chemical leaching and volatilization of mercury by bacteria. More than 85% of the mercury in sediment containing 0.11–37.4 mg/kg of mercury was efficiently extracted with 3 M HCl and 74 mM $FeCl_3$. Subsequent volatilization by bacteria resulted in the removal of 62.9–75.1% of mercury from mercury-contaminated Minamata Bay sediments. Methylmercury was also eliminated from soil at a high efficiency. Thus, this combined method of chemical and microbial treatments could be used for efficient removal of both organic and inorganic mercurials from natural sediments.

Introduction

Mercury pollution of the environment has become a serious environmental problem throughout the world. Minamata Bay in Japan has been heavily polluted by industrial wastes containing high concentrations of mercury. Minamata disease was caused by consumption of fish and shellfish that were heavily contaminated with methylmercury (Irukayama 1977). Although discharge of mercury into Minamata Bay from a chemical plant was stopped in 1968, the sediments had become contaminated by mercury at maximum concentrations of 262 mg/kg (Kumamoto Prefectural Government 1973) and 95 mg/kg (Kumagai and Nishimura 1978). World wide, mercury pollution has been reported in many places, including Quebec in Canada (Eyssen and Rudedy 1986; Wheatly et al. 1979) and the Amazon in Brazil (Malm et al. 1990; Nriagu et al. 1992). Major sources of mercury wastes are the gold-mining, agriculture, and chlor-alkali industries.

Mercury in the natural environment exists as mercury sulfide (HgS) (Gavis and Furgson 1972), which is extremely insoluble and chemically and physically stable. However, it has been postulated that small amounts of methylmercury are also formed from HgS in aerobic sediments (Fagerstrom and Jernelov 1971). Methylmercury accumulates in fish and shellfish and causes a potential risk of toxicosis to consumers. Because of this, the removal of mercury from mercury-contaminated environment is of primary importance.

A number of bacteria isolated from mercury-polluted environments have been shown to transform mercurial compounds into elemental mercury as mercury vapor (Nakamura et al. 1990; Nakamura et al. 1988). Some bacteria decompose organomercurials such as methylmercury and phenylmercury to elemental mercury through the action of two enzymes. An organomercurial lyase cleaves the carbon-mercury

bond, and a mercury reductase converts mercury ions (Hg^{2+}) to elemental mercury (Hg^0) (Begley et al. 1986; Nakamura and Silver 1994; Schiering et al. 1991) as follows

$$CH_3HgCl + H_2O \overset{\text{lyase}}{\rightarrow} CH_4 + Hg^{2+} + OH^- + Cl^-$$

$$Hg^{2+} + NADPH \overset{\text{reductase}}{\rightarrow} Hg^0 + NADP^+ + H^+.$$

Some other bacteria possess only mercury reductase, which can reduce inorganic mercurials, but not organic mercurials (Sahlman et al. 1984).

By exploiting these characteristics, a method to remove mercury from the Hg^{2+}-contaminated sediments and soils by using mercury-volatilizing bacteria has been developed (Barkay et al. 1992; Hansen et al. 1984). However, a method to remove HgS from sediment has not yet been developed, because there are no organisms that can volatilize HgS directly. The objective of the present study is to develop a method to remove HgS from Minamata Bay sediment through sequential treatments of chemical extraction and volatilization of mercury by bacteria.

Materials and methods

Sediment samples and methylmercury-amended soil

Sediment samples were previously collected from Minamata Bay in 1983 with an Ekman dredge sampler. These samples were dried overnight at 37 °C, passed through a 1 mm mesh sieve and used to leach mercury. Methylmercury chloride was added at the concentration of 20 mg/kg to field soil that had been passed through a 1 mm mesh sieve. This methylmercury-amended soil was also used for the mercury removal experiment.

Chemical leaching of mercury

Since most of the mercury in Minamata Bay is in the chemical form of HgS (Sakamoto et al. 1992) and it has been reported that HCl and copper solubilize HgS to Hg^{2+}, we attempted to leach mercury from Minamata Bay sediment using various acids and metals. The acids used in this study were HCl at various concentrations (M) between 0.1 and 6, 98% H_2SO_4, and 90% HNO_3. The metals used were $FeCl_3 \cdot 6H_2O$ at various concentrations (mM) between 37 and 2220, and 200 mM $CuCl_2$. Mercury in 0.5 g of Minamata Bay sediment was leached with 10 ml solution containing the various concentrations of acids and metals described above for 20 h at room temperature. The leached sediment was filtrated with Whatman filter paper No. 2 and dried at 37 °C. The contents of mercury in the sediments were analyzed using a flameless atom absorption spectrometer with a mercury analysis vaporizer (Rigaku SP-3, Nippon Instrument Tokyo, Japan).

Bacterial strains

Seawater samples from Minamata Bay were collected and the mercury-resistant bacteria were isolated using ZoBell agar media (Nakamura et al. 1990) supplemented with 40 mg/L of $HgCl_2$. The isolates were further screened for the ability to volatilize $HgCl_2$ and CH_3HgCl. The bacterium that could volatilize both $HgCl_2$ and CH_3HgCl at the highest level was isolated and used throughout this study.

Volatilization of mercurial compounds by bacteria

The volatilization of mercurial compounds by bacteria was determined by measuring mercury loss in liquid medium after incubation for 24 h at 30 °C. The following mercury compounds were used at the concentrations indicated (mg/kg): mercuric chloride, 1; methylmercuric chloride, 0.1; ethylmercuric chloride, 0.1; thimerosal 0.1; fluorescein mercuric acetate, 1; phenylmercuric acetate, 0.5; and ρ-chloromercuric benzoate, 0.1.

Results and Discussion

Isolation of bacteria capable of volatilizing mercurials

We isolated 204 mercury-resistant bacterial strains from Minamata Bay seawater, which were grown on ZoBell agar media containing 40 mg/kg of $HgCl_2$. Of these strains, 57 (27.9%) degraded methylmercury to elemental mercury (Hg^0). The ability to degrade methylmercury was highly strain dependent. The decrease in mercury ranged from 41.3 to 95.4%. One such bacterial strain (M-1) volatilized a variety of mercurials at the highest levels. The decreases in mercury from various mercury compounds by strain M-1 after incubation at 30 °C for 24 h were as follows: mercuric chloride (88.9%), methylmercuric chloride (95.4%), ethylmercuric chloride (83.8%), thimerosal (91.9%),

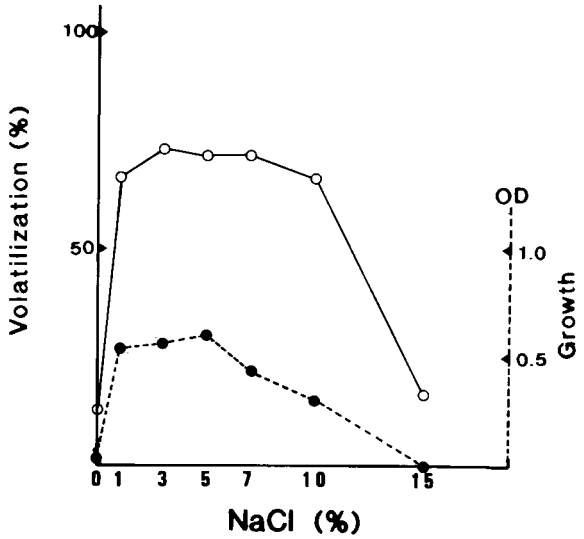

Figure 1. Effect of NaCl concentration on the bacterial volatilization of $HgCl_2$. The bacterial growth and volatilization were determined in liquid culture containing 1 mg/kg of $HgCl_2$ after shaking at 30 °C for 24 h. The bacterial growth was determined by measuring the optical density (OD) at 540 nm. ○, volatilization; ●, growth.

flluorescein mercuric acetate (74.6%), phenylmercuric acetate (5.7%), and ρ-chloromercuric benzoate (92.3%). We selected strain M-1 for the removal of mercury from the sediment of Minamata Bay following chemical leaching as described below. Strain M-1 was a Gram-negative rod and grew very well at both 10 and 37 °C. M-1 was identified to be *Pseudoalteromonas haloplanktis* by The National Collection of Industrial and Marine Bacteria Limited (NCIMB). The detailed characteristics of M-1 will be reported elsewhere.

Effect of NaCl on the volatilization of $HgCl_2$ by M-1

Since strain M-1 possessed a high capacity for volatilization of various mercurial compounds, the effects of NaCl on mercury volatilization were examined (Figure 1). M-1 failed to grow without NaCl, but good growth was observed at NaCl concentrations ranging 1 to 10%. The highest volatilization of $HgCl_2$ was observed with 3% NaCl, which is the concentration equivalent to that of natural seawater.

Table 1. Effect of HCl concentrations on extraction of mercury from sediments

HCl conc Added (M)	Mercury (mg/kg) in the sediments	Extraction %
None	23.94 ± 0.84[a]	0
0.1	24.00 ± 1.20	0
0.5	10.27 ± 0.80	57.1
1	8.78 ± 0.58	63.3
2	2.62 ± 0.19	89.1
3	1.61 ± 0.21	93.3
6	1.30 ± 0.02	94.6

[a]Mean ± SD, N = 5.
Mercury in 0.5 g of Minamata Bay sediment was leached with 10 ml of HCl at the concentrations indicated and 74 mM $FeCl_3 \cdot H_2O$. The mercury was determined by using atomic absorption spectrometer.

Chemical leaching of mercury from sediment and subsequent volatilization of mercury leached by strain M-1

It was previously reported that copper could leach HgS (Sakamoto et al. 1992) to Hg^{2+}. However we found that copper is toxic to strain M-1, so we used $FeCl_3$ instead of $CuCl_2$. It appears that when Fe^{3+} is reduced to Fe^{2+}, HgS may be changed to Hg^{2+} in acidic conditions. Although HCl, H_2SO_4, HNO_3 or $FeCl_3 \cdot 6H_2O$ alone were not effective in the extraction of mercury from the sediment (data not shown), we could leach mercury very effectively by using both HCl and $FeCl_3$ (Table 1). The amounts of mercury extracted from sediments increased with the increased concentrations of HCl.

The sediment samples (mercury contents 0.11–37.4 mg/kg) were leached in 60 ml impinger with 10 ml of 3 M HCl and 74 mM $FeCl_3$ for 20 h at room temperature. As a result, more than 85% of the mercury in the sediments was efficiently leached. The solution containing Hg^{2+} was neutralized with 3 M NaOH. Forty ml of 0.2 M phosphate buffer (pH 7.0) containing 5 mM thioglycolate were added to the neutralized solution. The resting cells of strain M-1 suspended in 0.2 M phosphate buffer (pH 7.0) were also added to the neutralized solution (Figure 2). The impinger was set in the device as shown in Figure 3. The mercury thus volatilized was completely trapped by 1.5 M H_2SO_4-0.2% $KMnO_4$ solution. Thioglycolate was essential for the volatilization of mercury by M-1. The effect of thioglycolate concentration was examined under the same conditions. The highest volatilization was ob-

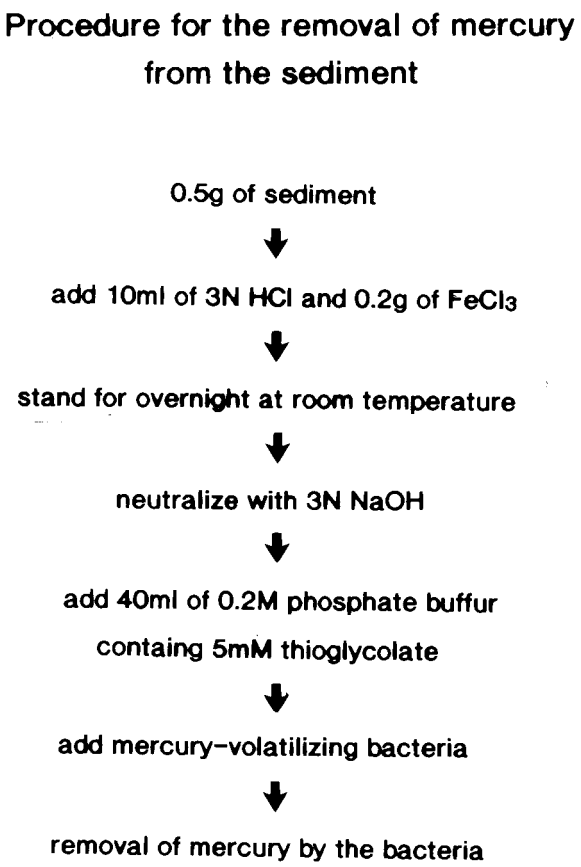

Figure 2. Procedure for the removal of mercury from the sediment.

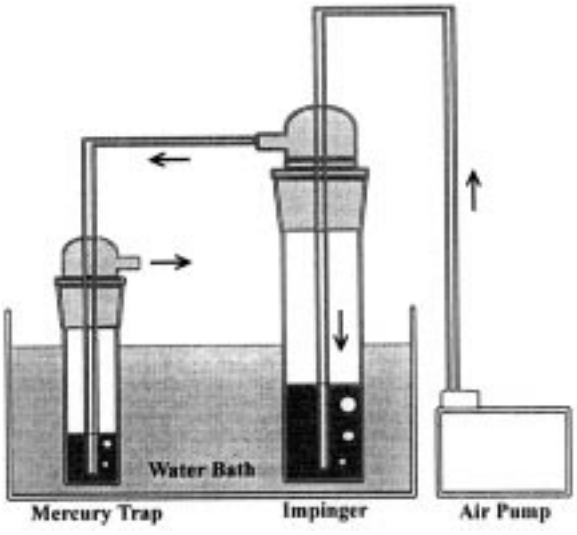

Figure 3. Device for the removal of mercury from sediment by bacteria. Mercury-resistant M-1 strain in impinger transforms mercury into mercury vapor. The volatilized mercury is trapped in 1.5 M H_2SO_4-0.2% $KMnO_4$ solution. The aeration rate is 1.2 L/min.

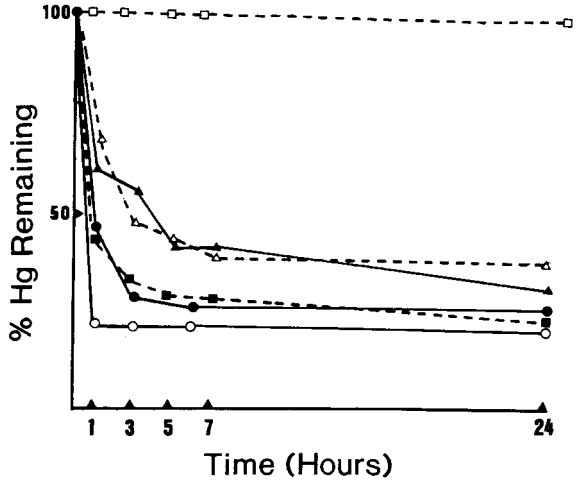

Figure 4. Removal of mercury from the sediment of Minamata Bay and the methylmercury-ammended soil by mercury-volatilizing bacteria. □, control (no bacteria with Minamata Bay sediment containing 31.5 mg/kg of mercury); ▲, Minamata Bay sediment (mercury content: 31.5 mg/kg); ●, (22.5 mg/kg); ■, (10.9 mg/kg); △, (10.9 mg/kg); ○, methylmercury-amended soil (22.9 mg/kg).

served at 5 mM thioglycolate. No volatilization was observed without thioglycolate or at the concentration of 50 mM (data not shown). Figure 4 indicates that 62.9–75.1% of mercury was removed from Minamata Bay sediments by this method. We also examined whether methylmercury could be removed by the same method. The mercury extracted from methylmercury-amended soil was treated by strain M-1 for 24 h, after which 78% of methylmercury was removed from the soil. When the HCl solution was neutralized with NaOH, large amounts of NaCl were formed in the solution. High concentrations of NaCl usually inhibit the volatilization of mercury by bacteria (Selifonova and Barkay 1994). M-1 grew very well at high concentrations of NaCl ranging from 1–10%, and was most successfully used for the removal of leached mercury ions in the presence of 3% NaCl. Although the major mercury compounds in Minamata Bay are present as HgS (Sakamoto et al. 1995), little is known as to whether the HgS in the sediments is stable or transformable to other chemical forms. However, it has been postulated that small amounts of methylmercury are also formed from HgS in aerobic sediments (Fagerstrom and Jernelov 1971). It is evident that the mercury contents of fish and shellfish in Minamata Bay are still a little higher than those in non-polluted areas near Minamata. It is conceivable that the immobilization of HgS occurs in Minamata Bay. In fact, it is reported that mercury-resistant *Thiobacillus ferroxidans* solubilized

mercury from cinnabar (HgS) and produced mercury vapor (Baldi and Olson 1987; Silver and Torma 1974). Since *Thiobacillus* species oxidize Fe^{2+} into Fe^{3+} under acidic conditions, it is possible that Fe^{3+} and acids produce solubilize mercury from cinnabar. One may then predict that methylation can take place through other microorganisms, as reported previously (Olson and Cooper 1976).

This is the first report describing a method that allows removal of HgS from natural sediments. However, despite repeated efforts, the removal of mercury from sediment by this method was limited to 60–75%. Tight adsorption of mercury by certain soil components such as clay may prevent the extraction and subsequent microbial attack. Alternatively, some mercurials in the sediments may be present as other, more complex forms than HgS.

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References

Baldi F & Olson GJ (1987) Effects of cinnabar on pyrite oxidation by *Thiobacillus ferroxidans* and cinnabar by a mercury-resistant strain. Appl. Environ. Microbiol. 53: 772–776

Barkay, T, Turner R, Saouter E & Horn J (1992) Mercury biotransformations and their potential for remediation of mercury contamination. Biodegradation 3: 147–159

Begley TP, Walts AE & Walsh CT (1986) Bacterial organomercuric lyase: overproduction, isolation, and characterization. Biochemistry 25: 7186–7193

GEM & Ruedy J (1986) Methylmercury exposure in northern Quebec I. Neurologic findings in adults. Am. J. Epidemiol. 118: 461–469

Fagerstrom T & Jernelov A (1971) Formation of methylmercury from pure mercuric sulfide in aerobic organic sediment. Water Res. 5: 121–122

Gravis J & Furguson JF (1972) The cycling of mercury through the environment. Water Res. 6: 989–1008

Hansen CL, Zwolinski G, Martin D & Williams JW (1984) Bacterial removal of mercury from sewage. Biotechnol. Bioengineering 26: 1330–1333

Irukayama K (1977) Case history of Minamata disease. In: Tubaki T & Irukayama K (Eds), Minamata disease (pp 1–59). Elsevier Science Publishing Inc., New York

Kumagai M & Nishimura H (1978) Mercury distribution in seawater in Minamata Bay and the particulate mercury. J. Oceanogr. Jpn. 34: 1013–1018

Kumamoto Prefectural Government (1973) White paper on environmental pollution, pp. 145–152 (Japanese)

Malm O, Pfeiffer WC, Souza CMM & Reuther R (1990) Mercury pollution due to gold-mining in the Medeira river baisin, Brazil. Ambio 19: 11–15

Nakamura K, Sakamoto M, Uchiyama H & Yagi O (1990) Organomercurials-volatilizing bacteria in the mercury-polluted sediment of Minamata Bay, Japan. Appl. Environ. Microbiol. 56: 304–305

Nakamura K, Sakata T, and Nakahara H (1988) Volatilization of mercury compounds by methylmercury-volatilizing bacteria in Minamata Bay sediment. Bull. Environ. Contam. Toxicol. 41: 651–656

Nakamura K & Silver S (1994) Molecular analysis of mercury-resistant *Bacillus* isolates from sediment of Minamata Bay, Japan. Appl. Environ. Microbiol. 60: 4596–4599

Nriagu JO, Pfeiffer WC, Malm O, Souza CMM & Mierle G (1992) Mercury pollution in Brazil. Nature 356: 389

Olson BH & Cooper RC (1976) Comparison of aerobic and anaerobic methylation of mercury chloride by San Fransisco Bay sediment. Water Res. 10: 113–116

Sahlman L, Lambeir AM, Lindskog S & Dunford HB (1984) The reaction between NADPH and mercuric reductase from *Pseudomonas aeruginosa*. J. Biol Chem. 259: 12403–12408

Sakamoto H, Tomiyasu T & Yonehara N (1992) Differential determination of organic mercury, mercury (II) oxide and mercury (II) sulfide, in sediment by cold vapor atomic absorption spectrometry. Anal. Sci. 8: 35–3

Sakamoto H, Tomiyasu T & Yonekura N (1995) The contents and chemical forms of mercury in sediments from Kagoshima Bay, in comparison with Minamata Bay and Yatsushiro Sea, Southwestern Japan. Geochem. J. 29: 97–105

Schiering N, Kabsch W, Moore MJ, Distefano MD, Walsh CT & Pfeiffer (1991) Structure of the detoxification catalyst mercuric ion reductase from *Bacillus sp* strain RC 607. Nature 353: 168–171

Selifonova OV & Barkay T (1994) Role of Na^{+} in transport of Hg^{2+} and induction of the Tn 21 *mer* operon. Appl. Environ. Microbiol. 60: 3503–3507

Silver M & Torma AE (1974) Oxidation of metal sulfides by *Thiobacillus ferooxidans* grown on different substrates. Can. J. Microbiol. 20: 141–147

Wheatly B, Barbeau A, Clarkson TW & Iapham L (1979) Methylmercury poisoning in Canada Indians-the elusive diagnosis. Can. J. Neurol. Sci. 6: 417–422