Journal of Industrial Microbiology (1995) 14, 343–348

An evaluation of *mer*-specified reduction of ionic mercury as a remedial tool of a mercury-contaminated freshwater pond

Erwan Saouter[1,3], Mark Gillman[1] and Tamar Barkay[2]

[1]*Center for Environmental Diagnostics and Bioremediation, University of West Florida, and* [2]*US EPA Environmental Research Laboratory, 1 Sabine Island Drive, Gulf Breeze, FL 32561, USA*

(Received 17 May 1994; accepted 7 January 1995)

Key words: Mercury contamination; Mercuric reductase; Microcosms; Remediation

SUMMARY

The potential for *mer*-mediated reduction/volatilization of ionic mercury as a tool in the decontamination of a freshwater pond was evaluated using laboratory incubations and a microcosm simulation. In flask assays inoculations with ionic mercury-resistant bacteria (10^5–10^7 cells ml^{-1}) isolated from the pond, significantly increased the rate of mercury loss (MANOVA, $P \leq 0.05$) relative to uninoculated controls. The effects of cell density, mercuric mercury concentration, addition of nutrients and supplementation with the sulfhydryl reagent β-mercaptoethanol on the rate of mercury loss, were investigated. Inoculation (by 10^5 cells ml^{-1}) of a flow-through microcosm that simulated the cycling of mercury in the contaminated pond, stimulated by more than 4-fold the formation of volatile elemental mercury. Thus, biological formation of volatile mercury may hold a promise as a remedial tool of contaminated natural waters.

INTRODUCTION

Regulatory action has efficiently decreased the release of heavy metals and metalloids to the environment in recent decades. However, numerous sites that were contaminated prior to the institution of preventive measures exist. In some instances, entire ecosystems, such as groundwater aquifers or stream floodplains, contain toxic metals at hazardous concentrations. In situ cleanup of such systems is a costly endeavor. Bioremediation may offer a less expensive solution [9]. Whereas some success has been reported for site decontamination of organic pollutants [8,15], similar approaches for metal removal are in their infancy [16].

Because metals and metalloids, unlike organic compounds, cannot be transformed to harmless elements (carbon dioxide and water), their remediation consists of separation and volume reduction rather than elimination. Biological treatments for the removal of metals may be achieved by passive adsorption to an organic matrix rich in binding sites [5,12] or by biotransformations resulting in chemical forms that can be readily separated and concentrated away from the cleaned matrix [11,14,19,25].

Mercury is one of the most toxic metals whose hazard is compounded by biomagnification and accumulation of methylmercury (MeHg) in the food chain [18]. Although the toxicity of mercury was recognized three decades ago and measures were taken to limit its discharge in the environment, a multitude of mercury-contaminated ecosystems exist. Decontamination of these sites is difficult and costly. We previously proposed that the stimulation of bacterial activities to reduce inorganic mercury (Hg[II]) to volatile elemental mercury (Hg^0) and degrade MeHg to Hg^0 and methane could be used to decrease MeHg concentrations available for bioaccumulation in contaminated waters [2]. These activities are specified by inducible enzymes that detoxify ionic mercury (mercuric reductase) and degrade organomercury (organomercurial lyase) and are encoded by the mercury resistance (*mer*) operon [24]. We have been testing this approach with samples collected in a contaminated freshwater pond, Reality Lake, located at the head stream of East Fork Poplar Creek in Oak Ridge, TN, USA [2].

MATERIALS AND METHODS

Isolation of mercury-resistant bacteria

A water sample collected in Reality Lake in the summer of 1991 was spread plated on Plate Count Agar (PCA; Difco Laboratories, Detroit, MI, USA) containing 10 μg ml^{-1} Hg(II) (as $HgCl_2$). Following 4-day incubation at 30 °C, single colonies were isolated and purified. Cultures were stored at −70 °C in 50% glycerol. For the work reported here, 19 isolates were resuscitated by plating thawed frozen stocks on PCA. Their resistance to Hg(II) was quantified using disk diffusion assays and dose response curves as described by Barkay and Colwell [1] and Weiss et al. [28], respectively. The response of strains isolated from Reality Lake was compared to that of reference strains. Reference strains were derivatives of *Pseudomonas*

Correspondence to: T. Barkay, US EPA, 1 Sabine Island Drive, Gulf Breeze, FL 32561, USA.

[3] Present address: Procter & Gamble, European Technical Center, Temselaan 55-100, B-1853 Strombeek-bever, Belgium.

Publication no. 906 of the US Environmental Research Laboratory, Gulf Breeze, FL 32561, USA.

Mention of trade names or commercial products does not constitute endorsement on recommendation for use.

aeruginosa PAO1 containing plasmids carrying *mer* operons. These included a strain resistant to Hg(II), a strain resistant to Hg(II) and MeHg, and a plasmid-less sensitive strain. Reality Lake isolates with a resistance level to Hg(II) similar to that of the PAO1 reference strain were selected for further testing. None of the 19 isolates was resistant to MeHg.

Some of the strains isolated from Reality Lake were characterized using API Rapid NFT strips (Analytab Products, Plainview, NY, USA) and further identified by gas chromatography-fatty acid methyl ester- and Biolog™-analyses (performed by Microbe Inotech Laboratories, St Louis, MO, USA).

Mercury analysis

Cold vapor atomic fluorescence (CVAF) was used to detect mercury in water samples as described by Saouter and Blattman [21]. The forms of mercury quantified were: total mercury (Hg_T): forms detected by CVAF after digestion of water samples with bromine monochloride and reduction with stannous chloride; dissolved mercury (Hg_{DIS}): forms detected by CVAF after filtration through 0.45-μm pore size nylon filters (Cole-Parmer® Instrument Co., Niels, IL, USA) and digested as above; total gaseous mercury (Hg_{TGM}): mercury purged from untreated water samples by bubbling with nitrogen gas; mercury in the headspace (Hg_{HSP}): volatile mercury in the headspace of microcosms (see below). In addition to the forms listed above, the term Hg(II) describes all species with the +2 valence. The term mercury is used when the form of the mercury is unknown.

Mercury reduction/volatilization flask assays

Resting cell assays for the determination of inducible mercuric reductase activities were performed as described by Weiss et al. [28]. Reality Lake water was collected from the inlet of the pond in polypropylene containers and shipped by overnight mail to the EPA Environmental Research Laboratory in Gulf Breeze where water was stored at 4 °C. This water was used for up to two weeks. The experimental system in these assays consisted of 50 ml water in 250-ml Erlenmeyer flasks stoppered with cotton plugs. Assays were performed in triplicate. Reality Lake water contains mercury in the $\mu g\ L^{-1}$ concentration range [2]. However, several hours after sampling, most of this mercury is not available for biological transformations due to adsorption to particulates and to the walls of storage containers [see 22 for a detailed discussion of this issue]. Therefore, the water was supplemented with a fresh dose of 1–2 μg Hg(II) L^{-1} (as $HgCl_2$). Flasks were incubated statically for up to 24 h at room temperature (approximately 22 °C), and samples for analysis of mercury (as Hg_T) remaining in the water were withdrawn periodically. Preliminary experiments indicated no significant difference (*t*-test, $P = 0.133$) between loss in flasks bubbled with air or incubated statically. Shaking of flasks during incubation resulted in a large loss of mercury by adsorption to the glass walls.

Cultures for inoculation of Reality Lake water were prepared by overnight growth in Luria-Bertani broth (10 g trypton [Difco]; 5 g yeast extract [Difco]; 10 g NaCl; pH 7.2–7.4). In some experiments, activity was induced by growth in 50 μM $HgCl_2$. Cells were collected by centrifugation and rinsed once with Reality Lake water prior to addition to flasks for the assays. Cell counts were obtained at inoculation and termination of the experiments by plating diluted samples on PCA supplemented with 50 μM $HgCl_2$. Colonies were counted after 2 days incubation at 30 °C. In most cases, the number of inoculated cells increased by up to one order of magnitude in 24 h of incubation. Enumeration of indigenous bacteria in Reality Lake water showed population densities of 10^3 CFU ml^{-1} for culturable counts [this study and Ref. 17] and 10^6 ml^{-1} for direct counts [13]. Yeast extract (0.01% [100 mg L^{-1}]; Difco) and β-mercaptoethanol (1 mM; Sigma Chemical Co., St Louis, MO, USA) were added to Reality Lake water when stimulation of mercury loss by nutrients and sulfhydryl reagents was examined. Rates of mercury loss were calculated from the slopes of regression curves describing loss of mercury over time (Loss = constant + a*time).

Microcosm experiments

The flow-through microcosm and its operation were described previously [22]. Four replicate microcosms were collected in March 1993 in Reality Lake, brought to the laboratory in Gulf Breeze within 12 h of sampling, and were operated for 13 days prior to inoculation of two microcosms. The turnover rate of water in microcosms was 8.5 h (similar to that of the pond); fresh Hg(II) (as $HgCl_2$) was added (1 $\mu g\ L^{-1}$) with input water. Microcosms were maintained at room temperature. Samples for Hg_T, Hg_{DIS} and Hg_{HSP} were collected periodically for analyses. Microcosm sampling and analyses were performed as described previously [22]. On day 13 and again on day 14, an isolate from Reality Lake, *Aeromonas hydrophila* KT20, was added to the water column of the microcosms through a sampling port in the lid of the microcosm to a final concentration of 10^5 cells ml^{-1}. The inoculum was prepared as described above for flask experiments. The flux of Hg^0 from microcosms was calculated as described by Saouter et al. [22].

Statistical analyses

The effects of different factors (time, inoculation, cell density, nutrient and β-mercaptoethanol additions) on mercury loss were estimated using analysis of variance (Multivariate General Linear Model; [26]).

RESULTS

Flask assays

The loss of mercury from Reality Lake water and the effect of inoculation with strains isolated from the pond were initially tested in simple laboratory incubations. Several Hg(II)-resistant bacteria isolated from Reality Lake were screened for the ability to remove mercury from Reality Lake water (Table 1). The inoculum size in these initial surveys was between 1.0×10^5 and 2.6×10^6 cells ml^{-1}. Some inoculated water samples as well as controls containing indigenous organisms demonstrated significant loss of mercury with time ($P \leq 0.05$) and inoculation with four of the seven test strains had a significant effect on the rates of mercury loss ($P \leq 0.05$).

TABLE 1

The effect of inoculation with mercury-resistant bacteria on loss of mercury from Reality Lake water

Inoculated strain	Rate of mercury loss[1] ($ng\ h^{-1}\ L^{-1}$)		
	A	B	C
RL sterile[2]	96[a]	93[a]	63[a]
RL water[3]		136[a,b]	58[a]
KT09	64[a]		
KT15	52[a]		
KT17	127[a]		123[a,b]
KT23	123[a]		156[a]
KT24	116[a]		
KT25	116		
KT20		159[a,b]	

[1] Rates are the slopes of curves describing mercury loss over time (loss = constant + a* time). A, B, and C are three independent flask experiments. Each model was estimated versus the control RL sterile with the following equation: loss = constant + a* time + c* strain (strain had only two modalities: control and every tested strain). Superscripts indicate: [a] – significant loss with time ($P \leq 0.05$); [b] – significant difference between inoculated and RL water ($P \leq 0.05$).
[2] RL – Reality Lake; sterile controls consisted of autoclaved and filtered samples. Because the two samples lost mercury at rates that did not differ significantly, the two were combined.
[3] RL water – water containing indigenous flora. A blank cell indicates that data are not available.

Strains KT17, KT23 and KT20 were the most active among the strains tested (Table 1) and therefore were employed in subsequent assays. These strains were tentatively identified as *Pseudomonas* sp. KT17, *Pseudomonas alcaligenes* KT23 and *Aeromonas hydrophila* KT20. Standard resting cell assays [28] of mercuric reductase showed inducible activities with similar initial rates for KT17 and KT23 (6.8×10^{-4} ng $cell^{-1}\ h^{-1}$) and a somewhat faster rate for KT20 (1×10^{-3} ng $cell^{-1}\ h^{-1}$). For comparison, a reference *P. aeruginosa* strain containing the *mer* operon of transposon 501 reduced Hg(II) at an initial rate of 2.4×10^{-4} ng $cell^{-1}\ h^{-1}$.

Treatments to stimulate the rate of mercury loss by mercury-reducing bacteria were carried out in shake flasks. The results are summarized in Table 2. Increasing Hg(II) concentration from 2 $\mu g\ L^{-1}$ (the in situ concentration in Reality Lake) to 2000 $\mu g\ L^{-1}$ resulted in a 2–3 orders of magnitude increase in the rate of mercury removal by cell suspensions of the three test strains (at 10^7 cells ml^{-1}). Activity leveled off between 200 and 2000 $\mu g\ L^{-1}$ Hg(II) for strains KT17 and KT20 and continued to increase linearly up to 2000 $\mu g\ L^{-1}$ for strain KT23. Thus, the mercury concentration at which reduction of Hg(II) reaches saturation is strain-specific.

Increasing the inoculum size from 10^5 to 10^6 cells ml^{-1} increased the rate of mercury loss from 123 to 1265 $ng\ h^{-1}\ L^{-1}$ for KT17 and from 156 to 368 $ng\ h^{-1}\ L^{-1}$ for KT23 (differences significant at $P \leq 0.05$). Some of this stimulation might have been due to a higher Hg(II) concentration in flasks inoculated with 10^6 cells ml^{-1}. The inoculum contained trace amounts of Hg(II) as a result of induction prior to harvesting.

The addition of nutrients (0.01% yeast extract) was expected to stimulate mercury loss as a result of increased bacterial metabolic activity, but was effective only in the presence of a high Hg(II) concentration (200 $\mu g\ L^{-1}$) (Table 2). Strain KT23 removed mercury at a rate of 100 $ng\ h^{-1}\ L^{-1}$ in unamended Reality Lake water and this rate was increased to 208 $ng\ h^{-1}\ L^{-1}$ by the addition of nutrients. Yeast extract had no effect when 2 $ng\ L^{-1}$ Hg(II) was employed.

In several water samples, no loss of mercury occurred even when a high number of active bacteria were added. It was postulated that bioavailability limited the access of mercury to intracellular mercuric reductases. The substrate for mercuric reductase is a thiolated Hg(II) (SR-Hg-SR; [27]), and the presence of sulfhydryl reagents is an absolute requirement in reductase assays [28]. With KT23 the presence of 1 mM β-mercaptoethanol increased rate of mercury loss from 1105 to 2935 $ng\ h^{-1}\ L^{-1}$, yet no effect was noted with strains KT17 and KT20. Thus, with some strains treatments that improve bioavailability of the substrate to the intracellular reductase can accelerate removal of mercury from contaminated waters.

The variable responses to the treatments indicate that the rate of mercury reduction/volatilization in Reality Lake water is determined by complex interactions among various factors. The data suggest that at the concentration of mercury in the pond (at least a hundred-fold below the concentration at V_{max}), metabolism of the active bacteria does not limit the rate of mercury loss. Rather, removal of mercury may be accelerated by increasing the density of the active biomass and/or the availability of mercury to bacteria which carry out the reduction.

Microcosm

A microcosm that simulated the cycling of mercury in Reality Lake [22] was inoculated with *A. hydrophila* KT20 to determine if the organism would affect production of gaseous mercury. A detailed description of this experiment is provided elsewhere [22]. Prior to inoculation, the cycling of mercury through the microcosm simulated trends observed with field measurements in Reality Lake [22].

On day 13 *A. hydrophila* KT20 was added to two of the four replicate microcosms. Inoculation resulted in a gradual increase in the flux of Hg^0 (Fig. 1). Activity reached a peak 18 h after inoculation, with a flux of approximately 23 000 $ng\ cm^{-2}\ d^{-1}$; more than 4-fold faster than uninoculated control microcosms, which remained at the preinoculation level (5000 $ng\ cm^{-2}\ d^{-1}$). Activity then declined due to dilution of the added bacterium in the flow-through microcosms. A repeated inoculation on day 14 resulted in another increase with a similar magnitude in the flux of Hg^0 and a subsequent decline. The microcosm experiment was terminated on day 16. Interestingly, the increase in Hg^0 flux was gradual although the active bacterium was inoculated by a batch addition of cells which had been induced by prior growth in the presence of Hg(II). This gradual increase was likely due to the phenomenon of super-saturation of Hg^0 in the micro-

TABLE 2

Factors affecting the rate of mercury loss following inoculation of Reality Lake water with mercury-reducing bacteria

Factor	Observed effect	Possible mechanism
Mercury concentration	Increased rate with an increase in Hg(II) concentration	Increased substrate concentration
Inoculum size	Higher rates when inoculum size was increased from 10^6 to 10^7	Stimulation due to increase in catalytic activity
Nutrient availability	Yeast extract stimulated rate only at a high Hg(II) concentration (200 $\mu g\ L^{-1}$)	Stimulation of bacterial metabolism and consequently mercury reduction
Reducing agents	Increased rate with some strains, no effect with others	Increased bioavailability of Hg(II) to reducing bacteria

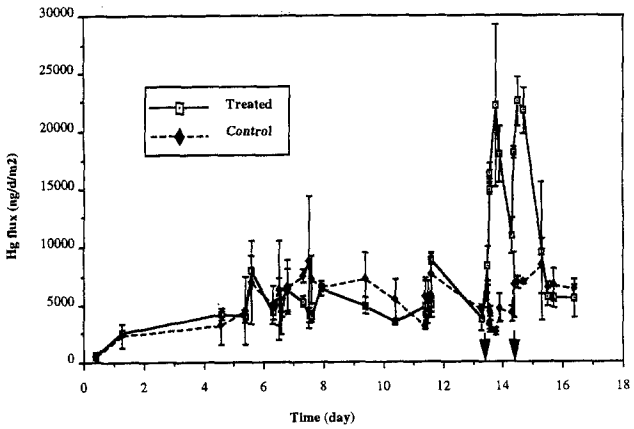

Fig. 1. The effect of inoculation with *Aeromonas hydrophila* KT20 on the flux of Hg^0 from microcosms simulating mercury cycling in Reality Lake. Arrows indicate addition of 10^5 cells ml^{-1} (final concentration in the microcosm). Bars represent the standard deviation of the means of duplicate microcosms.

cosm [22]. Indeed, Hg_{TGM} (volatile mercury in the water) concentration increased by more than 3-fold less than 3 h after inoculation (see below). Super-saturation means that the flux of Hg^0 was controlled by the rate of transfer through the water–air boundary rather than the rate of Hg^0 production in the water column.

Following inoculation, Hg_T and Hg_{DIS} concentrations in treated and control microcosms were similar (Hg_T — 1.17 ± 0.08 for treated and 1.25 ± 0.16 for control; Hg_{DIS} — 0.38 ± 0.09 for treated and 0.46 ± 0.08 for control). However, the difference in Hg_{TGM} following inoculation was dramatic with 93.8 ± 8.0 pg ml^{-1} in treated compared to 28.9 ± 9.3 for control microcosms, 2 h and 45 min after the first addition of *A. hydrophila* KT20. This difference in Hg_{TGM} was maintained throughout the inoculation period but levels declined (to 43.4 ± 0.8 for treated vs 46.0 ± 29.4 for control microcosms) as soon as the cells were washed out after the second inoculation. Thus, inoculation with a Hg(II)-reducing bacterium successfully increased the rate of gaseous mercury formation in a microcosm simulating Reality Lake.

DISCUSSION

The research described here was undertaken to test if the bacterial reduction of mercury could be exploited for remediation of a mercury-contaminated fresh water pond. The original experimental design [2] called for a gradual progression from simple to more complex test systems, beginning with flask incubations, through microcosms to field enclosures. Results presented in this manuscript show that, under certain conditions, inoculation with active bacteria removed mercury from Reality Lake water in flask experiments and increased the production of gaseous mercury in a microcosm simulating a contaminated freshwater pond.

Resting cell reductase assays showed that under optimal conditions, three Reality Lake isolates removed mercury at a rate of 10^{-3} ng Hg(II) $cell^{-1}\ h^{-1}$. Assuming that the average concentration of mercury in the inlet to Reality Lake is 2 $\mu g\ L^{-1}$ [2] and that the majority of this mercury is bioavailable to *mer* systems [23], an addition of 10^3 cells ml^{-1} should, in theory, remove a large proportion of the incoming mercury. Not surprisingly, the rate of mercury removal was lower than expected. Available data suggest that the rate of mercury reduction/volatilization in Reality Lake is limited by the availability of the substrate to microorganisms. Availability may be controlled by adsorption to particulate matter [22; Turner and Barkay, unpublished]. Increasing availability is one way by which the rate of Hg^0 flux from Reality Lake water could be improved. In this regard, the effect of β-mercaptoethanol on activity of strain KT23 in flask assays (Table 2) is encouraging.

The success of inoculation of microcosms with *A. hydrophila* KT20 was evaluated by the flux of Hg^0 through the water–air boundary and the production of Hg_{TGM} in the water column. Elemental mercury is the product of Hg(II) reduction. Because Hg(II) is the substrate for methylation, its removal as Hg^0 may reduce the methylation rate by substrate limitation [4] and the results of Xun et al. [29] suggest that MeHg production may be reduced to a proportionally greater extent than the decline in Hg(II) concentration. Although inoculation increased Hg^0 flux and Hg_{TGM} concentration by more than 4- and 3-fold, respectively, the evolved Hg^0 accounted for less

than 5% of the input mercury to the microcosm. Whether or not this small fraction had an effect on MeHg concentration in the microcosm is unknown. However, as suggested by flask experiments, removal of mercury as Hg^0 could be accelerated by increasing the number of cells, and enhancing bioavailability of mercury to reducing bacteria. Additional experimentation is obviously needed to test if treatments that improve the rate of mercury removal as Hg^0 are effective in reducing MeHg concentration in the microcosm and by inference, in Reality Lake.

We believe that *mer*-mediated reduction/volatilization as a tool in remediation of mercury is a serious proposition. However, attempting to remediate a contaminated pond is a rather ambitious goal. Cleaning up mercury from natural bodies of water is a difficult undertaking and not much success has been reported to date. On-site treatments, other than dredging and removing contaminated sediments, include liming to raise pH (which slows down MeHg formation) and addition of selenium which reduces accumulation of mercury in fish [6]. To the best of our knowledge, only one other attempt has been made to bioremediate a mercury-contaminated ecosystem. Rudd and Turner [20] tried to limit the rate of fish mercury accumulation in the English-Wabigoon River system by stimulating primary productivity. They reasoned that increased primary productivity would result in a faster growth rate of fish and consequently a dilution of mercury body burden. However, although primary productivity was increased by as much as 19-fold in field enclosures amended with inorganic nutrients, no significant reduction in tissue mercury concentration was observed.

Perhaps the most suitable use for *mer*-mediated reactions is in pollution prevention rather than in attempts to remove mercury from contaminated ecosystems. Hansen et al. [7] used reducing organisms in a sewage bioreactor to remove 85% of the input mercury. This goal could be achieved using genetically engineered microbes whose release to uncontained environments is currently strictly regulated. Brunke et al. [3] used microbial inoculants, natural and genetically engineered [10], to effectively remove Hg(II) from waste streams. The engineered strains produced higher levels of mercuric reductase than bacteria carrying a native *mer* operon. The containment of a process generating volatile mercury would have the added advantage of including a recovery step and possible recycling of the collected mercury.

ACKNOWLEDGEMENTS

We thank R. Turner (Oak Ridge National Laboratory) for advice, collection and shipment of water samples and assistance during field sampling and J. Moore for help with statistic analysis and his critical review of the manuscript. This work was sponsored by a Technology Transfer Agreement between the US EPA and the Electric Power Research Institute (RP3015-03) and by an agreement (RP3015-04) between the University of West Florida and the Electric Power Research Institute.

REFERENCES

1 Barkay, T. and R.R. Colwell. 1983. Altered mercury transport in a mutant of *Pseudomonas fluorescens* B69. J. Gen. Microbiol. 129: 2945–2950.
2 Barkay, T., R. Turner, E. Saouter and J. Horn. 1992. Mercury biotransformations and their potential for remediation of mercury contamination. Biodegradation 3: 147–159.
3 Brunke, M., W.-D. Deckwer, A. Frischmuth, J.M. Horn, H. Lünsdorf, M. Rhode, M. Röhricht, K.N. Timmis and P. Weppen. 1993. Microbial retention of mercury from waste streams in a laboratory column containing *merA* gene bacteria. FEMS Microbiol Rev. 11: 145–152.
4 Fitzgerald, W.F., R.P. Mason and G.M. Vandal. 1991. Atmospheric cycling and air-water exchange of mercury over mid-continental lacustrine regions. Wat. Air Soil Pollut. 56: 745–767.
5 Gardea-Torresdey, J.L., M.K. Becker-Hapak, J.M. Hosea and D.W. Darnall. 1990. Effect of chemical modification of algal carboxyl groups on metal ion binding. Environ. Sci. Technol. 24: 1372–1378.
6 Håkanson, L., P. Andersson, T. Andersson, Å. Nengtsson, P. Grahn, J-Å. Johnsson, C-F. Jönsson, H. Kvarnäs, G. Lindgren and Å. Nilsson. 1990. Measures to reduce mercury in lake fish. Final Report from the Liming-Mercury-Cesium Project. Uppsala University, Sweden.
7 Hansen, C.L., G. Zwolinski, D. Martin and J.W. Williams. 1984. Bacterial removal of mercury from sewage. Biotechnol. Bioeng. 26: 1330–1333.
8 Harkness, M.R., J.B. McDermott, D.A. Abramowicz, J.J. Salvo, W.P. Flanagan, M.L. Stephens, F.J. Mondello, R.J. May, J.H. Lobos, K.M. Carroll, M.J. Brennan, A.A. Bracco, K.M. Fish, G.L. Warner, P.R. Wilson, D.K. Dietrich, D.T. Lin, C.B. Morgan and W.L. Gately. 1993. In situ stimulation of aerobic PCB biodegradation in Hudson river sediments. Science 259: 503–507.
9 Hinchee, R.E., D.B. Anderson, F.B. Metting, Jr and G.D. Sayles. 1994. Applied Biotechnology for Site Remediation. pp. 485. Lewis Publishers, Boca Raton.
10 Horn, J.M., M. Brunke, W.-D. Deckwer and K.N. Timmis. 1994. *Pseudomonas putida* strains which constitutively overexpress mercury resistance for biodetoxification of organomercurial pollutants. Appl. Environ. Microbiol. 60: 357–362.
11 Karlson, U. and W.T. Frankenberger, Jr. 1989. Accelerated rates of selenium volatilization from California soils. Soil Sci. Soc. Am. J. 53: 749–753.
12 Kuyucak, N. and B. Volesky. 1988. Biosorbents for recovery of metals from industrial solutions. Biotechnol. Lett. 10: 137–142.
13 Liebert, C.A., T. Barkay and R.R. Turner. 1991. Acclimation of aquatic microbial communities to Hg(II) and CH_3Hg^+ in polluted freshwater ponds. Microb. Ecol. 21: 139–149.
14 Lovley, D.R. 1993. Dissimilatory metal reduction. Annu. Rev. Microbiol. 47: 263–290.
15 Madsen, E.L., J.L. Sinclair and W.C. Ghiorse. 1991. In situ biodegradation: microbiological patterns in a contaminated aquifer. Science 252: 830–833.
16 Means, J.L. and R.E. Hinchee. 1994. Emerging Technology for Bioremediation of Metals. pp. 148. Lewis Publishers, Boca Raton.
17 Nazaret, S., W.H. Jeffrey, E. Saouter, R. Von Haven and T. Barkay. 1994. *merA* gene expression in aquatic environments measured by mRNA production and Hg(II) volatilization. Appl. Environ. Microbiol. 60: 4059–4065.
18 Nriagu, J.O. 1979. The Biogeochemistry of Mercury in the Environment. pp. 696. Elsevier/North-Holland Biomedical Press, Amsterdam.

19 Oremland, R.S., N.A. Steinberg, A.S. Maest, L.G. Miller and J.T. Hollibaugh. 1994. Measurement of in situ rates of selenate removal by dissimilatory bacterial reduction in sediments. Environ. Sci. Technol. 24: 1157–1164.

20 Rudd, J.W.M. and M.A. Turner. 1983. The English-Wabigoon river system. V. Mercury and selenium bioaccumulation as a function of aquatic primary productivity. Can. J. Fish. Aquat. Sci. 40: 2251–2259.

21 Saouter, E. and B. Blattmann. 1994. Analyses of organic and inorganic mercury by atomic fluorescence spectrometry using a semiautomatic analytical system. Anal. Chem. 66: 2031–2037.

22 Saouter, E., M. Gillman, R. Turner and T. Barkay. 1995. Development and field validation of a microcosm to simulate the mercury cycle in a contaminated pond. Environ. Toxicol. Chem. 14: 69–77.

23 Selifonova, O., R. Burlage and T. Barkay. 1993. Bioluminescent sensors for detection of bioavailable Hg(II) in the environment. Appl. Environ. Microbiol. 59: 3083–3090.

24 Silver, S. and M. Walderhaug. 1992. Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria. Microbiol. Rev. 56: 195–228.

25 Summers, A.O. 1992. The hard stuff—metals in bioremediation. Curr. Opinion Biotechnol. 3: 271–276.

26 Systat: Statistic, Version 5.2 edition. Evanston, IL, 1992.

27 Walsh, C.T., M.D. Distefano, M.J. Moore, L.M. Shewchuk and G.L. Verdine. 1988. Molecular basis of bacterial resistance to organomercurial and inorganic mercuric salts. FASEB J. 2: 124–130.

28 Weiss, A.A., S.D. Murphy and S. Silver. 1977. Mercury and organomercurial resistances determined by plasmids in *Staphylococcus aureus*. J. Bacteriol. 132: 197–208.

29 Xun, L., N.E.R. Campbell and J.W.M. Rudd. 1987. Measurements of specific rates of net methyl mercury production in the water column and surface sediments of acidified and circumneutral lakes. Can. J. Fish. Aquat. Sci. 44: 750–757.