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Pilot plant for bioremediation of mercury-containing industrial wastewater

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Abstract Mercury is an extremely toxic pollutant that is currently being emitted mainly by low level industrial sources. It is distributed globally through the atmosphere, from where it precipitates onto the surface of the Earth, enters aquatic organisms, accumulates in fish and finally affects the health of human populations. Microbes have evolved a mechanism for mercury detoxification [mercury resistance operon (*mer*)] based on intracellular reduction of Hg^{2+} to non-toxic Hg^0 by the mercuric reductase enzyme and subsequent diffusional loss of Hg^0 from the cell. It was shown that Hg^0 produced by microbial detoxification can be retained quantitatively in packed bed bioreactors, in which biofilms of mercury-resistant bacteria are grown on porous carrier material. This review describes operation of this system on a technical, fully automated, scale, and its operation at a chloralkali electrolysis factory. It was shown to work with high efficiency under fluctuating mercury concentrations and to be robust against transiently toxic conditions. The gradient of mercury concentration in the technical scale system exerted a strong selective pressure on the microbial community, which resulted in a succession of mercury-resistant strains at high mercury concentrations and an increase in phylogenetic and functional diversity at low mercury concentrations. Clean-up of mercury-containing wastewater by mercury-resistant microbes is a simple, environmentally friendly and cost-effective alternative to current treatment technologies.

Introduction

Bioremediation of metals is directed at (1) precipitation and thus immobilization of inorganic contaminants, and (2) compartmentalization of metals to parts of the

environment where their potential harm is reduced (Barkay and Schaefer 2001). To this end, a vast diversity of microbe-metal interactions is being exploited, ranging from reduction for anaerobic respiration (Lovely 2000), to reduction for detoxification (Barkay 2000), as well as biosorption, bioleaching, bioaccumulation and biominer- alization (Gadd 2000).

In this context, mercury is unique because of the combination of the extreme toxicity—it is a metal with no known biological function—and low vapor pressure of elemental mercury, which is a liquid at room temperature. In contrast, most other heavy metals are needed by the cell, either as cofactors of enzymes or as electron acceptors for anaerobic respiration. Hence, sophisticated mechanisms exist to maintain steady-state concentrations for each of these metals, consisting of unspecific and specific transporters, efflux pumps and sequestering molecules (Nies 1999). Reduction occurs as a form of energy generation in some metals (e.g., dissimilatory iron reduction) and as a form of detoxification in others; besides mercury, also for chromate, arsenate and copper.

Only in the case of mercury is complete detoxification obtained by reduction of Hg^{2+} to Hg^0 and diffusional loss from the cell of Hg^0 . Volatilization of mercury from bacterial cells was discovered as a resistance mechanism more than 30 years ago (Summers and Silver 1972) and its genetic determinant, the *mer* operon, is an archaetyp- ical genetic system that has been studied in depth since then (for review, see Summers and Silver 1978; Misra 1992; Ji and Silver 1995; Osborn et al. 1997; Nies 1999; Barkay 2000). However, instead of being volatilized, elemental mercury can also be accumulated in a biore- actor under certain conditions because it is a liquid at room temperature and almost insoluble in water. Here, a bioreactor system is described that retains Hg^0 formed by microbial reduction of Hg^{2+} . We show its efficiency under various conditions, the scale-up to a full-scale process, the operation of a pilot plant at a chloralkali electrolysis factory and the microbial ecology of the mercury- reducing biofilm communities.

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Mercury toxicity

Mercury is the most toxic of all elements (Nies 1999). Because of this high toxicity, it has no beneficial biological function. The toxicity of mercury depends strongly on its redox state (Clarkson 1992). The most toxic form of mercury is the highly reactive anorganic mercury ion Hg^{2+} , which binds to the amino acid cysteine in proteins, thereby inactivating e.g., enzymes of the glutathione metabolism, and to N-atoms in nucleic acids. In contrast, the danger of elemental mercury (Hg^0) and organomercury compounds lies in their transport routes. Mercury vapor is easily inhaled, enters the blood stream in the lungs and is thus distributed throughout the body. Within cells, it is oxidised to reactive Hg^{2+} . The toxicity of monomethylmercury (MeHg^+) or dimethylmercury (Me_2Hg) is caused by its ability to penetrate membranes (e.g., human skin) within seconds and also to cross the blood-brain barrier. However, even lethal doses of MeHg^+ only cause symptoms many months after exposure. The reason is the slow demethylation of MeHg^+ to reactive Hg^{2+} by the cellular metabolism (Strasdeit et al. 2000). Symptoms of mercury poisoning are mainly neuronal disorders (inability to talk, see, smell, move), but also damage to the cardiovascular system, kidney, bones, etc. (Sigel and Sigel 1997).

Mercury pollution of the environment

About 1,000 tons of mercury were supplied in the United States in 1995; the major user was the chlor-alkali industry, followed by producers of electronic equipment, and measurement and control instruments. The total nationwide mercury emission estimate was 154 tons, of which 136 tons were emissions to air by combustion sources (coal, municipal waste, medical waste) (EPA 1997, 2000). In Europe, total mercury emissions in 1995 were estimated at 338 tons. Air emissions were derived mainly from low level sources, e.g., coal combustion in power plants and for residential heating, chlorine production, oil combustion, cement production, waste incineration, lead and zinc production.

The chlor-alkali industry is the major user of mercury. In Europe, about 55 factories use the so-called amalgam process, where mercury serves as an electrode (European Commission 2000). Process wastewater from chlor-alkali factories contains mercury in concentrations between 1 and 10 mg l^{-1} as well as high salt concentrations and traces of toxic compounds (chlorine, sodium bisulfite).

Mercury discharged into the environment undergoes complex physical, chemical and biological transformations. Again, the redox state of the element is critical for its fate. The two key processes are:

- 1 Long range transport of elemental mercury through the atmosphere, its photochemical oxidation to reactive Hg^{2+} (Ebinghaus et al. 2002) and subsequent deposition on soils, lakes, rivers and the sea. Even apparently

pristine environments like the Antarctic have been shown to have increased mercury levels (Braune et al. 1999; Muir et al. 1999; Macdonald et al. 2000).

- 2 Methylation of Hg^{2+} by sulphate-reducing bacteria (SRB) in anoxic habitats (e.g., eutrophic lakes with anoxic hypolimnia, anaerobic sediments), its uptake by aquatic organisms and accumulation in the food web. This results in high mercury concentrations in fish and chronic low level exposure of humans, the largest danger being damage to the brain and the cardiovascular system in unborn children (Clarkson 1992; Barkay 2000; Schrope 2001).

In the late 1950s and 1960s catastrophic mercury poisoning of whole populations directly exposed to industrial effluents (Minnamata) or consuming wheat treated with a methylmercury fungicide (Iraq) (Amin-Zaki et al. 1976) occurred. Today, many hot spots of mercury pollution still exist around the globe in mining areas and at factory sites. However, low level emissions from a variety of industrial sources are also a problem since they affect human populations worldwide and are hard to control using available technologies.

Microbial transformations of mercury

Mercury is a natural component of the Earth's mantle. Toxic mercury concentrations have therefore always been present locally in the biosphere, although the global pool of mercury participating in the biogeochemical cycle is thought to have increased substantially through industrial activities over the past century. Microorganisms are known to mediate four types of enzymatic transformations of mercury (see Barkay 2000 for a review):

- 1 reduction of Hg^{2+} to Hg^0
- 2 breakdown of organomercury compounds (including MeHg^+), resulting in formation of Hg^0
- 3 methylation of Hg^{2+}
- 4 oxidation of Hg^0 to Hg^{2+}

Reactions (1) and (2) are performed by the enzymes and proteins of the microbial mercury resistance (*mer*) operon and result in the production of Hg^0 . The detoxification (resistance) mechanism for mercury is based on the unique peculiarities of this metal: the electrochemical potential of $\text{Hg}^{2+}/\text{Hg}^0$ at pH 7 is +430 mV, which means living cells are able to reduce Hg^{2+} to the elemental form, which is non-toxic to bacteria. Secondly, the melting point/boiling point of mercury is extraordinarily low (melting point -39°C , boiling point 357°C), so that metallic mercury does not remain inside the cell but leaves it by passive diffusion and is then either volatilized into the air or precipitates due to its low solubility in water. In either case, the bacterial cell is effectively freed of toxic Hg^{2+} or organomercury compounds. The precise roles of these powerful detoxification mechanisms for the biogeochemical cycle of mercury need to be established,

since environmental mercury concentrations are usually well below the induction level of the *mer* operon (Barkay 2000). However, at high mercury concentrations, e.g., in polluted environments, mercury resistance is an important selective advantage for bacteria.

The cellular machinery encoded by the microbial *mer* operon provides specific uptake proteins (*merT*, *merP*, *merC*) that transport Hg^{2+} into the cytoplasm and so prevent damage to the cell. Inside the cell, Hg^{2+} is reduced with NADPH to Hg^0 by the enzyme mercuric reductase (*merA*), which is related to glutathione reductase (Schiering et al. 1991). The reaction takes place within minutes. The rate-limiting step for the overall detoxification reaction is probably uptake into the cell, rather than reduction of Hg^{2+} (Becker 1996; Danhamer 1995). The microbial reduction of mercury is a detoxification reaction that requires energy rather than producing it. Thus, in a bioreactor, the bacteria have to be supplied with nutrients. Some microbial *mer* operons contain the *merB* gene coding for a mercury lyase enzyme. These host bacteria are termed “broad spectrum mercury resistant” and are able to detoxify organomercury compounds, including MeHg^+ and Me_2Hg , which become demethylated.

Mercury reduction is an inducible trait; the transcription of the *mer* operon is suppressed if no mercury is present due to binding of the *merR* protein to the operator/promoter region of the *mer* operon. If mercury is present, it binds to the *merR* protein and causes it to leave the operator/promoter region, where transcription can then start. Truncation of the *merR* gene from the *mer* operon resulted in recombinant strains with constitutive expression of mercury resistance (Horn et al. 1994).

The *mer* operon is highly conserved among both Gram-positive and Gram-negative organisms and thus is probably of ancient evolutionary origin. It is usually located on transposable elements or plasmids and has been found in microbes of diverse phyla (Osborn 1997; Barkay 2000). Mercury-resistant microbes are ubiquitous in most habitats and enriched in mercury polluted sites.

Reaction (3)—methylation of mercury—is probably not a detoxification reaction, because it occurs at an extremely slow rate and the resulting monomethylmercury (MeHg^+) is also toxic to bacteria. Methylation of Hg^{2+} to MeHg^+ is performed by SRB, e.g., *Desulfovibrio desulfuricans*, in a narrow range of SO_4^{2-} concentrations in anoxic environments. Although concentrations of MeHg^+ in natural waters are extremely low (in the ng l^{-1} range), they are sufficient to cause accumulation of mercury in the food chain.

Recent evidence strongly suggests that Hg^{2+} , the substrate for methylation, enters methylating SRB by diffusion as soluble HgS forms (Benoit et al. 2001a, 2001b) that are favored in the presence of polysulfides at high pH. Under anaerobic conditions, methylation by SRB and demethylation by the *mer* operon-encoded resistance mechanism appear to occur simultaneously, the outcome depending on mercury concentration and redox potential (Schaefer et al. 2002). Under aerobic

conditions, however, demethylation by the *mer* operon-encoded mercuric lyase enzyme is the prevalent mechanism.

The ability to oxidize Hg^0 to Hg(II) (reaction 4) was described recently for *Escherichia coli* and two soil bacteria and was attributed to hydroxyperoxidases (Smith et al. 1998).

Retention of elemental mercury in packed bed bioreactors

Principle

Elemental mercury produced by microbial reduction diffuses out of the cells and can easily be volatilized into the atmosphere. Remediation technologies based on mercury volatilization have been explored (Fry et al. 1992; Saouter et al. 1994; Gadd 2000), but have never proceeded beyond laboratory scale, because collecting the volatilized mercury is tedious and expensive on a technical scale. Work performed at the National Research Institute for Biotechnology (Frischmuth et al. 1991; Brunke et al. 1993; Röhrich 1993) showed that the elemental mercury formed could also be retained in a packed bed bioreactor. The packed bed consisted of inert porous carrier material that was covered by a biofilm of mercury-resistant bacteria. The reactors were operated in upflow mode with hydraulic retention times of 15–30 min. Mercury accumulated in the carrier material and, after several weeks and months, microdroplets of mercury were visible by light microscopy and scanning electron microscopy (Fig. 1). Analytical electron microscopy demonstrated that these droplets consisted predominantly

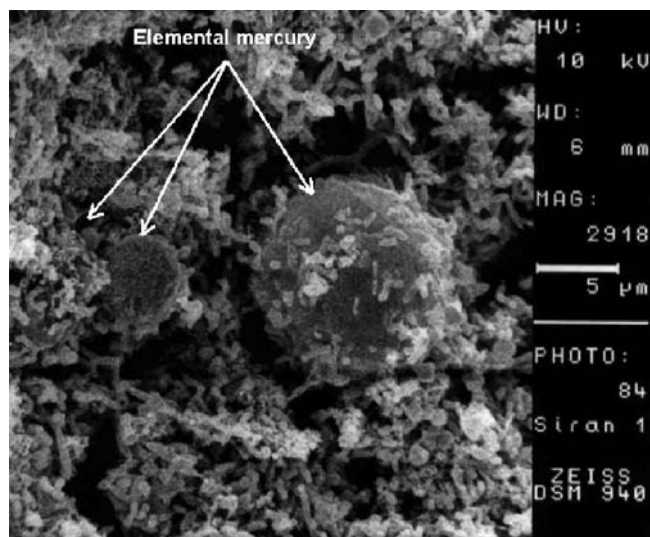


Fig. 1 Scanning electron micrograph of a microbial biofilm and mercury droplets (arrows) formed in a laboratory model reactor inoculated with *Pseudomonas putida* Spi3 on siran beads after several weeks of operation with synthetic wastewater

Table 1 Mercury gradients and mass balance in packed beds of bioreactors (20 cm³ bed volume) filled with ceramic carrier material and inoculated with a mercury-resistant strain of *Aeromonas hydrophila* after continuous operation for several months (length of operation indicated in brackets). Mercury inflow concentration between 1 and 10 mg Hg²⁺ l⁻¹, inflow volume between 1 and 10 bed volumes per hour. *Bi54t* Ceramic carrier (Feuerfest, Bochum, Germany; particle size 1.0–1.8 mm, porosity

75%, consisting of 50% Al₂O₃), *Siran* ceramic carrier (Schott Glaswerke, Mainz, Germany; particle size 1.0–2.0 mm, porosity 55–60%, consisting of SiO₂), *Biopor* ceramic carrier (Schott; particle size 0.5–1.0 mm, porosity 60%, consisting of silicate), *layers 1–4* four segments of equal size from the packed bed (1= bottom, 4= top), *dw* dry weight. See Röhrlich (1993) and Brunke et al. (1993) for more details

		Biomass (g dw/kg carrier)	Mercury (mg)	Mercury per biomass (mg Hg/g biomass dw)	Mass balance	Mercury (mg)
Bi54t (68 days)	Layer 1	63.0	87.6	794.6	Inflow	118.5
	Layer 2	37.5	18.5	281.9	Packed bed	108.3
	Layer 3	14.0	1.6	65.3	Effluent	1.8
	Layer 4	11.0	0.6	31.2	Recovery	92.9%
Siran (88 days)	Layer 1	74.0	151.6	745.0	Inflow	321.4
	Layer 2	53.2	110.2	753.2	Packed bed	305.7
	Layer 3	30.0	39.5	478.8	Effluent	2.6
	Layer 4	9.2	4.4	173.9	Recovery	96.0%
Biopor (103 days)	Layer 1	63.0	293.9	1,555.0	Inflow	439.2
	Layer 2	26.9	126.0	1,561.3	Packed bed	454.8
	Layer 3	19.8	21.6	363.6	Effluent	4.2
	Layer 4	15.0	13.3	295.5	Recovery	104.5%

of mercury (Brunke et al. 1993; Wagner-Döbler et al. 2000b).

Conditions required for quantitative accumulation of elemental mercury were: (1) completely submersed biofilm without contact to air and without air bubbling through it (which would result in stripping of mercury), (2) slow, constant flow rate of the wastewater, and (3) large porosity of the carrier material.

Mercury balance

Mercury balances were calculated for packed bed bioreactors (20 cm³ volume) with three different types of ceramic carriers, and biofilms of *Aeromonas hydrophila* HG32 as microbial catalysts (Table 1). Mercury inflow concentrations were between 0 and 10 mg l⁻¹ and volumetric loads were between two and four bed volumes per hour (Brunke et al. 1993; Röhrlich 1993). Interestingly, concentrations of mercury in the effluent were independent of inflow concentration and volumetric load, indicating an all-or-nothing mechanism within this range of parameters. At the end of each operation—after 68, 88 and 102 days, respectively—mercury was determined in the various layers of the packed bed (Table 1). The highest concentrations were observed in the bottom layer. A maximum of 1,555 mg Hg (g dry weight of biomass)⁻¹ was determined. The total amount of mercury recovered from the bioreactor and the effluent was between 93% and 104% of total inflow mercury. Thus, mercury was quantitatively retained in the bioreactor. Volatilization to air played no significant role in the process.

The solubility of elemental mercury in water is 60 µg l⁻¹ (Barkay 2000). This is the minimum concentration of mercury that can be obtained in the effluent of this type of mercury retention bioreactor. To reach the wastewater

discharge limit reliably, a polishing step, e.g., carbon filtration, is necessary.

Kinetics of mercury reduction

The enzymatic detoxification reaction for Hg²⁺ follows typical inhibition kinetics with respect to optimum concentration of mercury (Röhrlich 1993; Danhamer 1995; Becker 1996; Wagner-Döbler et al. 2002). The absolute value for the optimum mercury concentration depended on the buffer and medium used, the cell density and growth stage of the microbial culture, and on the characteristics of the bacterial strain under investigation. Using unbuffered mercury solutions, the maximum concentration where growth was observed for wastewater isolates and recombinant strains was between 1 and 10 mg Hg²⁺ l⁻¹ (von Canstein et al. 2002b; Wagner-Döbler et al. 2000b), while values up to 70 mg l⁻¹ were measured with buffered mercury solutions where Hg²⁺ might have been partly complexed (Frischmuth et al. 1991; Horn et al. 1994).

Carrier material

A large variety of different carrier materials were tested, e.g., *Siran*, pumice, activated carbon, wood chips, cellulose fibres, synthetic fibres and others. All worked, but small differences occurred with respect to the effluent mercury concentration, probably due to differences in the distribution and thickness of the biofilm on the carrier, the porosity of the packed bed and the general flow characteristics in the bioreactor. The microbial catalysts were also immobilized in alginate beads that could be operated with loading rates up to 20 bed volumes per hour (Frischmuth et al. 1991).

Hydraulic retention time, volumetric load, and length of operation

Mercury reduction takes place within minutes, and thus hydraulic retention times of 10–30 min were sufficient for complete reduction of Hg^{2+} in the inflow. The volumetric load of the bioreactor could be increased up to the point where elemental mercury and biofilm fragments were washed out. This was dependent on the flow velocity of the wastewater and the immobilization technique used for generating the biofilm. Generally, the bioreactors worked best at a constant volumetric load. Sudden rapid increases in flow rate resulted in transient wash-out of mercury, with subsequent adaptation of the bioreactor to the increased flow rate (von Canstein et al. 1999, 2001a).

Mercury is not accumulated in the bioreactor stoichiometrically like in processes based on absorption, but infinitely. The amount of catalyst (biomass) required is very small and can be kept constant. Growth is only required to replace dead bacteria, and can be controlled through the nutrient supply. Under ideal conditions, the length of operation is limited only by clogging of the packed bed due to accumulated mercury. In practice, clogging can also be caused by suspended solids in the inflow, dead microbial biomass as a result of adverse wastewater conditions, and too high feeding rate resulting in excess biofilm growth. Quantitative modeling of these processes in a packed bed is very difficult. Long term experiments with laboratory reactors showed that they could be operated for 450 days continuously without backflushing and without reduction in mercury retention efficiency (von Canstein et al. 2001a). Increased mechanical sensitivity was, however, observed in these reactors with time, and was found to be due to fine layers of sedimented material.

Monospecies or multispecies mercury-reducing biofilm?

It is the gut feeling of microbial ecologists that communities work better than pure cultures when it comes to bioremediation. However, in the case of mercury reduction the reaction carried out by the bacteria is a one-step enzymatic detoxification that is absolutely necessary for survival but not directly coupled to growth. In a completely mixed system, the principle of competitive exclusion would predict that strains with the highest detoxification rate have the largest selective advantage. They should therefore outcompete strains with lower detoxification rates in a mixed culture community and perform best if present as pure culture. Therefore, we compared the performance of bioreactors operated with pure cultures of mercury-reducing strains (monospecies biofilms), among them both environmental isolates and a genetically optimized strain, with bioreactors run with six phylogenetically and physiologically diverse mercury-reducing strains of Proteobacteria (multispecies biofilms). For each type of biofilm, three packed bed bioreactors were run in parallel. Four batches of factory wastewater

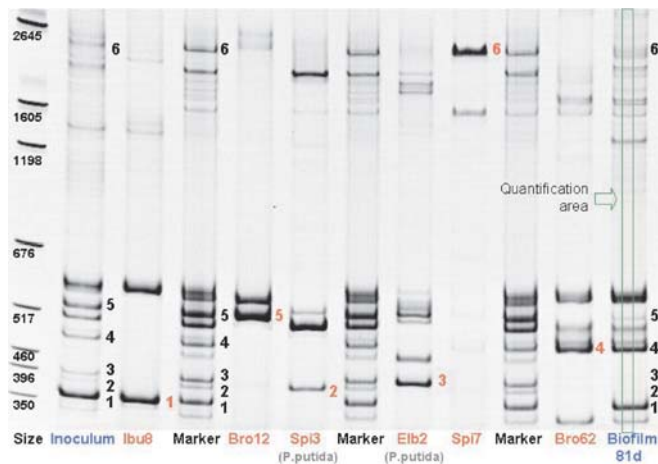


Fig. 2 Polyacrylamide gel showing DNA fingerprints based on the intergenic spacer region (ISR). Lanes: *Size* Molecular size marker, *Inoculum* inoculum community, *Ibu8*, *Bro12*, *Spi3*, *Elb2*, *Spi7*, *Bro62* single inoculum strains, *Marker* a synthetic mixed community, *Biofilm* a biofilm from the granules inside the reactor after 81 days of operation. 1–6 Diagnostic bands used to identify strains within the community. See von Canstein et al. (2002b) for more details

(ECI1–ECI4) were treated, which differed in their concentration of ionic mercury and chloride. Before entering the bioreactors, the wastewater passed through a 0.2 μm filter to prevent contamination. Biofilm composition was monitored throughout the experiment by community profiling of bioreactor effluents, using the PCR-amplified intergenic spacer between 16S- and 23S-rDNA (Fig. 2; von Canstein et al. 2002b).

The performance of a multispecies mercury-reducing biofilm community was clearly superior to all monospecies biofilms tested, with respect to both function (mercury outflow concentration) and stability (recovery after change), thus supporting the basic ecological axiom that diversity increases community stability and efficiency (Loreau and Hector 2001; Mulder et al. 2001). We assume that the various strains occupied different ecological niches within the bioreactor, since their concentration optima for mercury reduction as well as their growth characteristics were clearly different, and that their coexistence was mediated by differential growth and death rates at fluctuating wastewater mercury concentrations. In addition, direct mutualistic interactions might also occur between different strains resulting in mixed species microcolonies, where the activity of one organism protects the other (Moller et al. 1998; Nielsen et al. 2000). None of the high performance pure cultures, including the genetically improved mercury-resistant strain, reached high densities in bioreactors operated with factory wastewater (Felske et al. 2001; von Canstein et al. 2001b; 2002a; Wagner-Döbler et al. 2000b). In contrast, strains with medium level mercury resistance usually dominated those communities.

For the practical operation of mercury-reducing bioreactors in non-sterile industrial environments therefore, the

invasion of ubiquitous mercury-resistant bacteria is not a danger for the high performance inoculant strains, but increases biofilm diversity and contributes to the observed robustness and stability of the process under a wide range of physical and chemical conditions.

Design of a pilot plant for mercury retention from wastewater

A pilot plant was constructed that was able to treat 100 m³ wastewater per day, which is 50% of the mercury-containing wastewater in some factories and 100% in others. The plant was completely automated and consisted of a pre-treatment (pH adjustment to pH 7.0), nutrient amendment, a bioreactor with a volume of 1 m³, a polishing activated carbon filter, and measuring and control devices. Online monitoring of process parameters and control of valves and pumps were possible via remote control software and a telephone modem. The plant was placed in a mobile standard container (Fig. 3, Fig. 4, Wagner-Döbler et al. 2000a).

Mercury concentrations were monitored continuously in the wastewater inflow, the bioreactor outflow and the carbon filter outflow using the cold vapor technique and sample pre-treatment with KMnO₄. The bioreactor was protected from adverse wastewater conditions by automatically closing the inflow valve if parameters for mercury concentration, chlorine concentration, temperature or pH were detected that lay outside predefined ranges. In such cases, the wastewater entered a bypass and went back to the initial wastewater storage tank.

Inoculation and operation of the pilot plant

The plant was inoculated with seven different mercury-resistant strains of *Pseudomonas*, cultivated separately in 15 l vessels. These inoculum strains were naturally immobilized on the pumice carrier material. Full activity of the bioreactor was obtained 12 h after inoculation. Thus, no adaptation of the inoculum strains to the wastewater conditions at the factory site was necessary. A typical example of the retention efficacy of bioreactor and carbon filter is given in Fig. 5. The process was efficient at fluctuations in mercury inflow concentrations between 2 and 10 mg l⁻¹, chloride loads up to 50 g l⁻¹ (corresponding to a conductivity of 105 mS cm⁻¹) and transient temperature increases up to 47°C (Wagner-Döbler et al. 2000a). During the testing period of 8 months, 7,593.6 m³ wastewater were treated, containing a total of 29.3 kg Hg. The pilot plant removed 98% of the inflow mercury (28.8 kg) over the whole testing period; the effluent contained a residual total amount of 0.48 kg Hg. The average inflow mercury concentration was 4.8 mg l⁻¹. Since mercury inflow concentrations regularly fluctuated between 2 and 10 mg l⁻¹, the zone of maximum reduction activity in the packed bed oscillated between bottom and top, leading to a relatively even vertical

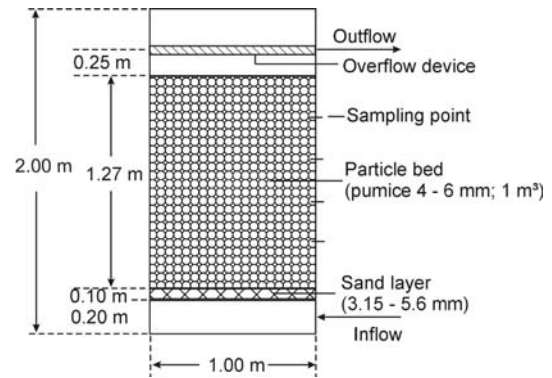


Fig. 3 Technical scale bioreactor (1 m³ volume) for treatment of up to 100 m³ of mercury-containing industrial wastewater per day

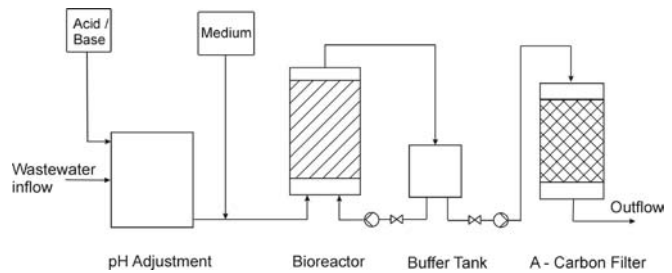


Fig. 4 Flow scheme of the pilot plant for continuous treatment of chloralkali electrolysis factory effluent

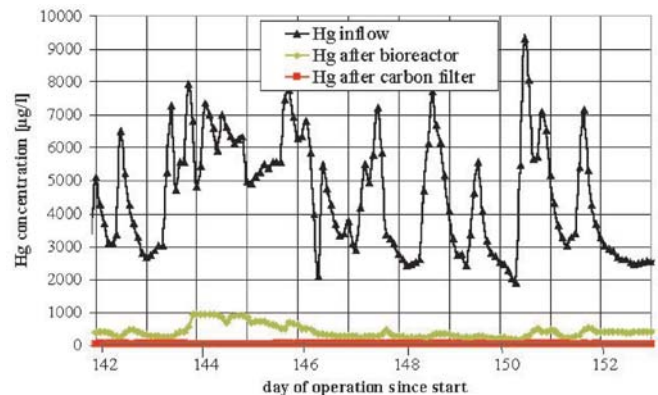


Fig. 5 Example of the performance of the bioreactor during the testing period at ECI Elektrochemie Ibbenbüren

distribution of mercury across the bed profile (von Canstein et al. 2002a).

Mercury-reducing microbial biofilms in technical operation

Mercury present in the wastewater exerted a strong selective pressure on the microorganisms that was largest close to the inflow and smallest at the outflow. Accordingly, the diversity of mercury-resistant strains increased towards the outflow. The microbial community within the

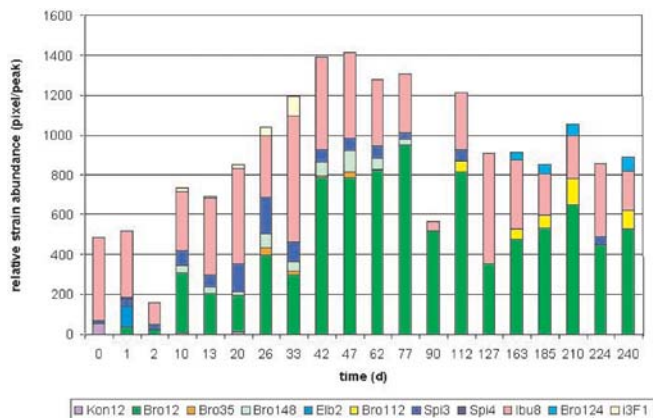


Fig. 6 Succession of mercury-resistant bacteria in the technical scale bioreactor based on analysis of ISR community fingerprints in comparison with fingerprints of effluent bacteria. Biofilm samples were taken at the bioreactor bottom above the inflow where high mercury concentrations were found. The biofilm was dominated by strain *Ibu8* and the invader *Bro12* during the whole testing period of 240 days. In addition, other mercury-resistant strains were detected for varying lengths of time. See von Canstein et al. (2002a) for more details

bioreactor showed a succession of mercury-resistant strains (Fig. 6). In the polishing activated carbon filter (which was continuously inoculated by the bioreactor effluent bacteria), an increase in diversity was observed over the 8 month testing period (von Canstein et al. 2002a). Compared to laboratory model reactors, the mercury-reducing biofilm of the technical scale reactor was stressed in several ways: exposure to high, fluctuating mercury and salt concentrations, high flow velocity, and an average temperature of 42°C, which is well above the optimum growth temperature of *Pseudomonas* (30°C). These parameters may have been among the driving forces selecting for better adapted strains for the bioreactor. Invasion of environmental mercury-resistant strains into laboratory bioreactors operated with non-sterile industrial wastewater had been observed previously (Wagner-Döbler et al. 2000b; von Canstein et al. 2001b). Neither in small scale laboratory reactors nor in the technical scale reactor could the succession in community composition be related to fluctuations in mercury inflow or outflow concentrations. Mercury retention efficiency remained high, irrespective of the composition of the microbial community. It was affected only by operational failures such as toxic wastewater loads, mechanical problems of the packed bed (clogging), or lack of nutrients.

An increase in species diversity as observed by community profiles based on the intergenic spacer region (a phylogenetic marker) could theoretically also have been caused by non-mercury-resistant invaders. This was excluded by analyzing mercury resistance in the dominant bioreactor effluent bacteria (von Canstein et al. 2002a). However, since mercury reduction is catalyzed by the *merA* enzyme, functional community diversity could be monitored directly and without the need for cultivation by

amplifying the *merA* gene from community DNA and separating the different amplicons on TGGE (A. Felske et al. MS submitted). The dominant invading strain was detected also on these *merA* community profiles, and could be shown to have a *merA* enzyme different from that of the inoculum strains.

Costs

Regenerable ion exchange columns are used for regular wastewater clean-up at the test site Elektrochemie Ibbbüren. The advantage of regenerable ion exchange columns is that the mercury-containing brine solution obtained after regeneration can be recycled into the mercury cell process. Thus, a truly closed cycle is obtained. A cost comparison was performed for investment and operating costs (Table 2). In both categories, microbial mercury reduction cost less than half of ion exchange columns. Moreover, it is more robust: a chlorine accident would irreversibly and expensively damage ion exchange columns, while the bioreactor has been demonstrated to recover within several days. In addition, the microbial system is also applicable to a range of other types of mercury-containing wastewaters.

Currently used clean-up technologies for mercury-contaminated soils, wastewater and groundwater

The best available technology for cleaning of mercury-contaminated soil and sediment is incineration at high mercury concentrations, and safe storage in landfills or in underground storage sites at low concentrations. To reach the European wastewater discharge limit for mercury (50 µg l⁻¹), the process effluent from chlor-alkali factories is currently cleaned by hydrogen sulfide precipitation, hydrazine precipitation or ion exchange columns (European Commission 2000). Clean-up of mercury-contaminated groundwater is performed by filtering it through activated carbon either in situ (barriers) or ex situ (Fry et al. 1992; Means and Hinchee 1994).

Alternative microbiological technologies for clean-up of mercury-contaminated matrices that are being investigated are mainly sorption and precipitation as HgS. Sorption of metal ions to biomass of bacteria, fungi and algae has been studied extensively. It is based on the sorption and ion exchange capacity of cell surface components for metal ions. Live biomass can be used, but dead biomass and various immobilization techniques are more often applied. However, in spite of large initial interest there has been no adoption of biosorption as a commercially viable treatment method to date (Gadd 2000). The most important reasons seem to be lack of specificity and lower robustness compared to ion exchange resins (Eccles 1999). Recently, recombinant strains have been constructed that accumulate mercury using the *mer* transport system and then sequester it to a

Table 2 Cost comparison between clean-up of chloralkali electrolysis wastewater using regenerable ion exchange columns and biological mercury retention. Calculation is based on treatment of $8 \text{ m}^3 \text{ day}^{-1}$ ($70,000 \text{ m}^3 \text{ year}^{-1}$), average mercury concentration

Process	Investment costs (Euro)	Operating costs (Euro/annum)	Total costs (Euro/annum)	Costs per removed kg Hg (Euro)
Regenerable ion exchange columns	1.3 Million	240,000	216,000	1,400
Microbial clean-up	0.5 Million	90,000	120,000	760

metallothionein (Chen and Wilson 1997a, 1997b; Chen et al. 1998), or express mercury-binding motifs in the cell (Bang and Pazirandeh 1999; Pazirandeh et al. 1998) or on the cell surface (Bae et al. 2000, 2001, 2002; Valls et al. 2000). Some of these systems have also been tested in bioreactors (Chang and Law 1998; Chang et al. 1998). These strains might potentially be useful for removing mercury from very dilute solutions. The *mer* operon has also been expressed in radioresistant *Deinococcus radiodurans*, which is able to tolerate extremely high doses of radiation and might be applicable to clean-up of radioactive waste sites (Brim et al. 2000; Daly 2000). Phytoremediation, e.g., the engineering of the combined power of plants (especially the rhizosphere) and microorganisms for clean-up of contaminated soils (de Lorenzo and Kuenen 1999; de Souza et al. 1999), has a particularly interesting application for mercury. Transgenic plants have been constructed that express *merA* and *merB* (Rugh et al. 1996, 1998; Bizily et al. 1999, 2000); the Hg^0 produced is released into the atmosphere.

Some remediation treatments used SRB as source of H_2S for precipitating metals as sulfides. This technology can be highly efficient for various metal ions (White et al. 1998). Its applicability for mercury is based on the extremely high solubility product of HgS (6.38×10^{-53} ; Weast 1984). However, SRB that produce H_2S also methylate a fraction of the total mercury to methylmercury, a process that is not completely understood at present and seems to depend, among other factors, on the concentration of SO_4^{2-} (Barkay 2000; Benoit et al. 2001a, 2001b). Methylmercury is the most toxic form of mercury (which often escapes attention since concentrations are in the range of ng l^{-1}), the compound is extremely volatile, and its analytical detection requires sophisticated sample pre-treatment. However, even these low concentrations are sufficient to cause accumulation in aquatic biota and represent a health problem for workers in bioremediation facilities. For these reasons, anaerobic treatment of mercury-contaminated matrices requires extensive safety measures.

Industrial applications of microbial clean-up technologies

In spite of the huge metabolic potential of microbes (Gadd 2000; Lovley 2000; Wackett and Bruce 2002) and the enormous market volume for remediation technologies (Eccles 1999), the number of industrial operations for

4 mg l^{-1} . Wastewater pre-treatment with NaOCl , NaHSO_3 and neutralization to pH 7 were included. Operating costs include chemicals for wastewater pretreatment, nutrients for bacteria, manpower, electricity, repair and replacement, disposal of mercury

clean-up of contaminated wastewater can be counted on one hand. There is a full scale facility for elimination of cyanide, zinc and copper in the United States, the Homestake Rotating Biological Contractors (Pümpel and Paknikar 2001). Cyanides are transformed to ammonia and carbonate by aerobic bacteria, mostly *Pseudomonads*. In a second step, ammonia is converted to nitrate. Zinc and copper are removed by adsorption to microbial biomass and precipitation as carbonates. The only plant in operation is at Homestake Mine, (Lead, S.D.) and treats up to $900 \text{ m}^3 \text{ h}^{-1}$ from one of the largest underground gold mines in the Western hemisphere.

All other full scale treatment systems are based on SRB. These bacteria produce H_2S , which then precipitates metals, mainly Sn, Cu, Pb, Ni, Zn, Fe, Cr and trace metals, from contaminated groundwater or industrial effluents. Sulfate and a number of organic contaminants are removed simultaneously. There is the Thiopaq system, which has been developed in the Netherlands by Paques in cooperation with Shell and the Budelco zinc refinery; the Metex anaerobic sludge reactor (Linde, Germany); and the Bio-Substrat anaerobic micro-carrier reactor (Dr. Fürst Systems and BKT Burggräf, Germany) (Pümpel and Paknikar 2001; Eccles 1999).

These applications all rely on the metabolic potential of complex microbial communities. There is no industrial application of genetically engineered microorganisms (Sayler and Ripp 2002). The cyanide- and mercury-removing bioreactors are the only ones based on a specialized microbial detoxification reaction.

Outlook

The plant for biological mercury remediation has now been operating for 2 years at a Czech electrolysis factory, where it is cleaning all mercury-containing process wastewater. To obtain stable performance, a wastewater pre-treatment system was installed, which oxidizes incoming mercury completely to Hg^{2+} . The inflow volume is kept at a constant rate by using a buffering tank that transiently stores excess wastewater. In this way, standby periods have been reduced practically to zero. To obtain long term operation without fresh inoculation, mercury and biomass are backflushed from the bioreactor periodically every few months, sedimented down and deposited.

Microbial mercury remediation as described above is applicable to all types of wastewater containing Hg^{2+} in

concentrations between 1 and 10 mg l⁻¹, such as soil wash water, gas scrubbers, and other washing solutions. It is based on a simple, highly effective, robust, self-optimizing biocatalyzer that can be operated continuously or in batch mode at ambient temperature without addition of extra chemicals in an environmentally friendly way.

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