

# Removal of Mercury from Chemical Wastewater by Microorganisms in Technical Scale

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The enzymatic reduction of Hg(II) to water insoluble Hg(0) by mercury resistant bacteria has been used for removal of mercury from wastewater in technical scale. Pure cultures of seven mercury resistant strains of *Pseudomonas* were immobilized on carrier material inside a 700 L packed bed bioreactor. Neutralized chloralkali electrolysis wastewater with a mercury concentration of 3–10 mg/L was continuously fed into the bioreactor (0.7 m<sup>3</sup>/h up to 1.2 m<sup>3</sup>/h). A mercury retention efficiency of 97% was obtained within 10 h of inoculation of the bioreactor. At optimum performance, bioreactor outflow concentrations were below 50 µg Hg/L, which fulfill the discharge limit for industrial wastewater. In combination with an activated carbon filter, outflow concentrations below 10 µg Hg/L were always obtained. The retention efficiency of the bioreactor was not affected by fluctuations in inflow conductivity (between 20 and 105 mS/cm), pH (between 6.5 and 7.5), or mercury concentration (between 3 and 10 mg/L) and was between 95% and 99%. Temperature increases up to 47 °C did not impair bioreactor performance. Standby periods up to 6 h could be tolerated without loss in activity. A simple, effective, and robust biotechnology for remediation of mercury polluted wastewater is thus demonstrated.

## Introduction

Mercury pollution of the environment by mining activities and industrial wastewaters has resulted in worldwide contamination of large areas of soils and sediments (1–4) and led to elevated atmospheric mercury levels (5). Because of a lack of suitable cleanup technologies, efforts to deal with polluted sites are directed toward the mechanical removal of contaminated material and its deposition elsewhere (6, 7). Such processes are costly and often result in remobilization of toxic mercury compounds during the dredging process (8).

Mercury is one of the most toxic elements. It binds to the sulfhydryl groups of enzymes and proteins, thereby inactivating vital cell functions (9). After discharge into the environment, mercury enters the sediments where it persists for many decades. It is taken up by aquatic organisms in the form of highly toxic methylmercury and is subsequently

biomagnified through the food chain. The health of top predators, e.g. birds, fish, seals, and man, is thereby threatened (10, 11). Mercury poisoning results in severe chronic disease or death (12). Therefore, the discharge of mercury into the environment needs to be prevented by efficient and cost-effective end-of-pipe treatment technologies for mercury emitting industries.

One of the core products of the chemical industry is chlorine gas, with a production of 9.2 million tons in Europe in 1998. Today, 60% of the European chlorine production result from chloralkali electrolysis by the so-called amalgam process (13), which is based on electrolytic dissociation of concentrated sodium or potassium chloride solutions with mercury as the cathode of the electrolysis cell. Wastewaters from this process are characterized by high mercury and salt concentrations. Current treatment procedures result in large volumes of mercury contaminated sludge, e.g. precipitation by hydrogen sulfide, or are expensive, e.g. ion exchange columns.

Mercury resistant bacteria are widespread in nature. Such bacteria reduce soluble Hg(II) to insoluble metallic Hg(0) by means of the cytoplasmic enzyme mercuric reductase, encoded by the *merA* gene (14, 15). Habitats containing high levels of mercury exist since ancient times (16), and therefore this microbial detoxification mechanism is thought to be a very old one (17). Here, we exploit it to remove mercury from chloralkali electrolysis wastewater and retain it within a bioreactor. Mercury reduced by the bacteria accumulates in the form of small droplets of metallic mercury within the microbial biofilm (18), from which it can ultimately be eluted and recycled back into the process.

We have previously shown that laboratory model reactors are capable of removing 90–97% of mercury from a chloralkali factory wastewater and that the mercury and salt concentrations commonly encountered in such wastewaters are not inhibitory to microbial mercury removal (19). However, good performance in the laboratory does not guarantee similar efficiency in large scale under process conditions, since important parameters are carefully controlled in the laboratory and fluctuations are usually avoided. To be applicable to an industrial cleanup problem, the microbiological process must be reliably scaled up without loss in efficiency. Moreover, it needs to be robust with regard to fluctuations in wastewater parameters typically encountered on-site a factory. Here, we describe the inoculation and operation of a pilot plant developed based on laboratory columns described previously (19). The pilot plant was capable of treating about 50% of the maximum amount of wastewater produced at the chloralkali factory which served as the first testing site. We show here the effect of large fluctuations in mercury concentration, salt concentration, temperature, and pH on microbial mercury retention and demonstrate the utility of the new microbiological treatment technology for the cleanup of mercury polluted wastewater at a technical scale.

## Experimental Section

**Design and Operation of Pilot Plant.** The pilot plant for continuous microbial mercury removal from chloralkali electrolysis wastewater is schematically shown in Figure 1. The pH of the incoming wastewater was neutralized to pH 7.0 ± 0.5 by addition of NaOH (20% w/w). This was done in two steps and regulated by an adaptive controller. For 1 m<sup>3</sup> of wastewater, appr. 1 L of NaOH was needed. Subsequently, medium was added to a final concentration of 50 mg/L sucrose and 10 mg/L yeast extract (300 mL of a medium

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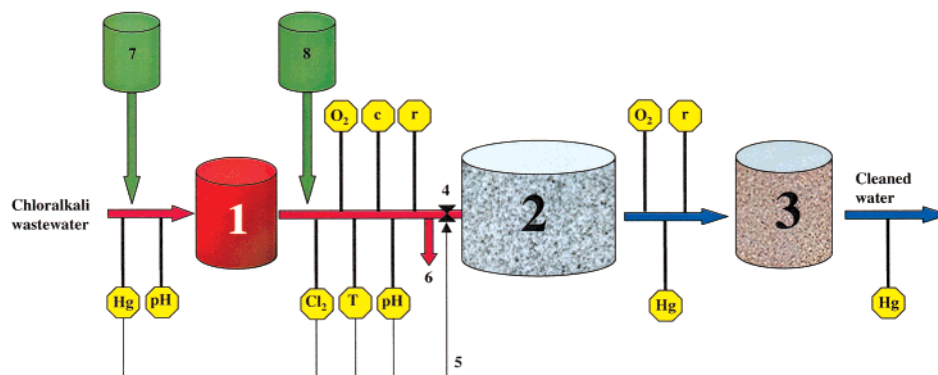


FIGURE 1. Scheme of pilot plant for microbial mercury remediation. Numbers refer to tanks or valves, yellow octagons to monitors. 1 neutralization tank; 2 bioreactor; 3 activated carbon filter, 4 bioreactor inflow valve; 5 control of bioreactor inflow valve; 6 bypass; 7 sodium hydroxide tank; 8 medium tank; Hg automated continuous mercury measurement; O<sub>2</sub> oxygen probe; c conductivity probe; Cl<sub>2</sub> chlorine probe; pH pH-probe; r redox potential probe; T temperature measurement.

solution containing 167 g/L sucrose and 33 g/L yeast extract per m<sup>3</sup> of wastewater). The neutralized, medium amended wastewater entered the bioreactor. The volume of the packed bed was 0.7 m<sup>3</sup>. As carrier material, pumice granules (Raab GmbH & Cie. KG, Luckenau, Germany) were used. Pumice consists mainly of SiO<sub>2</sub> (>70% w/w) and Al<sub>2</sub>O<sub>3</sub> (>10% w/w). We used a particle size of 4–6 mm, specific density 2.4 g/cm<sup>3</sup>, effective density 0.27 g/cm<sup>3</sup>, pore volume 80%. The bioreactor was operated in upflow mode at 0.7–1.2 m<sup>3</sup>/h. The bioreactor outflow ran through an activated carbon filter (1.0 m<sup>3</sup>). Mercury concentrations were determined in the wastewater inflow, the bioreactor outflow, and the activated carbon filter outflow. The bioreactor was protected from adverse wastewater inflow conditions by a bypass which automatically opened at inflow mercury concentrations above 10 mg/L, a pH value outside the allowed range of 7.0 ± 0.5, a wastewater temperature above 47 °C, and a Cl<sub>2</sub> concentration > 0.5 mg/L. A proportion of the factory wastewater entered the pilot plant continuously, and the cleaned or bypassed wastewater re-entered the factory wastewater treatment system. Conductivity, redox potential, chlorine concentration, and oxygen content were determined by electrode measurement in the bioreactor inflow, and redox potential and oxygen content were also determined in the bioreactor outflow. Chloride concentrations were determined in various wastewater batches using a kit from Dr. Lange (Düsseldorf, Germany).

**Cultivation of Inoculum.** The seven inoculant strains were 4 subspecies of *Pseudomonas putida* (Spi3, Spi4, Kon12, Elb2), 2 subspecies of *Pseudomonas stutzeri* (Ibü3, Ibü8), and 1 subspecies of *Pseudomonas fulva* (Spi11). Single colonies were picked from fresh plates and precultures (500 mL) inoculated and grown for 48 h at 30 °C in rich liquid medium (sucrose 10 g/L, yeast extract 10 g/L, NaCl 30 g/L, Hg(II) 5.0 mg/L, sodium phosphate buffer 0.25 M, pH 7.0). Precultures were transferred to seven 15 L fermentation vessels (Govanola, Switzerland) with 3 L of rich liquid medium (sucrose of 15 g/L, yeast extract 15 g/L, NaCl 30 g/L, Hg(II) 1.0 mg/L, neutralized to pH 7.0) and antifoaming agent (1 mL/L) (Ucolub N115, Fragol, Italy). A fed-batch fermentation was carried out for 4 days at 35 °C (aeration 0.25 vvm, stirring rate initially 100 rpm, later regulated to obtain 20% oxygen saturation), where 7 L of feed (sucrose 200 g/L, yeast extract 200 g/L, NaCl 30 g/L, Hg(II) 1.0 mg/L, neutralized to pH 7.0) was continuously added if the oxygen concentration in the bioreactor wastegas was 20%. Continuous pH-controlled supply of H<sub>3</sub>PO<sub>4</sub> prevented a drift of the pH of the culture to alkaline conditions during cultivation. Cultivation was stopped after 4 days by addition of 5 L of a precooled, oxygen-saturated NaCl solution (30 g/L) and subsequent storage of the inoculants at 4 °C until inoculation of the bioreactor 10 h later.

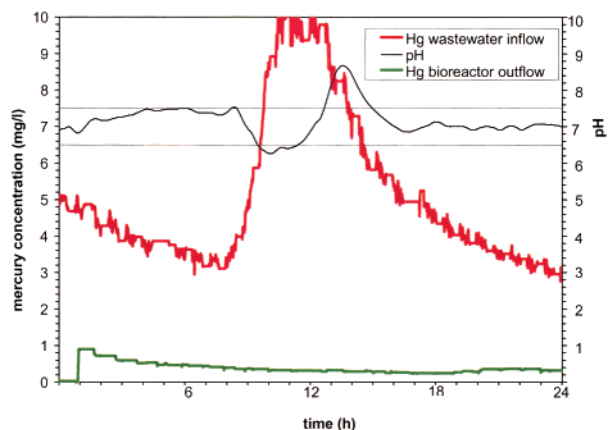


FIGURE 2. Mercury removal of the bioreactor after inoculation. Original process data recorded every minute are shown. Wastewater inflow rate 0.5 m<sup>3</sup>/h. The allowed pH range is indicated by solid black lines. Bypass operation occurred at 496 min for 12 min, from 570 to 685 min, and from 765 to 900 min.

**Inoculation of Pilot Plant.** The bioreactor was filled with autoclaved carrier material and the activated carbon filter was filled with activated carbon (Pool-Aktivkohle, 1–3 mm, Carbotech, Essen, Germany). Neutralized wastewater was continuously run through the pilot plant for several days. For inoculation, the wastewater inflow was stopped and the bioreactor pumped empty. The inoculum cultures were filled into the bioreactor (7 × 15 L). Neutralized wastewater (750 L) and 1875 mL of concentrated medium (sucrose 167 g/L, yeast extract 33 g/L) were pumped into the bioreactor until the carrier material was covered. Inoculum and medium were circulated through the fixed bed for 40 min (0.3 m<sup>3</sup>/h). Then, circulation was stopped, and neutralized wastewater inflow started at a flow rate of 0.5 m<sup>3</sup>/h and a final medium concentration of 0.4 g/L sucrose and 0.08 g/L yeast extract (corresponding to 2500 mL of medium per m<sup>3</sup> of wastewater). The first 1 m<sup>3</sup> bioreactor outflow was collected in a separate wastewater tank and sterilized before disposal. Then, the bioreactor outflow passed through the activated carbon filter. After 1 day, medium addition was reduced to a final concentration of 50 mg/L sucrose and 10 mg/L yeast extract (corresponding to 300 mL of medium per m<sup>3</sup> of wastewater).

**Mercury Measurement.** Mercury concentrations were determined by two automated continuous AAS instruments (Mercury Instruments, Karlsruhe, Germany) using the cold vapor technique. Sample oxidation was performed by KMnO<sub>4</sub> (0.5 g/L at 90 °C), and subsequently reduction of Hg(II) to Hg(0) was performed by addition of SnCl<sub>2</sub> (20 g/L). Metallic mercury was volatilized by air (80 L/h analyzer 1, 20 L/h

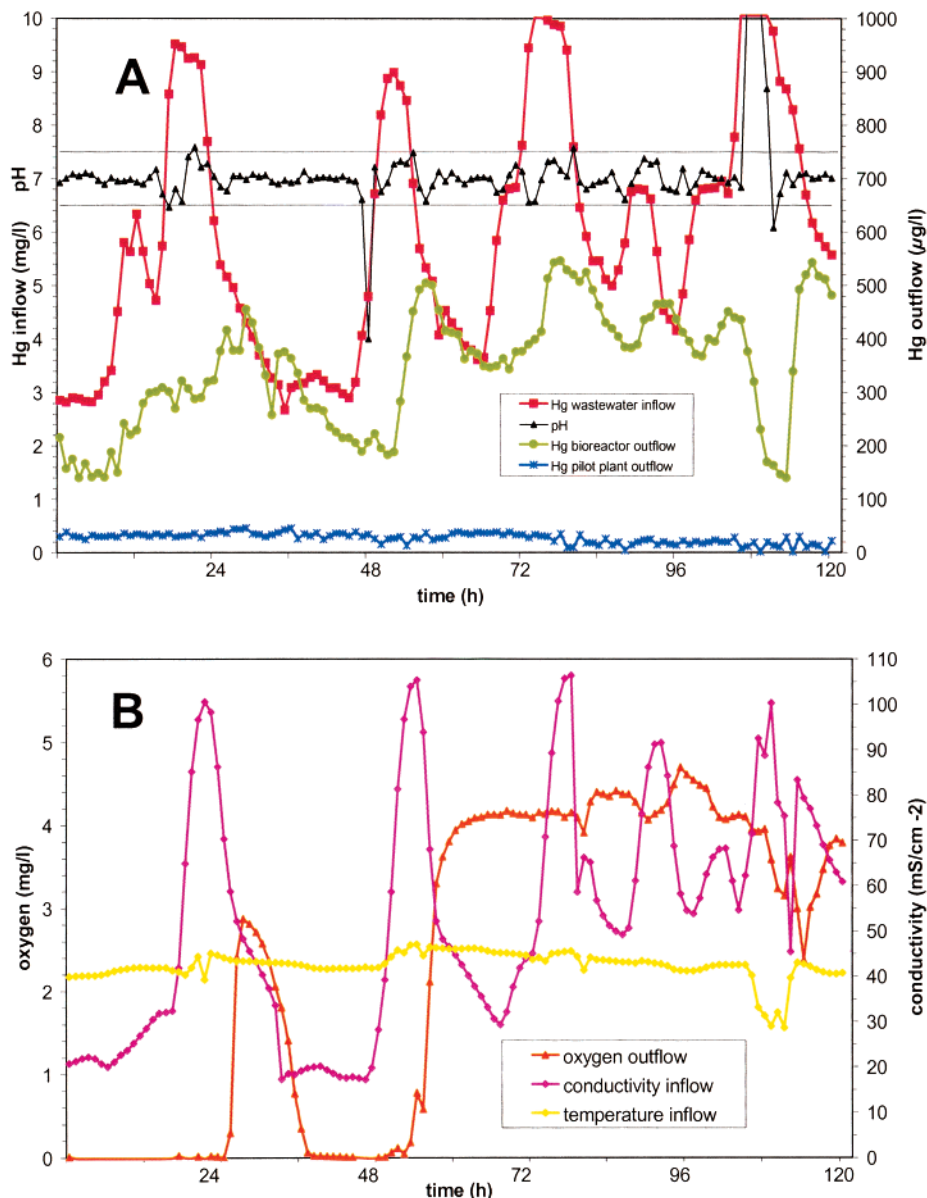


FIGURE 3. Wastewater fluctuations on site a chloralkali factory and their effect on the mercury retention efficiency and oxygen consumption of the bioreactor and the mercury outflow concentration after the activated carbon filter during a period of 5 days. Data are hourly mean values calculated from process data recorded every minute. Volumetric load 1.2 m<sup>3</sup>/h. (A) Concentration of mercury in the wastewater (left axis), in the bioreactor effluent (right axis), and in the activated carbon filter effluent (right axis). Effluent concentrations are shown on a 10-fold larger scale to make fluctuations more visible. The pH of the wastewater after neutralization is shown, and the allowed pH range is indicated by a dotted line. Bypass operation occurred at 18 h, 22 h, 49 h, 75–76 h, 81 h, and from 107 to 112 h. (B) Conductivity and temperature in the bioreactor inflow (right axis) and oxygen concentration in the bioreactor outflow (left axis).

analyzer 2) and determined by AAS spectroscopy at 253.7 nm. Every 60 min, an autozeroing was performed, and every 24 h, an automated calibration check occurred.

**Data Recording and Analysis.** Data were continuously recorded by the WIZCON (Version 7.5, PC-Soft, Israel) process control software package and stored on disk. Process data were transferred to the data analysis software package ACRON, Version 4.52 (MIS Kassel, Germany) from where they were exported to Microsoft Excel 97 and processed by standard procedures.

## Results

**Pilot Plant Design.** The automated pilot plant containing the bioreactor for mercury remediation is shown in Figure 1. Incoming wastewater at pH 2–3 was neutralized to pH 7.0 ± 0.5, supplemented with a small amount of nutrients to

provide energy to the bacteria, and then run through the packed bed bioreactor containing the mercury resistant bacterial catalysts as a biofilm on the carrier material. The effluent from the bioreactor was passed through an activated carbon filter to remove remaining traces of mercury. The bioreactor inflow valve automatically closed and a bypass opened if wastewater inflow parameters lay outside of pre-set ranges, namely a pH value above 7.5 or below 6.5, mercury inflow concentrations above 10 mg/L, a temperature above 47 °C, or chlorine concentrations above 0.5 mg/L. Treated or bypassed wastewater re-entered the factory wastewater treatment system. Conductivity, redox potential, and oxygen concentration as well as mercury concentrations in the wastewater inflow, at the bioreactor outflow, and in the activated carbon filter outflow were determined continuously.

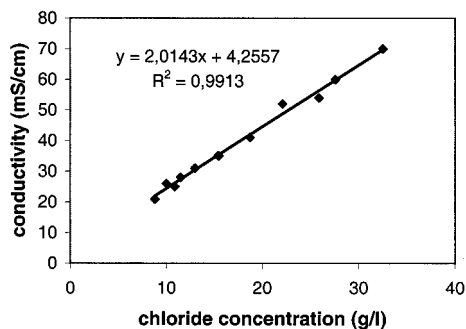


FIGURE 4. Correlation between conductivity and chloride content in chloralkali electrolysis wastewater.

**Start-Up of the Pilot Plant.** Mercury resistant bacteria were added to the bioreactor and allowed to adhere to the carrier material for 40 min. Then, wastewater entered the bioreactor for the first time. The initial mercury outflow concentration was 900  $\mu\text{g/L}$ , representing a retention efficiency of 82% at a mercury inflow concentration of 5 mg/L (Figure 2). Ten hours later, an inflow mercury peak of 9.5 mg/L was observed for about 3 h. The average mercury outflow concentration during these and the following 3 h was 306  $\mu\text{g/L}$ , representing a retention efficiency of 97%. Thus, full mercury removal efficiency was achieved immediately after inoculation, and no adaptation period of the bioreactor to on-site wastewater conditions was necessary.

**Fluctuations in Inflow Mercury Concentration, Conductivity, pH, and Temperature.** Figure 3 shows the effect of fluctuations in wastewater inflow parameters, inherent to the factory production process, on the performance of the pilot plant over a period of 5 days, 3 weeks after inoculation. Inflow mercury concentrations oscillated between 3 mg/L and 10 mg/L. During 108 to 112 h, peak values above 10 mg Hg/L were reached, which provoked a switch to bypass operation. The time interval between peaks was irregular. During the time period shown here, it was between 23 and 35 h. Peak inflow mercury concentrations usually lasted less than 4 h.

Simultaneously, oscillations in the conductivity of the wastewater were recorded, which roughly correlated with mercury inflow concentration. Analysis of the chloride content and conductivity of different batches of factory wastewater revealed a strict correlation between these two parameters (Figure 4). Thus, peak conductivities indicated high chloride concentrations. The maximum conductivity value observed was 105 mS/cm, which corresponds to appr. 50 g/L chloride.

The pH of the neutralized wastewater fluctuated irregularly, usually within the range of pH  $7.0 \pm 0.5$ . The bypass operated at hour 18, 22, 49, 81, and from 108 to 112 h due to failure of pH adjustment.

The average temperature of the wastewater after neutralization was 42.4 °C, with a maximum of 46.9 °C observed at hour 56. A minimum of 28.6 °C, corresponding to the preneutralization temperature, occurred at hour 112, due to prolonged breakdown of neutralization.

**Fluctuations in Bioreactor Mercury Outflow Concentration.** The concentration of mercury after the bioreactor showed oscillations similar to those of the mercury inflow concentration but strongly dampened (Figure 3A) and with a time lag of about 3 h. This was caused by the volume of the neutralization tank (1.2 m<sup>3</sup>) and the volume of the liquid-phase inside the packed bed of the bioreactor (0.7 m<sup>3</sup>), resulting in a residence time of appr. 2 and 1 h, respectively, at a flow rate of 0.7 m<sup>3</sup>/h. The minimum value for bioreactor mercury outflow concentration observed during the time period shown in Figure 3 was 139  $\mu\text{g/L}$  at an inflow

concentration of 3 mg Hg/L, representing a retention efficiency of 95.4%. Peaks of outflow mercury concentration were 454  $\mu\text{g/L}$ , 503  $\mu\text{g/L}$ , 544  $\mu\text{g/L}$ , and 542  $\mu\text{g/L}$ , which for the respective inflow peak mercury concentrations of 9.51 mg/L, 8.89 mg/L, 9.96 mg/L, and 9.76 mg/L represented retention efficiencies of 95.2%, 94.4%, 94.5%, and 94.5%. Thus, the retention efficiency of the pilot plant was not affected by oscillations in mercury inflow concentrations up to 10 mg/L. The increase in conductivity, and thus chloride concentration, which occurred simultaneously, did also not negatively affect mercury retention.

Microbial mercury retention was highly effective at an average wastewater temperature of 42 °C and was also not inhibited by peak temperatures of 47 °C.

**Effect of Standby Periods on Bioreactor Performance.**

During 6 h of bypass operation (hours 107 to 112 in Figure 3) the mercury concentration in the top phase of the bioreactor decreased due to continued microbial activity. When normal bioreactor operation was restored, microbial mercury retention continued at the same level as before. No adaptation period was needed, and no leaching of mercury from the bioreactor occurred after this standby period of 6 h.

**Oxygen Consumption.** The reduction of ionic mercury to metallic mercury, which was the mechanism responsible for mercury retention in the bioreactor, is an aerobic process requiring metabolic energy. Therefore, added nutrients and available oxygen were consumed within the bioreactor. Oxygen concentration in the wastewater inflow was 4.6 mg/L at the beginning of the reported time period in Figure 3 and gradually increased to 4.9 mg/L during day 4 and 5. During periods of low mercury inflow concentration, all oxygen in the inflow water was consumed (hour 1 to 25). However, high mercury inflow concentrations resulted in reduced oxygen consumption of the microbial biofilm, e.g. hours 27 to 35 and 57 to 121 in Figure 3. Here, the average outflow oxygen concentration was 3.9 mg/L. Thus, only 20% of the oxygen present in the wastewater inflow was consumed by the microbial biofilm during this time period. The observed reduction in respiratory activity was accompanied by an increase in mercury outflow concentrations but not by a reduction in the percentage mercury removal efficiency of the bioreactor.

Recovery of low oxygen and mercury outflow concentrations after periods of high mercury loads occurred spontaneously, without the need for additional measures (hour 36 to 56 in Figure 3). The time period between successive inflow mercury peaks between hour 53 and hour 112 was too short to allow complete recovery of the bioreactor, namely to reach outflow mercury concentrations of less than 200  $\mu\text{g/L}$  and to restore full respiratory activity of the microbial biofilm.

**Maximum Performance of Bioreactor.** Figure 5 shows the performance of the bioreactor after a period of stability of the important inflow parameters, i.e., pH and mercury concentration, at a wastewater inflow rate of 1.2 m<sup>3</sup>/h and an average pH of  $6.99 \pm 0.13$  2 months after inoculation. The average outflow mercury concentration over a period of 56 h was  $35.7 \pm 5.0 \mu\text{g/L}$  at an average inflow mercury concentration of  $3.8 \pm 1.8 \text{ mg/L}$ . Peak inflow mercury concentrations of 8.7 mg/L had no detectable effect on mercury outflow concentrations. During these 56 h, an average mercury retention efficiency of 99.0% was reached.

**Effectiveness of Activated Carbon Filter.** After the bioreactor outflow had passed through the activated carbon filter, mercury concentrations were below 10  $\mu\text{g/L}$ . For example, during the 3 days shown in Figure 5, the average mercury concentration after the carbon filter was  $6.3 \pm 2.9 \mu\text{g/L}$ . It was not affected by fluctuations in the inflow to the activated carbon filter, i.e., mercury concentrations in the outflow of the bioreactor.

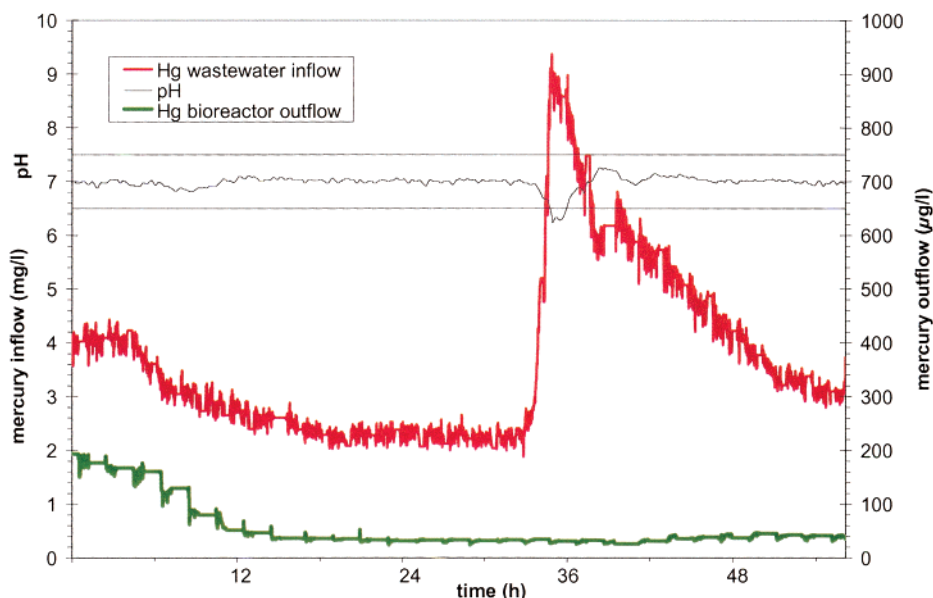


FIGURE 5. Disturbance free optimal performance of the bioreactor. Bioreactor mercury outflow concentration is shown on a 10-fold larger scale (right axis) than the mercury inflow concentration. Volumetric load 1.2 m<sup>3</sup>/h. Process data recorded every minute are shown, and the allowed pH range is indicated by solid lines. Bypass operation occurred at 35.8 h for 67 min. The mercury concentration after the activated carbon filter was  $6.3 \pm 2.9 \mu\text{g/L}$  during this period.

## Discussion

Microbes might be used to remediate metal contamination by removing metals from contaminated water or waste streams, sequestering metals in soils or sediments, or solubilizing metals to aid in their extraction. The mechanisms by which this is accomplished are either biosorption or enzymatically catalyzed changes in the metal redox state. Despite the magnitude of the pollution problem, bioremediation of metals is still primarily a research problem with little large-scale application of this technology (20, 21). Here, we used the reduction of ionic mercury to water insoluble metallic mercury, which is catalyzed by mercury resistant bacteria, as the mechanism by which to remove mercury from solution. While volatilization of the reduced Hg<sup>0</sup> from batch microbial cultures was used by other groups (22, 23), here we captured it as metallic mercury within the bioreactor. The active “mercury trap” constructed thus has the advantage of an extremely high efficiency per volume, since it is not saturated as conventional absorbers or ion-exchange resins. On the other hand, it is dependent on the catalytic activity of the bacteria in the biofilm. The bacteria have to be fed, and they also have to be protected from lethal conditions, e.g. toxic mercury concentrations, unfavorable pH and temperature, and high chlorine concentrations, which requires appropriate engineering solutions. To determine the utility of this type of technology for wastewater remediation, we therefore investigated the robustness of a technical scale bioreactor under fluctuating inflow conditions and exposure to various types of disturbances at a factory site.

The technical scale bioreactor (700 L bed volume) was about 4 orders of magnitude larger than model reactors (80 mL bed volume) investigated previously. It showed a retention efficiency for mercury which was similar or even better than that of the small model reactors. In fact, at optimum performance the pilot plant exhibited a higher retention efficiency than laboratory columns investigated previously. For example, it was possible to reach outflow mercury concentrations of 36 µg/L and a retention efficiency of 99% over a period of several days in the pilot plant bioreactor, compared to outflow concentrations of 200–300 µg/L and a retention efficiency of 90–97% for the laboratory columns (19). Entrance and outflow effects, a lower packing density,

and wall effects might have played a much larger role in the small laboratory reactors than in the pilot reactor.

The mercury concentration in the factory wastewater showed frequent oscillations with peak values close to 10 mg/L and occasionally above 10 mg/L (provoking bypass operation of the bioreactor). Therefore, this optimum performance could not be maintained continuously. In our previous laboratory column experiments, we had observed partial breakthrough of mercury at inflow concentrations between 7 and 9 mg/L, due to the toxicity of high mercury concentrations which killed part of the microbial biofilm. The pilot plant was able to treat mercury inflow concentrations of up to 10 mg/L with a retention efficiency of 95%. However, the toxicity of these high mercury concentrations resulted in reduced respiratory activity of the microbial community and increased mercury outflow concentrations. Recovery occurred spontaneously, but needed several days, and was sometimes overrun by the next mercury inflow peak. For continuous technical scale operation, the need for bypassing wastewater with high mercury inflow concentrations can be replaced by automated dilution of the inflow with cleaned wastewater from the bioreactor outflow or by implementation of a large buffering tank.

Compared to laboratory model reactors maintained under constant conditions, the mercury reducing microbial biofilm of the technical scale bioreactor was stressed in several ways, namely by exposure to fluctuating high mercury and salt concentrations, high flow velocity, and an average temperature of 42 °C. The growth optimum of *Pseudomonas* is at 30 °C, and only some of our inoculant strains showed slow growth at 40 °C (data not shown). Since full mercury retention efficacy was obtained from the day of inoculation we may conclude that the microbial community present had such a large activity, that even under the suboptimal conditions encountered in the pilot plant, almost complete mercury detoxification was achieved. It remains to be seen if a community shift occurred in the biofilm toward more temperature and/or salt tolerant mercury reducing strains.

The discharge limit for mercury for industrial wastewater (50 µg/L), even the limit of 10 µg/L which is demanded by some local water authorities, could be reliably reached by the pilotplant because of the combination of bioreactor and

activated carbon filter. This is of crucial importance for a potential industrial application of the microbial mercury remediation technology. The overall mercury capture of the bioreactor was 95% of inflow mercury, with the activated carbon filter retaining the remaining 5%. Effluent bacteria from the bioreactor colonized the activated carbon filter, so that it acted both as a passive absorber and a second bioreactor. Activated carbon alone has a loading capacity for Hg of 138 mg g<sup>-1</sup> (24).

The current alternatives to the mercury reduction based technology described here are sulfide precipitation (both chemically and biotechnologically), biosorption (using biomass or ion exchange columns), and bioaccumulation of mercury. Sulfide precipitation is used in technical scale for cleaning chloralkali electrolysis wastewater and is accomplishing mercury outflow concentrations below 50 or even 10 µg/L without problems. However, handling of the extremely toxic H<sub>2</sub>S requires extensive safety measures, and relatively large volumes of secondary mercury contaminated sludges, liquids, and solids are produced which need to be deposited on special waste dump sites. Recycling of the precipitated mercury is not possible with this technology. These principal drawbacks remain, if the source of H<sub>2</sub>S is microbial sulfate reduction, as proposed by White et al. (25). Moreover, under anaerobic conditions mercury is transformed to the extremely toxic and volatile methylmercury, a process which is strictly coupled to the rate of sulfate reduction (26). Therefore microbial sulfate reduction cannot safely be applied to remediate waste streams containing mercury.

Biosorption is potentially highly attractive because of the possibility to reuse cheap excess biomass (e.g. *Bacillus* biomass from industrial fermentations) for remediation of metal containing effluents. It is based on the absorption and ion exchange capacity of biomass for metal ions (27, 28). Some processes operating on an industrial scale have been described; however, since this is a passive process, saturation occurs at metal loadings around 10% of the dry weight, after which the metals need to be eluted and the biomass regenerated (27). For mercury, this technology is not competitive. By contrast, the state of the art technology for reliably reaching effluent concentrations below 10 µg/L are ion-exchange resins. Their initial capacity is 150 g Hg(II)/L. Most available resins cannot be regenerated and thus need to be deposited in waste storage sites and replaced after loading. Certain resins can be regenerated, and the extracted mercury can be recycled. However, high costs for the resins and the regeneration occur.

Bioaccumulation, i.e., active uptake of metals into the cell and later sequestering them by binding to metallothionein molecules or chelating agents, has not been observed for mercury (17). To construct a mercury bioaccumulator strain, Chen et al. (29, 30) engineered *E. coli* to express a Hg(II) transport system and a metallothionein simultaneously. This strain worked effectively at low concentrations of Hg(II) (0.2–4 mg/L), but breakthrough occurred after 33 bioreactor volumes of wastewater. Since these bacteria accumulate mercury within the cell, their capacity is limited by the number of metallothionein molecules present. They therefore act as life bioabsorbers. The same is true for engineered strains with improved surface adhesion properties (31–33). Such strains might represent an alternative to treat very dilute mercury-containing wastewater, e.g. to replace the activated carbon filter of our pilot plant.

In contrast to the above approaches, in the enzymatic reduction-based remediation technology described here, the microorganisms act as catalysts. Their capacity is theoretically unlimited. Growth is only required to replace dead biomass. In practical terms, the capacity of the bioreactor is probably determined by mechanical clogging which can, however, be

circumvented by various means. Therefore, the efficiency of a process based on an active enzymatic reaction cannot be described adequately by the loading efficiency of the biomass (34). A first estimate of the amount of mercury retained in the pilot plant bioreactor can be given based on its retention efficiency. During the first 8 months, 10080 m<sup>3</sup> of wastewater were cleaned. At an average retention efficiency of the bioreactor of 90% (including operational failures) and an average mercury inflow concentration of 5 mg/L this represents an amount of 45 kg of mercury retained within 1 m<sup>3</sup> of bioreactor volume, i.e., a loading efficiency of 45 g of mercury per liter of bioreactor volume. The biomass of the inoculum was 7 kg wet weight, appr. 700 g dry weight. Assuming an increase in biomass of about 40% during operation, it can be estimated that 45 kg of mercury have been extracted by 1 kg of biomass dry weight, representing a loading efficiency of 450%. Still, this is a conservative estimate, since the capacity of the pilot plant bioreactor was not exhausted after 8 months. Laboratory reactors have been running continuously for 18 months now. Measurement of the mercury content in the packed bed will be performed after completion of the pilot plant operation.

Since the microbial *mer* operon is small and well studied, it is particularly well suited for genetic engineering approaches to improve the performance of biocatalysts or to develop more optimal biotechnological processes. A modified *merA* gene was expressed in *Arabidopsis thaliana* and yellow poplar plants which consequently were able to take up dissolved ionic mercury, reduce it to Hg(0), and release it to the atmosphere as mercury vapor (35, 36). Recently, also *merB*, which codes for an organomercurial lyase enzyme that detoxifies organomercurials, was expressed in plants (37). It has been proposed that such approaches might accelerate phytoremediation of mercury contaminated soils (38, 39). However, for the treatment of large volumes of mercury contaminated wastewater, an end-of-pipe technology is more appropriate, since it allows to extract and recycle the mercury.

The mercury remediation technology described here offers a highly efficient way to extract mercury from polluted wastewater. It is environmentally friendly, since it works at ambient temperature, requires little electric energy and no extra chemicals. Operating costs are very low. For 100 m<sup>3</sup> of wastewater cleaned, about 17 Euro are required for nutrients to feed the bacteria. It proved to work effectively in technical scale and to be robust against a variety of stresses occurring at a factory testing site. The technology is applicable to wastewater from chloralkali electrolysis factories and to other types of mercury polluted water, e.g. groundwater, soil wash water, and mining wastes.

## Acknowledgments

We thank Dr. Ujma, Preussag AG, for establishing the cooperation to Preussag AG and for continuous support. We would like to thank Dipl.-Ing. R. Böckle and Dipl.- Ing. M. Golm, Preussag Wassertechnik, for constructive and creative cooperation during design, construction, and operation of the pilot plant. We are grateful to Dipl.-Ing. D. Sass, ECI, who opened the doors of ECI Elektrochemie Ibbenbüren to this experiment, and H. Hardemann and K. Bartel of ECI Elektrochemie Ibbenbüren, whose daily support made it possible. This work was funded by grant LIFE97ENV/D/000463 from the European Community.

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Received for review March 23, 2000. Revised manuscript received July 12, 2000. Accepted August 4, 2000.

ES0000652