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ORIGINAL PAPER

X. Deng · D. B. Wilson

Bioaccumulation of mercury from wastewater by genetically engineered *Escherichia coli*

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Abstract Genetically engineered *E. coli*, which express both a Hg^{2+} transport system and metallothionein, were tested for their ability to remove mercury from wastewater. The wastewater contained more than ten different ions, including 2.58 mg/l mercury, and its pH was 9.6. Mercury uptake was faster from the wastewater than from distilled water, probably because of the higher ionic strength, as the high pH had little effect on mercury accumulation. EDTA also stimulated mercury uptake rather than inhibiting it. A hollow-fiber bioreactor was used to retain induced cells for continuous mercury uptake. The cells removed more than 99% of the mercury in the wastewater and the final amount of mercury accumulated was 26.8 mg/g cell dry weight, while none of the other ions were removed from the water. These results indicated that the induced cells had a high affinity and specificity for mercury.

Introduction

Mercury is a toxic heavy metal that poses a serious threat to the environment, and has received increased attention in recent years (Spry and Werner 1991; Liu et al. 1999). Although there are several methods (chemical precipitation, ion exchange, and activated carbon adsorption) that can be employed to remove heavy metals from wastewater, accumulation by microorganisms is a promising approach because of its specificity and ability to function at low concentrations (Tsezos 1985; Belliveau and Trevors 1989). An *Escherichia coli* strain was produced by genetic engineering that can accumulate mercury effectively at very low concentrations (0–4 mg/l) (Chen and Wilson 1997a, b). It contains a mercury transport system (merP and merT) carried on plasmid pSUT and a glutathione-*S*-transferase pea metallothionein gene (GST-PMT) carried on plasmid pGPMT. Metallothioneins (MTS) are low-molecular-weight Cys-rich proteins that bind metal ions and sequester them in a biologically inactive form (Butt and Ecker 1987). It has been proved that genetically engineered *E. coli* cells that express both merP-merT and GST-MT are capable of accumulating mercury effectively at low concentrations (Chen and Wilson 1997b). Previous studies with this organism used solutions that were made with pure chemicals and deionized water. The presence of ions in a solution has been shown to interfere with metal uptake and ion measurements (Volesky and Holan 1995). In this study, wastewater taken directly from a contaminated site near Syracuse, NY, that contained sodium, potassium, magnesium, other ions, and organic matter, was used to test mercury removal (Table 1).

X. Deng
Department of Chemical Engineering, Xiamen University, Xiamen 361005, P.R. China

D.B. Wilson (✉)
Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 15853, USA
e-mail: dbw3@cornell.edu
Fax: +1-607-2556249

Materials and methods

Plasmids and bacterial strains

The bacterial strain used in this study was *E. coli* JM109, which simultaneously harbors two compatible plasmids, pSUTP containing the merP and merT genes, and pGPMT containing the GST-PMT fusion gene (Chen and Wilson 1997a).

Frozen cells were inoculated into Luria broth (LB) containing ampicillin (50 mg/l) and kanamycin (30 mg/l), grown overnight at 37 °C, diluted to an optical density at 600 nm (OD_{600}) of 0.1–0.3 with fresh LB containing ampicillin and kanamycin, and incubated at 37 °C with vigorous shaking (250 rpm). Isopropyl β-D-thiogalactoside (IPTG), 1 M solution in distilled water, was added to 1.0 mM concentration when the OD_{600} reached 0.5–0.7, and after 4 h the cells were harvested by centrifugation at 10,000×*g* for 10 min at 4 °C.

Batch uptake

Induced cells were harvested by centrifugation (10,000×*g* for 10 min, 4 °C), washed with 10 mM phosphate buffer, and resuspended either in wastewater or laboratory surrogates made with

10 mM phosphate buffer, pH 76, or distilled deionized water (ddH_2O) containing the indicated mercury concentration (1000 ppm mercury reference standard solution, Fisher Chemical, USA). After a 1-h incubation at 37 °C, the cells were harvested by centrifugation as above, dried, and digested with 70% nitric acid (trace-metal grade, Fisher Chemical, USA) for mercury analysis. For time-dependent accumulation, the cells were harvested at the indicated time by filtration of 10-ml samples on an 0.20-µm-pore-diameter nitrocellulose filter (Millipore).

Continuous HgII uptake

An Amicon H1MP01–43 hollow-fiber microfiltration cartridge (0.1 µm cut off) was employed as a bioreactor for continuous mercury bioaccumulation. Induced cells were washed and directly resuspended in 500 ml wastewater in a 1-l flask for the reservoir. The dry weight of cells was determined from the OD_{600} using the value of 0.35 g dry weight per liter of OD 1.0 that was found for these cells previously (Chen et al. 1998). The reservoir was closed during operation to make the flow rate of the permeate and the influent the same. The bioreactor was set up as described previously (Brady et al. 1994; Chen et al. 1998) and illustrated in Fig. 1. Bioaccumulation of mercury was monitored by measuring the mercury concentration in cell samples and in the effluent at various times as described below.

Mercurial ions may adsorb and diffuse into container walls, resulting in their loss (Noyes et al. 1976; Jenne and Avotins 1975). In our experiments, all tubing and glassware were routinely treated with a detergent solution, immersed in 20% nitric acid for at least 24 h, and finally rinsed three times with deionized water to minimize adsorption of mercury.

Ion analysis

All mercury analyses were done by the Fruit and Vegetable Analytical Laboratory of Cornell University by inductively coupled plasma atomic emission spectroscopy (ICP-AES). A Thermo Jarrell Ash IRIS Advantage ICP-AES system was used with a duo-view torch. The optical path was purged with nitrogen. A continuous yttrium internal standard was added in the amount of 2 ppm. The RF power was set at 1150 kW and the auxiliary argon flow was 0.5 l/min. The nebulizer was a GlassExpansion "Seaspray," which is a concentric type of nebulizer. The rinse water contained 5 ppm potassium dichromate in order to minimize memory effects due to the volatilization of mercury. Cell pellets were prepared for analysis as described for batch uptake.

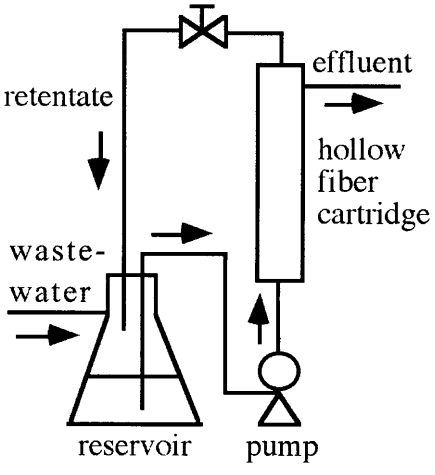

Fig. 1 Schematic diagram of the hollow-fiber bioreactor system

Results

Wastewater profile

Table 1 shows an analysis of the wastewater by ICP-AES. The mercury concentration of the wastewater was 2.58 mg/l, while sodium was the most abundant metal at 1550 mg/l, followed by potassium, magnesium, and calcium, whose concentrations were all higher than mercury. There were also some nonmetal elements such as sulfur, phosphorus, and silicon, and the pH of the wastewater was 9.6.

Time course of bioaccumulation

Genetically engineered cells (0.35 g dry weight/l) were resuspended in wastewater or in a laboratory mercury solution. The results (Fig. 2a) showed that the rate of mercury uptake from both solutions was rapid and the maximum accumulations were reached within 5 min. Furthermore, the induced cells accumulated mercury faster from wastewater than from the mercury solution, which implied that certain components stimulate uptake. To confirm this, we tested mercury accumulation by induced cells from solutions containing 0.5 mM EDTA or 0.1 M NaCl (Fig. 2b). Mercury accumulation was stimulated by both EDTA and NaCl.

Evaluation of bioaccumulation

The results of a 1-h incubation of cells with wastewater and mercury solutions are shown in Fig. 3. The amounts of mercury accumulated from wastewater (A and B) were 30% lower than those accumulated from the laboratory solutions (C and D). The decreased bioaccumulation is probably due to the other ions. Although previous research has shown that several ions do not significantly influence accumulation (Chen and Wilson 1997b; Kuyucak and Volesky 1989), the presence of more than ten ions and organic matter in the wastewater could lead to inhibition. An investigation of the accumulation of heavy metals by a variety of microorganisms showed that the amount of uranium accumulated by nearly all the

Table 1 Components of electrolyte wastewater

Element	Concentration (mg/l)
Ca	3.53
Fe	0.16
Hg	2.58
K	40.58
Li	0.46
Mg	21.75
Mo	0.14
Na	1550
Sr	0.36
Zn	0.11

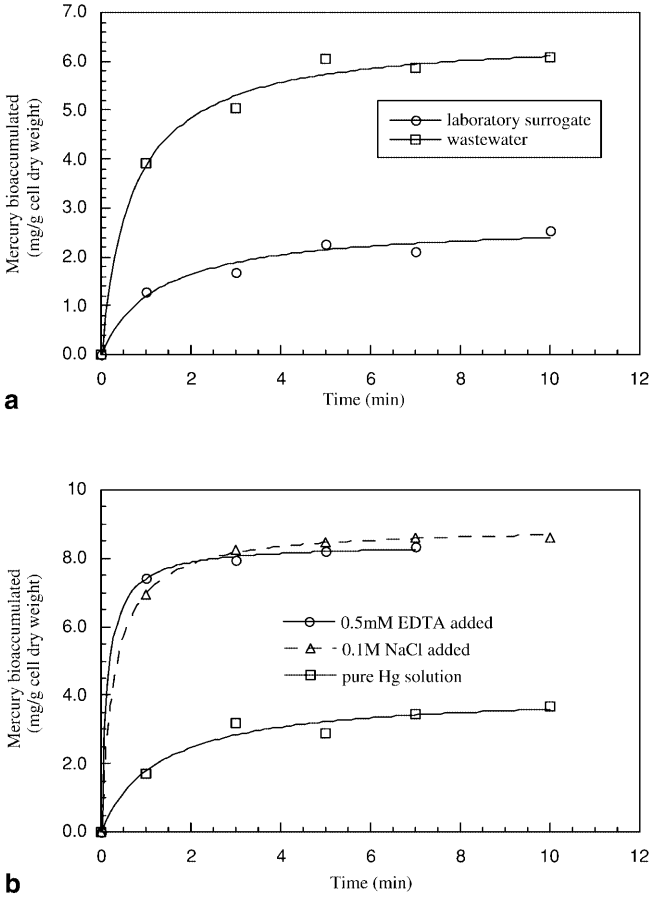

Fig. 2 **a** Time course of mercury uptake by genetically engineered *E. coli* JM109 from wastewater containing 2.58 mg/l mercury (□) and a solution containing 2.50 mg/l mercury made with ddH_2O (○). **b** Time course of mercury uptake by genetically engineered *E. coli* JM109 from solutions of ddH_2O containing 4 mg/l Hg^{2+} (□), 4 mg/l Hg^{2+} and 0.5 mM EDTA (○), and 4 mg/l Hg^{2+} and 0.1 M NaCl (△), respectively

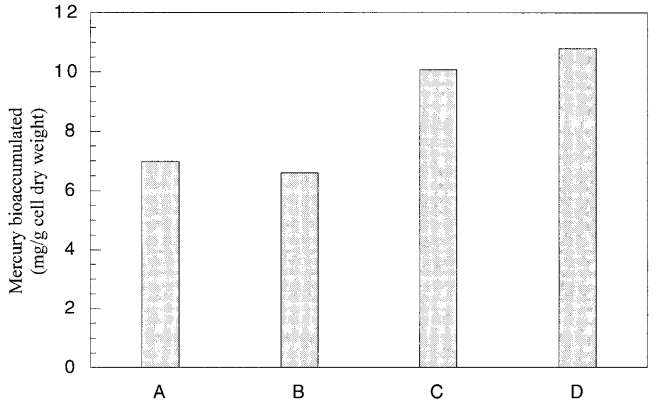

Fig. 3 Comparison of mercury bioaccumulation by genetically engineered *E. coli* JM109 from wastewater and surrogate solutions. Induced cells were resuspended in wastewater with the pH adjusted to 7.6 (*A*) and at the original pH (9.6) (*B*), solutions containing 2.5 mg/l mercury in ddH_2O, pH 7.6 (*C*), or phosphate buffer (pH 7.6) (*D*), respectively. The incubation time was 1 h at 37 °C

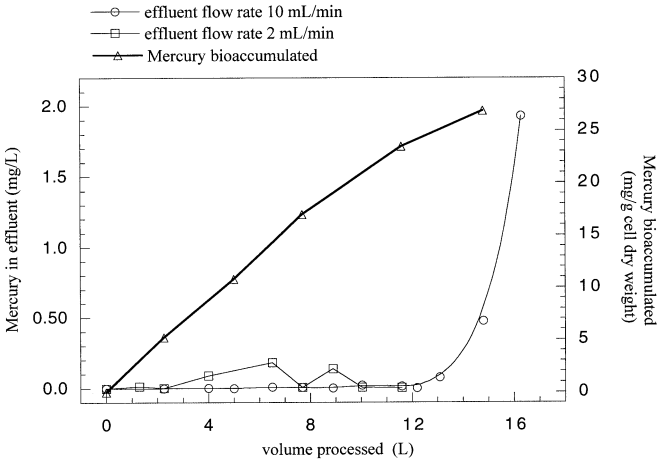

Fig. 4 Continuous mercury bioaccumulation from electrolyte wastewater by genetically engineered *E. coli* JM109 in a hollow-fiber bioreactor. The wastewater volume processed in experiment 1 was 11.65 l with a cell loading of 1.42 g cell dry weight and a 2 ml/min effluent rate (□). In experiment 2, the values were 16.3 l, 1.56 g cell dry weight, and 10 ml/min, respectively (○). Mercury bioaccumulated by the induced cells was monitored in the second experiment (△)

83 microorganisms tested from a solution containing 9 different metal ions was less than that from a solution containing only uranium (Nakajima and Sakaguchi 1986). In the present study, the high pH of the wastewater only slightly decreased the amount of mercury accumulated by induced cells (A and B), in agreement with previous results (Chen and Wilson 1997b).

Continuous bioaccumulation

Evaluation of mercury bioaccumulation needs to involve continuous-flow tests for industrial scale-up. Immobilization of cells can be liable to problems such as cell leakage and mass transfer barriers. Because of the fast kinetics of bioaccumulation, the overall accumulation rate is more often controlled by mass transfer of the sorbed solute (metal) to the active reaction site. Gel immobilization has been widely used with some success (Tsezos et al. 1989; Lewis and Kiff 1988), but intraparticle diffusion barriers usually make mass transfer more difficult and cell leakage hard to overcome. A practical alternative is cross-flow membrane filtration. A hollow-fiber microfiltration system was used to retain the cells in this study.

Two continuous mercury bioaccumulation experiments were run. One was run with a flow rate of 2 ml/min and 1.42 g cell dry weight of induced cells, while 10 ml/min and 1.56 g cell dry weight respectively were used in the other. As shown in Fig. 4, the first trial processed 11.65 l wastewater without any mercury release, while in the other mercury appeared after 13 l with 16.3 l of wastewater processed in total. Almost all the mercury in the wastewater was removed in both runs, and the flow rate did not make a significant difference because the mercury uptake rate was so rapid. The final

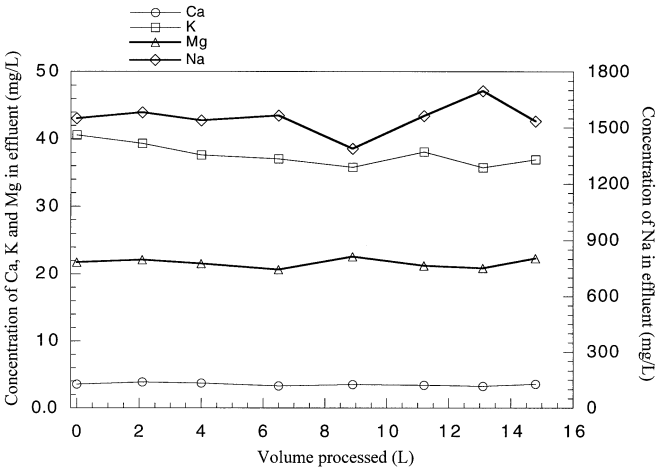

Fig. 5 Concentrations of some ions in wastewater during the mercury bioaccumulation process: ○ calcium, □ potassium, △ magnesium, ◇ sodium. Samples were analyzed as described in the Methods section

amount of mercury bioaccumulation after breakthrough was 26.8 mg/g cell dry weight.

In order to investigate the selectivity for mercury of the induced cells, the concentrations of some other ions in the wastewater were monitored (Fig. 5). The results showed that all these ions remained constant at the initial concentrations, indicating a high mercury selectivity.

Discussion

Previous biological processes have utilized biomass such as algae and dead bacterial cells to remove heavy metals because the materials were inexpensive and easy to handle. Unfortunately, biomass can only remove heavy metals effectively at a relatively high concentration, which is usually not lower than 10 mg/l (Chang and Hong 1994; Brady et al. 1994). The main reason for this is that the accumulation process is surface adsorption. In general, surface adsorption occurs on both inactive and active cells. The genetically engineered bacteria used in this study can accumulate extra mercury inside the cells in addition to the surface adsorption, because of the membrane-associated transport protein.

The first run in Fig. 4 showed no mercury breakthrough as the total amount of mercury (30 mg) run through the reaction was less than the capacity of the cells. In the second run, mercury breakthrough occurred after 33.5 mg had been absorbed and the cells were saturated. The value at saturation, 21.5 mg/g cells, is close to the 18 mg/g cells seen in a previous experiment run on a laboratory mercury solution (Chen et al. 1998).

In a previous study (Nakajima and Sakaguchi 1986), the best biosorbent for mercury was *Bacillus subtilis* IAM1026 among the 83 microorganisms tested. It led to a maximum adsorption capacity of 27.4 mg/g from a solution containing 8 mg/l mercury and eight other ions. Considering that the initial mercury concentration of the wastewater in this study was only 2.58 mg/l, the genetically engineered *E. coli* cells were as good as the best wild-type bacterium. Furthermore, the ability of the recombinant organisms to absorb 99.8% of the Hg^{2+} from the solution is a large improvement over the 51% seen with the best wild-type bacterium (Nakajima and Sakaguchi 1986), although the conditions under which the measurements were made were different.

The stimulation of the rate of Hg^{2+} uptake by EDTA that was observed in batch experiments is very surprising because EDTA should complex Hg^{2+} and this would be expected to inhibit Hg^{2+} transport. However, the result is consistent with a previous study that showed that EDTA did not inhibit Hg^{2+} uptake in a 1-h incubation (Chen and Wilson 1997b).

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