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Bioaccumulation of metals by Coryneform SL-1

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SUMMARY

An environmental isolate defined as Coryneform SL-1 has the capacity to remove large quantities of Pb, Ag, Cd, Hg, Ni, and Zn from aqueous solution. The organism effectively removed a mixture of Pb, Zn, Cd, Cu, and Ni from a simulated waste water. In examining the metal resistance of the organism it was found that agar-containing assay systems yield misleadingly high MIC values.

INTRODUCTION

A problem of growing international concern is the discharge of large quantities of toxic heavy metal directly into receiving water or into sanitary sewer systems where the metal can be tightly bound by sewage plant sludge. The magnitude of the problem is apparent in data from the Narragansett Bay Commission (Rhode Island, U.S.A.), which reported a peak of 956099 pounds of heavy metal released through its sewer system in 1981 [15]. Large metal processors are now required to install chemical pre-treatment systems to remove effluent metals but small operators need a simple inexpensive means of pre-treatment, and preferably a system which allows recovery of the metals.

There is an extensive literature on microorganism-metal relationship with a major emphasis on metal resistance mechanisms [11,14].

A recent review of metal resistance and accumulation by bacteria [2] provides an overview of recent efforts on the ecology, physiology, and genetics of metal resistance and accumulation, which demonstrates the versatility of bacteria in responding to toxic metal ions. Beyond this review there are certain key articles which underline the basic aspects of metal accumulation (bioaccumulation) by microorganisms.

The interaction of microorganisms with heavy metals can occur by several mechanisms [7] and can occur intracellularly, pericellularly (surrounding the cell), or extracellularly. Metal recovery from solution can require active metabolism or be independent of active cell growth [6,16]. Beveridge [3] and Beveridge and Koval [4] have studied the metal binding capacity of Gram-positive and Gram-negative cell walls and concluded that the peptidoglycan must be the more active site in the Gram-positive cell and the hydrophilic face of the outer membrane is the binding site for the Gram-negative cell. Solanellas and Bordons [13] described copper retention by a *Bacillus* at up to 3.8% of its cell dry weight; Kaplan et al. [10] describe the chelating properties of extracellular polysaccharides from *Chlorella*; Ghandour et al. [8] found uptake of silver ions by *E. coli* K12 with total accumulation of 67 μg/g dry weight of cells. It is apparent that metal bioaccumulation involves cellular binding with cell wall material, chelation by extracellular polysaccharides or active transport into the cell. Work by Mullen et al. [12] indicates that La^{3+} accumulates at the cell surface as needlelike, crystalline precipitates while silver precipitates as discrete colloidal aggregations at the cell surface or in the cytoplasm.

This project was designed to determine if a bacterial bioaccumulation system could be used to remove metals from effluent rinse waters of metal plating industries prior to discharge.

MATERIALS AND METHODS

Coryneform isolate and culture media. The bacterium used in this study was isolated from sediment of the Pawtuxet Cove, RI which contained 9.8 μg/g of Hg, 6.3 μg/g of Cd, 3.2 mg/g of Pb, 0.198 μg/g of Sn, and 0.135 μg/g of Zn [1]. The isolate was screened for resistance to heavy metals and carried on media supplemented with heavy metals. This isolate was initially identified as an *Arthrobacter* based on changes in cell form during the

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growth cycle from irregular rods to coccoid cells in older cultures, and on a variable Gram reaction. Rods frequently appeared as Gram-negative but with intracellular Gram-positive granules. We have recently re-examined the cellular morphology and biochemistry of the organism and have concluded it should be called a Coryneform since it did not key to a definite genus in this group. It is motile, oxidase-positive, nitrate reductase-positive, liquifies gelatin, hydrolyses starch, produces acid but not gas, on TSI, and H_2S -.; glucose was weakly fermentative which excludes *Arthrobacter*. Stock cultures were prepared from TSB (with an without metals), harvested from the mid-exponential growth phase, mixed with sterile glycerol to a final concentration of 10% (v/v), dispensed as 2-ml aliqots in small plastic vials, and stored at −70°C. All cultures were incubated at 30°C.

Metal bioaccumulation. Cells were grown in Trypticase soy broth (TSB, BBL) without added metal, harvested by centrifugation at 7500 rpm for 15 min. Cells were washed 3 times in pH 5.5 Pipes buffer [15] (Piperazine-*N,N'*-bis-2-ethansulfonic acid, Sigma) and resuspended in the same buffer at known mg/ml dry weight. The experiment was initiated by adding metal dissolved in deionized water to yield the following final concentration of each metal ion (mM): Cu, 0.08; Cd, 0.15; Cr, 0.96; Zn, 0.77; Ni, 0.85; Pb, 024; Ag, 0.47; and Hg, 0.25. Aliquots (5 ml) were withdrawn from the reactor at the indicated time intervals, and the cells removed by centrifugation. The residual metal in the aqueous phase was determined by atomic absorption spectrometry (Perkin and Elmer model 5000) using appropriate dilutions. Cell-associated metals were determined after digestion with boiling 4 N NaOH followed by 10 N HCl. The digest was brought to standard volume with deionized water.

Minimum inhibitory concentrations. Minimum inhibitory concentration (MIC) determinations were performed in Vogel-Bonner (V-B) broth [5], inoculated with a standardized (300 Klett Unit) suspension of the test organism. For plate assays V-B was solidified with washed agar, seeded with the test organisms and sterile Sensi Discs (BBL) loaded with different concentrations of metal were placed on the seeded agar. After incubation the diameter of the zone of inhibition was determined by measuring the zone at 3 axes and reporting the average to the nearest mm. For MIC determinations V-B medium was dispensed in tubes containing serial dilutions of a metal up to the concentration of metal causing precipitation.

Washed agar. One pound of Difco agar-agar was mixed with 20 l of warm (45°C) tap water. The agar was allowed to settle and the water decanted. This process was repeated 3 times during the first day. The water extraction was repeated once daily for 2 weeks. After extraction the agar was air dried and granulated before use.

Metal removal system. A simulated waste was prepared by dissolving 50 μM of Pb, 388 μM of Zn, 95 μM of Cd, 43 μM of Cu, and 430 μM of Ni in tap water. This was pumped at a rate of 3 ml/min into a New Brunswick Scientific BioFlow C30 containing 2.2 g of cells suspended in 900 ml of Pipes buffer at pH 5.5. The reactor content was agitated at 200 rpm to ensure rapid mixing. The reactor content was continuously pumped by a Watson-Marlow 501U pump running at 100 rpm to a B. Braun Bioprem fitted with a 90-mm Nuclepore 0.2-μM pore size membrane. The Bioprem drive was set for maximum velocity and the permeate flow valve was adjusted to deliver 3 ml/min. The cells retained were fed back to the reactor which provided a constant volume and cell density in the reactor during the experiment. Samples for metal analysis were collected from the permeate discharge line.

RESULTS AND DISCUSSION

Metal inhibition

The MICs, determined by growth in V-B medium, were 0.188 mM for Ag and 0.384 mM for Cr. A true MIC could not be determined for Cd, Cu, Ni, Pb, or Zn due to metal precipitation or color at their respective concentrations above 0.3 mM, 0.16 mM, 1.7 mM, 0.48 mM, and 1.5 mM. The organism was able to grow at these concentrations in V-B medium. Fig. 1 shows the results of the disc assay on TSA. In this assay the minimum inhibitory concentration for Ag was 2 mM and Cd was inhibitory at 4 mM. The difference in the inhibitory level of Ag by the two test systems supports the observation by Hallas and Cooney [9] that agar diminishes the toxic effect of metals.

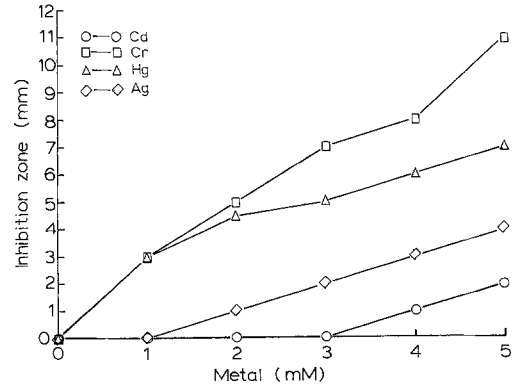

Fig. 1. Disc plate assay of metal toxicity to Coryneform SL-1 on V-B agar. Vogel-Bonner medium was solidified with washed agar; spread plates were seeded with Coryneform SL-1; BBL Sensi Discs contained the indicated concentration of metal. Inhibition zones represent the average of three diameter measurements, and are reported to the nearest mm.

Effect of cell mass on metal removal

As the cell mass was increased in the bioreactor there was an increase in the Cr taken from solution (Fig. 2). Beveridge [3] implicated the cell outer structures as the metal binding site which in turn is supported by these data in that increased cell mass would provide increased sites for binding. Recent studies [12] confirm binding at the cell surface, but in the case of Ag, the metal precipitated at the cell surface and occasionally in the cytoplasm. At a cell dry weight greater than 30–35 mg/ml there was a decrease in metal uptake (data not shown) due to the high viscosity of the cell suspensions which prevented proper mixing of cells and metal-containing aqueous phase. With other approaches not using a stirred reactor this problem may not occur.

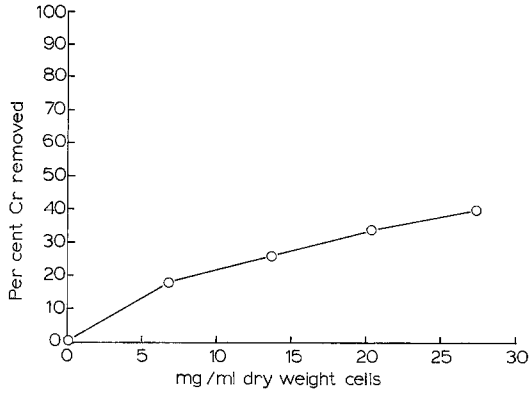

Fig. 2. Relationship between the dry weight of cells used in the bioreactor and percentage Cr removed from solution. Cells were suspended in Pipes buffer (pH 5.5), with agitation at 200 rpm for 1 h at 30°C.

Bioaccumulation of metal

Coryneform SL-1 was tested for metal accumulation with each individual metal (Table 1). After 10 min of reaction the percent metal accumulation ranged from 0 to 99% and after 130 min the range was 48 to 98%. In the cases of Ag and Hg, more metal was cell-associated at the shorter reaction time. This indicates a rapid metal binding followed by release of some metal back into solution, or a transformation of the metal. The Ag precipitation described by Mullen et al. [12], if it occurs with this organism, may not tightly bind all of the metal, allowing desorption back into the aqueous phase. In other projects (unpublished) we have found that the kinetics of metal uptake from solution by *Peudomonas* vary considerably for different metals. With this organism we were able to clear all of the metals from aqueous solution effectively with the exception of Cr and Cu. We have studied other bacteria that will completely remove Cu from solution but have yet to find an organism with greater capacity for Cr accumulation. In the cases of Ag, Cd, Cr, Cu and Ni the metals caused a color change in the bacterial pellets.

Model test system

We devised a model system for metal removal from water using a stirred reactor as described earlier. The simulated waste mixture was formulated at metal levels described by operators of plating operations. The metal waste was pumped into the bioreactor at a dilution rate of 0.2 over the course of 240 min. The effluent was analyzed for residual metal at the permeate outlet every 30 min.

Residual Pb, Cd and Cu were low and constant for the entire process. Cu was effectively removed from the metal mixture but not in studies which used single metals. The Zn and Ni concentrations in the effluent gradually in-

TABLE 1

Metal bioaccumulation by Coryneform SL-1 suspended in Pipes buffer

Metal	Initial conc. (μM)	Residual concentration[a] (μM)		Cell dry weight (mg/ml)	% Cellular accumulation	
		10 min	130 min		10 min	130 min
Pb	240	1.9	5	22.6	99	98
Ag	470	53	133	22.6	89	72
Cd	150	17	8	23.9	89	95
Hg	250	47	105	23.9	81	58
Cr	960	960	498	24.6	0	48
Cu	800	48	38	24.6	40	53
Ni	850	286	48	24.6	66	94
Zn	770	177	29	24.6	77	96

[a] Concentration of metal in aqueous phase.

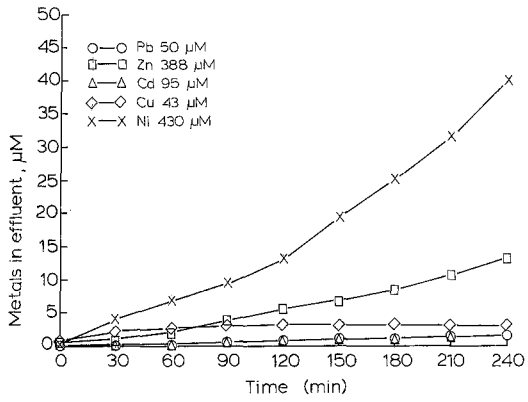

Fig. 3. Residual metal in effluent of treatment system fed with simulated metal waste. The bioreactor volume was 900 ml containing 24.7 mg/ml of Coryneform SL-1 suspended in Pipes buffer at pH 5.5. The flow rate was 3 ml/min which gave a $D = 0.2$, with agitation at 200 rpm and temperature at 30°C.

creased with time and were at 12 and 41 μM, respectively, after 240 min. Even as the system approached saturation with Zn and Ni the organism still removed 97% of the Zn and 90% of the Ni (Fig. 3).

The results indicate that bacterial cells can bind large quantities of metal and mixtures of metals from aqueous solution. In our experience, Cr is the most difficult of the metals studied to remove from aqueous solution. Although the stirred bioreactor approach used in this project may not be the best configuration for industrial application of bacterial metal binding capacity, one advantage of a bacterial system for metal removal is the ease with which the bacteria can be digested for recovery of the metal.

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