A BIOSYSTEM FOR REMOVAL OF METAL IONS FROM WATER

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ABSTRACT

The presence of heavy metal ions in ground and surface waters constitutes a potential health risk and is an environmental concern. Moreover, processes for the recovery of valuable metal ions are of interest. Bioaccumulation or biosorption is not only a factor in assessing the environmental risk posed by metal ions; it can also be used as a means of decontamination. A biological system for the removal and recovery of metal ions from contaminated water is reported here. Exopolysaccharide-producing microorganisms, including a methanotrophic culture, are demonstrated to have superior metal binding ability, compared with other microbial cultures. This paper describes a biosorption process in which dried biomass obtained from exopolysaccharide-producing microorganisms is encapsulated in porous plastic beads and is used for metal ion binding and recovery.
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INTRODUCTION

In today's industrial society, metals are among the most commonly used raw materials. They are introduced into the environment during mining and refining of ores and from other sources, such as the combustion of fossil fuels, industrial processes, the spraying of pesticides, and the disposal of industrial and domestic wastes. In the last two decades, metals have been mined and used extensively by mankind. The combination of a rapidly expanding industry and increases in domestic activities has caused significant environmental problems due to metal ion pollution and critical losses of non-renewable metal resources.(4)

The binding of metals to microorganisms in wastewater treatment plants was noted as early as 1965.(8) Since then, using microorganisms to accumulate metals from solution has been a very attractive topic, not only for water purification but also for the recovery of valuable or economically important metals.(1-3)

There are five predominant metal removal mechanisms by which microorganisms facilitate the removal of soluble metals from solution: 1) volatilization (methylation of metals by living microorganisms), 2) extracellular precipitation (immobilization of metals by the cells' metabolic products), 3) extracellular complexing and subsequent accumulation (chelation of metals with cells' siderophore systems or extracellular polymers), 4) binding to the cell surface (accumulation of metals by cell walls or membranes), and 5) intracellular accumulation (active transport of metal ions against a concentration gradient).

Although mechanisms 1, 2, and 5 are of interest, they require live/functioning bacteria and therefore are more difficult to maintain/operate and not as well suited to the development of practical large-scale processes as are mechanisms 3 and 4 that function well using decayed biomass. Microorganisms that use mechanism 3 have been shown to be superior in terms of metal binding compared with those that use mechanism 4 (1,5,9,10,15,20); however, commercial biosorption processes using such bacteria have not yet been developed.

The use of bioadsorbents in continuous or semi-continuous treatment systems has been attempted using immobilized cell cultures; however, most of these systems use living-functional microorganisms.(11-13) The chief limitations of these immobilized cell systems are those inherent in the use of living cells: susceptibility to cell death, contamination, low mechanical strength of immobilized particles, and the requirement for certain conditions and nutrients to maintain cellular activity. Indeed, metal ions are known to be toxic to microorganisms, including those species that are recommended for use in metal waste treatment systems.(16-19)
The use of dried biomass immobilized in various fashions has been reported recently in systems employing algae (5), bacteria (1,9,10,22), and fungal cells.(1,22) The results obtained using some of these systems confirm that this may well be the most practical application of bioadsorbents for the economical treatment of metal-contaminated wastewater, as this bioadsorbent technology is just beginning to be used commercially.(1-3)

Bioadsorbents clearly show great promise for use in removing and recovering metal ions from contaminated water, but much remains to be done. Although the presence of exopolymers has been shown to account for the superior metal binding abilities of some bacterial species (1,5,10,15,20), other research has illustrated that dried immobilized biomass constitutes a superior method of applying bioadsorbent technology.(9) However, no study has yet been reported in which the dried biomass obtained from exopolymer-producing microorganisms is immobilized and examined in bioadsorption experiments. This paper describes the metal ion binding characteristics of dried immobilized biomass obtained from the exopolysaccharide-producing microorganism Zoogloea ramigera 115 (Z.r.) and a mixed methanotrophic culture IGTMI.

MATERIALS AND METHODS

Bacterial Strains and Culture Media

The organisms used in this study were Z.r. 115 (ATCC 25935) and a mixed methanotrophic culture, IGTM1. The cultivation medium for Z.r. had the following composition (in grams per liter): glucose, 25; K2HPO4, 1; KH2PO4, 0.5; Casamino acid (Difco) 1.0; yeast extract, 0.5. Cultures of IGTM1 were maintained using nitrate minimal salts (NMS) liquid medium or plates, and a 1:1 air-methane mixture was used as the substrate.

The NMS medium had the following composition (per liter): KNO3, 1.0 g; MgSO4, 1.0 g; Na2MO4.2H2O, 0.5 g; trace element solution, 1.0 ml; FeEDTA solution (4%), 0.1 ml; 5% stock PO4 solution, 10.0 ml.

The trace elements solution for NMS had the following composition (per liter): CuSO4.5H2O, 200 mg; FeSO4.7H2O, 500 mg; ZnSO4.7H2O, 400 mg; H3BO3, 15 mg; CoCl2,H2O, 50 mg; MnCl2.4H2O, 20 mg; NiCl2.6H2O, 10 mg; and EDTA, 250 mg.

The phosphate stock buffer solution (pH 6.8) had the following composition (per liter): KH2PO4, 26.0 g; and Na2HPO4, 33.1 g.

The solid medium was prepared by adding 15 g of agar to the liquid medium.
The organisms were cultivated at 23° to 30°C. Cultures were harvested following 3 to 14 days of incubation after viscosities ≥600 centipoise were achieved.

**Exopolysaccharide Production**

The exopolysaccharides produced by Z.r. were monitored by measuring the viscosity of the culture medium, and the exopolysaccharide was purified and quantified according to the procedures described by Norberg and Enfors.(16) A spectrophotometric technique (6) was also used to determine the concentration of exopolysaccharide.

**Immobilization of Dried Biomass**

Biomass (cells plus exopolysaccharides) was harvested by centrifugation at 10,000 g for 10 minutes and dried under vacuum in a rotary evaporator at 65°C. The dried biomass was ground to a uniform fine powder using a mortar and pestle.

The immobilization of dried biomass was typically accomplished by blending 100 g of dry cell powder into a solution containing 100 g of polysulfone per liter of dimethylformamide (DMF). Various biomass-to-DMF ratios were also investigated. The biomass-polysulfone-DMF mixture was transferred through a No. 14 silica tube by a pump and added drop-by-drop into a stirred flask containing distilled water. About 450 to 500 beads (3 to 4-mm diameter) occupying a volume of approximately 15 ml could be formed from 10 ml of the biomass-polysulfone-DMF mixture. The complete leaching/removal of DMF from the beads was accomplished by washing with several changes of water, over a 16-hour period.(9) The resulting beads were stored either at room temperature as dry beads or at 4°C in a pH 6.0 buffer: Na₂HPO₄, 1 g/L; KH₂PO₄, 3.5 g/L.

**Metal Ion Solutions**

The following metal salts were used to prepare aqueous solutions ranging in concentration from 0.001 ppm to 10 ppm: AuCl₃, CdCl₂, CoCl₂·6H₂O, CrCl₃, CuSO₄·5H₂O, HgCl₂, Ni(NO₃)₂·6H₂O, P₆(NO₃)₂, SnCl₂, V₂O₅, ZnSO₄, NaAsO₂, Na₂CrO₄·4H₂O, Na₂MoO₄·2H₂O, H₂SeO₃, Na₂WO₄·2H₂O. Stock solutions of 100 and 1000 ppm were prepared of individual metal ion species as well as mixtures of several metal ions. The pH of stock solutions was adjusted to 4.0 using 1 N HCl.

**Analytical Procedures**

An atomic absorption spectrophotometer (AAS) (Model 403, Perkin-Elmer Co.) and an inductively coupled plasma emission spectroscopy (ICP-AES) (Atom Scan 25, Thermo Jarrell Ash Co.) were used for metal concentration analyses. Samples had to be diluted to 0.1 to 5.0 ppm for accurate analysis. To remove the interference from the suspended particles, samples were centrifuged...
at 600 rpm for 5 minutes and filtered by a 0.45 µm filter before injection into the AAS or the ICPAES.

**Metal Adsorption Studies**

Batch studies were performed using 14 ml (containing 1 g biomass, dry weight) of fresh beads in a 250-ml Erlenmeyer shake flask containing 100 ml of 10 ppm metal solution (pH 6.0). The flask was shaken overnight at a speed of 160 rpm at room temperature. The beads were allowed to settle, and the supernatant was decanted and filtered; the metal ions were then analyzed by AAS or ICPAES.

Continuous-flow metal binding studies were performed using 14 ml of beads (containing 1 g biomass dry weight) in a 25 cm x 2.5 cm column. From 100 ml to 20 liters of metal ion solution at various concentrations were then passed over the column at room temperature. Various flow rates were investigated, but the typical flow rate was 100 ml/h.

**RESULTS AND DISCUSSION**

The solubility of metal ions is a function of both the pH of the solution and the concentration of metal ions. These facts can be exploited to precipitate metal ions from solution; at certain ranges of pH and metal ion concentration, however, precipitation does not occur, as shown in Figure 1. Nearly all of the experiments were performed under conditions in which metal ion binding results because of biosorption rather than precipitation.

![Figure 1. SOLUBILITIES OF METAL HYDROXIDE AS A FUNCTION OF pH (Streaked Area Indicates the pH and Concentration Range for Most Experiments)](image)

Because this work focused on the use of microorganisms that produce water-soluble exopolysaccharide, the chief concern with the use of the polysulfone system was that these exopolysacchar-
rides would leach out of and diminish the metal binding capacity of the polysulfone beads. To evaluate this concern, metal binding experiments were performed using polysulfone-encapsulated biomass obtained from Escherichia coli (E. coli), Z. r. 115, IGTMI, and exopolysaccharide purified from Z. r. 115 according to published techniques. A mixed metal ion solution containing 10 ppm each of Cd, Co, Cu, Ni, and Zn was used in 16-hour binding experiments at 23°C and a pH of 6.0. One gram (dry weight) of biomass encapsulated in polysulfone beads was used for each test that were performed and analyzed as described in the Materials and Methods section above. Figure 2 shows the results of these tests. Biomass beads prepared from whole cultures of Z. r. 115 or IGTMI yield clearly superior metal binding results compared with biomass derived from E. coli (a culture that produces relatively little exopolysaccharide) or the polysulfone control. These results also indicate that biomass beads prepared from purified exopolysaccharide are inferior to beads prepared from whole culture, suggesting that the water-soluble exopolysaccharide can indeed be leached from polysulfone beads but the encapsulation of whole cultures (bacterial cells plus exopolysaccharide) could overcome this problem. This point was investigated further by examining the supernatants of metal ion binding experiments and by water-washing experiments. A spectrophotometric assay was used to detect the presence of exopolysaccharide in the supernatant of the metal binding tests. This assay confirmed that exopolysaccharide was leaching from biomass beads containing purified exopolysaccharide but not from beads prepared using whole cultures. Furthermore, washing tests were performed in which biomass beads were exposed to a continuous stream of water for 48 hours, after which their metal binding ability was retested. The metal binding ability of water-leached biomass beads prepared from whole cultures was undiminished, whereas biomass beads prepared from purified exopolysaccharide exhibited drastically reduced metal binding ability; this is only marginally better than the polysulfone bead control (data not shown).

The Effect of pH on Metal Binding

Previous research concerning the metal ion binding ability of Z. r. or its purified exopolysaccharide has shown that pH has a profound effect, and the manipulation of pH has been advocated as a control mechanism in biosorption processes using Z. r. biomass. (16-19) The response of polysulfone-encapsulated biomass to pH was investigated by performing titration experiments and by evaluating metal ion binding ability at various pHs. Titration experiments were performed by equilibrating 14-m1 aliquots of polysulfone beads, beads containing Z. r. 115 biomass, and beads containing IGTMI biomass in a 50-mM phosphate buffer (pH 7.0) overnight. The beads were recovered by centrifugation, washed in distilled deionized water, and then put in 100 ml of distilled water and titrated with 1 N NaOH or HCl to pHs of 12.0 and 1.0. The results (Figure 3) show that the polysulfone control and Z. r. 115 beads yielded nearly identical results and displayed essentially very small buffering capacities. However, the encapsu-
Figure 2. THE METAL BINDING ABILITIES OF DIFFERENT SELECTED BACTERIAL STRAINS
**Figure 3.** TITRATION CURVES OF POLYSULFONE-BIOMASS BEADS
lated IGTMI biomass displayed a more gradual change in pH value, responding to each drop of NaOH or HCl solution added.

The metal ion binding ability of biomass beads as a function of pH was also investigated: Polysulfone control, Z.r. 115 beads, and IGTMI beads were incubated overnight with a standard 10-ppm solution of Cd, Co, Cu, Ni, and Zn at various pHs; then metal binding/precipitation was measured by centrifuging the solution and determining the concentration of metal ions in the supernatant. The pHs of the solutions were measured after incubation (24 hours) as the equilibrium pH. The result of the metal ion precipitation measurement of the polysulfone control is shown in Figure 4, which shows that essentially no precipitation occurs in the polysulfone control up to pH 6.0, whereas almost all of the metal ions precipitate at a pH of 8.0 and above. The results of metal ion binding/precipitation measurements of biomass-containing beads (Figures 5 and 6) illustrate that the Z.r. 115 and IGTMI encapsulated beads outperformed the beads that contain only polysulfone (Figure 4) at every pH, indicating that the biosorption of metals can be accomplished over a wide pH range using polysulfone-encapsulated biomass beads. Furthermore, these data illustrate that biomass beads have a considerably greater affinity for the binding of copper than the other four metal ions used in this study.

**The Effect of Temperature on Metal Binding**

The effect of temperature on metal ion binding was tested using Z.r. 115. The results, shown in Figure 7, show that the metal binding ability of Z.r. beads decreased when the temperature was higher than 40°C. This result differed from those of other researchers (1), who showed that the binding ability of biomass increased as the temperature increased. High temperature would cause the exopolysaccharide to dissolve or leach, which may explain why the results reported here differ from those of previous research.(1)

**The Kinetics of Metal Binding**

The kinetics of metal ion binding were investigated using biomass beads reacted in a shake flask for 15, 30, 60, and 120 minutes and overnight using the standard batch experiment procedure. The supernatants were then analyzed for their metal concentration. The results shown in Figure 8 suggest that the majority of metals are bound in less than 15 minutes. More than 90% of the metal ions were adsorbed in 30 minutes, and after 60 minutes the metal ion binding process was essentially complete. Samples that were reacted overnight were treated as 100% saturated. Based on this information, subsequent batch mode metal binding experiments were performed using contact times of 60 minutes. The kinetics of metal ion binding were also investigated using columns of biomass beads operated at various flow rates. Five 100-ml portions of metal solution were passed through five identical columns that contained 14 ml of beads,
Figure 4. METAL BOUND/PRECIPITATED BY POLYSULFONE AS A FUNCTION OF pH
Figure 5. Percent Metal Bound/Precipitated by Z.I. 115 Beads as a Function of pH.
Figure 6. METAL BOUND/PRECIPITATED BY IGMTM1 BEADS AS A FUNCTION OF pH
Figure 7. THE EFFECT OF TEMPERATURE ON THE METAL BINDING ABILITY OF Z.r. 115 BEADS
Figure 8. METAL BOUND BY Z.r. 115 BEADS AS A FUNCTION OF CONTACT TIME
using the following times: 15, 30, 60, 90, and 120 minutes. Samples reacted overnight in shaking flasks were treated as 100% saturated. The results, shown in Figure 9, demonstrate that more than 90% of the metal ions were adsorbed at a flow rate of 100 ml/30 minutes.

**Interference of Common Metal Ions (Na, K, Mg, and Ca) and EDTA**

The interference of common metal ions (Na, K, Mg, and Ca) on the binding of heavy metal ions was investigated by preparing 10-ppm metal solutions in a pH 6.0 phosphate buffer solution (which contained 7 ppm Na and 23 ppm K), 10 ppm and 1000 ppm of Mg solutions, and 10 ppm and 1000 ppm of Ca solutions. The metal binding ability of Z.r. 115 beads in these solutions was compared with a control experiment that contained no buffer or other metal ions. The results, shown in Figure 10, demonstrated that the interferences of the pH 6.0 buffer, 10 ppm Ca, and 10 ppm Mg solutions were minor (0% to 10%), but the 1000 ppm Mg or Ca solutions caused as much as 65% to 75% interference. The interference of 25-mM EDTA on metal ion binding was evaluated using the standard batch procedure; 70% to 90% inhibition of metal ion binding was observed for both Z.r. 115 and IGTMI biomass beads.

**Treatments for Disinfection and Storage of Beads**

Experiments were performed to determine the effect of drying, autoclaving, and freezing on polysulfone beads containing Z.r. 115 biomass. Encapsulated biomass beads were prepared in the usual way, and a sample was dried for 48 hours at 103°C to remove all moisture. Similarly, a sample of beads was autoclaved at 121°C and 25 atm for 20 minutes. These beads were then included in typical metal binding shake flask experiments. Control experiments were performed using untreated polysulfone beads containing Z.r. 115 biomass. The performance of the dry beads was not quite as good as the control, but they had a tendency to float and were not fully wetted, so these results may be an underestimate of the binding ability of dried beads. Redissolved frozen beads and autoclaved beads were tested and found to have the same binding ability as the fresh beads (data not shown). The results obtained with autoclaved beads were unexpected based on the results of the temperature study, shown in Figure 7. The reason for the difference may be that the beads for this experiment were autoclaved using a minimum volume of water and the metal binding test was subsequently performed at room temperature. On the other hand, the temperature study was performed using 14 ml of beads in 100 ml of solution. In any event, these results suggest that biomass beads can be successfully dried, autoclaved, or frozen while retaining their metal binding ability.

**Effect of Biomass Concentration**

A batch of dried Z.r. 115 biomass was prepared and polysulfone beads were made that contained a range of concentrations of
Figure 9. Metal bound by Z.E.I. 115 beads as a function of column flow rate.
Figure 10. INTERFERENCE OF COMMON METALS ON THE BINDING ABILITY OF Z.r. 115 BEADS
biomass: 10, 50, 100, 200, and 300 grams per liter of DMF-polysulfone solution (containing 100 g of polysulfone in 1 liter of dimethylformamide). Ten ml of each type of bead were tested by the standard procedure. The results of these experiments (Figure 11) show that the amount of metal ions bound increased linearly with increased concentrations of biomass, but the curve began to flatten out when the biomass concentration was about 300 g/L. The results shown in Figure 11 were then converted to the capacity diagram (Figure 12), and they showed that the metal binding capacity (mg of metal bound/g of dry cells weight) was constant (except for Cu) for biomass concentrations up to about 200 g/L but declined at higher biomass concentrations. These experiments indicate that a concentration of 50 to 200 g biomass/L yields beads that effectively use the maximum metal binding capacity of the biomass. As mentioned, a biomass concentration of 100 g/L was used for the majority of experiments.

Binding Tests With Other Heavy Metals

In addition to the study of the binding of Cd, Co, Cu, Ni, and Zn from mixtures containing 10 ppm of each of these ions, the binding of Au, Cr, Hg, Pb, Sr, and V by encapsulated Z.r. 115 biomass from a mixture containing 10 ppm of each ion was examined. A shaking flask binding experiment using a pH of 6.0 was performed. After overnight incubation at room temperature, the pH had shifted to 5.3 and the amount of metal bound was Au, ≥99%; Cr, ≥99%; Hg, ≥95%; Pb, ≥95%; Sr, 43%; and V, 90%. (The concentrations of Au, Cr, Hg, and Pb ions after bioadsorption were below the limits of detection for the conditions employed in these experiments). This degree of metal ion binding compares very favorably with the results previously obtained with other metals. In general, therefore, all of the metal cations tested showed good bioadsorption using polysulfone/biomass beads.

The binding of metal anions was also investigated using a solution containing 10 ppm each of NaAsO₂, Na₂CrO₄-4H₂O, Na₂MoO₄-2H₂O, H₂SeO₃, and Na₂WO₄-2H₂O. No significant binding of any of these anions was observed.

Multiple Cycles of Binding and Elution

The elution of metal ions bound to biomass beads was investigated using 0.1N HCl (9), as well as the ability of these beads to subsequently be used to bind additional metal ions. Metal ion binding experiments were performed using a 10-ppm mixed metal ion solution according to the standard protocol; then the beads (14 ml) were placed in 100 ml of 0.1N HCl (pH 1.6) and shaken overnight at a speed of 160 rpm at room temperature. The beads were then removed by centrifugation and the metal ion concentration in the resulting supernatant was determined. For reuse, the beads were washed five times with 100-ml portions of distilled de-ionized water to remove the HCl and then exposed overnight to 100 ml of pH 6.0 buffer.
Figure 11. METAL BINDING ABILITY OF Z.r. 115 BEADS AS A FUNCTION OF BIOMASS CONCENTRATION
Figure 12. METAL BINDING CAPACITIES OF Z.r. 115 BEADS AS A FUNCTION OF BIOMASS CONCENTRATION
Multiple Cycles of Binding and Elution

Multiple cycles of binding and elution of metal ions using biomass beads were tested; the results are shown in Figure 13. The metal binding efficiency in this experiment was nearly equal for each of the nine cycles. This indicates that the amount of metal ion that is irreversibly bound to the biomass beads was minor and not cumulative such that the majority of the metal ion binding capacity of the beads was available for each cycle of binding. Since no loss in binding efficiency was apparent, the maximum number of times biomass beads can be reused is unknown but certainly exceeds nine cycles and may be significantly higher. Other researchers using a similar approach demonstrated as many as 75 cycles of binding, and elution can be performed without significant losses in efficiency.(9)

Bioadsorption Studies With Low-Concentration Solutions

Experiments with large volumes of water contaminated with low concentrations of metal ions (0.1 ppm) showed that the encapsulated biomass beads had a good ability to accumulate the metal ions, which could subsequently be easily eluted in a concentrated form. The experiment was set up by using three columns connected in series (10-ml, 20-ml, and 20-ml beads). Ten liters of 0.1 ppm mixed metal ion solution was passed at a speed of 100 ml/h through the columns. Then, each column was eluted with 100 ml of 0.1 N HCl at the same speed. The control was treated using the standard procedure. The efficiency of metal binding was assessed based on the quantity of metal eluted from the columns because the quantity of metal ions in the effluent was below the limits of detection. The results showed that greater than 90% of each of the five metal ions could be absorbed by the first two columns (30 ml beads total) and essentially no metal binding to the third column occurred (data not shown). These results illustrate that biomass beads can be used effectively to remove metal ions from dilute solutions, subsequently recovering the bound metals in a concentrated form (200 ml or 2% of the original volume). Further experiments with elution conditions might well achieve an even greater concentration of recovered metal ions.

Separation and Purification of a Mixed Cadmium and Nickel Solution

An experiment was performed to simulate the use of exopolysaccharide-containing biomass beads to simultaneously decontaminate a mixed metals-contaminated wastewater and recover the individual metal ions in a concentrated and purified form. Five liters of water containing 0.2 ppm each of Cd and Ni ions was passed through a biomass bead column at a flow rate of 100 ml/h. The bound metals were subsequently eluted using 100-1 portions of eluant solutions under investigation at IGT. Approximately 90% of the metal ions were removed from solution yielding clean water. The bound metal ions were subsequently recovered at 89% and 76% purity for Cd and Ni, respectively, in a volume of 100 ml
Figure 13. THE PERFORMANCE OF IGMT1 BEADS THROUGH MULTIPLE CYCLES OF METAL BINDING AND ELUTION
CONCLUSIONS

Currently, more than 90% of the wastewater treatment systems for heavy metal removal use caustic and/or sulfide precipitation (chemical treatments). These systems possess a very important operating deficiency: They produce a metal sludge as the product of the chemical precipitation. The sludge is the focal point of substantial regulation; the producer of this sludge must retain responsibility for its safe disposal for a period of 10 years.(3) This responsibility for toxic sludges, therefore, is a compelling factor driving industry to search for improved metal abatement technologies.

The use of biomass encapsulated in polysulfone is a good alternative to current practices because it is an efficient, convenient, and reliable metal-removal method that eliminates the disposal problem by allowing metals to be purified and recovered. The use of biosorption by industry has already occurred, but the use of immobilized biomass from exopolysaccharide-producing microorganisms has just begun to be investigated. Initial results are highly promising and may well yield an efficient and effective process for the removal and recovery of metal ions from wastewater.

REFERENCES CITED


IMMOBILIZED BED OF BIOMASS-POLYSULFONE BEADS

○ Cd: 1 mg
○ Ni: 1 mg

+ 0.1 L 76% NICKEL
+ 0.1 L 90% CADMIUM

5 L CONTAMINATED WATER

CLEAN WATER

Figure 14. SEPARATION AND PURIFICATION OF MIXED Cd AND Ni SOLUTION


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