REMOVAL OF HEAVY METAL IONS FROM CONTAMINATED WATER BY CHLORELLA VULGARIS

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SUMMARY

Several aspects of the binding of heavy metal ions with Chlorella vulgaris in aqueous solutions were investigated. In particular, it was found that several heavy metal ions may be removed successfully from solution by adsorption onto the cell walls of the algae, or by precipitation with macromolecules that are probably proteins derived from the algae cells. Some operating conditions which were studied included pH, salt effects, the effect of competing metal ions, and the mass of algae used. Specific attention was given to the binding of Hg^{2+} (very strong binding occurs), which is suspected of binding to sulfhydryl groups on the algae surface. The binding of other metal ions, including Pb^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cr^{3+}, Al^{3+}, and Ca^{2+}, also were investigated, and results with these ions indicated that the binding to the algal cells was very pH dependent. With these metals the binding is reversed by lowering the pH.

It was shown that ionic strength alone is not a limiting factor when working with salt containing solutions, but that competition of certain ions such as Ca^{2+} of Mg^{2+} for binding sites or interactions with complexing ligands may limit the use of Chlorella vulgaris for metal removal operations in some waters.

The UO_2^{2+} ion also was bound very strongly to algal cells, and the successful removal of UO_2^{2+} from contaminated waters taken from

the Ambrosia Lake area in northern New Mexico indicated that the use of Chlorella vulgaris may be an attractive alternative to the use of ion-exchange resins in water clean-up operations. In one sample, the UO_2^{2+} concentration was reduced from about 20 ppm to 1.4 ppm using 10 mg/ml algae (dry weight). These results indicate that the use of , Chlorella vulgaris is promising for large scale use in removal of heavy metal ions from mining or industrial wastewaters.

INTRODUCTION

The development of effective, low-cost water purification and recovery techniques for the removal of heavy metal ions from water supplies is important from both environmental and economic standpoints. The interactions of some microorganisms with heavy metal ions in aqueous solutions may result in an active uptake or binding of the free metal ions to the cell. Complexation or precipitation of metal ions by substances derived from the organism also may occur. Many reports on the abilities of several microorganisms including bacteria, fungi and various algae to accumulate heavy metal ions indicate promise for industrial exploitations in the microbiological recovery of Pb^{2+}, Cd^{2+}, Zn^{2+}, UO_2^{2+}, Ni^{2+}, and other metal ions from contaminated waters (Ferguson and Bubela 1974; Kollman and Lynch 1981; Brierly and Brierly 1981; Nakajima, Horikoshi, and Sakuguchi 1981; Khummongkol, Caterford, and Fryer 1982; Galuh, Keller, and Malki 1983; Wook and Wang 1984). The success of the biosorption process depends on the organism, conditions of pH, metal ion and salt concentrations, and the presence of competing metal ions or complexing ligands in the solution.

The organism Chlorella vulgaris, a weedlike green algae grown locally and inexpensively, is known to interact with several important heavy metal ions in aqueous solutions (Ferguson and Bubela 1974; Khummongkol, Caterford and Fryer 1982). To further test the application of Chlorella vulgaris to the biotechnological removal of a variety of heavy metal ions from contaminated waters, we experimentally studied several realistic operating parameters. In a specific application study, we can report the

successful removal of uranium from contaminated ground waters from the Ambrosia Lake area of New Mexico.

Mechanisms for the biological removal of metal ions from aqueous solutions may be divided into three categories: (1) adsorption onto cell walls, (2) intracellular uptake, and (3) chemical transformations of metal ions by organisms, including complexation or precipitation by biologically derived materials. The first and third mechanisms are of particular interest because they may occur under conditions normally toxic to living cells. Evidence suggests that adsorption of metal ions on the surface of either living or dead microorganisms is an ion-exchange process involving amine, carboxyl, hydroxyl, sulfate, sulfhydryl, imidazole, or amide functional groups (Kollman and Lynch 1981; Driscoll, Hassett, and Shecher 1982; Walsh and Garnas 1984). Processes that compete with adsorption of metal ions on the cell surface may be complexation or precipitation by biologically derived solution species (Bird and Haas 1931; Ferguson and Bubela 1974; Laegreid et al. 1983). The latter behavior is typical of metal ion-humic or fulvic acid interactions (Saar and Weber 1980; Saar and Weber 1982). We present evidence that this occurs with Chlorella vulgaris and may also play an important role in the distribution of metal ions in algae-treated waters.

MATERIAL AND METHODS

Chlorella vulgaris was cultivated outdoors in growth media containing KH_2PO_4 (0.3 g/1), $MgSO_4 \cdot 7H_2O$ (0.3 g/1), KNO_3 (2.0 g/1), and urea (0.6 g/1) made up in tap water. The pH was maintained near 5.5 with carbon dioxide. The cells were harvested by centrifugation, dialyzed against distilled water to remove salts, and lyophilized. In metal uptake experiments, this material was resuspended in the desired solution. Adjustments of pH were made with concentrated NaOH or HNO_3.

Most metal ion uptake experiments were performed in 0.05 M sodium acetate, a complexing media chosen in part to help prevent the precipitation of metal hydroxides. Experiments were typically performed by suspending a known mass of algae and adjusting the pH. The

appropriate metal ion(s) was then introduced into an aliquot and the suspension was mixed continuously or at frequent intervals. Prior to metal ion analysis, the pH was re-checked and recorded. After the required time, the reaction mixture was centrifuged to remove algal cells, decanted immediately, and the supernatent was analyzed for the appropriate metal ion. The results of several kinetic studies indicated that in general, equilibrium is reached before one hour with an initial metal ion concentration of 1.00×10^{-4}M. The reaction of p-Hydroxymercuribenzoate with algae cells was fast, within two minutes. In most metal ion removal experiments, an equilibration time of two hours is allowed. Precipitation experiments were performed using the supernatant from an algae suspension. Metal ion analyses were performed on the supernatants by Flame Atomic Absorption Spectrophotometry (FAAS) or Direct Current Plasma Emission Spectrophotometry (DCP).

An Instrumentation Laboratory 457 atomic absorption/atomic emission (air-acetylene flame) or a Spectrametrics Spectraspan V direct current plasma emission spectrophotometers was used. Several analyses with DCP were performed using a multielement cassette for the simultaneous determination of Pb^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Al^{3+}, Cr^{3+}, Mg^{2+}, and Zn^{2+}. Where possible, matrix matching was used to minimize possible ionization interferences. The method of standard additions was used to quantitate initial concentrations of uranium in ground water samples; background scans of the emission spectrum around the uranium peak at 424.2 nm showed less than 5 percent interference. The precision of either method was within 1-3 percent at the 10^{-4} to 10^{-6}M levels. Other instrumentation used was a Spex Fluorolog spectrofluorimeter, a Perkin Elmer model 320 uv-visable spectrophotometer, a Perkin Elmer model 283-B infrared spectrophotometer, and a BAS model CV1 voltage generator equipped with an X-Y recorder and a voltmeter.

RESULTS AND DISCUSSION

The Effect of pH on the Removal of Metals from Algae Suspensions

The amounts of metal ions removed by adsorption on algal cell walls, or by precipitation due to interaction with material derived from the

algae cells, may be strongly dependent on pH, depending upon the particular metal ion. Figure 1 shows the pH dependence of the simultaneous removal of Pb^{2+}, Cd^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Cu^{2+}, Ni^{2+}, and Co^{2+}, each at an initial concentration of $1.00 \times 10^{-4}M$ from aqueous solutions containing algae at 5 mg/ml (dry weight). Diagrams of this type are useful in determining optimum pH conditions for metal removal. Reversal experiments, in which equilibrium mixtures of metal ions and algae are acidified, indicated that the binding of Pb^{2+}, Cd^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Cu^{2+}, Ni^{2+}, and Co^{2+} was reversible and that these ions, adsorbed at pH values ranging from 2-7, may be quantitatively desorbed by lowering the pH.

Examination of figure 1 shows that the removal of most of the metal ions by the algae suspension is very pH dependent. It is also apparent from this figure that selective removal of metal ions can be accomplished by judicious adjustment of pH. At pH 3.5, Al^{3+}, and Pb^{2+} can be rather selectively removed. In the pH 6-7 range most of the metal ions tested can be significantly removed under these conditions.

We have found that some metal ions are not only bound to the surface of the algae cell, but also to macromolecules that are solubilized when the lyophilized algal cells are suspended in solution. Several experiments showed that the clear supernatant solution resulting from a centrifuged algae suspension, which we refer to as "algae supernatant," has the capacity to react with certain metal ions to produce a precipitate. The formation of this precipitate results in a decrease of the metal ion concentration in the solution. Spectral analysis (UV, fluorescence, IR) give indications that the metal-containing precipitate contains proteinaceous material. The IR spectrum of the uranyl-precipitate very closely resembles that described by Nakajima et al. (1981), who claim it is that of uranyl ion adsorbed on the cell walls of Chlorella regularis. A more accurate mechanism for the removal of uranyl ion, which may be precipitated extensively (figure 2), is perhaps bioprecipitation or bioflocculation (in 0.05M sodium acetate), rather than bioadsorption. The pH studies in figure 2 with UO_2^{2+} showed that UO_2^{2+} was very strongly removed by either the algae suspension
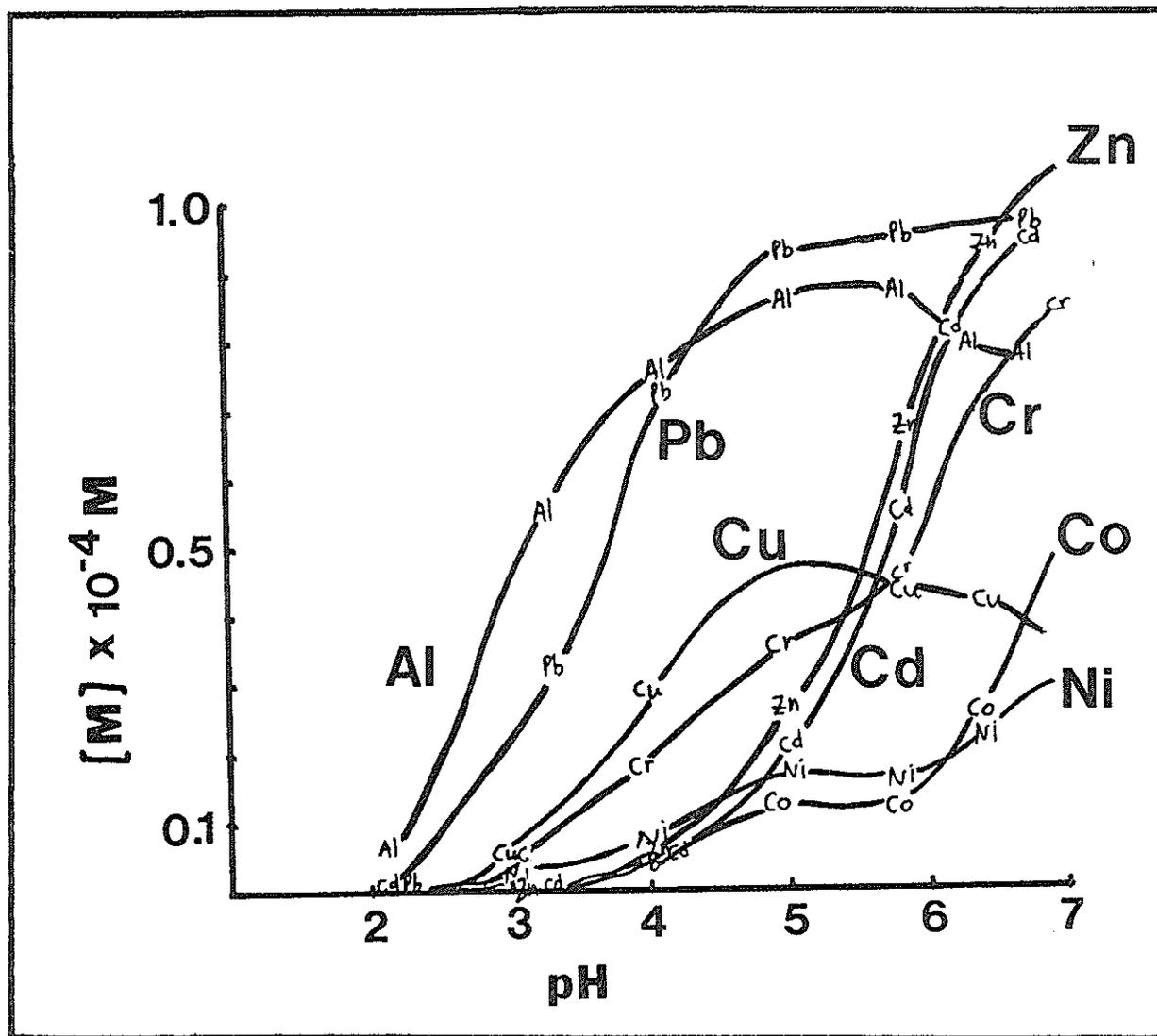
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Fig. 1. The Effect of pH on the Removal of Eight Different Metal Ions from aqueous Solutions by <u>Chlorella vulgaris.</u> Algae (5 mg/ml, dry weight) was reacted at the appropriate pH's with solutions which were initially $1.00 \times 10^{-10}M$ in Pb^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, and Ni^{2+} (from an equimolar stock solution of the metal acetates) in .05 sodium acetate. After two hours, the mixtures were centrifuged and the supernatants were analyzed for each metal. The ordinate represents the concentrations of metal ions which are removed from the solution, i.e., the initial concentrations minus the final concentrations of each metal in the solution, and includes both the effect of adsorption by the cells and precipitation by macromolecules (see text).
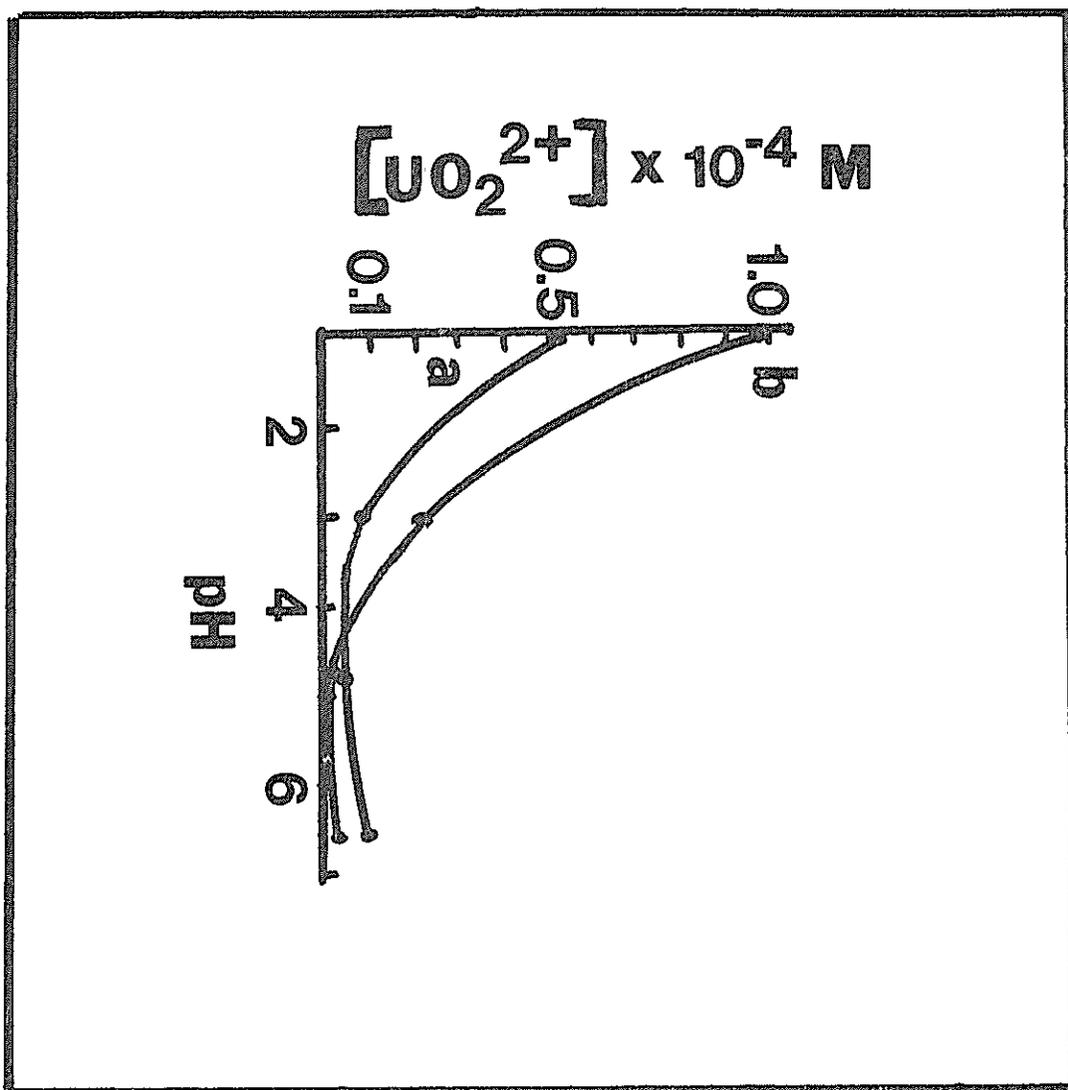
Fig. 2. The Effect of pH on the Removal of UO_2^{2+} by Adsorption and Precipitation. Algae suspensions (a), 5 mg/ml (dry weight), were reacted with 1.00×10^{-4} M $UO_2(C_2H_3O_2)_2$ in 0.05 M sodium acetate at pH's in the 1-7 range. Curve (b) is obtained from the corresponding reactions of $UO_2(C_2H_3O_2)$ with the supernatant obtained from centrifugation of 5 mg/ml (dry weight) suspensions of algae. The ordinate represents the final concentration of UO_2^{2+} measured in the solutions after centrifugation.

or the algae supernatant in the pH 4-6 range. Below this range, from pH 1-3, it becomes possible to differentiate the amounts of UO_2^{2+} bound to the cells and in precipitated form. Although it is experimentally difficult to measure the amount of precipitated metal ion in an algae suspension, experiments with algae supernatants give first approximations to the amounts of metals that are removed by the soluble macromolecules. Subtracting the quantity of metal ion precipitated in a supernatant experiment from the total quantity of metal ion removed in the corresponding algae-suspension experiment gives a calculated value for the amount bound to the cells. Some metals for which precipitation by the algae supernatant can be significant are Pb^{2+}, Cu^{2+}, UO_2^{2+}, Hg^{2+}, Fe^{3+}, and Al^{3+}.

In several experiments with Hg^{2+}, it was desired to minimize the effect of precipitation due to Hg^{2+} interactions with the algae supernatant while maintaining strong binding to the cells. It was found that a pH 2 Hg^{2+} is not precipitated by the algae supernatant while greater than about 80 percent of the metal (at an initial concentration of $1.00 \times 10^{-4}M$) is bound to the cells using an algae concentration of 1.5 mg/ml.

The Effect of Salts on the Removal of Metal Ions by Chlorella vulgaris

The extent of metal ion removal is affected by the presence of certain salts in solution. Experiments showed that the removal of Pb^{2+} or Zn^{2+} (5 mg/ml algae, pH 5.5) was not inhibited in solutions containing up to 1.0 M concentrations of KNO_3. This suggests that ionic strength alone has little effect on adsorption of the metal ions. Other salts, however, may play a major role. Under the same conditions as above, increasing Ca^{2+} or Mg^{2+} concentrations resulted in an interference with the removal of Pb^{2+} or Zn^{2+}. This suggests that high concentrations of Ca^{2+} and Mg^{2+} may successfully compete for binding sites otherwise available to the heavy transition metal ions.

The presence of complexing ligands also may inhibit metal ion binding. Experiments with increasing sodium acetate concentrations (same conditions as above) also resulted in decreasing amounts of Pb^{2+} and Zn^{2+} removed, as might be expected on the basis of competing metal ion

complexation by acetate. Other anions such as carbonate, bicarbonate, chloride, and sulfate may also cause interferences in the removal of heavy metal ions, and further investigations into the effect of these ions are in progress.

The Effect of Algae Mass on the Removal of Heavy Metal Ions

A comparison of the binding of several heavy metal ions to algae cells suggests that distinct categories of metal ion binding sites exist. These include: (1) very strong binding metal ions such as Hg^{2+} and possibly UO_2^{2+}; and (2) metal ions which are more weakly bound and which exhibit pH dependent binding. These include Pb^{2+}, Cd^{2+}, Zn^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Cr^{3+}, Cu^{2+}, and Fe^{3+}. In the case of reaction with a very strong binding metal ion, such as Hg^{2+}, concentrations of algae at about 1 mg/ml were sufficient to remove essentially all of the metal ion from solutions containing $1.00 \times 10^{-4}M$ concentrations of metal ion. This suggests that specific binding sites are responsible for the strong interactions.

Competition effects may occur in the algae supernatant, where complexation and precipitation equilibria may be altered by the presence of other metal ions. Results show that the amount of Pb^{2+} which was precipitated by the algae supernatant was decreased by more than 50 percent when equimolar amounts of Zn^{2+} and Cd^{2+} were present. This suggests that Zn^{2+} and Cd^{2+} not bound to the algae may preferentially form soluble complexes with the components in the supernatant which would otherwise precipitate Pb^{2+}.

The Removal of Uranium from Contaminated Ground Waters with Chlorella vulgaris

Metal ions originating from uranium milling operations may enter ground water supplies. In addition to UO_2^{2+}, other important heavy metal ions which may be present are vanadium, MoO_4^{2-}, Co^{2+}, Zn^{2+}, arsenic, Cu^{2+}, Ni^{2+}, and Pb^{2+} (Dreesen et al. 1982). Two uranium ground water samples used in these experiments were derived from mining operations in the Ambrosia Lake area of New Mexico. Each sample had a pH of 7.6; the initial uranium concentrations were 7.1 and 2.5 x $10^{-5}M$ (about 20 and 7 ppm U_3O_8). Figure 3 shows the dependence of UO_2^{2+} removal from the ground waters as a function of mass algae,
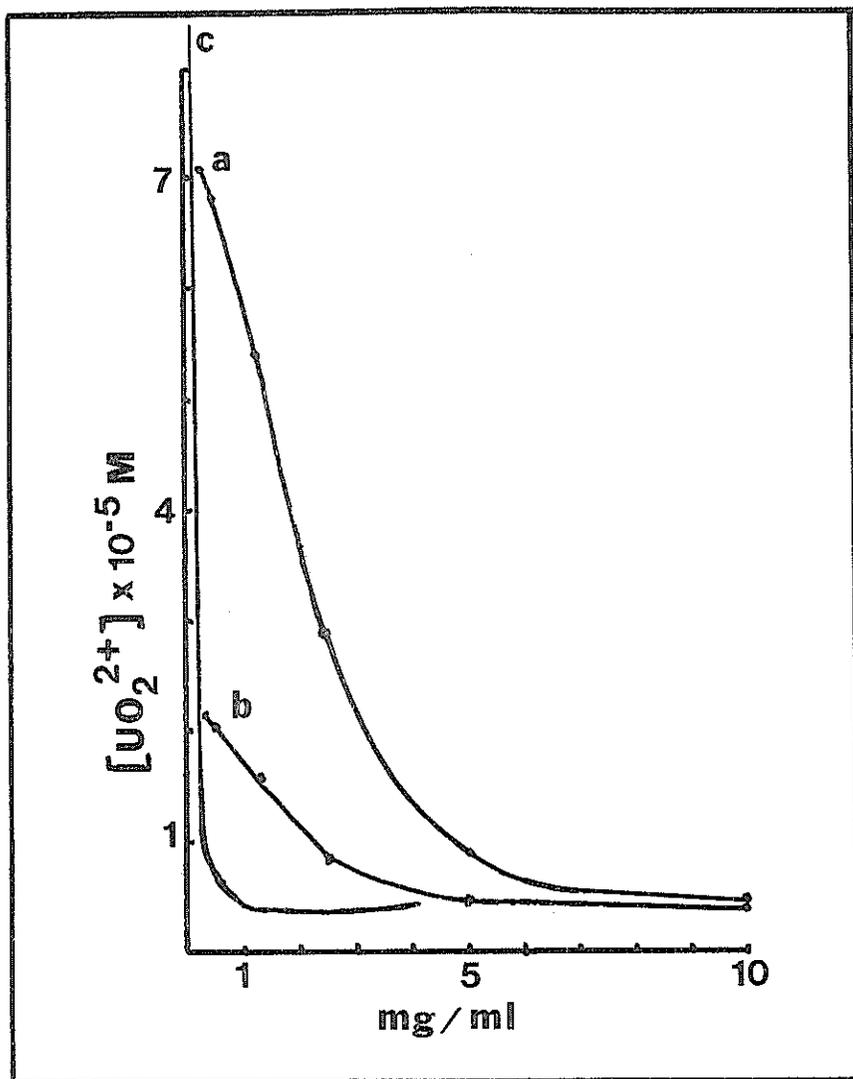
$[UO_2^{2+}] \times 10^{-5}$ M

mg/ml

Fig. 3. The Effect of Algae Mass on the Removal of UO_2^{2+} from Natural Groundwater Samples and from Solutions of 0.05M Sodium Acetate at pH 5.0. The UO_2^{2+} containing solutions were allowed to react with different amounts of algae in the 0.05 - 10 mg/ml range, and after two hours, the suspensions were centrifuged and the free UO_2^{2+} concentrations were measured. Curve (a) and (b) represent two different groundwater samples taken from the Ambrosia Lake area of Northern New Mexico. Each was at pH 7.6; no pH adjustments were made. Curve (c) represents a solution of 0.05M sodium acetate at pH 5, containing 1.00×10^{-4}M $UO_2(C_2H_3O_2)_2$. The initial UO_2^{2+} concentrations were about 7.1×10^{-5}M and 2.6×10^{-5}M, in curves (a) and (b), respectively. The final concentrations in the groundwater samples were about 1.4 ppm.

and for comparison the removal of UO_2^{2+} from 0.05 M sodium acetate. Inspection of the slopes of these curves shows that interfering substances may affect the efficiency of UO_2^{2+} removal from the ground water samples as compared to the sodium acetate, but with larger concentrations of algae, a similar removal level of uranium from these solutions is approached, about 1.4 ppm. However, these experiments clearly show that the algae is effective in removing uranium from natural water samples.

CONCLUSIONS AND PROJECTIONS

Chlorella vulgaris is very effective in the removal of several important heavy metal ions from metal ion-containing waters. Success with UO_2^{2+} removal from ground water samples indicates that algae treatment of uranium-contaminated waters may be promising on a large scale. The high binding capacity for UO_2^{2+} combined with the low cost of algae production (less than 50¢/lb) may make Chlorella vulgaris an attractive alternative to the more expensive ion-exchange resins (up to $100/lb) that are now being used in many clean-up operations. In the event that metal ion recovery is not practical by desorption or dissolution, simple combustion may provide a relatively efficient way of recovering the bound metal ions.

Evidence has been presented that the precipitation of some heavy metals from algae supernatants may be an important path for the removal of these ions. We believe that metal ion removal by precipitation in this manner can be further exploited for the removal of heavy metal ions from contaminated waters. Currently, we are investigating the use of dialysis bags to contain the algae and the associated macromolecules that are responsible for the precipitation. The results of preliminary experiments in which a dialysis bag containing an algae suspension was submerged in a solution containing Pb^{2+} indicate that Pb^{2+} was removed very rapidly from the outside solution, with no precipitation outside the dialysis bag occurring. These results suggest that the molecules responsible for precipitation of metal ions from the algae

supernatant are macromolecules and are being retained within the dialysis bag. The development of methods of this type may enable the facile removal of metal ions from contaminated waters without the necessity of centrifugation.

A very real advantage of using the system for removal of metal ions from industrial or mining wastewaters is that the system can be used under conditions that might normally be toxic to living cells. Since the process we are observing is not dependent upon a living organism, it can be used under a variety of conditions.

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