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BIOSORPTION/HEAVY METAL IONS FROM INDUSTRIAL/MINING WASTE WATERS

Technical Completion Report
Project No. 1423624

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ABSTRACT

The interactions between algal biomass and various metal ions were investigated. For most of the ions examined, uptake is dependent upon pH, with cations bound most strongly above pH 4 and complex anions bound most strongly at lower pH values. However, the binding of gold(III), silver, and mercury(II) ions is relatively insensitive to pH. Heat-treatment of the biomass was found to have little effect on binding capacity. As with other ion-exchange matrices, the extent of metal-ion removal is strongly influenced by the solution composition -- in particular, the presence of competing ligands. However, unlike conventional strong-acid cation-exchange resins, the algal biomass displays low affinity for Ca^{2+} and Mg^{2+} and, thus, may be especially well-suited for hard-water treatment applications. Algal biomass can be readily immobilized in silica to produce a durable chromatographic support which shows substantial longevity in lab-scale operations.

Key words: algae, biomass, heavy metal ions, water treatment

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PROJECT OBJECTIVE

In recent years, increased attention has been focused upon pollution of water supplies by heavy metal ions, which, in rather low concentrations, can lead to acute and chronic illness in humans and other animals. State and federal laws have been enacted to control further contamination of surface and groundwater supplies. Industries have been required to install pollution devices that remove heavy metals prior to effluent discharge. Chemical and energy costs associated with water treatments such as carbon adsorption, flocculation-sedimentation, reverse osmosis and ion-exchange are a major expense. These costs become magnified when nearly complete removal of minor concentrations of heavy metal ions is required. In addition, many ground- or surface waters are already contaminated with heavy metal ions, which in many instances limits the use of these waters. Thus, the development of rapid, widely applicable, low-cost methods for the removal and recovery of toxic metal ions from industrial and mining waste waters as well as from naturally occurring water is a high priority. The goal of this project was to evaluate the potential for use of algal biomass in the removal of toxic metal ions from polluted waters.

BACKGROUND

The binding of metal ions to microorganisms and the application of this phenomenon to water-treatment are rapidly growing areas of interest. A survey of the pertinent scientific literature reveals two distinct approaches to the problem: 1) use of living organisms, and 2) use of nonviable biomass. Metal-ion binding to live cells can occur either through surface adsorption or intracellular accumulation. This assertion is supported by the work of Khummongkol *et al.* (1982), who showed that uptake of metal ions by live cultures can not be accurately described by a model which assumes adsorption only. Binding to non-viable cells, however, is presumed to occur exclusively through surface adsorption.

1) *Live Microorganisms*. Galun *et al.* (1983a, 1983b) have reported the biosorption of UO_2^{2+} to the mycelium of growing *Penicillium digitatum*; they propose that the ion is bound to chitin and cellulose in the cell wall. The presence of Fe^{3+} was observed to strongly inhibit uptake of uranium.

Preston *et al.* (1972) examined the concentrations of various metal ions bound to certain marine algae (seaweed) found in British Isles coastal waters. They observed elevated levels of Cd^{2+}, Cu^{2+}, Fe^{2+}/Fe^{3+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Ag^{+}, and Zn^{2+}. Trollope and Evans (1976) measured the levels of Cu^{2+}, Fe, Pb^{2+}, Ni^{2+}, and Zn^{2+} in freshwater blooms of the algal *Tribonema* near zinc smelters. These investigators saw marked variations in the metal ion concentrations, which were attributed to variations in the age and physical condition of the cultures, as well as to local environmental variation. This study points out a potential limitation associated with the use of live algae in water-treatment, namely that the degree of metal ion removal will very likely be strongly dependent on the health of the algae.

Laube *et al.* (1979) demonstrated that Cd^{2+} and Cu^{2+} were accumulated

significantly from Ottawa River sediments by Anabaena, Ankistrodesmus braunii, and Chlamydomonas. Their results indicated that 1) algae could conceivably mobilize toxic ions from otherwise stable deposits, thereby introducing the ions into the food chain, and 2) that there are binding sites for these ions on the algae significantly stronger than the binding sites in the sediments.

Rebhun and Ben-Amotz (1984) showed that the uptake of Cd^{2+} from a seawater medium by the phytoplankton Chlorella stigmatophora increases with the Cd^{2+} concentration in the medium. In cultures containing between 0.1 - 10 ppm Cd^{2+}, chlorophyll synthesis was inhibited, but there was no apparent decrease in the rate of growth.

Les and Walker (1984) studied the removal of Cu^{2+}, Zn^{2+} and Cd^{2+} (between 0.1 - 200 ppm) by living Chroococcus paris. The degree of binding was observed to increase as the pH was raised from 4.0 to 7.0, and the bound ions were removable with EDTA. However, concentrations of the metal ions exceeding 1 ppm were found to be toxic to the cultures.

Brierly and Brierly (1981) found that living cultures of th benthuic alga Chama and the filamentou algae Spirogyra and Oscillatoria did not significantly decrease the ppm concentrations of uranium (VI) or Mo(VI) from mine waters (pH 7.8, containing bicarbonate ion). However, disruption of the cells greatly improved the binding capacity.

Filip et al. (1979) have evaluated, on a lab scale, a system for removal of metal ions from wastewater lagoons that involved growing the algae in the contaminated water, then collecting the metal-saturated algae by filtration. Metal ion toxicity was a problem for certain species. the degree of removal was strongly dependent on the concentration of biomass, hence highly dependent on local environment and climatic conditions. Jennet et al. (1979) and Gale and Wixon (1979) have studied the effectiveness of algae meander streams in

adsorbing metal ions from lead- and zinc-mining and milling efluents in the lead-mining district of Missouri. Contaminated water was circulated through shallow canals containing benthic algal mats. In the most favorable cases, Pb^{2+} levels dropped from an initial 3 ppm to 50 ppb. Although all of the species screened for use bound Hg^{2+} strongly, the uptake of Pb^{2+} and Cd^{2+} was variable. It was found necessary that the algae by growing vigorously for effective metal-removal to occur.

It should be apparent that all water-treatment systems which employ live microorganism suffer from non-trivial difficulties associated with control and maintenance of growth in polluted waters.

2) Non-viable biomass. Methods for water-treatment that employ non-viable cells are not complicated by the problem of attempting to maintain growth under adverse circumstances. (In fact, Horikoshi et al. (1979) found that heat-killed cells display a binding capacity for U(VI) three times greater than that measured for living cells.) Instead, the biomass is treated merely as another reagent, a surrogate ion-exchange resin. The binding, or biosorption, of metal ions by the material results from coordination of the ions to various functional groups on the cell wall or cell membrane. These chelating groups--contributed by carbohydrates, hydroxyl, phenolic, phosphate, amino, thiol, and thioether moieties. A number of research groups, besides our own, are working in this area. We present a brief synopsis of their major contributions here; a summary of our work appear below (See Progress Report).

Tsezos et al. (1980) and Tsezos and Volesky (1981, 1982a,b; 1983) studied the binding of U(II) and Th(IV) to non-living Rhizopus arrhizus, a common fungi. They proposed that both ions are bound initially to amino groups present in chitin. The resulting complex then hydrolyzes, forming insoluble hydroxy species which precipitate in pores on the cell surface.

Ferguson and Bubela (1974) examined the biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} to frozen or freeze-dried preparation of Ulothrix, Chlamydomonas and Chlorella vulgaris. The degree of binding, they observed, was greater at pH 7 than at pH 3. They found that NaCl and $Mg(NO_3)$ inhibited only the binding of zinc, suggesting that selective adsorption of Pb^{2+} or Cu^{2+} was possible.

Nakajima et al. (1981, 1982) studied the binding of various ions to freeze-dried Chlorella regularis. They observed selective accumulation of ions from an equimolar solution (1×10^{-4}M in each ion at pH 5.0) which decreased in the following order:

$$UO_2^{2+} >> Cu^{2+} >> Zn^{2+} > Ba^{2+} \approx Mn^{2+} \geq Cd^{2+} > Ni^{2+} \approx Sr^{2+}.$$

The removal of UO_2^{2+} was unaffected by the presence of other ions, but Cd^{2+} uptake was strongly inhibited by equal concentrations of UO_2^{2+} or Cu^{2+}. The same authors also described a procedure for immobilizing C. regularis in polyacrylamide for use as a chromatographic matrix.

METHODOLOGY

Metal-Binding Assays

We currently employ two distinct procedures to assess the metal-binding properties of our algal preparations: One is a "batch" technique; the other is a "column", or chromatographic, technique. In the batch procedure, a known quantity of lyophilized (freeze-dried) Chlorella vulgaris is first washed two or three times, by suspension and centrifugation, in 0.05M HOAc, pH 2.0. This treatment removes soluble biomolecules that bind metal ions and complicate the interpretation of results. The washed material is then resuspended in a known volume of the solution of interest. After a sufficient contact time, the sample is centrifuged, and the supernatant liquid is analyzed for residual metal ion(s). The difference between the initial and final concentrations

represents the amount bound to the algae. The algal material collected by centrifugation can then be resuspended in a fresh aliquot of the metal-containing solution, if the effect of repeated exposure (saturation) is to be examined. Alternatively, one can desorb the bound metal ions by resuspending the algae in a suitable eluant. If need be, the amount of metal ion bound may be measured directly by digesting the algae with aqua regia or hydrogen peroxide and then analyzing the resulting solution.

The batch configuration is employed in many of our preliminary experiments because of the ease with which solution parameters can be manipulated. For pH studies, aliquots of the algal suspension can be readily adjusted to the desired pH values prior to introduction of the metal ion(s). The degree of interference of metal ion building caused by other ions is easily ascertained by adding various amounts of the species in question to otherwise identical aliquots of an algal suspension.

It would be useful to pack algae directly in a column in order to make an "algae filter" through which waters containing metal ions could be passed. Unfortunately, Chlorella packs so tightly that water will not flow through a column containing only algal biomass. Therefore, it is necessary to immobilize the algal cells in a support in order to obtain acceptable flow characteristics. The resulting material is then granulated and sieved to obtain a preparation having particle sizes between 40- and 100-mesh. This material functions extremely well as a chromatographic matrix. We have found the "column" methodology useful for investigating certain questions relevant to the use of algae in water-treatment applications, since it bears a closer resemblance to a practical contact system than does the batch system.

Immobilization of Algal Biomass

Immobilization in Polyacrylamide. 1.5g dry algal biomass is washed once in 0.01N HCl and once in 0.7% NaCl. Acrylamide (4.25g) and N,N'-bis-methylene acrylamide (0.25g) are dissolved in water, and this solution is then mixed with the algal suspension. Polymerization is initiated by addition of 1.0ml 10%(V/V) N,N,N',N'-tetramethylethylenediamine (TEMED) and 1.0ml of 5%(W/V) ammonium persulfate. The reaction mixture is carefully overlain with water. Polymerization occurs within several minutes and is considered to be complete after an hour. The resulting material is forced through a 40-mesh sieve, then washed on a 100 mesh sieve, and stored at 4°C.

Preparation of Chlorella-Silica Polymer. 30% (w/v) sodium silicate (30%, w/v) was added dropwise with stirring to 20% (v/v) H_2SO_4 until the pH reached 5.0. Dry Chlorella pyrenoidosa or C. vulgaris was then added and stirred to yield a uniform suspension. Polymerization occurred within minutes. After one hour, the gel was broken into small pieces and rinsed with deionized water until addition of Ba^{2+} no longer precipitated $BaSO_4$ in the rinsings. After drying for 12-16 hours at 110°C, the resulting material was gently ground with a mortar and pestle and then sieved, to yield a particle size between 40 and 100 mesh.

The algal content can be varied up to approximately 90% (calculated on a dry weight basis). A variation of the above protocol is employed to obtain material at the upper end of this range. Specifically, addition of sodium silicate is halted at pH 3.6, and dry algae is added until polymerization occurs spontaneously. Otherwise, the procedure is unchanged.

Metal Ion Analysis

Metal ion analyses are performed by either of two atomic spectroscopic methods: atomic absorption spectrometry (AA) and direct-current argon plasma

(DCP) emission spectroscopy. Flame atomic absorption spectrometry permits the analysis of a large number of samples, containing low levels of ions, with high precision and accuracy. Replacement of the flame with an electrothermally heated carbon-furnace lowers the limits of detection still further. DCP emission spectroscopy complements atomic absorption, permitting ready analysis of certain elements that are difficult to measure by AA: uranium, boron, phosphorus, sulfur, aluminum, vanadium, chromium, etc. Both techniques enable most elements to be measured at levels of a ppm or less.

RESULTS

Metal-Ion Binding Diversity

It was found that algal biomass has affinity for a large variety of metal ions. The list, presented in table 1, includes "hard" cations such as Al^{3+} and Be^{2+} as well as "soft" ones such as Hg^{2+} and Ag^{+}. Certain anions are bound as well, so that algal biomass can, in principle, function as either a cation- or anion-exchanger. The binding diversity probably stems from the diversity of functional groups on and within the algal cell. The lipid, carbohydrate, and protein components combine to create a spectrum of distinct binding sites which differ in affinity and specificity.

Dependence of Binding Upon pH

We have found that metal ions can be divided into three general classes based upon the pH-dependence of their binding to algal biomass. The first class is comprised of metal ions which are tightly bound at pH $\geq$ 5 and which can be removed (or are not bound) at pH $\leq$ 2. Most cations fall into this class: Al^{+3}, Cu^{+2}, Pb^{+2}, Cr^{+3}, Cd^{+2}, Ni^{+2}, Co^{+2}, Zn^{+2}, Fe^{+3}, Be^{+2}, and UO_2^{+2}. The second class is comprised of metallic anions which display the opposite behavior of class I metal ion, i.e., they are strongly bound at pH $\leq$ 2 and weakly bound or not bound at all at pH values near 5. Ions in class II

include $PtCl_4^{-2}$, CrO_4^{-2}, $Cr_2O_7^{-2}$, SeO_4^{-2} and MoO_4^{-2}, and VO_3^-. The third class of metal ions includes those metal ions for which there is no discernible pH-dependence for binding: Ag^+, Hg^{+2}, and Au^{+3}. These three ions are the most strongly bound of all metal ions tested. Figure 1 illustrates data for the three classes of metal ions.

Thermal Stability

Figure 2 demonstrates that the binding of either gold, copper, or silver ion by Chlorella vulgaris biomass is virtually unaffected by boiling for five minutes. This finding proves that the uptake is occurring independently of any transport process and thus represents simple adsorption of the ions to macromolecular components on or within the cell. It is clear, from electron micrographs of C. vulgaris preparations that have been exposed to Au^{3+}, that a large percentage of the cells are permeable to metal ions. This is probably the result of lysis which occurs during the drying step (either lyophilization or spray drying).

Effect of Boiling on Uptake of Au^{3+}, Ag^+, and Cu^{2+} by C. vulgaris Biomass

Algae, either boiled for five minutes or untreated, was suspended in 4.0 ml of 2×10^{-4}M metal ion (in 0.5M NaOAc, pH 5.0) at a concentration of 5 mg/ml. After a one hour contact time, the algae was collected by centrifugation, and the supernatant was analyzed for residual metal ion. The algal pellet was resuspended in a fresh 4.0 ml aliquot of metal-ion solution. This procedure was repeated for a total of either four (Cu^{2+}) or five (Ag^+, Au^{3+}) exposures. The total amount of metal ion bound is plotted in Figure 2 for each exposure: a) Au^{3+}, b) Ag^+, c) Cu^{2+}.

Competition

The manner in which a given metal ion interacts with algal biomass is strongly influenced by competition -- either competition with other ions for

the limited number of binding sites on/within the algal cell or competition between the biomass and other ligands in solution for the metal ion. Viewed in its most basic terms, the pH-dependence for uptake of metal cation is determined, at the low end, by how well the cation competes with protons for chelating groups on the biomass and, at the high end, by how well the biomass competes with hydroxide ion for complexation of the cation.

We have uncovered numerous other instances in which the degree of removal of a certain species from solution is strongly dependent on the solution composition. A striking example of competition between two ions for binding space on C. vulgaris biomass is provided by the data for Hg^{2+} and Ag^{+} (figure 3). When present alone, either ion is bound completely. However, if both are present, the uptake of Ag^{+} is severely depressed. By contrast, the presence of mercury has almost no effect on the binding of Al^{3+} (data not shown). Presumably, silver and mercury, both being large "soft" ions, have a preference for similar liganding groups (such as those containing N,S); whereas, Al^{3+}, a small "hard" cation, prefers "hard" ligands (e.g., oxygen-containing groups, such as hydroxyls or carboxyls).

The ability of competing ligands to depress binding of metal ions is amply illustrated by the chloride inhibition data presented in figure 4. Removal of each of the ions shown is reduced to some extent by the presence of Cl^{-}. Those most strongly affected are Hg^{2+}, Pb^{2+}, and Cu^{2+}, ions known to form strong chloro-complexes.

The effect of bicarbonate ion on the uptake of UO_2^{2+} by C. vulgaris biomass (figure 5) is another excellent example of competition by other ligands present in solutions. Above pH 6, the algal biomass is unable to bind significant amounts of uranyl ion, either from actual mine waters known to contain HCO_3^{-} or from lab samples to which bicarbonate had been added. If, on

the other hand, the pH was reduced below 6, then nearly all of the UO_2^{2+} could be removed from the solutions. Figure 6 demonstrates that this is an ion-specific phenomenon. With the exception of phosphate, no other anion tested significantly inhibited binding of UO_2^{2+}.

The uptake of gold is likewise strongly influenced by the composition of the sample: $AuCl_4^-$ is strongly bound from near pH 0 to pH 8. However, removal of the $Au(CN)_2^-$ complex is strongly pH-dependent, exhibiting a maximum near pH 3 (see Figure 1b).

We can conclude from these studies that whether or not algal biomass is applicable to the treatment of a given wastewater will depend on the precise composition of the water. The biomass is no different in this respect than any other ion-exchange matrix and, in fact, has certain advantages over conventional ion-exchange resins.

Low Affinity for Ca^{2+} and Mg^{2+}

C. vulgaris biomass displays relatively little affinity for the hard-water ions Ca^{2+} and Mg^{2+} (figure 7). Batchwise exposure of the algal (5 mg/ml) to 1.0×10^{-4}M metal solutions resulted in removal efficiencies of only 25% and 15%, respectively. In this respect, the biomass more closely resembles a chelating ion-exchange resin than a conventional strong-acid cation exchange resin. This finding suggested that biomass might be well-suited to the treatment of hard waters, a hypothesis which was largely confirmed by an experiment which measured the uptake of copper ion in the presence of increasing levels of Ca^{2+} and Mg^{2+}. The results (figure 8) show that, while there is initially some loss of binding capacity as the hardness of the water is increased, above 400 ppm total hardness there is little further decrease. This stability suggests the existence of two populations of copper-binding sites: one class of weak, non-specific sites (susceptible to

competition by Ca^{2+}) and a second class of stronger chelating groups (selective for Cu^{2+}). Under these conditions, use of the algal biomass would be preferred over the use of a standard strong-acid resin. Whether or not it can compete with available chelating resins must await a more complete economic analysis, which would include such considerations as production cost, binding capacity and durability.

Immobilization of Algal Biomass in Silica

We have devised a procedure for embedding algal cells in a silica matrix. The resulting material is durable, retains the metal-binding properties of the alga, and functions superbly as a chromatgraphic support.

Figure 9 illustrates for Au^{3+} that the degree of uptake is proportional to the algal content of the polymer. This result implies that all of the binding sites on the immobilized algal cells are accessible to the solvent. That the binding is due entirely to the algal material is clear from the silica control, which displays a negligible degree of binding.

To investigate the durability of the material, an aliquot of the alga-silica polymer was subjected to 26 cycles of binding and elution of gold ion over the course of six days. Within experimental error, there was no decrease in binding capacity (figure 10). When the binding ability was investigated at intervals over a longer period (figure 11), a significant loss of capacity was evident as early as day 10, increasing to approximately 33% after 28 days. This result was not unexpected, since some decrease in binding capacity with time is to be expected for any ion-exchanger.

Selective Binding and/or Elution of Metal Ions

The variations in pH-dependence for binding to algal biomass seen among the various metal ions, as well as differences in their coordination chemistries, can be used in certain cases to achieve selection binding or

elution of certain ions. We were able to selectively recover Cu^{2+}, Zn^{2+}, Hg^{2+} and Au^{3+} from a solution containing all four of these ions (figure 11). The solution initially at pH 6, was loaded onto a column of immobilized C. vulgaris. Zn^{2+} and Cu^{2+} were eluted first with a pH gradient. The mercury was then eluted at pH 2 with 2-mercaptoethanol, and the bound gold ion was recovered with the same reagent at pH 5.0. This experiment illustrates how solution parameters can be manipulated to magnify selective interactions.

Mechanism of Au(III) Binding to C. vulgaris Biomass

The interaction of Au^{3+} with Chlorella is marked by some interesting chemistry. Binding of the ion to the biomass is accompanied by the concomitant release of three equivalents of chloride ion. This was determined by titration of the algal supernatant with $AgNO_3$ to a silver chromate endpoint and confirmed by measurements with a chloride-specific electrode. This result suggested that either: 1) three of the chlorides on the Au^{3+} ion are being replaced by algal ligands, or 2) that the Au^{3+} is reduced upon binding to Au(I), which then forms a linear, bidentate complex, to which the algal matrix contributes one ligand.

That the latter explanation was in fact the correct one was suggested by an experiment in which some of the bound gold ion was stripped from the Chlorella using a concentrated solution of bromide. The resulting extract exhibited negligible absorbance at 380nm, a wavelength at which $AuBr_3^-$ has a pronounced absorbance peak (Figure 13). From this, it was concluded that Au(III) is reduced to Au(I) upon binding to Chlorella.

Bound gold ion can be reduced further to elemental gold under some conditions. This reduction is demonstrated by the appearance of a peak which grows in at 550 nm in the visible spectrum of gold-saturated algal biomass (figure 14). This peak is characteristic of colloidal gold. Examination of

this material by electron microscopy reveals the presence of small gold crystals both on and within the cell. The rate of reduction of bound gold ion was examined as a function of the degree of saturation. It was found that the rate of reduction increases with the amount of gold bound initially. Moreover, below a certain threshold value (approximately 0.055 mole Au per gram Chlorella) of bound gold, the reduction is not observed. These results suggest that reduction of gold to the atomic state does not occur at the sites of highest affinity and that the phenomenon is associated primarily with the sites of lower affinity.

Treatment of Waste Waters

The metal-binding properties of Chlorella vulgaris were tested on two actual water samples. One was a waste solution obtained from a jewelry manufacturer. With a pH of 10.2, this solution contained gold (at $6.3X10^{-3}M$), silver ($1.9X10^{-1}M$) and copper ($2.7X10^{-3}M$) as their cyano complexes. Employing a 10X dilution of this solution water, it was found that three exposures to C. vulgaris biomass at the natural pH of the water removed 95% of the copper and silver and only 10% of the gold. Following adjustment to pH 3, four exposures to the Chlorella then reduced the gold concentration to just 25% of the original value.

Another sample, obtained from a mining concern in Minnesota, contained significant concentrations of copper, nickel, cobalt, and zinc. This water (1.275 liters) was passed through a column of Chlorella immobilized in silica. Analysis of the eluate revealed that the treatment reduced the nickel ion content of the water from the initial value of 1.1 ppm to 140 ppb or less. The column was not saturated at this point.

CONCLUSIONS

Our research supports the contention that algal biomass possesses several

properties which make it a logical candidate for use in water-treatment applications.

1. Algal biomass has affinity for a wide range of ions. The list includes both cations and anions. In principle, algal biomass could be employed in either cation-or anion-removal processes.

2. Metal ion uptake is unaffected by heat-treatment (boiling or 150°C,2 hours), proving that it is strictly the result of adsorption processes and does not involve transport. Living cells are not required.

3. The metal ions most tightly bound by the algal cells are gold (either Au(I) or Au(III), mercury(II), and silver(I), in that order.

4. Au(III) undergoes reduction to Au(I) upon binding. Under certain conditions, it is further reduced to Au(0). Thus, recovery of bound gold in an application setting will require re-oxidation, if some of the gold is present in the elemental form.

5. The uptake of some species by algal biomass is pH-dependent, with cations binding most strongly between pH 5 and neutrality and anions tending to bind more strongly at pH values below 5.

6. Binding of a given ion can be reduced, or even eliminated, by certain interfering substances. Thus, the presence of mercury will severely limit binding of silver. This results from competition of ions for a limited number of binding sites on and within the algal cell. Alternatively, a competing ligand may form a stable complex with the ion in question, thereby restracting binding. For example, bicarbonate ion eliminates UO_2^{2+} uptake; excess cyanide ion depresses binding of Au(I); and chloride ion reduces binding of Hg^{2+} and Pb^{2+}. These observations lead to the conclusion that algal biomass will not be equally suited to every water-treatment application. Indeed, no ion-exchange resin can be. In this regard, algal biomass exhibits relatively

low affinities for Ca^{2+} and Mg^{2+}, a property which may make it particularly well-suited to the clean-up of hard waters.

7. Differences in the binding behavior as well as the chemistry of the various metal ions can be exploited to achieve selectivity in binding to, and/or elution from, algal biomass.

8. In the laboratory setting, when suitably immobilized, algal biomass appears to be sufficiently durable for use in water-treatment application.

RECOMMENDATIONS

In the laboratory, algal biomass has clearly demonstrated potential for application to the removal and recovery of metal ions form waste waters and mining process-streams. Our recommendations for further development of this novel technology are listed below.

Waste Water Screening

An extensive screening program should be undertaken in order to identify those types of waste waters for which the application of immobilized algal biomass is particularly appropriate. Each sample should be treated with immobilized algal biomass and with a representative sampling of commerical ion-exchange resins as well. The performance of the biomass in relation to the conventional ion-exchangers would provide insight into the relative cost-effectiveness of the algal treatment method.

Large-scale Production of Immobilized Algal Biomass

To date, only lab-scale quantities of the alga-silica material have been produced. A typical preparation affords only 100g of product, and roughly half that amount is rendered unsuitable (too fine) by the granulation process. Obviously, implementation of the algal-biomass-treatment technology will require development of a method for large-scale production of immobilized

biomass. Since the cost of the biomass is expected to be relatively high, the immobilization process will need to be very efficient, resulting in minimal loss of algal cell mass.

Pilot-plant Scale Operation

Having identified several suitable waste waters, pilot-plant-scale operations should be installed on-site. Evaluation of the performance of algal biomass under these conditions would afford a more realistic appraisal of such factors as: 1) durability of immobilized algal biomass; 2) effectiveness; 3) ease-of-operation; and 4) cost of operation.

REFERENCES

Brierly, C. L. and Brierly, J. A. (1981). Biological Process for Concentrating Trace Elements from Uranium Mine Wastes, Technical Completion Report No. 140, New Mexico Water Resources Research Institute, New Mexico.

Ferguson, J. and Bubela, B. (1974). "The Concentration of Cu(II), Pb(II) and Zn(II) from Aqueous Solutions by Particulate Algal Matter," Chemical Geology 13, 163-186.

Filip, D. S., Peters, T., Adams, V. D. and Middlebrooks, E. J. (1979). "Residual Heavy Removal by an Algae-Intermittent Sand Filtration System," Water Res. 13, 305-313.

Gale, N. L. and Wixon, B. G. (1979). "Control of Heavy Metals in Lead Industry Effluents by Algae and Other Aquatic Vegetation," Proc. Int. Conf. Management and Control of Heavy Metals in the Environment, London.

Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S. M. and Siegel, B. Z. (1983b). "Recovery of Uranium (VI) from Solutions Using Precultured Penicillin Biomass," Water, Air and Soil Polution 20, 221-232.

Galun, M., Keller, P., Malki, D., Feldstein, H., Galun, E., Siegel, S. M. and Siegel, B. Z. (1983a). "Removal of Uranium (VI) from Solutions by Fungal Biomass and Fungal Wall-Related Biopolymers," Science 219, 285-286.

Horikoshi, T., Nakajima, A. and Sakaguchi, T. (1979). "Uptake of Uranium by Chlorella regularis," Agric. Biol. Chem. 43, 617-623.

Jennett, J. C., Hassett, J. M. and Smith, J. E. (1979). "Control of Heavy Metals in the Environment Using Algae," Proc. Int. Conf. Management and Control of Heavy Metals in the Environment, London.

Khummongkol, D., Cantèrford, G. S., and Fryer, C. (1982). "Accumulation of Heavy Metals in Unicellular Algae," Biotechnol. and Bioeng. 24, 2643-2660.

Laube, F., Ramamoorthy, S., and Kushner, D. J. (1979). "Mobilization and Accumulation of Sediment-Bound Heavy Metals by Algae," Bull, Environ. Contam. 21, 763-770.

Les, A. and Walker, R. W. (1984). "Toxicity and Binding of Copper, Zinc, and Cadmium by the Blue-Green Alga, Chroococcus paris," Water, Air and Soil Pollut. 23, 129-139.

Nakajiima, A., Horikoshi, T., and Sakaguchi, T. (1981). "Studies on the Accumulation of Heavy Metal Elements in Biological Systems XVII. Selective Accumulation of Heavy Metal Ions by Chlorella regularis," Eur. J. Appl. Microbiol. Biotechnol. 12, 76-83.

Nakajiima, A., Horikoshi, T., and Sakaguchi, T. (1982). "Recovery of Uranium by Immobilized Microorganisms," Eur. J. Appl. Microbiol. Biotechnol. 16, 88-91.

Preston, A., Jefferies, D. F., Dutton, J. W. R., Harvey, B. R. and Steele, A. K. (1972). "British Isles Coastal Waters: The Concentrations of Selected Heavy Metals in Sea Water, Suspended Matter and Biological Indicators-A Pilot Survey," Environ. Pollut. 3, 69-82.

Rebhun, S. and Ben-Amotz, A. (1984). "The Distribution of Cadmium Between the Marine Alga Chlorella stigmatophora and the Sea Water Medium," Water Res. 18, 173-178.

Trollope, D. R. and Evans, B. (1976). "Concentration of Copper, Iron, Lead, Nickel and Zinc in Freshwater Algal Blooms," Environ. Pollut. 11, 109-116.

Tsezos, M. (1980). Biosorption of Uranium and Thorium, Ph.D. Dissertation, McGill University, Montreal.

Tsezos, M. and Volesky, B. (1981). "Biosorption of Uranium and Thorium," Biotechnol. Biveng. 23, 583-604.

Tsezos, M. and Volesky, B. (1982a). "The Mechanism of Uranium Biosorption by R. arrhizus," Biotechnol. Bioeng. 24, 385-401.

Tsezos, M. and Volesky, B. (1982b). "The Mechanism of Thorium Biosorption by R. arrhizus," Biotechnol. Bioeng. 24, 955-969.

Tsezos, M. and Volesky, B. (1983). "The Role of Chitin in Uranium Adsorption by R. arrhizus," Biotechnol. Bioeng. 25, 2025-2040.

TABLE 1

DIFFERENT METAL IONS WHICH BIND TO ALGAE

TEST METAL ION	% REMOVED	INITIAL CONCENTRATION
Au(III)	100	1.0×10^{-4} M, pH 2–7
Ag(I)	100	
Hg(II)	100	
U(VI)	100	1.0×10^{-4} M in 0.05 M Sodium Acetate at pH 5.0
Cu(II)	90	
Be(II)	80	
Al(III)	80	
Pb(II)	75	
Cd(II)	60	
Ni(II)	40	
Zn(II)	40	
Pt(II)	90	1.0×10^{-4} M, pH 2.0
Cr(VI)	84	

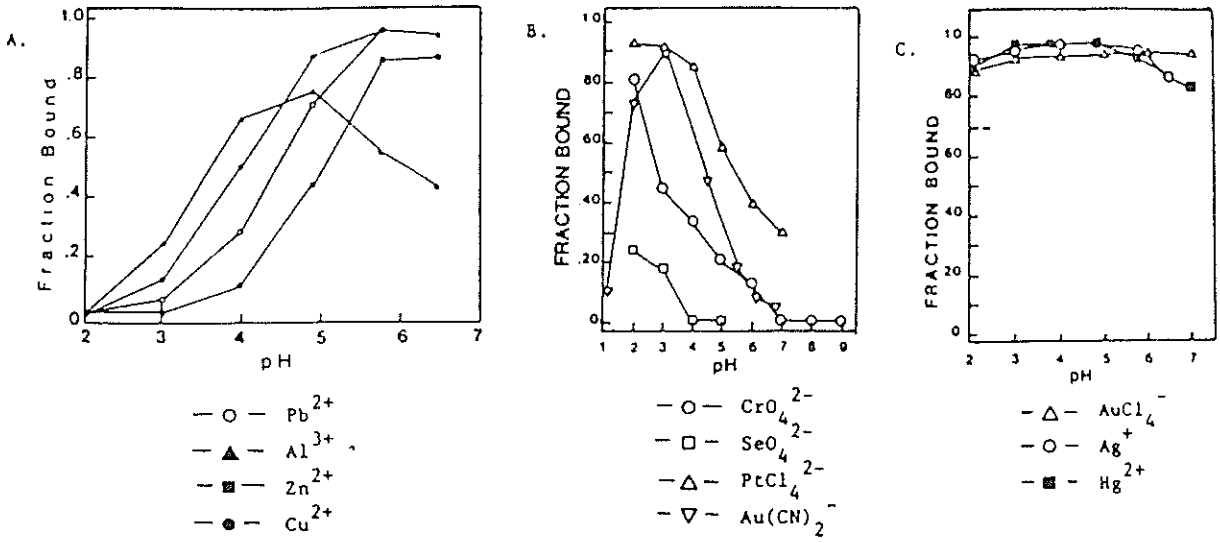

Figure 1. Effect of pH on the Uptake of Various Metal Ions by C. vulgaris Biomass. Washed C. vulgaris was suspended, at 5 mg/ml, in 1 x 10^{-4} M solutions of metal ion at the indicated pH values. After equilibration, samples were centrifuged, and the supernatants were analyzed for residual metal ion.

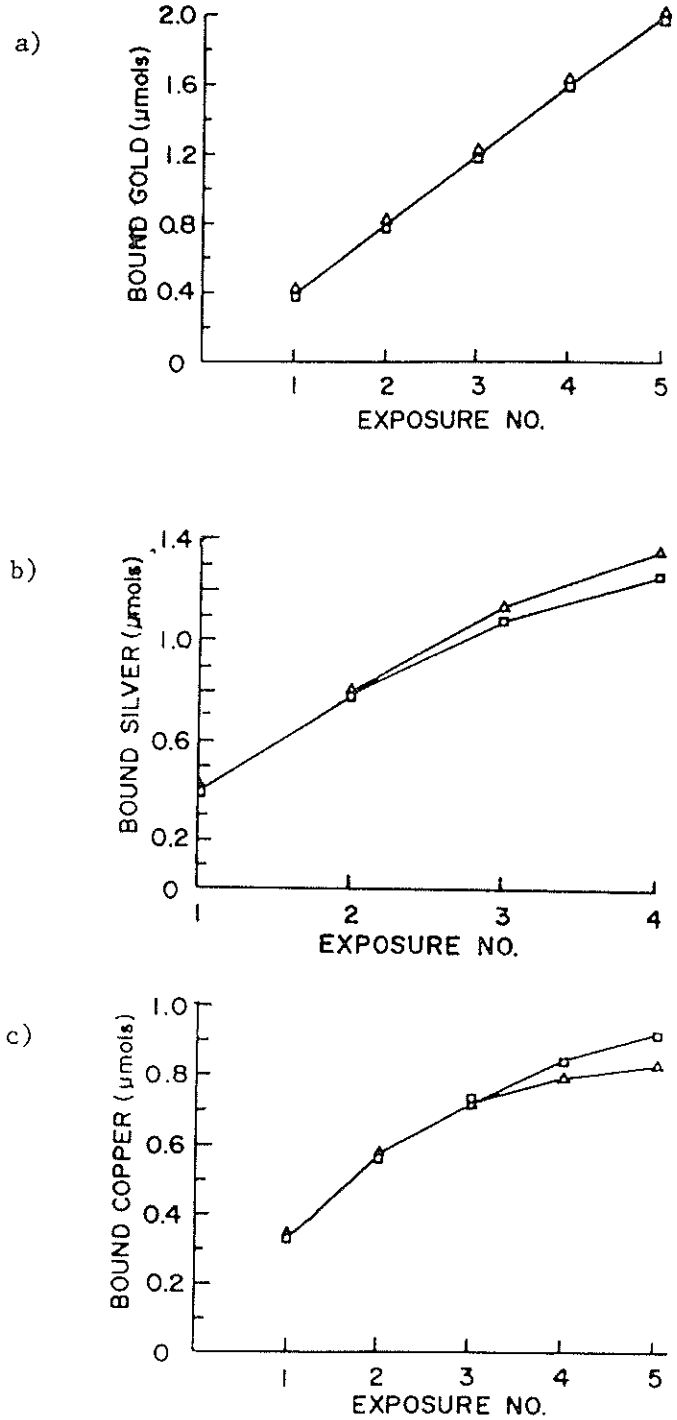

Figure 2. Effect of Boiling on Uptake of Au^{3+}, Ag^{+}, and Cu^{2+} by C. vulgaris Biomass. Algae, either boiled for five minutes (-□-) or untreated (-Δ-), was suspended for one hour in 2.0×10^{-4} M metal ion at a concentration of 5 mg/ml. At the end of the contact period, the supernatant was analyzed for residual metal ion. This procedure was repeated for a total of either four or five exposures. a) Au^{3+}, b) Ag^{+}, c) Cu^{2+}.

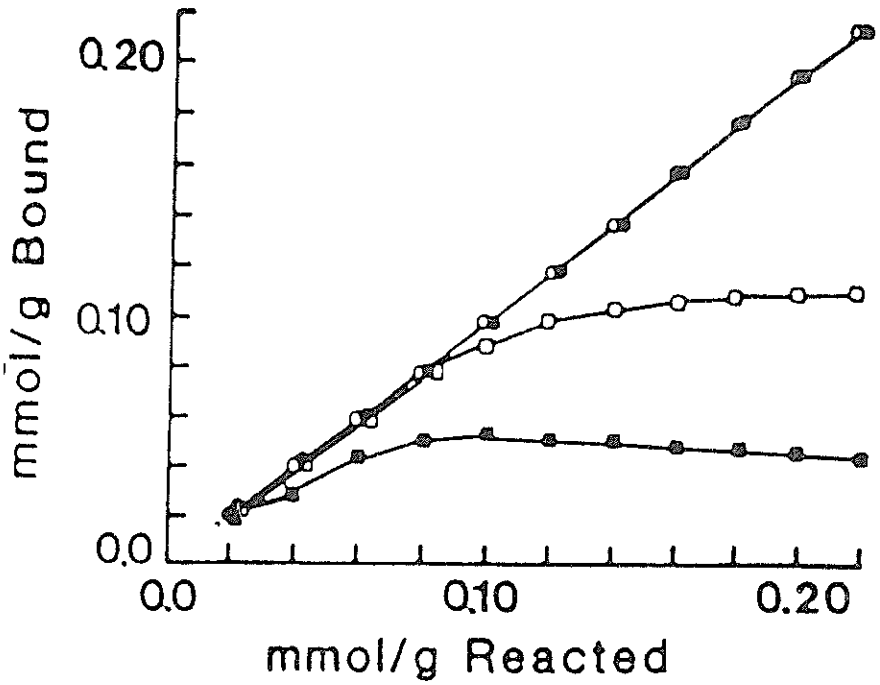

Figure 3. Competition between Mercury(II) and Silver(I) Ions for Binding Sites on C. vulgaris Biomass. Algal biomass was reacted repeatedly with fresh portions of 0.1 mM silver(I) nitrate, 0.1 mM mercury(II) nitrate, or a solution containing 0.1 mM of each metal salt. All solutions contained 0.05 M sodium acetate, pH 5.0, and the algal concentration was 5 mg/ml. The supernatant solutions were analyzed for residual metal ion(s) after each exposure. (-○-), binding of mercury in absence of silver; (-●-), binding of mercury in presence of silver; (-□-), binding of silver alone; (-■-), binding of silver in presence of mercury.

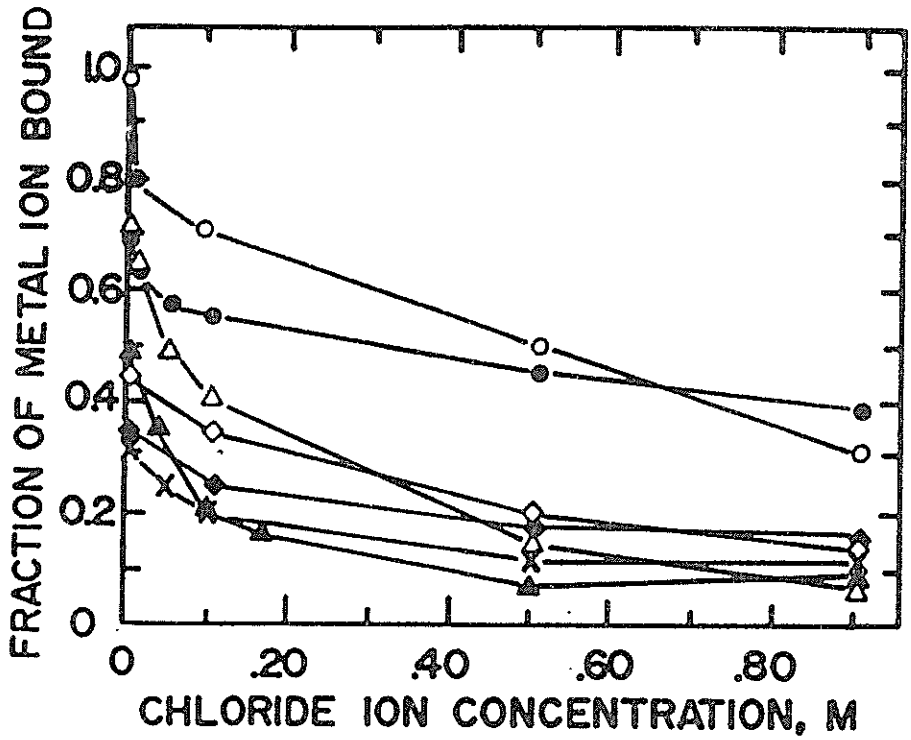

Figure 4. Effect of Chloride Ion on Removal of Various Ions from Aqueous Solutions by Chlorella vulgaris. Chlorella (5 mg/ml) was incubated with solutions of the individual metal ions (1.0×10^{-4}M) in 0.05 M NaOAc, pH 5.0, in the presence of differing concentrations of Cl^-. After two hours, the algae was removed, and the remaining solution was analyzed for metal ion. The ions studied were: Hg^{2+} (-o-), Cu^{2+} (-●-), Pb^{2+} (-Δ-), Cd^{2+} (-▲-), Ni^{2+} (-◇-), Zn^{2+} (-◆-), and Co^{2+} (-x-).

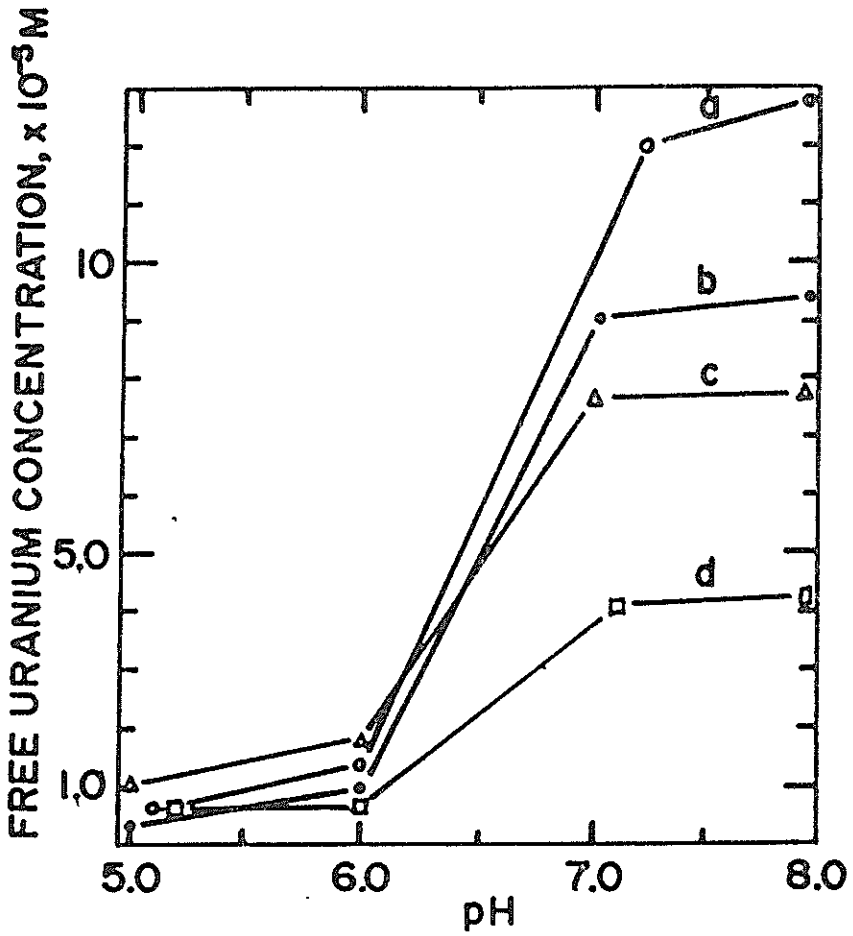

Figure 5. The Effect of pH on the Removal of Uranium Ion by Algae from Mine Waters and Sodium Bicarbonate Solutions. Algae suspensions in mine waters (curves a,c, and d) and 0.05 M sodium bicarbonate (curve b) were adjusted to the indicated pH values (original pH was 8). The suspensions were allowed to react for 2 hours, at which time they were centrifuged, and the amount of uranium ion remaining in the supernatant was determined. It is apparent that sodium bicarbonate strongly inhibits the binding of uranium ion to algae above pH 5 or 6. Algae-free control samples showed that pH adjustment alone did not result in any precipitation of uranium ion.

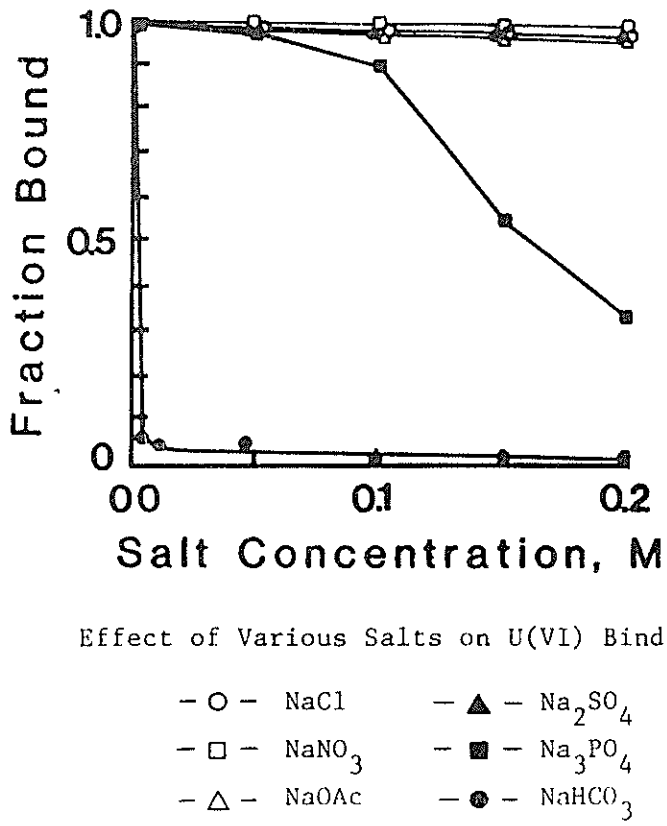

Figure 6. Effect of Various Salts on the Binding of U(VI) by Chlorella vulgaris Biomass. Washed C. vulgaris was resuspended in 1 x 10^{-4} M uranyl acetate in the presence of the compounds shown above, at the indicated concentrations. After equilibration, the algae was removed, and the supernatant was analyzed for U(VI). The algal concentration was 5 mg/ml.

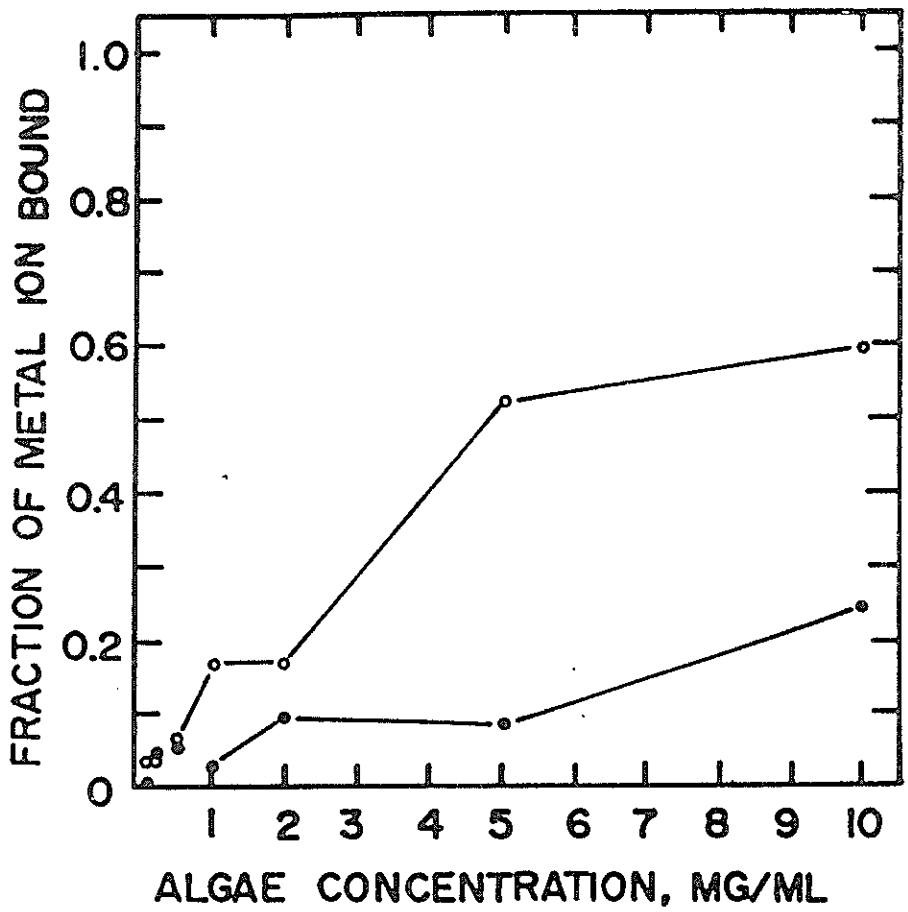

Figure 7. Binding of Ca^{2+} and Mg^{2+} to Chlorella vulgaris. Differing amounts of algae were combined with solutions of either Ca^{2+} or Mg^{2+} in 0.05M NAOAc, pH 5.0. The algae concentrations ranged from 0.1 mg/ml up to 10 mg/ml, and the initial metal ion concentration was 1 x 10^{-4}M. After a two-hour incubation period, the algae was removed by centrifugation, and the SN was analyzed for either Ca (-o-) or Mg (-•-).

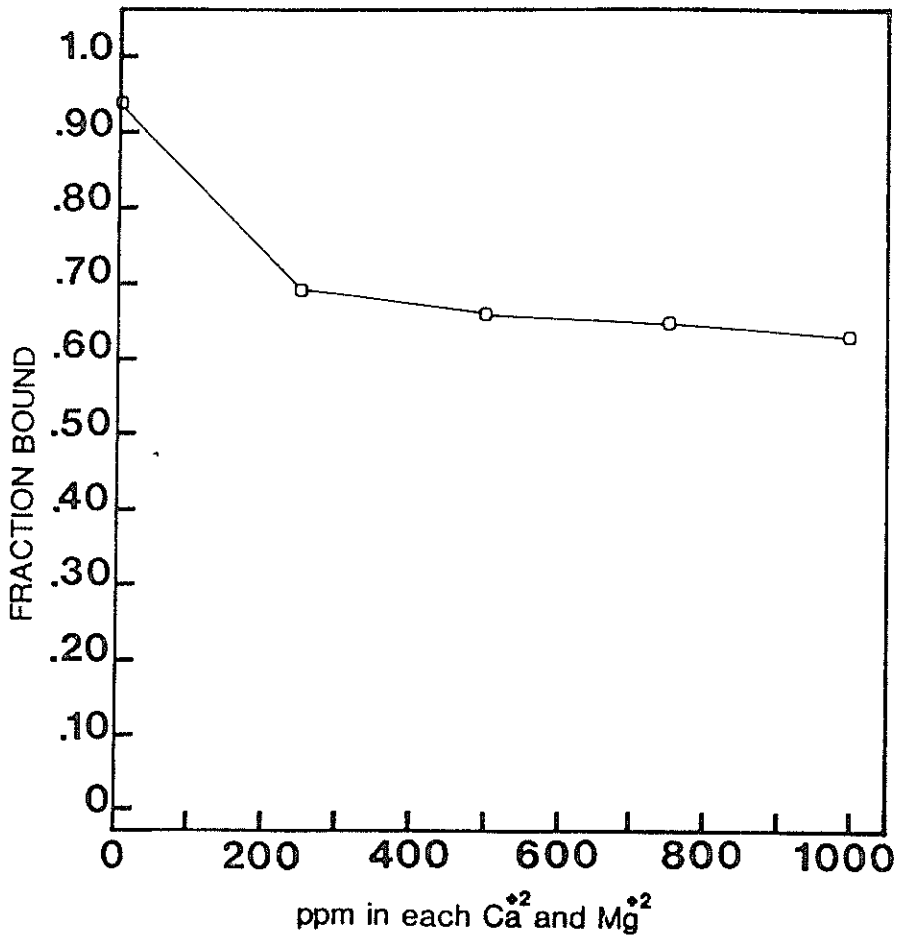

Figure 8. Effect of Calcium and Magnesium Ions on the Uptake of Cu^{2+} by C. vulgaris Biomass. Algal biomass, at a concentration of 5 mg/ml, was exposed to 1×10^{-4} M Cu^{2+} in the presence of varying amounts of Ca^{2+} and Mg^{2+}. After equilibration, the algal material was removed by centrifugation, and the supernatant was analyzed for residual Cu^{2+}.

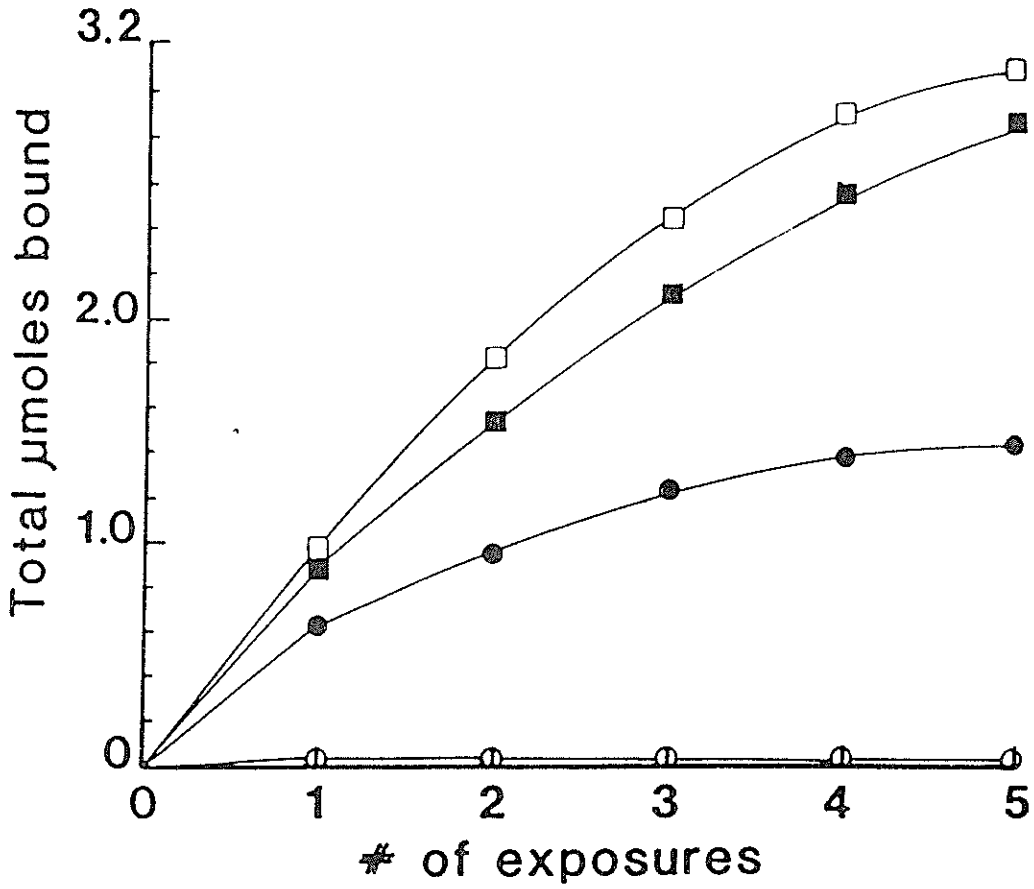

Figure 9. Relative gold-binding capacities of C. vulgaris, Chlorella-silica, and silica alone. Silica (○), 44% Chlorella-silica (●), 86% Chlorella-silica (■), and Chlorella alone (□) were suspended at 2.0 mg/ml in 5.0 ml of 0.20 mM $AuCl_4^-$ for a total of five exposures.

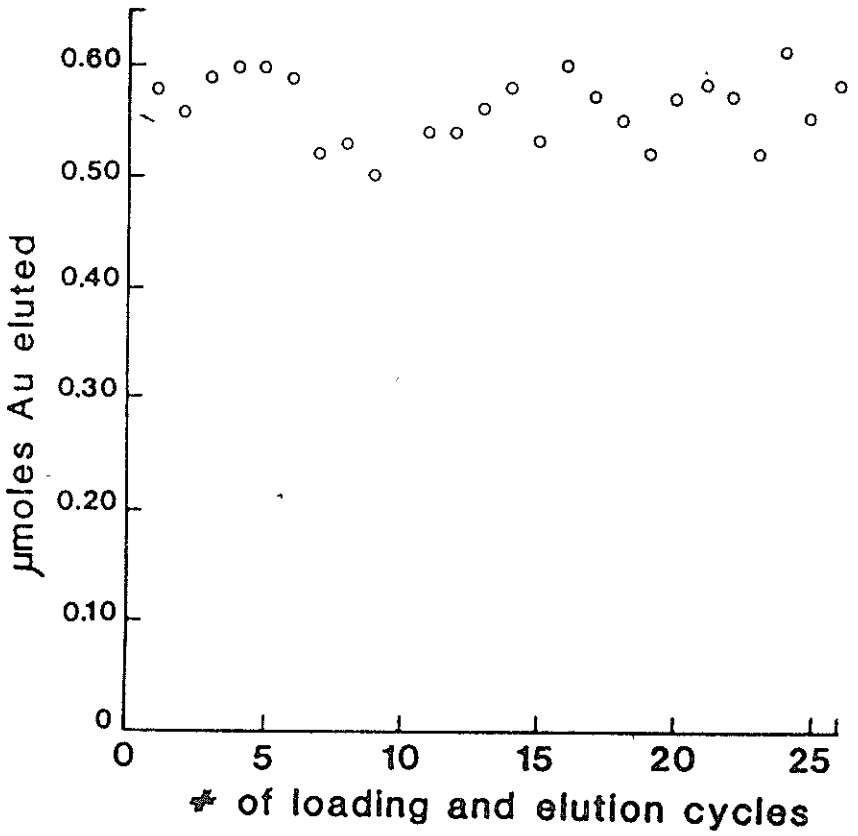

Figure 10. Recycling of the alga-silica: repeated binding and elution of gold ions. A small column of the immobilized Chlorella (50 mg polymer, dry weight) was loaded with 10.0 ml of 0.10 mM $AuCl_4^-$ and then stripped with 10.0 ml of 0.10 M thiourea. Values are presented as micromoles of gold recovered for each cycle. Mass balance calculations showed that all gold that was bound during the loading step was recovered during stripping. When not in use, the column was stored at room temperature in 0.01 M hydrochloric acid.

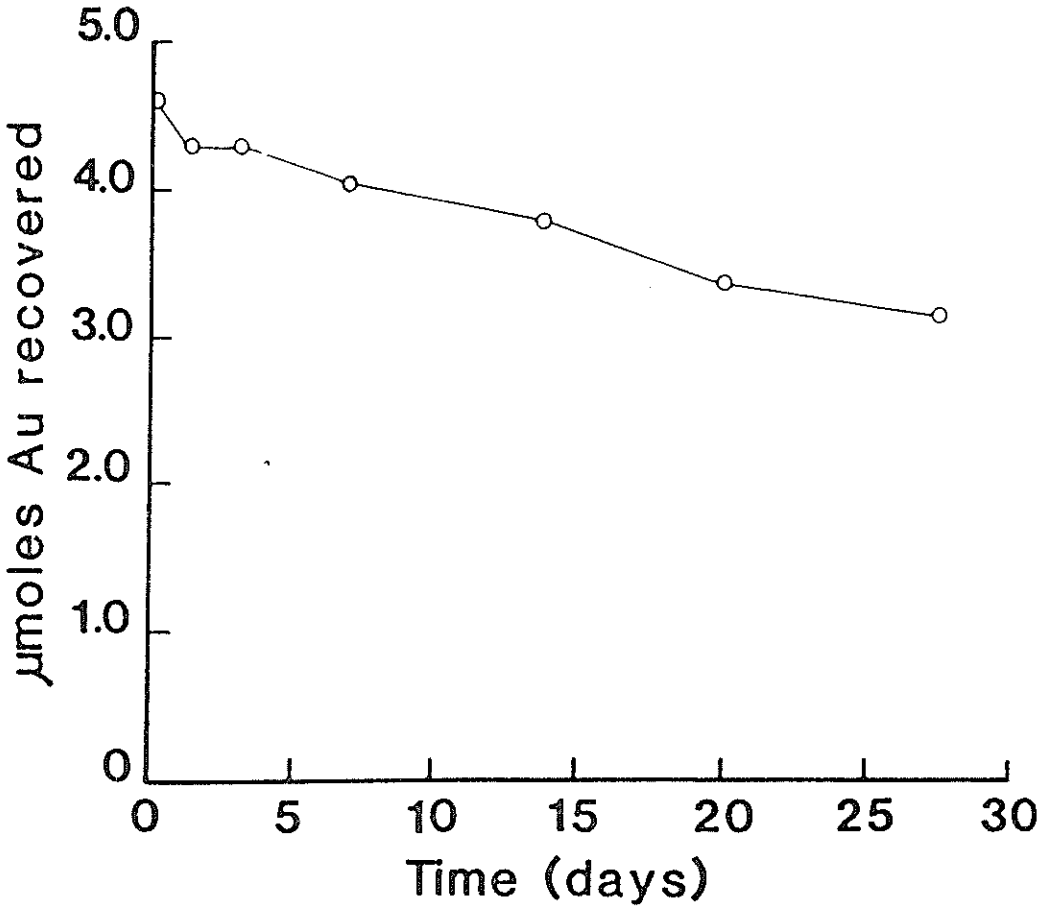

Figure 11. Longevity of an alga-silica column with respect to binding and elution of gold ions. A small column of Chlorella-silica was loaded with 50.0 ml of 0.50 mM $AuCl_4^-$ and subsequently stripped with 10.0 ml of 0.10 M thiourea. The data is presented as micromoles of gold recovered from the column in the thiourea eluant. Mass balance calculations indicated that all of the ion that was bound during the loading step was recovered in the elution procedure.

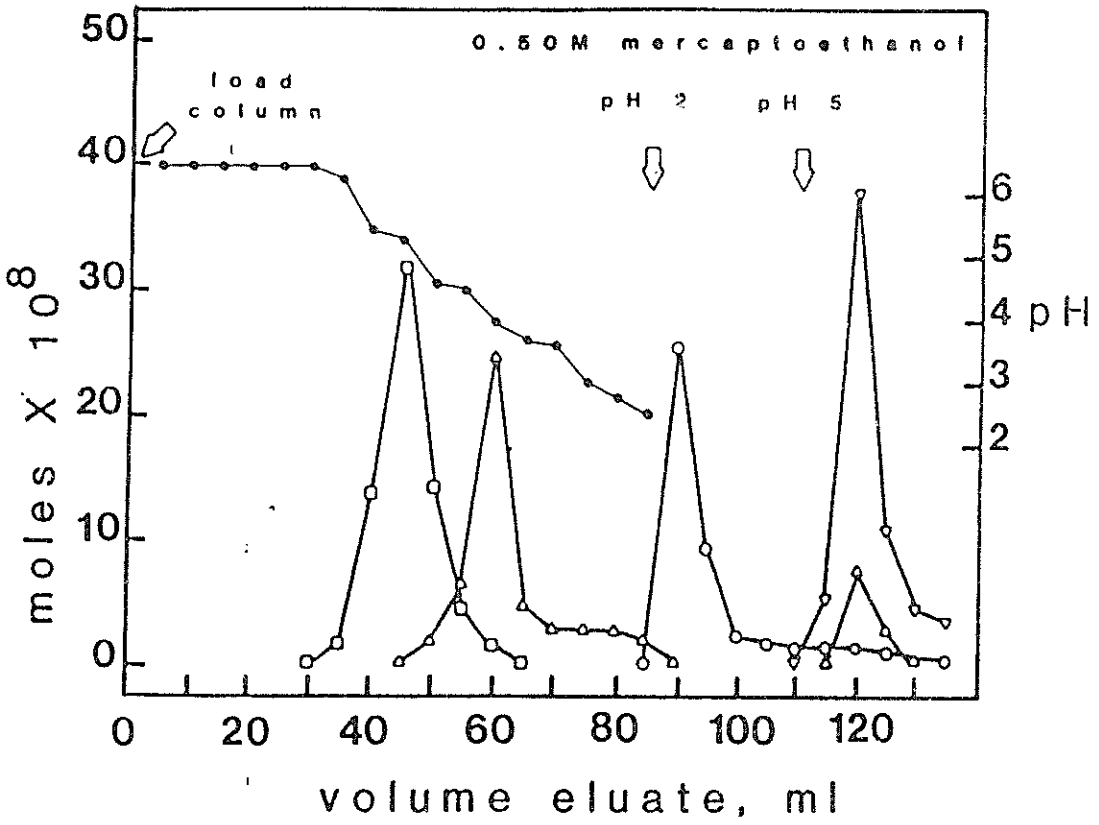

Figure 12. Selective Elution of Four Metal Ions from Immobilized Chlorella. 5.0 ml of a solution containing Cu^{2+}, Zn^{2+}, Au^{3+}, and Hg^{2+} (all at 1.0×10^{-4} M) in 0.05 M NaOAc, pH 6.0, was loaded onto a 5.0 cm x 0.8 cm column of immobilized C. vulgaris (60% algae by wet weight) at a flow rate of 0.5 ml/minute. The column was then washed with 25 ml of 0.05M NaOAc, pH 6.0. A pH gradient (--) was generated by successive elution with 10 ml 0.05M NaOAc, pH 5.0; 10 ml 0.05M NaOAc, pH 4.0; 15 ml 0.05M HOAc, pH 3.0; 10 ml 0.05M HOAc, pH 2.5; and 10 ml 0.05M HOAc, pH 2.0. At this point, the column was eluted with 25 ml 0.50M 2-mercaptoethanol, pH 2.0, followed by 25 ml of 0.50M 2-mercaptoethanol, pH 5.0. Fractions were collected throughout the elution scheme and analyzed for each of the four ions. The amount of each ion recovered is plotted vs. the elution volume.

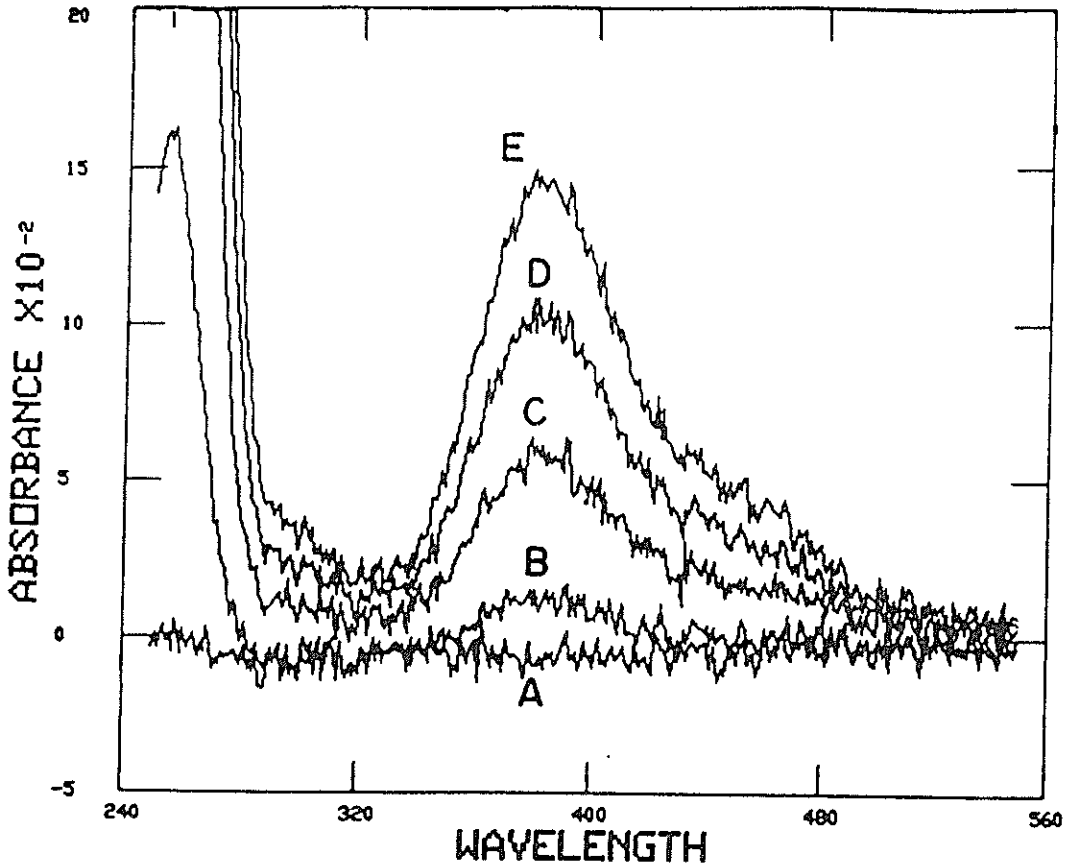

Figure 13. Elution of Au(I) from C. vulgaris by NaBr. Washed Chlorella was suspended, at 1.5 mg/ml, in 0.01M HNO_3. $AuCl_4^-$ was added to afford a concentration of $1.0x10^{-4}$M. Analysis of an aliquot of the suspension confirmed that all of the $AuCl_4^-$ was bound under these conditions. After five minutes, the algae was collected by centrifugation, then resuspended in the same volume of 1M NaBr in 0.01M HNO_3. After 15 minutes, this mixture was centrifuged, and the supernatant was analyzed for gold, by atomic absorption, and for $AuBr_4^-$ specifically, by UV-vis spectroscopy. The atomic absorption analysis indicated a gold ion concentration of $7.9x10^{-5}$M. The UV-visible spectrum of the NaBr eluate is shown above in trace A. Traces B-E correspond to known solutions of $AuBr_4^-$ at concentrations of 1.0, 2.0, 3.0, and $4.0x10^{-5}$M, respectively. Clearly, the concentration of $AuBr_4^-$ in the eluate is well below $1.0x10^{-5}$M, indicating that the gold ion was eluted in the +1 oxidation state, as Au(I)Br.

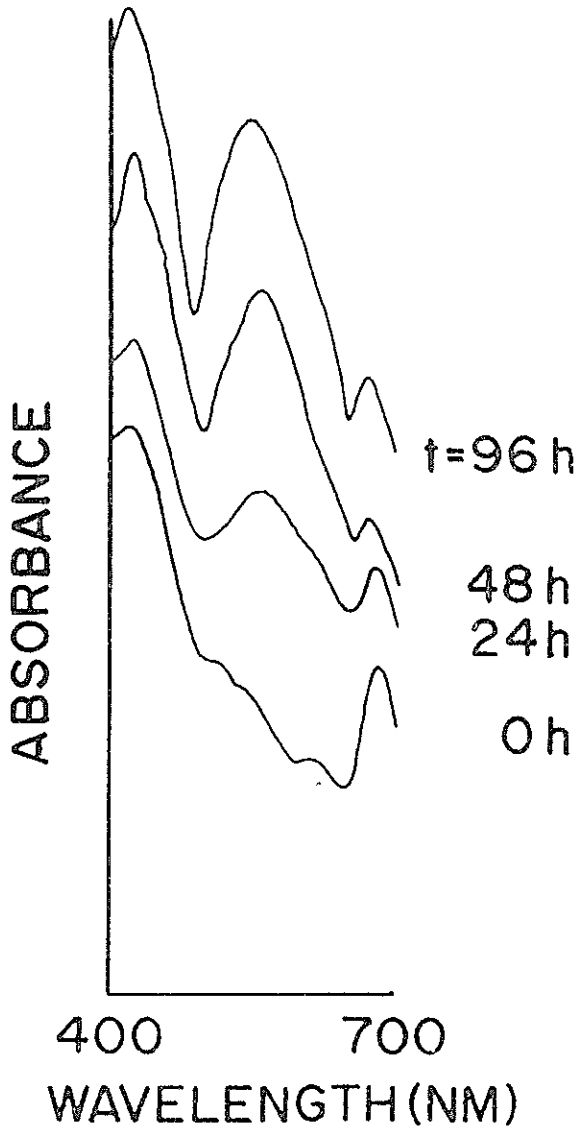

Figure 14. Reduction of Gold Ion on the Surface of C. vulgaris. Washed Chlorella, at a concentration of 0.5 mg/ml, was exposed to 1.0×10^{-3} M $AuCl_4^-$. The algal material was then washed free of excess gold and allowed to sit in distilled water in capped test tubes. At the indicated times, an aliquot of the material was suspended in glycerol, and the visible absorption spectrum was obtained. The peak which grows in at 550 nm is characteristic of colloidal gold.