REMOVAL OF HEAVY METALS FROM WATER WITH MICROALGAL RESINS
I. PROCESS DEVELOPMENT

CLF Technologies, Inc.
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Agreement No. 1425-5-CR-81-20290

Water Treatment Technology Program Report No. 74

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Contamination of water supplies with metals is a constant area of concern. Nationally and internationally, the challenge to remediate hazardous metal-containing waste streams from present or former mining operations, industrial sites, and groundwaters is immense. The technology under study (bioresin) uses immobilized non-living biomass to strip metals from dilute solutions. During this bench-scale project, cultures of biomass were isolated and sufficient material to produce bioresins was generated. Methods for immobilization have been developed. Four bioresin materials were tested, including materials derived from two different species of microalgae. Bioresins derived from one biomass type were found to be highly effective in binding Cu^{2+}, Ni^{2+}, and Pb^{2+}. Efficient recovery of Cu^{2+} was achieved by elution with dilute HCl. More that 50 bed volumes of 10 ppm Cu^{2+} could be passed through a column before breakthrough of Cu^{2+} was achieved. Other experiments evaluated the effects of other cations, such as Na^{+} and Ca^{2+}, which had little effect within certain limits when applied in 44-fold molar excess. Overall, these results appear promising and encourage us to believe that further testing and development are warranted.

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U.S. Department of the Interior

The mission of the Department of the Interior is to protect and provide access to our Nation's natural and cultural heritage and honor our trust responsibilities to tribes.

Bureau of Reclamation

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Table of Contents

List of Tables

List of Figures

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1. Summary

Contamination of water supplies with metals is a constant area of concern. Nationally and internationally, the challenge to remediate hazardous metal-containing waste streams from present or former mining operations, industrial sites, and groundwaters is immense. The technology under study (bioresin) uses immobilized non-living biomass to strip metals from dilute solutions.

In recent years, there has been increasing interest in the use of biomass from microbial sources, particularly the microalgae, to absorb heavy metal ions as part of remediation efforts. The key advantage of this type of biomass is the wide range of functional groups present. These groups occur in a variety of macromolecules, including polysaccharides, proteins, peptides and nucleic acids. Superimposed upon this is the tremendous evolutionary diversity among the microalgae. This diversity further reinforces the variety of available macromolecules that may be utilized in ion-exchange processing of metals from surface waters, groundwaters, or pollutant streams. Conventional precipitation technology is still used in many applications for metals remediation, but is expensive, labor intensive, and ineffective below ppm levels. Conventional ion exchange does not equal the potential from these biologically-based exchangers because total dissolved solids in the waste waters (such as Ca^{2+} - or Mg^{2+} -hardness) interferes with the processing of the toxic ions of interest. Work with biomass has indicated potential to overcome these problems associated with conventional approaches. CLF Technologies has a proprietary collection of microbial cultures derived from contaminated sites. These cultures were used to produce bioadsorbents.

Conventional precipitation technology relies upon the addition of chemical agents to form a precipitate, followed by the collection and processing, and, typically, burial of a sludge which contains toxic metals. Even from a single site, material handling required annually can exceed 10 million pounds of material. The sludge needs to be pumped, dewatered, trucked, buried, and monitored. Energy utilization and cost for each of these steps is substantial because of the amount of material to be handled. Because of the large number of sites, there is substantial energy use now and in the future to handle this material. The proposed new technology should serve to minimize these needs.

During this bench-scale project, cultures of biomass were isolated and sufficient material to produce bioresins was produced. Methods for immobilization have been developed. Four bioresin materials were tested, including materials derived from two different species of microalgae. Bioresins derived from one biomass type were found to be highly effective in binding Cu^{2+}, Ni^{2+}, and Pb^{2+}. Efficient recovery of Cu^{2+} was achieved by elution with dilute HCl. More that 50 bed volumes of 10 ppm Cu^{2+} could be passed through a column before breakthrough of Cu^{2+} was achieved. Other experiments evaluated the effects of other cations, such as Na^{+} and Ca^{2+}, which had little effect within certain limits when applied in 44-fold molar excess. Overall, these results appear promising and encourage us to believe that further testing and development are warranted.

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2. Background and Objectives

Study Goal

The goal of this study is to develop new metals remediation technology, and to build upon the scientific advances of recent years in that area.

Adsorption by Live Microalgae

The microalgae which exist in the freshwater environment and the oceans are important in global ecology, extremely efficient, and taxonomically diverse (Brown and Zeiler, 1993). These microalgae (phytoplankton) in the oceans live in an environment which comprises more than 70% of the earth's surface and is responsible for at least 32% of global photosynthesis (Whittaker, 1975). Through 500 million years of evolution and within this large competitive environment, these microalgae have developed a myriad of polymers which can scavenge the metals of interest, many of which are essential nutrients utilized at low concentrations in microalgal metabolism. In fact, these polymeric materials in the particulate fraction of the world's oceans and lakes regulate the distribution of heavy metal ions in natural waters. The propensity for sequestration of heavy metals by cell walls of these unique and diverse microbes makes them an ideal source of the complex multifunctional polymers which can be used to sequester many different metals through adsorption or ion-exchange processes. This tendency to absorb toxic metals is a problem in microalgal biomass cultures destined for food use. Microalgae are so efficient at scavenging of metals from influent water, from contaminants in nutrients, or from atmospheric deposition into open ponds, that the biomass produced sometimes can contain amounts at the upper limit of metal content for food use (Kajan et al., 1992).

It should be pointed out that the concentrations of the required elements for microalgae in the natural environment are very low, and are sometimes responsible for the dominance of one phytoplankton group over another (Wetzel, 1983). For instance, copper is often present at only 1-2 ppb in lakes, yet is a required nutrient (Wetzel, 1983). Microalgae phytoplankton that produce polymers in their cell walls with high affinity for copper would be expected to sometimes have a competitive advantage over other phytoplankton which do not produce these polymers. The required elements are sometimes the same as those causing pollution problems, or are interspersed in the periodic table among those elements. Concentration factors for Zn, for example, are reported up to 19,000:1 (O'Kelley, 1974). A significant bonus is that uptake in the natural environment typically proceeds in the presence of high concentration of divalent (Ca^{2+}, Mg^{2+}) or monovalent (Na^{+}, K^{+}) cations in both freshwater and marine environments. This selectivity for heavy metals is of particular value in dealing with wastewaters which also have significant amounts of such ions.

These microalgae can be cultivated inexpensively as biomass (Brown and Zeiler, 1993) and provide a ready source of these complex polymeric mixtures for inclusion by immobilization into adsorption columns for a biotechnological cleanup of contaminated sites.

Previous Work with Bioresins Derived from Microalgae

Laboratory experiments have been done by investigators worldwide in recent years demonstrating and confirming the adsorption of metals. A few key papers are reviewed here. Experiments have been done with live or non-living microalgae. Sequestration by live microalgae (see above) does not necessarily preclude concomitant binding by the non-living parts of the cell (wall material). When interpreting studies it is often unclear whether cellular or cell wall material is responsible for binding of heavy metals.

The use of non-living immobilized microalgal biomass is receiving continuing attention. However, commercialization is still not realized. Several laboratory studies have pointed the way

toward a practical process. For example, chromium was adsorbed on microalgal biomass and then recovered by elution at pH 1 (Pappas et al., 1990). Another study took a similar approach with cadmium, and demonstrated adsorption and quantitative elution at pH 2 in several microalgae (Fehrmann and Pohl, 1993). A commonly available strain of microalgae, *Stichococcus bacillaris,* was killed and methods were described for immobilization of the biomass on silica gel (Mahan and Holcombe, 1992). The resulting adsorbent was tested and found to be effective for Pb adsorption at 25, 50 and 100 ppm. Quantitative elution was achieved with 0.012 N HCl. The adsorbent was tested through 20 adsorption elution cycles, and its capacity declined 15%.

Some testing work has been done on heavy metals in groundwater from contaminated sites using immobilized algae. The work supported by the DOE Office of Environmental Restoration and Waste Management and spearheaded by Dr. Dennis Darnall, who incorporated as Bio-Recovery Systems, Inc., of Las Cruces, NM (Feiler and Darnall, 1991). The project was a bench-scale treatability study using immobilized algae to adsorb Hg, Cr and U from contaminated groundwaters. The bed volume of the immobilized algae adsorbent columns was 5 mL. The performance of the adsorbent met project goals. 160 ppb U was treated and the effluent concentration was reduced to 10 ppb. Waters with 323 ppb of Cr were reduced to less than 50 ppb in the effluent. U was eluted with 1.0 N H_2SO_4 and Cr with 3 N NaOH with excellent efficiency. For Hg, 30 ppb was reduced to less than 2 ppb in the effluent. Dr. Darnall is deceased, and the report on the work was released posthumously in 1992 in a report dated 1991 (Feiler and Darnall, 1991). See the discussion of related patents below (Commercial Potential section).

Work by an EPA scientist (U. S. EPA, 1990, Barkley, 1991) also served as a test of the immobilized algae produced by Darnall. After a variety of trials comparing different algal preparations, the results were obtained in an in-situ test with two 0.4 liter columns in series, the first for bulk Hg removal, and the second for polishing. Influent ground water ranged from 330-1,000 ppb. More than 500 bed volumes of water were passed through the columns before the discharge limit of 10 ppb was exceeded. The groundwater contained relatively high dissolved solids (11,000 ppm). Sodium thiosulphate was used to strip Hg from the column in some of the laboratory trials. Other laboratory results referred to in the paper included the reduction in Cd in well water from 660 ppb to 1 ppb, with the column regenerable with 0.3 N H_2SO_4. Lead was reduced from 27 ppm to below the detection limit of 0.1 ppm for 300 bed volumes.

It is clear, however, that testing for each type of site will be necessary before any remedial action is possible. If more than one metal contaminant is present in a potential water supply or wastewater stream, this also presents challenges in resin development, selection and process design. A specialized industrial infrastructure will need to develop to respond to these challenges and provide technical solutions that fit site requirements. The present proposal attempts to map out a strategy to extend and develop past efforts and develop a capability to solve these very challenging environmental problems.

3. Conclusions

Technical Accomplishments

- All activities in the experimental plan were completed (see Table 1).
- Cultures of biomass were isolated, purified and sufficient material to produce bioresins was cultivated and harvested.
- Methods for immobilization on silica gel and with polymers were developed. Four bioresin materials were tested, including materials derived from two different species of microalgae.
- The scientific advancements of recent years including testing of new bioresin materials have been extended by this study.

Scientific Conclusions

- Bioresins derived from one biomass type were found to be highly effective in binding Cu^{2+}, Ni^{2+}, and Pb^{2+}.
- Efficient recovery of Cu^{2+} was achieved by elution with dilute HCl.
- Column capacity was more that 50 bed volumes of 10 ppm Cu^{2+} passed through a column before breakthrough was achieved.
- Cations such as Na^{+} and Ca^{2+} had little effect within certain limits when applied in 44-fold molar excess.

Recommendations

- Results appear promising and encourage us to believe that further testing and development are warranted.
- Future work will focus on providing information to allow assessment of whether this technology can be adapted to situations such as particular waste sites with heavy metal contamination.

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4. Equipment and Methods

Cultures for Biomass Production - Isolation and Purification

Water samples were taken from a mining site in Gilpin County, Colorado, and numerous isolates obtained. Additional cultures were derived from water samples taken by Harry Jong of the Bureau of Reclamation from the Summitville Mine site on March 13 and 14, 1996. These samples were taken 200 feet downstream from the discharge point at the South Fork near the bridge on the site. The rationale for favoring former mining sites is that these may harbor microbes with superior metal-binding capacities, although this supposition is unproven. Twenty-two individual isolates are now in culture, representing a broad taxonomic grouping. Initial isolations were done in Bold's Bristol Medium (BBM), a standard freshwater formulation (Sigma Chemical Co. Product B5282) with 2 mM $NaHCO_3$ added (Nichols, 1973). The pH was adjusted to 7.0 and buffered with 5 mM HEPES buffer (N-[2-Hydroxyethyl]piperazine-N'[2-ethanesulfonic acid]). Isolations were done from colonies which grew in petri dishes with media solidified with 1% purified agar. These cultures will be used as a source of biomass for the production of bioresins. Cultures were grown at 25-27^{o} C, at a light intensity of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided continuously. Some dishes contained antibiotics (penicillin G and erythromycin) at 60 mg/L to aid in the production of bacteria-free cultures.

Biomass Production

A solenoid, needle valve and multi-event timer were adapted for the automated introduction of 5% CO_2 / 95% air into the cultures. The enhanced CO_2 in air regime provides for an order of magnitude enhanced yield from these cultures because CO_2 is the carbon source for growth. This system was assembled, leak tested, and programmed for introduction of short gas pulse every 90 minutes. This allowed for the economical use of the compressed gas as well as avoidance of inhibition of the cultures due to pH depression which often results from over-gassing.

Batches of 4 liters were grown (Fig. 15). Cultures were incubated at 25-27 ^{o}C under approximately 100 $\mu E\ m^{-2}\ s^{-1}$ illuminance from cool-white fluorescent lamps, and continuously stirred with a magnetic stirrer. After 15-17 days, cultures were harvested by centrifugation at 1560 x G for 5 min, washed twice with distilled water and lyophilized. Some cultures are harvested by filtration onto a mesh screen and lyophilized.

Bioresin Production

The algae were immobilized on silica gel using standard methods (Mahan and Holcombe, 1992) which involved wetting a mixture of silica gel and biomass with ultrapure water, then drying at 105 ^{o}C for 20 min, then repeating this process twice. Algae were also immobilized on proprietary polymers from our manufacturing partner, Innovative Design Center, Inc., of Denver.

Bioresin Testing

Columns were tested according to the protocol given in Table 9. The protocol was designed to allow a rapid evaluation of column efficiency. Loading of each eluant or sample was controlled by the use of a peristaltic pump operated at 1 or 2 mL/min, and calibrated by measuring the eluted volume (Figs. 13 and 14). Eluants were introduced from reservoirs through a multiport valve operated manually. Semi-automatic operation was effected by the use of an interval timer with relay which provided power to the peristaltic pump. Wetted surfaces were glass, polypropylene, teflon, and silicone rubber. Total volume collected from each experiment was 41.28 mL. A Typical test solution containing 10 ppm metal was provided to the column. The column was regenerated and bound metal was eluted with 0.012 N HCl. The eluant was

tested for quantitative recovery. The column was flushed with ultrapure water, as indicated (Table 9).

Atomic Absorption Spectrophotometric Analyses - Copper, Nickel and Lead.

Metals were analyzed with a model 5100 PC Perkin-Elmer Atomic Absorption (AA) Spectrophotometer with Zeeman furnace module, complete computer automation, model AS-60 autosampler, and motorized 6-lamp turret.

A hollow cathode multi-element lamp was used for Cu and Ni analyses, and an electrodeless discharge lamp was used for Pb analyses. Sample volume loaded into the autosampler (polystyrene autoanalyzer cuvettes) was 1 mL, with 20 μL being introduced into the graphite furnace for each analysis. Instrumental parameters for Cu are given in Table 6, Ni in Table 7, and Pb in Table 8. Metals differed in time intervals and temperature of the furnace program, wavelength, slit width and lamp used.

All column fractions prepared for AA were diluted 250 or 500-fold in 0.8 % HNO_3 with 0.32% of the non-ionic detergent Triton X-100. These additives were sufficient to keep metals in solution and allowed for efficient operation of the pipetter in the AS-60 autosampler that dispenses the standards and samples into the AA graphite furnace. The Perkin-Elmer software automatically measures the area under the atomic peaks (Figs. 2, 4, 5, 6, 10, and 17), subtracts the blank, and plots a calibration curve (Figs. 3-5). Calibration solutions were diluted from AA Certified Standards # C-6024 for Cu (990 ppm), N-4137 for Ni (990 ppm), and L-4885 for Pb (1010 ppm) from Sigma Chemical Company, St. Louis, MO. Dilutions for standards were 1:1000 followed by 0.5-2.5:100.

5. Results

Cultures

Cultures of microalgae were established from water samples from mining sites in Colorado. The cultures were grown in 4 liter batches and amounts of biomass were harvested to allow for an assessment of metal-binding ability. Several batches from three different cultures were prepared.

Atomic Absorption Spectrophotometric Analyses - Copper, Nickel and Lead.

The model 5100 PC Perkin-Elmer Atomic Absorption (AA) Spectrophotometer using Zeeman corrected graphite furnace methodology was used to analyze for Cu, Ni and Pb. The instrument records the peaks generated by atomic and background signals during operation of the furnace. The areas under the peaks for Cu (Fig. 2), Ni (Fig. 4a) and Pb (Fig. 5a) are calculated by the instrumental software, and constructed into calibration curves for Cu (Fig. 3), Ni (Fig. 4b) and Pb (Fig. 5b). Two replicate readings were taken for each sample, one for each standard.

Typical atomic and Zeeman peaks for the 5 second read time are given for analytical standards (Figs. 2 and 17). Zeeman correction peaks for Cu are typically just slightly smaller than the atomic peaks for clean solutions without significant matrix effects. Zeeman background peaks for Ni and Pb were smaller (Fig. 17).

Column Testing Protocol

Columns were tested according to the protocol given in Table 9. The protocol was designed to allow a rapid evaluation of column efficiency. Other details are given is Section 4 (Equipment and Methods).

Binding of Cu and Column Capacity for Cu

A column with silica gel as the support was used for biomass from culture SDW001a (7.4% biomass content), and was extremely efficient, as shown in the AA peaks (Fig. 6) and in the calculated data (Table 2). The loading and flush solution (Eluant A) was completely free of Cu^{2+} (at this analytical level). It also appeared that essentially complete recovery of copper was achieved in these trials. Other controls in the same experiment demonstrate that two trials with water as the loading sample had no Cu^{2+} signal (Table 2). This was followed with two virtually identical 10 ppm Cu runs, followed by another run with ultrapure water as the loading sample in which no evidence of carry-over of Cu occurred. Calculations indicated that more than 11 bed volumes of 10 ppm copper solution was taken up (Table 2).

The breakthrough of Cu^{2+} for a bioresin column based on silica gel was evaluated by taking 1.5 mL fractions from an experiment in which 10 ppm Cu was continuously introduced into the column (Fig. 7). No Cu was detected in the column effluent until 50 bed volumes had been passed through at this concentration. Based on data derived from the Dept. of Energy-supported experiments (Appendix 2), the author (L. M. Brown, unpublished) calculated that at least 42 bed-volumes of Cu should be bound by this type of biomass. Thus, these results are in good agreement with the data obtained with the non-immobilized, non-living biomass. A total of 421 μg of Cu^{2+} was bound by this column after correction for dead volume and the amount passed through the column after breakthrough (Fig. 7).

Efficiency of Binding of Cu by Silica- and Polymer-Based Bioresins

In confirmation of earlier trials with silica-based bioresins (biomass SDW001a), efficiency of binding and elution were high (Figs. 8, 9). Silica gel without microalgae was a poor adsorbent for Cu^{2+} (Fig. 9). Polymeric resins with immobilized microalgae at a content of 10 % of (SDW001a) biomass were considerably less effective in sequestering Cu^{2+} (Fig. 9, Fig. 10).

On average, more Cu passed through the column than was bound even at this moderate loading. Examination of the individual runs for efficiency reveals that on each succeeding trial (Table 4), capacity decreased for this material; however total recovery of copper increased (Table 4). It may be that certain sites were being saturated, and that Cu was not being removed from those sites with the eluant used. By the end of the experiment, the easily exchangeable sites (for Cu) gave good recovery, but poor capacity. The behavior of this material has also been studied at higher biomass content (58%) in Fig. 9. In that experiment, binding efficiency was increased to 87%, but 13% passed through the column. Recovery of Cu exceeded 90%, and the performance was more reproducible than the formulation with 10% biomass content.

Other Bioresins and Cu-Binding

The binding and elution of Cu^{2+} from silica bioresin with biomass SDW001a was highly efficient (Figs. 8 and 9) using the standard protocol (Table 9). However binding of Cu^{2+} by biomass SDW017 on silica gel was less efficient (Fig. 11). This lesser binding efficiency for Cu was not noted previously for non-immobilized biomass material of SDW017 (see DOE-supported studies, Fig. 18 and 19). However, since the reduction for copper was only 20%, it may not have been easily detectable because of the large standard deviations which occurred in these experiments due to the difficulties in centrifugation of the native biomass (Fig. 18). The results suggest that assay of native biomass for Ni-binding may be more meaningful.

Reproducibility and Column Longevity for Cu-binding

Even after 23 cycles of loading, elution with 0.012 N HCl followed by water, efficiency of binding of 10 ppm Cu exceeded 97 % for the first 11.4 mL (114 μg) applied to the column, even in the presence of excess Na^{+} and Ca^{2+} (Table 5). However, metals were not present in all 23 loading cycles (some were controls with ultrapure water). Typical performance data after 15 cycles with this column are illustrated in Fig. 8.

Binding of Ni and Pb

Binding of Ni^{2+} and Pb^{2+} by silica bioresin SDW001a was high and equal to that of Cu-binding capacity under the conditions tested (Fig. 11). However, Ni and Cu (see above for discussion of Cu) were not bound as well by biomass SDW017. These experiments were performed by the standard protocol (Table 9).

Competition of Na and Ca with binding of Cu

Other experiments with silica bioresin column SDW001a evaluated the effects of other cations, such as Na^{+} and Ca^{2+} had little effect (2.6% reduction in complete binding) on the first 114 μg (1.6 μmol) when applied in 44-fold molar excess (Table 5). The Na and Ca were added at twelve-fold the column capacity for Cu. Continuing infusion of this mixture (10 ppm Cu^{2+}, 100 ppm Na^{+}, and 100 ppm Ca^{2+} as Cl^{-} salts) resulted in some breakthrough with 65±3% and 43±2% binding of Cu in sequentially applied 69 μg aliquots of this mixture (Fig. 12).

6. Analysis of Results and Commercial Viability of the Project

Analysis of Results

The capacity for Cu is illustrated in Fig. 7 and is calculated to be about 421 μg (6.63 μmol) which is equivalent to 211 μmol/g biomass. However, careful examination of Fig. 7 indicates that this bioresin may not have been completely saturated at the conclusion of this experiment. Thus, this should be considered as a minimum value. Literature values indicate that capacities for algal biomass sources have been recorded as <300 μmol/g (Mahan and Holcombe, 1992) and 300-500 μmol/g (de Carvalho et al, 1995) for heavy metal accumulation.

Biomass types had differing binding capacities for heavy metals (Fig. 11). Lead was effectively bound by both biomass types tested, but copper, and especially nickel, were less effectively bound. Other workers have also shown biomass-preparation specific differences in binding. Some biomass binds copper in preference to cadmium (de Carvalho et al. 1995), but other preparations bound cadmium in preference to copper (Leusch et al. 1995). These differences in binding probably reflect chemical differences in biomass composition. Such differences can be potentially exploited for treatment of particular metals, or combinations of metals.

The key features of the present results are the reproducibility, and the selectivity in the presence of other interfering ions. Process economics along with the potential for developing unique bioresins from unique materials will determine whether these results will have practical application. The potential for commercial viability is discussed in the following sections.

Commercial Viability

Technical Advantages

Conventional precipitation technology is still used in many applications for metals remediation, but is expensive, labor intensive, and ineffective below ppm levels (Feiler and Darnall, 1991, Leitz et al., 1995). Conventional ion exchange does not equal the potential from these biologically-based exchangers because total dissolved solids in the waste waters (such as Ca^{2+} or Mg^{2+} hardness) interferes with the processing of the toxic ions of interest. Work with biomass has indicated potential to overcome these problems associated with conventional approaches (Feiler and Darnall, 1991). The rationale for the use of biomass derived from microalgae is based on the biological properties of this material. It appears that this selectivity is based on the polymers present in the cells, particularly in the cell wall. The selectivity described for the living biomass often carries over to the non-living material, which can be more easily handled as an ion exchanger.

Advantages in Energy Consumption

Conventional precipitation technology relies on the addition of chemical agents to form a precipitate, followed by collection and processing and typically burial of a sludge which contains toxic metals. For example, copper is precipitated as insoluble cupric sulfide with polymers to encourage flocculation. The copper-containing sludge is collected and further dewatered in a filter press. Even from a single site, the amount of material handling required annually amounts to 10 millions of pounds of material (Leitz et al., 1995). The sludge needs to be pumped, dewatered, trucked, buried, and monitored. Energy use and cost for each of these steps is a factor because of the amount of material to be handled. Moreover, the energy required for producing and delivering the large amount of precipitant chemicals is also a factor. Because of the large number of sites, there is substantial energy use now and in the future to handle this material. The proposed new technology should serve to minimize these needs. It is expected that energy

consumption will be reduced by simplifying the process and avoiding pumping and sludge handling and transport

Advantages in Process Economics

One example of a metal contaminated water is at the Summitville Mine site in Colorado. Treatment of copper-containing waste is based on combining sodium sulfide and ferrous sulfate to produce ferrous sulfide, which is then allowed to react to produce insoluble cupric sulfide. The production of >1 million lb of sludge per year in one unit is a significant problem for this operation. The cost of operation for one of these units is $6.60 per 1,000 gal treated (Leitz et al., 1995) (see also Fig. 16 and Appendix 2). Another unit at the site produces >5 million lb of sludge per year at a water processing cost $46/1000 gal. A third unit, treats another 110 gpm at $25/1000 gal.

In contrast, Darnall estimated operating cost for a bioadsorption plant (using immobilized non-living algae) at $0.25-5.00/1000 gal, depending on local conditions and process details (Feiler and Darnall, 1991). Those calculations were based on a 600 gpm unit (see also Fig. 16 and Appendix 2).

Also, while best efforts are being made to store the sludges associated with metals removal using conventional technology, cautious concerns have been expressed about long-term stability of the massive amounts of sludge produced (Leitz et al., 1995). Any sludge minimization through bioresin use will be of significant benefit.

Commercial Potential and Market Considerations

Contamination of water supplies with metals is a constant area of concern. Nationally and internationally, the challenge to remediate hazardous metal-containing waste streams from present or former mining operations, industrial sites and groundwater is immense. Under the DOE jurisdiction alone, there are 3,700 sites covering over 26,000 acres in 26 states and territories, many of which are contaminated with toxic concentrations of metals (U. S. Department of Energy, 1994). Colorado has many former mining sites which require expensive and cumbersome cleanup operations such as the Summitville mine (Leitz et al., 1995). There are heavy metals released in conjunction with these industries because of the magnitude of production and nature of the resources. Some of these metals can make their way into water supplies and remain a continuing problem in states such as Colorado

This project is aimed at being responsive to the need for improvement of existing chemical treatment processes for industrial and municipal wastewater pollution control. A particular need is seen for processes for removing heavy metals such as chromium, arsenic, lead, and mercury from industrial waters or soil washing effluents. The use of bioresins with unique properties seeks to fill the need for economically viable recovery of metals for reuse and safe disposal.

A patent search has been conducted to determine whether there are patents in force which would preclude or limit the development of this technology. While there are several patents which deal with related approaches, none is so broad as to preclude the development of new technology. The most relevant of these is a composition patent that used the binding of microbial cells, especially algae, with natural polymers derived from seaweed, precipitating particles, and heat treating the particles at 300-500 ^{o}C (Greene et al., 1991). Another approach used a silica gel-based composition or an immobilization on glass wool (Darnall et al., 1991). The adsorbents were effective for gold, silver, mercury, or platinum or oxanions such as molybdate and vanadate. Another patent described the extraction of uranium from seawater using colonies of microalgae and other microorganisms (Paschke et al., 1981). Ground samples of brown algae can be used to remove gold from industrial or natural waters. (Volesky et al., 1988). Gold can be recovered from cultures of cyanide-producing microalgae and other microorganisms (Kleid et al., 1995).

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Table 1. Activities completed under this contract.

Activities Completed under Contract No. 1425-5-81-20290, Water Treatment for Metals with Microalgal Bioadsorbents
✓ Immobilize algae on silica gel and produce columns
✓ Test efficiency of Uptake
✓ Compare columns from two different algae, Investigate immobilization strategies
✓ Develop methods for regeneration
✓ Test Regeneration Efficiency
✓ Analyze applicability to practical problems

Table 2. Raw data for sequence of experiments in which water or 10 ppm Cu^{2+} were loaded on silica gel column with immobilized microalgal biomass (SDW001a). These data are in the sequence of trials done with this column. Sequence demonstrates that there was no evidence of carry-over of Cu between runs.

Run #	Sample in Elution Step # 1	Pooled Eluant	[Cu] (μg/L) in diluted pooled eluant
1	H_2O	A	-0.2
		B	1.0
		C	-0.3
2	H_2O	A	0.3
		B	-0.2
		C	0.1
3	10 ppm Cu^{2+}	A	-0.1
		B	21.0
		C	-0.2
4	10 ppm Cu^{2+}	A	-0.2
		B	20.0
		C	0.4
5	H_2O	A	-0.2
		B	-0.1
		C	-0.2

Table 3. Adsorptive capacity of the biomass. These data are calculated from Fig. 9.

Resin Material	mg Cu^{2+} adsorbed/g biomass	mg Cu^{2+} adsorbed/g resin
Silica Gel with Immobilized Microalgal Biomass	>2.55	>0.189
Silica Gel without Microalgal Biomass	-	0.06

Table 4. Experiment with polymeric resin with immobilized microalgal biomass in which 6.88 mL of 10 mg/L copper is loaded on columns according to the elution protocol in Table 9. These are the detailed data contributing to the means reported in Fig. 9. Resins containing microalgal biomass have been prepared from CLF culture #SDW001a. Note that the capacity of the columns to bind Cu decreases with each trial, indicating a possible saturation of sites.

Cu elution from test columns (percentage of total Cu^{2+} added to columns)					
% Bound	% Not Bound (eluant a)	% Eluted by HCl (eluant b)	% Eluted by H_2O (eluant c)	% Recovered from column	# bed volumes of 10 mg/L Cu^{2+} adsorbed
78	22	24	0	46	6.3
50	50	23	0	73	4
35	65	28	4	98	2.8

Table 5. Summary of competition experiments in which a bioresin column (0.72 mL) composed of biomass derived from CLF culture SDW001a is bound to silica gel. Binding results represent triplicate determinations ±SD. Cu^{2+} is bound to this column in experiments conducted at 2 mL/min. A forty-four-fold molar excess of other cations (Na^{+} an Ca^{2+}) resulted in only a 2.6% reduction in efficiency of binding of Cu. Added Na and Ca at twelve-fold the capacity for Cu (prior to breakthrough - see Fig. 7) did not substantially affect Cu-binding, indicating selectivity for Cu.

	Control				Competition by Na and Ca						
Metal	**Metals added (ppm)**	**µmoles added to column**	**µmoles bound by bioresin**	**Binding efficiency for Cu (%)**	**Metals added (ppm)**	**µmoles added to column**	**Molar ratios of added metals**	**µmoles bound by bioresin**	**Binding efficiency for Cu (%)**	**µmoles in excess of column capacity for Cu**	**Ratio of amount added to column capacity for Cu**
Cu	10	1.57	1.57	100 ± 0	10	1.57	1	1.53	97.4 ± 1.1	0	0.3:1
Na	0	0			100	43.3	28			37.6	7.6:1
Ca	0	0			100	25.0	16			19.3	4.4:1

Table 6. Instrumental operating parameters used for the Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer graphite furnace with Zeeman correction for Cu.

Instrumental					
Element: Cu		Peak Storage: 1/sample			
Instrument Model 5100		Technique: HGA	Software Version 7.10		
Wavelength 324.8 nm peak	Slit 0.7 Low	Signal Measurement: Peak Area	Read Delay:0	BOC Time: 2	Lamp: Multi-element hollow cathode, Cu, Ni, Fe
Calibration					
Solution	*Concentration (μg/L)*	*Autosampler Location*	*Volume (μL)*		
Blank	0	1	20		
Standard 1	5.0	2	20		
Standard 2	14.8	3	20		
Standard 3	19.8	4	20		
Standard 4	24.8	5	20		
Samples		Various	20		
Calibration Type: Linear:	Matrix Modifier: none				
Furnace Time/Temperature Program					
Step	*Temperature ℃*	*Ramp Speed*	*Hold Time (s)*	*Gas Flow mL/min*	*Read Step*
1	130	1	60	300	
2	1000	1	45	300	
3	2300	0	10	0	Yes
4	2600	1	6	300	
5	20	1	20	300	
Injection Temp: 20 °C	Pipette speed: 100%				

Table 7. Instrumental operating parameters used for the Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer graphite furnace with Zeeman correction for Ni.

Instrumental					
Element: Ni		Peak Storage: 1/sample			
Instrument Model 5100		Technique: HGA	Software Version 7.10		
Wavelength 232.0 nm peak	Slit 0.2 Low	Signal Measurement: Peak Area	Read Delay:0	BOC Time: 2	Lamp: Multi-element hollow cathode, Cu, Ni, Fe
Calibration					
Solution	*Concentration (μg/L)*	*Autosampler Location*	*Volume (μL)*		
Blank	0	1	20		
Standard 1	5.0	2	20		
Standard 2	9.9	3	20		
Standard 2	14.8	4	20		
Standard 3	19.8	5	20		
Standard 4	24.8	6	20		
Samples		Various	20		
Calibration Type: Linear:	Matrix Modifier: none				
Furnace Time/Temperature Program					
Step	*Temperature ℃*	*Ramp Speed*	*Hold Time (s)*	*Gas Flow mL/min*	*Read Step*
1	130	1	60	300	
2	1400	1	45	300	
3	2500	0	10	0	Yes
4	2600	1	6	300	
5	20	1	20	300	
Injection Temp: 20 °C	Pipette speed: 100%				

Table 8. Instrumental operating parameters used for the Perkin-Elmer Model 5100 Atomic Absorption Spectrophotometer graphite furnace with Zeeman correction for Pb.

Instrumental					
Element: Pb		Peak Storage: 1/sample			
Instrument Model 5100		Technique: HGA	Software Version 7.10		
Wavelength 283.3 nm peak	Slit 0.7 Low	Signal Measurement: Peak Area	Read Delay:0	BOC Time: 2	Lamp: Single-element electrodeless discharge
Calibration					
Solution	*Concentration (µg/L)*	*Autosampler Location*	*Volume (µL)*		
Blank	0	1	20		
Standard 1	5.1	2	20		
Standard 2	10.1	3	20		
Standard 3	15.2	4	20		
Standard 4	20.2	5	20		
Standard 5	25.2	6	20		
Samples		Various	20		
Calibration Type: Linear:	Matrix Modifier: none				
Furnace Time/Temperature Program					
Step	*Temperature ℃*	*Ramp Speed*	*Hold Time (s)*	*Gas Flow mL/min*	*Read Step*
1	120	1	30	300	
2	800	1	45	300	
3	1800	0	6	0	Yes
4	2600	1	6	300	
Injection Temp: 20 °C	Pipette speed: 100%				

Table 9. Protocol for loading and elution of metals from bioresin columns. In the experiments described in this table, flow rate was 2 mL/min. In some early experiments, flow rate was 1 mL/min. At least 3 system volumes of eluant are added at each step. One or two steps are pooled in each eluant tube.

Elution Step	Pooled Eluant Tube	Eluant Component	Time (min)	Volume of Eluant (mL)	# Dead Volumes of Column System	Pooled Volume in Eluant Tube
1 (loading)	A	10 ppm Cu^{2+} (loading solution)	3.375	6.88	3	
2 (flush)	A	H_2O	3.375	6.88	3	13.76
3 (acid stripping)	B	0.012 N HCl	3.375	6.88	3	
4 (flush)	B	H_2O	3.375	6.88	3	13.76
5 (flush)	C	H_2O	6.75	13.76	6	13.76

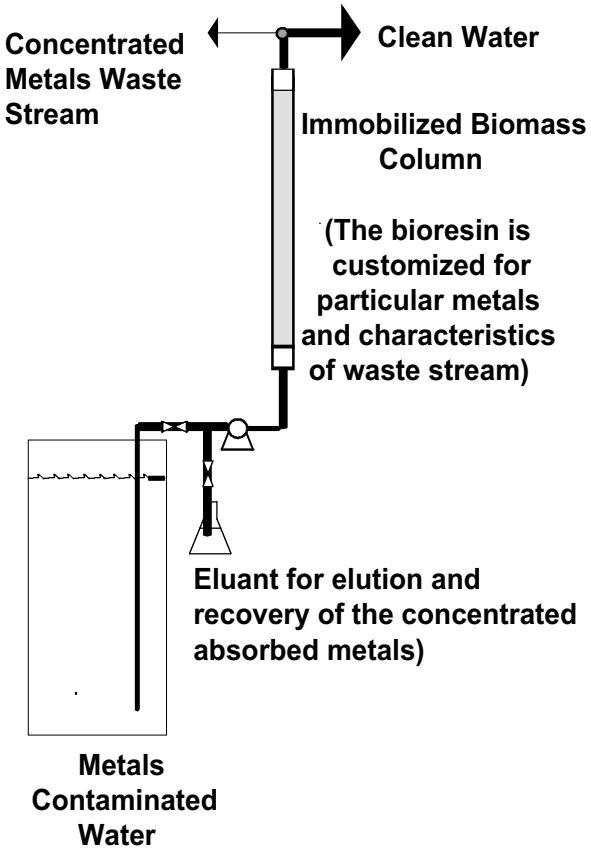

Figure 1. Schematic of immobilized biomass system for sequestration and recovery of heavy metals.

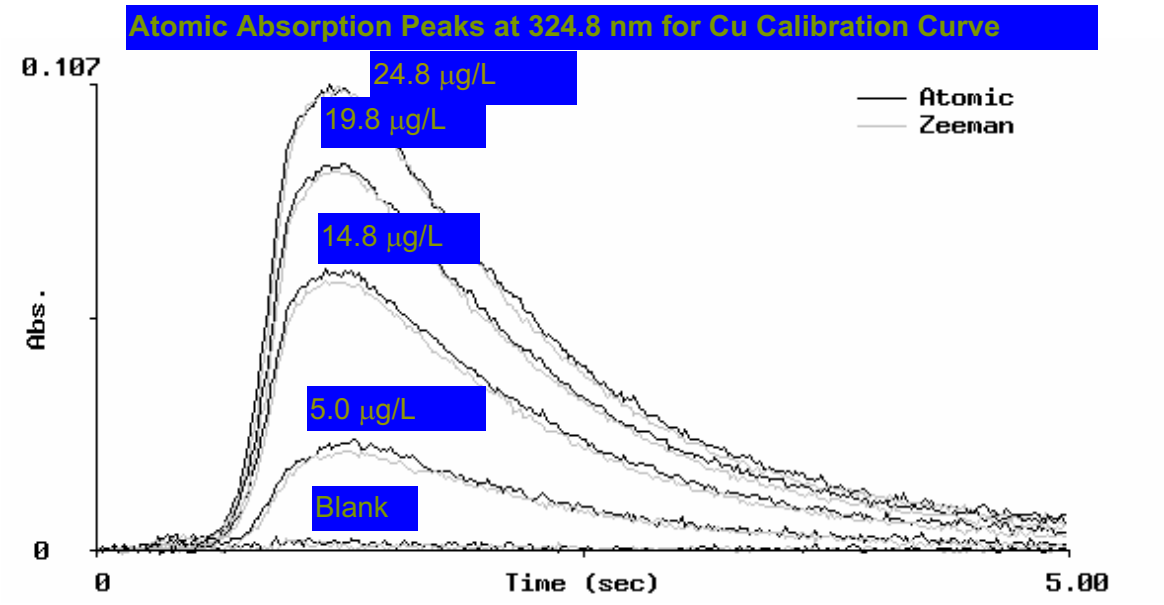

Figure 2. AA peaks for calibration curve, 0-24.8 ppb Cu. Both atomic and Zeeman correction spectra are shown for each standard. Blank corrected peak areas for these standards are plotted in Fig. 3. The slightly smaller Zeeman peaks are characteristic of pure Cu solutions with little or no matrix interference.

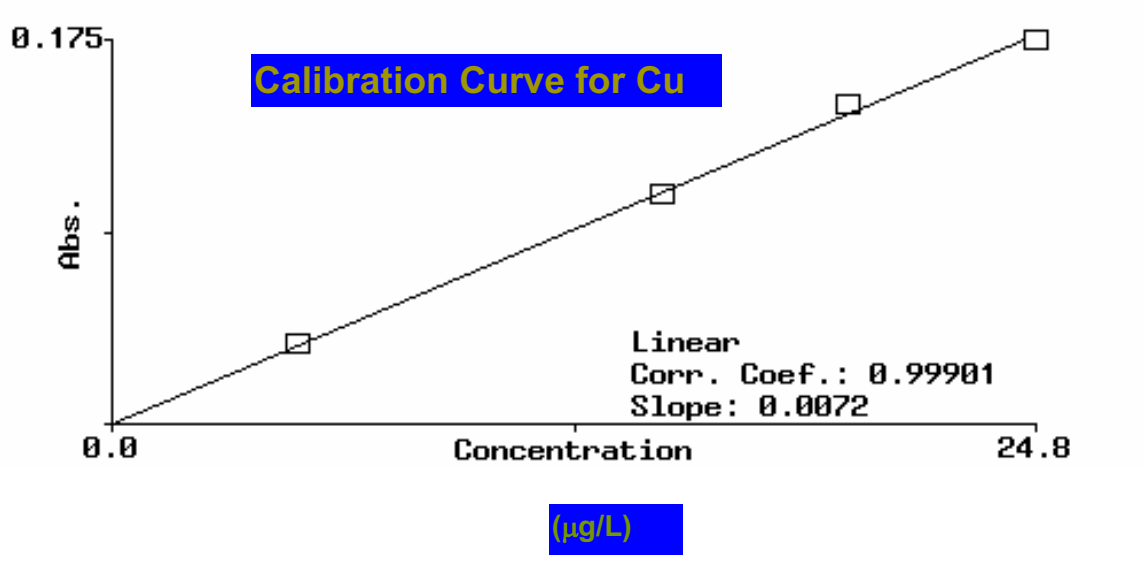

Figure 3. Calibration curve for copper. Blank-corrected peak areas for each measurement from Fig. 2. This instrument provides a reproducible linear response for Cu. A calibration is done for each day's analysis.

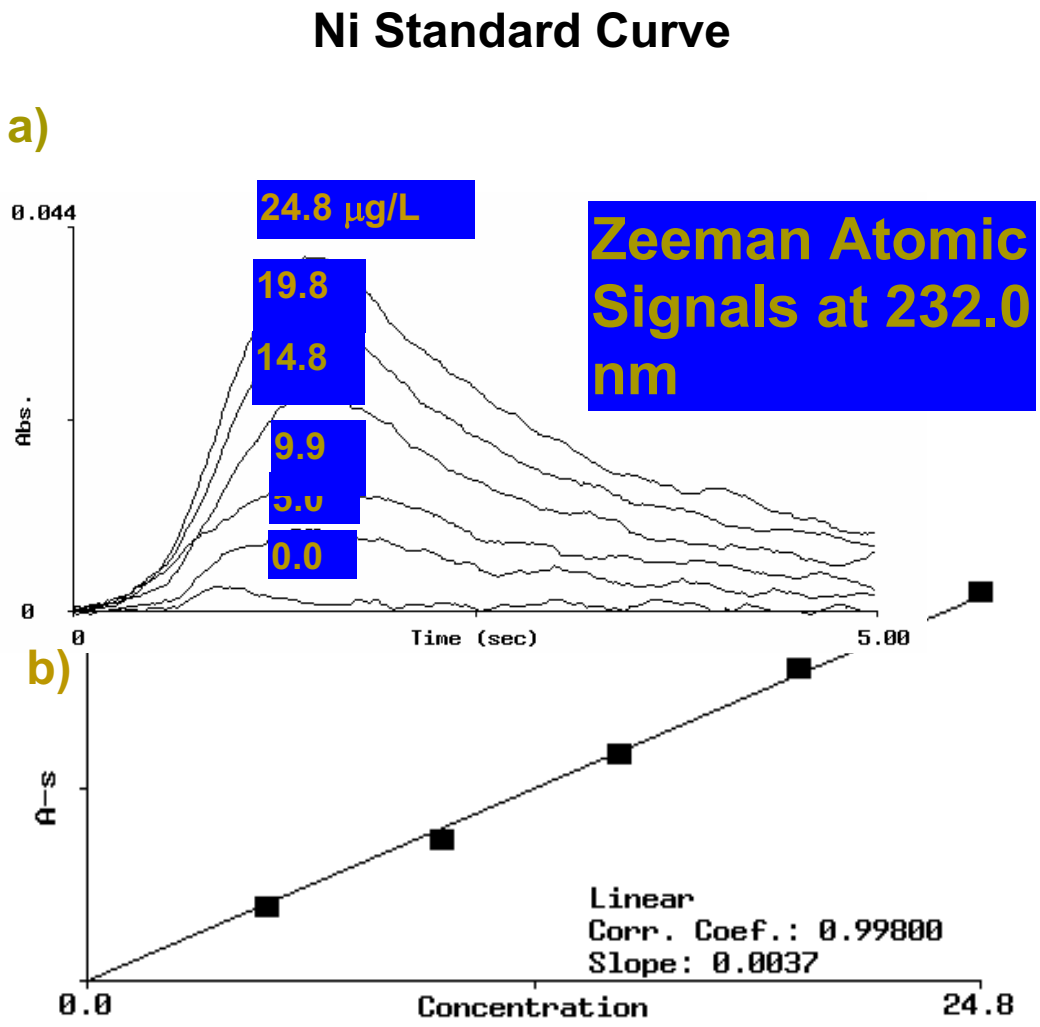

Figure 4. **a)** AA peaks for standard curve, 0-24.8 ppb Ni. Atomic spectra are shown for each standard. **b)** Calibration curve for Ni. Blank-corrected peak areas for each measurement from (a).

This instrument provides a reproducible linear response for Ni. A calibration is done for each day's analysis.

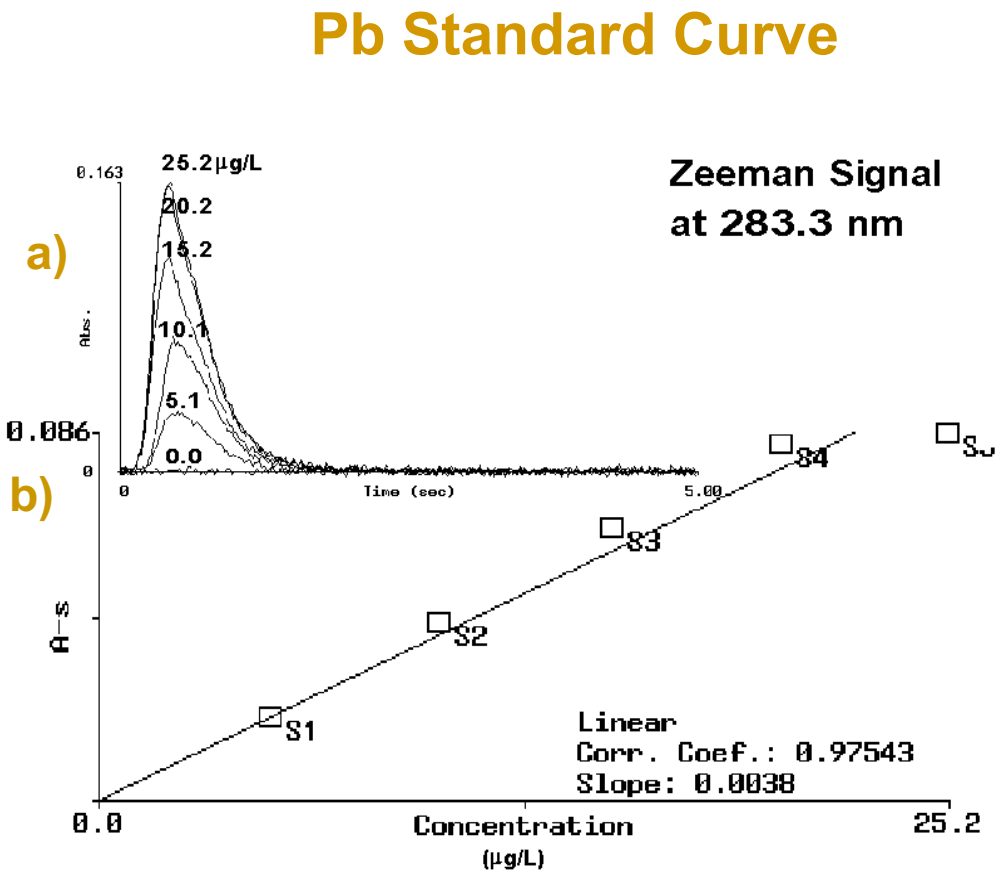

Figure 5. **a)** AA peaks for standard curve, 0-25.2 ppb Pb. Atomic spectra are shown for each standard. **b)** Calibration curve for Pb. Blank-corrected peak areas for each measurement from (a). This instrument provides a linear response for Pb. A calibration is done for each day's analysis.

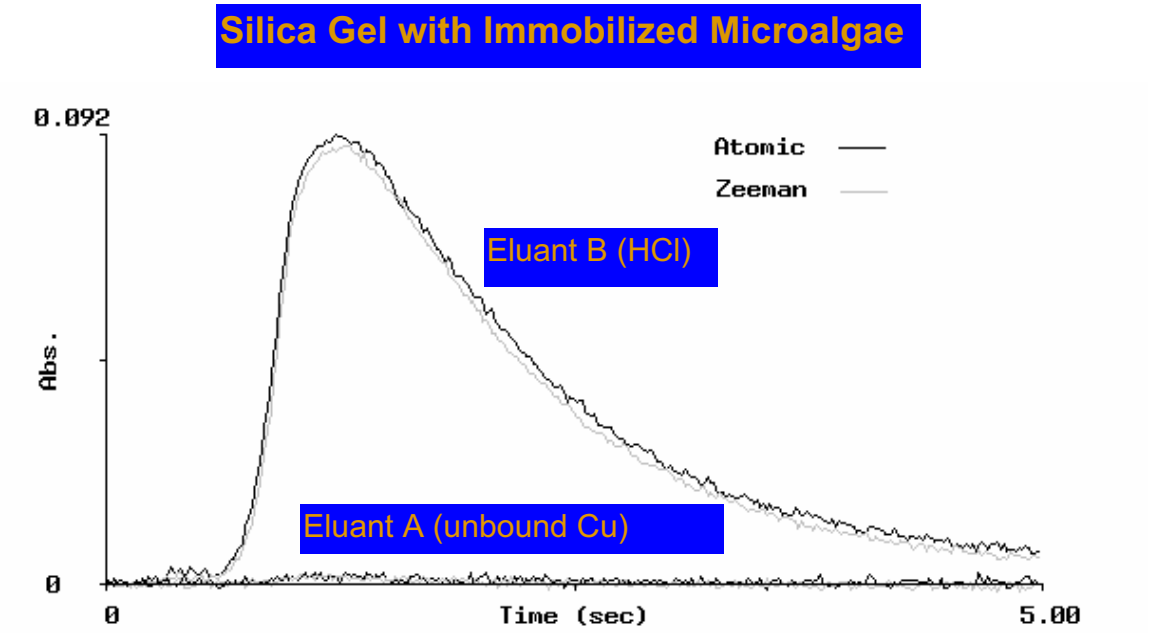

Figure 6. AA scans of a sample derived from a Cu^{2+} solution of 10 mg/L passed through a column of microalgae immobilized on silica gel. The solutions are diluted 250x. Eluant A represents the 6.88 mL Cu solution pooled with 6.88 mL of H_2O used to flush the Cu solution through the column. Eluant B represents the 6.88 mL of HCl pooled with 6.88 mL of H_2O, which eluted all of the bound Cu from the column. Compare with Fig. 10 in which much of the Cu is not effectively bound to the bioresin in Eluant A. The slightly smaller Zeeman peaks are characteristic of pure Cu solutions with little or no matrix interference.

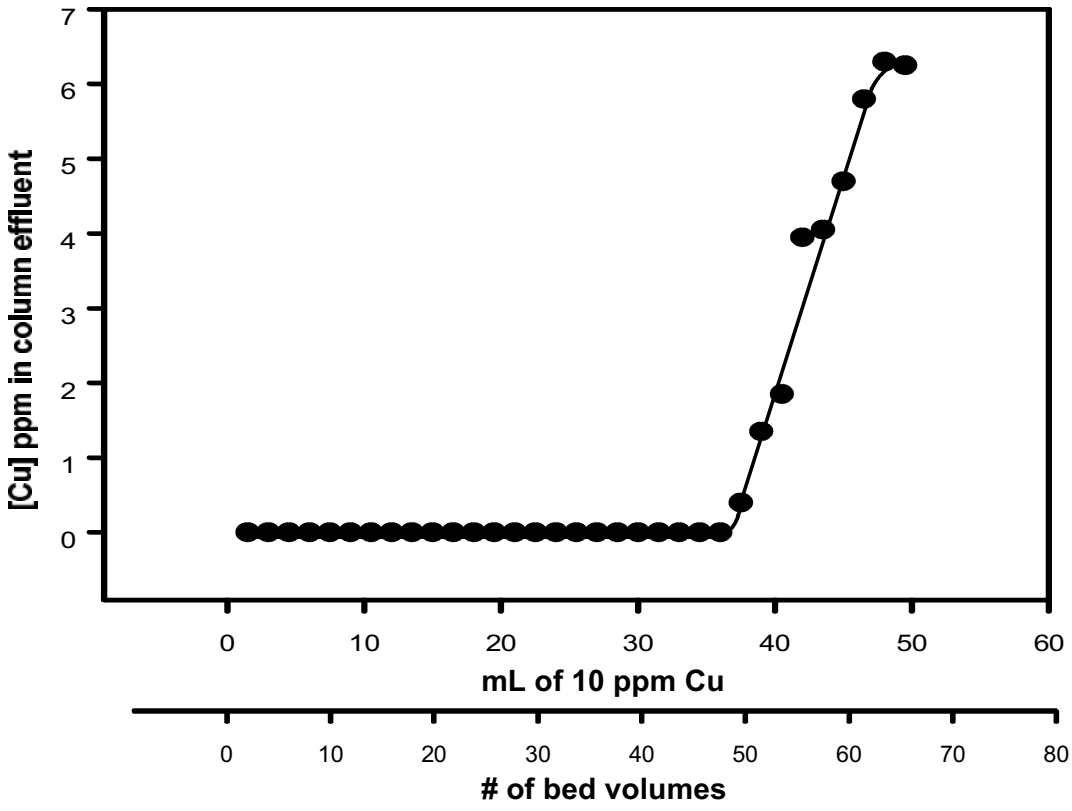

Figure 7. Column capacity test for Cu^{2+}. A solution of 10 ppm $CuCl_2$ is added to a bioresin of SDW001a biomass at a loading of 7.4%. The column effluent was monitored for Cu, and, as shown, Cu was detectable only after at least 50 bed volumes of 10 ppm Cu had passed through the column (volumes are gross column volume of adsorbent, not void volume of the column). This test represents the 19th cycle for this column.

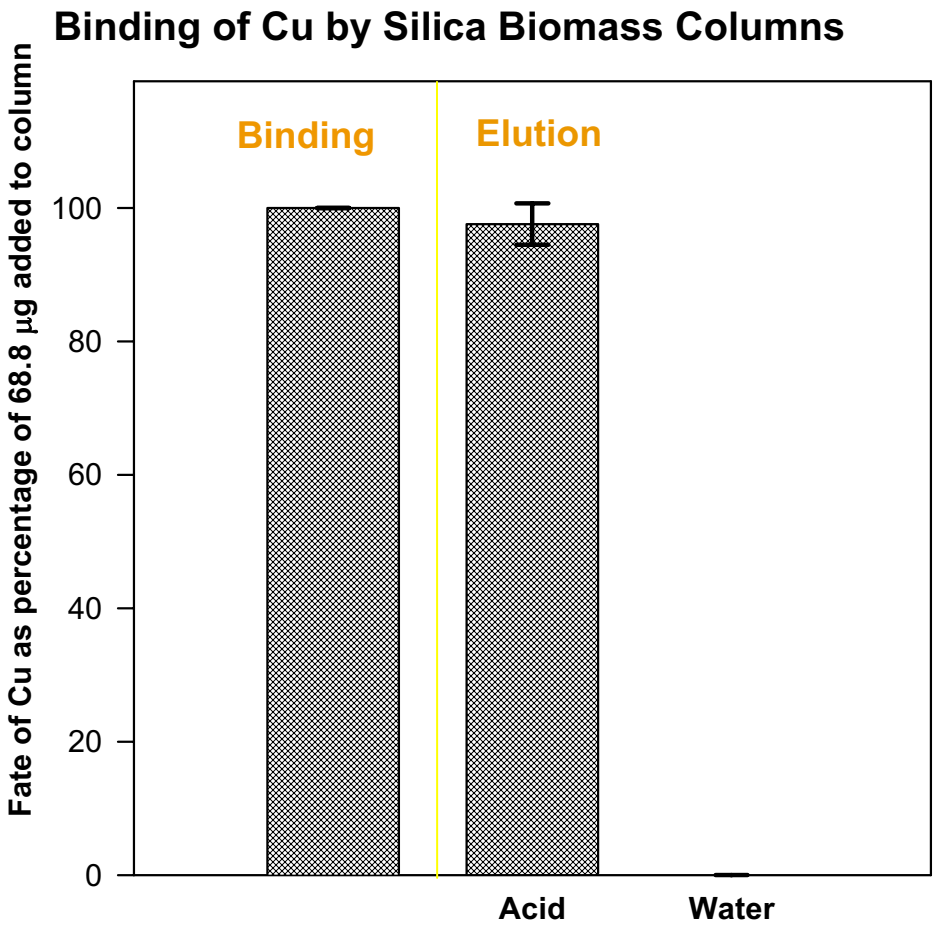

Figure 8. Binding and elution of Cu^{2+} in experiment done just before trial performed by using same column as Fig. 7. A solution of 10 ppm of Cu is added to a bioresin of SDW001a biomass at a loading of 7.4%. Aliquots of 6.88 mL were added to a column with 0.72 mL bed volume at a flow rate of 2 mL/min. Triplicate determinations were made for each metal (±SD). No Cu was detected in the effluent. This test represents the 16th-18th cycle for this bioresin

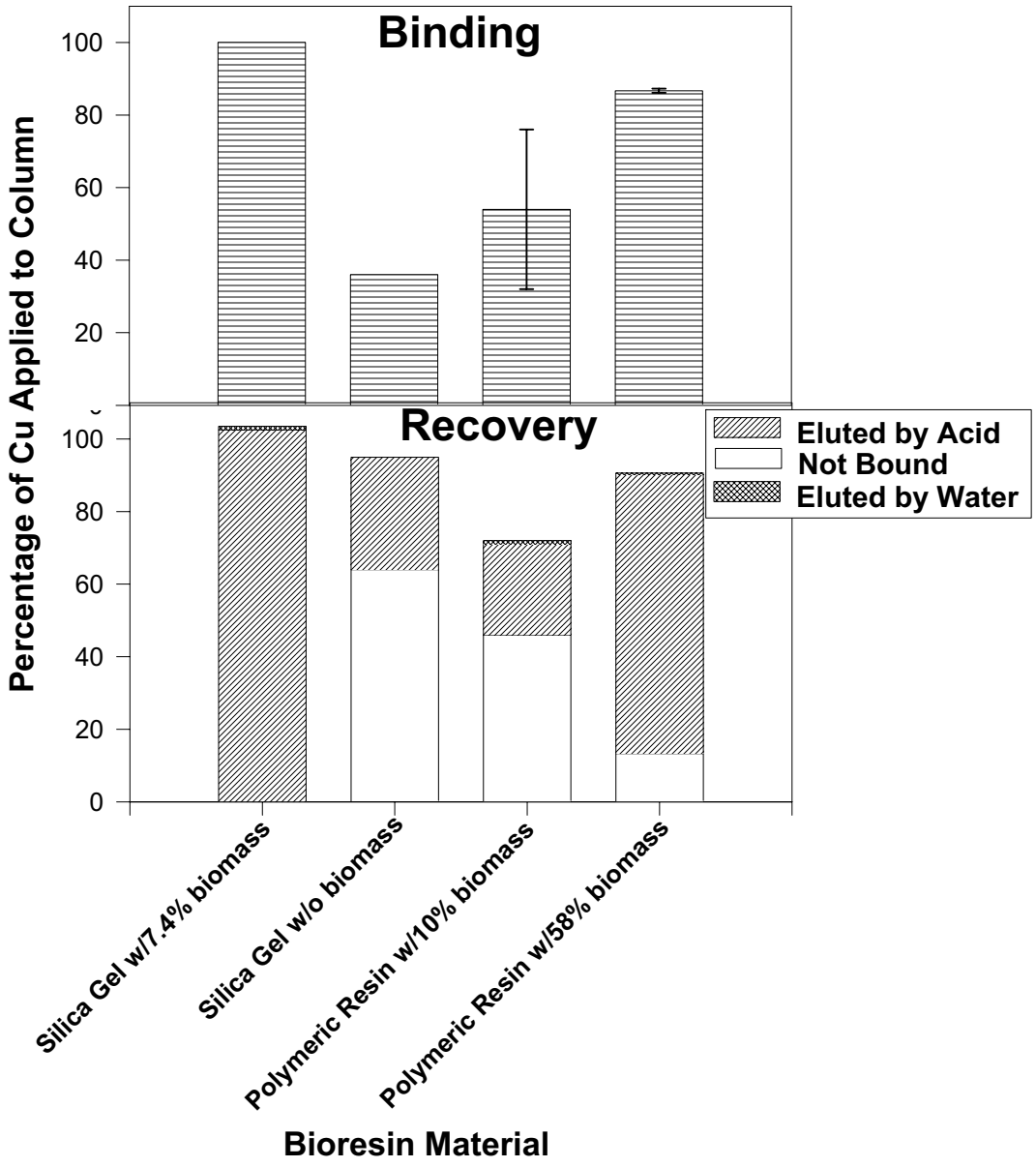

Figure 9. Experiment in which 6.88 mL of 10 mg/L copper is loaded on columns according to the elution protocol in Table 2. Those resins which contain microalgae have been prepared from CLF culture #SDW001a. Note the excellent binding characteristics of the silica gel bioresin with microalgae. The Cu is completely eluted with dilute acid. Compare to the control silica gel without microalgae in which binding of copper is poor. Polymeric resin at 10% loading with immobilized biomass has an intermediate adsorptive capacity, but exhibits an inconsistent response and poor recovery of copper. Improvements were made in the performance of the polymeric resin by increasing the biomass percentage to 58%. (rightmost bar) (n=3, ±SD for polymeric resin, n=1 for silica gel, n=2 for silica gel w/biomass).

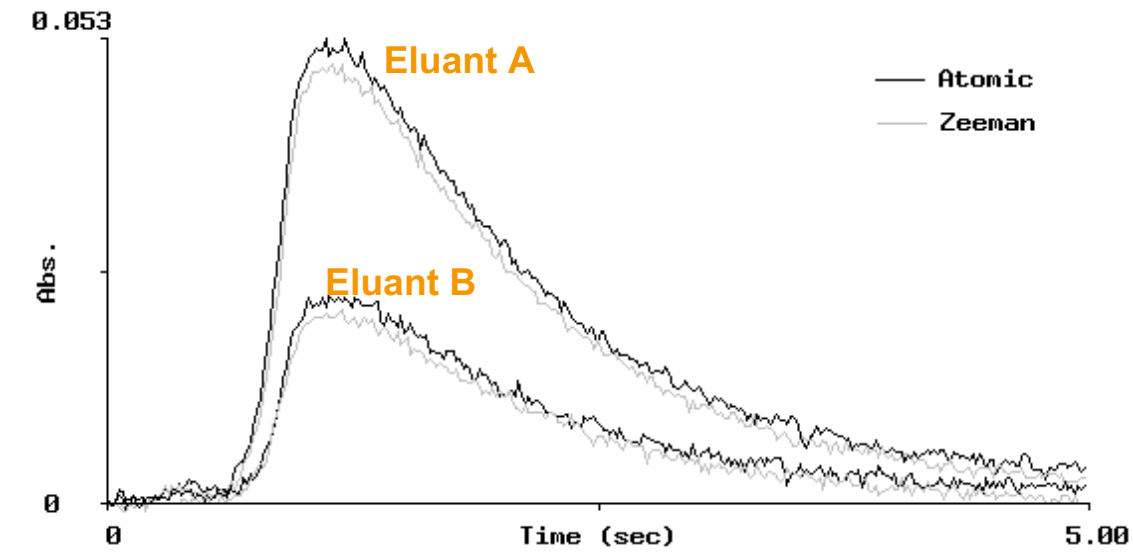

Figure 10. AA scans of a sample derived from a Cu^{2+} solution of 10 mg/L passed through a column of microalgae immobilized on a polymeric resin (10% loading). The solutions are diluted 250x. Eluant A represents the 6.88 mL Cu solution pooled with 6.88 mL of H_2O used to flush the Cu solution through the column. Note that most of the Cu is in this fraction, indicating very poor binding. Eluant B is the 6.88 mL of HCl pooled with 6.88 mL of H_2O which eluted any bound Cu from the column. This figure represents one trial from the experiment described in Fig. 9.

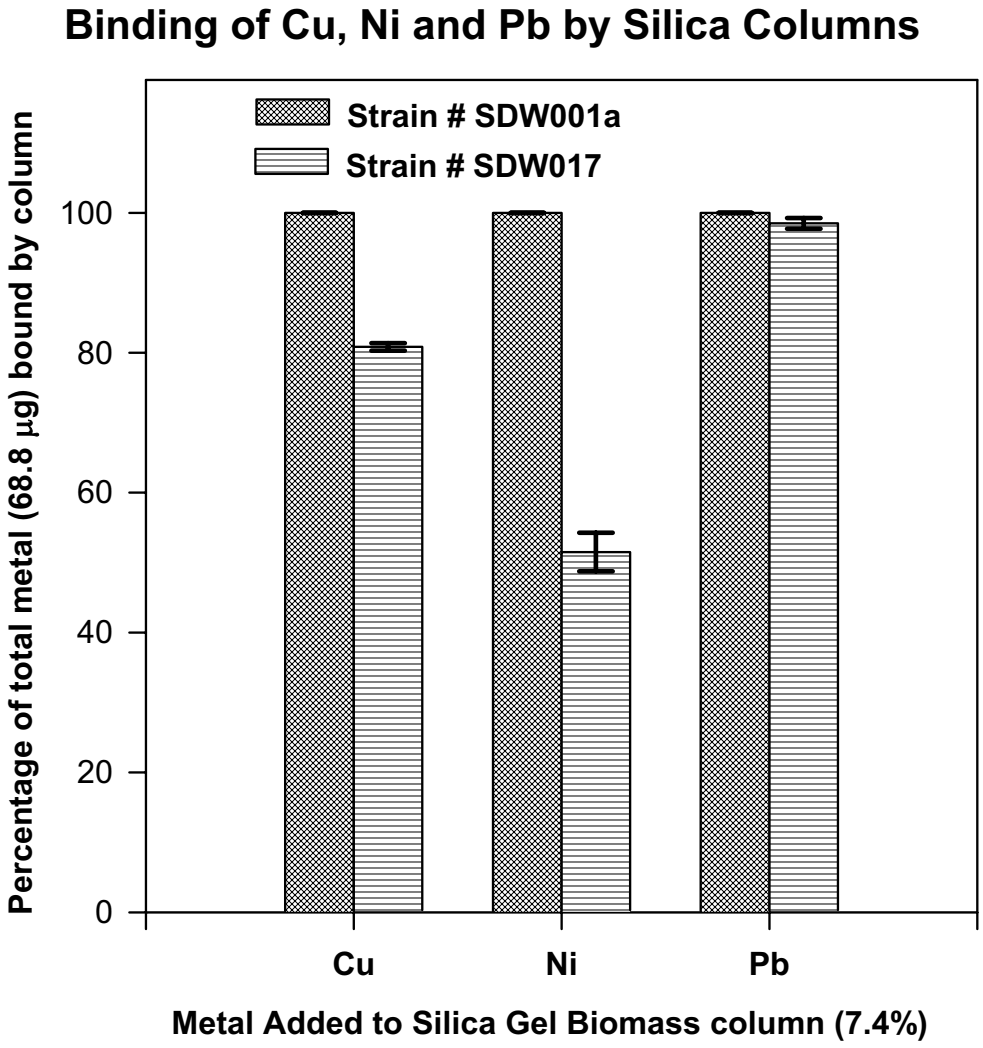

Figure 11. Binding of Cu^{2+}, Ni^{2+}, and Pb^{2+}. A solution of 10 ppm of each metal is added to a bioresin of SDW001a or SDW017 biomass at a loading of 7.4%. Aliquots of 6.88 mL are added to a column with 0.72 mL bed volume at a flow rate of 2 mL/min. Triplicate determinations were made for each metal (±SD). No metal was detected in the effluent for SDW001a, but SDW017 proved to be less efficient for Ni and Cu.

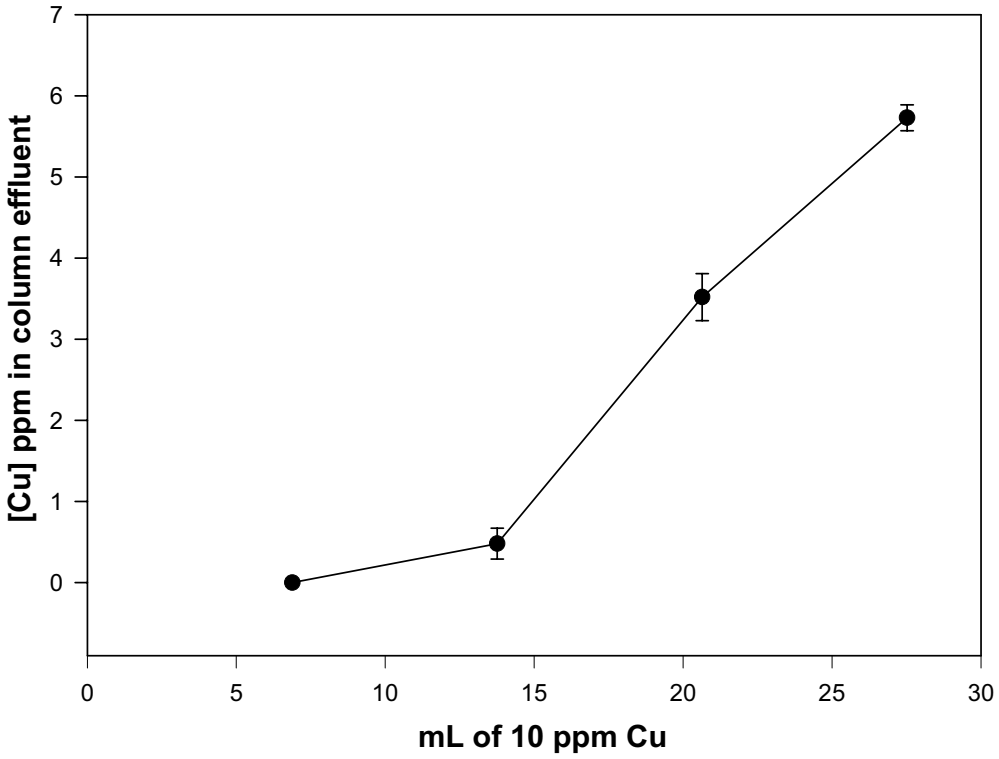

Figure 12. Competition experiments in which a bioresin column (0.72 mL) composed of biomass derived from CLF culture SDW001a bound to silica gel. Binding results represent triplicate determinations ±SD. Cu^{2+} is bound to this column in experiments conducted at 2 mL/min. A forty-four-fold molar excess of other cations (Na and Ca^{2+}) resulted in only a 2.6 % reduction in efficiency of binding of Cu for first two data points. See Table 5 for other calculations on this experiment. System dead volume is 2.3 mL.

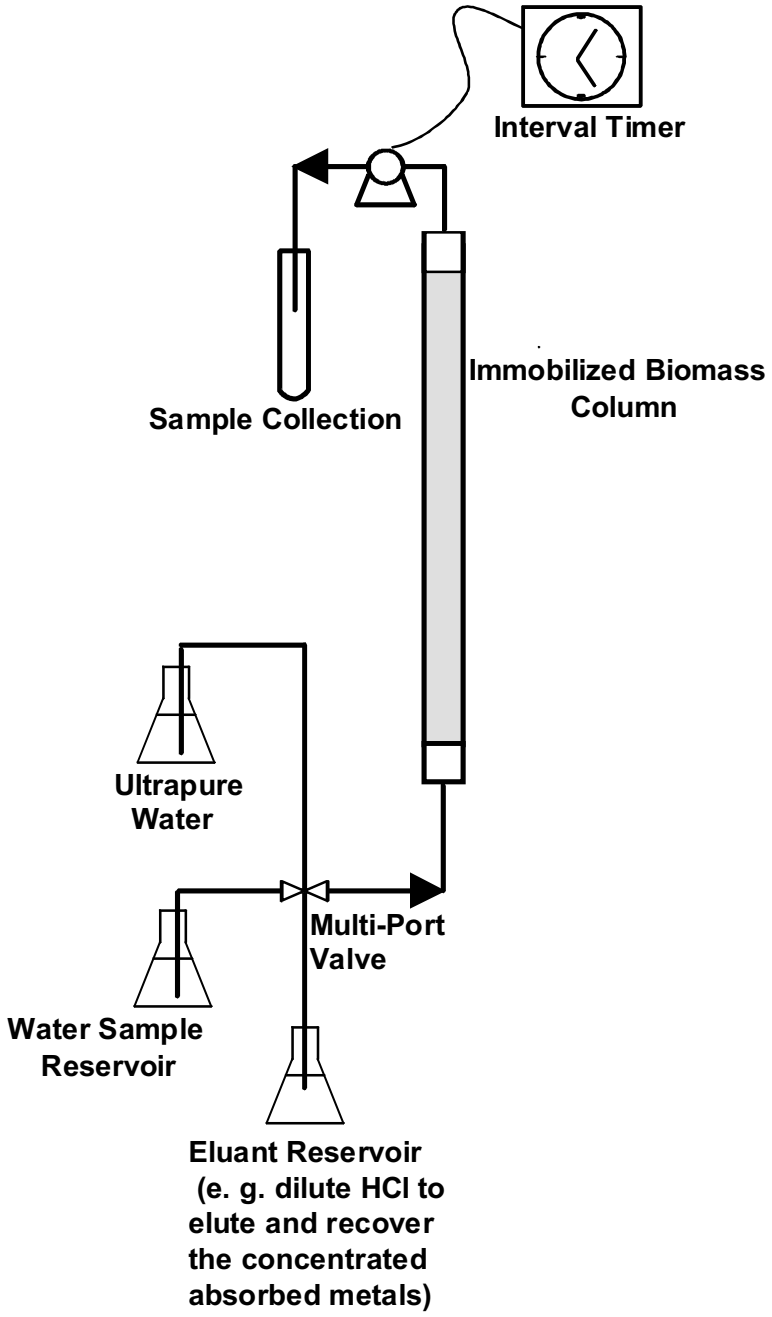

Figure 13. System used to evaluate the efficiency of bioresins. system includes eluant reservoirs, column, peristaltic pump, and timer. The timer controls the operation of the pump. See photos of components in Fig. 14.

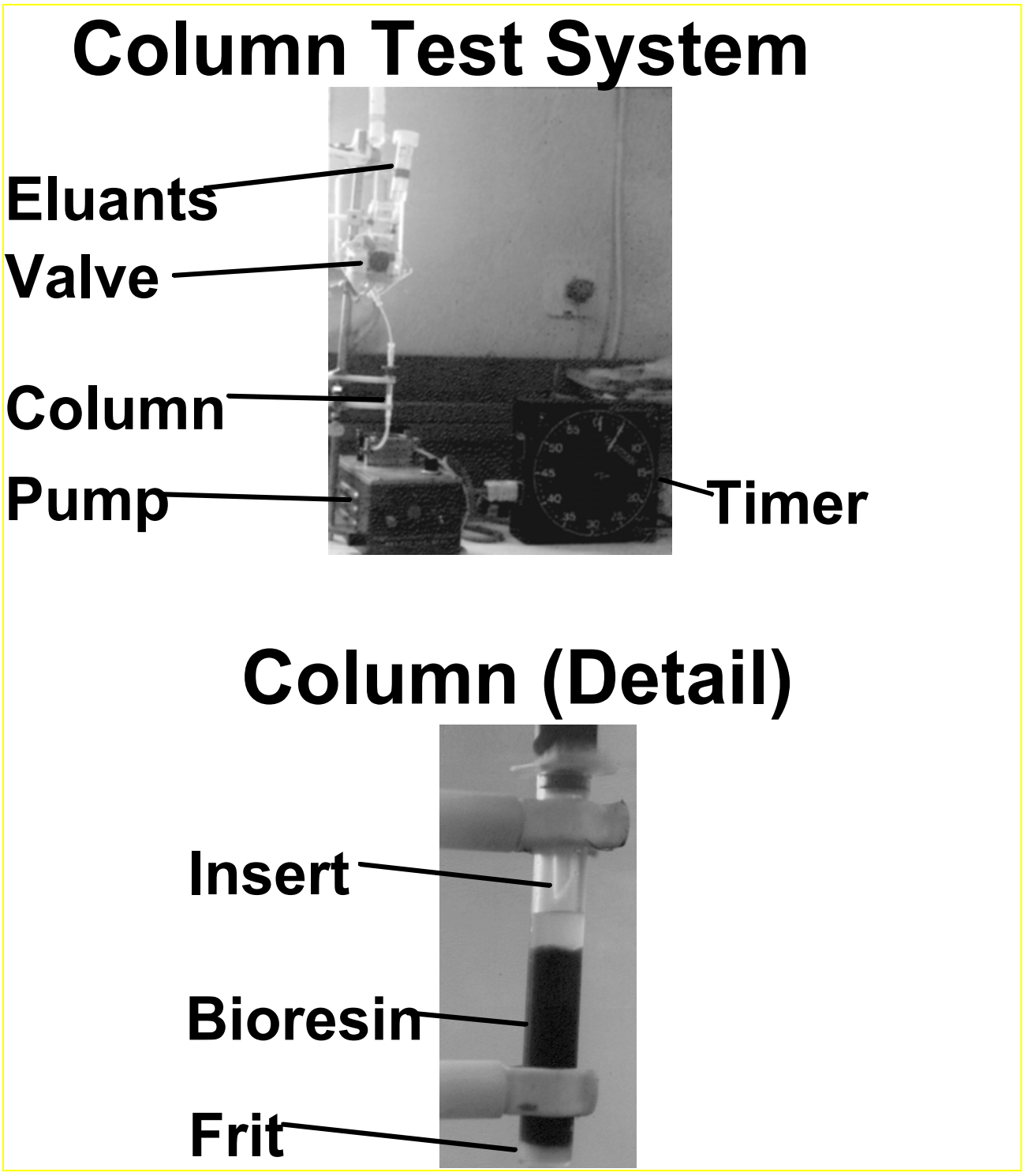

Figure 14. System used to evaluate the efficiency of bioresins. Upper photo shows the complete system, including eluant reservoirs, column, peristaltic pump, and timer. The timer controls the operation of the pump. The lower photo shows the detail of the polypropylene column containing silica-based bioresin. System is pictured diagrammatically in Fig. 13.

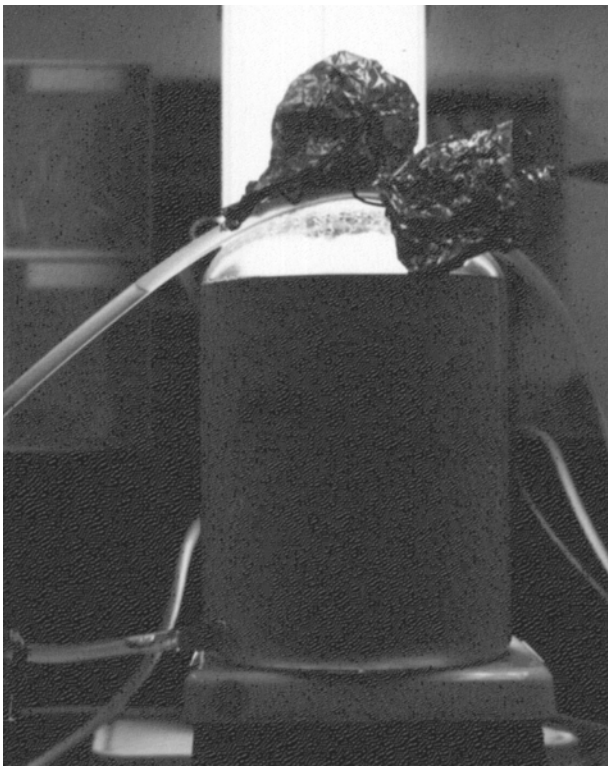

Figure 15. Culture system (4 liter) used for the production of biomass showing fluorescent lamp, sparger, tubing, and magnetic stirrer.

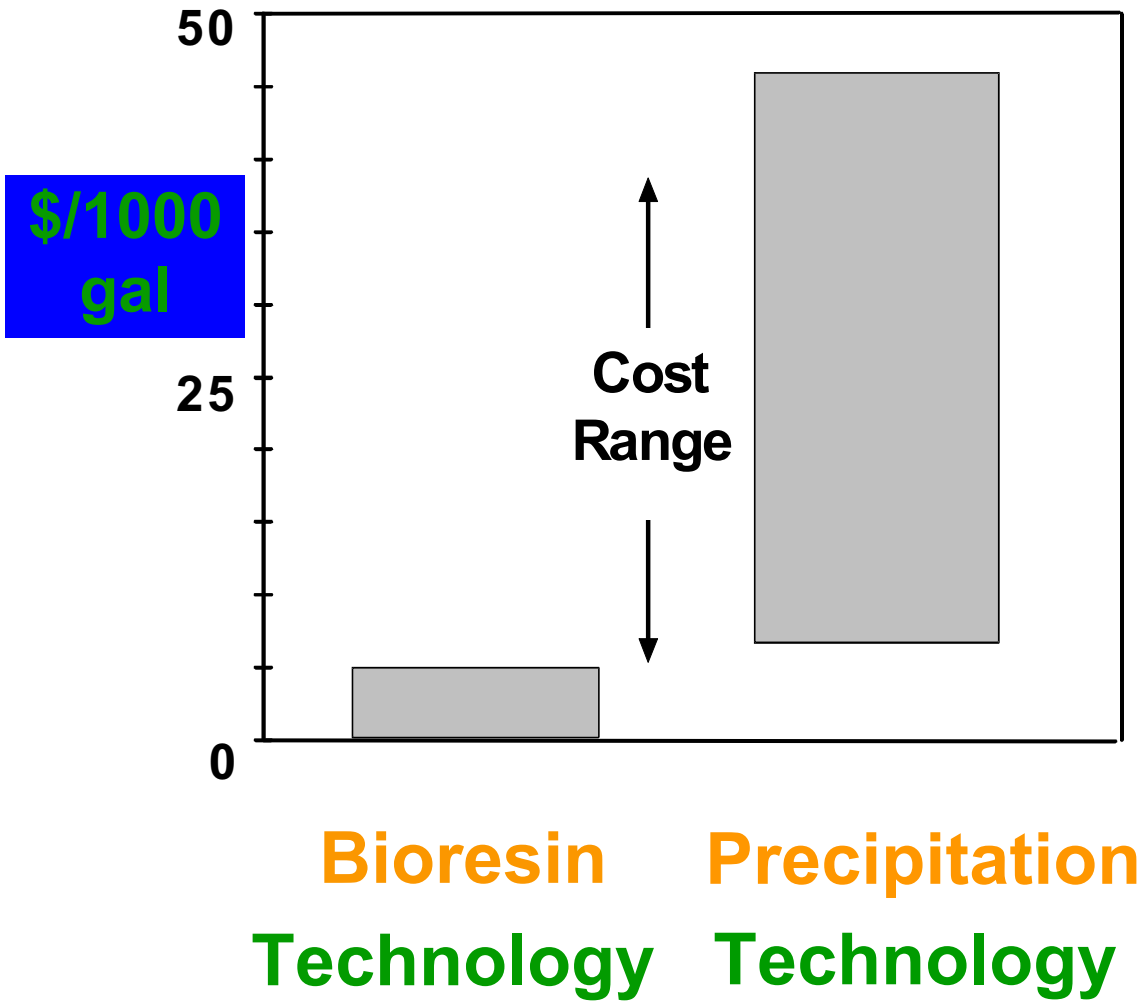

Figure 16. Operating cost ranges for precipitation technology calculated from Leitz et al. (1995) compared to values calculated for bioresin technology by Feiler and Darnall (1991). These are preliminary estimates. Capital costs were not considered.

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Appendix 1. Data Records

The following tables represent data records used to calculate means included in the report.

Data used to construct Table 4		
Possible µg 114.3	µg	% binding
Sample 1	45.85	
	66.39	98.3
Sample 2	45.85	
	66.05	97.9
Sample 3	45.85	
	63.98	96.1

Data used to construct Fig. 7 500x dilution							
Fraction	ppb	Fraction	ppb	Fraction	ppb	Fraction	ppb
1	0	11	0	21	0	31	11.6
2	0	12	0	22	0	32	12.6
3	0	13	0	23	0	33	12.5
4	0	14	0	24	0	34	
5	0	15	0	25	0.8	35	
6	0	16	0	26	2.7	36	
7	0	17	0	27	3.7		
8	0	18	0	28	7.9		
9	0	19	0	29	8.1		
10	0	20	0	30	9.4		

Data used to construct Fig. 8		
Fraction	µg in fraction	Bound or released
1a	0	100
1b	65.19	94.8
1c	0	0
2a	0	100
2b	69.48	101.0
2c	0	0
3a	0	100
3b	66.73	97.0
3c	0	0

Data used to construct Fig. 9

	7.4% Biomass	Silica Gel	Polymeric 10%	Polymeric 58%
% binding	100	36	78.2	87
	100		50.1	87
			35	86
% Eluted by acid	105	31	23.9	75
	100		23.0	74
			27.7	82
% Eluted by water	0	0	0	0
	2		0	1
			3.7	0
Not bound	0	64	21.8	13
	0		49.9	13
			65.0	14

Data used to construct Fig. 11 % of metal (68.8 μg) bound by column		
	SDW001a	SDW017
Cu	100	81
	100	80
	100	81.5
Ni	100	54.5
	100	49
	100	51
Pb	100	99.5
	100	98
	100	98

Data used to construct Fig. 14 - These are operating costs only - capital was not considered. ($M=10^6$)				
Reference	**From Leitz et al. 1995**			**From Feiler and Darnall 1991**
System	Metals Removal Plant	Portable Interim Treatment System		Bioresin Plant (estimate)
Flowrate	691 gal/min	92 gal/min		600 gal/min
Mgal/yr	363 Mgal/yr	48.3 Mgal/yr		315 Mgal/yr
cost/yr	$2.4 M/yr	$2.24 M/yr		$0.078-1.58 M/yr
cost/1000 gal	$6.60/ 1000 gal	$46/1000 gal		$0.25-5.00/1000 gal

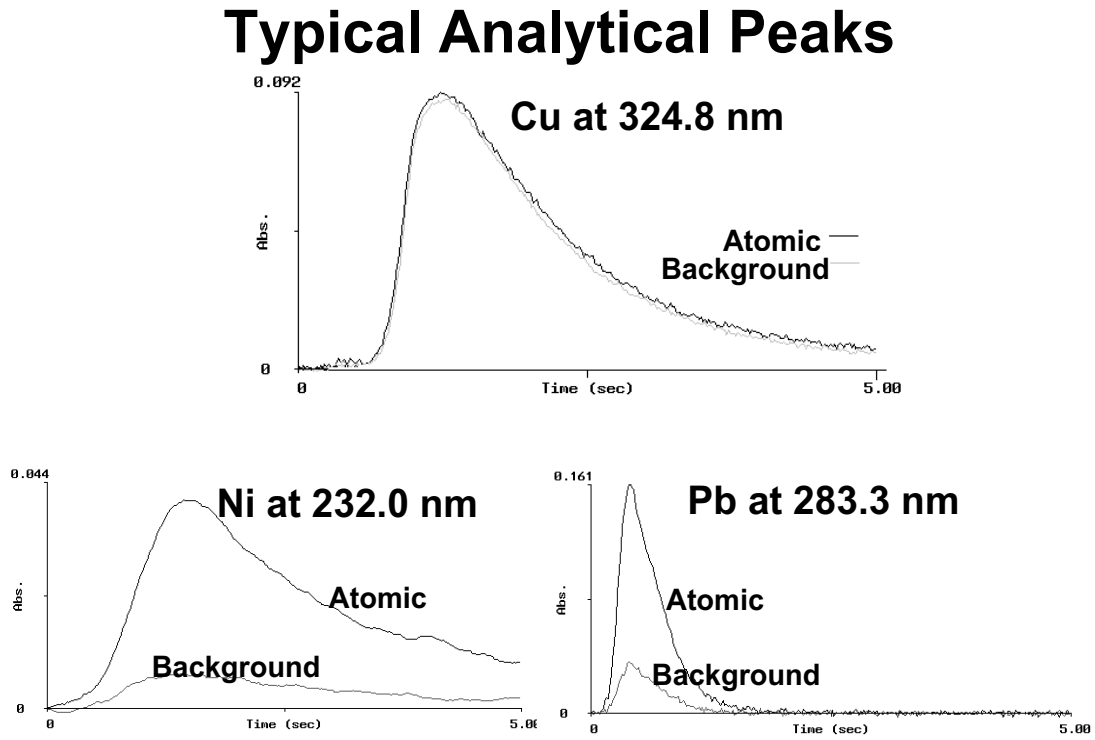

Figure 17. Atomic and Zeeman background peaks for standards of Cu, Ni. and Pb. The area of the Zeeman peak are characteristic of each element. Variations in the areas of the Zeeman background peak are indicative of the need for any corrections for matrix effects. No matrix corrections were found necessary in this work.

Appendix 2. Work supported by the U. S. Department of Energy

Recovery of Metals from Waste Streams with Microalgal Bioadsorbents
United States Department of Energy
Date: October 1, 1995. - Sept. 30, 1996
Innovative Concepts for Transportation and Environment Program, Grant No. DE-FG48-95R810557

The results are included in Figs. 18 and 19 and are included because the work complements the results reported under the present contract.

Summary of Results

The biomass (5 mg of lyophilized material in triplicate for each of three biomass cultures) was washed in 1 mL of ultrapure water and centrifuged six times (10,000x*g*). A solution of 1 mL of 10, 100 or 250 ppm Cu^{2+} was then added to each biomass sample in triplicate and the samples were incubated at room temperature for 1 h with gentle agitation. The results indicate that there were no significant differences among the strains in uptake of copper at each concentration (Fig. 18). At 10 ppm, more than 98% of the copper was sequestered. Lesser percentages were sequestered at the higher concentrations. When these data are plotted on the basis of mg Cu/g of biomass versus [Cu], it becomes apparent that the maximum binding capacity is about 10-14 mg/g and is similar for the three strains of microalgae (Fig. 19).

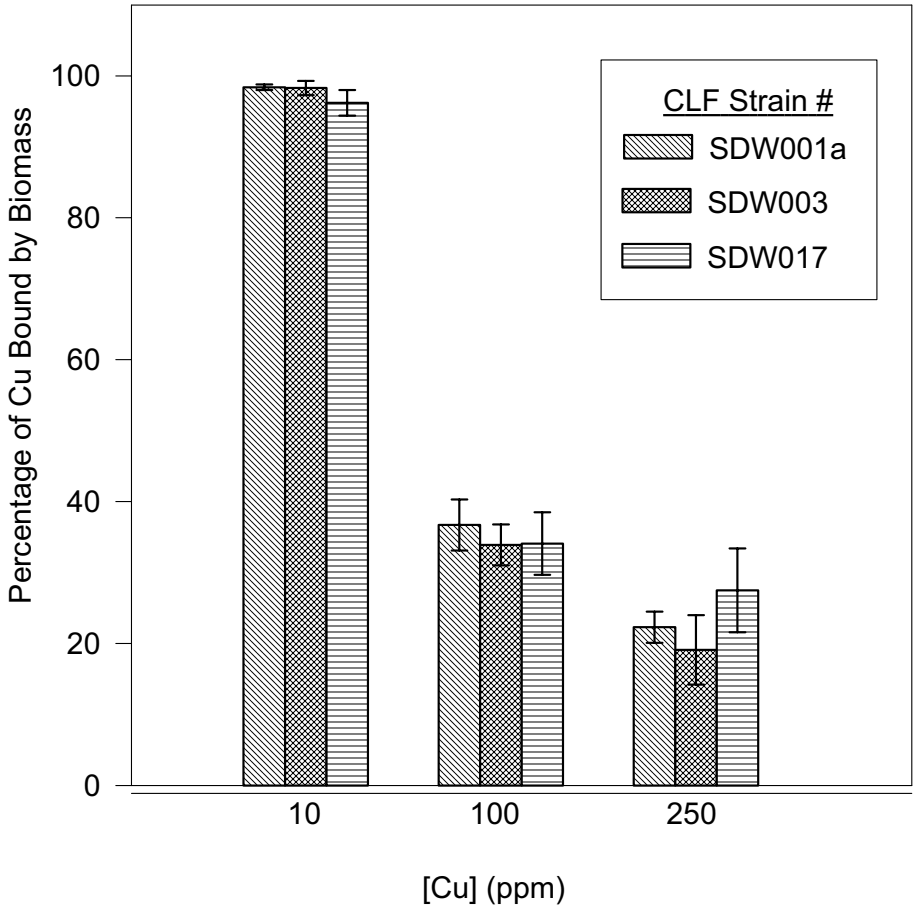

Figure 18. Binding of Cu^{2+} by non-immobilized biomass (calculated by weight). Binding of Cu by non-immobilized biomass. This experiment was conducted with biomass which was exposed to Cu for 1 hr with gentle agitation. Three strains were used at the concentrations indicated. Means ±SD are shown (n=3). At 10 ppm, more than 98% of the copper was taken up. This biomass was not immobilized in these experiments. The work was supported by the U. S. Dept. of Energy. See also Fig. 19.

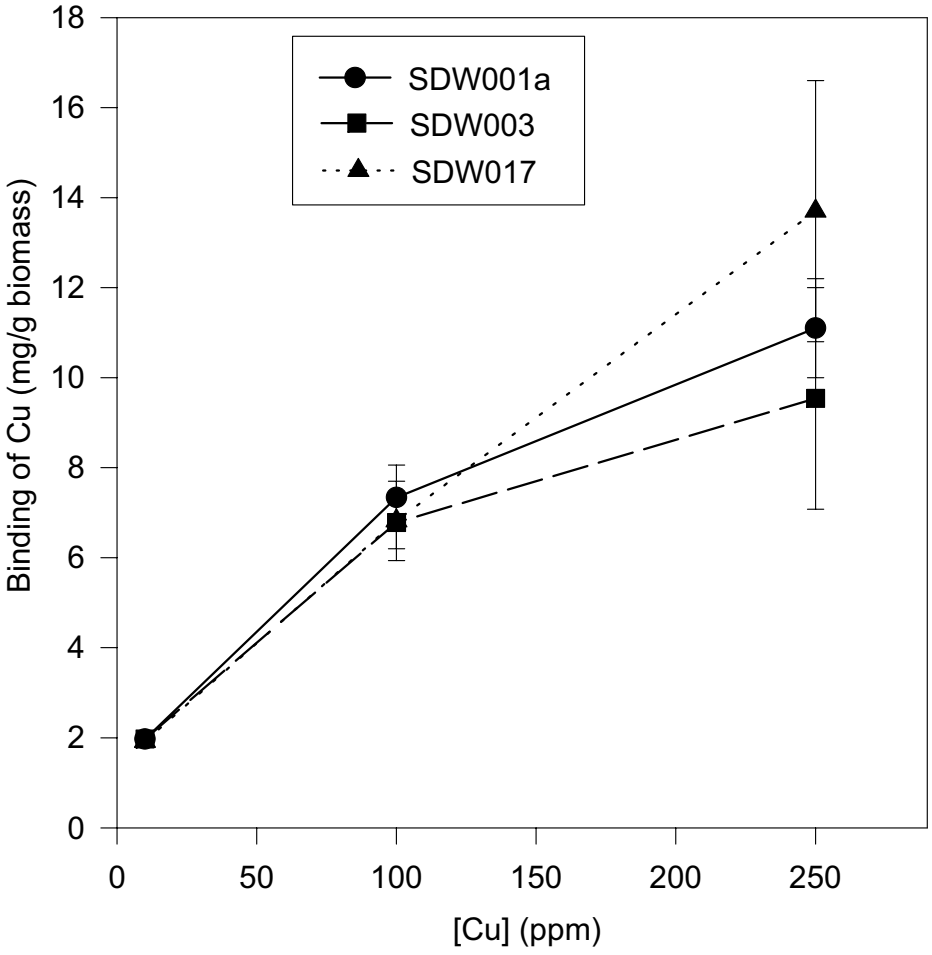

Figure 19. This experiment was conducted with biomass which was exposed to Cu^{2+} for 1 hr with gentle agitation. Three strains were used at the concentrations indicated. Means ±SD are shown (n=3). Same data as Fig. 18, but plotted on the basis of mg/g. Results show that maximum binding appears to be about 10-14 mg Cu/g biomass for all three strains Biomass was not immobilized in these experiments. The work was supported by the U. S. Dept. of Energy.