Metal uptake by algae

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Abstract

Metal uptake by microorganisms has been studied for some years. Research indicates that algae have the ability to accumulate trace metals by biosorption and bioaccumulation. Biosorption is a term designated for all of the passive interactions with metals. In certain sites of the algae cell wall, adsorption reactions occur between the cell wall and the metal ion. Depending on the binding site (functional group on the binding site) and the type of metal, any of several various adsorption reactions can occur. Bioaccumulation reactions, on the other hand, are active reactions in which the metal ions are directly absorbed into the algal cell. The mechanism behind these reactions is generally a defensive one; meaning that the cell is absorbing the metal for the sole purpose of avoiding being poisoned. It is important to note that there is a striking difference in the time for each of these processes. The biosorption process has been shown to be rapid and frequently selective. Biosorption occurs in a matter of minutes while bioaccumulation occurs on a much longer time scale. Uptake of metals by algae may be a viable cost-effective biological pretreatment for the wastewater and water industries. Thus, a study was conducted to identify algae that can accumulate copper and arsenic via rapid biosorption. Common green algae Scenedesmus abundans and Chorella vulgaris were used for determining metal uptake (copper and arsenic) in batch experiments. Results of the experiments indicated that the algae biosorption could be modeled by the conventional Freundlich and Langmuir isotherm models. Algae morphology studies indicated that the algae cells were impacted due to the presence of both metals as evidenced by clumping or loss of cell clusters. Results indicate that algae uptake of metals may be a viable method of pretreatment of metal contaminated wastewater.

Keywords: arsenic, copper, algae, biosorption, metals, Scenedesmus abundans, Chorella vulgaris.
1 Introduction

In recent decades there has been an increasing interest in heavy metals and metalloids, not only because they pose a danger to humans, animals, plants, and other living organisms, but also because they cannot be removed from contaminated soils or water cost effectively. Metals and metalloids occur naturally in the environment with an array of oxidation states and coordination numbers, and this diversity is related to their toxicity [1].

Literature indicates that certain species of algae uptake heavy metals such as copper, cadmium, chromium, lead, and nickel. The uptake of metals by algae in aqueous solutions occurs when algae release a protein called metallothioneins. When metallothioneins are released, the alga begins to chemically bind the metal to itself as a defense mechanism to remove the metal from its regular cellular activity [1]. Karna et al. [2] reported that the noxious effect of metals to algae seems to be related to the production of reactive oxygen species (ROS) and the consequential unbalanced cellular redox status. Algae respond to heavy metals by the synthesis of low molecular weight compounds such as carotenoids and glutathione, and the initiation of several antioxidants, as well as enzymes including superoxide dismutase, catalase, glutathione peroxidase and ascorbate peroxidase [2]. When metals come in contact with algae at high concentrations, damage to the cells of the algae occur because (ROS) levels exceed the capacity of the cells.

Metal uptake by algae is typically brought about by bioaccumulation and biosorption. The accumulation of metals and metalloids by algae may occur by any or an amalgamation of the following processes: (1) active transport along the cell membrane, (2) entrapment by cellular components (3) adsorption, (4) cation exchange or complexation [3]. Bioaccumulation is an increase in the concentration of a chemical in a microorganism over time, compared to the chemical's concentration in the environment [3]. During this process of active transport, energy is required; proteins help molecules migrate to the plasma membrane. Matsuto et al. [4] found that within the cell membrane compounds are moved against their concentration gradient and migrated through the membrane by carrier and transport proteins. The complex chemical makeup of cell walls of certain species of algae has a high affinity for metal ions and carries a net negative charge. This is mainly due to carboxylic, phosphoric and sulfhydryl groups. Biosorption is an energy-independent process, which causes metal ions to bind along the cell wall of the algae [1, 2, 3, 4]. This process entails the use of either living or dead cells and includes the mechanisms of adsorption and cation exchange.

Both arsenic and copper are of significant environmental concern and are toxic to a variety of living organisms from humans to bacteria. The U.S. Environmental Protection Agency (USEPA) regulates both metals. Arsenic is a widely distributed contaminant in soils and sediments and many watersheds experience severe arsenic loading. Over 99% of the naturally occurring arsenic occurs in rocks [5]. Arsenic is typically associated with sulfur compounds especially arsenopyrite. The common arsenic bearing minerals are
Arsenopyrite (FeAsS), Realgar (AsS), Enargite (CuAsS4) and As2S3. Thus the primary source of arsenic in soils and sediments is the parent material from which they originate. Anthropogenic inputs include the industrial use of arsenic trioxide; milling and smelting operations of lead, zinc, copper and gold ores; production of fly and bottom ash from coal combustion; and the applications of inorganic arsenic compounds as pesticides and herbicides. Copper is extensively used in industrial applications such as anti-corrosion and decorative coatings on metal alloys and also as an algaecide.

Current waste removal practices typically include expensive, maintenance intensive treatment processes such as chemical precipitation, ion exchange or membrane processes. Biological removal of these metals have been investigated by a number of researchers. The use of algae for metal uptake may have potential from a cost alternative and environmentally friendly process standpoint.

The results of a study performed by Suhendrayatna et al. [6] exposed Chlorella sp. to concentrations of arsenite ranging from 0 to 100 µg As cm⁻³ the maximum concentration being 100 µg As cm⁻³. The cell growth of Chlorella sp. was not affected by the arsenite until it was exposed to concentrations higher than 50 µg As cm⁻³. At concentrations greater than 50 µg As cm⁻³, the cell growth of the species was suppressed. Beceiro-González et al. [7] concluded that Chlorella sp. retained approximately 50% of arsenite from a solution. During a contact time of up to forty-eight hours it was observed by Taboada-de la Calzada et al. [8] that there were no improvements in the accumulation of arsenite over a long period of time as most of the biosorption was rapid and occurred in the first fifteen minutes by the Chlorella sp.

Harris and Ramelow [9] studied the use of Chlorella sp. and Scenedesmus sp. for biosorption of gold, copper, cadmium, and zinc. The authors indicated that metal uptake occurs quickly initially due to adsorption or ion exchange. It was found that approximately 90% of sorption took place within fifteen minutes and that the remnants adsorbed at a slower rate. Experiments indicated that the metal binding ability of Scenedesmus sp. was much greater than that of Chlorella sp. Terry and Stone [10] reported on the biosorption of cadmium and copper contaminated water by Scenedesmus abundans. It was shown that both living and non-living S. abundans removed cadmium and copper from water, with better removal rates with the living algae. Algae concentration was not a factor in the metal biosorption process. The study indicated that biosorption by algae could be a viable mechanism for metal waste treatment and also indicated that the method would work for multi-component metal systems.

This study was conducted to determine which algae species can biosorb copper and arsenic rapidly so that this may be a viable treatment method for metal removal from water or wastewater. An extensive literature review was conducted to determine which algae species have high tolerance for metals. It was concluded that Chlorella vulgaris and Scenedesmus abundans were the two species that had potential for high uptake of copper and arsenic. Experiments were thus conducted to test the abilities of Scenedesmus abundans and Chlorella vulgaris in removing copper and arsenic from water.
2 Experimental methods

The select algae *S. abundans* (UTEX LB 1358) and *C. vulgaris* (UTEX 265) were obtained from the Culture Collection of Algae at the University of Texas and grown a modified Bristol's solution [11] at 16:8 L:D cycle at a temperature of 25°C. The algae were counted using a Sedgwick-Rafter Counting Chamber under a Leitz Laborlux S-phase-contrast microscope, and expressed as number of cells per milliliter. Chlorophyll a was determined by filtration of a 50-ml subsample through an acetate filter (Whatman HA 0.45 um) and extraction using 100% DMSO and determined spectrophotometrically with a Milton Roy Spectronic Genesys 5 Spectrophotometer [12]. Algal biomass as ash-dry biomass was determined by filtering the cultures on pre-ash (550°C) glass fiber filters (Whatman GF/C) using a Metler Toledo precision balance.

Copper in the form of copper chloride was added in concentrations of 5, 10, 15 and 20 mg/L. Arsenic in the form of sodium arsenite was added in concentrations of 1, 5, 10, 20, 50 and 100 mg/L. Both chemicals were obtained from Fisher Scientific.

Metal removal experiments were conducted in batch tests in 250 ml acid washed Erlenmeyer flasks. Flasks were filled with 200 mL water containing *S. abundans* or *C. vulgaris*. Copper or arsenic was added to the flasks in concentrations mentioned above. Flasks were placed in an environmental shaker and samples were obtained at certain time intervals to analyze for residual metal concentrations. Residual metal concentrations in the water were obtained by filtering the sample through 0.22 mm Millipore filters to remove algae and then acid preserved. Metal analyses were carried out in a Perkin-Elmer 460 atomic absorption spectrophotometer. Flasks were tested for residual chlorophyll a, cell counts and algae biomass. Algae morphology was also observed using a Leitz Laborlux S-phase-contrast microscope. All experiments were conducted in duplicate and average values are presented in the results.

3 Results and discussion

Experiments with copper were conducted for a total of 36 hours using *S. abundans* at a concentration of 24 mg/L. The copper concentrations were measured over time and are presented in Figure 1. The graphs indicate that there was rapid decrease in copper concentration in the first two hours followed by some increase in the concentration after that. It is further evident that the batch tests had reached equilibrium in 36 hours.

Many researchers have indicated that biosorption of metals can be modeled by conventional isotherm models such as the Langmuir and Freundlich isotherm models [13]. Qiming et al. [13] indicated that the biosorption of cadmium, copper and lead by nine marine macro algae could be modeled by the Langmuir isotherm model. In general heavy metal uptake by freshwater algae has been reported to range from 0.5 to 1.0 mmol/g and for marine algae 1.0-1.5 mmol/g. It was indicated that marine algae sorption capacities were high for metals and at times exceeded capacities of activated carbon and natural zeolite.
Thus data was further plotted to determine if the biosorption of copper followed the common Freundlich Isotherm model or Langmuir isotherm used in traditional sorption studies. The Freundlich Isotherm modeled the data better than the Langmuir model. The Freundlich relationship is given as follows:

\[ q = K_p C_e^{1/n} \]

where

- \( q \) = equilibrium concentration of metal in mg/L
- \( C_e \) = equilibrium concentration of metal in mg/L
- \( K_p \) = constant
- \( n \) = constant

The plot obtained for the data is shown in Figure 2.

![Figure 1: Copper concentration in mg/L as a function of time for experiments with *S. abundans*.](image)

Algae cell counts and chlorophyll concentrations were measured at the end of the experiments. Cell clumping was observed visually in all flasks at all copper concentrations. This cell clumping could have impacted the cell numbers. Cell clumping observed is presented in Figure 3.

Algae cell counts and chlorophyll \( a \) data is presented in Figure 4. The data indicates that chlorophyll \( a \) concentrations were not impacted significantly with change in copper concentration. However microscopic observations indicated that the cell morphology was drastically impacted at all copper concentrations with the four-cell coenobia separating into single cells. Cell clumping was also observed visually in all flasks at all copper concentrations. This explains the
variation in the cell numbers. Similar results were obtained for the *C. vulgaris* studies with copper.

![Graph showing Freundlich Isotherm calculations for copper uptake.](image)

**Figure 2:** Freundlich Isotherm calculations for copper uptake.

![Clumping of algae at the bottom of reactor flasks.](image)

**Figure 3:** Clumping of algae at the bottom of reactor flasks.

Arsenic experiments indicated similar results for both algal species. Most of the arsenic sorption occurred during the first hour of the experiment as indicated in Figure 5. This plot shows typical arsenic concentrations remaining with time for select arsenic tests. The data also indicated increases in arsenic concentration with time, indicating that desorption processes may occur if the contact time with algae is too long. This type of arsenic excretion was also observed by Suhendrayatna et al. [6]. These authors also indicated that arsenic accumulation decreased with increase on arsenate concentration. This was not observed in the tests conducted for this study.

The data thus indicates that the sorption of arsenic is rapid and occurs within the first hours.
Algae biomass and chlorophyll $a$ at the end of the experiments are presented in Figures 6 and 7. The algae biomass did not vary significantly with increasing arsenic concentrations and results were similar for both algae species.

![Figure 4: Cell # and chlorophyll $a$ concentration in S. abundans versus copper concentration.](image1)

![Figure 5: Arsenic concentrations with time for S. abundans studies.](image2)
Figure 6: Algal biomass versus arsenic concentration.

Figure 7: Chlorophyll a concentration versus arsenic concentration.

The plots indicated that both species were impacted similarly by the presence of arsenic. Higher concentrations of arsenic did not significantly impact the chlorophyll a concentration or the biomass. Morphology studies however indicated cell clumping with *S. abundans* losing its four cell configuration. This was consistent for all arsenic concentrations. Visual inspection of the flasks
during the experiments indicated algae clumping with more pronounced effects for *S. abundans* than for *C. vulgaris*.

The data was further plotted to determine if a conventional adsorption model could be used to represent the data. The data could be represented using the Freundlich isotherm model.

4 Conclusions

The use of algae for removal of metal from water or wastewater is an attractive treatment alternative. Results from these studies indicate that high percentages of metal could be removed by two common species of algae *Scenedesmus abundans* and *Chorella vulgaris*. Significant differences in metal removal were not observed by the two species. However, morphology studies indicated that the *Scenedesmus abundans* was impacted by the presence of the metals as evidenced by loss of its four cell structure. Results from the batch studies further indicated that the major metal removal process was biosorption which could be modeled by conventional engineering sorption models such as the Freundlich isotherm model. Results also indicated that metal removal was rapid and occurred during the first hour. Longer contact times were detrimental as the algae released the metal as a detoxification mechanism. This study indicates the potential of the use of algae for metal removal. Final disposal of algae could include treatment or metal extraction or incineration.

References


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