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Effects of Supplements on the Bioaccumulation of Lead in *Anabaena* spp.

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Heavy metals are known to be insidious toxic pollutants in the natural environment and their presence in aquatic systems is a current major concern. Since the primary producers, green algae and cyanobacteria, are able to accumulate large quantities of metals, these contaminants can be efficiently passed along in the food chain. However, the ability to accumulate metals from solution also indicates their potential use as sequestering agents in metal recovery systems.

Previous studies on the mechanisms of metals uptake have demonstrated that the process generally takes place in two distinct stages (Matzku and Broda 1970; Norris and Kelly 1977). The first stage has been described as a passive adsorption of ions and it is likely that a number of different functional groups are involved in this process (Crist et al. 1981). This type of metal uptake is rapid and thought to be unrelated to the energy economy of the cell. The second stage of uptake is slower and involves active transport mechanisms requiring cellular energy. Hart and Scaife (1977) studied the effect of light on the uptake of cadmium of Chlorella pyenoidosa and found that cells in the dark could not accumulate this metal. Since the energy intensive active transport may be a significant mechanism, particularly in long-term metal uptake, media enrichments might increase the efficiency of the metal uptake process. In addition, such enrichments might also increase the culture growth providing additional cell surfaces for the passive adsorption process.

A descriptive model of long-term metal uptake, including experiments in media enrichments, has been lacking in previous research. Understanding the process of metal uptake and its relation to cell growth over longer periods of time is important to both the development of recovery systems and the production of metal transport through the ecosystem. The objectives of this research are to investigate (1) the effects of carbon dioxide and wastewater enrichments on the simultaneous culture and lead uptake in Anabaena during a 30-day culture period and (2) the effects of energy supply on the lead uptake.

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MATERIALS AND METHODS

Cultures of Anabaena sp (NB-19, isolated in Florida) were grown at 25 degrees in 100 ml of AA medium (Allen and Arnon 1955) or in AA/WW medium (AA plus wastewater, 1:1). The wastewater component, after primary treatment, contained the following material in mg/ml: phosphate, 1.0; ammonia, 6.0; reducing sugars, 0.1; Fe, 2.5. Other heavy metals present in concentrations less than 1.0 mg/l were: Cd, Cr, Ca, Pb, Hg, Ag, Zn, As, Ba and Mn. Although the precise composition of the wastewater is not known, it was used in the enrichment experiments because it represents an abundant potential resource for microbial metal removal systems.

Standard inoculum of mid-log pre-culture was 0.1 mg per 100 ml for growth/uptake experiments and 0.3 mg per 100 ml for energy experiments. The cultures were maintained in continuous shaking at 100 rpm in a controlled environment chamber under incandescent illumination (at 33 cm). For the carbon dioxide enrichment experiments, cultures were sparged with 3% carbon dioxide for 3 h/d at a rate of 150 ml/min. Controls were bubbled with air. Growth was determined by dry weight measurements of harvested biomass.

Cultures for the energy effects experiments were grown in AA plus nitrate (0.4 g/l) with carbon dioxide enrichment. Glucose cultures were enriched with 4.8 g/l glucose. Dark cultures were covered with foil.

Nine trials, each in triplicate, were performed for all growth/uptake experiments. Culture media without algae served as a control for any potential leaching of the metal from solution. Algae cultures without lead were used as controls for any potential source of lead contamination as well as indicators of algae growth rate without lead. All lead solutions were prepared by dilution certified standard lead nitrate (Fisher Scientific Co.) with deionized water and adding it to the culture medium to at a final concentration of 30 mg/l. Media solutions were adjusted to a pH of 8.5.

Algal cultures were separated from the media by centrifuging at 18 x g. Both algal biomass and media were acid hydrolyzed according to the Standard Methods of Examination of Wastewater (1971). The hydrolysates were analyzed for lead concentration with a Perkin-Elmer 500 atomic absorption unit.

RESULTS AND DISCUSSION

Metal leaching controls (media plus lead without algae) showed that the leaching effect was less than 1%. Lead removed from the media was accounted for in the hydrolysates of the algal biomass.

The carbon dioxide and wastewater supplements increased the lead uptake rate and the two combined produced the highest level of lead uptake (Fig. 1 and 2). The AA/WW data without carbon

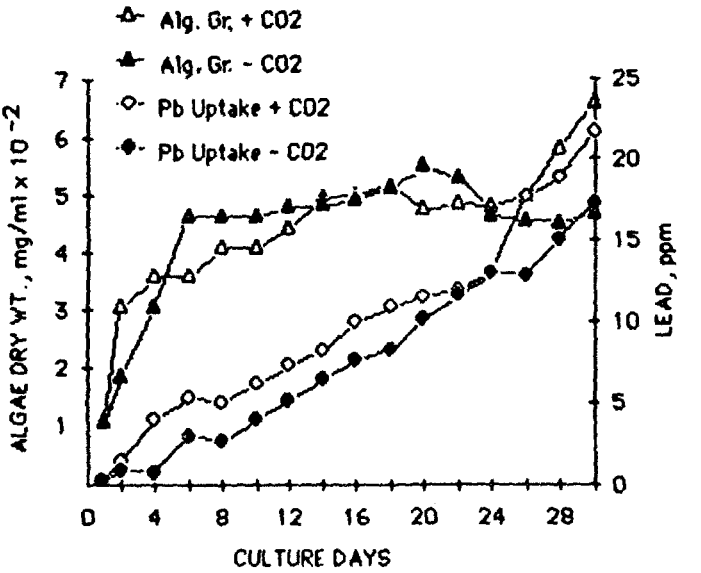

Figure 1. Lead uptake and algal growth in Allen/Arnon medium with and without carbon dioxide enrichment.

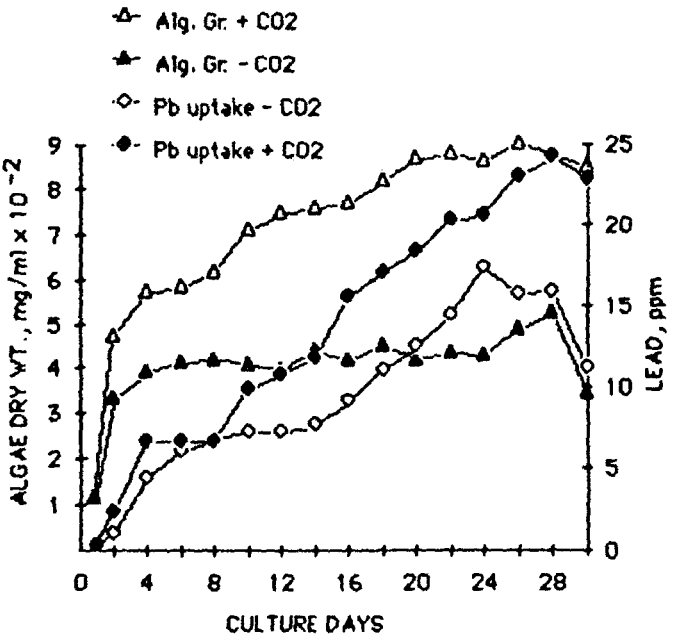

Figure 2. Lead uptake and algal growth in Allen/Arnon plus wastewater medium with and without carbon dioxide enrichment.

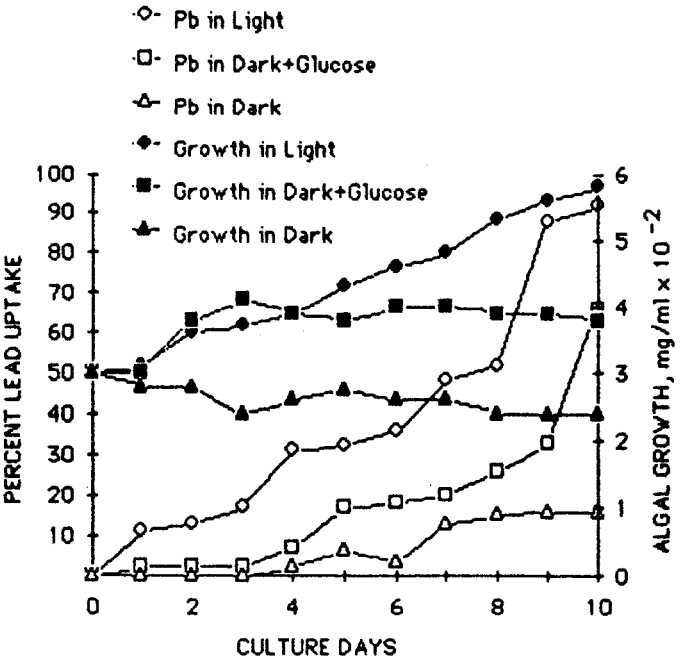

Figure 3. Effects of energy supply on algal growth and lead uptake.

dioxide (Fig. 2) showed that lead uptake occurs during stationary phase, indicating that actively growing cells are not required for uptake. In addition, there was sufficient biomass available for day 6 for the 20 ppm that was removed from the media by day 30. It is, therefore, apparent that an adequate number of cell sites were available in biomass of approximately 4 mg of algae (day 6) for the total uptake of 2 mg leach achieved in 30 days. Passive adsorption on the cell surfaces did not ,therefore, seem to be the primary mechanism in long-term lead uptake with Anabaena, NB-19.

Enrichment significantly increased lead uptake after day 6. However, there was a lack of correlation between biomass production and lead uptake. Carbon dioxide increased lead uptake but not culture growth between days 6-22 (Fig. 1). Analysis at the 26th day of culture in Figure 2 showed that enrichments of both wastewater and carbon dioxide produced approximately 100% increase in biomass with only 28% increase in lead uptake. Although increasing the number of cells by media enrichment did produce an increase in lead uptake, this may represent a simultaneous effect involving the energy economy of the culture, rather than the number of cell sites available for uptake.

Enrichment effects of carbon dioxide and wastewater, when calculated separately showed that the enrichments in the presence of lead did not increase cell growth. If lead was absent (controls), there was significant growth increases and the combined effects of the enrichments were additive. This may indicate that a competition exists for available energy to fuel

both the metabolic processes for cell growth and lead transport mechanisms. When the combined enrichments were applied, both cell growth and lead uptake increased, indicating that an energy threshold may have been reached.

Figure 3 demonstrates that the requirement of energy for lead uptake. In these experiments a larger inoculum of algal biomass was used in order to provide a sufficient number of cells for initiation of lead uptake. Availability of light seems to provide a better energy source for metal uptake than does glucose. In the glucose-supplemented culture, lead uptake seemed to be competitive with culture growth. The low-level lead uptake that occurred in the dark culture without glucose may have been due to passive lead adsorption to the algal cell surfaces or to the products of cell lysis.

It has been observed that the long-term removal of lead from aqueous media may be less dependent on cell number or actively growing cultures than on the energy available to the system. In general, the efficiency of lead recovery systems can be increased by supplements, which elevate the energy content of the culture. Wastewater may represent an inexpensive and abundant energy resource of such systems.

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