0273-2289/94/4546—0847$03.60

Biosorption of Nickel in Complex Aqueous Waste Streams by Cyanobacteria†

SUSAN LORA CORDER[1] AND MARK REEVES*[,2]

[1]Engineering Development Section, Chemical Technology Division, Oak Ridge National Laboratory,‡ Oak Ridge, TN; and [2]Present Address: Oak Ridge National Laboratory, PO Box 2008, Building 4500N, Mail Stop 6251, Oak Ridge, TN 37831-6251

ABSTRACT

A study was undertaken to determined if a suitable biosorbent could be found for removal of nickel at low concentrations (<20 parts per million [ppm]) from a chemically complex wastewater effluent generated by electroplating operations. Algae and cyanobacteria were chosen as candidate biosorbent materials because they are easy to grow and they have the ability to withstand processing into biosorbent materials. Several species were screened for nickel-biosorption capacity initially, and three species of cyanobacteria were selected for further study based on their performance in the scoping tests. When compared to live controls, autoclaving improved the binding capacities of all three species, but usually biosorption data from experiments with live cells were more consistent. None of the three species was able to bind nickel efficiently in actual effluent samples. Further experimentation indicated that sodium ions, which were present in high concentrations in the effluent, were interfering with the ability of the cells to bind nickel. Adsorption isotherm plots for biosorption of nickel by two species of *Anabaena* in $NiCl_2$-deionized water solutions were prepared.

Index Entries: Cyanobacteria; biosorption; nickel; algae; wastewater.

*Author to whom all correspondence and reprint requests should be addressed.

‡Managed by Martin Marietta Energy Systems, Inc., for the US Department of Energy under contract No. DE-AC05-84OR21400.

INTRODUCTION

The presence of toxic metals, usually in ionic form, in aqueous wastes is a widespread environmental and waste management problem at industrial, military, mining, and other sites throughout the world. The presence of toxic metals in industrial effluents, such as those from electroplating or other commerical processes, and in groundwater is a particularly vexing problem.

A variety of suitable methods exists for removal of metal pollutants from such wastes when they are present in high concentrations (e.g., parts per thousand [ppt] or higher). Identifying practical and cost-effective means of removing such contaminants at lower concentrations in the range of 10 ppm or less has proved much more difficult. Processes that are otherwise suitable at higher concentrations are often either ineffective or cost prohibitive when applied to more dilute wastes. Another factor driving the search for a useful treatment methodology for dilute metal wastes is the recent trend by various regulatory agencies to lower the allowable levels of certain metals in treatment plant effluents and in drinking water. This trend has in recent years led to a growing interest in the application of biosorbent technology for the removal of trace amounts of toxic metals from dilute aqueous wastes.

There are a number of promising biosorbent candidates for removing targeted metals and radionuclides from dilute waste solutions. Biomaterials —including algae, bacteria, fungi, higher plants, and products derived from these organisms—have been demonstrated to remove certain chemical species with great efficiency. The degree of specificity of the various biosorbents for the chemical to which they have been applied seems to vary as does the binding capacity for the various metal ions.

Several suitable, nonbiological methods for removing high levels of metals from wastes exist, but often these methods leave behind as residue trace levels of metals that can be significant from a regulatory viewpoint. The current study was prompted by the need to develop an efficient and cost-effective method for removal of nickel in trace amounts (<20 ppm) from a treated electroplating effluent generated by a process operation on the US Department of Energy's (DOE) Oak Ridge Reservation (ORR). Because nickel is a common component of such wastes, we searched for an algal or a cyanobacterial species that demonstrated a potential use as a biosorbent for nickel in dilute solution. The decision to focus on algae and cyanobacteria was made because

1. They are generally rugged organisms and therefore should easily stand up to processing into useful biosorbents;
2. They are grown easily and cheaply; and

3. In previous studies they have shown a potential for use as biosorbents for other metals.

There is a small body of literature dealing with the interactions of algal and cyanobacterial cells with nickel and with other toxic metals in dilute solution. It is important to keep in mind that because the terms *biosorption, bioaccumulation,* and *uptake* are often used interchangeably, there are sometimes mistaken impressions about the mechanisms involved in the removal of dissolved metals from solution by microorganisms. For the purposes of this paper, only those literature citations dealing with nonmetabolic bioaccumulation, for which *biosorption* is probably the best term, were considered. However, in some cases, the cited authors have measured only a removal phenomenon without regard to mechanistic considerations, and in other cases both active and passive uptake were considered during the same investigation. The reader then should be aware that there may be some overlap between active and passive mechanisms of metal accumulation. A brief review of the recent literature follows.

A recent review by Benemann and Wilde (*1*) summarizes information on the use of bioaccumulation by microbes, including algae and cyanobacteria, for removal and recovery of heavy metals from solution.

Many species of algae have been examined for their ability to bind metals. Chan et al. (*2*) investigated the potential for using *Chlorella pyrenoidosa* and *Chlorella* HKBC-C3 as biosorbents for removing copper and nickel from an electroplating sewage effluent. These investigators found that copper was removed at a similar rate by these two species and that rate was fairly high (>68% removal in 72 h). Nickel was also removed at a similar rate by both species, although that rate was considerably lower (<20% removal in 72 h) than that seen for copper. Wong and Pak (*3*) also investigated biosorption of copper and nickel by several species of *Chlorella* in both free-cell and immobilized forms. Their results demonstrated that differences exist among these species in their tolerance of certain concentrations of copper and nickel without their growth rates being affected and that the removal efficiencies of these species for both metals was partially dependent upon the actual concentration of the metal in solution.

Aksu et al. (*4*) observed that dead cells of *Chlorella vulgaris* and *Zoogloea ramigera* were good biosorbents for copper and that both pH and temperature were important in determining how well copper could be bound by these species (pH optimum = 4.0–4.5; temperature optimum = 25°C). It was also determined that the initial binding rate was dependent upon initial concentration of the metal ion in solution and that the optimum concentration was slightly different for the two species tested. A related study identified both the temperature and pH optima for binding

of copper, zinc, iron, chromium, and lead ions by *C. vulgaris* (*5*). The removal of lead ions from dilute solutions by *C. vulgaris* in a batch reactor has been accomplished by this research group (*6*).

Another recent report has documented the bioaccumulation of cobalt, zinc, and manganese by *Chlorella salina* immobilized in alginate microbeads (*7*). These authors found a rapid, energy-independent uptake mechanism, which was followed by a slower, energy-dependent uptake phase. Under similar conditions, immobilized cells accumulated greater amounts of all three metals than free cells because of an increased active phase of uptake. Some binding by the alginate matrix was also noted.

In another study using heat-killed green algae as biosorbents (*8*), the authors first tested the algae against a variety of metals in pure solution and then ranked the metals in order of affinity shown by the algal cells for binding them. Lead, iron, and copper were taken up most readily, but biosorption was noted for every element tested. No notable differences were found among the three species tested in their abilities to bind these metals.

The mechanism of metal binding to the surfaces of algal cells is not understood, and it probably varies among species. One group has demonstrated the importance of carboxyl groups on the surfaces of algae in furthering metal-binding activity (*9*).

Mann et al. (*10*) noted that acidophilic microorganisms like *Euglena* that grow in the low pH, heavy-metal-laden discharges from mine-tailing-leaching operations are efficient scavengers of the metal ions present in the discharges. These authors measured concentrations of iron, aluminum, zinc, manganese, cadmium, titanium, and nickel that were 103–105 times higher in bulk algal samples from these environments than in the wastewaters themselves.

Another investigation of bioaccumulation of algae by several algal and cyanobacterial species, including *Euglena* (*11*), revealed that there is a wide range of concentration factors (ratio of metal concentration in cells to concentration in surrounding medium) among these species ($0–3 \times 10^3$) and that pH was the most important environmental variable in determining how well a species accumulates nickel. Most of the tested species had an optimum pH of 8.0 for nickel accumulation. This work also included an investigation of nickel binding by the cyanobacterial genera *Synechococcus* and *Oscillatoria*. Although the cyanobacterial species were found to be more sensitive to nickel toxicity than were the green algae used in this study, the former proved to have good binding capacities for nickel under most conditions tested.

Little work has been done exclusively with cyanobacteria as metal-binding biosorbents. One report from the literature (*12*) deals with the binding of copper, zinc, and cadmium by *Chroococcus paris*. These investigators found that all three metals were bound rapidly by this organism and that the amount of metal bound increased with increasing pH, in the

range of 4–7. Binding curves for all metals followed the Langmuir adsorption isotherm model. Another group of investigators found that the uptake of copper by *Nostoc calcicola* (*13*) was characterized by a metabolism-independent phase (adsorption) and a metabolism-dependent phase (uptake); this has been noted by other investigators (*12,14–17*) using a variety of cyanobacterial species and metal ions. No specific processes presented in the literature other than reference (*11*) were found involving cyanobacteria as a biosorbent for nickel in aqueous wastes.

In certain environments and for specific applications, the use of algae as biosorbents is approaching the practical industrial scale. One such commercial product (*18*) consists of nonliving biomass (primarily algae) that has been immobilized in a polymeric matrix to produce a biological ion-exchange resin. Another such product, called BIO-FIX (*19*), is a bead-shaped resin containing biomass blended from sphagnum and algae. Each of these products has demonstrated its efficacy in removing heavy metal ions from solutions and its satisfactory reuse through numerous loading-elution cycles.

The goal of this study was to identify a suitable candidate biosorbent for removal of nickel from dilute wastes from an electroplating process. The techniques used and the results of the study follow.

METHODS

Cultivation of Test Organisms

Following a search of the available literature, several organisms were selected for initial screening for nickel binding capacity. These included the cyanobacteria *Anabaena cylindrica* (University of Texas Culture Collection [UTEX] strain B629), *Anabaena flos-aquae* (UTEX 1444), *Nostoc* sp. (American Type Culture Collection [ATCC] strain 27895 [a kind gift from R. J. Mehlhorn of Lawrence Berkeley Laboratory]), and *Synechococcus leopolensis* (UTEX 625); and the green algae *Chlorella regularis* var. *minima* (UTEX 1807), *Chlorella vulgaris* (UTEX 397), *Scenedesmus obliquus* (UTEX 393), and *Stichococcus bacillaris* (a kind gift from B. D. Faison, Oak Ridge National Laboratory, Oak Ridge, TN). Stock cultures of these organisms were maintained on agar slants at room temperature with incidental light. To produce cell quantities sufficient for testing as biosorbents, cells from the agar slants were inoculated aseptically into small Erlenmeyer flasks containing an appropriate growth medium (for cyanobacteria, Kratz and Myers medium D [*20*]; for green algae, Bold's basal medium [*21*]). The liquid cultures were incubated at room temperature, and after visible growth was achieved, 10-mL aliquots were transferred to growth flasks increasingly larger in size. All flasks were incubated on an orbital shaker rotating at 160 rpm with fluorescent lamps providing continuous light.

The cells were incubated for 5–8 d, or until they had reached about the midlogarithmic phase of the growth cycle.

Cell Sample Preparation

After the cells were harvested, they were poured into tapered, sterile tubes and centrifuged at 2000 rpm (325*g*) for 15 min. The supernatant was decanted, and the resulting pellets were washed once with deionized water, recentrifuged, then combined into one test tube, and resuspended in ~20 mL of deionized water. The resulting cell suspension was added to challenge solutions containing nickel. In a separate set of experiments, the cells were pretreated by autoclaving at 120°C and 100 kPa for 10 min to kill the cells before the washing step.

During the initial scoping experiments, the total cell mass added to nickel challenge solutions (dry weight basis) was measured, but not standardized. Varying, but known, cell masses were added to challenge solutions containing 10 mg/L of nickel in deionized water. This concentration of nickel was selected because it approximated the concentration of nickel present in the ORR waste stream. The test samples, 40 mL in volume, were allowed to mix for 1–24 h in 50-mL screwcap tubes on a test-tube mixer. At time intervals varying from 1 to 24 h, 3-mL aliquots were removed and centrifuged at 2000 rpm (325*g*) for 15 min; the supernatant was then analyzed for nickel content (*see* Analytical Procedures). The equilibrium times and binding capacities (g Ni sorbed/g dry cell mass, based on the difference in liquid concentration of nickel before and after exposure to the biosorbent) were calculated from the resulting data.

After the initial scoping experiments were completed, a standardized approach was developed for testing the organisms selected for further study. In this approach, the dry mass of cells added to nickel solutions was determined by withdrawing aliquots of cells and drying the cells to constant weight in preweighed aluminum pans at 100°C. The volume of cell suspension needed to produce a target concentration (1 g dry wt equivalent cells/L nickel solution) was then calculated, and this amount was used in the experiments to determine equilibrium time and adsorption isotherm kinetics.

Testing to determine the potential for sodium interference with sorption of nickel was performed by preparing the nickel-containing challenge solutions in a solution of sodium sulfate (4000–6000 mg Na_2SO_4/L deionized water) instead of preparing them in deionized water.

Adsorption Isotherm Kinetics

The first step in determining the kinetics of biosorption of nickel by cyanobacteria was to determine precisely the equilibrium time, defined for the current purpose as the amount of time required for the cells of different species to bind the maximum amount of nickel. This was deter-

mined by exposing the cells to a solution containing 10 mg Ni/L deionized water and measuring the remaining nickel concentration, typically at 1, 2, 4, and 24 h after initial exposure. When the nickel concentration had remained steady for two successive readings, it was assumed that the lower of the two time points represented the equilibrium time (rate of sorption equals rate of desorption).

Adsorption isotherm plots were prepared from data collected in the following manner. Challenge solutions were prepared in disposable 15-mL screwcap tubes. It was previously determined that binding of nickel to the walls of the tubes was negligible. The standard dry weight equivalent of cells (1 g/L) was introduced into challenge solutions containing concentrations of nickel ranging from 10–200 mg/L in a deionized water matrix. The samples were placed on a test-tube mixer at room temperature and agitated until equilibrium was achieved (determined as described previously), after which time the samples were centrifuged and the supernatant was analyzed for nickel content.

Equilibrium adsorption isotherms were prepared by plotting mass nickel sorbed per mass of dry cells as a function of equilibrium concentration of nickel in solution. The data were plotted, and both Langmuir and Freundlich isotherm curves were fitted to the data; the best fit was noted in each case.

Analytical Procedures for Nickel

Nickel analyses were performed by flame atomic absorption spectroscopy using a Perkin Elmer Model 1100 B Atomic Absorption Spectrometer. A cell-free control with deionized water substituted for the cell suspension was used at each nickel concentration; its nickel content was taken as the initial concentration in the challenge samples.

RESULTS AND DISCUSSION

The first step in this work was to identify from the available literature and cultures on hand a suite of algal and cyanobacterial species worthy of further investigation for their ability to biosorb ionic nickel. An initial literature survey resulted in selection of four green algal species (*Chlorella regularis, Chlorella vulgaris, Scenedesmus obliquus,* and *Stichococcus bacillaris*) and four cyanobacterial species (*Anabaena cylindrica, Anabaena flos-aquae, Nostoc* sp., and *Synechococcus leopolinsis*). A quick screen of samples of all these species in terms of their ability to bind nickel in pure solution in deionized water revealed that the green algae were inferior to the cyanobacteria in this respect. The three species that showed the highest binding capacities, *A. cylindrica, A. flos-aquae,* and *Nostoc* sp., were further screened to determine the binding capacities of living and heat-killed (autoclaved) cells.

Table 1
Initial Screening of Algae for Nickel-Binding Capacity

Organism	Cells, g/L	Ni, mg/L	Equilibrium time, h	Binding capacity, g Ni sorbed/g dry cell mass
Anabaena cylindrica, live	3.4	10	2	0.002
Anabaena cylindrica, autoclaved	1.1	10	2	0.007
Anabaena flos-aquae, live	0.9	10	2	0.006
Anabaena flos-aquea, autoclaved	0.4	10	2	0.007
Nostoc sp. live	1.0	10	2	0.003
Nostoc sp. autoclaved	1.0	10	2	0.009

The results of these screening experiments are shown in Table 1. It is interesting to note that, in each case, autoclaving the cells increased their capacity to bind nickel in solution. In the case of *A. cylindrica*, autoclaving increased the binding capacity by more than threefold.

No published data on nickel biosorption by cyanobacteria are suitable for comparison with the data from these experiments. The best comparison can be made with data on nickel biosorption by *Scenedesmus* (*11*). This green alga was found to have a maximum binding capacity for nickel of 6.12×10^{-5} g/g dry wt of cells, which is considerably lower than the highest binding capacity, 0.009 g/g dry wt of cells for autoclaved *Nostoc* sp., noted in this study.

While performing these experiments, it was noted that data from tests using autoclaved cells were somewhat less consistent from day to day than were those from tests using live cells. This occurred even though the autoclaved cells usually showed higher binding capacities than their live counterparts.

Adsorption Isotherms for *A. flos-aquae* and *A. cylindrica*

Two of the organisms identified by the current study as being relatively proficient in biosorption of nickel in aqueous solution were selected for further analysis by plotting equilibrium adsorption isotherms for their interactions with ionic nickel in pure aqueous solution. Equilibrium adsorption isotherms were determined for *A. cylindrica* and *A. flos-aquae* using both live and autoclaved cells of each species. These data

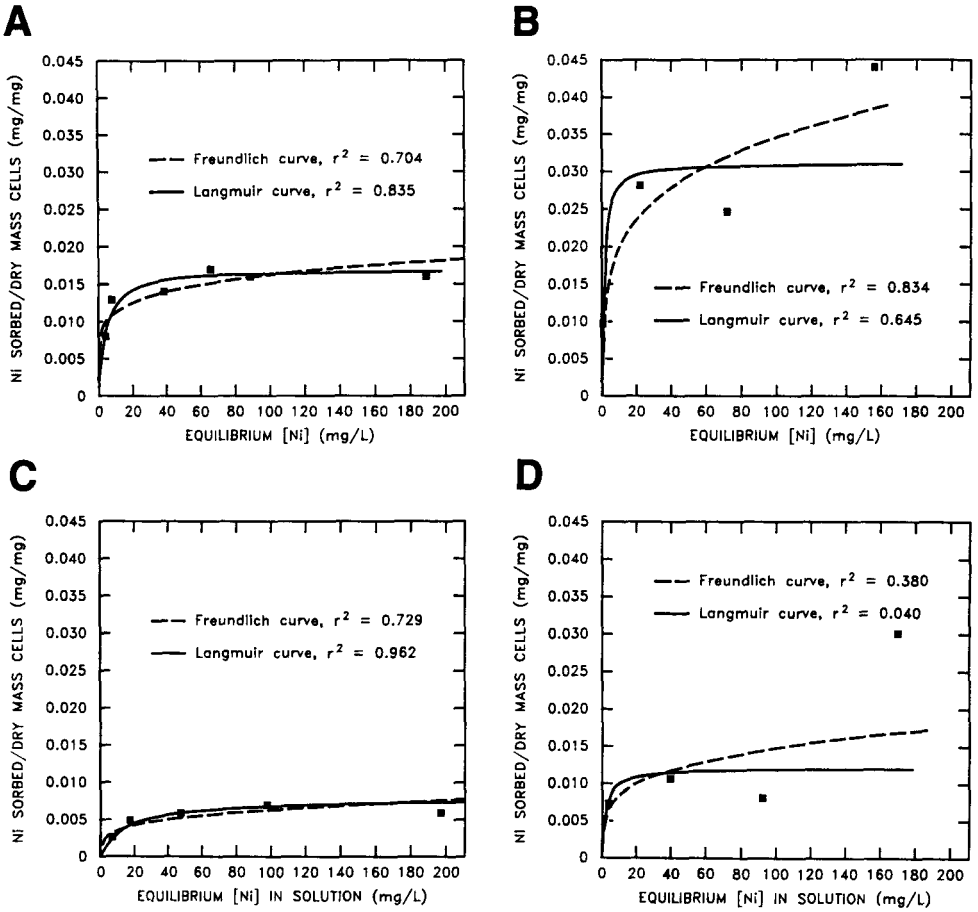

Fig. 1. Adsorption isotherm profiles for live *Anabaena flos-aquae* (A), autoclaved *A. flos-aquae* (B), live *A. cylindrica* (C), and autoclaved *A. cylindrica* (D). Curves shown represent fitting to both Langmuir and Freundlich profiles; r^2 values are given to show the best fit to the particular data set in question (1.0 = perfect fit).

are shown in Fig. 1(A–D). Figure 1a shows the equilibrium adsorption isotherm for *Anabaena flos-aquae* (live cells) at standardized conditions (as described under *Methods*). As shown in Fig. 1A, the cells became saturated with nickel at an equilibrium concentration of ~50 mg/L and demonstrated a maximum binding capacity of ~0.016 g Ni sorbed/g dry cell mass. In contrast, the autoclaved cells (Fig. 1B) were able to sorb 0.04 g Ni/g dry cell mass, with a saturation concentration of nickel equal to ~20 mg/L.

Similar data for *Anabaena cylindrica* are shown in Figs. 1C and 1D. The maximum binding capacity of nonautoclaved cells of this species was 0.007 g Ni sorbed/g dry cell mass, with nickel saturation being achieved at ~70 mg/L in the solution. Autoclaved *A. cylindrica* demonstrated a binding capacity of 0.012 g Ni sorbed/g dry cell mass; the saturation point was reached at a nickel concentration of ~30 mg/L.

The isotherm data show a number of interesting features. In live cells of both species, the Langmuir isotherm model provides a better representation of the data than does the Freundlich model, but this situation is reversed when autoclaved cells are used—the Freundlich model then provides the better fit. It is unclear if this finding has any significance in terms of application of the biosorbent. Perhaps the mechanisms of sorption are different for live cells and autoclaved cells, though we have no direct evidence to support this supposition. Another consistent observation during this study was that isotherm data from live cells tended to be more "consistent" than those from autoclaved cells; that is, data from autoclaved cells were more difficult to reconcile with either isotherm model than were data from live cells.

Biosorption of Nickel in Actual Wastewater Samples

Anabaena flos-aquae, the live cells of which demonstrated a higher binding capacity for nickel in surrogate samples than any other live cyanobacteria tested, was used in a series of tests to determine its usefulness in removing nickel from actual samples of the ORR wastewater. This wastewater contained 7.1 mg/L nickel, along with a host of other chemicals at various concentrations, and its pH was 5.5. A sample containing live *A. flos-aquae* was introduced to the wastewater, thus resulting in a final dry cell mass equivalent of 1 g/L wastewater. The organism's binding capacity under these conditions was determined to be 0.002 g Ni/g dry cell mass (2.0 mg Ni removed/L wastewater) at 1 h, with no further biosorption noted at 3 h. This was considerably lower than the binding capacity of 0.006 g Ni/g dry cell mass measured for this organism against a surrogate wastewater containing only nickel at 10 mg/L.

The complex chemical nature of the wastewater led to the hypothesis that the nickel was perhaps complexed with some organic molecule in the wastewater; if this were true, it could affect the organism's ability to bind nickel. An alternative hypothesis was that, because of the high concentrations of some other cations in this effluent, interference with nickel binding by competing ions could be occurring. To test these hypotheses, the wastewater was spiked with $NiCl_2$ to achieve a final nickel concentration of 20.2 mg/L. Samples of *A. flos-aquae* were introduced, and aliquots were removed and analyzed at 1, 2, 3, 4, and 24 h. The binding capacity of the cells under these conditions was identical to that seen in unspiked wastewater. This observation was taken to be an indication that the poor nickel binding seen in the actual wastewater was caused by the presence of interfering ions, not by the nickel being present in a chemical complex.

Interference Testing with *A. flos-aquae* and Sodium Sulfate

Table 2 represents the results of preliminary testing of the hypothesis that sodium, which was shown by previous analysis to be present in samples of the waste water effluent at ~4000–6000 mg/L, could be inter-

Table 2
Interference of Sodium Sulfate with Nickel Binding

Organism	Exposure time, h	Initial Ni in solution, mg/L	Ni after exposure to biosorbent, mg/L	Binding capacity, g Ni sorbed/g dry cell mass
A. flos-aquae autoclaved[a]	1	8.9	7.9	0.0010
	2	8.8	7.7	0.0011
	3	8.8	7.7	0.0011
Nostoc sp., live[b]	1	8.8	8.8	0.0000
	2	8.8	8.7	0.0001
	3	8.8	8.3	0.0005
	24	8.8	7.5	0.0013
Nostoc sp., autoclaved[b]	1	10.5	4.7	0.0058
	2	10.5	5.1	0.0054

[a]Sodium concentration in tests with *A. flos-aquae* was 5280 mg/L.
[b]Sodium concentration in tests with *Nostoc* sp. was 4000 mg/L.

fering with the abilities of cyanobacteria to bind nickel. The waste solution from the plating operation, as received in our laboratory, had already undergone initial treatment steps to remove gross amounts of certain toxic components. Consultation with the engineers responsible for this initial treatment process revealed that sodium sulfate was added in fairly high concentrations to the wastewater at one stage of the process. We elected to use the same salt for our testing to determine whether sodium could interfere with nickel binding by the candidate biosorbents.

Sufficient amounts of autoclaved cells of *A. flos-aquae, A. cylindrica,* and *Nostoc* sp. to provide 1 g/L in the final suspension were added to a solution of nickel in a sodium sulfate matrix (actual concentrations of nickel and sodium noted in the Table 2). In all three species, the ability to bind nickel was significantly reduced by the presence of sodium in the surrogate waste solution. Autoclaved cells of *Nostoc* sp. were affected least by the sodium in the solution, showing a binding capacity of 0.006 g Ni sorbed/g dry cell mass after 1 h. This is more than 30% less than the binding capacity for this species in the absence of sodium (0.009 g Ni sorbed/g dry cell mass). Autoclaved cells of *A. flos-aquae,* which had a binding capacity at 2 h in the absence of sodium of 0.007 g Ni sorbed/g dry cell mass, had a binding capacity at 2 h for nickel in the sodium matrix of 0.001 g Ni sorbed/g dry cell mass (a reduction of over 85%).

It is apparent by a comparison of these data to those in either Table 1 or Fig. 1 that the presence of sodium in the nickel-containing surrogate

waste solutions significantly reduced the ability of the cyanobacterial cells to bind nickel. These results are not definitive, however, as the actual ORR wastewater contains other ions that could be potential competitors with nickel for binding sites on the surfaces of the cyanobacterial cells. These other ions, however, are present in much lower concentrations than sodium. Further testing will be required to determine whether any of the other chemical species present in the wastewter are impediments to nickel binding by cyanobacteria.

CONCLUSIONS

The results of this study demonstrate that the species of filamentous cyanobacteria tested have the potential for application as efficient biosorbents for nickel in aqueous wastes. They show an affinity for nickel that is considerably higher than can be found in the literature for other organisms, such as green algae. There are current limitations to their application, however, as shown by the severe interference of sodium, and possibly other, ions, with the binding of nickel. This lack of specificity for nickel is one significant problem that will have to be solved before widespread deployment of these organisms as biosorbents can be accomplished.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Thomas Klasson in preparing the adsorption isotherm data and that of T. Klasson and Jonathan Woodward in reviewing this manuscript. This work was sponsored by the US Department of Energy's Office of Technology Development.

REFERENCES

1. Benemann, J. R. and Wilde, E. W. (1991), *Literature review on the use of bioaccumulation for heavy metal removal and recovery, vol. 2,* Westinghouse Savannah River Co. Report No. WSRC-TR-90-175-VOL. 2.
2. Chan, S. S., Chow, H., and Wong, M. H. (1991), *Biomed. Environ. Sci.* **4(3),** 250.
3. Wong, M. H. and Pak, D. C. H. (1992), *Biomed. Environ. Sci.* **5(2),** 99.
4. Aksu, Z., Sag, Y., and Kutsal, T. (1992), *Environ. Technol.* **13(6),** 579.
5. Aksu, Z. and Kutsal, T. (1990), *Environ. Technol.* **11,** 979.
6. Aksu, Z. and Kutsal, T. (1991), *J. Chem. Tech. Biotechnol.* **52,** 109.
7. Garnham, G. W., Codd, G. A., and Gadd, G. M. (1992), *Environ. Sci. Technol.* **26(9),** 1764.
8. Mahan, C. A., Majidi, V., and Holcombe, J. A. (1989), *Anal. Chem.* **61(6),** 624.

9. Gardea-Torresdey, J. L., Becker-Hapak, M. K., Hosea, J. M., and Darnall, D. W. (1990), *Environ. Sci. Technol.* **24,** 1372.
10. Mann, H., Fyfe, W. S., Kerrich, R., and Wiseman, M. (1989), *Biorecovery* **1(3),** 155.
11. Wang, H.-K. and Wood, J. M. (1984), *Environ. Sci. Technol.* **18(2),** 106.
12. Les, A. and Walker, R. W. (1984), *Water, Air, Soil Pollut. (Netherlands)* **23(2),** 129.
13. Verma, S. K. and Singh, S. P. (1990), *Curr. Microbiol.* **21,** 33.
14. Campbell, P. M. and Smith, G. D. (1986), *Arch. Biochem. Biophys.* **244,** 470.
15. Peterson, H. G., Healey, F. P., and Wagemann, R. (1984), *Can. J. Fish Aquat. Sci.* **41,** 974.
16. Shehata, F. H. A. and Whitton, B. A. (1982), *Br. Phycol. J.* **17,** 5.
17. Singh, D. P. (1985), *J. Gen. Appl. Microbiol.* **31,** 277.
18. Jeffers, T. H., Ferguson, C. R., and Bennett, P. G. (1991), Biosorption of metal contaminants using immobilized biomass: a laboratory study, Bureau of Mines (Washington, DC) Report No. BUMINES-RI-9340.
19. Darnall, D. W. (1986), *Biosorption of heavy metal ions from industrial/mining waste waters (technical completion report)*, New Mexico Water Resources Research Institute (Las Cruces) Report No. WRRI-210.
20. Kratz, William A. and Myers, J. (1955), *Am. J. Bot.* **49,** 282.
21. Starr, R. C. (1964), *Am. J. Bot.* **51,** 1013.