Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Biosorptive Flotation in Metal Ions Recovery

K. A. Matis[a]; A. I. Zouboulis[a]; I. Ch. Hancock[b]
[a] DEPARTMENT OF CHEMISTRY, ARISTOTLE UNIVERSITY, THESSALONIKI, GREECE [b] DEPARTMENT OF MICROBIOLOGY, THE UNIVERSITY NEWCASTLE-UPON-TYNE, UNITED KINGDOM

To cite this Article Matis, K. A. , Zouboulis, A. I. and Hancock, I. Ch.(1994) 'Biosorptive Flotation in Metal Ions Recovery', Separation Science and Technology, 29: 8, 1055 — 1071
To link to this Article: DOI: 10.1080/01496399408005617
URL: http://dx.doi.org/10.1080/01496399408005617

SEPARATION SCIENCE AND TECHNOLOGY, **29**(8), pp. 1055–1071, 1994

Biosorptive Flotation in Metal Ions Recovery

K. A. MATIS* and A. I. ZOUBOULIS
DEPARTMENT OF CHEMISTRY
ARISTOTLE UNIVERSITY
GR-540 06 THESSALONIKI, GREECE

I. CH. HANCOCK
DEPARTMENT OF MICROBIOLOGY
THE UNIVERSITY
NEWCASTLE-UPON-TYNE NE2 4HH, UNITED KINGDOM

ABSTRACT

Heavy metals can be removed from dilute aqueous solutions in many ways. Among the innovative ones may be classified a process consisting of biosorption followed by flotation. A metal cation, cadmium, was examined; the metal was abstracted by microorganisms belonging to the *Actinomycetes*, i.e., *Streptomyces clavuligerus* and *Streptomyces griseus*, which have a filamentous morphology, and hence present a flocculent character. Dissolved-air flotation was the technique applied on a laboratory scale without the addition of any flotation surfactant. The parameters investigated in the batch mode were contact time, recycle ratio, solution pH, Cd concentration, biomass addition, and use of a frother (ethanol). Promising results were obtained; in certain cases an almost quantitative cadmium abstraction, followed by higher than 90% biomass recovery, was found.

INTRODUCTION

Although adsorption as well as biosorption is usually operated in columns (packed-, fixed-, fluidized-, or expanded-bed), the operation of stirred tanks using a suspended biomass was also suggested (1). In the latter case a subsequent solid/liquid separation stage from the suspension is required. Obviously, filtration can be applied. However, this process

* To whom correspondence should be addressed.

may face filter blocking problems, especially with materials in the fine or ultrafine size range, while sedimentation is a relatively slow process when dealing with biological materials which are usually of low density. Perhaps this is the reason why centrifugation is often practiced as a separation process, but it requires a large energy input per unit mass of the cells separated. Many attempts have been made to develop energy-saving innovative separation techniques. Biotechnology, a current evolution of science and technology with a great future, has indeed an immediate requirement of new and alternative separation methods, efficient and of low cost.

Flotation, which originated in mineral processing, constitutes a viable selective separation process, especially for the treatment of fine particles (2). In the present case, the dissolved-air flotation technique was used. Both it and its application to wastewater treatment were recently reviewed during an Advanced Study Institute (3). One of the advantageous uses of the flotation method is in the selective recovery of metal ions (4, 5). Heavy metals, of course, can be removed from dilute aqueous solutions by applying other techniques, some of them more conventional than flotation (such as precipitation, ion exchange, etc.). Although the flotation of algae, bacteria, yeast, and other microorganisms has been examined in a number of recent articles and reviews (see, for example, Refs. 6–8), the flotation of a metal-loaded biomass has been examined for only limited cases (see, for example, Refs. 9 and 10). Smith has commented that much is still unknown regarding the specific details of microorganism flotation (11).

The main aim of the present article is to examine a combination of the two aforementioned processes, i.e., biosorption and flotation, into a unified operation that could be termed "biosorptive flotation." Cadmium, a serious pollutant, was the metal under examination; it has virtually no biological function and it is highly toxic. On the other hand, *Actinomycetes* were selected as biosorbents because of their filamentous morphology and flocculent properties. Hence, they may be suitable for separation through flotation. Another innovation of this work is the use of a dispersed instead of a packed-bed reactor, i.e., the application of microorganisms without any preliminary immobilization into granulated products, as has been reported for other cases (12). Therefore, relatively free access of metal ions to the cell wall was allowed, in order to allow for effective binding.

Biosorption of Metals

The highly toxic and variable conditions encountered in many waste and process waters, which sometimes take extreme values, usually preclude the use of living systems and dictate the application of nonliving systems for metals removal. Live and dead biomasses have rather equal biosorptive capacities (13). However, the use of a dead biomass eliminates the problem of toxicity and the economic aspects of nutrient supply and culture maintenance. The difficulty of the subsequent solid/liquid separation stage was also stressed in Ref. 13. Therefore, flotation is suggested as an alternative separation process.

It is apparent that the waste microbial biomass produced during many industrial fermentation processes, like antibiotics production, but also in biological wastewater treatment processes, may well present a relatively economic metal-sorbing material (14). *Actinomycetes* form a large group of bacteria, naturally occurring in soil, which surpass all other microorganisms in their capacity to produce biologically active substances (15). Microbial biosorption of metals from aqueous solutions is a method of increasing significance. The industrial potential of biosorption depends on such factors as loading capacity, efficiency and selectivity, ease of metal recovery, and equivalence to traditional treatments in performance, economics, and immunity from interference by other effluent components or operating conditions (16). Biosorptive treatments need not necessarily replace existing methodologies, but may act as "polishing" systems for processes that are not completely efficient.

The favorable properties of gram-positive bacteria (like the *Actinomycetes*) have been discussed (17). Anionic polymers, including teichoic acids, play important roles in the cell wall function and are widely distributed in cell walls (15). A combination of mechanisms is said to be involved in biosorption (complexation, ion exchange, coordination, adsorption, chelation, microprecipitation, etc.) (5). The biosorption of toxic (like Cd, Cu, Cr) or precious (Au, Ag) metals by *Actinomycetes* was presented in a number of articles (see, for example, Refs. 18 and 19). The selective accumulation of uranium by a large number of microorganisms (including 20 *Actinomycetes*) was also reported (20).

EXPERIMENTAL

A dissolved-air flotation apparatus was used for batchwise experiments (21). The transparent flotation cell had a total volume of 1.5 L, it was cylindrical (9.3 cm diameter), and it had no baffles. Synthetic mixtures of 1 L volume, containing the cadmium solution and the biomass concentration in deionized water, were kept in dispersion during the preliminary conditioning time (10 minutes) with a flat-bladed impeller (100 rpm). Stirring was slowed down to 60 rpm during flotation. Water saturated with air in the saturator and kept under a pressure of 400–500 kPa was introduced to the base of the cell via a specific nozzle arrangement and a solenoid valve. When the pressure was released to the atmosphere, fine air bubbles, appropriate for solid/liquid separation, were generated. A 30% recycle ratio was usually applied, i.e., an additional 300 cm^3 volume of saturated water was added in the cell beyond the initial 1 L suspension. Figure 1a is a schematic illustration of the applied process.

Unless otherwise stated, the initial solution had a 1×10^{-4} M (or 11.3 mg/L) cadmium concentration and was prepared from $CdNO_3$. A study of the metal aqueous speciation showed that the cadmium hydroxide appeared at a pH value of about 8.5 (22). Chemical analysis by AAS of the remaining solutions (after flotation) was used in order to assay the unremoved cadmium. The flotation process was tested collectorless. A small amount of ethanol was added (0.5% v/v) in order to obtain a more stable foam layer. The solution pH was adjusted by using hydrochloric acid or sodium hydroxide solutions.

The preliminary stage of biosorption was carried out in a beaker, stirred magnetically at a low rate for about 600 seconds because it is a rather fast process. A minimum energy requirement was anticipated. After flotation, gravimetric analysis of the floated biomass solids was conducted following a careful collection of the foam produced under vacuum, subsequent filtration using membrane filters of 0.45 μm diameter (an extremely slow microfiltration process), and drying the separated biomass under IR lamps. The results were expressed as recovery percent (Re%) in the usual manner on a dry biomass basis. The desorption experiments were also performed by pH modification. The biomass used in this case was collected from previous flotation experiments and was found to contain (after digestion) 5.57 mg Cd/g of biomass.

Two bacterial strains belonging to the genus *Streptomyces* (16) were used. The first one, *S. clavuligerus*, kindly supplied by Smithkline Beecham (Worthing, UK); these bacteria were harvested and heat sterilized at the production site. The second strain was *S. griseus*, a fast-growing

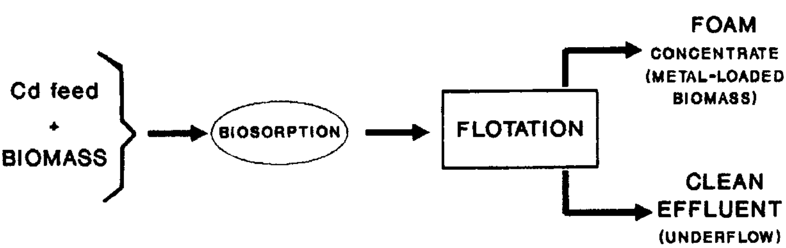


FIG. 1a Biosorptive flotation flow sheet.

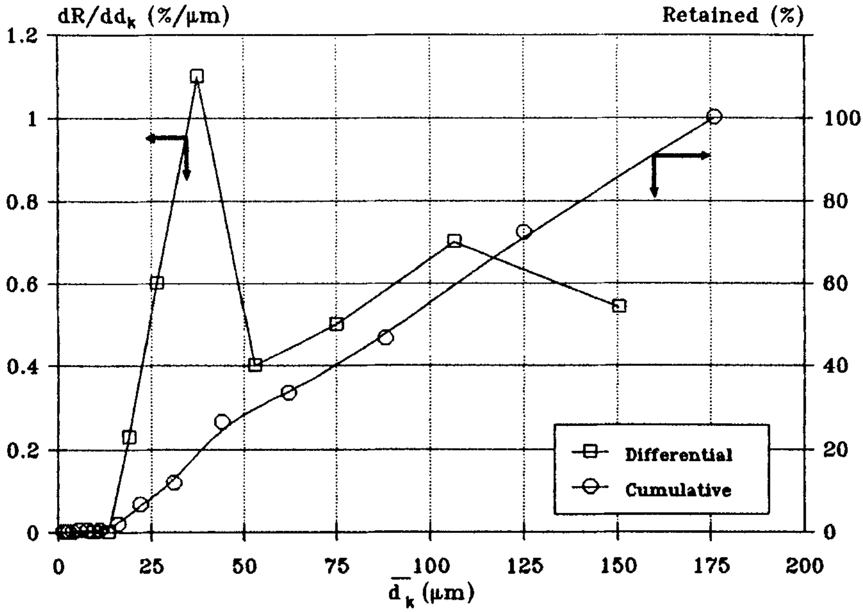


FIG. 1b Particle size analysis for unwashed *S. griseus*; cumulative and frequency distribution (average of 24 runs).

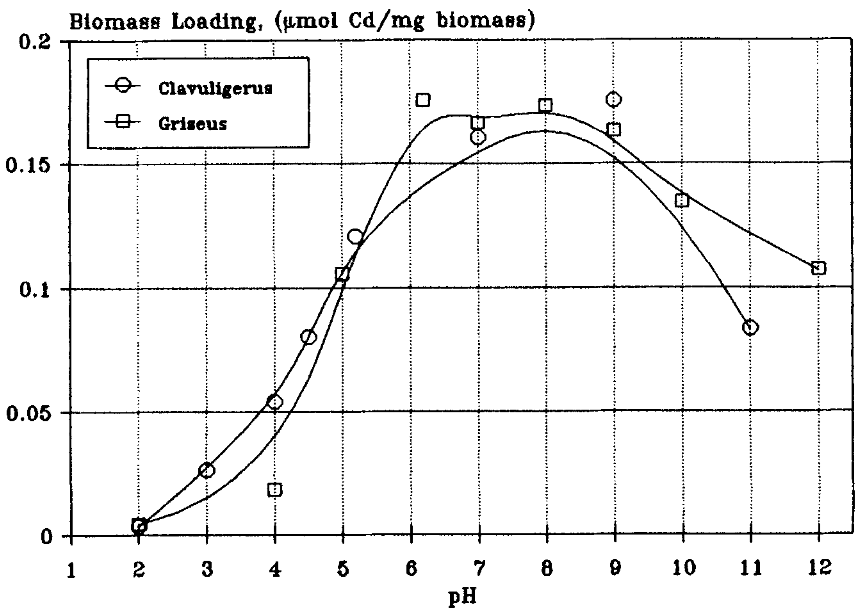


FIG. 1c Cadmium loading of unwashed biomass against the solution pH; comparison of the two samples for 0.3 g/L initial biomass concentration.

strain from the culture collection of the Microbiology Department, University of Newcastle-upon-Tyne, initially isolated from contaminated soil. It is worth noting that the usual practice of microbiologists is the isolation of bacteria from contaminated soil sites (23). These bacteria were harvested by continuous centrifugation (at about 10,000*g*) and killed by autoclaving (at 394 K for 1.2 ks). Microbiological hazards were not foreseen for the dead biomass.

Figure 1b presents the size distribution of *S. griseus*, showing that the biomass "particles" belong to the fine range (about 20–150 μm). Two maxima of the differential curve (around 35 and 110 μm) were observed. The flotation processing of fines has been discussed (24). Foam flotation was also applied for the separation of NaY-type zeolites from a dilute aqueous suspension (1 g/L); the zeolites had a particle size range of −5.5 +1.9 μm and had been previously used for the ion exchange/removal of zinc ions (10 mg/L) from solution (25). This process presents several similarities with the present one.

The biomass was previously homogenized using a hand homogenizer with a 76 μm clearance before the beginning of the experiments. *S. griseus* batches contain mainly filamentous bacteria under controlled growing conditions. A biomass (washed) concentration of 1 g/L has proved satisfactory in order to remove cadmium effectively.

Both unwashed and washed biomaterial samples were tested in order to compare their behaviors during flotation. It was discovered during preliminary experiments that the initial biomass samples contained approximately 70% by weight of soluble components due to culture medium residues. These had to be removed by washing with water after autoclaving and before drying. Unfortunately, the natural pH values of the bacteria suspensions varied. These pH values presumably depend on the nature of counterions associated in the bacterial cell surface, mainly phosphodiester.

Figure 1c presents selected preliminary experiments related to cadmium bio-abstraction; the results were calculated following dissolved-air flotation under the same conditions. Biosorption started around pH 5.

RESULTS AND DISCUSSION

The recycle ratio constitutes an important parameter of dissolved-air flotation. Its value can be manipulated in order to alter the volume of air entering the flotation cell, and hence the number of fine bubbles available for effective flotation. Figure 2a shows that even with a 10% recycle ratio, satisfactory results were obtained. In the following figures, both biomass recovery and cadmium removal are presented. However, the two separate

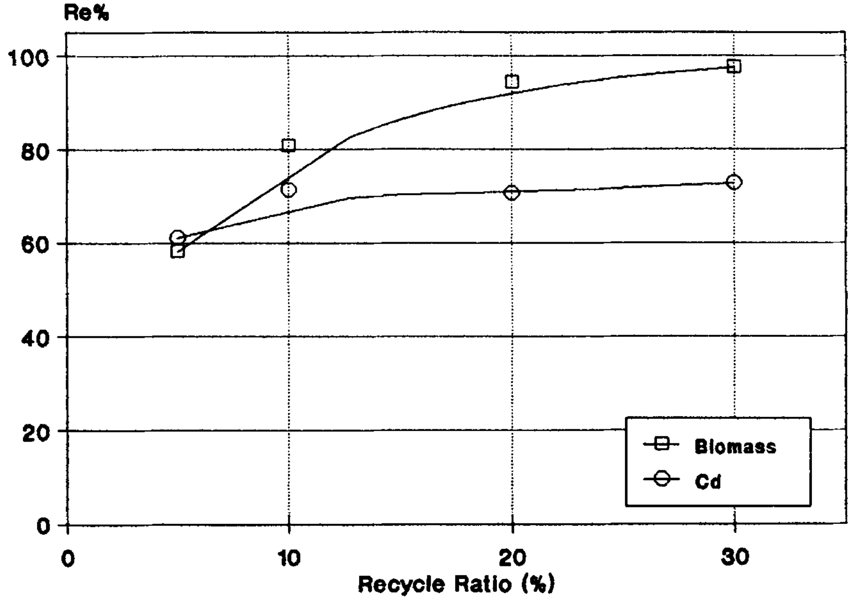


FIG. 2a Dissolved-air flotation of 0.3 g/L unwashed *S. griseus*; effect of recycle ratio in the presence of 0.5% ethanol at natural pH 6.2.

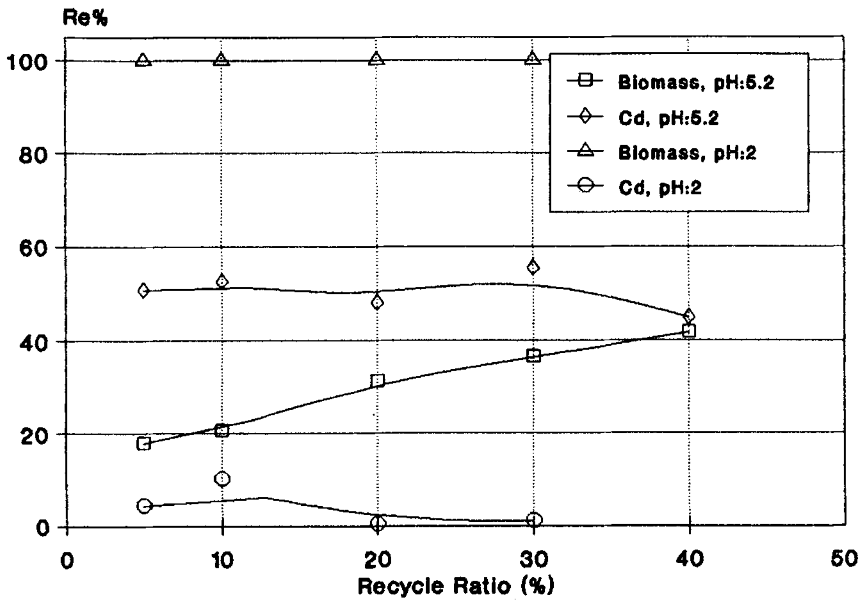


FIG. 2b Experiments with 0.3 g/L unwashed *S. clavuligerus* at pH 5.2 and comparison with pH 2.

stages of the system (metal biosorption and flotation) have not often been operated at their respective optimum conditions, which may be different in each case.

Preliminary experiments with an unwashed biomass proved that separation by flotation is effective even in the presence of dissolved culture medium material. This was demonstrated with *S. griseus*, where a flotation recovery of over 90% was observed in certain cases. This may mean that the initial pretreatment (washing with water) was not actually necessary, and that the culture medium residues did not depress flotation separation. Although the initial concentration of unwashed biomass in this case was also 1 g/L, the actual concentration was only 0.3 g/L, the remainder being soluble components which do not take part in the biosorption process.

Figure 2b presents a comparison between two different pH values (2 and 5.2) for unwashed *S. clavuligerus*. Cadmium removal was almost nil at pH 2, while at the same time all the biomass was floated very effectively and almost 100% flotation recovery was found. Metal removals were improved at pH 5.2 (around 50%), but biomass floatability greatly deteriorated, decreasing to approximately 40%. Nevertheless, the recycle ratio does not seem to be a critical parameter.

For Fig. 3 the preliminary contact time of the Cd/biomass system was varied from under 600 seconds up to 86.4 ks. Flotation of the biomass was not greatly affected by this parameter; however, there was some influence on cadmium removal which reached constant removal after half an hour. The mediocre metal removal (on the order of 40%) could be explained by the relatively lower biomass concentration (0.3 g/L instead of 1 g/L) actually present in these experiments. A pH value of 4 was selected for these experiments; it is the limit for the beginning of cadmium biosorption under these conditions (later confirmed).

Scatchard plots of cadmium abstraction by *Streptomycetes* (26) supported the existence of more than one type of metal binding site on the biomass surface and participating simultaneously during biosorption. It was also reported in experiments with *Citrobacter* sp. and cadmium that insoluble metal phosphate was precipitated on the cell surface as a result of phosphate production by the phosphatase-catalyzed hydrolysis of glycerol-2-phosphatase (14). In the case of gram-positive bacteria, the metal binding properties are largely due to specific polymers (like peptidoglycan, teichoic or teichuronic acids, etc.) existing on the cell wall structure. Therefore, they exhibit high biosorption capacities, which is an important factor for any industrial application of these bacteria as biosorbents. Another important advantage of these kinds of bacteria (like the *Actinomy-*

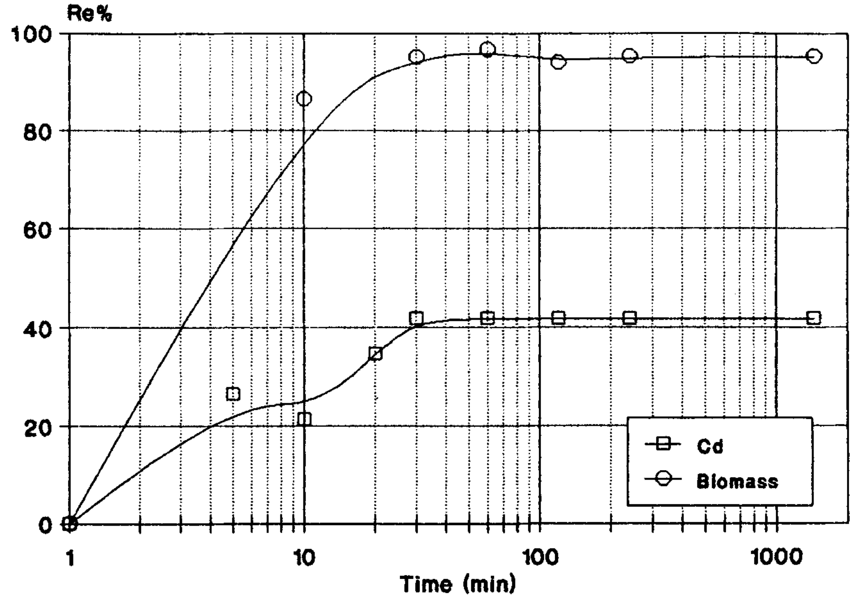


FIG. 3 Influence of initial contact time ot metal solution/biosorbent on dissolved-air flotation; 0.3 g/L washed *S. clavuligerus* at pH 4.

cetes) seems to be the ease with which their wall properties can be controlled (20).

Although it is possible in theory for much greater metal concentrations to be accumulated inside the cell than on its surface, in the case where accumulation of metals by translocation into microbial cells is sought, the sorbed metal can only be subsequently recovered from the cell by destroying it. Hence, the recycling of used biomass does not seem to be possible (14).

The great influence of solution pH on cadmium biosorption/flotation is clearly observed in Fig. 4 where a comparison between the presence and the absence of ethanol (frother) is shown. The main conclusion based on this figure is that the presence of ethanol is not significant in small (laboratory) scale experiments, for it was found to improve the flotation results to only a small degree. No influence of the ethanol presence in metal binding or biomass degradation was also noticed (26). Although different surfactants have been examined in the literature for other cases (11), the flotation process in the present article could operate effectively even collectorless. Biomass floatabilities starting from high values in acidic pH

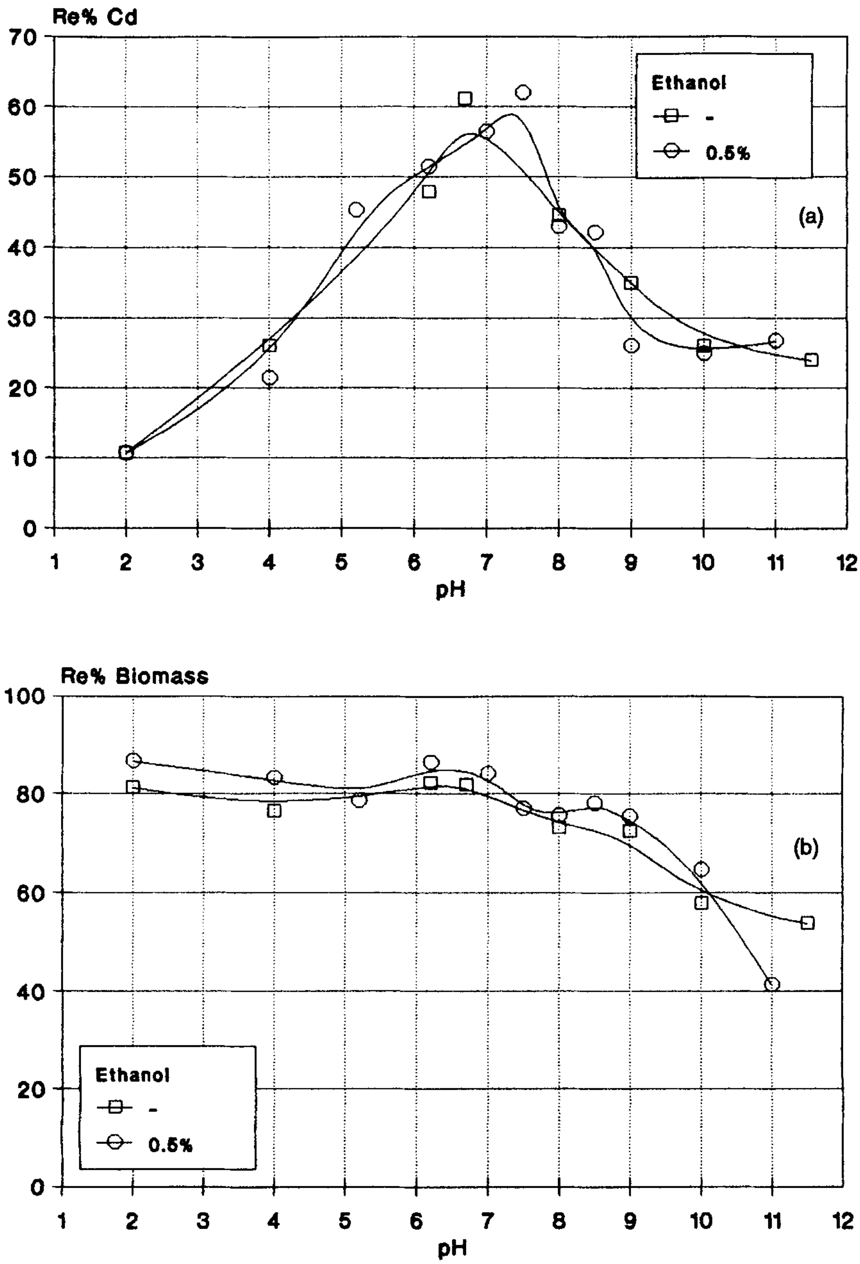


FIG. 4 Influence of ethanol addition on dissolved-air flotation using washed *S. griseus* (0.3 g/L) against the solution pH: (a) cadmium removal and (b) biomass recovery.

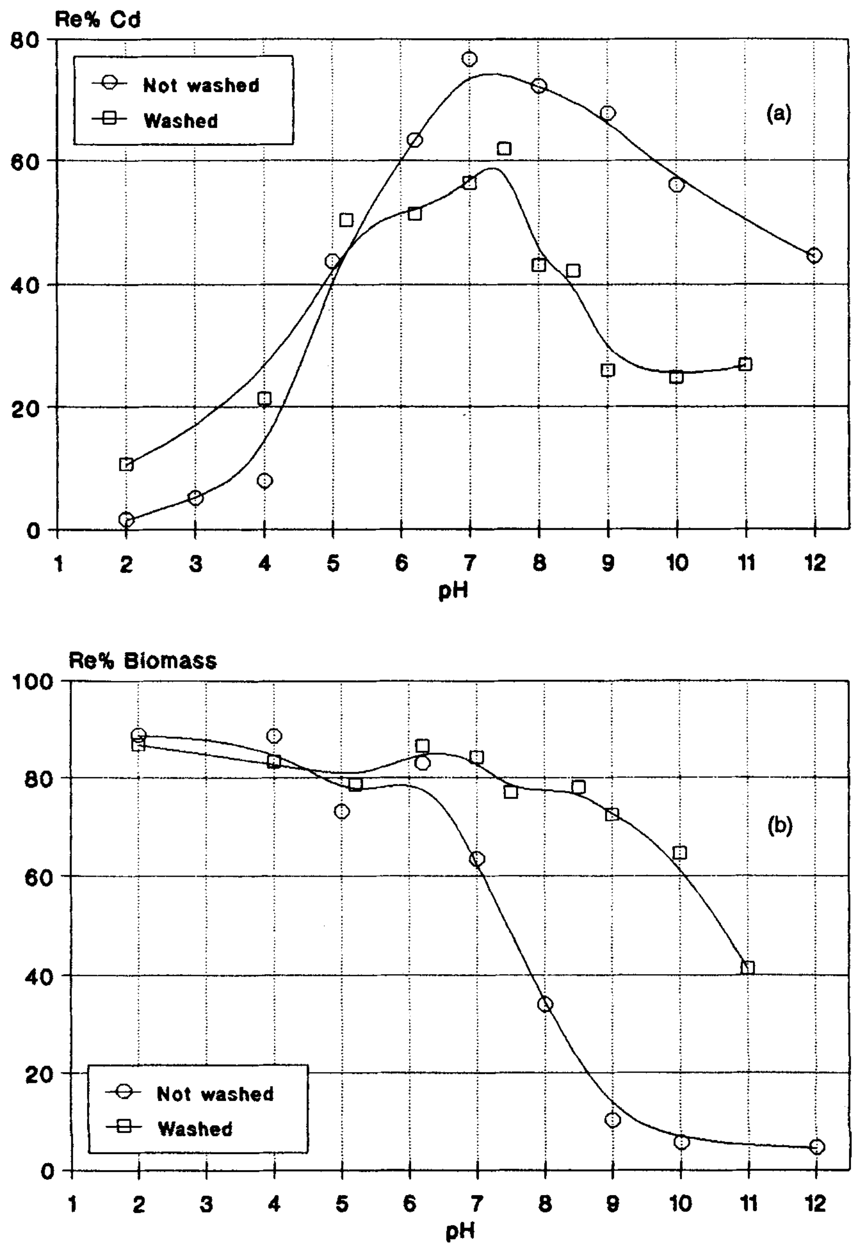


FIG. 5 Comparison of washed and unwashed biomass on dissolved-air flotation using 0.3 g/L *S. griseus* against the solution pH: (a) cadmium removal and (b) biomass recovery.

media were shown to deteriorate in alkaline pH regions, while cadmium removal reached a maximum at approximately neutral pH values.

During preliminary experiments the flotation behaviors of the two bacteria strains were not similar; the effective flotation of *S. clavuligerus* was more dependent on acid pH values than that of *S. griseus* (26). A pH value of 7 represents the upper limit without the application of a flotation surfactant for effective biomass recoveries to be obtained.

Similar observations can be made from Fig. 5 where a comparison between washed and unwashed *S. griseus* samples is made. The main difference between them appeared at a pH of about 7.5. Foreign organic and inorganic substances may accompany the microorganisms. The literature states that these substances may function as surfactants, frothers, or modifiers (11). Cadmium removals were somehow improved, due possibly to its coprecipitation with insolubles. Meanwhile, flotation diminished due to the interference of coexisting soluble material.

When the initial cadmium concentration was varied, metal removals was effectively improved, reaching almost 100% by increasing the addition of biomass (Fig. 6a). This appeared especially for the *S. griseus* where a

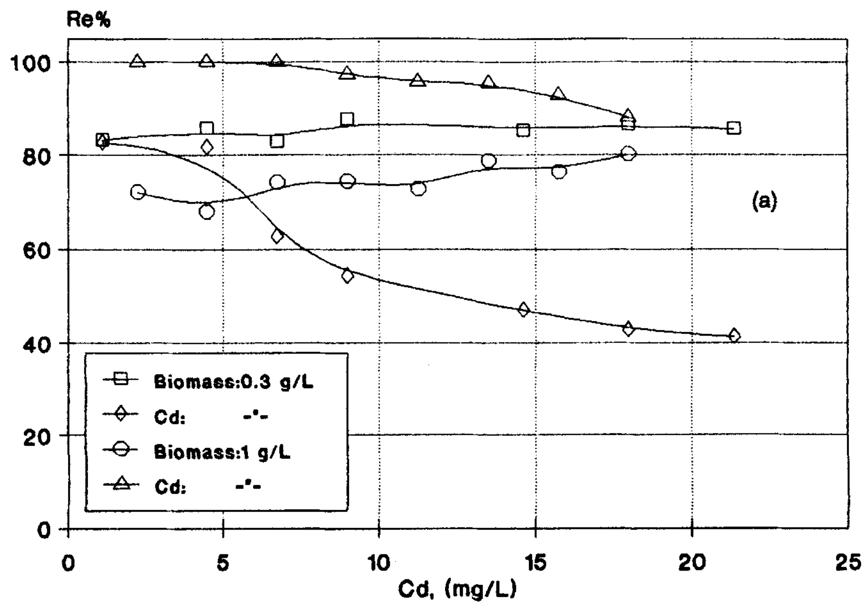
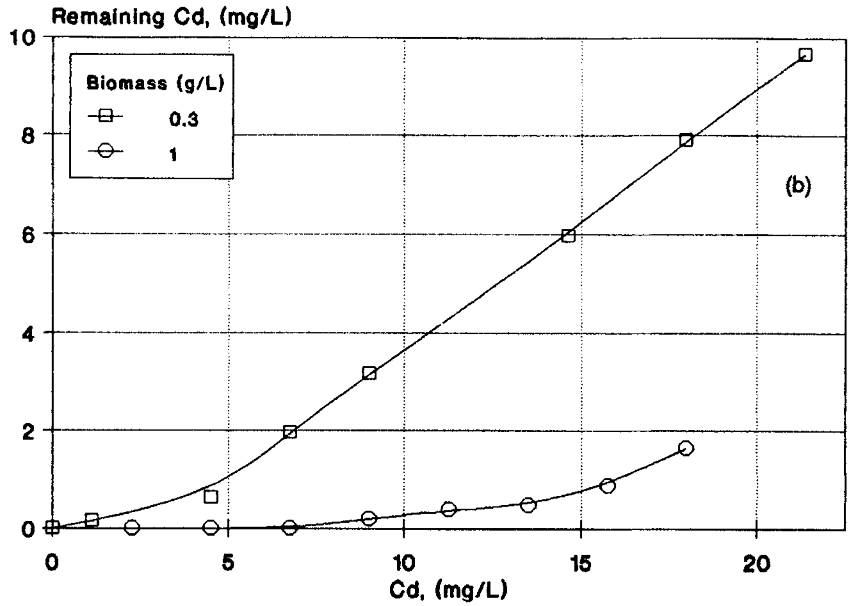
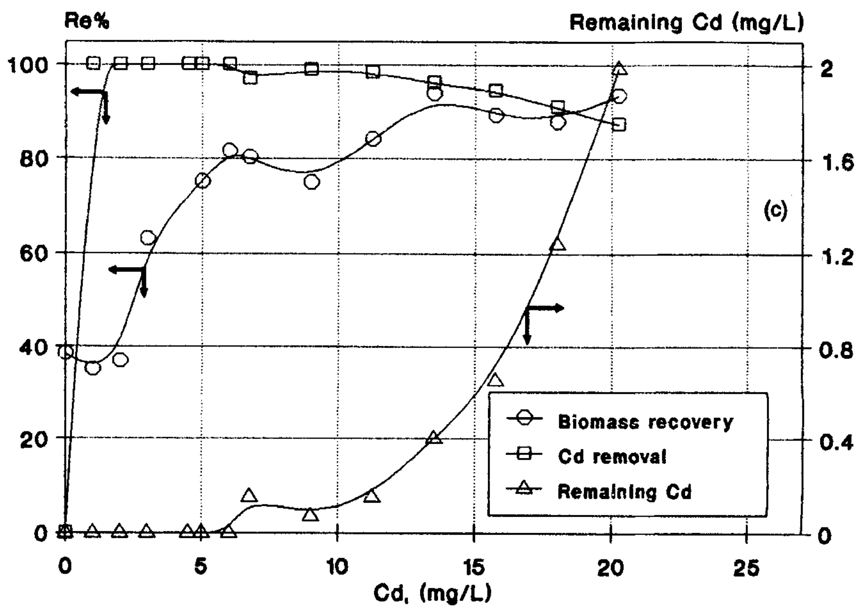


FIG. 6 Influence of initial cadmium concentration on dissolved-air flotation using washed biomass: (a) *S. griseus* at two different biomass concentrations (pH 6.2), (b) remaining cadmium under the same conditions as (a), and (c) *S. clavuligerus* at pH 7 and 1 g/L initial concentration.


Remaining Cd, (mg/L)
Biomass (g/L)
0.3
1
(b)
Cd, (mg/L)


Re%
Remaining Cd (mg/L)
(c)
Biomass recovery
Cd removal
Remaining Cd
Cd, (mg/L)

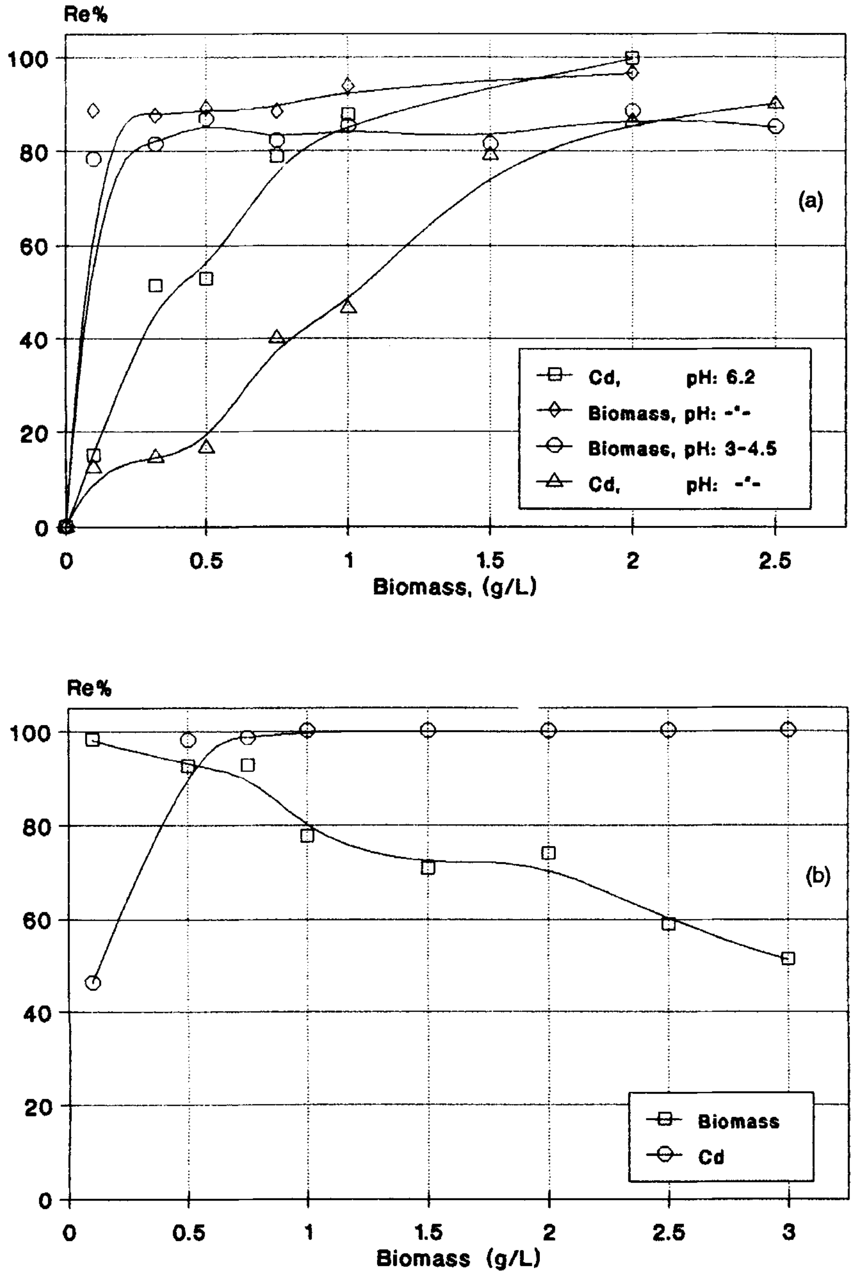


FIG. 7 Effect of washed biomass feed concentration (in g/L of metal solution) on the flotation process: (a) *S. griseus* at two different pH values (6.2 and 3–4.5), (b) *S. clavuligerus* at natural pH 7.

low biomass addition (0.3 g/L) gave decreased removals (down to 40%) with an initial cadmium concentration of around 20 mg/L. On the other hand, biomass recoveries were of the order of 80%, but they decreased slightly for higher biomass concentrations (1 g/L). The remaining Cd concentrations are shown in Fig. 6(b), which points out the great differences between the two biomass concentrations used. On the other hand, high cadmium removals were obtained (over 90% for all cases) for *S. clavuligerus* (Fig. 6c), while higher biomass recoveries occurred when the initial cadmium concentration was increased to over 5 mg/L. In the same figure the remaining cadmium concentration can be observed, and the advantages of working with a 1 g/L biomass is apparent.

Figure 7 shows that the initial cadmium concentration was not significant alone, but it was significant in combination with the added biomass concentration. *S. griseus* was found to float better at two different pH values (6.2 and 3–4.5). *S. clavuligerus* recoveries at pH 7 decreased when the biomass quantities (initially present in the solution) were increased. Cadmium removals were substantially increased, surpassing 90%, as expected, with higher biomass additions.

The elution of the metal from the floated biomass is of great importance for a number of reasons, such as the stability of the cadmium-laden biomass and the possibility of pollution prevention. Figure 8 shows that in a

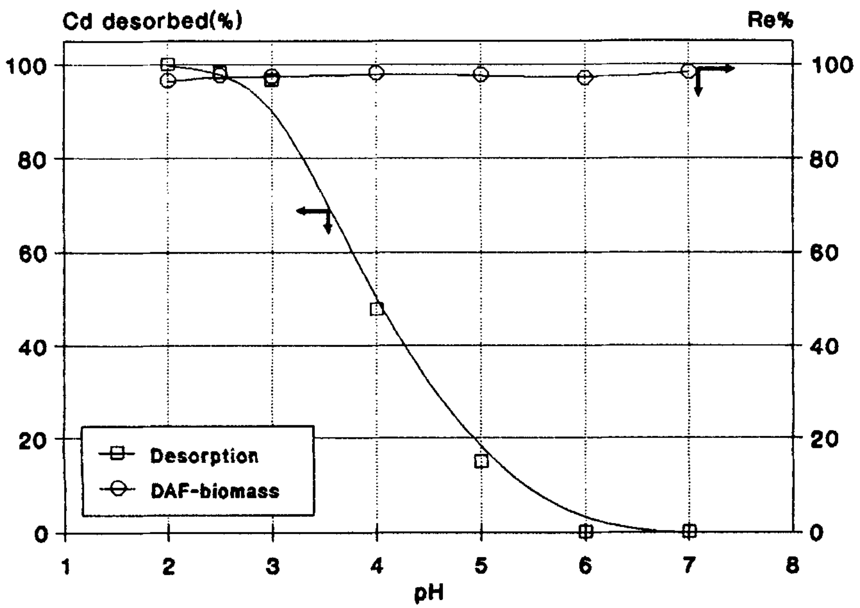


FIG. 8 Desorption experiments of cadmium from washed *S. griseus* and reflotation of the resulting biomass at various pH values.

highly acidic pH range (pH 2–2.5), the metal again transfers to the aqueous solution to a significant degree. This pH range is not of interest for biomass recycle because the groups at the cell surface would be expected to be destroyed. However, over pH 5, the binding of cadmium onto the biomass surface is quite stable.

The biomass used was treated (by pH modification) in order to desorb the abstracted cadmium. It was used again in flotation experiments (Fig. 8), where it gave high floatabilities up to pH 7. This conclusion was important because it points out that following the elution stage, the biomass can be recycled back in the beginning of the process in order to be used again for metal biosorption, i.e., in a number of adsorption/desorption cycles. Obviously, a complexing agent could be also applied for an effective elution stage. Further research is warranted in this area because the biomass should be retained in a suitable form for reuse. Additionally, eluates from the metal-laden biomass, which will be 10 to 100 times more concentrated solutions, could be subsequently treated by another known metal recovery method (for instance, electrochemical), hence integrating the whole process and recycling the metal for the market.

CONCLUDING REMARKS

Biosorption of metals from dilute aqueous solutions, as several effluents or leachates are, constitutes an important method as shown in the present work for cadmium. The subsequent separation of the resulting metal-loaded microbial cells from suspension is also significant. For density reasons, among others, flotation seems to be a satisfactory alternative solution.

Biosorptive (collectorless) flotation is a promising method for cadmium removal from dilute aqueous solutions which contain an initial concentration of about 10 mg/L. Dissolved-air flotation, the generation technique of bubbles, was shown to be an efficient and fast separation method. pH was a critical parameter, also affecting metal speciation. The coexistence of soluble material was not found to depress flotation recovery of the biomass, except at alkaline pH values. Both biosorption and flotation should be operated at optimum conditions.

ACKNOWLEDGMENT

This work was conducted as part of the European Community—Environment Programme (Contract EVWA-CT92-0003).

REFERENCES

1. P. J. Jackson, A. P. Torres, and E. Delhaize, U.S. Patent 5,120,441 (June 9, 1992).
2. A. I. Zouboulis, K. A. Kydros, and K. A. Matis, *Sep. Sci. Technol.*, *27*(15), 2143 (1992).
3. A. I. Zouboulis, K. A. Matis, and G. A. Stalidis, in *Innovations in Flotation Technology* (P. Mavros and K. A. Matis, Eds.), Kluwer Academic, Dordrecht, Netherlands, 1992, p. 475.
4. N. K. Lazaridis, K. A. Matis, G. A. Stalidis, and P. Mavros, *Sep. Sci. Technol.*, *27*(13), 1743 (1992).
5. A. I. Zouboulis, K. A. Matis, and N. K. Lazaridis, *Ibid.*, *25*(4), 463 (1990).
6. J. K. Edzwald, *Water Sci. Technol.*, *27*(10), 67 (1993).
7. N. Ohmura, K. Kitamura, and H. Saiki, *Biotechnol. Bioeng.*, *41*, 671 (1993).
8. K. H. Bahr and K. Schuegerl, *Chem. Eng. Sci.*, *47*(1), 11 (1992).
9. Z. Sadowski, Z. Golab, and R. W. Smith, *Biotechnol. Bioeng.*, *37*, 955 (1991).
10. R. W. Smith and Z. Yang, in *Proceedings, XVII International Mineral Processing Congress*, Vol. 5, Bergakademie Freiberg, FRG, 1991, p. 131.
11. R. W. Smith, *Min. Process. Extract. Metal. Rev.*, *4*, 277 (1989).
12. B. Volesky, *Trends Biotechnol.*, *5*, 96 (April 1987).
13. C. L. Brieley, J. A. Brieley, and M. S. Davidson, in *Metal Ions and Bacteria* (T. J. Beveridge and R. Doyle, Eds.), Wiley, Chichester, UK, 1989, p. 359.
14. M. N. Hughes and R. K. Poole, *Metals and Microorganisms*, Chapman and Hall, London, 1989, p. 328.
15. A. Boularbah, J. L. Morel, G. Bitton, and A. Guckert, *Environ. Toxicol. Water Qual.*, *7*, 237 (1992).
16. G. M. Gadd, *J. Chem. Technol. Biotechnol.*, *55*(3), 302 (1992).
17. I. C. Hancock, in *Trace Metal Removal from Aqueous Solution* (R. Thompson, Ed.) (Special Publication 61), Royal Society Chemistry, London, 1986, p. 25.
18. A. A. Pradhan and A. D. Levine, *Water Sci. Technol.*, *26*(9–11), 2145 (1992).
19. H. Babich and G. Stotzky, *Appl. Environ. Microbiol.*, *33*(3), 681 (1977).
20. A. Nakajima and T. Sakaguchi, *Appl. Microbiol. Biotechnol.*, *24*, 59 (1986).
21. G. A. Stalidis, K. A. Matis, and N. K. Lazaridis, *Sep. Sci. Technol.*, *24*(1&2), 97 (1989).
22. M. Pourbaix, *Atlas of Electrochemical Equilibria in Aqueous Solutions*, NACE, Brussels, 1974, p. 417.
23. I. Naumova, *Microbiol. Sci.*, *5*(9), 275 (1988).
24. K. A. Matis and P. Mavros, *Sep. Purif. Methods*, *20*, 163 (1991).
25. A. I. Zouboulis, D. Zamboulis, and K. A. Matis, *Sep. Sci. Technol.*, *26*(3), 355 (1991).
26. I. Ch. Hancock and K. A. Matis, Unpublished Data.

Received by editor September 17, 1993