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REMOVAL OF METAL IONS FROM SIMULATED WASTEWATER BY *SACCHAROMYCES* YEAST BIOMASS: COMBINING BIOSORPTION AND FLOTATION PROCESSES

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ABSTRACT

Aqueous solutions containing heavy metals can be successfully treated by a combination of biosorption and flotation, in order to remove (or recover) the contained metals. Nonliving biomass of yeast *Saccharomyces*, which is a solid industrial by-product, was found to be a suitable biosorbent of metal ions (zinc, copper, and nickel). It was found also possible to reuse it after the appropriate desorption treatment. Electrokinetic behavior of biomass as well as elution and multiple-cycles operation were investigated. The dispersed-air flotation technique, which was selected for generation of bubbles, was subsequently examined for solid/liquid separation, in order to harvest the metals-loaded biomass downstream. The main parameters affecting the flotation process were studied, such as the solution pH, the concentration of flotation collector (surfactant), the preliminary biomass modification, and the biomass concentration. The biosorptive flotation method

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was found promising for remediation applications of wastewaters containing toxic metals.

Key Words: Biosorption; Toxic metals removal; Flotation; Yeast; Biomass.

INTRODUCTION

Several biomass types, such as filamentous fungi, algae, yeast, and bacteria, have been reported to remove heavy metals from aqueous solutions (1). The interactions between metal ions and microorganisms present potential applications for the remediation of metal-contaminated waters generated in various industries (2). Furthermore, the separation of metals from waste solutions would be beneficial, as the removed metal may be subsequently desorbed from biomass and recovered for reuse. Additionally, the release of toxic metals into the environment would be restricted. The use of waste biomass, which is produced in several industries as biosorbent, is expected to increase the economic competitiveness of microbial-based remedial technologies. Yeast biomass represents an important source of biosorbents because it is not expensive material, it can be easily recovered at the end of fermentation processes, and it is produced as waste by-product in large quantities.

The application of commonly used solid/liquid separation methods, such as sedimentation or centrifugation, presents several problems when used in the field of wastewater treatment. This is mainly due to the small size and density of solid particles involved in the processes. In sedimentation, large settling tanks are necessary because of extensive retention times. Centrifugation is considered as relatively expensive method, regarding the power demand per quantity unit of microorganism cells recovered, particularly in small-scale applications. Therefore, flotation was alternatively investigated as a solid/liquid separation method, for instance during the recovery of yeast, by using a countercurrent bubble/foam column (3).

Flotation, nowadays a well-established unit operation for the selective separation of minerals, has been also practiced during the last years for the harvesting of biological materials (4). The process of flotation includes several techniques, such as foam or bubble fractionation, foam separation, and froth flotation, which was already examined for the separation of proteins, either in baker's yeast fermentation (*Saccharomyces cerevisiae*) (5) or from human placental extract (6). The recovery of valuable proteins from biological sources by using innovative separation techniques is an important issue because of the respective commercial potential.

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Flotation was also examined as a solid/liquid separation method for metal-loaded biomass particulates (suspension of actinomycetes of *Streptomyces* gene). It was found to be a satisfactory method for the removal of cadmium, a priority pollutant (7). The term *biosorptive flotation* was suggested for the combined process. The method involves the preliminary abstraction of metal ions using proper biomass, followed by a subsequent flotation stage for the separation of metal-loaded particles from the dispersion. The ability of microorganisms, alive or dead, to remove metals from aqueous solutions is a commonly observed phenomenon that has already found certain industrial applications (1). In specific cases, biosorbents were reported to present higher efficiency than ion-exchange resins, as, for instance, when applied to plating wastewaters for the removal of toxic metals (8).

Yeast has been used by humans for over six thousand years for a variety of applications, and it is presently one of the most important commercial microorganisms. Nevertheless, its significance and industrial utilization could be further increased. Nonliving yeast cells may be used in many ways and forms for removal of metals (9,10). The applications of yeast in biosorption processes vary from highly polluting cases, such as uranium (11), to worth-recovering valuable metals, such as silver (12). Metabolism-independent binding of metal ions to fungi and yeast cell walls is usually a rapid process, and relatively large amounts of metals may be bound and hence removed (13). The applicability of the concept of hard and soft metal ions for predicting the characteristics of their biosorption in a biological context (*Saccharomyces cerevisiae*) was also examined (14). A foam separation technique was also attempted to separate living *Saccharomyces carlsbergensis* cells from their broth without the addition of any surface-active agents, and a preliminary model for cell separation in a batch column was developed (15).

As the cost of biomass production for biosorption applications would be possibly economically prohibitive on an industrial scale, the use of waste microbial biomass has been considered as an alternative option. Among the several biomass sources, that of industrial fermentation yeast, which is a readily available biological by-product, was examined in the present work as an appropriate sorbent material for the treatment of a toxic metals mixture, simulating an actual wastewater. Dispersed-air flotation was subsequently applied for the efficient separation of metal-laden yeast biomass, and the main parameters influencing the effectiveness of this process as initial pH, collector, and biomass concentration, were investigated.

EXPERIMENTAL PART

The following aqueous mixture of metals in deionized water was examined in all experiments: Zn 50, Cu 10, Ni 2, Ca 100, and Na 100—units expressed in

mg/L. Nitric salts were used for the preparation of stock metal solution. The studied metals are commonly found in most metal-laden wastewaters. The used concentrations are considered as average values from different sources. Therefore, this mixture of metals can simulate an actual wastewater (16). The addition of Ca and Na ions was done as a simulation for tap water of medium hardness.

In this investigation as biosorbents two different yeast samples of *Saccharomyces* biomass were examined, considered as industrial by-products (solid wastes). The biomass samples were supplied by two brewing industries: Federation Brewery (Gateshead, U.K.) and Unicer (Lisbon, Portugal), the latter provided through Dr. F. Rodrigues (INETI, Lisbon). Selected determined physical properties of the used biomass are presented in Table 1. The first sample was referred to as *Saccharomyces cerevisiae* and the second sample as *Saccharomyces carlsbergensis.* Nonliving material was used throughout the experiments at 1 g/L (dry weight) concentration. The biomass batches, harvested mainly in the form of cell aggregates, were properly inactivated by autoclaving. They were also washed extensively with deionized water in order to remove the various soluble residuals, carried over. The efficiency of separation by flotation was found to be influenced negatively by the extensive foaminess of used culture medium (3). The latter was also strongly affected by the presence of extracellular proteins, which were excreted by yeast cells during cultivation. The cloudy supernatant was then removed by decantation, and the remaining biomass slurry was kept in the refrigerator up to the appropriate time. The biomass suspension for the biosorptive flotation experiments was prepared by using a hand homogenizer (Jencons, U.K.) with a clearance of 45 μm.

Table 1. Selected Physical Properties of Used Biomass Samples

Biomass Type	Origin	Water Content[1] (%)	pH (Natural)[2] —	Solubles in Water[3] (%)	Inorganics Content[4] (%)
Saccharomyces cerevisiae (Yeast-1)	Lisbon Portugal	73.26	5.6–5.8	22.0	5.56
Saccharomyces carlsbergensis (Yeast-2)	Gateshead U.K.	64.28	6.3–6.5	27.0	15.21

[1] An appropriate amount of biomass was put in oven (105°C) up to constant weight.
[2] For a suspension of 1 g/L biomass.
[3] An appropriate dry weight of biomass was suspended in water (1 g/L), stirred for 1 h, and separated by filtration. The filter was dried, and the difference of weight represents the solubility.
[4] An appropriate amount of biomass was put in oven (550°C) up to constant weight.

The pH values of biomass suspensions were adjusted by nitric acid or sodium hydroxide solutions (in deionized water), as required. A cationic polyelectrolyte (Zetag 92, Allied Colloids, U.K.) was also examined for pretreatment of biomass. Dodecylamine (denoted hereafter as DA), a common primary amine, was applied as cationic surfactant or collector reagent during the flotation experiments in order to enhance the floatability of biomass. Following preliminary experiments DA was used at a final concentration of 3×10^{-4} M (unless otherwise stated). Stock solutions of DA were prepared in ethanol, which is a convenient flotation frother; the concentration of ethanol during flotation was 0.6% v/v.

The application of biomass was performed in a CSTR-type (i.e., dispersed). Previous experience has shown that when applying biomass as sorbent material by using such a dispersed system, the main advantage (in comparison with immobilized biomass systems) is the shorter contact time required to obtain equilibrium, which is in the range of few (five) min. This observation was examined also for the studied biomass (*Saccharomyces*) during preliminary experiments, and the same result has been found (i.e., there is an influence of contact time but only within the first 5 min of contact time, then the sorption system reaches the equilibrium stage). Therefore, it has been decided to keep the contact time for the following experiments to 15 min (i.e., to provide certain excess of contact time in order to secure reaching the equilibrium stage).

The used batch dispersed-air flotation cell (column) had a Schott D_4 fritted funnel in the base as air diffuser, 600 mL volume, and 60 cm height. The flotation retention time was kept constant at 10 min. The air flowrate was 200 cm^3/min (or 0.267 cm/s superficial velocity) at an excess pressure of 1 atm. Foamates were removed by sucking from the top of the column. The conditioning time after biosorption and before flotation was 15 min, allowing the contact/sorption of flotation collector to biomass particulates. Therefore the total duration of process was 40 min.

The residual metal concentrations were analyzed by Atomic Absorption Spectroscopy in the resulting solution (after biosorption and flotation). The removal of metals and the recovery of biomass were expressed as a percentage in the usual way. Electrokinetic measurements of biomass under different experimental conditions were carried out by using a Rank Brothers Mark II microelectrophoretic apparatus. The experiments were repeated at least two times (the average value is shown in the figures), and the experimental error was kept between $\pm 5\%$.

Biomass pretreatment (considered as a kind of preliminary modification) was performed for selected experiments by the addition in the biomass suspension of 10 mg/L Zetag polyelectrolyte, followed by vacuum filtration for biomass separation, using a membrane filter (0.45 μm). The filter cake was subsequently washed by the eluant.

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From a previous investigation, EDTA was tested as a possible eluant (desorbing agent) of cadmium-loaded *Streptomyces clavuligerus* biomass (17). However, during the application of eluted biomasss in subsequent reuse cycles, certain deterioration of results was recorded for metal removal and, particularly, for biomass flotabilities. During this study the elution of metals from biomass following biosorption and flotation was carried out by using 100 mL of a mixture of sodium sulfate (1.0 M) and tri-sodium citrate (0.1 M), as defined during preliminary experiments. Elution was performed on the filter cake, formed by vacuum filtration of collected foamates. The effect of eluant mixture on behavior of biosorptive flotation was also examined. The effectiveness of eluted biosorbent was examined in subsequent biosorptive flotation cycles.

RESULTS AND DISCUSSION

Experiments with Yeast-1 Sample

The remediation of heavy metal ions from a variety of wastewaters is focused nowadays on the aid of microbes. The contact process between the sorbents and the solution to be treated are typically performed in continuous-stirred tank reactor (CSTR) equipment or in columns (packed or fluidized bed). Nevertheless, both operational modes have certain disadvantages. In CSTR operation, the subsequent solid/liquid separation (usually performed by coagulation, settling, and/or filtration processes) can be problematic and time consuming, particularly in the fine or ultrafine particle-size range. Moreover, settling is generally a slow process when dealing, for example, with separation of low density (such as biological) materials, while filtration may also face blocking problems of filters, especially with material of ultrafine size (18). In column operation, high-pressure loss can be usually encountered. Therefore, the application of flotation was examined as a solid/liquid separation process.

The biological uptake of metals is occurring by two mechanisms or a combination of them: 1) an active, called bioaccumulation, and 2) a passive, called biosorption. The first one is taking place by viable cells, with the characteristic of intracellular metal accumulation, and it is mainly conducted by ion channels/pumps and endocytosis. The second one is taking place by nonviable cells with the characteristic of metal ions binding onto the surface by several mechanisms, usually acting simultaneously, such as surface complexation, coordination, ion exchange, adsorption, and microprecipitation (19,20).

The use of viable microorganisms has reported to increase, in some cases, the metal ion uptake toward nonviable microorganisms. Even though, dead microbes have some obvious advantages over living ones, such as 1) no need for nutrients, 2) ability to be stored as simple reagents, 3) lack of toxic effects from

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the effluents, and 4) greater ability for desorption of metal ions under extreme conditions (19). Therefore, the application of nonliving yeast biomass was examined for the removal of metals mixture.

The sample of yeast-1 was applied initially without the presence of dodecylamine surfactant. The removal of metal ions by biosorptive flotation was found to be appreciable depending on initial pH values of biomass suspensions, as shown for the three examined metals in Fig. 1. Copper was first removed from the acidic pH value of 5.5, due to its aqueous speciation and partial precipitation as hydroxide (21). Zinc and nickel were more efficiently separated at pH values higher than 8.0. Taking into consideration the different initial concentration of metals in feed solution, the percentage of removal is perhaps misleading. Following biosorption, rather good (over 90%) floatabilities of metal-loaded biomass were obtained at pH range 7.0–8.0. The biosorption of nickel (without the application of flotation) was found elsewhere to be low by a similar biomass; it was also observed during desorption tests that the process was mainly irreversible (20). Furthermore, it was concluded that it is necessary to control carefully the pH of contact solutions. In another case, uranium biosorption by *S. cerevisiae* was found to be dependent both on metal concentration in the initial solution and also on the pH value of suspension (11).

The previous experiments were repeated by adding an appropriate amount of cationic surfactant, acting as flotation collector (DA 3×10^{-4} M), as shown in

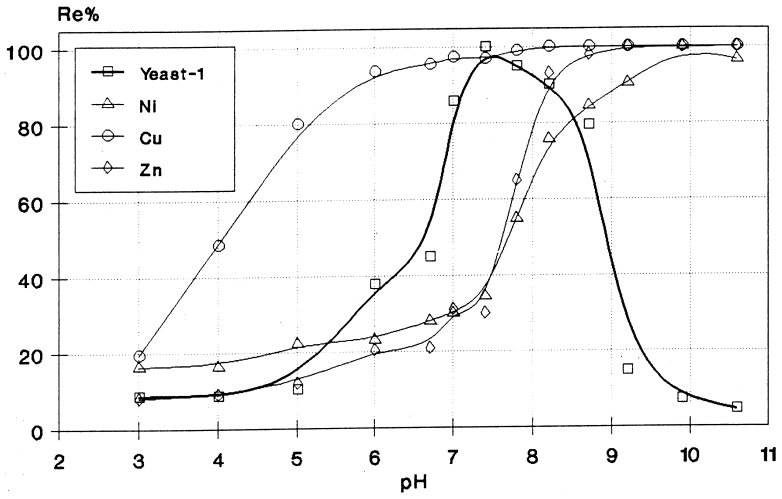

Figure 1. Effect of solution pH on the removal of metals (copper, zinc, and nickel) during biosorption and collectorless flotation (both expressed as Re%), with unmodified yeast-1 biomass(1 g/L, concentration).

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Fig. 2. In this case, yeast flotabilities were increased (up to 100%) at acidic pH values, but they were observed to decrease at pH values higher than 9.0. The comparison between Figs. 1 and 2 showed a different flotation behavior for acidic pH environments, due to presence (or not) of the surfactant.

Electrokinetic measurements, of unmodified biomass were conducted in parallel for different conditions, against the initial pH values of biomass suspension; they are shown in Fig. 3. Plain biomass samples present a point of zero charge at pH value less than 3.0 (the limit of this study) and negative zeta-potentials for the examined pH range (3.0–11.5). The presence of metals mixture and/or surfactant (3×10^{-4} M) was found to increase these values toward less negative ones. The influence of cationic polyelectrolyte (Zetag 10 mg/L) was noticed to increase further the zeta-potentials towards zero values at pH value over 8.0, whereas for lower pH values and in the acidic region a charge reversal toward positive zeta-potentials was observed. A study of zeta-potential for actinomycetes biomass has been previously reported with comparable observations (7). Specific microorganisms, such as *S. cerevisiae,* when killed by heat drying and treated by grinding, were reported to increase their ability to accumulate a range of metal cations; chemical modification might also change the specificity of biosorption (20). For this reason, waste yeast prepared as granular biosorbent was treated with hot alkali, but it was found incapable of accumulating an alkaline-earth metal (calcium) or an oxyanion (chromium). Therefore, such modifications are not considered always as economically attractive (9).

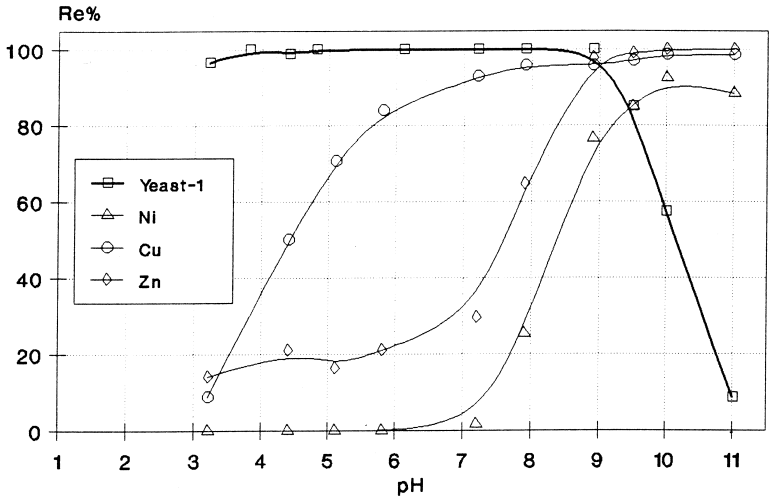

Figure 2. As in Fig. 1, but in the presence of 3×10^{-4} M dodecylamine surfactant (and 0.6% v/v ethanol).

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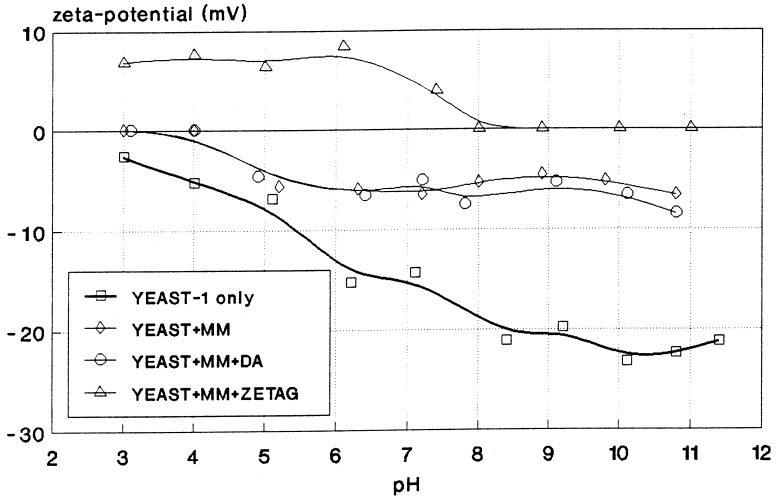

Figure 3. Electrokinetic measurements, expressed as zeta-potential, of (unmodified) yeast-1 biomass; influence of solution pH in presence of metals mixture (MM), cationic surfactant (DA), and/or polyelectrolyte (Zetag).

Experiments with Yeast-2 Sample

The second yeast sample was initially examined unmodified and without any surfactant present. Although it performs comparatively with the previously examined yeast-1 sample for the removal of metals, regarding the recovery of biomass by flotation, quite mediocre results were found, being around 70% at pH range 7.0–8.5 (Fig. 4). The comparison between the two *Saccharomyces* yeast batches in absence of flotation collector showed that the sample of yeast-1 presents higher floatabilities. Selected experiments were repeated with the simultaneous addition of polyelectrolyte, which was introduced in biomass suspension during the biosorption stage, causing flocculation of biomass. Collectorless floatabilities were further improved, reaching almost 100% at pH values over 9.0 (Fig. 5).

The presence of surfactant (3×10^{-4} M DA) caused an increase, as expected, of biomass recovery by flotation up to maximum removal (nearly 100%) at pH values around 7.0–9.0, followed by significant decrease in higher alkaline pH environments (pH values higher than 10.0), as well as in acidic pH range (pH values lower than 7.0), as shown in Fig. 6. This behavior is very different from that found for the yeast-1 sample, where in acidic pH values almost quantitative biomass separation by flotation was obtained. However, it has to be noted that at the acidic pH range, biosorption takes place in small extent; there-

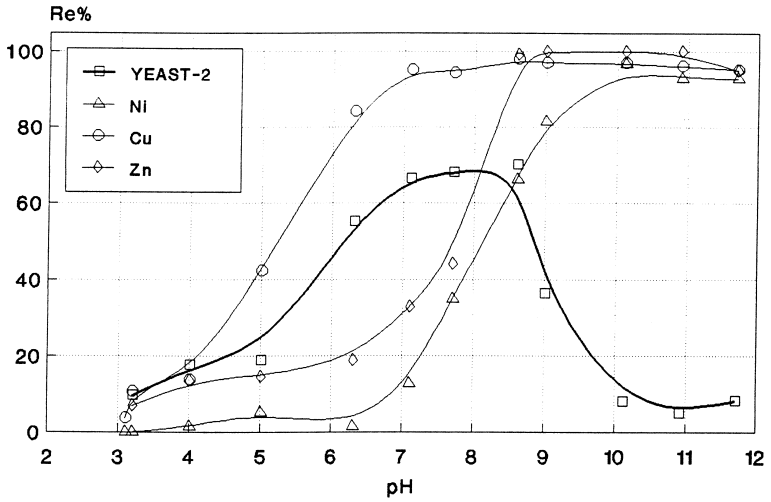

Figure 4. As in Fig. 1, but using yeast-2 biomass, unmodified.

fore, this pH region is not presenting any practical interest, for removal of metals.

The need for surfactant presence in order to improve flotation is shown illustratively in Fig. 7, where a comparison between the floatabilities of two metals-laden biomass types was presented.

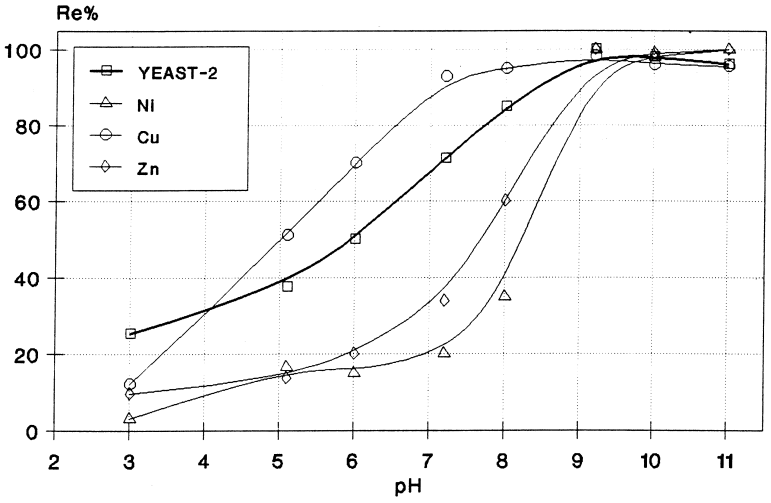

Figure 5. As in Fig. 4, but with the preliminary addition of polyelectrolyte (10 mg/L Zetag).

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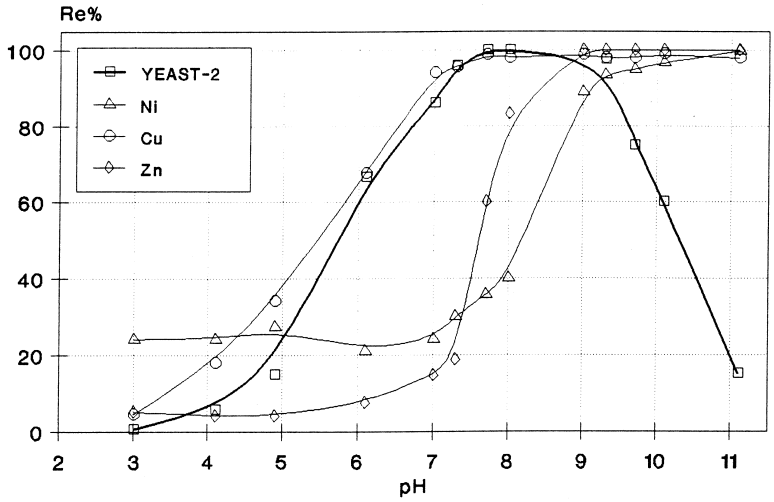

Figure 6. As in Fig. 4, but with the presence of flotation collector (DA).

Figure 8 presents the influence of yeast concentration on biosorptive flotation from 0.5 to 6 g/L at pH 7.0, whereas the remaining experimental conditions were kept constant. The removals of zinc and nickel were increased with the biomass concentration, whereas the addition of constant DA concentration (3×10^{-4} M) caused a decrease of yeast recovery by flotation to values less than 50%,

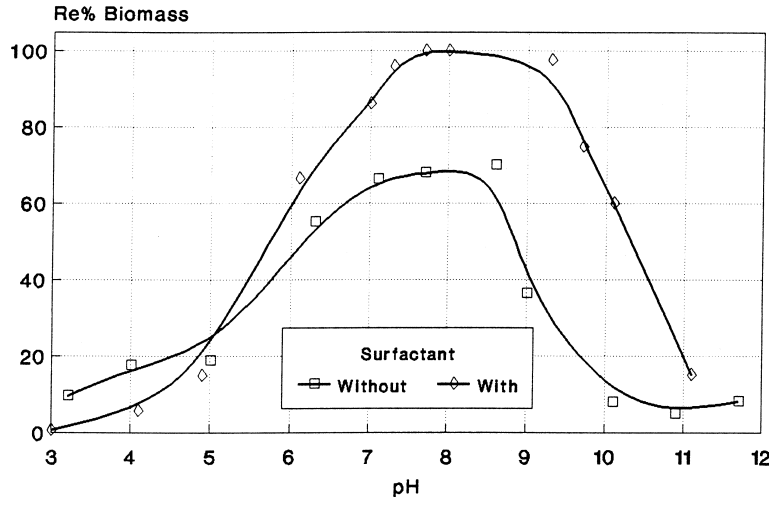

Figure 7. Comparison of flotation collector presence, regarding yeast-2 biomass floatability.

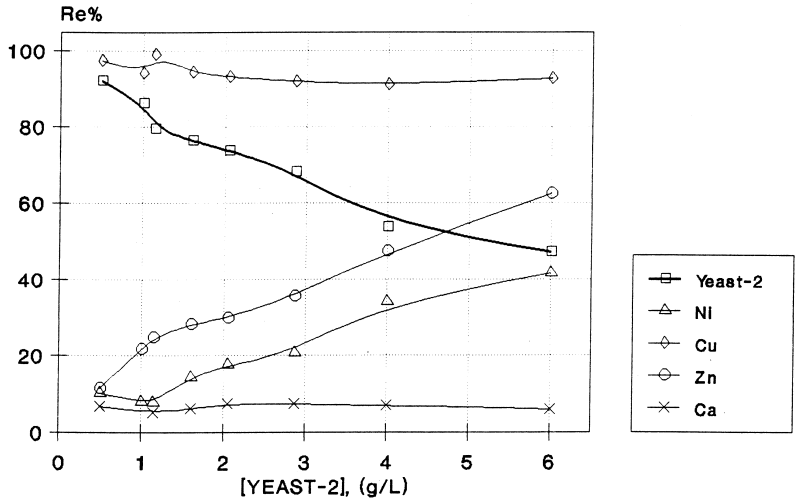

Figure 8. Influence of yeast-2 biomass concentration at pH 7.0.

indicating this surfactant concentration was not enough to effectively float higher biomass concentrations. Therefore, by using 6 g/L biomass concentration, the removal of metals can be appreciably improved at pH 7.0 (more than 60% of zinc and 40% of nickel); a slight decrease of copper removal was noticed, perhaps accompanying that of biomass floatability decrease. Calcium was also analyzed, and its removal was found to be less than 10%, although calcium acts antagonistically with the other metal cations toward the same biomass sorbing sites.

In yeast biomass, the cell wall is a multilaminate, microfibrillar structure consisting of up to 90% polysaccharide, with mannan and glucan being the main structural polymer in *S. cerevisiae.* Other important components include proteins, lipids, and pigments, and this diversity is reflected by the presence of a range of distinct potential metal complexation sites, such as carboxylate, phosphate, sulfydryl, and amine groups (14).

Experiments were also carried out to examine, among others, any possible influence of eluant on removal of metals and on biomass floatability. Figure 9 presents the pH influence on modified the yeast-2 sample in the presence of DA. Maximum flotation recovery (over 90%) was obtained for a pH range of 8.0–9.0. Copper was again found to remove first from the pH of 6.0, showing possibly a certain selectivity in comparison with the other toxic metals. It was argued that most cell wall components were responsible for some copper accumulation (9). Nevertheless, in another comparison of metal sequestering capability for several biomass strains, including *S. cerevisiae*, no clear selectivity was found between the removed metal ions (22).

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The effect of surfactant addition was subsequently studied for the neutral pH of 7.0 (Fig. 10); the addition of 5×10^{-4} M DA caused slightly higher flotation recoveries (over 90%).

Improving the Integrating Process

Sorption is exclusively responsible for metal accumulation by nonviable biomass (10). Yeast cell walls appear to be the major cell component responsible for metal binding, particularly for aqueous solutions containing low metal concentrations. The most efficient metal binding occurs when the yeast cell wall is intact (9). Therefore, during desorption it is imperative that minimal damage occur to the cell walls of biomass, if the latter is going to be reused in subsequent cycles. The sorbed metal has to be eluted from the biomass under the application of mild conditions after each run. Immobilized *S. cerevisiae* was previously reported in the literature to be tested in successive adsorption-desorption cycles (up to 8). A binding equilibrium of 70% for Cu was obtained in batch reactors within 20 min of contact, and the bound copper was subsequently desorbed by elution with hydrochloric acid (23). The examination of multiple cycles (five or six) operation at pH 7.0 was performed, although it has to be stressed that pH 7.0 was not the optimum value found for efficient flotation of biomass. Nevertheless, the pH value of 7.0 was selected in order to avoid a final pH regulation of treated effluent. Each cycle included biosorption, flotation,

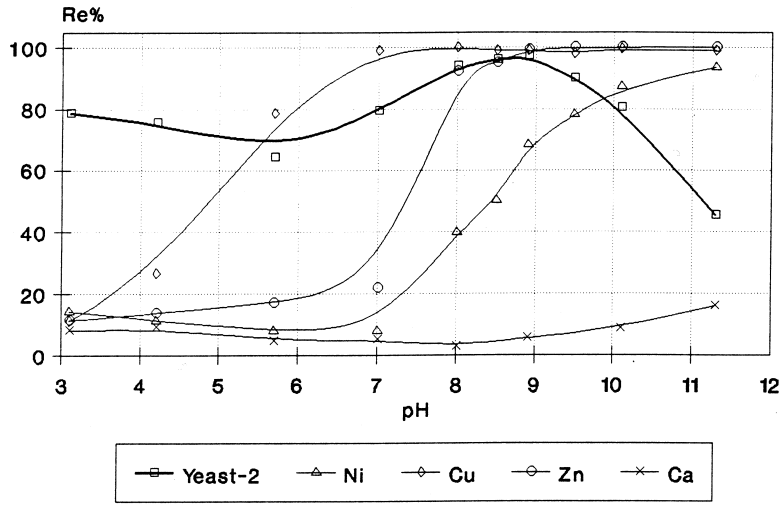

Figure 9. Yeast-2 sample: modified (pretreated) biomass in presence of surfactant.

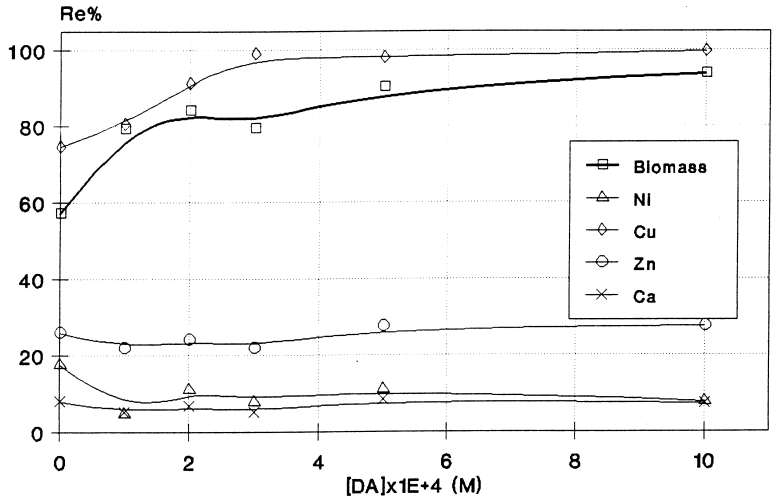

Figure 10. Effect of dodecylamine concentration on the biosorptive flotation process at pH 7.0 and 1 g/L of modified biomass, concentration (yeast-2) sample; the behavior of calcium was also analyzed.

and elution of yeast biomass (1 g/L feed). The metals were obtained from the later stage as a concentrated (around 10 times) solution, suitable for recovery of them, by applying for example electrolysis (24).

The addition of surfactant (at least) in every other cycle was found necessary in order to obtain sufficient solid/liquid separation by flotation, as presented in Figs. 11 and 12. The removal of metals by biosorption was rather unaffected. Therefore, this biomass could be reused in a consecutive number of operational cycles.

Biological materials, such as the studied biosorbents, are usually of relatively complex nature. Therefore, the creation/design of new synthetic materials similar to them, applied for the removal of metal ions from dilute aqueous solutions (as most of wastewaters can be considered) can be approached mainly as *modification* attempts of natural biosorbents in order to enhance the sorption/removal of toxic metals. The integrated synthesis route for new materials similar to biosorbents is not considered an economically viable procedure, such as in the case of synthetic versus natural (inorganic) zeolites.

Biosorbents prepared from biomass of limited reuse value (by-products), as in the present case of the studied yeast biomass, are expected to be much cheaper than similar synthetic materials; therefore, they may represent interesting alternative sorbents. The modification of biosorbents, usually performed by the application of chemical reagents, is an interesting issue, which is currently under examination.

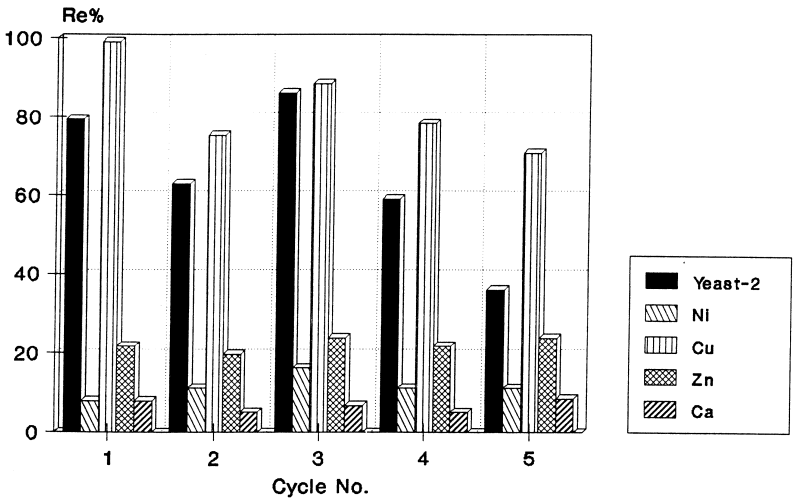

Figure 11. Repeated five cycles operation of biosorption/flotation/elution using modified yeast-2 biomass (1 g/L); DA was added only during the first and third cycle.

In conclusion, satisfactory results have been found regarding the removal of toxic metal cations from an aqueous mixture, simulating an industrial wastewater, by an interesting process combining biosorption and flotation and using as biosorbent a suspension of *Saccharomyces* yeast biomass. In an environmental biotech-

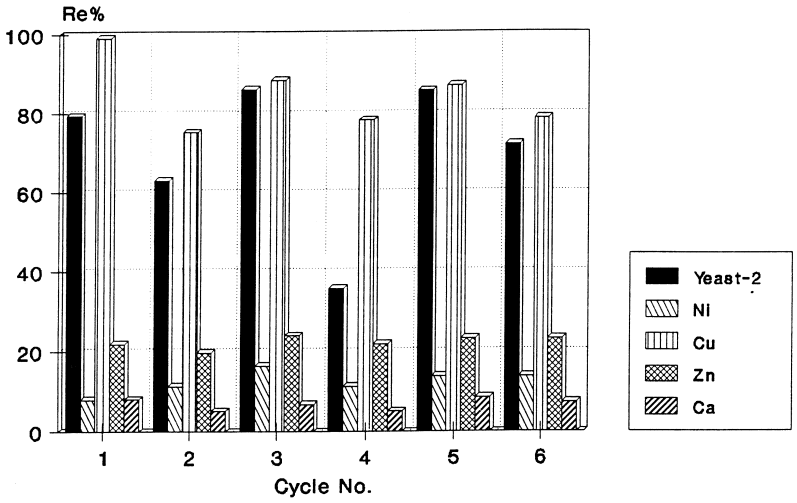

Figure 12. Repeated six cycles of operation; DA addition during the first, third, and fifth cycles.

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nology context, waste yeasts have been useful sorbents for the remediation of metal-containing liquid effluents.

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