Journal of Industrial Microbiology (1995) 14, 240–246
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Considerations on the use of commercially available yeast biomass for the treatment of metal-containing effluents

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(Received 4 April 1994; accepted 18 August 1994)

Key words: Yeast; Copper; Silver; Ion selective electrodes; Metal complexation; Metal uptake

SUMMARY

Three strains of *Saccharomyces cerevisiae* and one strain of a *Candida* sp. obtained from different industrial sources were screened for uptake of silver and copper. Considerable differences in metal uptake capacities were found between the different strains of *S. cerevisiae* and between *S. cerevisiae* and the *Candida* sp. used. Copper uptake capacities ranged from 0.05 mmol g^{-1} dry wt to 0.184 mmol g^{-1} dry wt while values of 0.034 mmol Ag g^{-1} dry wt and 0.193 mmol Ag g^{-1} dry wt biomass were observed. Use of ion-selective electrodes (ISEs) enabled the detection of copper complexing agents (possibly proteins and carbohydrates) released by yeasts into the surrounding medium. In contrast, these compounds had no silver complexation abilities. Langmuir and Scatchard transformations of metal adsorption isotherms suggested differences in the mechanisms involved in metal uptake by the various yeasts. The differences between strains of *S. cerevisiae* were due possibly to differences in cell wall composition. Different methods of preparation of biomass (fresh, air, oven and freeze-dried) had little effect on metal uptake in comparison with fresh biomass. Storage of fresh waste biomass at 4 °C for 20 days had no effect on metal biosorption capacities. It was also observed that individual batches of waste biomass produced from different fermentation runs had consistent metal uptake capacities. The implications of the above results on the use of waste yeast biomass for treatment of metal-containing effluents are discussed.

INTRODUCTION

The potential for using microorganisms in the treatment of metal-containing liquid effluents has been studied intensively and many organisms including bacteria, fungi and algae have been found to remove metals from solution [8]. The mechanisms of metal uptake have also been examined and appear to vary depending on the organism and metal in question [4,23,25]. Mechanisms involved may be metabolism-dependent or independent [11] and both living and dead (denatured) biomass is capable of metal uptake [3].

Despite this attention, there are few microbially based metal removal processes in commercial use and it is clear that microbial treatment systems must compete economically and perform as well as the metal treatment systems already in place. To reduce the cost of producing microorganisms specifically for metal treatment processes the use of biomass from industry has been proposed [9,17] and clearly yeast biomass would be a good candidate for study. Numerous studies have investigated the nature and extent of yeast/metal interactions and have shown the potential of yeast for metal recovery/removal [7,10,19] but, to date, limited work has been carried out on actual industrial strains for biotechnological purposes. Industrially produced yeasts are likely to differ greatly in their metal biosorption capacities as the production methods and strains used vary widely. For example, the medium in which organisms are grown can alter metal uptake capacity [24] and recently Avery and Tobin [3] demonstrated that a laboratory strain of *Saccharomyces cerevisiae* removed less strontium from solution than a strain used in the brewing industry. Therefore, the aim of this work was to determine the metal biosorption capacities of commercially produced yeast biomass obtained from different sources and to examine some of the basic factors that should be considered for future commercial use of biomass in metal treatment operations.

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MATERIALS AND METHODS

Cultures used

Yeasts 1, 2 and 3 were identified by the supplying breweries as *Saccharomyces cerevisiae*, while yeast 4 was identified by the supplying industry as a *Candida* sp.

Washing and drying of yeast

Yeast biomass was obtained directly from the industrial sources and immediately centrifuged (4900 × *g* for 10 min) in an RC2-B Sorvall centrifuge (Du Pont, Delaware, USA) at 4 °C. The pellet (20 g fresh wt) was resuspended in 1 L deionized distilled water (4 °C) and centrifuged again as before. The supernatant was removed and the pellet washed twice more. Biomass thus obtained was either left fresh, or placed in a freezer at −50 °C overnight before freeze-drying using an LSL Secfroid freeze-drier (LSL Secfroid SA, Aclens, Switzerland). Yeast 1 was also dried by oven drying at 55 °C or air drying at room temperature.

Treatment of dried biomass

All dried biomass was ground with a mortar and pestle and passed through a sieve of 300-μm aperture diameter. Scanning electron microscopy (Fig. 1) and light microscopy indicated that, for each drying treatment, cells were still intact after grinding.

Metal adsorption isotherms

Solutions containing from 0.1 mM to 3.0 mM of copper or silver were prepared from deionized distilled water and the nitrate salts of the appropriate metal (BDH Chemicals, Poole, UK). The metal solutions were adjusted to pH 4 using 0.5 M HNO_3 or NaOH. This pH value was chosen to avoid precipitation of metals, in particular Cu^{2+}[15], which would interfere with biosorption assays. Aliquots (25.0 ml) of each metal solution were pipetted into 250-ml Erlenmeyer flasks. Biomass (50 mg dry wt) was added to each flask and the flasks incubated on an orbital shaker table at 200 r.p.m. and 27 °C for 1 h. After incubation the biomass was centrifuged at 17 300 × *g* for 10 min in an RC2-B Sorvall centrifuge. The supernatant was removed and diluted with deionized distilled water. Metal analysis was carried out using a Varian AA-475 Atomic Absorption Spectrophotometer (Varian Techtron, Springvale, Australia). Metal accumulation by the biomass was determined by the difference in metal content between flasks containing no biomass (control) and flasks containing biomass (test). This centrifugation method of determination of metal accumulation was compared with another method in which the metal content of the biomass was measured directly by digestion using a modified method described in procedure B of the Analytical Methods Committee [1]. The pellet obtained from the centrifugation of the biomass/metal solution was resuspended in 50 ml of deionized distilled water and centrifuged as before. The supernatant was discarded and the biomass washed twice more. In a thick walled test tube, 2 ml of concentrated H_2SO_4 (BDH Chemicals) was added to the biomass (50 mg dry wt). The test tube was heated gently and

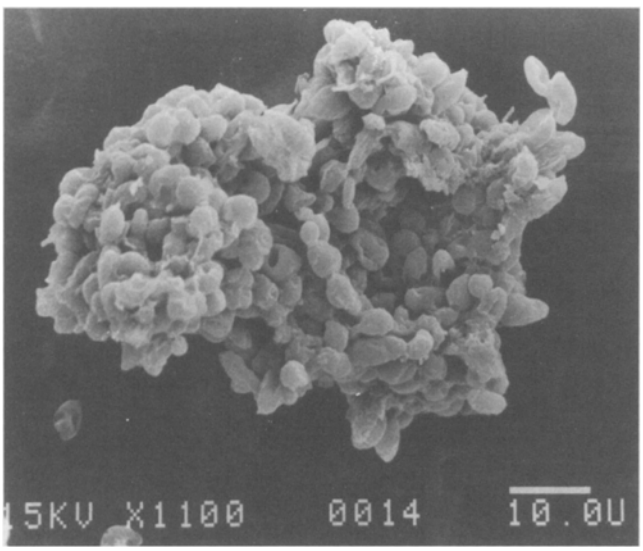

Fig. 1. Scanning electron micrograph of freeze-dried yeast 1 showing clumping of cells. Bar = 10 μm.

5 ml H_2O_2 (30% w/v, BDH Chemicals) added dropwise. The resultant solution was a clear liquid containing digested biomass. After appropriate dilution with deionized distilled water the metal content of the digest was determined.

A separate experiment was carried out to determine the effect of biomass particle size on metal uptake. Freeze-dried biomass (yeasts 1 and 4) was incubated as above in deionized distilled water (adjusted to pH 4 using 0.5 M HNO_3 or NaOH) for 1 h, after which time half the flasks containing each type of biomass were sonicated at lowest power for 30 s using an Insonator model 500 sonicator (Ultrasonic Systems Inc., New York, USA). Light and electron microscopy confirmed that the sonication treatment produced a suspension of single cells whereas biomass not subjected to sonication remained clumped (Fig. 1). Silver or copper was added to all flasks to a final concentration of 0.5 mM. Flasks were incubated for another hour before biomass removal and metal supernatant determination. All experiments were performed in duplicate.

Release of metal complexing agents

Freeze-dried biomass was incubated as above in either deionized distilled water or copper solution (1.0 mM), at pH 4. The biomass was centrifuged and the supernatant filtered through 0.45-μm low protein binding filters (Gelman Sciences, Ann Arbor, MI, USA). Aliquots of a standard copper solution were added sequentially to the filtrate until a final concentration of 3.0 mM was reached. After each addition the amount of copper in solution was determined using the copper ion selective electrode (Orion Scientific Instruments, Boston, USA), conditioned according to the manufacturer's instructions, and a graph plotted of copper added (μM) versus copper detected (μM). Controls consisted of deionized distilled water or copper solution (1.0 mM Cu) to which known amounts of metal were added to a final concentration of 3.0 mM. The filters used were found not to bind detectable amounts of copper and all experiments were performed in duplicate.

Protein and total carbohydrate determination

Biomass was incubated as above in deionized distilled water supplemented with either 0, 1.0 mM or 3.0 mM copper or silver (pH 4). The cells were then filtered through a 0.45-μm low protein binding filter and protein measured in the filtrate by the Folin–Ciocalteau method [13]. Carbohydrate in the same filtrate was measured by the phenol/sulphuric acid method [13]. All assays were performed in duplicate.

Scanning electron microscopy

Yeast 1 biomass which had undergone different types of drying was mounted on separate aluminium stubs and coated with 20–30 nm of gold using a Polaron SEM Coating Unit E5100 (Polaron Equipment Ltd, Watford, UK). The stubs were examined using a Jeol 35-C scanning electron microscope (Jeol Ltd, Tokyo, Japan) at 15 kV and ×1100 magnification.

RESULTS

Adsorption isotherms

For estimation of the amount of metal taken up by the biomass the two methods used (sulphuric acid/hydrogen per-

oxide digestion and centrifugation) were found to give similar results, and so the easier method (centrifugation) was used in all subsequent experiments.

Results were initially plotted as metal adsorption isotherms. For most biomass types metal uptake (q_e) increased with metal concentration until a saturation level was reached (Fig. 2(A,B)). A different uptake pattern was observed with copper for yeast 1, where a further increase in metal adsorption occurred at higher metal concentrations. This effect was observed previously with copper uptake by *Cladosporium resinae* and *Penicillium italicum* and reflects an 'unfavourable adsorption' process [6]. One hour was the chosen time period for incubation as previous experiments showed that equilibrium was reached well within this time (results not shown). Maximum and minimum silver uptake levels were

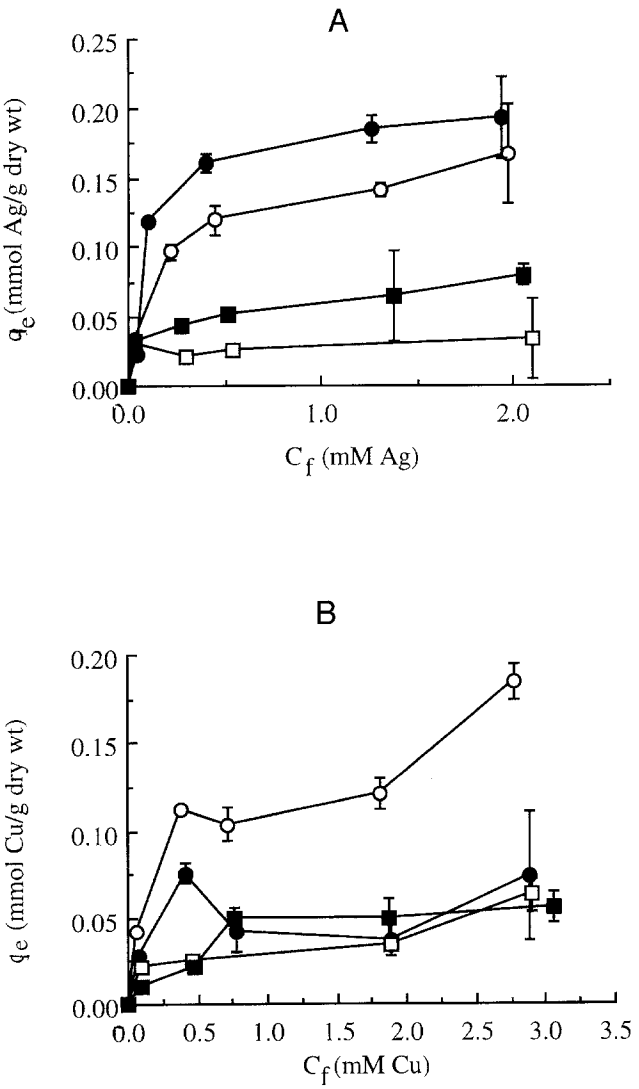

Fig. 2. Silver (A) and copper (B) adsorption isotherms for four different types of freeze-dried industrially produced yeast biomass. Yeast 1 (○), yeast 2 (•), yeast 3 (□), yeast 4 (■). C_f represents the final concentration of metal left in solution after the biosorption process reached equilibrium. The amount of metal taken up by the biomass (mmol g^{-1} dry wt) is represented by q_e. Mean values ± standard errors of the mean of duplicate flasks are shown.

0.193 mmol g^{-1} dry wt (yeast 2) and 0.034 mmol g^{-1} dry wt (yeast 3) respectively, while copper uptake varied from 0.185 mmol g^{-1} dry wt (yeast 1) to 0.056 mmol g^{-1} dry wt (yeast 4). Reciprocal Langmuir plots of silver uptake, using data transformed from adsorption isotherms in Fig. 2, revealed differences in uptake characteristics between the different yeasts (Fig. 3). Yeasts 1 and 3 gave a linear relationship between reciprocal values for q_e and C_f, while a nonlinear relationship was observed for the other yeasts. Scatchard transformations were all nonlinear, although silver uptake by yeast 1 showed only slight deviations from linearity (Fig. 4(A,B)). For copper uptake all Langmuir and Scatchard transformations were nonlinear except yeast 1, which conformed well to the Langmuir model (results not shown).

Release of metal binding components by yeast

After incubation of the different yeasts in deionized distilled water in the absence or presence of 1.0 mM copper, more copper was added in known quantities to the filtrate and the amount of metal present determined using the copper ISE. The results for yeast 2, when compared to the relevant control, showed that not all of the copper added to the filtrate was detected by the ISE. Amounts of metal detected by the ISE were 83 per cent of the total added after incubation of biomass in distilled water alone (Fig. 5(A)) and 61 per cent of that added after incubation of biomass in 1.0 mM Cu (Fig. 5(B)). Pre-incubation of the other yeasts in solutions containing 1.0 mM of copper also resulted in subsequent interference of metal analysis by ISE (Fig. 5(B)).

Clearly, compounds released by the yeasts during incubation for 1 h were interfering with the ISE method and the effect was increased by pre-incubation with high metal concentrations (1.0 mM Cu). This is significant as ISEs will only measure the amount of free (uncomplexed) metal in solution and complexed metals are not detected [5]. An identical procedure performed for silver demonstrated that the released compounds had no silver complexation abilities as no interference of silver analysis using the Ag ISE was found (results not shown).

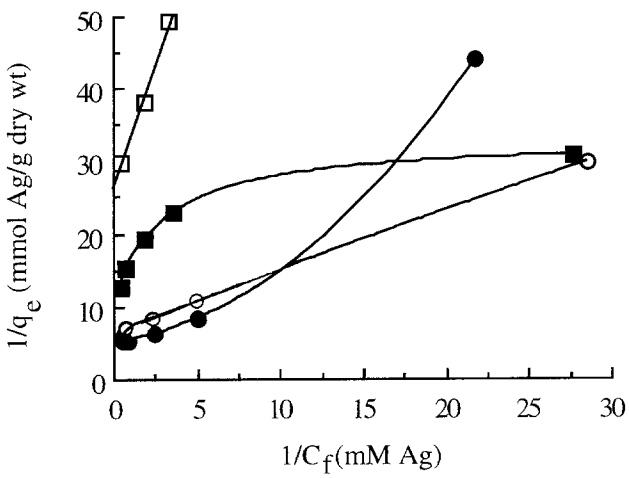

Fig. 3. Langmuir transformations of silver adsorption isotherms from Fig. 2(A). Yeast 1 (○), yeast 2 (•), yeast 3 (□), yeast 4 (■).

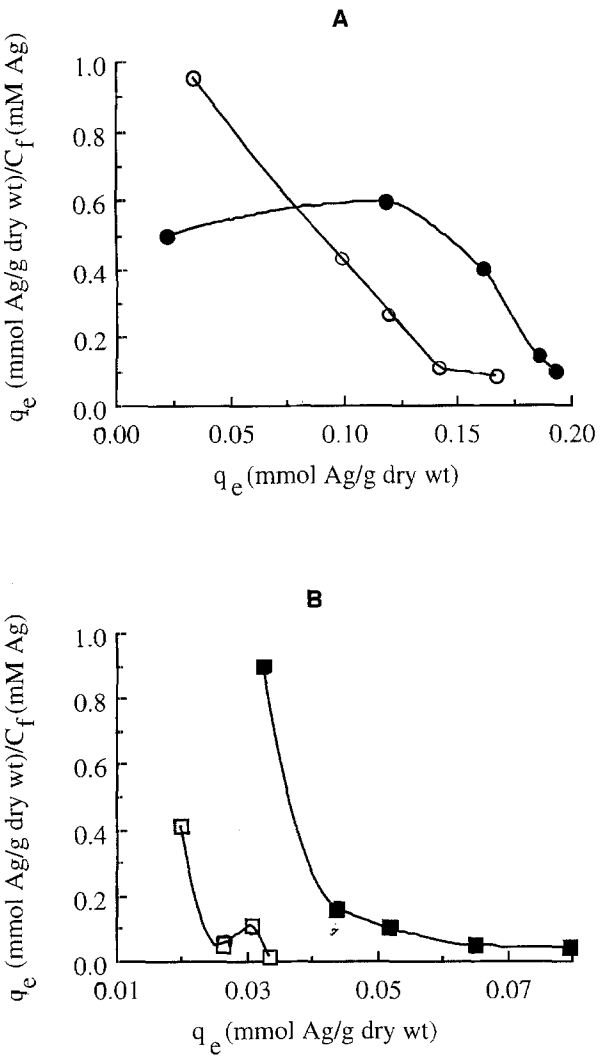

Fig. 4. Scatchard plots of silver adsorption isotherms from Fig. 2(A). (A) Yeast 1 (○), yeast 2 (•). (B) Yeast 3 (□), yeast 4 (■).

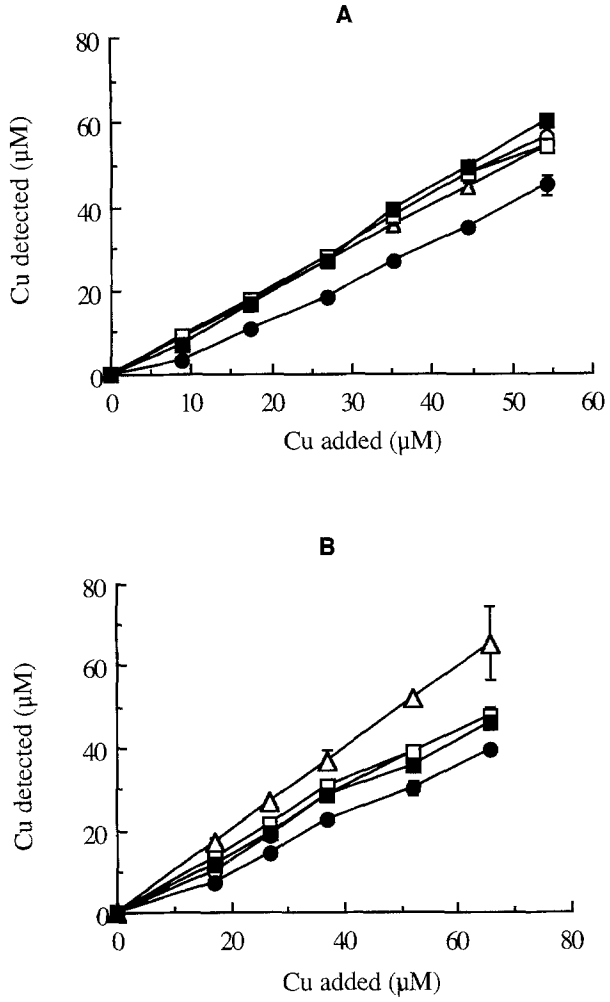

Fig. 5. Copper detection by ion-selective electrode. Freeze-dried biomass was incubated in deionized distilled water in either the absence (A) or presence (B) of 1.0 mM Cu and removed by centrifugation. The supernatant was filtered and known amounts of copper added. Copper in solution was measured by use of an ion selective electrode after each addition of metal. Yeast 1 (○), yeast 2 (•), yeast 3 (□), yeast 4 (■). Addition of copper solution to deionized distilled water (control) is represented by (△). Mean values ± standard errors of the means from duplicate assays are shown.

Protein and total carbohydrate analyses performed on the yeast supernatants revealed that significant amounts of these compounds were released by most types of biomass, the extent of release depending on experimental conditions used (Tables 1 and 2). After pre-incubation in water, yeast 2 released large amounts of protein (12.1 mg per 50 mg dry wt biomass). Incubation of the same yeast in 1.0 mM or 3.0 mM copper solutions caused a decrease in protein release. Smaller amounts of protein were found in supernatants of the other yeast strains after incubation in water (1.05–4.2 mg protein per 50 mg dry wt biomass). When the copper concentration was increased from 1.0 mM to 3.0 mM an increase in protein release occurred for these yeasts. Protein determination in silver-containing supernatants was difficult to estimate due to interference with the assay by silver.

Carbohydrates were found in most yeast supernatants after incubation in water alone. Amounts released ranged from 0.08 mg per 50 mg dry wt biomass (yeast 3) to 0.75 mg per 50 mg dry wt biomass (yeast 1) while no carbohydrate release could be detected from yeast 4. The effect of metals on carbohydrate loss from yeast varied depending on the metal and

TABLE 1

Release of protein (mg protein per 50 mg biomass dry wt) by yeast biomass after incubation in deionized distilled water, 1.0 mM Cu or 3.0 mM Cu. Means ± standard errors from duplicate flasks are shown

	H_2O	Cu (1.0 mM)	Cu (3.0 mM)
Yeast 1	4.20 ± 0.00	4.50 ± 0.40	5.70 ± 0.40
Yeast 2	12.10 ± 0.20	5.60 ± 0.30	9.20 ± 0.00
Yeast 3	1.20 ± 0.20	2.30 ± 0.30	3.90 ± 0.50
Yeast 4	1.05 ± 0.00	0.90 ± 0.20	2.30 ± 0.30

TABLE 2

Release of carbohydrates (mg carbohydrate per 50 mg biomass dry wt) by yeast biomass after incubation in deionized distilled water, 1.0 mM Cu, 3.0 mM Cu, 1.0 mM Ag or 3.0 mM Ag. Means ± standard errors from duplicate flasks are shown

	H_2O	Cu 1.0 mM	Cu 3.0 mM	Ag 1.0 mM	Ag 3.0 mM
Yeast 1	0.75 ± 0.02	0.65 ± 0.03	0.70 ± 0.10	0.64 ± 0.03	0.62 ± 0.07
Yeast 2	0.11 ± 0.00	7.24 ± 0.09	6.65 ± 0.83	5.64 ± 0.91	5.71 ± 0.37
Yeast 3	0.08 ± 0.04	4.31 ± 0.10	3.58 ± 0.44	4.59 ± 0.00	4.24 ± 0.00
Yeast 4	0	0.67 ± 0.13	0.36 ± 0.10	0	0.37 ± 0.05

biomass type. For yeasts 2, 3 and 4 an increase in carbohydrate release occurred after incubation with copper (1.0 mM and 3.0 mM), whereas carbohydrate release from yeast 1 did not change with incubation in either metal. Incubation with silver (1.0 mM) resulted in increased carbohydrate loss from yeasts 2 and 3 but had no effect on yeasts 1 and 4. On increasing the silver concentration from 1.0 mM to 3.0 mM yeast 4 released carbohydrate, but no further increase in carbohydrate loss at the higher Ag concentration was observed for the other organisms.

The scanning electron micrograph of freeze-dried yeast cells shows that the yeast cell wall is damaged and as a result cell components could be released either from the wall or from the cell interior (Fig. 1).

There was no significant difference in Ag uptake between yeast 1 and yeast 2 after incubation in 2.10 mM Ag solution (Fig. 2(A)). Yeast 1, however, displayed the best Cu uptake (Fig. 2(B)) and was waste biomass produced by industry while yeast 2 was bought by the same industry as a starter culture for the process. Therefore it was decided to use yeast 1 in further investigations. The percentage metal removal by yeast 1 was determined for both Ag and Cu at 0.1 mM. This yeast showed extremely high removal for both Ag and Cu (99% and 89.3% respectively).

Yeast preparation

The silver and copper adsorption isotherms of fresh, freeze-dried, oven-dried and air-dried yeast 1 were compared. No significant differences were found in metal uptake between any of the different preparations (results not shown). All the preparations showed an increase in uptake with increasing metal concentration. As the particles of dried biomass had a diameter of 0.3 mm or less, samples of freeze-dried biomass types 1 and 4 were sonicated to produce single cells. These suspensions were then incubated in 0.5 mM Ag or Cu to see if the increase in surface area between sonicated and non-sonicated cells could increase metal uptake. No significant increase in metal biosorption was obtained by sonication (results not shown) although differences in metal uptake were observed between both types of biomass and between both metals. Storage of fresh yeast 1 biomass at 4 °C for 20 days did not change the silver uptake capacity of the yeast (results not shown). Freeze-dried biomass from six different fermenter runs, were found to have constant silver and copper uptake capacities (results not shown).

DISCUSSION

Yeasts 1, 2 and 3 were strains of *S. cerevisiae* and the results demonstrate that the different strains have widely varying metal biosorption capacities. The flocculation characteristics of yeasts are important in the choice of strain used in fermentation processes and the surface properties of the organism are involved in determining these characteristics. In particular, lectins (surface proteins) are involved in yeast flocculation [22]. It is likely that these different cell surface properties influence the nature of interaction between yeast and metal obtained and explain, in some part, the different metal uptake capacities of the strains. The different cell wall composition of *Candida* spp. compared to *Saccharomyces* spp. [21] may explain the lower metal uptake observed for yeast 4.

Other factors that could influence the metal uptake capacities of the yeasts examined include culture age [27] and growth medium composition [24,26]. The different treatments employed on completion of an industrial fermentation, for example autoclaving, must also be considered as these may cause alterations in the cell wall which would subsequently affect metal uptake.

The Langmuir and Scatchard plots give indications of the metal binding processes involved. Silver and copper binding by freeze-dried yeasts 1 and 3 conformed to the Langmuir adsorption isotherm suggesting a simple non-interactive monolayer binding to the cell surface. However, the Langmuir model was not obeyed by the other yeasts examined, for either copper or silver uptake, indicating a more complex multilayer adsorption. All Scatchard plots were curved to some degree suggesting multiple binding sites were involved in the process. These results are consistent with results found for other types of biomass [23] which indicate that the cell surface is a complex, heterogeneous matrix containing an array of possible different metal binding sites. More recently, prediction of these interactions in *S. cerevisiae* has been attempted using the hard and soft principle developed by Pearson [20] and further refined by Nieboer and Richardson [18]. Although complex, the experimentally derived interactions were found to be generally consistent with considerations of the hard and soft principle [4].

A further complication in analysing the biomass/metal interactions involved could be the release of metal binding agents into solution by organisms. Our results demonstrate that significant amounts of both protein and carbohydrates are pre-

sent in the yeast supernatant after incubation either in water or metal solutions. The amount of material released is dependent on the organism and type of incubation and the effect is variable. However, ISE analysis suggested that in all cases metal binding compounds were released into the surrounding medium. The phenol–sulphuric acid assay used detects a variety of carbohydrate materials including sugars and nucleic acids and all these compounds are known to either bind metals or contain possible metal binding sites [2,16]. Proteins are also well known for their metal complexation abilities [2,16]. Metal binding groups on these released materials will compete for metal with binding sites on the biomass and this competition is likely to obscure interpretation of results obtained. The release of large amounts of metal binding compounds may reduce the amounts of metal bound by the biomass. Such a relationship was evident for yeast 2, which displayed poor copper removal and good silver removal, while released materials appeared to have high affinities for copper but not for silver. No silver binding compounds were released by any of the biomass types used. In a previous study, protein release from *S. cerevisiae* appeared to be responsible for decreases in copper uptake ability [14]. Observations reported here support these findings but also suggest that other released materials could interfere with metal biosorption processes.

From the initial screening process, yeast 1 was chosen for further study as it had high metal binding capabilities and at present is a discarded fermentation end-product. Some basic studies were made on the feasibility of using this biomass as a metal biosorbent on the basis of ease of preparation and storage. Different studies on biosorption have used many different methods of biomass preparation including freeze-dried, fresh and oven-dried material [3,12]. Dried biomass is easier to granulate and store than fresh biomass and we were interested in the effect of different drying procedures on metal uptake. The results showed that drying had no significant effect on the biosorption process and that decreasing particle size from 300 μm (sieved diameter) to single cell diameter did not influence the final metal uptake. These results indicate that the biomas could be dried and therefore stored for long periods without decreasing metal removal efficiency. Also, if immediate drying of the biomass was not possible, the results demonstrate that storage of fresh biomass at 4 °C for 20 days would not decrease metal biosorption. The biomass could then be used either in dried or fresh form for effluent metal removal. Finally, the constant metal removal ability of different batches of yeast 1 is promising, in that once a suitable source of waste biomass is identified, its metal uptake characteristics are unlikely to vary, thus ensuring a continuous supply of metal biosorbent.

This work shows that it is necessary to screen different types of biomass from industry for determination of their potential use as biosorbents. Preliminary investigations indicate that not all biomass types have good biosorbent characteristics but that others are worthy of further investigation. Compounds (proteins and carbohydrates) released by yeasts may interfere with the analysis and interpretation of results and any interference should be allowed for when predicting metal/biomass interactions. The factors affecting and the mechanisms involved in metal uptake by waste yeast 1 are currently under investigation.

ACKNOWLEDGEMENTS

Paul Simmons gratefully acknowledges the provision of an Eolas postgraduate studentship. A large part of this work was funded by a U.C.D. Presidents Research Award. The authors would also like to thank David McFadden for supplying waste yeast biomass.

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