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A method to increase silver biosorption by an industrial strain of *Saccharomyces cerevisiae*

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Abstract Ag^+ biosorption by an industrial strain of *Saccharomyces cerevisiae* was investigated. Older (96 h old) biomass had half the biosorption capacity of younger (24 h old) biomass (0.187 and 0.387 mmol Ag^+/g dry mass respectively). Comparisons of cell walls isolated from biomass of either age indicated that chemical composition and Ag^+ biosorption capacity varied little over the time span examined and that cell walls from either age of culture had small Ag^+ biosorption capacities compared to whole cells of a similar age. Silver-containing precipitates were observed both on the cell wall and within the cell, indicating that intracellular components sorbed Ag^+. The concentration of these precipitates within the cell appeared visually to decrease with age in Ag^+-exposed cells. Incorporation of L-cysteine into the growth medium resulted in biomass with increased silver biosorption capacities, protein and sulphydryl group content. Increasing the concentration of L-cysteine in the growth medium from 0 to 5.0 mM increased silver biosorption from 0.389 to 0.556 mmol Ag^+/g dry mass Isolated cell walls of biomass grown in supplemented media also showed a possible link between silver biosorption capacities, protein and sulphydryl group content. No precipitates were observed in silver-exposed biomass that had been grown in the presence of 5.0 mM L-cysteine.

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Introduction

Microorganism/metal interactions have been reported widely in the literature and may be divided into energy-dependent (bioaccumulation) and energy-independent (biosorption) processes (Gadd 1986, 1990). Both mechanisms may be utilised to remove metals from industrial effluents such as those produced by the photographic and electroplating industries (Brauckmann 1990). However, to be of any real significance, microbe based technologies must compete on both operational and economical terms with existing metal-removal treatment systems. The commercial applicability of microbial metal-removal processes could be increased by a combination of different approaches including the use of waste biomass from industry and either chemical or physiological manipulation of biomass to improve its metal-biosorption abilities. Studies have shown that several different types of waste biomass have good metal-biosorption characteristics (Avery and Tobin 1992; Luef et al. 1991; Volesky and May-Philips 1995) but, to date, little progress has been made in identifying the mechanisms and cell components involved in metal binding, though recent work concerning the biosorption of metals by yeast has highlighted the importance of cell wall protein in binding cobalt, copper and cadmium (Brady et al. 1994). Also certain types of cellular proteins, called metallothioneins, are able to bind large amounts of metals due to the presence of cysteine residues. The sulphydryl groups of these amino acid residues have been identified as major metal-binding components of the protein (Hamer 1986). Therefore, increasing the protein and/or sulphydryl group content of yeast may increase the metal biosorption capacity of the resultant biomass.

Given the above arguments, this work attempted to identify the cell components involved in Ag^+ biosorption by yeast cells and, through deliberate physiological manipulation, increase the ability of the cells to remove

this metal from solution. The strain of yeast used has previously been shown to have good metal-binding characteristics (Simmons et al. 1995). Silver was chosen for this study as the photographic development industry produces waste containing high levels of this element (Brauckmann 1990) and because silver is regarded as being toxic to microorganisms (Failla et al. 1976). Removal of this metal from solution would prevent potential damage to the environment and achieve re-cycling of a valuable metal.

Materials and methods

Culture

The *Saccharomyces cerevisiae* strain used was supplied and identified by a brewery located in Dublin, Ireland. The organism was routinely maintained on Oxoid malt extract/agar (Oxoid Ltd., Hampshire, UK) and incubated at 27° C.

Chemicals

All chemicals used were BDH AnalaR (BDH Chemicals, Poole, Dorset, UK) unless otherwise specified.

Production of *S. cerevisiae* biomass

Malt extract broth (Oxoid, 30 ml) was inoculated with culture grown on malt extract/agar (Oxoid) and incubated on an orbital shaker at 200 rpm and 27° C for 16 h. An aliquot of this suspension (approximately 0.2 ml) containing 10^7 cells was added to 100 ml malt extract broth in a 250 ml conical flask and incubated as before for 24 h or 96 h. After incubation, the biomass was centrifuged in a Sorvall RC2-B centrifuge (DuPont, Delaware, USA) for 10 min at 4900 *g*, washed in three 50-ml lots of deionised water and freeze-dried using a LSL Secfroid freeze-drier (LSL Secfroid, Aclens, Switzerland).

In certain experiments the above procedure was repeated except that the 100-ml malt extract broth contained either no supplement, 1.0 mM L-cysteine (Sigma Chemical Co., St. Louis, USA), 2.5 mM L-cysteine or 5.0 mM L-cysteine. All of the supplements were added to the flasks of sterile media by filtration through 0.2 μm low-protein-binding filters (Gelman Sciences, Ann Arbor, Mich., USA) to the required final concentration.

The effect of L-cysteine supplementation on the growth of *S. cerevisiae* in malt extract broth

Biomass was grown in flasks containing various concentrations of supplement as described above. Every 3 hours, 0.1 ml culture was taken from each flask and the number of cells per millilitre estimated using a counting chamber (Gelman Hawksley, Lancing, Sussex, UK) and light microscope. After incubation (27 h) the contents of each flask were centrifuged in a RC2-B Sorvall centrifuge and washed in deionised water as above. The contents were freeze-dried in pre-weighed Universal bottles and the dry weight of biomass produced by 100 ml media estimated. Data shown represents the means and standard deviations of duplicate determinations from two independent experiments.

Recovery of live cells

Freeze-dried cells (10 mg) were incubated in 10 ml sterile deionised water for 30 min. Aliquots (0.1 ml) of the yeast suspension were plated out on malt extract/agar and incubated at 25° C for 24 h. No colonies were recovered from any of the freeze-dried cell suspensions.

Isolation of cell walls

The cell walls of fresh biomass were isolated and washed according to the method of Catley (1988). Glass beads (0.45–0.50 mm diameter, B. Braun, Melsungen, Germany), deionised water and a 75 ml Braun homogeniser bottle were cooled to 0° C in melting ice. Cells, deionised water and beads were then mixed in a 1:2:1 ratio (wet mass:v:v) to a total volume of 30 ml. The cells were then broken using a Braun MSK homogeniser. Complete breakage of whole cells was confirmed by light microscopy. The suspension was filtered through coarse sintered glass and the retained material washed with two 5 ml lots of cooled deionised water. The filtrate was centrifuged at 3000 *g* for 10 min at 4° C. Contaminating cytoplasmic material was removed by vortexing the cell wall pellet with absolute ethanol (Merck, Darmstadt, Germany) in a 1:10 ratio (packed cell walls:wash) for 10 min followed by centrifugation (3000 *g* for 10 min, 4° C). This step was repeated once more. Isolated cell walls were similarly treated three times with a 1:1 (v:v) chloroform:methanol mixture and then a 1:1 (v:v) ethanol:ether mixture. The cell walls were then freeze-dried.

Chemical analysis of cells and cell walls

The total carbohydrate content of whole cells and cell walls was determined by the phenol/sulphuric acid assay (Herbert et al. 1971). The protein content of whole cells and cell walls was determined by the biuret assay (Herbert et al. 1971). The relative amounts of mannan and glucan in the cell walls was determined by the carbazole method of McMurrough and Rose (1967). Chitin in the cell wall was determined by the Elson-Morgan reaction (Herbert et al. 1971). The sulphydryl group content of whole cells and isolated cell walls was determined by the 5,5′-dithio-bis(2-nitrobenzoic acid) method of De Nobel et al. (1990). Data shown represent the means and standard deviations of duplicate determinations from two independent experiments.

Ag^+ biosorption assay

Silver nitrate in solution forms Ag^+ and NO_3^- (Smith and Carson 1977). Ag^+-containing solutions (pH 5.6) were prepared from this salt using deionised water. The pH was adjusted using 0.5 M HNO_3. This pH value was chosen to avoid silver precipitation, which can occur in basic solutions (Smith and Carson 1977). Aliquots (25 ml) of each concentration were placed in separate 250-ml conical flasks. Whole cells or isolated cell walls (50 mg dry mass) were placed into each flask and the flasks incubated on an orbital shaker at 200 rpm and 27° C for 1 h. After incubation, the contents of each flask were centrifuged in a Sorvall RC2-B centrifuge for 10 min at 17 300 *g*. The supernatant was diluted with deionised water and the silver content determined by atomic absorption spectrophotometry using a Varian AA-476 atomic absorption spectrophotometer (Varian Techtron, Springvale, Australia). Ag^+ biosorption was estimated by determining the difference in metal content between control flasks without biomass and test flasks containing biomass. Data shown represent the means and standard deviations of duplicate determinations from two independent experiments.

Transmission electron microscopy and X-ray microanalysis

Freeze-dried biomass (50 mg dry mass) was incubated in 25 ml 1.0 mM Ag^+ for 1 h and centrifuged as above. The pellet was washed with three lots (25 ml each) of deionised water and suspended in 0.2 ml 1.2% (w/v) Oxoid technical agar in a 1.5-ml Eppendorf centrifuge tube. When firm, the agar was sliced into strips 1–2 mm in depth and 5 mm in diameter. The strips were fixed in 2.5% (w/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 1 h and then washed in six changes of buffer. The strips were then placed in 1% (w/v) osmium tetroxide (Aldrich chemicals, Dorset, UK) for 1 h and washed as before. Sections were dehydrated in an ascending series (50%–95%) of ethanol (Merck) concentrations before embedding in epon and polymerising at 60° C for 24 h. Sections were cut using an LKB ultratome and placed on copper grids before coating with carbon. Sections were examined in a Jeol 2000 transmission electron microscope (Jeol Co., Tokyo, Japan) equipped with a Link AN10/5 X-ray microanalysis system (Link Systems Ltd., High Wycombe, UK). The counting time was 100 s.

Results

The effect of age on Ag^+ biosorption by whole cells and isolated cell walls of laboratory-cultured biomass

Ag^+ biosorption isotherms were constructed for both 24-h-old and 96-h-old biomass for initial concentrations of 0.1–3.5 mM Ag^+. Freeze-dried laboratory cultured biomass, harvested at either age, displayed different silver biosorption capacities (Fig. 1a). Biomass that was 24 h old, had a maximum biosorption capacity of 0.387 mmol Ag^+/g dry mass as opposed to 0.187 mmol Ag^+/g dry mass for 96-h-old biomass.

Ag^+ biosorption isotherms for 24-h and 96-h-old cell walls were constructed as described above (Fig. 1b). Isolated cell walls removed less Ag^+ from solution than the same mass of whole cells of the same age, although the difference in biosorption capacity between cell walls of either age was small (approx. 0.015 mmol Ag^+/g dry mass).

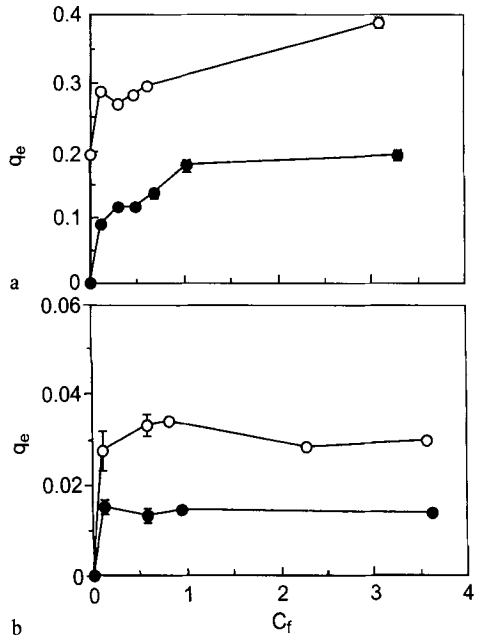

Fig. 1 (a, b) Ag^+ biosorption by (○) 24-h-old and (●) 96-h-old (a) whole cells and (b) isolated cell walls of *S. cerevisiae*. C_f (mM Ag^+) the concentration of metal left in solution after the biosorption process has reached equilibrium. q_e Ag^+ biosorption by the biomass (mmol Ag^+/g dry mass)

Table 1 Chemical analysis of whole cells and isolated cell walls of *S. cerevisiae* at 24 h and 96 h. *CHO* carbohydrate

Preparation	Content (% dry mass, w/w)	
	24 h old	96 h old
Cell walls		
Protein	15.7 ± 1.7	10.9 ± 2.1
Total CHO	82.6 ± 4.5	90.9 ± 1.8
Mannan	38.6 ± 2.1	39.8 ± 0.8
Glucan	43.0 ± 2.3	49.9 ± 1.1
Chitin	1.0 ± 0.1	1.3 ± 0.1
Protein:total CHO	0.19	0.12
Whole cells		
Protein	36.6 ± 2.0	28.7 ± 1.3
Total CHO	40.7 ± 3.6	56.3 ± 1.9
Protein:total CHO	0.90	0.51

Chemical analysis of whole cells and cell walls of laboratory cultured *S. cerevisiae*

Chemical analysis of the whole cells and cell walls of both 24-h and 96-h biomass was undertaken (Table 1). The protein:carbohydrate ratio (w/w) in whole cells of the yeast decreased from 0.90 in 24-h-old biomass to 0.51 in 96-h-old biomass. An analysis of the cell walls of biomass of both ages revealed small changes in the chemical content. The amount of protein in the cell wall decreased with age, while the carbohydrate content increased over the same period.

The effect of L-cysteine supplementation on the growth of *S. cerevisiae* in malt extract broth

Supplementation of malt extract broth with concentrations of L-cysteine above 1.0 mM had a marked effect on the growth of *S. cerevisiae* (Fig. 2). Increasing the concentration of L-cysteine to 2.5 mM decreased the number of cells after 27 h growth from 1.25×10^8 ml^{-1} in the control flasks to 8.25×10^7 ml^{-1}. In flasks containing 5.0 mM L-cysteine, the cell concentration decreased to 6.2×10^7 ml^{-1}. The dry mass of biomass obtained from control, and growth medium supplemented with 1.0, 2.5 and 5.0 mM L-cysteine was 301, 303, 175.5 and 124.5 mg/100 ml growth medium respectively.

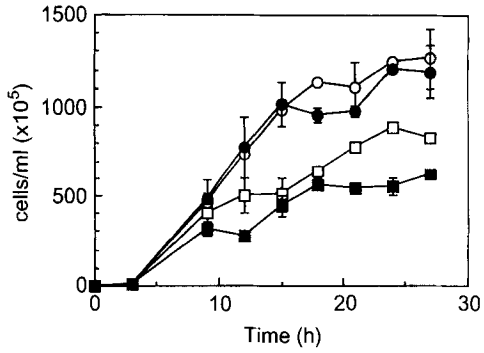

Fig. 2 Growth of *S. cerevisiae* in malt extract broth supplemented with L-cysteine. ○ Broth alone, ● broth + 1.0 mM L-cysteine, □ broth + 2.5 mM L-cysteine, ■ broth + 5.0 mM L-cysteine

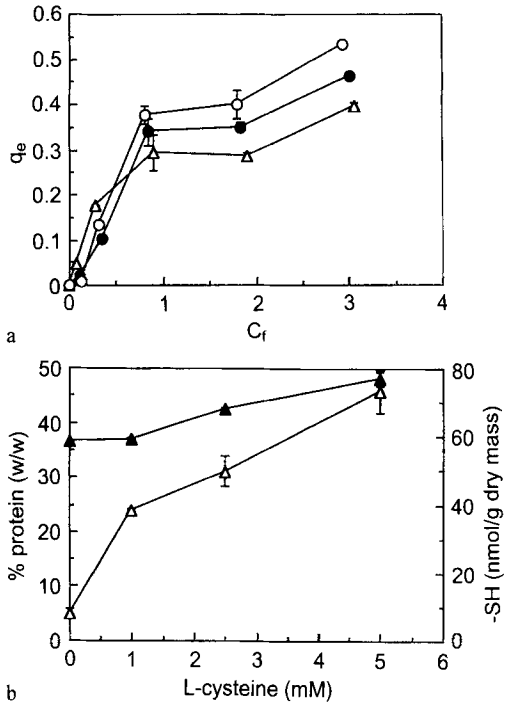

Fig. 3a Ag^+ biosorption by *S. cerevisiae* grown in the presence of L-cysteine. △ Broth alone, ● broth + 1.0 mM L-cysteine, ○ broth + 2.5 mM L-cysteine. C_f, q_e as in Fig. 1. **b** Protein content (▲) and sulphydryl (-SH) content (△) of cells grown in the presence of differing concentrations of L-cysteine

The effect of L-cysteine supplementation on Ag^+ biosorption by whole cells and isolated cell walls of *S. cerevisiae*

Ag^+ biosorption isotherms (0.1–3.5 mM initial Ag^+ concentration) were constructed for biomass that had been grown in 1.0 mM and 2.5 mM L-cysteine (Fig. 3a). Ag^+ removal from 3.5 mM Ag^+ solution was also determined for biomass grown with 5.0 mM L-cysteine. Isotherms for control biomass (no L-cysteine supplementation) were constructed for comparison. Ag^+ biosorption was greater for biomass grown in

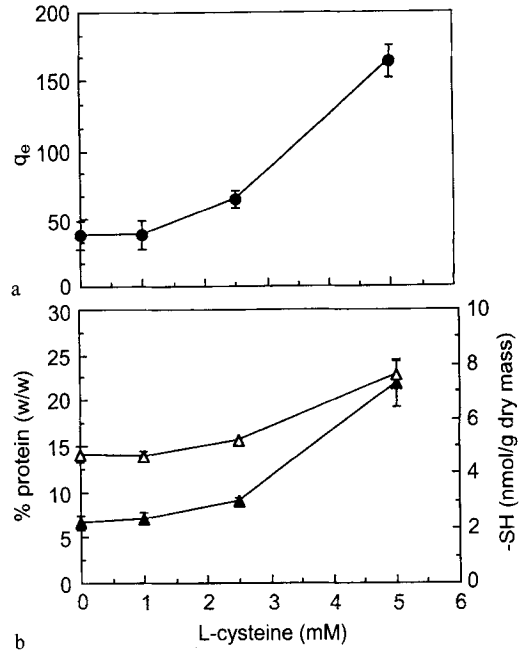

Fig. 4a Ag^+ biosorption by cell walls of *S. cerevisiae* isolated from biomass grown in the presence of differing concentrations of L-cysteine. q_e Ag^+ biosorption by the cell walls (μmol Ag^+/g dry mass). Initial Ag^+ concentration was 1.0 mM. **b** The effect of supplementation of growth medium with L-cysteine on the protein content (▲) and sulphydryl (-SH) (△) content of the resultant cell walls

supplemented medium. Increasing the concentration of supplement in the growth medium resulted in biomass with increased biosorption capacity. At the highest concentration of Ag^+ examined, Ag^+ biosorption for biomass supplemented with 0, 1.0 mM, 2.5 mM and 5.0 mM L-cysteine was 0.389, 0.462, 0.535 and 0.556 mmol Ag^+/g dry weight respectively. At the lowest concentration of Ag^+ examined, the pattern of biosorption reversed and biomass from growth medium with 2.5 mM L-cysteine had the lowest Ag^+ removal capacity.

Isolated cell walls of biomass from supplemented growth medium were tested for silver biosorption capacity at 1.0 mM Ag^+ (Fig. 4a). Cell walls from control and 1.0-mM-supplemented medium showed no difference in biosorption capacity, while cell walls from 2.5-mM-supplemented medium had an increased silver biosorption capacity of 69.2%. Cell walls from biomass grown in the presence of 5.0 mM L-cysteine removed approximately five times more silver from solution than cell walls from biomass grown in the absence of cysteine.

Effect of L-cysteine supplementation on protein content and sulphydryl content of whole cells and isolated cell walls of *S. cerevisiae*

Increasing the L-cysteine supplementation in the growth medium above 1.0 mM increased the protein

content of both whole cells and isolated cell walls (Figs. 3b, 4b). Protein content increased by 5.9% in 2.5-mM-supplemented whole cells and by 11.4% in 5.0-mM-supplemented whole cells. Cell walls isolated from 2.5-mM- and 5.0-mM-supplemented medium showed increased protein contents of 2.4% and 15.3% respectively. Whole cells from biomass supplemented with 1.0 mM L-cysteine had an increased sulphydryl group content of 375% relative to the control, while biomass from medium supplemented with 2.5 mM and 5.0 mM L-cysteine had an increased sulphydryl group content of 523% and 816% respectively, relative to the control (Figs. 3b, 4b).

Increases in sulphydryl content for the isolated cell walls were less than for whole cells and there were no increases at all for cell walls isolated from biomass grown in medium supplemented with 1.0 mM L-cysteine. An increased sulphydryl content of 10.6% and 61.7% respectively was recorded for cell walls isolated from medium supplemented with 2.5 mM and 5.0 mM L-cysteine.

Electron microscopy and X-ray microanalysis

Electron micrographs of silver-exposed biomass (both 24 h old and 96 h old) revealed differences in Ag^+ biosorption. Both pictures demonstrated the presence of the typical double-layer wall structure of yeast, a dark outer mannoprotein layer enveloping an inner glucan layer. Laboratory-cultured biomass exposed to silver also contained black precipitates, located both on the cell wall and within the cell. Younger (24 h old) biomass (Fig. 5) had a higher concentration of precipitates than older (96 h old) biomass (Fig. 6). Biomass not exposed to Ag^+ did not contain any precipitates (results not shown). When the precipitates were analysed by X-ray microanalysis (Fig. 7), peaks indicating the presence of silver and sulphur were observed. X-ray microanalysis of cells not exposed to silver ions did not have peaks due to silver or sulphur (results not shown). Cu and Cr peaks present were due to background contamination from the copper grids and the microscope. Biomass grown in the presence of 5.0 mM L-cysteine and subsequently washed, freeze-dried and exposed to 1.0 mM Ag^+ solution was not observed to contain any precipitates as described above (results not shown).

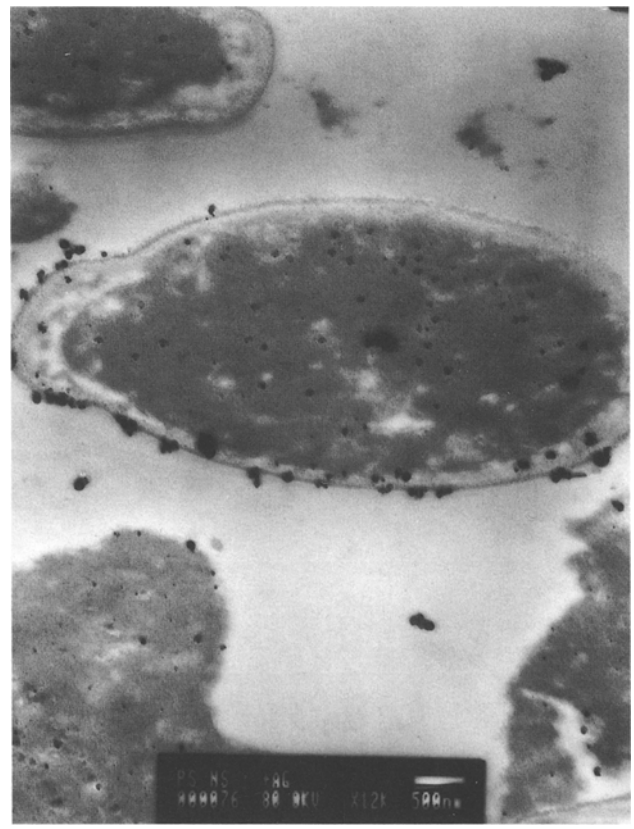

Fig. 5 Transmission electron micrograph of a freeze-dried 24-h-old laboratory-cultured *S. cerevisiae* cell after exposure to Ag^+ solution (1.0 mM). *Bar* 500 nm

Discussion

The effect of culture age has been shown to be important for determining the ability of yeast biomass to remove metals from solution. Volesky and May-Philips (1995) have shown that biosorption of uranium by cells of *S. cerevisiae* was markedly affected by culture age and that younger cells (12 h) were able to remove 4.6 times more uranium from solution than older cells (24 h). The results of the work carried out here were in agreement with the above observations and it was found that younger biomass (24 h) could remove twice as much silver from solution as older biomass (96 h).

The cell walls of microorganisms are known to be important for metal binding (Yazgan and Ozcengiz 1994; Gadd 1986). In addition to this, changes in yeast cell wall composition and ultrastructure with culture age have also been noted before. For instance, Cassone et al. (1979) reported that the cell wall of *Candida albicans* was affected by extended periods of starvation. Therefore, it was decided to investigate whether changes in cell wall composition with age were responsible for the decrease in Ag^+ biosorption capacity of the biomass.

The Ag^+ biosorption capacity and chemical composition of cell walls from either age varied only by a small degree. Although cell walls isolated from younger biomass could remove more silver from solution than cell walls isolated from older biomass, the amount removed in either case was only approximately 10% of that amount removed by an equivalent mass of whole cells of the same age and the difference in Ag^+ biosorption capacity between either age of cell wall was small. If cell walls are assumed to be approximately

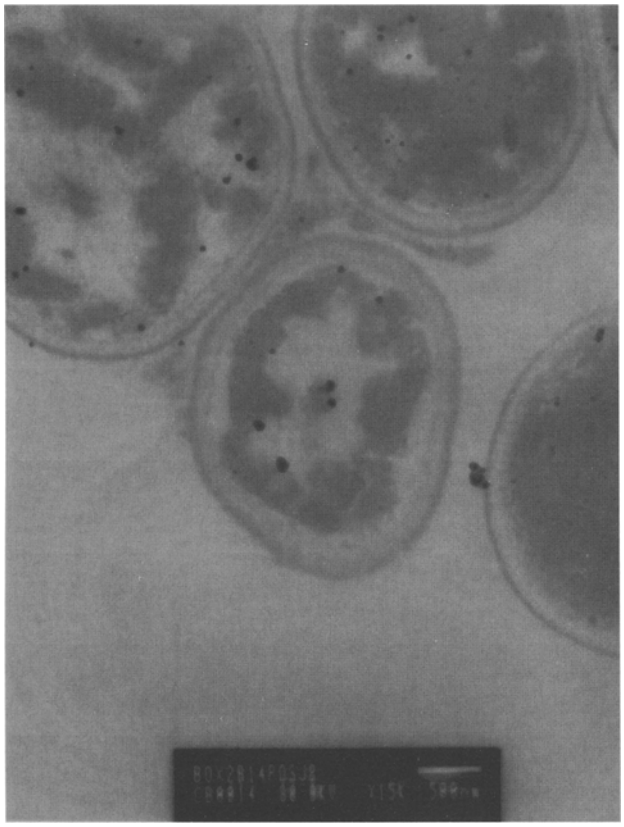

Fig. 6 Transmission electron micrograph of a freeze-dried 96-h-old laboratory-cultured *S. cerevisiae* cell after exposure to Ag^+ solution (1.0 mM). *Bar* 500 nm

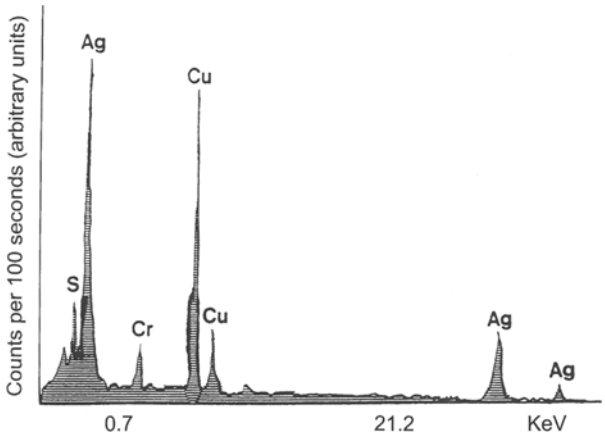

Fig. 7 Typical X-ray microanalysis spectrum of precipitate from Ag^+ exposed laboratory-cultured *S. cerevisiae* (Figs. 5, 6)

25% of the dry mass of whole cells (Catley 1988) then silver biosorption by cell walls of 24-h-old cultures should only be 0.008 mmol Ag^+/g dry mass of whole cells (i.e. approximately 2% of total biosorption when q_e is 0.387 mmol Ag^+/g). A similar calculation can be performed for cell walls isolated from 96-h-old culture. The cell wall composition found here was similar to those reported by other authors and indicated that the procedure used was able to isolate the cell wall intact (Northcote and Horne 1952; Killick 1971). Overall, it appeared that the cell walls played a minor role in Ag^+ biosorption by this yeast and that changes in Ag^+ biosorption capacity with culture age were due to differences in intracellular composition. Further evidence of this was obtained using transmission electron microscopy and X-ray microanalysis.

Electron-dense precipitates were observed both inside the cell and on the cell wall and, upon examination by X-ray microanalysis, these precipitates were found to contain silver and a small sulphur peak. The presence of these two peaks within the silver precipitate indicated that the silver may have bound to a sulphur-containing molecule and precipitated around it. It appeared visually that the concentration of these precipitates within the cell decreased with culture age, as would be expected from their relative Ag^+ biosorption capacities.

Precipitates containing silver and sulphur have previously been described around cells of *Candida albicans* grown in the presence of silver (Kierans et al. 1991), while *S. cerevisiae* grown on the same medium had deposits of elemental silver on or around the cell wall. These deposits were attributed to the reduction of Ag^+ to Ag^0 by volatile and non-volatile reducing substances produced by the growing cells (Kierans et al. 1991). Even though freeze-dried cells were not viable (as determined by the lack of recovery when reconstituted and inoculated onto malt extract/agar) there may have been residual enzyme activity present in the dried cells, which may have reduced Ag^+ to Ag_2S. It is difficult to define the precise nature of the interactions between Ag^+ and the components of the cell responsible for the formation of the precipitates. Mullen et al. (1992) also reported the presence of colloidal silver deposits around the cell walls of *Aspergillus niger* although their presence within the cell was also reported. Silver ions are known to be able to penetrate the cytoplasmic membrane of microorganisms (Ivanov and Fomchenkov 1992) and the presence of silver-containing precipitates within the cell was not unexpected. Although the decrease in protein content may not be highly significant, there could be a tentative link between cell protein content and biosorption capacity because of the large metal-binding capacity of protein (Brady et al. 1994; Oda et al. 1988; Hunt 1986) and the large amount of cellular protein present. However, the role of other cellular constituents (e.g. nucleic acids and carbohydrates) cannot be excluded. The results indicated that there were possible links between the ability of the cells to bind Ag^+, total cell protein and sulphur in the cell and it was decided to grow yeast with L-cysteine as this amino acid is known to affect the protein content of *S. cerevisiae* (Killick 1971). Also, if this amino acid was incorporated directly into cell protein the content of sulphydryl groups within the biomass would increase. Both these factors are important as cell protein contains N and S centres and, as Ag^+ is a soft metal, it will

bind preferentially to groups containing these atoms (Nieboer and Richardson 1980).

Biomass from L-cysteine-supplemented-media had increased Ag^+ biosorbent properties compared to biomass from non-supplemented media. In addition, the biosorption potential of the biomass, from each medium, was related to the initial concentration of L-cysteine. The increased biosorption potential of the biomass appeared to be correlated with the sulphydryl group content, indicating that either L-cysteine and/or sulphydryl groups had been incorporated into the biomass. The largest increase in cell sulphydryl group content was for biomass from media supplemented with 1.0 mM L-cysteine; increasing the concentration of L-cysteine further only resulted in smaller increases. Interestingly, at concentrations above 1.0 mM, L-cysteine-supplemented growth media appeared to stimulate protein synthesis. Killick (1971) observed a similar occurrence with cells of *S. cerevisiae* that had been grown in media containing 10 mM L-methionine (a sulphur-containing amino acid). These cells had increased protein content although the increase (about 3.5%) was less than was found in this study.

A comparison of sulphydryl group content and protein content of biomass grown in supplemented media and cell walls from the same biomass was made. This was carried out to determine the location of the incorporated sulphydryl groups and their effects on the chemical composition of the cell components. The results indicated that most of the sulphydryl groups incorporated in the biomass were located intracellularly and not on the cell wall.

Amino acids in media can be toxic at high concentrations (Killick 1971; Slaughter 1988) and, in accordance with this, growth of our strain of *S. cerevisiae* decreased with increasing concentrations of cysteine in the medium. If cysteine supplementation were to be used to increase metal-uptake properties of waste industrial biomass, then the effect of cysteine on the actual commercial process, e.g. alcohol production, would need to be examined.

Overall, the results of this work indicated that the age of the cell is important in determining Ag^+ binding capacity and that intracellular components bind more Ag^+ than the cell wall. Addition of sulphur-containing amino acids to the growth medium resulted in biomass with enhanced silver biosorption capacity by increasing cell protein levels and/or altering levels of sulphur-containing compounds within the cell.

Interestingly, the precipitates observed in Ag^+-exposed laboratory-cultured biomass were absent in biomass grown in the presence of 5.0 mM L-cysteine and subsequently exposed to Ag^+. This indicated that growth of this yeast in the presence of this amino acid may change the mechanism by which Ag^+ is sequestered from solution.

The effects of sulphur-containing amino acids on metal uptake by fungi (and other microorganisms) are worthy of further investigation and may prove to enhance the economical application of the biosorption phenomenon. Further investigations into physiological manipulations of the biomass to increase its metal biosorption, and their effect on the mechanism of silver biosorption are currently underway.

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