Appl Microbiol Biotechnol (1995) 43:579–584

MINI REVIEW

K. J. Blackwell · I. Singleton · J. M. Tobin

Metal cation uptake by yeast: a review

Received: 10 August 1994 / Accepted: 27 August 1994

Abstract This review addresses metal uptake specifically by yeast. Metal uptake may be passive, active or both, depending on the viability of the biomass, and is influenced by a number of environmental and experimental factors. Uptake is typically accompanied by a degree of ion exchange and, under certain conditions, may be enhanced by the addition of an energy source. Intracellularly accumulated metal is most readily associated with the cell wall and vacuole but may also be bound by other cellular organelles and biomolecules. The intrinsic biochemical, structural and genetic properties of the yeast cell along with environmental conditions are crucial for its survival when exposed to toxic metals. Conditions of pH, temperature and the presence of additional ions, amongst others, have varying effects on the metal uptake process. We conclude that yeasts have contributed significantly to our understanding of the metal uptake process and suggest directions for future work.

Introduction

The study of the interactions between metals and fungi has long been of scientific interest. In an environmental context, accelerating pollution by toxic metals, metalloids, radionuclides and organometal(loid)s has influenced research towards the biotechnological potential of utilising microorganisms for metal removal and/or recovery from the biosphere. Yeasts possess an acknowledged potential for accumulating a range of metal cations. This review will address all aspects of this metal uptake process.

K. J. Blackwell · J. M. Tobin (✉)
School of Biological Sciences, Dublin City University, Dublin 9, Ireland.
Fax: 0353-17045412

I. Singleton
Department of Industrial Microbiology,
University College Dublin, Dublin 4, Ireland

Metal uptake

Fungal metal uptake is essentially a biphasic process consisting of a metabolism-independent and metabolism-dependent step. The initial biosorption step is rapid (Avery and Tobin 1992; Brady and Duncan 1994a), typically only a few minutes in duration (White and Gadd 1987) and is independent of temperature (Mowll and Gadd 1984), metabolic energy (de Rome and Gadd 1987), the presence of a metabolisable energy source and the presence of metabolic inhibitors (White and Gadd 1987; Mowll and Gadd 1984). Almost always this initial binding is thought to involve the microbial cell wall, although extracellular polymers may be responsible in some cases. Binding is attributed to ion-exchange, adsorption, complexation, precipitation and crystallisation within the multilaminate, microfibrillar cell wall structure (Remacle 1990; Volesky 1990; Tobin et al. 1984). Biosorption is exclusively responsible for metal accumulation by non-viable biomass (Tobin et al. 1984; Avery and Tobin 1992) owing to the absence of metabolic activity necessary for intracellular metal accumulation. Brady and Duncan (1994a) found that if the metal to biomass ratio was below 100 nmol/g then metal accumulation was almost entirely dependent on biosorption of metal ions to the cell wall. The second step, designated bioaccumulation, is a slower metabolism-dependent step, influenced by factors such as temperature and the presence of metabolic inhibitors (de Rome and Gadd 1987; Mowll and Gadd 1984; Okorokov et al. 1977). Greater amounts of metal may be accumulated by this means in some organisms, especially yeasts (Avery and Tobin 1992; Norris and Kelly 1977).

Suggested mechanisms for transport of metal ions into microbial cells include lipid peroxidation, complex permeation, carrier mediation, ion channels/pumps and endocytosis (Ford and Mitchell 1992). Transport is inhibited by low temperature but does not cease altogether (Norris and Kelly 1977). Transport systems encoun-

tered in microorganisms are of varying specificity through which both essential and non-essential ions may be taken up (Mowll and Gadd 1984; White and Gadd 1987). Most mechanisms of metal transport appear to rely on the electrochemical proton gradient across the cell membrane, which has a chemical and electrical potential, both of which are responsible for driving transport of ionised solutes across membranes (Gadd 1993). Various metabolic inhibitors that interfere with membrane potential have been shown to reduce metal accumulation (White and Gadd 1987; Mowll and Gadd 1984; Norris and Kelly 1977). In several organisms, notably filamentous fungi, transport may not be as significant a component of total uptake as general biosorption (Gadd 1990b).

Bioaccumulation is enhanced by the presence of a metabolisable substrate such as glucose (Okorokov et al. 1977; Norris and Kelly 1977; Avery and Tobin 1992), becomes evident within minutes (Norris and Kelly 1977; Avery and Tobin 1992) and continues for a number of hours (Avery and Tobin 1992). Localisation of cations in yeasts, in the presence of a metabolisable substrate, is primarily determined by the active transport in response to electrochemical proton gradients, generated by membrane-bound H^+–ATPases, across the cytoplasmic and vacuolar membranes (Jones and Gadd 1990). Metal accumulation by cells in the presence of glucose can be up to threefold greater than in the absence of glucose (Norris and Kelly 1977; Avery and Tobin 1992). A period of cell starvation (30 min to 20 h) prior to metal addition is included in many experimental protocols. Brady and Duncan (1994a) cited the absence of this step to explain the lack of increased metal uptake in the presence of glucose. Non-metabolisable analogues of glucose, although absorbed by the cell, do not stimulate metal uptake (White and Gadd 1987), indicating that metal uptake is not linked to substrate transport across cell membranes. Glucose-enhanced metal uptake has been linked to external metal concentration. Gadd and Mowll (1983) found glucose stimulated Cd^{2+} uptake up to 0.05 mM external Cd^{2+}, but above this concentration there appeared to be little difference with or without glucose.

Viability of biomass during the course of experiments is important when considering metabolism-dependent metal uptake (Gadd and Mowll 1983). White and Gadd (1987) observed a progressive reduction in viability (to 50%) of cells incubated over a range of zinc concentrations up to 100 μM. Viability loss correlated with indicators of zinc toxicity such as inhibition of H^+ efflux and K^+ uptake but not with Zn^{2+} uptake. No loss of viability was observed for cells of *Aureobasidium pullulans* up to Cd^{2+} concentrations of 0.5 mM (Mowll and Gadd 1984) whereas *Saccharomyces cerevisiae* cells experienced rapid viability loss (90% decrease within 5 min) at the same Cd^{2+} concentration (Gadd and Mowll 1983). At 0.05 mM Cd^{2+} loss of viability was rapid in the presence of glucose but Cd^{2+} uptake was double that of cells incubated in the absence of glucose (where cells remained viable), indicating that Cd^{2+} was accumulated by a mechanism normally used to sequester essential non-toxic cations. Partial inhibition of Cd^{2+} uptake in yeast by Ca^{2+} supports this view (Norris and Kelly 1977).

There is ambiguity concerning whether live or inactivated biomass is the better metal sorbent. Different yeast strains were reported to accumulate varying amounts of metal (Junghans and Straube 1991) with uptake by living cells only slightly higher than that of dead (boiled) cells. Volesky et al. (1993) observed Cd^{2+} uptake in excess of 625 μmol/g cells for aerobic baker's yeast compared to dry non-living yeasts, which did not accumulate more than 180 μmol/g Cd^{2+}. Sr^{2+} uptake by strains of *S. cerevisiae* did not exceed 40 μmol/g whereas denatured biomass accumulated between 100 μmol/g and 200 μmol/g at similiar external Sr^{2+} concentrations (Avery and Tobin 1992). Live biomass did prove to be a more efficient metal sorbent than denatured biomass at low (10 μmol) external concentration.

Concomitant with metal uptake, ion release from biomass (viable and inactivated) is frequently observed. Release of K^+, H^+, Ca^{2+} and Mg^{2+} (Belde et al. 1988; Avery and Tobin 1992, 1993; Norris and Kelly 1977; Okorokov et al. 1983; Brady and Duncan 1994b) has been most studied. There are varying reports on whether a stoichiometric relationship exists between ion release and metal uptake. Numerous studies refer to K^+ release by yeast in response to metal uptake. Two K^+ ions were released for each Co^{2+} ion taken up in freshly prepared cell suspensions, but the lack of K^+ exchange by K^+–deficient cells and during the latter stages of prolonged Co^{2+} uptake suggested K^+ loss is not always a feature of Co^{2+} accumulation (Norris and Kelly 1977). The same study observed extensive K^+ loss following Cd^{2+} uptake but no simple stoichiometric relationship was apparent. Gadd and Mowll (1983) also reported the absence of a simple stoichiometric relationship between Cd^{2+} uptake and K^+ release. K^+ release was attributed to membrane disruption by Cd^{2+} binding to organic ligands, and was more marked in the presence of glucose. Cu^{2+} and Cd^{2+} accumulation induced extensive loss of cellular K^+ and Mg^{2+} but little loss of Ca^{2+} (Brady and Duncan 1994b). Li^+ accumulation was accompanied by a stoichiometric efflux of K^+ (Perkins and Gadd 1993). Also two K^+ ions were released for each Cd^{2+} ion accumulated intracellularly (Mowll and Gadd 1984) suggesting K^+ efflux occurs to maintain ionic balance across the membrane. White and Gadd (1987) observed rapid, almost instantaneous K^+ efflux at Zn^{2+} concentrations below 50 μM, followed by a phase of uptake which restored K^+ levels to those observed initially. At Zn^{2+} concentrations above 50 μM, K^+ efflux was not reversed and was followed by a phase of equilibrium or slow efflux. No stoichiometric relationship was evident. At non-toxic Cu^{2+} concentrations the rate of K^+ efflux by *S. cerevisiae* in the presence of glucose was twice that of

Cu^{2+} influx (de Rome and Gadd 1987). K^+ efflux was uniphasic with little K^+ efflux observed in the presence of factors that decreased Cu^{2+} uptake into cells. Levels of intracellular K^+ were critical for maximal Cu^{2+} accumulation–cells with high levels of internal K^+ (approx. 139 mM) took up more Cu^{2+} than cells with lower levels (approx. 82 mM).

The total charge released during Sr^{2+} adsorption to live biomass was approximately 45% of that taken up (Avery and Tobin 1992). Greater amounts of Mg^{2+} were released by denatured yeast (5 to 40 fold greater) but Ca^{2+} and H^+ displacement was reduced. Cytoplasmic levels of Ca^{2+} and Mg^{2+} declined in response to Sr^{2+} uptake. Further Mg^{2+}, but not Ca^{2+}, loss from the vacuole correlated with stimulated Sr^{2+} uptake in the presence of glucose. Similarly Gadd and Mowll (1983) observed decreases in cellular Mg^{2+} in the presence of Cd^{2+}–90% of cellular Mg^{2+} was lost within 5 min. In contrast Co^{2+} and Cd^{2+} uptake did not appear to affect cellular Mg^{2+} levels (Norris and Kelly 1977).

Intracellular fate of toxic metals

The diversity of intracellular organelles and biomolecules provides a wide range of potential binding sites (Gadd 1990a). Large amounts of metal can remain associated with the cell wall. The major fraction of Hg^{2+} in *S. cerevisiae* was tightly bound to the cell wall (Murray and Kidby 1975) by proteins associated with cell wall glucan. Hg^{2+} did penetrate to the cytoplasm but only a minor fraction was present as low-molecular-mass components, suggesting that the majority was associated with high-molecular mass moieties such as polypeptides or cellular organelles. One quarter of the accumulated Mn^{2+} ions were found to be wall-associated, with the remainder in the yeast protoplast (Okorokov et al. 1977). Volesky et al. (1993) detected no Cd^{2+} deposits on the outer wall of yeast cells (Cd^{2+} was preferentially located intracellularly) and observed that some cells exhibited no deposition at all. Protein and carbohydrate fractions of yeast cell walls were involved in binding Cu^{2+}, Cd^{2+} and Co^{2+} ions (Brady and Duncan 1994c). Isolated components of yeast cell walls (mannans, glucans and chitin) were observed to accumulate greater quantities of metal than intact cell walls. This, together with a 30% reduction in metal-accumulating capacity when the protein fraction of cell walls was removed by enzymatic digestion, suggests that the outer mannan-protein layer of the cell wall is more important than the the inner glucan-chitin layer in heavy metal cation accumulation (Brady et al. 1994). Deliberate chemical modification of the yeast cell walls was found not to increase metal-accumulating capacity (Brady and Duncan 1994c).

Many cytoplasmic biomolecules have the ability to bind metals. In addition to the cysteine-rich polypeptides of the "metallothionein" family, another group of molecules present in certain fungi and yeasts are the short, cysteine-containing γ-glutamyl peptides. Synthesis of these molecules can be induced by the presence of heavy metals (Gadd 1993). Cellular metabolites such as nucleotides, RNA (Cabral 1992; Huang et al. 1990), inorganic phosphate (Volesky et al. 1993) and sulphydryl moieties (Scot and Palmer 1990) all possess significant metal-binding capacity. In general, cytosolic metal concentrations are lower than vacuolar or membrane/organelle fractions (Avery and Tobin 1992; White and Gadd 1987; Nieuwenhuis et al. 1981).

The vacuole has an important role in the regulation of the cytosolic concentration of metal ions both for essential metabolic functions and for detoxification of potentially toxic metal species. Many ions including inorganic phosphate and monovalent and divalent cations are preferentially located in vacuoles (Gadd 1993). Localisation of metal ions in the vacuole enables low non-toxic cytosolic concentrations to be maintained. 70% Sr^{2+} and 90% Mn^{2+} accumulated intracellularly by *S. cerevisiae* was present in the vacuole, with the remainder in the cytoplasm (Nieuwenhuis et al. 1981). Approximately 60% of cellular Zn^{2+} was compartmented in the vacuole, 5% in the cytoplasm with the remainder in the membrane/organelle fraction (White and Gadd 1987). Electron-dense metal deposits of Cd^{2+} were predominantly accumulated within vacuoles of *S. cerevisiae* (Volesky et al. 1993). Deposits became larger with increased time of exposure, and metal appeared to be deposited as cadmium phosphate. Avery and Tobin (1992) found that the relative importance of the cytosolic and vacuolar compartments in intracellular localisation of Sr^{2+} was dependent on whether uptake was active or passive. In non-metabolising yeast intracellular, Sr^{2+} was predominantly located in the cytoplasm. Although Sr^{2+} uptake in the presence of glucose resulted in elevated levels of the cation in the cytoplasm, increases were more evident in the vacuole. Metal-uptake experiments conducted on isolated vacuoles of *S. cerevisiae* established the presence of an energy-dependent Zn^{2+} transport system to establish and maintain a Zn^{2+} gradient across the vacuolar membrane (White and Gadd 1987). Stimulatory/inhibitory studies indicated that ATPase activity, and the presence of a proton gradient were necessary for Zn^{2+} to be accumulated.

Toxicity of heavy metals

When metals with no known biological function compete with, or replace a functional metal, toxicity results (Hughes and Poole 1989). Toxic metals can exert harmful effects principally as a consequence of their strong coordinating abilities (Gadd 1993). Toxic effects include the blocking of functional groups of biologically important molecules, the displacement and/or substitution of essential metal ions from biomolecules, conformational modification, denaturation and inactivation of

enzymes and disruption of cellular and organelle integrity (Gadd 1993). On a macro scale, effects include reduction in growth rates, extension of lag phase and perturbations in morphology and physiology (Hughes and Poole 1989).

The cell wall is an obvious initial site of action for toxic metal species. As a result of metal-binding, membrane damage can occur due to the loss of mobile cellular solutes leading to an increased permeability of the cell to external material. Loss of cellular K^+, Mg^{2+} and Ca^{2+} ions upon metal binding has been observed by several authors (Avery and Tobin 1992; Norris and Kelly 1977; Gadd and Mowll 1983; de Rome and Gadd 1987).

Indirect mechanisms of toxicity may involve free radicals which are deleterious to cells. Major targets of radicals are cell membranes where lipid peroxidation is initiated with consequent loss of cell integrity. Many aerobic organisms possess protective metalloenzymes containing either Mn, Fe or Cu/Zn, which eliminate radicals produced by normal metabolism (Gadd 1993).

Organometals are generally more toxic to living organisms than the corresponding free metal ions (Gadd 1993). Alkylmetal toxicity depends on the number and nature of alkyl groups present, with toxicity reflecting the lipophilicity of the species. Even a single methyl group introduced onto a metal or metalloid can elicit dramatic changes in volatility and lipid solubility (Hughes and Poole 1989). Methylmercury is many times more toxic than Hg^{2+}, and, because of ready diffusion through biological membranes, it is the most significant form of mercury in both salt and fresh water (Ford and Mitchell 1992; Hughes and Poole 1989). Other organometallic compounds present in the biosphere include organotins (fungicides) and organoleads (petroleum additives). Effects of these compounds include inhibited growth and respiration, and blocking of ATPase activity (Hughes and Poole 1989). Arsenic, selenium, thallium and antimony can also be methylated by a variety of microorganisms as a means of removal by volatilisation (Ford and Mitchell 1992).

Survival of microorganisms in the presence of toxic metals depends on intrinsic biochemical and structural properties, physiological and/or genetic adaptation, environmental modification of metal speciation, availability and toxicity. Cell wall construction is crucial for survival, and in yeast is composed of several layers bearing anionic groups to which metal cations bind. Up to 90% of the yeast wall is polysaccharide complexed with proteins, lipids and other substances. The yeast cell wall presents a multilaminate, microfibrillar structure–an outer layer of glucans, mannans or galactans and an inner microfibrillar layer, the crystalline properties of which are conferred by the parallel arrangement of chains of chitin or cellulose or non-cellulosic glucan, with a continuous transition between both layers (Remacle 1990). The adsorptive capacity of the yeast cell wall for heavy metals is not determined mainly by its protein component, but rather by the structural organisation of the entire protein-carbohydrate complex and by the degree of dissociation of the negatively charged functional groups and their accessibility to the metals (Davidova and Kasparova 1992). Phosphodiester and carboxyl groups confer the electrical surface potential to the wall. Significant variations are observed in fungal wall features depending on stages of the life cycle and on culture conditions (Remacle 1990). The structural features of the cell wall provide a mechanism to immobilise metals and prevent their entry into the cell. Ono et al. (1988) observed that Hg^{2+}–resistant mutants possessed a greater cell wall binding capacity than parental strains. Training by repeated culture on elevated metal concentrations resulted in increased resistance to Co^{2+}, Cu^{2+} and Cd^{2+} through reduced uptake (Belde et al. 1988; White and Gadd 1986) and altered intracellular distribution (White and Gadd 1986). Resistance to Co^{2+} and Ni^{2+} by a mutant yeast strain was due to a reduction in uptake of these metals via a Mg^{2+} transport system (Joho et al. 1991).

Other methods employed to prevent metal cations entering the cell include extracellular precipitation of metals as sulphides or hydroxides by means of excreted metabolites. Sulphate-reducing bacteria utilise the production of H_2S to precipitate metals in an insoluble form (Ford and Mitchell 1992; Hughes and Poole 1989). Extracellular polysaccharides bind large quantities of metal because of the negatively charged groups present (Scot and Palmer 1990).

Many resistance mechanisms result from genetic adaptation. Most, but not all are determined by genes located on plasmids. One mechanism that is not plasmid-encoded is the yeast copper metallothionein, which is the main internal detoxification mechanism for Cu^{2+} (Butt and Ecker 1987). The best understood plasmid-encoded systems are those conferring resistance to mercuric compounds, arsenate and cadmium. Organomercurial compounds are enzymatically converted to volatile elemental mercury (Hughes and Poole 1989). Arsenate resistance is achieved via an inducible ATP-driven transport system that serves to eject toxic ions from the cell. Cadmium resistance is also based on an efflux system. The cadmium efflux system may be able to transport zinc, although this has not been directly demonstrated (Hughes and Poole 1989). Efflux of Cd^{2+} from Cd^{2+}–loaded cells of *A. pullulans* was significantly enhanced by the addition of glucose (Mowll and Gadd 1984). Most of the intracellular Cd^{2+} was removed from the cell after a short period of time. Sr^{2+} and Mn^{2+} were found to be actively removed from cells of *S. cerevisiae* (Nieuwenhuis et al. 1981) the rate of efflux of Sr^{2+} being three times that of Mn^{2+}.

Environmental factors

The influence of pH on metal uptake by yeasts (Junghans and Straube 1991; Brady and Duncan 1994a), algae (Wang and Wood 1984) and bacteria (Nakajima and

Sakajuchi 1993) is very similar. Extremes of pH generally decrease the rate and extent of metal uptake. At a pH below 2.0 metal uptake by yeasts was undetectable (Brady and Duncan 1994a; Junghans and Straube 1991). At low pH, H^+ ions compete with metal ions for cellular binding sites and reduce potential metal interaction with cells (Gadd 1993). Variations in external pH can affect the degree of protonation of potential ligands that contribute to metal binding (Tobin et al. 1984). A pH between 4.0 and 8.0 is widely accepted as being optimal for metal uptake (Brady and Duncan 1994a; Fourest and Roux 1992) for almost all types of biomass. Hydrolysis reactions occur with nearly all the metallic cations, and because of the diversity of the hydroxide complexes that can be formed in solution, the resulting chemical behaviour of a given metal can be a complicated function of pH and concentration (Baes and Mesner 1976). Hydroxide complexes are often polynuclear, reducing the free metal ions available for sequestration. Increased pH can result in precipitation of metal hydroxides or oxides, with the same result.

Temperature effects are confined to metabolism-dependent metal accumulation (Norris and Kelly 1977). At low temperature (0–5° C), little or no metal is sequestered through metabolic processes by viable biomass. Most laboratory experiments are carried out in the temperature range 25–35° C, which has been reported optimal for metal accumulation (Brady and Duncan 1994a). Metal binding through biosorptive action is unchanged across the temperature range 4–25° C (Norris and Kelly 1977; White and Gadd 1987).

Cations and anions additional to the ion of interest have a generally detrimental impact on metal uptake (Tobin et al. 1987). Decreased metal uptake is thought to be a response to increased competition between like charged species for binding sites, with preference given to particular ions (Norris and Kelly 1977; Gadd 1993; Gadd and White 1987). K^+ and Mg^{2+} were found to inhibit Zn^{2+} uptake by *S. cerevisiae* whereas Na^+ and Ca^{2+} did not. It was concluded that inhibition of Zn^{2+} uptake was related to K^+ and Mg^{2+} accumulation by the cell (Ca^{2+}, Na^+ were not taken up). Reduction of net surface charge by bound cation may also be involved (Borst-Pauwels and Theuvenet 1984). In contrast, the presence of Ni^{2+} enhanced Zn^{2+} uptake at concentrations of 100 μM compared to 20 μM Zn but its mode of action was unknown (White and Gadd 1987). Sr^{2+} and Ca^{2+} uptake by yeast was inhibited by monovalent cations (K^+, Rb^+, Cs^+, Na^+, Li^+) at equimolar concentrations, and correlated with uptake of these cations (Roomans et al. 1979). Norris and Kelly (1977) observed Co^{2+} and Cd^{2+} uptake to be severely inhibited by cations of similar size. Ca^{2+} (the closest in ionic radius to Cd^{2+}) was found to depress Cd^{2+} uptake strongly. Mowll and Gadd (1984) reported similar findings and proposed that Cd^{2+} was accumulated via a Ca^{2+} transport system.

Avery and Tobin (1993) related uptake inhibition to metal chemistry. Inhibition effects of the hard ion SO_4^{2-} were most marked on uptake of the hard metals Sr^{2+} and Mn^{2+}, whereas greater relative effects on adsorption of the softer cations Cu^{2+} and Cd^{2+} correlated with complexation by the soft anion $S_2O_3^{2-}$. Inhibition of the borderline metal Zn^{2+} by SO_4^{2-} and $S_2O_3^{2-}$ was found to be approximately equal.

Dissolved and particulate matter in the environment and in growth media, generally reduces metal toxicity by complexation and binding (Babich and Stotzky 1977; Gadd 1993). Proteins (Huang et al. 1990), humic acids (Wood and Wang 1983) and cellular metabolites released as a consequence of induced membrane damage (Cabral 1992) bind appreciable quantities of free ions, making them unavailable for uptake.

Metal uptake is modestly inhibited by elevated ionic strength (Brady and Duncan 1994a,c). Metabolic inhibitors affect metabolism-dependent accumulation (Norris and Kelly 1977; White and Gadd 1987; Mowll and Gadd 1984) through interference with membrane transport systems. The oxidation/reduction potential of an environment affects metal speciation and solubility. Insoluble metal sulphides form in reducing environments, which exhibit little or no toxicity (Gadd 1993).

In conclusion, yeasts have contributed to our understanding of metal uptake and toxicity, and how metal resistance may be achieved in eukaryotic cells. In a biotechnological context, yeasts may be useful in the treatment of metal-containing effluents. Future work in the area should be directed towards identifying compounds in yeast cell walls responsible for metal binding. Subsequently either genetic or physiological manipulation could be used to enhance metal uptake characteristics and give the phenomenon greater specificity.

References

Avery SV, Tobin JM (1992) Mechanisms of strontium uptake by laboratory and bewing strains of *Saccharomyces cerevisiae.* Appl Environ Microbiol 58:3883–3889

Avery SV, Tobin JM (1993) Mechanisms of adsorption of hard and soft metal ions to *Saccharomyces cerevisiae* and influence of hard and soft anions. Appl Environ Microbiol 59:2851–2856

Babich H, Stotzky G (1977) Reduction in the toxicity of cadmium to microorganisms by clay minerals. Appl Environ Microbiol 33:696–705

Baes CF, Mesner RE (1976) The hydrolysis of cations. Wiley, New York

Belde P, Kessels B, Moelans I, Borst-Pauwels G (1988) Cd^{2+} uptake, Cd^{2+} binding and loss of cell K^+ by a Cd-sensitive and a Cd-resistant strain of *Saccharomyces cerevisiae.* FEMS Microbiol Lett 49:493–498

Borst-Pauwels G, Theuvenet A (1984) Apparent saturation kinetics of divalent cation uptake in yeast caused by a reduction in the surface potential. Biochim Biophys Acta 771:171–176

Brady D, Duncan JR (1994a) Bioaccumulation of metal cations by *Saccharomyces cerevisiae.* Appl Microbiol Biotechnol 41:149–154

Brady D, Duncan JR (1994b) Cation loss during accumulation of heavy metal cations by *Saccharomyces cerevisiae.* Biotechnol Lett 10:543–548

Brady D, Duncan JR (1994c) Binding of heavy metals by the cell walls of *Saccharomyces cerevisiae.* Enzyme Microb Technol 16:633–638

Brady D, Stoll AD, Starke L, Duncan JR (1994) Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*. Biotechnol Bioeng 44:297–302

Butt T, Ecker D (1987) Yeast metallothionein and applications in biotechnology. Microbiol Rev 51:351–364

Cabral J (1992) Limitations of the use of an ion-selective electrode in the study of the uptake of Cu^{2+} by *Pseudomonas syringae* cells. J Microbiol Methods 16:149–156

Davidova E, Kasparova S (1992) Adsorption of metals by yeast cell walls. Mikrobiologiya 61:1018–1022

Ford T, Mitchell R (1992) Microbial transport of toxic metals. In: Environ Microbiol, Wiley-Liss,,New York, pp 83–101

Fourest E, Roux JC (1992) Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. Appl Microbiol Biotechnol 37:399–403

Gadd GM (1990a) Biosorption Chem Ind 13:421–426

Gadd GM (1990b) Heavy metal accumulation by bacteria and other microorganisms. Experientia 46:834–840

Gadd GM (1993) Interaction of fungi with toxic metals. New Phytol 124:25–60

Gadd GM, Mowll JL (1983) The relationship between cadmium uptake, potassium release and viability in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 16:45–48

Huang C, Huang P, Morehart A (1990) The removal of Cu(II) from dilute aqueous solutions by *Saccharomyces cerevisiae*. Water Res 24:433–439

Hughes MN, Poole RK (1989) Metals and micro-organisms. Chapman & Hall, London

Joho M, Tarumi K, Inouhe M, Tohoyama H, Murayama T (1991) Co^{2+} and Ni^{2+} resistance in *Saccharomyces cerevisiae* associated with a reduction in the accumulation of Mg^{2+}. Microbios 67:177–186

Jones RP, Gadd GM (1990) Ionic nutrition of yeast–physiological mechanisms involved and implications for biotechnology. Enzyme Microb Technol 12:1–17

Junghans K, Straube G (1991) Biosorption of copper by yeasts. Biol Metals 4:233–237

Mowll JL, Gadd GM (1984) Cadmium uptake by*Aureobasidium pullulans*. J Gen Microbiol 130:279–284

Murray AD, Kidby DK (1975) Sub-cellular location of mercury in yeast grown in the presence of mercuric chloride. J Gen Microbiol 86:66–74

Nakajima A, Sakaguchi T (1993) Accumulation of uranium by basidiomycetes. Appl Microbiol Biotechnol 38:574–578

Nieuwenhuis B, Weijers C, Borst-Pauwels G (1981) Uptake and accumulation of Mn^{2+} and Sr^{2+} in *Saccharomyces cerevisiae*. Biochim Biophys Acta 649:83–88

Norris PR, Kelly DP (1977) Accumulation of cadmium and cobalt by *Saccharomyces cerevisiae*. J Gen Microbiol 99:317–324

Okorokov L, Lichko L, Kadomtseva V, Titovsky V, Kulaev I (1977) Energy-dependent transport of manganese into yeast cells and distribution of accumulated ions. Eur J Biochem 75:373–377

Okorokov L, Lichko L, Andreeva N (1983) Changes of ATP, polyphosphate and K^+ contents in *Saccharomyces carlsbergensis* during uptake of Mn^{2+} and glucose. Biochem 6:481–488

Ono B, Ohue H, Ishihara F (1988) Role of cell wall in *Saccharomyces cerevisiae* mutants resistant to Hg^{2+}. J Bacteriol 170:5877–5882

Perkins J, Gadd GM (1993) Accumulation and intracellular compartmentation of lithium ions in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 107:255–260

Remacle J (1990) The cell wall and metal binding. In: Volesky B (ed) Biosorption of heavy metals, CRC, Boca Raton, Fla

Rome L de, Gadd GM (1987) Measurement of copper uptake in *Saccharomyces cerevisiae* using a Cu^{2+}–selective electrode. FEMS Microbiol Lett 43:283–287

Roomans G, Theuvenet A, Van Den Berg T, Borst-Pauwels G (1979) Kinetics of Ca^{2+} and Sr^{2+} uptake by yeast. Effects of pH, cations and phosphate. Biochim Biophys Acta 551:187–196

Scot JA, Palmer SJ (1990) Sites of cadmium uptake in bacteria used for biosorption. Appl Microbiol Biotechnol 33:221–225

Tobin JM, Cooper DJ, Neufeld RJ (1984) Uptake of metal ions by *Rhizopus arrhizus* biomass. Appl Environ Microbiol 47:821–824

Tobin JM, Cooper DG, Neufeld RJ (1987) Influence of anions on metal adsorption by *Rhizopus arrhizus* biomass. Biotechnol Bioeng 30:882–886

Volesky B (1990) Biosorption by fungal biomass. In: Volesky B (ed) Biosorption of heavy metals. CRC, Boca Raton, Fla

Volesky B, May H, Holan ZR (1993) Cadmium biosorption by *Saccharomyces cerevisiae*. Biotechnol Bioeng 41:826–829

Wang H, Wood JM (1984) Bioaccumulation of nickel by algae. Environ Sci Technol 18:106–109

White C, Gadd GM (1986) Uptake and cellular distribution of copper, cobalt and cadmium in strains of *Saccharomyces cerevisiae* cultured on elevated concentrations of these metals. FEMS Microbiol Ecol 38:277–283

White C, Gadd GM (1987) The uptake and cellular distribution of zinc in *Saccharomyces cerevisiae*. J Gen Microbiol 133:727–737

Wood JM, Wang H (1983) Microbial resistance to heavy metals. Environ Sci Technol 12:582–590