Bioremediation of industrial effluents containing heavy metals using brewing cells of *Saccharomyces cerevisiae* as a green technology: a review

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Abstract The release of heavy metals into the environment, mainly as a consequence of anthropogenic activities, constitutes a worldwide environmental pollution problem. Unlike organic pollutants, heavy metals are not degraded and remain indefinitely in the ecosystem, which poses a different kind of challenge for remediation. It seems that the "best treatment technologies" available may not be completely effective for metal removal or can be expensive; therefore, new methodologies have been proposed for the detoxification of metal-bearing wastewaters. The present work reviews and discusses the advantages of using brewing yeast cells of *Saccharomyces cerevisiae* in the detoxification of effluents containing heavy metals. The current knowledge of the mechanisms of metal removal by yeast biomass is presented. The use of live or dead biomass and the influence of biomass inactivation on the metal accumulation characteristics are outlined. The role of chemical speciation for predicting and optimising the efficiency of metal removal is highlighted. The problem of biomass separation, after treatment of the effluents, and the use of flocculent characteristics, as an alternative process of cell–liquid separation, are also discussed. The use of yeast cells in the treatment of real effluents to bridge the gap between fundamental and applied studies is presented and updated. The convenient management of the contaminated biomass and the advantages of the selective recovery of heavy metals in the development of a closed cycle without residues (green technology) are critically reviewed.

Keywords Chemical speciation · Electroplating wastewater bioremediation · Heavy metal biosorption · Incineration · Metal selective recovery · Yeast flocculation

1 Introduction

Anthropogenic activities are largely responsible for the release of heavy metals into the environment. Examples of industries associated with the discharge of metals are mining, metallurgy, electroplating and other surface finishing industries, leather and tanning industries, energy production and pigment and battery manufacturing. These industrial activities generate effluents containing various heavy metals at concentrations that do not meet the wastewater limit discharge criteria; in these effluents, metals can be present at concentrations that may create a serious environmental hazard and a threat to water supplies.

High toxicity has been observed with several metals, such as Cd, Cu and Hg. Even at low concentrations, heavy metals can be accumulated through the food chain and

become hazardous to animals and public health. Therefore, it is not surprising that stricter regulations have been progressively introduced by governments with the purpose of limiting the discharge of heavy metals and reducing metallic pollution.

Unlike organic pollutants, metals and their salts are not degraded or destroyed, but rather they remain indefinitely in the environment. Therefore, the release of heavy metals in the environment is a matter of concern and poses a different kind of challenge for remediation. In developing countries, municipal sanitary sewers are not equipped to treat toxic wastes, such as industrial effluents containing heavy metals. As a result, heavy metals should be removed, in a "previous step", from these metal-laden effluents before they are released into the water body or sent to a municipal treatment plant.

Physicochemical technologies, such as chemical precipitation/neutralisation, activated carbon adsorption, ion exchange resins, reverse osmosis, solvent extraction and electrochemical technologies have been used to treat effluents containing heavy metals. However, these processes cannot be environmental friendly, fully efficient (precipitation/neutralisation and electrochemical technologies) or present very high costs (membrane technologies and ion exchange resins) when applied to large volumes of wastewater containing low metal concentrations (1–100 mg/L). The recent work of Naja and Volesky (2010) provides a detailed description of these technologies in the treatment of industrial effluents containing heavy metals.

The disadvantages presented by the available "best treatment technologies" associated with the increase in environmental regulations have compelled the search for innovative, environmentally friendly, low-cost and efficient processes for the detoxification of metal-bearing wastewaters over the last two decades. Different types of microorganisms, such as algae, bacteria, filamentous fungi and yeast cells have the ability to remove, concentrate and immobilise different metals, providing a basis for the development of new methodologies to remove inorganic pollution (Gavrilescu 2004; Volesky 2003; Wang and Chen 2009). Microorganisms are small in size and have a high surface area-to-volume ratio; as a consequence, they display a large contact area that can interact with metal ions present in solution. Due to their heavy metal removal efficiency, environmental suitability, simplicity and mode of operation analogous to well-established ion-exchange technology, waste microbial biomass has been identified as a promissory biotechnological approach in the fight against metal pollution.

Yeast biomass is obtained in large amounts as a by-product of the brewing industry, and its use remains largely unexploited; therefore, its disposal is frequently an environmental problem (Ferreira et al. 2010). In the present work, the use of brewing yeast cells of *Saccharomyces cerevisiae* in the bioremediation of effluents loaded with heavy metals is reviewed. Due to the autoaggregation properties of brewing yeast strains, they can be quickly and easily separated from the treated effluent; this intrinsic property avoids the use of cell immobilising techniques or solid–liquid separation processes. After treating the effluent, the convenient management of the contaminated biomass and the selective recovery of metals to ensure the minimisation of waste production and low operating costs become important. Heavy metals are a valuable resource for different industrial applications. The recovery of metals, if obtained with high yields and purity, allows them to be reintroduced into the industrial process (closing the cycle of metals—green technology) or sold. In both cases, the recycling of metals leads to a reduction in the costs of the bioremediation process; these issues are also reviewed in the present work.

2 Advantages of using brewing yeast biomass

S. cerevisiae and its relatives in the *Saccharomyces sensu stricto* complex are the most useful and industrially exploited microorganisms (Vaughan-Martini and Martini 1998). Traditionally associated with the production of alcoholic beverages (such as beer, wine and cider), this yeast is currently used in the production of renewable fuels (bio-ethanol), food ingredients and pharmaceuticals. Another use of *S. cerevisiae* cells is in environmental applications. Among the different types of biomass that can be used in the bioremediation of heavy metals, *S. cerevisiae* cells from brewing seem to be well suited, for the reasons described below.

Availability Readily available microbial biomass can be used as a primary criterion in the selection of biomass to be used during the bioremediation process (Volesky 1990). *S. cerevisiae* cells can be found in large amounts because they are a by-product of large fermentation industries. *S. cerevisiae* biomass is the second major by-product (after spent grain) of the brewing industry; during fermentation, the yeast biomass increases three to sixfold. In a typical lager fermentation, approximately 2.6 kg of surplus yeast solids are produced per cubic meter of beer produced (Huige 2006). Other sources of *S. cerevisiae* biomass from fermentation industries are wine (including sparkling wine), distilled liquor and bio-ethanol production. Contrary to the biomass obtained from the pharmaceutical industries, yeast

cells from fermentation industries are stable and are not subject to the drastic treatments associated with the recovery process of the primary product. For instance, fungal biomass used in the pharmaceutical industry is subject to treatment with solvents; these treatments can affect metal removal performance and cause concerns about its subsequent use (Volesky 1990).

Ability to remove heavy metals The yeast biomass from brewing can accumulate a large range of metals, namely Ag (I), Cd(II), Cr(III), Cs(I), Cu(II), Ni(II), Pb(II), Sr(II) and Zn (II), at a variety of pHs (Avery and Tobin 1992; Chen and Wang 2008; Ferraz et al. 2004; Han et al. 2006; Machado et al. 2009; Marques et al. 1999; Soares et al. 2002; Zhao and Duncan 1997; Zouboulis et al. 2001).

Price The biomass from brewing has received little attention as a profitable product. The hops used in beer production give the yeast a bitter taste. Spent brewer's yeast is generally sold, after heat inactivation, as an inexpensive product to the animal feed industry (Ferreira et al. 2010; Huige 2006). Therefore, the surplus yeast produced from fermentation industries can be obtained at a low price.

Safe organism S. cerevisiae strains are described as "generally recognised as safe" organisms by the US Food and Drug Administration, which means that these cells can be freely manipulated without public concern; this fact increases the feasibility of using yeast biomass in bioremediation processes.

Model system Yeast cells, particularly *S. cerevisiae*, are a suitable model for performing fundamental studies because they are eukaryotic cells that can be easily cultured and manipulated and have a completely sequenced genome (Goffeau et al. 1996). The use of omics technology can provide a wide range of knowledge about the mechanisms of metal accumulation and the impact of these metals on the cells. Additionally, it is possible to improve the biosorption properties of heavy metals by genetic manipulation (see section 3.2).

Flocculation characteristics Traditionally, the brewing industry has used flocculent yeast strains. These strains can aggregate into multicellular masses (flocs) and settle rapidly in suspension media. The usefulness of this property in the bioremediation of heavy metals is described below (section 8.3).

Together, these properties make yeast biomass from brewing a very promising weapon in the fight against metal pollution.

3 Improvement in metal removal by yeast cells

3.1 Inactivating processes and chemical modification of the yeast surface

Techniques for inactivating and processing *S. cerevisiae* cells include lyophilisation (Bustard and McHale 1998), autoclaving (Göksungur et al. 2005; Suh and Kim 2000), heat treatment (Avery and Tobin 1992; Machado et al. 2009; Özer and Özer 2003) and chemical treatments with alkaline solutions (Ashkenazy et al. 1997; Göksungur et al. 2005; Lu and Wilkins 1996), ethanol (Ghorbani et al. 2008; Göksungur et al. 2005), formaldehyde (Strandberg et al. 1981) and acetone (Ashkenazy et al. 1997). The way yeast cells are treated/inactivated can affect the metal-binding capacity of the cells. Treatment with formaldehyde has been shown to increase uranium accumulation in yeast cells (Strandberg et al. 1981). Autoclaving or the treatment of yeast cells with NaOH or ethanol has increased Cd(II) and Pb(II) biosorption; the highest values have been obtained with ethanol-treated cells (Göksungur et al. 2005). Heat treatment followed by ethanol treatment has also increased Cd biosorption capacity by twofold (Ghorbani et al. 2008); this enhanced capacity has been explained by an increase in the accessibility of the metal ions to the metal-binding sites on the biomass (Ghorbani et al. 2008; Göksungur et al. 2005). Alternatively, it has been reported that hot alkali yeast treatment can moderately decrease Cu(II) accumulation when compared with native (untreated) yeast cells (Brady et al. 1994a); other authors have also reported that dead biomass has lower Sr(II) (Avery and Tobin 1992) or Pb(II) uptake (Suh and Kim 2000; Suh et al. 1998) than the respective live cells. In contrast, heat treatment can enhance the exposure of additional functional groups implicated in the biosorption of heavy metals. Machado et al. (2009) have shown that a mild heat treatment (45°C) of the brewing biomass improves the Ni(II)- and Zn(II)-binding capacities when compared with live (untreated) yeast cells. With this moderate-heat treatment, no appreciable structural or molecular changes occurred, as evaluated by fluorescence microscopy, scanning electron microscopy and infrared spectroscopy. Brewing yeast cells lose membrane integrity during thermal inactivation treatment. The disruption of the membrane allows for the passive intracellular metal accumulation. The exposure of additional metal-binding sites present inside the cells enhances metal removal and also explains the increase in the accumulation capacity of the dead biomass when compared with the same live cells (Machado et al. 2009). Mild heating (45°C) seems to be an appropriate process to inactivate yeast cells for subsequent use in the bioremediation process; with this treatment, the cells did not lose their flocculation ability (Machado et al. 2008).

An improvement in the metal removal efficiency of yeast strains with modified surfaces has also been described. Bingol et al. (2004) reported an increase in chromate anion removal due to the modification of the yeast surface with cetyl trimethyl ammonium bromide. Other studies have shown that yeast surfaces that have been modified with poly(amic acid) via the reaction of pyromellitic dianhydride and thiourea (Yu et al. 2007) or with ethylenediaminetetraacetic dianhydride (Yu et al. 2008), led to increases in Pb(II) and Cd(II) or Pb(II) and Cu(II) biosorption, respectively.

3.2 Genetically engineered microorganisms

The potential use of *S. cerevisiae* yeast cells for the treatment of metallic pollution can be enhanced by genetic manipulation. Therefore, yeast modifications have been performed to improve the efficiency or selectivity of metal removal. One possibility was to use yeast cells with an increased capacity for intracellular metal accumulation due to over-production of metal-binding proteins in the cytoplasm, such as glutathione and cysteine-rich proteins (Kuroda and Ueda 2010). *S. cerevisiae* cells expressing *Arabidopsis thaliana* phytochelatin synthase showed enhanced intracellular arsenic (III) accumulation, which resulted in a sixfold increase in As removal (Singh et al. 2008).

Another method involved the cell surface design of microorganisms using recombinant DNA techniques. In "cell-surface engineering", also called "molecular display (arming) technology", it is possible to anchor various functional metal-binding proteins/peptides on the yeast cell surface (Shibasaki et al. 2009). The foreign peptide to be expressed is generally fused to a mannoprotein that is anchored covalently onto the yeast cell surface (Kondo and Ueda 2004; Salem et al. 2008; Shibasaki et al. 2009). These yeast cells, which display the heterologous proteins on the cell surface, have exhibited an enhanced selective metal removal by a metabolism-independent process; in this case, metal recovery from yeasts does not require cell disruption, which allows for subsequent reuse (Kuroda and Ueda 2010; Salem et al. 2008). Cell-surface-engineered yeasts, which display a histidine oligopeptide (hexa-His), have the capability to chelate divalent heavy metals and have shown the ability to remove three to eight times more copper than parent yeast cells; additionally, the genetically engineered microorganisms (GEM) was more resistant to copper than was the parental strain (Kuroda et al. 2001). Yeast cells that displayed a histidine or a cysteine-rich sequence on their surface have presented a twofold increase in capacity to remove Cd(II) and Zn(II) when compared with the parental strain (Vinopal et al. 2007). In another approach, a yeast metallothionein (YMT) was displayed on the cell surface; the modified yeast strain was more effective at removing Cd(II) than the parental strain (Kuroda and Ueda 2003). To increase the level of Cd(II) removal, YMT was displayed by means of an *a*-agglutinin-based system; the ability of the cells to remove Cd(II) gradually improved with the increasing number of tandem metallothionein repeats (Kuroda and Ueda 2006). In another approach, a short, metal-binding peptide harbouring the metal fixation motif of the bacterial Pb(II)-transporting P1-type ATPases was fixed on the yeast cell wall. The genetically engineered strain showed higher (up to fivefold) Pb(II) removal ability than the parental strain; additionally, the Pb(II) removal capacity was not impaired by the presence of a threefold molar excess of either Cd(II) or Zn(II) (Kotrba and Ruml 2010). To overcome the problem of cell separation from the treated effluent, a transformed strain with dual features was obtained, and it enhanced the removal of copper and improved the ability to self-aggregate in response to binding to and accumulation of copper ion (Kuroda et al. 2002). Besides the selective removal of toxic metals described above, genetically modified yeast cells have also been designed to selectively remove rare metal ions, such as molybdenum (Nishitani et al. 2010).

Presently, these genetically engineered yeast cells cannot be seen as a real alternative for detoxifying actual industrial effluents or removing rare metals because of the existence of legal and socio-political barriers, particularly those pertaining to the release of GEM into the environment.

4 Factors influencing heavy metal removal

Metal removal by yeasts is affected by several physico-chemical properties of the solution, namely the pH, the redox potential (E_h), the concentration of metal ions and complexing agents (organic and inorganic) and the affinity of these agents for metal ions. Some of these factors can act at different levels, such as on the affinity of yeast cells for heavy metals and on the metal speciation in solution. Chemical speciation can be defined as the different physicochemical forms of a metal in solution that make up its total concentration (Florence 1983).

Extremes of pH often decrease the accumulation of heavy metals in yeast cells. *S. cerevisiae* is able to remove heavy metals from wastewater between pH 5.0 and 9.0 (Brady and Duncan 1994); in addition, pH values between 5 and 6 are optimal for Cu(II), Cd(II), Pb(II), Ni(II), Zn(II) and Cr(III) biosorption by yeast cells (Brady and Duncan 1994; Cui et al. 2010; Ferraz and Teixeira 1999; Han et al. 2006; Junghans and Straube 1991; Mapolelo and Torto 2004; Özer and Özer 2003; Parvathi and Nagendran 2007; Vasudevan et al. 2003; Zouboulis et al. 2001). The pH of a solution affects the percentage of ionised groups on the yeast cell wall. In the case of live cells, the pH can also

affect the cell physiology and metabolism. At pH 6.0, the main chemical groups on the biomass surface that can participate in the removal of metal cations (carboxyl, phosphate, sulphydryl, hydroxyl and nitrogen-containing groups) are already completely (carboxylic acids) or partially (phosphate and amine groups) deprotonated (Martell and Smith 2004). On the other hand, at low pH values, the functional groups on the yeast cell wall are progressively more protonated and metal accumulation decreases. The opposite effect is observed with the removal of Cr(VI); at very acidic conditions (pH 1.0–2.5), the removal of Cr(VI) is enhanced (Bingol et al. 2004; Goyal et al. 2003; Krauter et al. 1996; Machado et al. 2010d; Wu et al. 2011; Zhao and Duncan 1998). This different behaviour is due to the fact that at pH <5, the dominant species of Cr (VI) is $HCrO_4^-$, and the yeast cell surface is surrounded by H^+ ions, which enhances the $HCrO_4^-$ interaction with biomass-binding sites by electrostatic forces (Machado et al. 2010d; Özer and Özer 2003; Parvathi and Nagendran 2007). Additionally, pH values can affect metal speciation. An increase in the pH can result in the precipitation of metal hydroxides. Consequently, less soluble amounts of metal will be available for accumulation in the yeast cells (Sheng et al. 2004).

The redox potential can also affect the speciation of a given element. For example, chromium exists as Cr(VI) or Cr(III) according to the E_h of the solution. These two different oxidation states of Cr originate different chemical species of Cr(III) or Cr(VI) as a function of the pH and have different bioremediation efficiency profiles (Machado et al. 2010b; Machado et al. 2010d). Additionally, these two oxidation states of Cr exhibit significant differences in terms of toxicity; in trace amounts, Cr(III) is an essential element for humans (it is required for sugar and lipid metabolism) while Cr(VI) acts on live cells as a mutagen and a carcinogen (Mertz 1993). Due to the high toxicity of Cr(VI), a low limit (0.1 mg/L) for effluent discharge has been established (Machado et al. 2010d). In addition, effluents containing both Cr(III) and Cr(VI) require a different strategy for removal, as discussed below (section 7.2).

Organic and inorganic ligands present in the effluents can affect the removal of heavy metals by yeast cells. Generally, organic and inorganic ligands present in solution can complex with the metal ions. A reduction in metal accumulation by *S. cerevisiae* cells is expected if the affinity constants of the metal–ligand complexes are higher than the affinity constant of the metal-biosorption sites on the yeast cell wall (Avery and Tobin 1993; Kapoor and Viraraghavan 1995). The detrimental effect of ligands is dependent on the relative affinity of each metal for the biomass versus the soluble ligands (inorganic and organic) present in the effluent, as well as on the amounts of ligands and biomass. No significant negative influence of the ligands on the bioremediation efficiency is expected when the affinity constants of each metal for the biomass are much higher than the affinity between the metal and the ligands (at least ten times). If this difference is smaller (a factor of ten or less), the relative concentrations between the biomass and the soluble ligands will influence the efficiency of the bioremediation process.

Effluents from electroplating industries usually contain other chemical species, such as organic (ethylenediaminetetraacetic acid (EDTA)) or inorganic ligands (chlorides, cyanides, fluorides, carbonates, phosphates and sulphates) (Naja and Volesky 2010). For instance, fluorides are used for pickling certain metal alloys and in some chromium electrodeposition baths (Schlesinger 2004) while sulphates are present when Cr(VI) is reduced to Cr(III) with a sulphuric acid solution of bisulphite (Wang and Li 2006). A detailed assessment of the impact of inorganic ions on the removal of heavy metals (Cr(III), Cu(II), Ni(II) and Zn(II)) from electroplating effluents by brewing yeast cells has been performed by Machado et al. (2010a). The affinity of heat-inactivated brewing yeast biomass for copper, nickel and zinc is much higher (two decades or more) than for carbonates, chlorides, fluorides and nitrates; this means that these ligands do not compete with brewing yeast cells for the metal ions considered, even if present in large excess. In practice, this means that $MHCO_3$, MCl, MF and MNO_3 species, corresponding to reactions 2, 3, 4 and 5, respectively (Fig. 1), are not formed, and the biosorption of copper, nickel and zinc in yeast biomass should not be influenced. For similar reasons, sulphates do not seem to affect the removal of copper or zinc ions by yeast biomass. Conversely, the affinity constants between the nickel ions and sulphates or biomass only differ ten times. This means that effluents containing molar concentrations of sulphates that are much higher than the concentration of nickel ions can compete with the yeast cells and reduce the bioremediation efficiency (reaction 6). However, this effect can be more or less pronounced depending on the amount of biomass used, as mentioned above; an increase in the biomass concentration alleviates the detrimental effect produced by the sulphates in nickel bioremediation by yeast cells. The presence of fluorides or phosphates in solution can impair the removal of Cr (III) by yeast cells (reactions 4 and 7 of Fig. 1, respectively). The magnitude of the affinity constant between Cr(III) and fluorides is about a hundred thousand times higher than observed between Cr(III) and the biomass; therefore, effluents containing molar concentrations of fluorides similar to Cr(III) will compete with yeast cells and will reduce or inhibit the bioremediation efficiency (Machado et al. 2010a). This theoretical

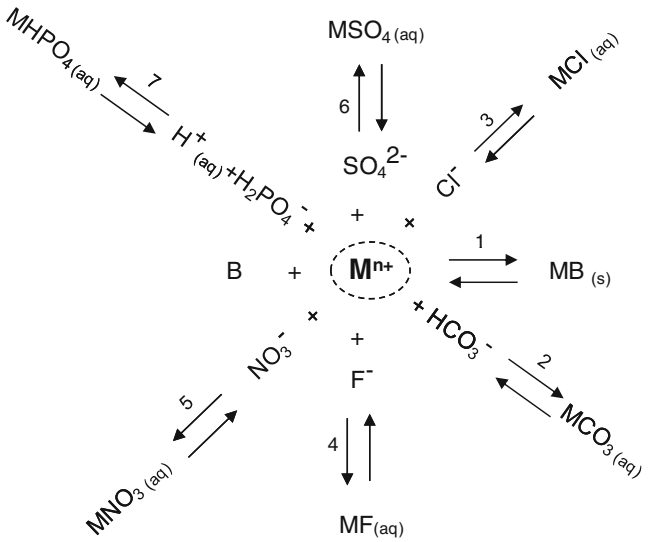

Fig. 1 Diagrammatic representation of metal–ligand interactions. M^{n+} metal ions, *B* biomass (yeast cells). For simplicity of representation, the charges of the complexes have been omitted. Yeast cells are able to remove heavy metals from the industrial effluent (reaction 1). The presence of ligands in the effluents (reactions 2–7) can reduce the efficiency of the process by competing with the biomass for metal removal. Source: Machado et al. (2010a)

prevision was confirmed using an effluent containing multiple elements (Cu(II) and Ni(II) and Cr(III)). The presence of fluorides severely decreased the removal of Cr(III) by the yeast biomass; on the other hand, a higher level of removal of Cu(II) and Ni(II) was observed (Machado et al. 2011a). In the presence of fluorides, Cr(III) was primarily complexed with fluoride and unavailable for yeast accumulation. Because less Cr (III) was biosorbed, more yeast-binding sites remained available to remove other metals present in the solution. This point is particularly remarkable for biomass with a lower affinity for certain metals, such as Ni(II) (Machado et al. 2011a).

The literature also describes the inhibition of gold accumulation by the unicellular algae *Chlorella vulgaris* (Greene et al. 1986) due to the presence of chloride, bromide and iodide. Conversely, Diniz and Volesky (2005) have shown that chloride and nitrate do not affect La(III) sorption by the brown seaweed *Sargassum polycystum* while sulphate decreased metal removal. The presence of sulphate and nitrate anions drastically reduced Co(II) uptake by the freshwater cyanobacterium *Oscillatoria angustissima* (Ahuja et al. 1999). The inhibition of metal uptake is more pronounced when ligands with strong complexing activity, like EDTA, mercaptoethanol, cyanide or thiourea, are present in solution (Greene et al. 1986). The presence of EDTA totally inhibits Cd(II) and Pb(II) uptake by the fungus *Rhizopus arrhizus* (Tobin et al. 1987) and the inhibition of heavy metals uptake by the seaweed biomass (Ramelow et al. 1992); in the last case, other organic ligands had a lesser (sodium citrate) or no (sodium lauryl sulphate) significant effect.

Besides the metals of interest, the presence of other cations (Ca, Mg and Mn) in solution can reduce heavy metal accumulation by biomass due to a competition effect (Han et al. 2006; Mapolelo et al. 2005; Norris and Kelly 1977). Additionally, the accumulation performance of a given metal can be influenced by the presence of other heavy metals (Ferraz and Teixeira 1999; Han et al. 2006; Marques et al. 1999) because of the different affinities of the biomass for heavy metals.

5 Metal removal

Heavy metal removal by yeast cells can be performed via two main mechanisms: a passive step, usually called biosorption, and a metabolism-dependent step, known as bioaccumulation. While biosorption is characteristically a surface phenomenon, bioaccumulation involves the passage of metallic species through the yeast cell membrane and its intracellular accumulation.

5.1 Biosorption

5.1.1 General description

Generally, biosorption can be defined as the interaction of the sorbate (atom, molecule or ion) with the biosorbent (the solid surface of a biological matrix); this results in accumulation of the sorbate at the biosorbent interface and, consequently, a reduction of the sorbate concentration in the solution (Gadd 2010). When referring to the control of heavy metal pollution, biosorption is the first step of metal removal. It is a rapid event (few minutes) and is brought about by live and dead cells (Brady and Duncan 1994; Machado et al. 2009; Soares et al. 2002). This initial metal removal is attributed to the interaction of the yeast cell wall with the metal ions; it is metabolism independent and does not require the presence of a metabolisable energy source (Blackwell et al. 1995).

Biosorption is the exclusive mechanism for metal removal by non-viable yeast cells because the lack of metabolic activity impairs intracellular metal accumulation (Avery and Tobin 1992). However, in the case of yeasts with cell membranes permeabilised by the action of detergents (Gadd 1990), HCHO or $HgCl_2$ (Strandberg et al. 1981), metals can be intracellularly accumulated by a metabolic independent process. Similarly, brewing yeast cells that have been heat killed at 45°C lose membrane integrity, which enhances metal removal (Machado et al. 2009; Machado et al. 2008).

5.1.2 Yeast cell wall and metal biosorption

The *S. cerevisiae* cell wall has a thickness of 100–200 nm and represents 10–25% of the dry mass of the cell (Aguilar-Uscanga and François 2003). The cell wall surrounds the periplasmic space and cytoplasmic membrane, determines the morphology of the cell, confers mechanical stability, prevents lyses in hypotonic environments and plays a role in the retention of periplasmic enzymes (Klis 1994). The cell wall of *S. cerevisiae* presents a layered structure, composed of an amorphous inner layer and a fibrillar outer layer.

The inner layer consists mainly of β-glucan (a polymer of glucose) and is responsible for the mechanical strength of the cell wall. β-Glucans are composed of β(1→3)- and β(1→6)-linked glucose residues; β(1→3) glucan is the major structural component of the wall (30–45% of the wall mass) while β(1→6) glucan is relatively minor (5–10%) but very important for cross-linking. Chitin (linear chains of β(1→4)-linked *N*-acetylglucosamine) are present in small amounts in the wall (1.5–6%) and are predominantly located in the bud scars, although some are dispersed throughout the wall (Aguilar-Uscanga and François 2003; Cabib et al. 2001).

The outer layer is predominantly composed of α-mannan (a polymer of mannose). α-Mannan is highly glycosylated and associated with proteins (mannoproteins) and corresponds to 30–50% of the wall mass (Aguilar-Uscanga and François 2003; Klis 1994; Klis et al. 2002). The cell wall contains more than 20 different covalently anchored glycoproteins. These proteins seem to form an external protein coating that surrounds the inner polysaccharide layer (Klis et al. 2010). Several macromolecules present on the yeast cell surface allow the yeast cells to recognise matting partners, flocculate, form biofilms and grow pseudohyphally and invasively. Additionally, the external layer influences the global electric charge, hydrophobicity and wall porosity for the macromolecules (De Groot et al. 2005; Klis 1994).

The presence of the macromolecules, described above, allows yeast cells to display several functional groups that can interact with heavy metals. The external layer of the cell wall, which is mannoproteic in nature, seems to be more important for metal accumulation than the innermost layer of glucans (Brady et al. 1994b). Among the functional groups that have been implicated in surface metal accumulation by yeast cells, carboxyl, amino, amide, hydroxyl, sulphydryl and phosphate groups should be highlighted. It has been reported that blocking of the amino, carboxyl or hydroxyl groups of the yeast cell wall reduces the accumulation capacity of Cu(II) (Brady et al. 1994a; Wang 2002); these facts strongly indicated that these functional groups participate in Cu(II) binding. The involvement of carboxyl, phosphate and amino groups in lead biosorption has been described (Ashkenazy et al. 1997; Parvathi et al. 2007). The use of infrared analyses has shown that dead brewing yeast cells exposed to Cu, Ni and Zn displayed a decrease in the peaks of fingerprinting regions. These data suggest the participation of carboxyl, amino, hydroxyl and amide groups of protein and carbohydrate fractions (most likely from mannoproteins, glucans and chitin) of the cell wall in yeast metal uptake (Machado et al. 2009).

Different mechanisms have been described for the biosorption of heavy metals, such as ion exchange and complexation. Biosorption by a complexation mechanism can be explained according to the hard and soft acids and bases (HSAB) concept. In this theory, metals are considered as hard or soft acids while ligands are characterised as hard or soft bases. Therefore, in a competitive situation, hard acids tend to form complexes with hard bases and soft acids with soft bases. Additional information about HSAB theory can be found in a recent review (Naja et al. 2010).

As a consequence of the culture age, growth and nutritional conditions, changes in the composition and architecture of the yeast cell wall influence metal biosorption. Younger biomass (with a growth time of 12–24 h) were able to remove 4.6 times more uranium and twice as much silver than older biomass (96 h of growth) (Simon and Singleton 1996; Volesky and May-Phillips 1995). On the other hand, distinct cultural conditions entail different cell sizes (and consequently different surface areas for metal binding) and wall compositions. Yeasts cultured in a growth media supplemented with cysteine displayed a greater biosorption capacity for Pb(II), Cu(II), Cd(II) (Engl and Kunz 1995), Cr(VI) and Fe(II) than cells grown in a non-supplemented medium (Goyal et al. 2003). The addition of cysteine to the growth medium most likely increased the levels of sulphydryl groups involved in metal binding. The impact of different growth-limited conditions on Cd(II), Cu(II) and Ag(I) biosorption capacity revealed that K-, Mg- and C-limited conditions favour Cd(II) removal while K-limited cells bound the greatest amount of Cu(II). Phosphorus-limited cells displayed two different behaviours: they bound the greatest amount of Ag(I) and had the lowest sorption capacity for Cd(II). Sulphur-limited conditions had a negative impact on Cu(II) and Ag(I) removal (Dostalek et al. 2004).

5.2 Bioaccumulation

In the case of live yeast cells, after the initial biosorption step, a second, metabolism-dependent step (bioaccumulation) can occur. The bioaccumulation of heavy metals is slower, occurs only in metabolically active cells and is influenced by the temperature (inhibited by low temperature) and the presence of metabolic inhibitors (Blackwell et

al. 1995). Bioaccumulation can be greatly enhanced (threefold) in the presence of an external metabolisable energy source, such as glucose (Avery and Tobin 1992), and could be desirable for heavy metal removal from effluents (Mapolelo and Torto 2004; Stoll and Duncan 1996).

Another possibility for treating effluents can involve the application of a selected consortium of metal-resistant cells; this approach combines biosorption and continuous metal accumulation with the removal of organic loads from the effluent (Malik 2004). Recently, a *S. cerevisiae* mutant (*pmr1*Δ), which is hypersensitive to heavy metals due to increased uptake and has the enhanced ability (compared with wild type) to remove Mn(II), Cu(II) and Co(II) from synthetic effluents, has been described (Ruta et al. 2010). Similarly, over-expression transporters that are responsible for the influx of arsenite (Fps1p or Hxt7p) in *S. cerevisiae* displayed an enhanced (three- to fourfold greater) accumulation of the contaminant (Shah et al. 2010).

The transport of metal ions into yeast cells has varying specificity. Metals can be essential for growth and metabolism (such as Na, Mg, K, Mn, Fe, Co, Ni, Cu and Zn) or non-essential; in the latter case, these metals can compete with the essential metals for transport systems. Generally, divalent cations appear to enter the cell as a result of an electrochemical gradient generated by the activity of H^+-ATPase of the plasma membrane (Gadd and Sayer 2000). In yeast cells, metals can also be also internalised via pores or channels (Gadd 2009).

The intracellular accumulation of metals leads to cell death unless a detoxification process is triggered. Several mechanisms can be involved in normal metal homeostasis. These mechanisms embrace sequestration by metal-binding molecules (such as the tripeptide glutathione (L-γ-glutamyl-L-cysteinyl-glycine) and low molecular weight, cysteine-rich proteins (metallothioneins)), compartmentalisation in the vacuole and regulation of transport and efflux mechanisms (Gadd 2010; Gadd and Sayer 2000).

5.3 Assessment of the performance of metals removal

The evaluation of metal removal performance by specific types of biomass can be performed using kinetics and equilibrium studies. Kinetics studies allow us to obtain information about the temporal evolution of metal accumulation by the biomass and establish the equilibrium time for adsorption isotherms; from a practical point of view, these studies are useful because they provide information on the contact time required by the biomass to remove metals from the effluents. Rapid metal accumulation by the microbial biomass is desirable to provide a short contact time between the metal bearing solution and the biosorbent material.

The equilibrium biosorption of the metals by yeast biomass follows an adsorption isotherm. The application of mathematical biosorption models, such as Langmuir and Freundlich adsorption isotherms, to equilibrium studies allows us to determine two important parameters: the maximum possible amount of metal ion adsorbed per unit weight of biomass and the affinity constant between the binding sites of the biomass and a specific metal at a defined pH value. For a more detailed description of the quantification of metal-biomass interactions, please see a recent review by Naja et al. (2010).

6 Chemical speciation as a tool for predicting and optimising metal removal

Chemical speciation studies can be a useful and powerful tool for the prediction and improvement of heavy metal removal efficiency by yeast biomass (Machado et al. 2010a).

The efficiency of bioremediation process on metal ions from effluents is dependent on both the chemical speciation of the metal ions present in the effluent and the accumulation parameters of the biomass used (Machado et al. 2010a). For this purpose, previous information about the accumulation parameters of the biomass should be available or determined according to well-described models.

As mentioned above (section 4), the major driving force behind the efficient bioremediation treatment of an effluent containing metal ions is the relative affinity of each metal for the biomass versus the presence of several soluble ligands (inorganic and organic ones) in the effluent, which can compete with the biomass (Fig. 1). For a specific metal–biomass interaction, a higher affinity constant results in more efficient bioremediation removal.

Chemical speciation studies are usually performed by considering the total concentrations of metals and ligands present in solution, as well as the metal hydroxide solubility product constants and all of the affinity constants between the heavy metals and the ligands. For a known concentration of metal, these studies predict the fractions of the metal that remain soluble in the effluent (as free metal ions and associated with the different ligands) and associated with the biomass (Fig. 2). In this way, computer chemical simulations, where the amount of biomass is manipulated, can predict the best concentration of biomass that should be used to obtain a suitable biosorption of metals under specific conditions.

Metal chemical speciation has been used to study the biosorption of Cd, Cu, Ni, Pb and Zn by the marine brown alga *Fucus spiralis*. In this study, the predicted (theoretical) sorption values were compared with the experimental

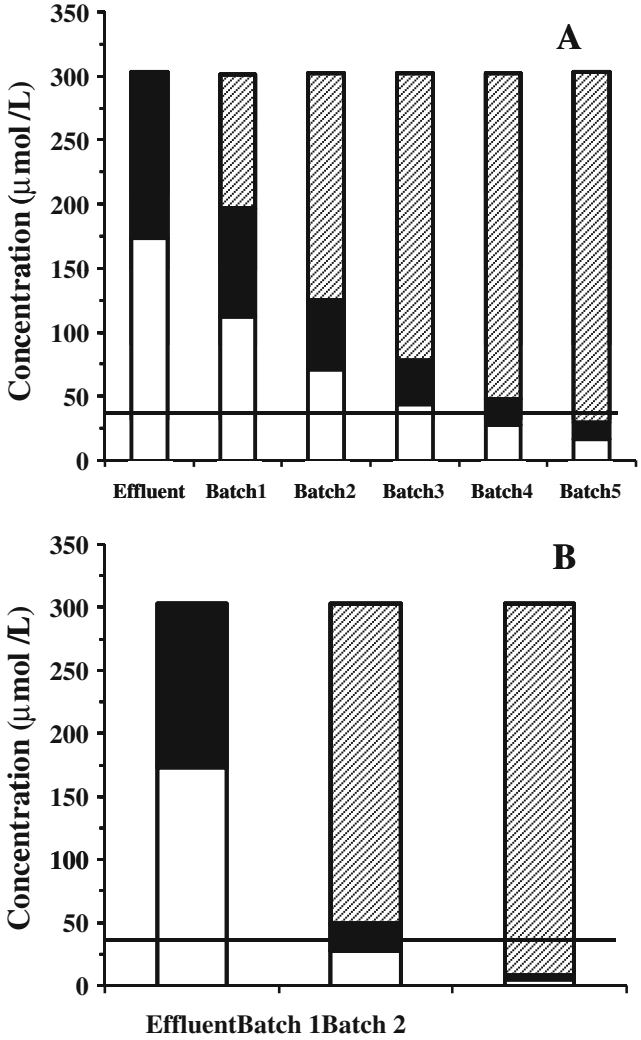

Fig. 2 Simulation of the influence of yeast biomass concentration on the nickel removal from a real electroplating effluent. Biomass concentration was optimised to treat a real effluent with a minimum number of batches: **a** 1.5 or **b** 12 g (dry weight)/L of yeast biomass. The electroplating effluent contained nickel above the limit discharge criteria and sulphate as the main inorganic ligand. Free nickel ions (*white part of the bar*); nickel associated with inactivated *S. cerevisiae* NCYC 1364 (*dashed part of the bar*); nickel associated with sulphate (*black part of the bar*). The effluent was treated when the concentration of free nickel ion plus nickel associated with sulphates was equal to or below the Portuguese wastewater discharge criteria for nickel (34 μmol/L) (*solid line*). Adapted from Machado et al. 2010c

values (Romera et al. 2008). The influence of the pH and chloride concentration on the sorption capacity of the macroalga *Cystoseira baccata* (Herrero et al. 2005) or of sulphate-reducing bacteria (de Vargas et al. 2004) was also simulated by chemical speciation. Recently, Machado et al. (2010a) predicted the impact of inorganic ligands (carbonates, chlorides, fluorides, phosphates, nitrates and sulphates), which can be found in real electroplating effluents, on the metal bioremediation efficiency of brewing yeast cells of *S. cerevisiae* and compared that with experimental data.

The treatment of real effluents containing multiple elements is a complex task due to the possible presence of multi-ligands and multi-metal complexes. Metal ions can compete for the same biomass-binding sites. Therefore, the displacement of one metal species by another that has a higher affinity for yeast-binding sites can occur (Brady et al. 1994a). For an efficient bioremediation process, the biomass concentration, when compared with the total amount of metals, should be in excess to have sufficient available biomass sites for all metals, including those with a lower affinity for the biomass. Copper, chromium, nickel and zinc are the metal ions most widely found in electroplating effluents; the order of the affinity constants between *S. cerevisiae* yeast cells and these metal ions are as follows: Cu>Cr≅Zn>Ni (Ferraz and Teixeira 1999; Machado et al. 2009).

As mentioned above, chemical speciation studies can be used to optimise the experimental conditions needed in the bioremediation process. For this purpose, chemical speciation simulations, where the concentration of biomass was manipulated, have been performed to predict the suitable amount of yeast cells needed to reduce the number of batches required to efficiently remove the heavy metals present in real electroplating effluents (Machado et al. 2010a; Machado et al. 2010b). Figure 2 shows that the chemical speciation studies have predicted that 1.5 g dry weight l^{-1} of yeast biomass would only allow the bioremediation of the effluent after the fifth batch while 12 g dry weight l^{-1} of yeast cells can treat the effluent after the second batch (Fig. 2). These results clearly show how chemical speciation can be an important tool for predicting and optimising the best experimental conditions for removing heavy metals from effluents.

7 Treatment of effluents

7.1 Treatment with yeast cells

Flocculent brewer's yeast biomass has a high potential of application for the bioremediation of industrial effluents because it combines the ability to remove metals with easy and rapid biomass separation. Basically, two approaches can be considered: (1) the use of yeast biomass to treat the effluent or (2) the use of yeast cells in a polishing step after the chemical treatment (such as metal alkaline precipitation or electrolytic recovery) of the raw effluent to remove the bulk of the metal.

Previously, an important issue to consider is which type of cells should be used: live or non viable yeast cells. The advantages of using dead biomass for industrial applications have been emphasised by some authors. Dead yeast cells are immune to the toxic effects of heavy metals, do not

require a nutrient supply, are less affected by extreme pH and temperature conditions and are easy to store as dried biomass (Gadd 1990; Simmons et al. 1995). Biomass and/or metal recovery is also easier to perform with dead biomass (Bishnoi and Garima 2005) (see below, section 9).

Most experiments that have used *S. cerevisiae* cells have been performed in synthetic solutions containing only one heavy metal element (Cojocaru et al. 2009; Machado et al. 2008; Matis et al. 2003; Özer and Özer 2003; Zouboulis et al. 2001); therefore, these studies are far from a commercial context. As a rule, real effluents are more complex and much more difficult to treat. Recently, the difficulty in treating real effluents due to the complex composition of the effluents was recognised; the efficiency of copper removal in real electroplating wastewater was lower than that obtained with synthetic wastewater (aqua solutions) (Cojocaru et al. 2009).

Another important point is related to the type of metals present in the effluent. *S. cerevisiae* biomass has a much lower affinity for nickel than for copper (five times less) and chromium or zinc (two times less) (Machado et al. 2010a). This means that the efficiency of bioremediation of effluents containing nickel, among the other three metal ions (chromium, copper and zinc), will be determined by the nickel affinity (Machado et al. 2010a).

It is of great importance to bridge the gap between the theoretical studies performed with synthetic solutions and the real application of yeast cells in the treatment of industrial effluents. In this sense, Duncan et al. have attempted to treat real electroplating effluents with *S. cerevisiae* biomass using three different electroplating effluents containing the following metals: (a) Cd (16 mg/L) and Zn (260 mg/L); (b) Cr (71 mg/L) and Zn (460 mg/L); or (c) Cr (4 mg/L), Cu (30 mg/L) and Ni (15.6 mg/L). These effluents were treated in a batch mode using live cells (Stoll and Duncan 1996). Another study used formaldehyde cross-linked yeast cells that were fixed in bed-columns to treat an electroplating rinse effluent containing 30 mg/L Cr (VI) (Zhao and Duncan 1998). In both cases, treatment of the effluents was unsuccessful. Recently, an electroplating effluent containing a high nickel concentration (18 mg/L) was treated with flocculent brewing heat-inactivated biomass in a batch mode. In this study, after the second batch of yeast biomass, the treated effluent attained the quality criteria for industrial effluent discharge according to the U.S. Environmental Protection Agency, corresponding to a nickel removal of ~85% (Machado et al. 2010a).

7.2 Treatment with a hybrid process

As reported above, another approach to treat real effluents loaded with multiple heavy metals combines chemical precipitation with the subsequent use of a biotechnologically based process (use of yeast cells).

Frequently, industrial effluents have very low pH values (approximately pH 2); therefore, these effluents cannot be directly discharged without pH correction. Simply adjusting the pH of the industrial wastewaters containing heavy metals to 6, several objectives can be met: (1) pH 6 is close to neutrality, and this pH value does not affect the environment and meets the wastewater discharge criteria; (2) at this pH value, if effluents contain large amounts of Cr (III) and Cu (II), they partially precipitate as metal hydroxides (Machado et al. 2010b; Stoll and Duncan 1997); consequently, more biomass will be available to remove other metals like Ni and Zn; (3) at this pH, the efficiency of the biotechnologically based process is improved because the main chemical-binding groups on the yeast cell surface are already totally deprotonated (as previously discussed in section 4).

Stoll and Duncan (1997) have described the treatment of an acidic (pH 1.5) electroplating effluent containing excess of Cd (20–80 mg/L), Cr (60–70 mg/L) and Zn (1,000–3,500 mg/L), according to US-EPA criteria (US-EPA 1984). After chemical treatment (pH adjustment to ~6) and settling of the sludge, the Cd (20–60 mg/L) and Zn (300–800 mg/L) remained in solution. Immobilised, non-viable S. *cerevisiae* biomass was used in continuous-flow stirred bioreactors to remove the remaining heavy metals; however, full treatment of the effluent was not achieved.

Recently, two real effluents containing multiple elements (Cu, Ni and Zn; or Cr, Cu, Ni and Zn) have been effectively treated using a hybrid technology (Machado et al. 2010b). This process combined chemical precipitation at pH 6.0 with a subsequent biotechnologically based process (use of heat-killed flocculent yeast cells of *S. cerevisiae*, in batch mode). The raw effluents had a pH ~2 and had metal (Cr (mainly in the form of Cr(VI)), Cu, Ni and Zn) concentrations above the limits for discharge allowed by the US-EPA and Portuguese law. In the case of the effluent containing Cr, Cu, Ni and Zn, Cr(VI) was previously reduced to Cr(III) before the pH adjustment. As a consequence of the increased pH, a significant amount of the metal(s) (81 % of the Cu) was removed from the first effluent, and approximately 40% of Cr(III) and Cu was removed in the second effluent. Subsequent treatment with inactivated yeast cells, after the third batch of biomass, lowered the metal concentrations below the legal limits of discharge according to the US-EPA and Portuguese law; removals of ≥89%, 91%, 92% and 94 % were attained for Ni, Cu, Cr and Zn, respectively (Machado et al. 2010b).

For effluents containing both Cr(III) and Cr(VI), two successful strategies can be pursued: (1) reducing Cr(VI) to Cr(III), adjusting the pH to 6.0 and treating with a serial batch of yeast biomass (Machado et al. 2010b); (2) selectively removing Cr(VI) (98 %) by yeast biomass at pH 2.3, increasing the pH of the effluent to 6.0 and using

serial batches of yeast cells for the bioremediation of the industrial effluent (Machado et al. 2010d).

8 Yeast separation from treated effluent

8.1 Solid–liquid separation processes

Different reactor configurations, such as continuously stirred tank reactors, fixed-bed reactors and fluidised bed reactors, have been proposed for the bioremediation of industrial wastewater loaded with heavy metals. A description of these configurations can be found in the book by Volesky (2003).

Microbial biomass has a small size and density. As a result, when yeast cells are used in stirred tank reactors, which operate in batch or continuous mode, a solid/liquid separation stage is required for the effluent. Unit operations like sedimentation, filtration or centrifugation can be used to separate the cell biomass from the treated effluent. However, these procedures tend to be time consuming or expensive. Sedimentation requires large settling tanks and extensive retention times to obtain a cell-free supernatant while filtration and centrifugation have high costs associated with capital investments for equipment, maintenance and power demands. These facts hinder or limit their use in remediation technologies. In the case of filtration, filter blockage can occur (Soares 2011). Flotation has also been described to aid in separation (Zamboulis et al. 2011). This separation process is based on the affinity of cells to air bubbles; bubbling air through the medium leads to an accumulation of cells on the surface where they can be removed. Although this cell separation process is relatively fast and efficient, it requires the addition of ionic surfactants and the generation of bubbles (which requires energy input) to raise the particles to the surface.

8.2 Yeast immobilisation

Different cell immobilisation techniques have been proposed to overcome the problem of solid–liquid separation and the limitation of the type of reactor to be used in the treatment of effluents. Immobilisation techniques include the encapsulation of cells in a polymer gel matrix, adsorption to an inert support, covalent bonding to carriers and cross-linking of cells (Soares 2011). The immobilisation of yeast cells presents several advantages: it facilitates handling, helps the solid/liquid separation process and improves scale-up, and it expands the use of the cells in packed or fluidised-bed reactors.

In addition to the costs associated with the support material and skilled supervision, the immobilisation of yeast cells also presents some disadvantages, including mass transfer limitations, unsuitability at high pH, a decrease in cell viability due to the immobilisation process, a decrease in the metal adsorption properties of the biomass and poor kinetic characteristics as compared with the same biomass in a non-immobilised form (Cassidy et al. 1996; Tsezos 1990).

8.3 Use of flocculent cells

Flocculation is a spontaneous and reversible process of aggregation of yeast cells into multicellular masses, called flocs, which rapidly settle in the medium where they are suspended (Fig. 3). Yeast flocculation has been traditionally exploited in the brewing industry; however, this characteristic is also useful in the production of other alcoholic beverages (wine, cachaça and sparkling wine) and renewable fuels (bio-ethanol) and in modern biotechnology processes (production of heterologous proteins) (Soares 2011). In the last several years, the exploitation of this property in the bioremediation of heavy metals has also been attempted. In this context, flocculation emerges as an off-cost process of solid–liquid separation in synthetic and industrial effluents containing heavy metals (Machado et al. 2008; Soares et al. 2002)

According to the lectin-like mechanism, which is widely accepted as the *S. cerevisiae* flocculation model, a specific lectin-like protein (also called flocculin, adhesin or zymolectin), which is only present on the flocculent cells, recognises and interacts with the surface carbohydrate residues of the α-mannans (receptors) of neighbouring cells (Soares 2011). Recently, it has been shown that calcium ions are directly involved in the binding of flocculin to the carbohydrate receptor and not indirectly involved in the stabilisation of the conformation of the flocculation lectin (Veelders et al. 2010).

Yeast flocculation is a complex process influenced by multiple factors, including the chemical characteristics of the medium and the expression of specific *FLO* genes (which encode the flocculation lectins), including *FLO1*,

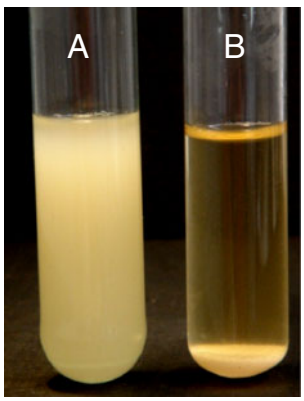

Fig. 3 Culture of a nonflocculent strain (*A*) and a brewing flocculent strain (*B*) of *S. cerevisiae*

FLO5, *FLO9*, *FLO10, FLO11* and *Lg-FLO1*. In addition to the *FLO* genes, recessive, flocculation activators and suppressor genes have been described (Soares 2011).

Flocculent cells of *S. cerevisiae* can aggregate in single or multi-element solutions containing the most common heavy metals found in industrial wastewaters (Zn(II), Cu (II), Cd(II), Ni(II) or Cr(III)) (Machado et al. 2008; Soares et al. 2002), except for Pb(II), which inhibits yeast flocculation (Gouveia and Soares 2004); however, small amounts of calcium (an element usually found in effluents) are enough to alleviate the inhibition induced by Pb. This means that flocculation of yeast cells occurs in industrial effluents. Flocculation is an easy, eco-friendly, fast and efficient process of cell separation, and it avoids the costs of equipment and energy demands associated with solid–liquid separation; more than 95% of the cells can be settled within five minutes (Machado et al. 2008). Flocculent yeast cells can be used in the treatment of effluents under different reactor configurations of suspended biomass; they can operate in batch or continuous mode without the risk of biomass washout; these reactors can be employed on a large scale with low operational and maintenance costs.

In addition, it was found that flocculent yeast cells have a higher ability to accumulate Cu(II) than non-flocculent cells. The most likely explanation is that heavy metals occupy the lectin Ca-binding sites and consequently increase metal removal (Soares et al. 2002).

9 Regeneration or destruction of yeast biomass?

After the industrial effluents have been treated with yeast cells, the problem of heavy metal pollution has only partially been solved. In fact, heavy metals were only transferred from the liquid phase (effluent) to the yeast biomass. Like in the alkaline treatment, this procedure creates a problem with the appropriate disposal of this toxic yeast biomass. Different strategies can be used regarding toxic biomass management: (1) metal desorption from the biomass, (2) biomass dissolution and (3) biomass incineration.

Metal desorption from the yeast cells can lead to the regeneration of biomass and simultaneous reclamation of the valuable metals. Theoretically, this process is desirable to reduce costs. As a result, the majority of the work on this subject has centred on testing different desorption agents. However, in the case of live cells, due to intracellular accumulation, metals can only be recovered by destructive processes (Bishnoi and Garima 2005). In an analogous process to ion exchange resins, metals and biomass are easier to recover when dead biomass is used. Ideally, the desorption agent should be cheap, induce minimal damage to the biomass (the biomass should recovery its original biosorption capacity without physical changes in order to be reused several times) and should efficiently desorb the maximum amount of metals with a minimum volume of the eluant; the complete cycle (biosorption and desorption) should concentrate the metal solution by a factor of 100 or more (Naja et al. 2010).

The methods for biomass regeneration consist of diluted (0.1 mol/L) mineral (HCl, HNO_3 and H_2SO_4) (Ferraz et al. 2004; Kordialik-Bogacka 2011; Padmavathy 2008; Strandberg et al. 1981; Wilhelmi and Duncan 1995; Wilhelmi and Duncan 1996) and organic acids (CH_3COOH) (Ferraz et al. 2004), bases (Na_2SO_4, Na_2CO_3, $CaCO_3$, NaOH, KOH), salts (KCl, $CaCl_2$) (Kordialik-Bogacka 2011; Strandberg et al. 1981; Wilhelmi and Duncan 1996) and a chelating agent (EDTA) (Ferraz et al. 2004; Kordialik-Bogacka 2011; Strandberg et al. 1981; Yu et al. 2008). However, some practical difficulties have been observed, including the production of damage to the yeast cells because of the desorption process, particularly when concentrated acids or prolonged exposure times are used, a reduction in yeast metal uptake ability during the successive biosorption/desorption cycles and, in some cases, a decrease in capacity to concentrate the metals in a small volume of solution (Ferraz et al. 2004; Kordialik-Bogacka 2011; Strandberg et al. 1981). These drawbacks strongly compromise the feasibility of desorption.

When biomass is inexpensive, like brewer's yeast cells, heat dissolution with strong acids or incineration followed by acid digestion of the ash is advantageous because it generates a concentrated solution of heavy metals. This concentration step is particularly important because it can determine the feasibility of metal recovery. In this context, the combustion of microorganisms and the subsequent recovery of the metals from ashes by acid leaching appear to be one of the best approaches. A fast and efficient reduction in the biomass contaminated with heavy metals by incineration at 550°C has been described; this process resulted in a 83% and 98% decreases in the biomass after 15 min and 4 h, respectively (Machado et al. 2010c). Incineration significantly reduces the amount of residue. The ashes that have been produced can be immobilised in an inert support or land filled. Alternatively, microwave-assisted acid digestion of the ashes containing heavy metals has been described (Machado et al. 2010c); with this procedure, a concentrated metal solution (~170 times the concentration of metals initially present in the effluents) was successfully achieved (Machado et al. 2010c). The metals can then be selectively recovered from the acid solution by separation processes, as described below (section 10).

Biomass dissolution, with strong acids and heat, can also be performed. However, accounting for the amount

of biomass to be digested, high volumes of acids will be required; this procedure results in more diluted heavy metal acid solutions (compared with the digestion of the ashes described above) and increases the costs of the process.

10 Selective recovery of metals

Compared with the large research efforts that have been dedicated to the development of biologically based alternatives for removing metals from industrial effluents, very limited attention has been given to metal recovery.

The development of methodologies to recover heavy metals is of great importance because it contributes to reducing the costs associated with the global process of purification of metal-bearing effluents. One of the most striking environmental benefits of secondary metal (and metal salt) production is the reduction in the amount of energy needed to produce a ton of metal (Dewulf et al. 2011). In addition, metal recycling also reduces mining and beneficiation activities that disturb ecosystems (Dewulf et al. 2011).

Industrial wastewaters are frequently loaded with heavy metals in a concentration high enough to pose serious environmental damage; however, these concentrations are too low to be directly recovered. Therefore, these heavy metals can be removed (concentrated) by yeast biomass and subsequently recovered for recycling; this procedure closes the cycle of metal use. The recovered heavy metals can be sold or re-used in industrial process. This is an important issue given the increase in metal prices and exhaustion of reserves. Additionally, financial benefits can be achieved no matter what the final destination of the metals.

The removal of Cu (>99%), by electrolysis at a controlled potential, from a mixture of Cu, Ni and Zn (Suzuki et al. 1995) or from a solution containing Bi, Cd, Co, Cu, Ni and Zn (Aydin et al. 1998) has been described. The selective electrodeposition of Cu, Pb, Cd and Zn with high purity (>99 mol%) from a synthetic solution of the metals in the chloride form has been shown (Doulakas et al. 2000). On the other hand, a different approach (sulphide precipitation) has been described by Fukuta et al. (2004) (quoted by Tokuda et al. (2008)); with this strategy, 94.5%, 65.9% and 75.9% of the Cu, Ni and Zn were selectively recovered. Further studies that also used sulphides improved the selective recovery of the same metals more than 95% (Tokuda et al. 2008). In a similar way, Cu, Ni and Zn were selectively precipitated with CaS from a simulated plating solution containing the metals at 100 mg/l. In this process, Cu was precipitated with a purity of 99.5% while Zn and Ni showed lower purities of 81.4% and 18.2%, respectively (Soya et al. 2010). The selective recovery of Cr, Cu, Ni and Zn was also attempted by sulphidation and Cr oxidation with H_2O_2 treatment of a plating sludge (Kuchar et al. 2010). Using this strategy, Cu and Zn were recovered with selectivities of 96.6% and 91.5% while Ni and Cr were recovered with lower selectivities (64.3% and 30%, respectively). The sequential precipitation of Al, Cd, Co, Cu, Fe, Mn, Ni and Zn as hydroxides or sulphides present in acid mine drainage has been described with a recovery ranging from 47.8% (Ni) to 100% (Zn) (Tabak et al. 2003). It is important to note that the use of sulphides implies the application of adequate safety procedures because this chemical species is toxic and corrosive. Additionally, the transport, storage and production of sulphides are expensive (Huisman et al. 2006).

Alternatively, heavy metals (Cu, Ni and Zn) in the acid solution obtained from the acid digestion of ashes from yeast cells used in the bioremediation of industrial effluents were sequential and selectively recovered with a high yield (>99% for all metals) and purity (92%, 99.4% and 99.9% for Ni, Zn and Cu, respectively) (Machado et al. 2010c). The process involves Cu electrolysis (copper is recovered as metal) followed by alkaline precipitation (Ni and Zn are sequentially recovered as metal hydroxides at pH 14 and 10, respectively).

Similar to the case of metal removal by yeast biomass, chemical speciation studies are useful in the design of the best conditions for metal recovery (Machado et al. 2010c). This strategy has been used to design a process for the selective recovery of four metals (Cr, Cu, Ni and Zn) after copper recovery by electrolysis (with a yield of 97.6% and a purity of ≥99.8%). The simultaneous oxidation of Cr(III) to Cr(VI) and increase in the pH to 14 was performed; under these conditions, the chemical simulations predicted that Ni should be precipitated as $Ni(OH)_2$, and Zn and Cr would remain in solution as $Zn(OH)_4^{2-}$ and CrO_4^{2-}, respectively. In fact, experimental data have shown that 87.9% of the nickel is recycled as a precipitate of $Ni(OH)_2$. A subsequent decrease in the pH of the solution to 10 resulted in 82.7% recovery of the total amount of Zn as $Zn(OH)_2$ while 95.4% of the Cr(VI) remained in solution as chromate (Machado et al. 2011b).

11 Closing the loop: the green process

The use of *S. cerevisiae* allows us to develop an environmentally friendly (green) process for depurating metal-bearing effluents by minimising the environmental hazard of these wastes. Additionally, a reduction in the energy consumption is achieved because of the flocculation characteristics of the biomass. As a consequence, the costs

associated with the solid–liquid separation of the biomass from the treated effluents are decreased. The bioremediation process can be completed because the metals that have been immobilised in the biomass can be selectively recovered and recycled. All of these points contribute to a more sustainable future.

For an effluent at pH ~2 that contains Cr(III), Cr(VI), Cu, Ni and Zn, the following primary steps of the global process can be used (Fig. 4): (a) reduction of Cr(VI) to Cr (III) and subsequent pH adjustment to 6; (b) bioremediation of the effluent by heat-inactivated flocculent brewing yeast cells; (c) separation of the biomass from the treated effluent by flocculation; (d) incineration of the contaminated biomass; (e) microwave-assisted acid digestion of the ashes; (f) recovery of copper from the concentrated acid solution by electrolysis at a controlled potential; (g) simultaneous oxidation of chromium(III) to chromium(VI) and alkalinisation of the solution up to pH 14, which leads to nickel recovery as $Ni(OH)_2$; (h) reduction of the pH of the solution to 10, which leads to zinc recovery as $Zn(OH)_2$ while chromium(VI) remains in solution as chromate.

This strategy combines the minimisation of the environmental hazard of industrial wastes containing heavy metals with an efficient and nearly closed cycle for the selective recovery of the different metals. Copper, nickel and zinc can be resold as pure copper or nickel and zinc hydroxides, respectively; alternatively, metals can be sent back and reused in the electroplating process.

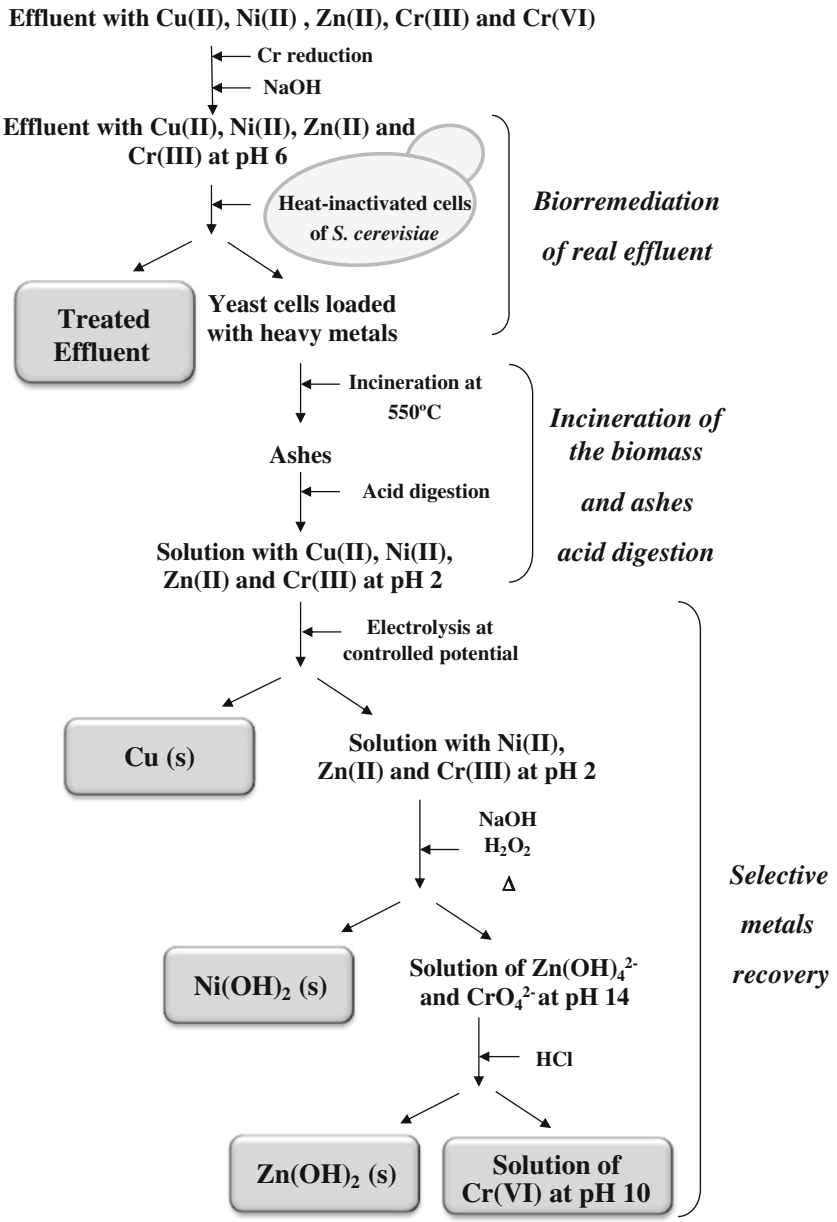

Fig. 4 Diagrammatic representation of a green process proposed for the bioremediation of heavy metals from industrial effluents and the selective recovery of metals. Adapted from Machado et al. (2010c) and Machado et al. (2011b)

12 Conclusions

S. cerevisiae yeast cells occur in large amounts as a by-product of industrial fermentations, such as from the brewing industry. The exploitation of the use of brewing yeast cells in the detoxification of industrial wastes containing heavy metals constitutes a new opportunity for the valorisation of this biomass. Heat-inactivated flocculent brewing yeast cells of *S. cerevisiae* have been shown to be effective in the treatment of real industrial wastes (Machado et al. 2010a; Machado et al. 2010b; Machado et al. 2010d). In addition, we can take advantages of the auto-aggregation properties of these yeast cells; flocculation overcomes the need for cell immobilisation or the use of a solid–liquid separation technique. Due to the flocculent properties of the yeast cells, sedimentation tanks, which are already available in the wastewater treatment plants of the major electroplating industries, can be used. The use of flocculent yeast cells can feasibly be engineered on a large scale and avoids the capital investment when changing from the conventional (chemical precipitation) to a biotechnologically based process, with or without a previous alkaline treatment step (hybrid process). The employment of a stirred batch tank reactor has the following advantages: simple design, operational stability and low operational and maintenance costs. Together, these are important characteristics of a low cost and competitive technology. However, if desired, flocculent yeast cells can also be used in continuous mode, in columns, without the risk of biomass washout.

Ideally, the bioremediation of industrial effluents should be linked with a selective recovery of the metals. Using a careful strategy as described above, it is possible to develop a nearly closed cycle (effluent-free process) for selectively removing and recovering different heavy metals with high yield and purity. The selective recovery of metals allows for companies to sell or recycle them for industrial processes without the generation of wastes. This strategy can be easily implemented and constitutes a positive approach to wastewater treatment because it combines the minimisation of environmental liabilities with financial benefits from reselling or recycling the metals.

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