Abstract  *Saccharomyces cerevisiae*NCYC 1190 cells accumulated (after 1 h) lead and cadmium at similar levels, and to a lesser degree also copper. During heavy metal accumulation, there was a considerable loss of viability of copper-treated cells (about 99% in the first 20 min of contact with the metal), and a less pronounced lethal effect on cadmium- and lead-treated cells (about 66% and 46% after 1 h of contact with cadmium or lead, respectively) was detected. During copper accumulation, a leakage of UV-absorbing compounds and inorganic phosphate was observed; this did not occur with lead, whereas with cadmium a small amount of leakage of inorganic phosphate was detected. The filtrates of copper-treated cells contained copper-binding molecules. The copper-binding capacity of the filtrates increased with time according to the release of inorganic phosphate and UV-absorbing compounds. These compounds can bind an appreciable quantity of metal ions, making them unavailable for cell uptake and thus reducing the efficiency of heavy metals removal by yeast cells.

Introduction

Industrial countries are increasingly concerned with the release of large amounts of toxic heavy metals, coming from industrial waste waters, into the environment. Lead, cadmium and mercury are considered the “big three” heavy metals, when their toxicity and consequently their environmental impact are taken into account (Volesky and Holan 1995); in addition to these metals, copper is extensively used in industry and the increasing levels of this metal in the environment are cause of concern.

The use of biological materials as an alternative to classical physicochemical methods for the removal of heavy metals from water has received much attention in recent years, due to their effective performance and low cost (Gadd 1990; Volesky and Holan 1995). Different groups of microorganisms [algae, fungi (yeasts and molds) and bacteria] have been used for “collecting” many heavy metals (for a survey on microbial biomass used for metal binding see Volesky and Holan 1995). Yeast cells have been reported to be a mediocre metal biosorbent, when compared with algae and fungi (Volesky and Holan 1995). However, yeasts are an inexpensive source of biomass, since they are a by-product of large-scale fermentations. Yeasts accumulate a broad range of heavy metals to varying degrees under a wide range of external conditions (Avery and Tobin 1992; Brady and Duncan 1994a; Blackwell et al. 1995). Thus, they are economically attractive for the treatment of a wide variety of metal-bearing industrial effluents. Metal uptake by yeasts typically occurs in two steps: a metabolism-independent and a metabolism-dependent step. The first step (initial biosorption) is rapid and takes place during the first minutes of contact with the metal (Avery and Tobin 1992; Brady and Duncan 1994a). It is independent of metabolic energy (DeRome and Gadd 1987), temperature and metabolic inhibitors (Blackwell et al. 1995). The second step, designated bioaccumulation, is slow, metabolism-dependent and influenced by temperature and the presence of metabolic inhibitors (Blackwell et al. 1995).

Although many heavy metals (e.g. Cu, Fe, Zn, Co, Mn) are essential for metabolism at lower concentrations, many of them are toxic to organisms above specific threshold concentrations (Gadd 1993). A loss of viability (Ross 1977; Gadd and Mowll 1983; Joho et al. 1983; Gadd et al. 1984) and the release of $K^+$, $H^+$, $Ca^{2+}$
and Mg\textsuperscript{2+} (Norris and Kelly 1977; Gadd and Mowll 1983; Gadd et al. 1984; Kessels et al. 1985; De Rome and Gadd 1987; Belde et al. 1988; Avery and Tobin 1992; Brady and Duncan 1994b), as well as organic compounds (Joho et al. 1984; Ohsumi et al. 1988; Brady and Duncan 1994b) from the biomass during metal accumulation have been reported. However, the consequences of heavy metal toxic effects on the ability of yeast cells to remove metals have not been well-studied.

The purpose of this work was to follow the viability of \textit{S. cerevisiae} yeast cells and the leakage of low molecular mass compounds (UV-absorbing compounds and inorganic phosphate) during lead, cadmium and copper accumulation. Additionally, the complexing properties of low molecular mass compounds were studied, and the possible competition for metals between cells and these compounds and their influence on the overall heavy metal recovery process are discussed. These are important issues when considering the use of live yeast biomass in the removal of heavy metals from industrial effluents.

### Materials and methods

#### Strain and culture conditions

The ale-brewing strain of \textit{Saccharomyces cerevisiae} NCYC 1190 was used in this work. The strain was obtained from the National Collection of Yeast Culture (NCYC), UK. It was routinely maintained at 4 °C on YEPD agar slants, containing (per litre of water): yeast extract (Difco), 10 g; peptone (Difco), 20 g; glucose (Merck), 20 g; agar (Difco), 20 g.

The pre-cultures were prepared by inoculating a loop of yeast (from agar slant) in 40 ml of YEPD broth in 100-ml Erlenmeyer flasks. The cells were incubated 24 h at 28 °C on an orbital shaker (Braun Certomat S) at 150 rpm. Cultures were obtained by inoculating 0.5 l of YEPD, in 1-l Erlenmeyer flasks at a ratio of 1:50 (pre-culture/fresh growth medium). The flasks containing the cultures to remove metals have not been well-studied.

The ISE measurements consisted of titrations with Cu\textsuperscript{2+} ion. As titrant, 1.574×10\textsuperscript{-2} M of Cu(NO\textsubscript{3})\textsubscript{2} was used. The readings were registered after stabilization of the potential to less than 0.1 mV min\textsuperscript{-1}. From the total ([Cu\textsubscript{t}]) and free ([Cu\textsubscript{f}]) copper ion concentrations at each point of each titration curve, the complexation fraction is calculated from the equation re-